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Contents

Editorial

Welcome to this the sixteenth volume of the *Journal of Cetacean Research and Management*. This volume contains 8 papers covering a wide range of conservation and management issues.

Two issues of the Journal will be published in 2017 – this volume and the next.

To submit a manuscript to the Journal, please contact in the first instance Jessica Peers (e-mail: *jessica.peers@iwc.int*). A *Guide for Authors* and other information is available online at *http://www.iwc.int/jcrm*

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G.P. DONOVAN
Editor

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

[8] *http://iwc.int/sanctuaries.*

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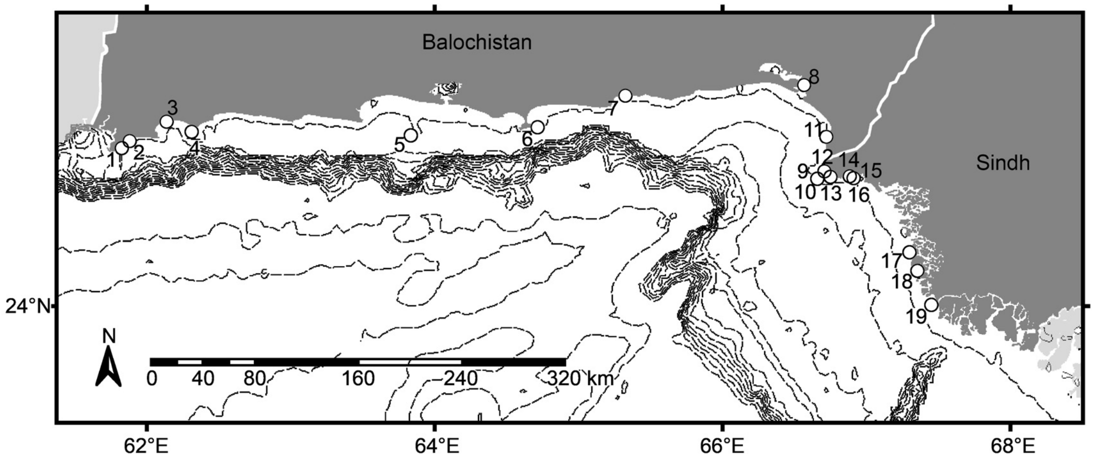

Fig. 1. Map of the Pakistani coast showing the locations where the cetacean remains were found. Iran to the west and India the east are shown as a lighter shade of grey; the border between the two coastal provinces of Pakistan (Balochistan and Sindh) is marked. Bathymetric contours are shown (at 10m, 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; $\boxed{M}$ = Male (amplifying X and Y); M = Male with reduced confidence (amplifying Y, but not X); $\boxed{F}$ = 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	$\boxed{M}$	–
PAK 6	03/01/08	15	D^{0}	BD	$\boxed{M}$	–
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	$\boxed{F}$	–
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	$\boxed{M}$	–
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	$\boxed{F}$	–
PAK 29	07/05/08	19	HBD	HBD	$\boxed{M}$	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

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

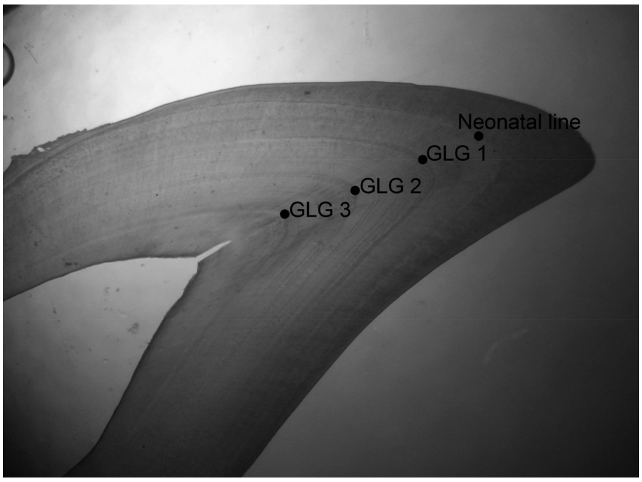

Fig. 2. Stained tooth section of humpback dolphin (PAK 14), showing three clear Growth Layer Groups (GLG), the neonatal line, and several less pronounced accessory lines in the dentine.

