Assessment of beach-cast cetaceans in Pakistan: implications for conservation and management

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ABSTRACT

Until recently, little was known about the distribution and species occurrence of marine cetaceans in Pakistani waters, an area which needed to be addressed exigently given its inclusion in the Indian Ocean Whale Sanctuary. Boat-based surveys (2005–09) carried out along the coast of Pakistan identified 12 species of cetaceans. Although these surveys can be very useful for providing information on species presence and distribution, estimates of the age and sex of these groups can be more uncertain. Consequently, this present study undertook complementary beach-based surveys over the same period across all accessible regions of the Pakistani coast and created a community reporting scheme for stranded and beach-cast remains of cetaceans. Tissue samples and/or skeletal material were collected over three years from 37 individual specimens, with DNA successfully extracted from 24. Using molecular techniques, a total of seven species were identified and there was an indication that the majority of the samples were from males. An analysis of teeth collected from 12 beach-cast odontocetes showed an age range between neonatal and 17 years. The results of this study corroborate the presence of species observed during the boat-based surveys and identified a further three species. The data also provide additional information on age and sex. A comparison with similar studies suggests that the stranding rate is low in Pakistan. No mass strandings occurred during the seven year monitoring period. The results indicate that beach-based surveys are effective for gathering data on species presence in regions where resources are limited, the terrain is harsh and availability of data is low. Ultimately, the results of this work will help with assessing the conservation status and management requirements of the region's cetaceans, both locally and internationally with respect to the Indian Ocean Whale Sanctuary.

KEYWORDS: STRANDINGS; CONSERVATION; INDIAN OCEAN; SURVEY – SHORE-BASED; AGE DETERMINATION; SEX RATIO; GENETICS; INDIAN OCEAN HUMPBACK DOLPHIN; SPINNER DOLPHIN; INDO-PACIFIC FINLESS PORPOISE; BOTTLENOSE DOLPHIN

INTRODUCTION

Until recently, the only information available on cetaceans in Pakistani waters was a preliminary list of species based on a wide variety of *ad hoc* reports, reviewed in Gore *et al.* (2012). They confirmed occurrences, by boat-based surveys, of 12 species of cetacean, among which the most commonly observed were: spinner dolphins (*Stenella longirostris*); Indian Ocean humpback dolphins (*Sousa plumbea*); Indo-Pacific finless porpoises (*Neophocaena phocaenoides phocaenoides*) and bottlenose dolphins (*Tursiops* sp.). However, acquiring accurate estimates of the age and sex of these cetaceans is difficult using boat-based studies. Collins *et al.* (2002) reviewed reports on cetaceans beach-cast in Oman. Alfonsi *et al.* (2013) and Thompson *et al.* (2013) highlighted the use of DNA to confirm cetacean species identification and closer to Pakistan, Jayasankar *et al.* (2008) used molecular techniques to identify cetacean species in India. Reports on cetacean strandings in remote and/or previously undocumented regions (e.g. Collins *et al.*, 2002; Meirelles *et al.*, 2009; Norman *et al.*, 2004) have provided information on species lists and sex ratios, and on species that are rarely recorded (e.g. Gore *et al.*, 2007b; Thompson *et al.*, 2012).

The present study aimed to confirm species and sex of the remains of stranded cetaceans using molecular techniques and to provide an estimate of age for odontocetes by analysing teeth. The results are compared with those from boat-based surveys that occurred during the same period (Gore *et al.*, 2012). Pakistan's territorial seas are included within the Indian Ocean Whale Sanctuary[8] and the findings of this work are intended to help with the assessment of the conservation status and management requirements of the region's cetaceans.