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 9.3yr^{-1} and 0.035km^{-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

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[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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Annual and monthly fluctuations in humpback whale (*Megaptera novaeangliae*) presence in Skjálfandi Bay, Iceland, during the feeding season (April–October)

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ABSTRACT

Humpback whales are among the most common whale species occurring in Skjálfandi Bay, North Iceland. Since 2001, 309 photographed humpback whales have been identified and catalogued. The percentage of whales re-sighted from year to year has been always less than 27% and only 49 out of 309 identified individuals were sighted in the Bay in two or more years, indicating that the humpback whales occurring in Skjálfandi Bay have various feeding areas. Kernel Density Estimations indicated distribution changes within the bay and throughout the season. In early summer, humpback whale densities were higher in the southern and western part of Skjálfandi Bay, whereas as the season progressed whale concentration increased further north in the direction of the open sea.

Generalised Linear Models (GLMs) were used to analyse a 10 year time series of monthly humpback whale Sightings Per Unit of Effort (SPUE; sightings per minute) during the summer season (May–October). Whalewatching boats were used as research platforms. The total effort was 136,503 minutes. 1,401 sightings of humpback whales were recorded. GLMs show significantly higher SPUE in 2006 (0.0132), 2011 (0.0111) and 2012 (0.0246) when compared to the start of the time series. September showed a significantly lower SPUE (0.0024) when compared to the baseline month (May). An independent dataset derived from logbook data provided by commercial whalewatching operations was used to derive the percentage of humpback whale positive surveys (surveys where the species was seen) over a longer time series (1995–2012). These data were analysed using Generalised Additive Models (GAMs) and show an increase on the percentage of positive surveys in the period 2006–09, a slight decrease in 2010 and a new increase in 2011–2012. Annual trends in positive surveys for two additional species of locally abundant cetaceans, the harbour porpoise and the white-beaked dolphin, were explored for comparison purposes. No trend was found for porpoises while the percentage of positive surveys for white-beaked dolphins showed a general decrease after 2002.

Fluctuations in SPUE and percentage of positive surveys may be due to varying environmental conditions within the bay, with potential implications for whalewatching operations. Given the scientific value of these conclusions, this study also supports the value of integrating whalewatching vessels as opportunistic platforms for cetacean research.

KEYWORDS: HUMPBACK WHALE; PHOTO-ID; SIGHTINGS PER UNIT EFFORT; ICELAND; WHALEWATCHING; WHITE-BEAKED DOLPHIN; HARBOUR PORPOISE

INTRODUCTION

The humpback whale, *Megaptera novaeangliae* (Borowski, 1781), inhabits all oceans of the world except polar pack ice zones (Chittleborough, 1965) and is among the most common cetacean species occurring in Skjálfandi Bay, North Iceland. Between the winter (November–April) and the summer (May–October) seasons they migrate over long distances, switching between their warmer tropical breeding areas and their feeding grounds in higher latitudes (Norris, 1977; Pomilla and Rosenbaum, 2005). In the eastern part of the North Atlantic, the distribution of the species during their feeding season ranges from Iceland and Scotland to the Barents Sea (Stevick *et al.,* 2003a). There are two known breeding grounds in the North Atlantic, located in the tropical waters of Cape Verde (Baker *et al.,* 1990; Katona, 1986; Kellogg, 1929; Vigness-Raposa *et al.,* 2010; Wenzel *et al.,* 2009) and also in the Caribbean (Balcomb and Nichols, 1982; Whitehead, 1982).