MATERIALS AND METHODS

Study areas

The survey area incorporated the coast of Pakistan (Fig. 1). To the west the Balochistan coast extends for 800km and is dominated by high rocky cliffs interspersed with long sandy shores (Majid, 1988). In the east by contrast, the coast of Sindh is dominated by low lying sandy-muddy shores and by creeks and deltaic tributaries frequently colonised by mangrove forest (Meynell, 1999; Quraishee, 1988). Pakistan has a sloping continental shelf which is only 3km wide at the western border with Iran, widens steadily moving eastwards and extends to some 160km offshore near the border with India. Along the coast the terrain is harsh, with a wide temperature range between 0–48°C and in Balochistan it is

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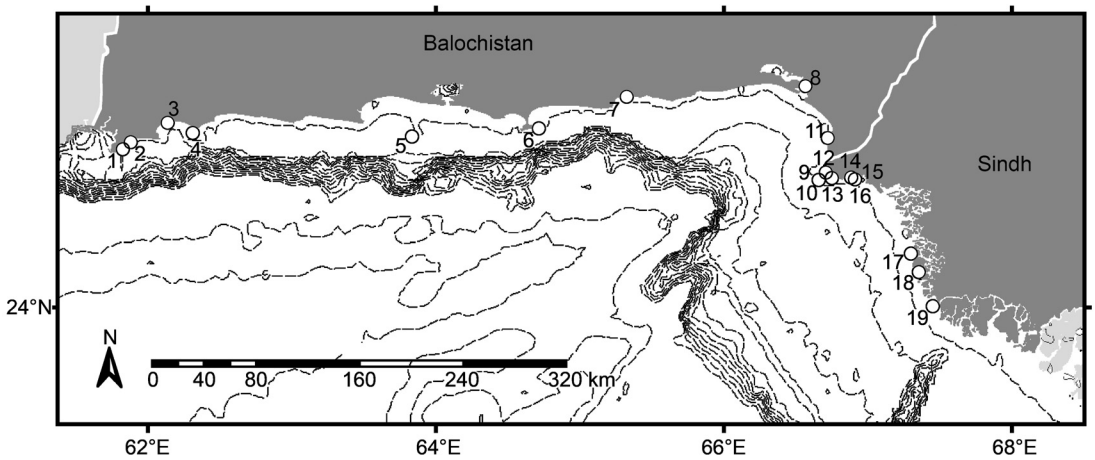

Fig. 1. Map of the Pakistani coast showing the locations where the cetacean remains were found. Iran to the west and India the east are shown as a lighter shade of grey; the border between the two coastal provinces of Pakistan (Balochistan and Sindh) is marked. Bathymetric contours are shown (at 10m, 50m, 100m, 200m, 300m, 400m, 500m, 600m, 700m, 800m, 900m, 1000m, 2000m, 3000m and 4000m). Based on the results of the genetic analyses 1: SD/BD(1); 2: LBCD(2); 3: BW(1); HBD(2), LBCD(2), BD(2); 5:HBD(2); 6:SD/BD(1); 7:LBCD(1); 8:BW(2); 9:SD(1); 10:LBCD(1); 12:PSD(1); 13:SD/BD(1); 14:BW(1); 15:BD(1); 16:BD(1); 17:BW(1); 19:HBD(1); where genetic data were not available (see Results) 4: FP(3), D(2); 5: D(2); 11: W(1); 13: D(1), W(1); 14: W(1); 16: FP(1); 18: D(1); the number in parenthesis indicates the number of specimens of each species recovered. FP = Indo-Pacific finless porpoise; BD = bottlenose dolphin; SD = spinner dolphin; HBD = Indian Ocean humpback dolphin; LBCD = long-beaked common dolphin; PSD = pan-tropical spotted dolphin; BW = Bryde's whale; D = unidentified dolphin species; W = unidentified whale species. Note that data point 5 is on an offshore island.

very arid with strong windstorms. Beyond the shelf edge off Balochistan, the seabed drops away rapidly to depths exceeding 1,000m off the western part of this coast, extending into the deeper waters of the Oman Abyssal Plain. Conversely, off the Sindh coast, the continental slope falls away gradually.