Although the humpback whale is thought to be philopatric (Boye *et al.,* 2010; Mitchell, 1974; Palsbøll *et al.,* 1997; Whitehead, 1982), female humpback whales have been found 10,000km away from their natal breeding sites in the Southern Hemisphere (Stevick *et al.*, 2011). This was considered an indication of behavioural flexibility in migration patterns of a typically philopatric species as the females were probably exploring new breeding grounds due to changeable environmental conditions.

The worldwide abundance of the species strongly decreased during the whaling periods in the 1800s and early 1900s (Tønnessen and Johnsen, 1982). The International Whaling Commission (IWC) protected humpback whales from commercial whaling in the North Atlantic in 1955 (Best, 1993). Since the cessation of humpback whaling, North Atlantic populations show signs of recovering (Stevick *et al.,* 2003b). Between 1986 and 2001, aerial surveys in coastal Icelandic waters revealed an increase in humpback whale numbers of about 12% and estimated up to 4,928 (CV 0.463) individuals (Pike *et al.,* 2009). Mark-recapture abundance estimates by Smith *et al.* (1999) and Stevick *et al.* (2003a) approximate a North Atlantic population size of 10,600 (95% CI 9,300–12,100) and 11,570 (95% CI 10,290–13,390) individuals, respectively, based on data collected during surveys during the mid–1990s. Multiple partial-area surveys from 1996 to 2001 estimated about 3,246 (CV 0.512) humpback whales in the eastern North Atlantic (Øien, 2009). Abundance has been constantly increasing in later years and

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the current IUCN (International Union for the Conservation of Nature) Red List category for the humpback whale is Least Concern, although some subpopulations, for example in the Arabian Sea, remain Endangered (Reilly *et al.,* 2008). During shipboard and aerial surveys around Iceland and the Faroe Islands, Paxton *et al.* (2009) estimated abundances of 10,521 (95% CI: 3,716–24,636) in 1995 and 14,662 (9,441–29,879) in 2001. This trend has been also observed by Víkingsson *et al.* (2015) where results from aerial and shipboard surveys between 1986 and 2009 indicated an increase in humpback whale numbers up to the year 2001 with a slight decrease thereafter. Studies conducted in 2007 by Pike *et al.* (2010) gave evidence for stagnating numbers in the North Atlantic. Conventional distance sampling analysis estimated 11,572 humpback whales (95% CI 4,502–23,807) which is similar to the number estimated in the mid 1990s by Stevick *et al.* (2003b).

Photo-identification is a popular method for studying cetacean movements and population size. This technique is particularly appropriate for the individual identification of humpback whales given that they show a unique black and white pattern on their flukes' ventral surface. Moreover, the shape of the dorsal fin, which is variable in size, as well as markings such as scars, can also be used for individual identification (Clapham and Mayo, 1987; Katona and Whitehead, 1981). Photo-identification matches have been registered between humpback whales that migrated between Iceland and the Caribbean (e.g. Martin *et al.,* 1984; Smith *et al.,* 1999; Stevick *et al.,* 2003), and one humpback whale that migrated between Iceland and the Cape Verde islands (Jann *et al.,* 2003). These show that the whales feeding in Icelandic waters possibly originate from two different breeding grounds.

Whalewatching started in Iceland in Skjálfandi Bay, Húsavík, in 1995 (Rasmussen, 2014). Since 2001, whalewatching boats have been used as research platforms and humpback whales observed in Skjálfandi Bay were photographed and catalogued by personnel from the Húsavík Whale Museum. In 2003 an additional and extended data collection scheme started focusing on behavioural and habitat preferences of all the local cetacean species.

In this study photo-identification was used to investigate the number of new and re-sighted humpback whales in Skjálfandi Bay between 2001 and 2012 and seasonal patterns were examined in humpback whale distribution within the bay through Kernel Density Estimations. In addition, potential temporal trends were assessed in the Sightings Per Unit Effort (SPUE) and percentage of positive surveys (i.e. percentage of trips in which the species was seen) of humpback whales in the same area and similar time period (2003–12 and 1995–2012 respectively). Trends in the percentage of positive surveys of harbour porpoises (*Phocoena phocoena*) and white beaked dolphins (*Lagenorhynchus albirostris*) were also analysed (1995–2012).

METHODS

Data collection took place in Skjálfandi Bay, northern Iceland (see Fig. 1). Whalewatching boats 'Gardar', 'Bjössi Sör' and 'Náttfari', operated by North Sailing (*www.northsailing.is*), were used as research platforms. A dedicated data collection scheme was carried out over the whalewatching season (May–October) from 2003 to 2012. Additional data were provided from the Húsavík Whale Museum, in the form of a Humpback Whale Catalogue, which contains all humpback whale individuals photoidentified in Skjálfandi Bay since 2001. In 2001 and 2002 data collection was limited to photo-identification materials along with data on effort and environmental factors. These data were used to calculate sighting and resighting rates of individual humpback whales in Skjálfandi Bay.

Standard protocols for the collection of photo-identification data were followed as described in Bertulli *et al.* (2013). Between one and three trained observers worked on board and recorded effort and cetacean presence. Sighting data includes: the time and location of the sightings, the species and number of individuals and the animals' main behaviour. Effort data includes environmental conditions

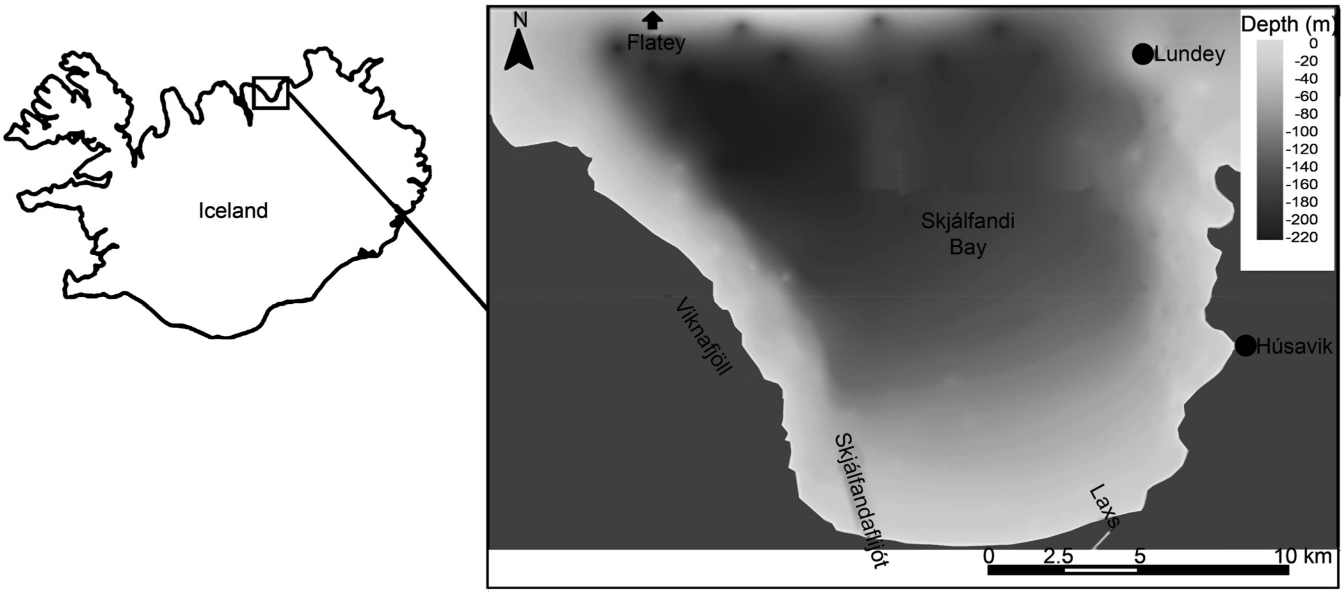

Fig. 1. Research area of Skjálfandi Bay.