Beach-based surveys

Monitoring of strandings took place between 2004 and 2011, with quantitative beach-based surveys undertaken at points along the entire coastline between 2005 and 2008 to collect cetacean remains. From these remains, identification of species, age, sex and cause of death were made when possible. Soft tissue and skeletal material were obtained for age determination and genetic analysis for identifying sex, and to confirm species which was especially important in cases where the remains did not allow identification in the field. Survey sites were distributed across the length of the coastline, except for some areas which were inaccessible such as remote desert and mangrove locations or areas in Balochistan under military restrictions. For effort-based surveys, band transects were used to scan beaches for stranded cetacean remains. Teams of trained surveyors walked in line abreast the full length of each beach with individuals approximately 4m apart so that each surveyor scanned 2m to either side. A local team was trained over the monitoring period to identify beach-cast cetacean remains using information from papers by Geraci and Lounsbury (1993), Jefferson *et al.* (1993) and Reeves *et al.* (2002) for reference.

Community reporting scheme

To complement field surveys and involve local communities, a reporting scheme was also established using fishermen and community leaders in 74 fishing villages along the entire coast. It was requested that the research team was alerted, usually by funded telephone calls, in the event of stranded cetaceans being found. Samples were collected from cetacean strandings during effort-based surveys and incidental reports from local communities.

Sampling

The condition of the remains were categorised according to Rage (2002). Remains in good condition were inspected for any indication of the cause of death; however, most of the remains were considerably degraded and classed as condition 3 to 5. Field-based examination of external and skeletal/cranial morphology provided identification for some species, but positive species identification of severely degraded specimens could only be ascertained through DNA analysis, particularly where the training and experience of team members needed support.

When remains were found the date, time, state of decomposition and, where possible, species, sex and age class were recorded (Table 1). Tissue samples and skeletal specimens (including teeth) were collected when possible and stored in analytical grade 100% ethanol. Samples were kept out of direct sunlight in a laboratory at an ambient room temperature of 27–33°C. Subsequently, tissue samples and teeth were analysed at the Molecular Ecology Laboratories at Durham University, UK to confirm and/or identify the sex and species. The teeth were analysed at the Age Dynamics Laboratory, Denmark, to ascertain the age of the individuals. Morphometric measurements, meristics (such as tooth counts) and photographs collected in the field were used where possible to supplement the genetic analyses.

DNA extraction

DNA was extracted from soft tissue (n = 34) using standard phenol-chloroform protocols, as described in Hoelzel (1998). Where soft tissue was not available (n = 3), DNA was extracted from teeth. Sandpaper was used to clean the teeth of surface contaminating DNA. A variable speed *Dremmel* drill was used at slow speed to enter the pulp cavity of each

Table 1

A list of the data obtained from beach-cast cetaceans, giving a reference number and date. Details on the location where a sample was found are given in Fig. 1. Initial ID (species identification and estimated age) was based on visual examination *in situ*, whereas 'ID based on mtDNA' was based on mtDNA control region sequences 406–456bp in length. For sex: Y = Yes; N = No; [M] = Male (amplifying X and Y); M = Male with reduced confidence (amplifying Y, but not X); [F] = Female (amplifying X only); F = Possible female (not amplifying X or Y but amplification of *ca*. 400bp of mtDNA, see Methods); U = Unknown sex and/or species; Age from GLGs = age estimated from the annual deposits of the growth layer groups, + sign denotes the final age plus ≤ 11 months; *indicates that only teeth were available for these samples; superscripts indicate [0]individual as a calf, [1]individual was female, [2]individual was male, [3]the exact date the specimen was collected is not known.

Ref. Number	Date	Location	Initial ID	ID based on mtDNA	Sex	Age from GLGs
PAK 1	20/01/07	16	FP	–	U	–
PAK 2	01/11/09	4	FP[1]	–	U	–
PAK 3	18/11/09	4	FP	–	U	–
PAK 4	05/12/09	4	FP	–	U	–
PAK 5	07/12/06	16	BD	BD	[M]	–
PAK 6	03/01/08	15	D[0]	BD	[M]	–
PAK 7	22/05/08	7	BD	LBCD	F	–
PAK 8	18/03/09	2	SD	LBCD	M	–
PAK 9	19/03/09	9	SD	SD	M	–
PAK 10	19/03/09	10	SD	LBCD	M	–
PAK 11	13/02/06	6	HBD	SD/BD	M	–
PAK 12	15/05/08	17	U	BW	[F]	–
PAK 13	21/02/09	4	D	–	U	15
PAK 14	21/02/09	4	HBD	HBD	M	3+
PAK 15	07/05/08	8	W	BW	[M]	–
PAK 16	DD/MM/08[3]	8	W	BW	M	–
PAK 17	20/09/09	3	W[2]	BW	M	–
PAK 18	01/04/08	11	W	–	U	–
PAK 19	18/01/06	14	BW	BW	M	–
PAK 20	18/01/06	14	W	–	U	–
PAK 21*	19/11/06	13	W	–	U	8
PAK 22	19/11/06	13	D	SD/BD	F	6
PAK 23*	19/11/06	13	D	–	U	13+
PAK 24	16/02/07	5	D	HBD	M	–
PAK 25	16/02/07	5	D	HBD	F	–
PAK 26	16/02/07	5	D	–	U	–
PAK 27	16/02/07	5	D	–	U	–
PAK 28	07/03/08	12	D	PSD	[F]	–
PAK 29	07/05/08	19	HBD	HBD	[M]	3
PAK 30*	08/05/08	18	D	–	U	17
PAK 31	12/02/09	4	D[0]	–	U	0+
PAK 32	21/02/09	4	D	LBCD	M	14
PAK 33	21/02/09	4	D	BD	M	5
PAK 34	21/02/09	4	D	LBCD	M	2+
PAK 35	21/02/09	4	D	BD	M	0+
PAK 36	23/03/09	2	D	LBCD	M	–
PAK 37	20/03/09	1	D	SD/BD	M	–

tooth and *ca*. 1g of displaced powder was collected for DNA extraction. Powder was digested overnight in 0.5ml of buffer (50mM Tris pH7.5; 500mM EDTA; 100mM NaCl and 1% w/v SDS) with 50µl of proteinase K (20mg ml^{-1}). Digestions were constantly agitated and incubated at 50°C. QIAquick PCR purification columns (Qiagen, BmbH, Germany) were used to perform DNA extraction. To prevent aerosol contamination, the procedure was conducted in a dedicated laboratory under a laminar-flow hood, separate from laboratories performing PCR reactions and working with high concentrations of modern DNA. All equipment and reagents used were regularly sterilised and decontaminated. Disposable gloves and protective clothing were also worn throughout the procedure.

Species identification

Amplifications of 520bp from the mitochondrial DNA control region were performed in a 20µl final reaction volume containing approximately 0.5µg of template DNA, 1.25U of Go*Taq* Flexi DNA polymerase, 10× buffer (Promega), 0.2mM dNTPs, 2mM $MgCl_2$ and 0.2µM of each primer; TRO (L15812) 5' CCT CCC TAA GAC TCA AGG AAG 3' (developed at the Southwest Fisheries Science Centre; see Zerbini *et al.*, 2007) and D (H16498) 5' CCT GAA GTA AGA ACC AGA TG 3' (see Rosel *et al.*, 1994). The PCR profile included initial heating at 95°C for 2 minutes, followed by 35 cycles of 95°C for 40 seconds, annealing temperature of 60°C for 40 seconds and 72°C for 1 minute, and a final 72°C extension for 10 minutes. Sanger sequencing was undertaken using PCR product run on an ABI automated sequencer.

To identify species, a web-based phylogenetic analysis tool *DNA Surveillance*[9] was used as described in Ross *et al.* (2003). This method involves an online database of 121 reference cetacean mtDNA control region sequences to identify species based on their clustering within a neighbour-joining phylogenetic reconstruction.