such as cloud cover, sea state, precipitation, wind direction, glare, swell height and visibility. Time spent on effort started when leaving the harbour and continued until entering the port again. For every tour the route was tracked, with GPS-positions, determined every five minutes using GPSmap 60CSx GARMIN. Additional GPS coordinates were set when approaching a whale. Once humpback whales were sighted, photos were taken, using an EOS 60D CANON 28–135mm camera for individual identification (Clapham and Mayo, 1987; Katona and Whitehead, 1981). When several humpback whales were spotted at the same time they were considered a single sighting of several individuals. When a whale was first observed alone but more individuals approached the area, new sightings were recorded. Although the number of trips with observers increased over the years, the procedure of collecting data was standard over the years.

In addition the whalewatching company provided access to their logbooks, which contained the species and number of sighted cetaceans for each whalewatching tour from 1995 onwards. The logbook data were used solely to calculate the percentages of positive surveys of humpback whales, harbour porpoises and white-beaked dolphins in Skjálfandi Bay from 1995 to 2012.

To analyse the number of whales sighted in Skjálfandi Bay, cetacean sightings per unit effort (SPUE) were calculated using the formula:

$$Sightings\ per\ Unit\ Effort = \frac{Number\ of\ Sightings}{Minutes\ Effort}$$

$$SPUE = \frac{n}{LT}$$

Where n corresponds to the total number of sightings, LT is the total amount of time spent on surveys (total effort = minutes actively spent for searching).

To determine differences in the numbers of sightings and resightings per year, t-tests were performed using SigmaPlot 11.0.

To assess temporal trends in humpback whale SPUE, Gaussian Generalised Linear Models (GLMs) were used. SPUE was used as response variable and both year (2003–2012) and month (May–October) were used as explanatory variables (factors). The original SPUE data were Squared Root transformed to fulfil normal distribution requirements. Available data for 2003, 2004 and the month of October were limited, since humpback whales were recorded only one month during 2003 (August) and 2004 (July) and only two years during October (2010 and 2012), which compromised model validation. Subsequently 2003, 2004 and October were excluded from the temporal analyses. GLM models were optimised using a backwards selection, finally accepting the one with the lowest AIC (Akaike Information Criterion).

Trends in the percentage of positive surveys for three common cetacean species in the area (humpback whale, harbour porpoise and white-beaked dolphin) were investigated using Gaussian Generalised Additive Models (GAMs). The response variable was the yearly percentage of positive surveys (1995–2012) and year was introduced in the model as a continuous explanatory variable.

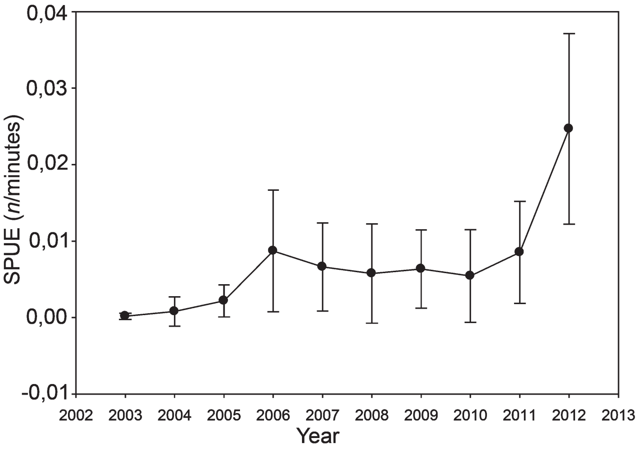

Fig. 2. Mean humpback whale SPUE and standard deviations from 2003 to 2012 (number of sightings/minute).

All models were validated by checking that no large ‘hat’ values (indicating influential data points) were detected, no over-dispersion was found, and serious patterns did not remain in the residuals. All models were performed in R.3.1.