Sexing

Samples were sexed by PCR using methods described in Fain and LeMay (1995). ZFX and SRY specific primers were: (P15EZ) 5' ATA ATC ACA TGG AGA GCC ACA AGC T 3'; (P23EZ) 5' GCA CTT CTT TGG TAT CTG AGA AAG T 3' and (Y53-3c) 5' CCC ATG AAC GCA TTC ATT GTG TGG 3'; (Sry Y53-3d) 5' ATT TTA GCC TTC CGA CGA GGT CGA TA 3'. Reactions were carried out in a 20–30µl final reaction volume containing approximately 0.5µg of template DNA, 1.25U of Go*Taq* Flexi DNA polymerase with 10× buffer (Promega), 0.2mM dNTP, 1.5–1.8mM $MgCl_2$ and 0.2µM of each primer. The PCR profile was 94°C for 3 minutes, followed by 35 cycles at 94°C. A multi-tube gradient annealing temperature ranging from 51–60°C for 45 seconds was also carried out on particularly degraded samples. An elongation step at 75°C for one minute, was followed by a final 72°C elongation step for 5 minutes.

Ageing

To assess age, odontocete teeth were analysed as outlined in Lockyer (1993; 1995). The teeth were sorted by size; large teeth were first trimmed using an Isomet circular diamond saw. A thick central wafer in the plane from crown to root of about 2.5–3mm thickness was made. Where necessary, a thin section of about 150µm was cut centrally from eight of the 12 specimens. All other teeth were mounted similarly on a block and sliced with the Isomet circular diamond saw. The resulting tooth wafers were fixed in 10% neutral buffered formalin for several hours, and then rinsed further for several hours in water. Subsequently, the samples were decalcified in RDO (a proprietary brand decalcifying agent produced by Apex Engineering, Illinois, USA) for a period of several hours as specified by the manufacturer, rendering the teeth to a rubbery texture. These were rinsed for several hours in water and then mounted on the freezing stage of a microtome using Cryoembed, a water soluble mountant, followed by

[9] *http://www.dna-surveillance.fos.aukland.ac.nz:23060/page/whales/title*

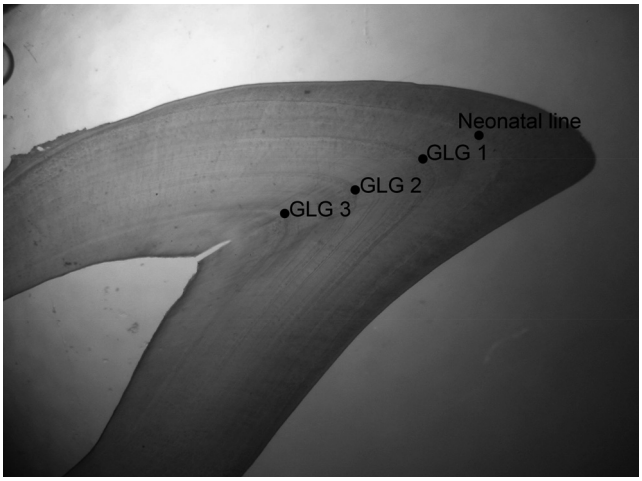

Fig. 2. Stained tooth section of humpback dolphin (PAK 14), showing three clear Growth Layer Groups (GLG), the neonatal line, and several less pronounced accessory lines in the dentine.

sectioning 25µm thicknesses close to the centre of the tooth. The foci for sectioning were the crown, root and as much of the pulp cavity as possible. The sections were stained in histo-cassettes using ripened Ehrlich's acid haematoxylin stain for 15 minutes, then rinsed in water and blued in weak ammonia solution for a few seconds. Consequently, selected sections were floated onto slides that were previously coated with 5% gelatine. After drying, the slides were mounted with DPX under a glass cover slip and the DPX hardened over several days. Finally, all sections (including untreated sections from eight of the animals) were examined under a low power binocular microscope at magnifications from ×10–40 using transmitted plain white light to determine the number of Growth Layer Groups (GLG) in the dentine (Fig. 2). The sections were read several times by CL, who did not have access to any additional data for these specimens. The final age was taken as the median count subject to further checking with reference to image analyses when required. On these occasions, a *Nikon Coolpix* 4500™ camera was used on the microscope to capture images of the teeth.

RESULTS

Species occurrence

Beach-based surveys in Sindh covered 24.8km from six sites ranging between Hawkes Bay and Khobar Creek. In Balochistan, there were 18 sites covering 11.3km ranging between Jiwani to Mubarak Village. Much of Sindh and Balochistan are very remote or under military exclusion; so no repeat surveys were undertaken. All of the specimens examined during the present study were found dead. Samples from a total of 37 stranded cetaceans were collected between December 2005 and July 2008 (Table 1; Balochistan $n = 19$, 60%, and Sindh $n = 18$, 40%) and found at sites noted in Fig. 1. The strandings rate was approximately $9.3 yr^{-1}$ and $0.035 km^{-1}$ of coastline.

Fourteen specimens were found during standardised effort-based (beach) surveys and 23 were reported by fishing communities. Field-based species identifications included bottlenose dolphins (*Tursiops* sp.), long-beaked common dolphins (*Delphinus capensis tropicalis)*, spinner dolphins (*Stenella longirostris*), Indian Ocean humpback dolphin, Indo-Pacific finless porpoise and Bryde's whale. Six of these animals (four Indo-Pacific finless porpoise and two Bryde's whales) were relatively fresh with a stranding condition of 2, while the remaining specimens were highly decomposed with a stranding condition of 3–5, making it difficult to determine positive species identification from external morphology alone (Table 1).

DNA extraction

Many of the samples yielded only degraded DNA, but nevertheless 520bp of mtDNA was successfully amplified from 24 specimens (Table 1). Readable sequences ranged from 406–456bp in length.

Species identification

Phylogenetic analysis using the DNA surveillance web facility was performed on the sequences derived from these 24 specimens. Where species identification in the field conflicted with the molecular identification, the latter was taken as correct. Species identified were 4 bottlenose dolphins (*Tursiops* sp.), 6 long-beaked common dolphins, 1 spinner dolphin, 4 Indian Ocean humpback dolphins, 1 pan-tropical spotted dolphin (*Stenella attenuata*) and 5 Bryde's whales (Table 1). For three specimens, species identification could not be completely resolved using this method (see Discussion below).

Sexing

Due to high DNA degradation and low concentrations of nuclear DNA, multiple PCR attempts were required. Four of the 24 individuals proved to be male with a further 15 as putative males (Table 1). Two individuals were female, and the remaining three samples did not amplify ZFX or SRY fragments; consequently, these three individuals were recorded as putative females.

Ageing

The age estimated for the 12 sets of dolphin teeth ranged from neonate to 17 years, with the majority of the individuals being less than 10 years old (Table 1). The teeth generally showed clear GLGs, as illustrated in Fig. 2. While accessory lines occurred, these did not interfere with the clear GLGs or reading the age of the specimens. The species (as determined by identification in the field and DNA analysis) in which ageing was possible included 3 Indian Ocean humpback dolphins, 2 bottlenose dolphins, 1 bottlenose or spinner dolphin, 2 long-beaked common dolphins and 4 unidentified dolphin species (Table 1).

DISCUSSION

Due to high intra-specific variability in the mtDNA control region and the recent radiation of some delphinid taxa (including *Stenella*, *Tursiops* and *Delphinus*) it can be difficult to distinguish between species (Dizon *et al.*, 2000). Bootstrap support values are often very low, thus reducing confidence in identification. Along the Sindh and Balochistan coast, the large variation in temperature, very strong drying wind and wet monsoon conditions[10] are the

[10] *www.pmd.gov.pk/.*

likely cause for the substantial amount of DNA degradation observed in the samples collected. Such conditions would be beneficial to microorganisms and their metabolites degrading DNA. Furthermore, exposure of surface tissues to ultra-violet radiation will shear DNA. When storage methods are sub-optimal post-sampling, DNA can continue to degrade (Burger *et al.*, 1999). The DNA degradation observed in this study is likely to be a combined result of environmental conditions pre-sampling and sub-optimal sample storage post-sampling.