To visualise humpback whale distribution patterns within the bay the most recent year in the data set 2012 was used as an example, GPS positions of the sightings were plotted using ArcGIS 10.1. Kernel Density Estimation, a method for estimating the probability distribution of a random variable (Silverman, 1986), was then performed to investigate the intensity of use of the study area across months. Kernel Density Estimation represents a uniformly consistent, continuous estimator of an unknown probability measure by a series of densities, often used in ecological analysis. Thus, the probability with which animals stay in a specified geographical area (i.e. ranging patterns) can be estimated (Rodgers and Kie, 2011).

RESULTS

The number of minutes spent on effort differed over the years, starting at a low level in 2003 (2,748 minutes) and continuing on a high, slightly increasing level from 2005 (11,522 minutes) onwards, peaking in 2012 (21,188 minutes). The monthly peak value was 7,711 minutes in July 2011 (Table 1). A total of 1,401 sightings of humpback whales was reported, of which 519 took place during 2012. In 2003 the SPUE was the lowest recorded (average: 0.0002), whereas 2006, 2011 and 2012 showed a higher SPUE (see Fig. 2).

GLM models identified both year (p = 0.003) and month (p = 0.02) as significant variables influencing SPUE (squared rooted). The deviance explained by the best model was 60.1% (Table 2). When looking at individual years and months, 2006 (p = 0.04), 2011 (p = 0.02) and 2012 ($p < 0.001$) showed significantly higher SPUE than the year used as baseline (2005) while the month of September had a significantly lower SPUE (p = 0.008) than the baseline month (May). Note that the years 2003 and 2004 and the months of April and October were not considered in the GLM due to lack of data (see Methods).

Results from GAMs highlighted a significant influence of year in the percentage of positive surveys of humpback whales (p = 0.02), showing an increase in the period 2006–

Table 1

Survey effort for 2003 to 2012 during summer research seasons. For each year, both monthly and total effort data, expressed in minutes, are reported.

	May	June	July	August	September	October	Total effort
2003	245	184	1,373	946	0	0	2,748
2004	0	0	1,698	1,996	0	0	3,694
2005	0	3,046	4,201	3,497	778	0	11,522
2006	1,360	4,340	3,585	3,478	175	0	12,938
2007	2,164	4,488	4,449	3,279	470	0	14,850
2008	2,308	4,229	3,022	4,321	2,018	0	15,898
2009	4,277	4,441	3,673	2,848	526	0	15,765
2010	1,806	4,758	5,165	4,294	2435	624	19,082
2011	863	4,702	7,711	5,003	539	0	18,818
2012	4,677	4,408	4,620	2,886	2,486	2,111	21,188

Table 2

Comparison of the three GLM models considered in the present study where the response variable is humpback whale SPUE.

GLM model	Explanatory variables	Deviance explained (%)	AIC
GLM1	Year	41	–138.4719
GLM2	Month	19	–131.702
GLM3	Year, month	60	–146.029

09, a slight decrease in 2010 and a new increase in 2011 and 2012 (Fig. 3). The percentage of positive surveys of white-beaked dolphins was borderline significantly influenced by year ($p = 0.047$) with a higher percentage in the period 1999–2003 and a later decline (Fig. 3). No yearly effect was observed in the percentage of positive surveys for the harbour porpoise ($p = 0.6$).

Regarding the ranging preferences within the bay from April to October in 2012, Kernel Density reached highest values in the western part of the bay, which represents the deepest area. Additional high densities were recorded in regions closer to the shore (Fig. 4).

The few sightings of humpback whales in April were spread over the whole bay, but mostly close to the shore. In May and June the whales preferred the western and the southern part, but were also present in an area at approximately 8km distance from Húsavík harbour in the middle of the bay. Humpback whales occurred throughout Skjálfandi Bay in July, with the exception of the southernmost areas. In August, September and October the whales were mostly restricted to the northwestern part of the bay.

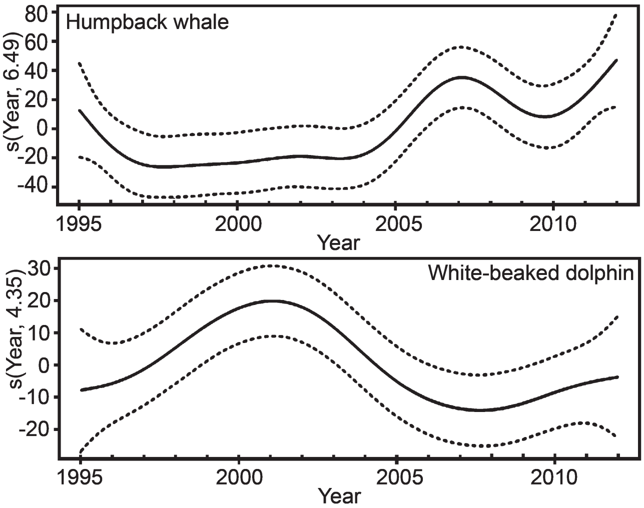

Fig. 3. Smoothed effect of year on percentage of positive surveys for humpback whale and white-beaked dolphin. The Y-axis represents the trend (positive or negative) in percentage of positive surveys in relation to year. Dotted lines are the approximate 95% confidence limits.

The total number of photo-identified humpback whales increased annually from 7 individuals (2001) to 39 individuals (2009). After a slight decrease in 2010 (33 individuals), 42 humpback whales were photo-identified in 2011. The peak value was reached in 2012 with 77 photo-identified individuals (Table 3). The rate of resightings always remained under 27%. The first resighting was recorded in 2004, the highest resighting rate (26%) was reached in 2007 and the two lowest (7% in both cases) in 2005 and 2006. The majority of the re-captures (77%) involved individuals that had been photographed in Skjálfandi Bay the year before their first resighting.

When the numbers of newly identified individuals are plotted against the total number of whale encounters per year (no data collection took place in 2003), the discovery curve increases without any signs of flattening (Fig. 5), which suggests that humpback whales in Skjálfandi Bay do not belong to a closed population.

DISCUSSION

Interannual occurrence of humpback whales in Skjálfandi Bay is prone to fluctuations. Our data show annual and seasonal variation; significantly higher SPUEs in 2006, 2011 and 2012 when compared to the baseline year (2005), increasing percentage of humpback whale positive surveys in the period 2006–09 and after 2010, and significantly lower SPUEs in September when compared to the baseline month (May). Furthermore, the discovery curve, representing the number of new identified humpback whales in relation to the total number of catalogued individuals, has not reached a plateau yet, indicating that the humpback whales observed in the bay do not form a closed population and that every year new individuals visit the bay.

The high SPUEs recorded in recent years are in agreement with previous studies in the North Atlantic. For example, based on aerial surveys, Pike *et al.* (2009) recorded an increase in humpback whale abundance of about 12% in northeastern and eastern Iceland (including Skjálfandi Bay)