The identification of *Tursiops* species was of particular interest as two species are found in the Indian Ocean, *T. truncatus* and *T. aduncus* (Hale *et al.*, 2000). From qualitative observations during boat-based surveys (Gore *et al.*, 2012) it was noted that bottlenose dolphins were relatively large and that there were no other obvious morphometric differences between individuals seen inshore and offshore. Nevertheless, in the present study it was not possible to distinguish between these species. In the case of the Indo-Pacific finless porpoises, species could not be identified using molecular techniques as the DNA was highly degraded, possibly post-sampling due to relatively fresh stranding state. However, given the distinct morphology of the Indo-Pacific finless porpoise (compared to other species in the region) and the condition of the remains, we were certain of the species from the visual inspection.

While cetacean population genetic structure is unlikely to be confirmed using cetacean carcasses alone (Bilgmann *et al.*, 2011), confirming species is important, especially where remains are too decomposed to be of use for identification. DNA analysis showed that four of 24 samples that yielded DNA were misidentified in the field (Table 1), which could be attributed to progressive training and/or the fact that these carcasses were heavily degraded. There were three species confirmed using DNA that had not been observed during the boat-based surveys by Gore *et al.* (2012). These were pan-tropical spotted dolphin, long-beaked common dolphin and Bryde's whale. A further two species, a juvenile male sperm whale, *Physeter macrocephalus* (Gore *et al.*, 2007a) and a young female Cuvier's beaked whale, *Ziphius cavirostris* (Gore *et al.*, 2007b) were confirmed from skeletal remains. Braulik *et al.* (2010) listed 14 species from Iranian waters, which included all of those identified in the present study. In neighbouring India, Jayasankar *et al.* (2008) identified five of the six species of marine cetaceans (Indian Ocean humpback dolphin, spinner dolphin, bottlenose dolphin, long-beaked common dolphins and Indo-Pacific finless porpoise) using molecular identification techniques. By contrast, using boat-based surveys, Gore *et al.* (2012) found that spinner dolphins (which were typically found offshore along the Balochistan coast) and Indian Ocean humpback dolphins and Indo-Pacific finless porpoises (most frequently found near-shore along the Sindh coast) were the more abundant species. This highlights that beach surveys are an important and effective method for determining species presence/absence and certain life history information such as sex and age of stranded animals.

The results of the present study suggest that the majority of strandings were young males. Pichler (2002) reported sex related mortality in New Zealand's Hector's dolphin (*Cephalorhynchus hectori*). He found mortalities in South Island were largely male and likely prone to fishing related mortality, whereas 78% in North Island were female and likely to be non-fishing related. Of the 24 samples sexed in the present study, 4 were male and 2 were female. Further to this, 15 males and three females were putatively identified (see Table 1) giving a putative total of 19 males and five females. These sex related findings are interesting; however, without additional research and a larger sample size it is not possible to say whether or not this indicates specific mortality events that males are more prone to, such as interactions with fisheries or travelling inshore more frequently.

Reeves *et al.* (2002) have noted that Indo-Pacific finless porpoise may live to 33 years plus, Indian Ocean humpback dolphins to 40 years plus, spinner dolphins to 20 years plus, Indo-Pacific bottlenose and long-beaked common dolphin *ca*. 40 years and bottlenose dolphin 40–50 years for males and 50 years plus for females. Ageing cetaceans from visual inspection alone can only provide a very rough estimate, whereas an analysis of odontocete teeth can narrow the age to a much more accurate figure. The oldest cetacean aged in the present study was an unidentified dolphin species at 17 years old, whilst the youngest animals were neonatal (n = 2). Nine of the 12 (69%) cetaceans were estimated to have been less than ten years old. For the neonatal animals, the age class was confirmed by the fact that the teeth of these animals had not yet erupted. In the case of the neonatal bottlenose dolphin, there was no evidence of milk found in the stomach, suggesting that it had not suckled before stranding although it may have vomited due to the stress of stranding (Alonso *et al.*, 1999). From these results and assuming that senescence does not vary considerably between geographic regions, old age was not the cause of death of these individuals.

Mass strandings have occurred in neighbouring Iran (Braulik *et al.*, 2010) and Oman (Collins *et al.*, 2002) and while they do occasionally occur in Pakistan (Kiani *et al.*, 2011), none took place during the seven year monitoring period of 2004–11 for the present study. The seas off Pakistan are subject to naval exercise and seismic surveys (e.g. Howden, 2003), which contribute to noise pollution in the local marine habitat and have been linked elsewhere to cetacean strandings (Tyack, 2009; Tyack *et al.*, 2011; Weilgart, 2007). Chaghtai and Saidullah (2001) reported on toxic algal blooms in the Indus creek system at Korangi, Manora channel and the continental shelf off Pakistan, which could also contribute to strandings. Other potential causes of cetacean mortality, and ultimately stranding, include: disease (Ross, 2002; Van Bressem *et al.*, 2009); boat collisions (Carrillo and Ritter, 2010; Laist *et al.*, 2001); interactions with fisheries (Crespo *et al.*, 1997; Leeney *et al.*, 2008; Read *et al.*, 2006) and inter-/intra-species interactions (Parsons and Jefferson, 2000; Ross and Wilson, 1996). As the remains were often highly degraded, it was not possible to determine the cause of death for most of the individuals in the present study.

Beach surveys also provide the opportunity to discuss perceptions and raise awareness of cetaceans with local fishermen and coastal communities (see Gore *et al.*, 2012 for further details). This is particularly useful in countries such as Pakistan where resources are limited. Given that the

majority (62%) of the cetacean remains were discovered by community members, this approach was regarded as highly successful in the present study. An obvious drawback is that this approach can result in a clustering of strandings around inhabited locations. However, despite there being more fishing communities along the Sindh coast than the Balochistan coast, similar numbers of remains were found in both provinces. This might indicate a higher number of strandings along the Balochistan coast or a better success rate in reporting strandings, but further research is needed to confirm this. It is important to note that although these animals were likely to have come from the Indian Ocean, tides and currents may have brought the carcasses from other territorial waters (Peltier *et al.*, 2012).

From a more global perspective, stranding studies have been undertaken in a wide variety of locations. For example, using 72 years of data, Norman *et al.* (2004) reported strandings of 951 individual cetaceans of 23 species over 4,243km of the Pacific NW coast of USA. This coast has similar characteristics to the coastline of Pakistan, as the continental shelf on the NW coast of the USA is very wide in Washington (56% strandings) and narrow in Oregon (44% strandings). In contrast, off Pakistan there appears to be a lower proportion of strandings in Sindh (40%) where the continental shelf is wide relative to Balochistan (60%) with a narrow shelf. Overall, strandings in Pakistan amounted to approximately $9.3yr^{-1}$ and $0.035km^{-1}$ of coastline. Comparing superficially, Norman *et al.* (2004) reported $13.2yr^{-1}$ and $0.22km^{-1}$ of coastline for the Pacific NW. Along the coastline of Brazil, Meirelles *et al.* (2009) reported cetacean strandings of $19.4yr^{-1}$ and $0.44km^{-1}$. The rate for Oman was very high at $241.7yr^{-1}$, which includes mass strandings (Collins *et al.*, 2002).

The results of the present study will enhance knowledge of Pakistan's cetaceans and contribute to local and international conservation and management efforts. Specifically, these results and the action plan for cetacean conservation in Pakistan (Gore, 2008) will provide a basis for legislative action on the part of NGOs in Pakistan (including WWF-Pakistan and the IUCN-Pakistan), the Convention on Biodiversity Working Group within Pakistan's Ministry of Environment, the Marine Fisheries Department of the Government of Pakistan and the Ministry for Environment of the Government of Pakistan. This is especially important, given that to date, none of the cetaceans reported in this work have been gazetted as protected species in either the Sindh or the Balochistan province.

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