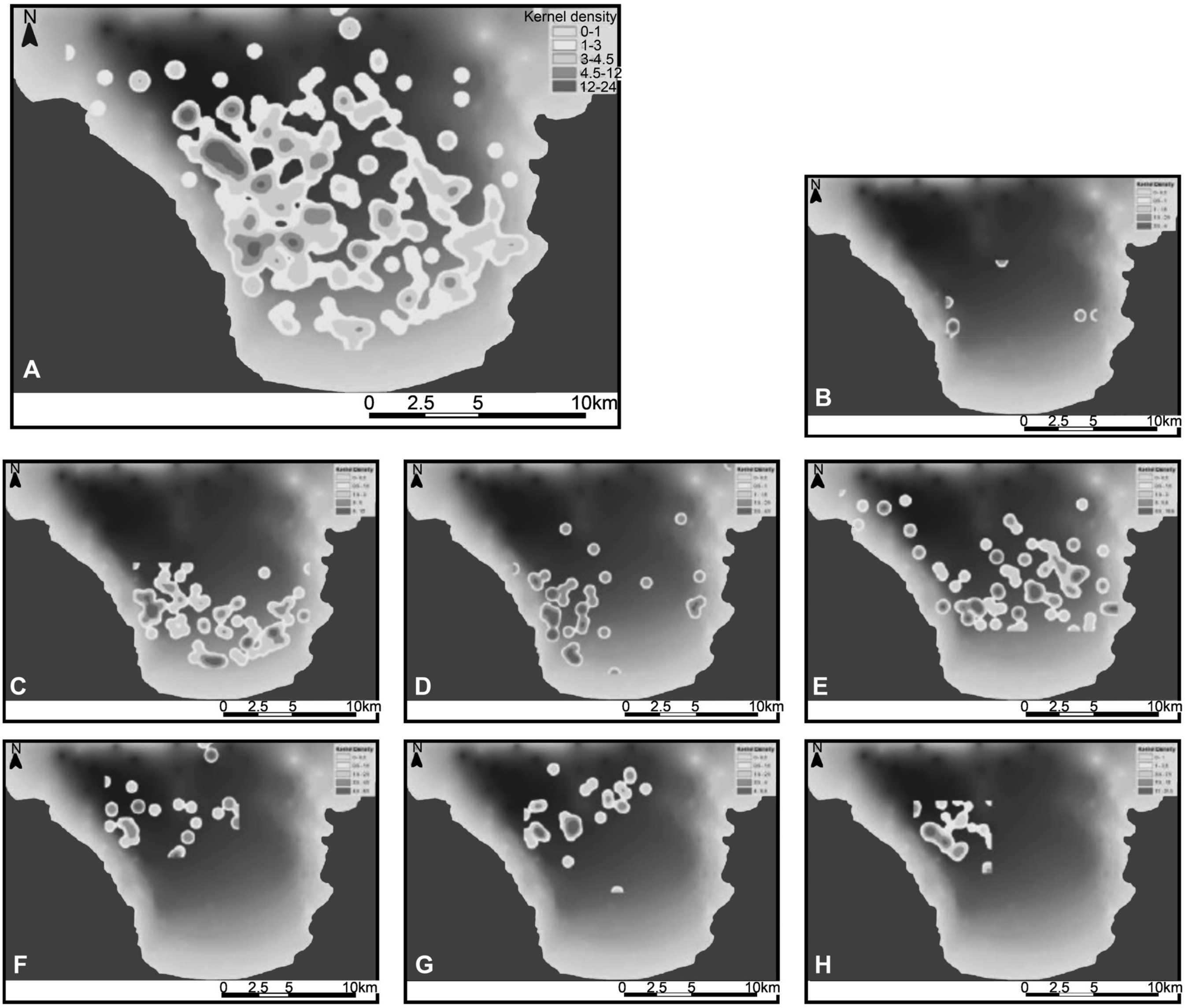

Fig. 4. Kernel density of humpback whale encounters in Skjálfandi Bay during summer research season 2012 (April to October). (A) Overview April–October, (B) April, (C) May, (D) June, (E) July, (F) August, (G) September, (H) October. Darker areas, mainly in western part of bay, correspond to sighting hot spots.

Table 3

Number of identified humpback whales in Skjálfandi Bay from 2001 to 2012. Sightings and resightings are noted in total numbers, the values in brackets are percentages. No data was available for 2003. (ID = total number of identified individuals; *N* = number of new identified individuals).

Year first seen	ID	*N*	No. of whales seen in each subsequent year 2005	2006	2007	2008	2009	2010	2011	2012	No. resighted ≥ 1 year
2001	7	7	–	–	1 (14.3)	–	–	–	–	–	1 (14.3)
2002	4	4	1 (25.0)	–	–	–	–	–	–	–	1 (25.0)
2004	5	5	–	–	–	–	–	–	–	–	–
2005	15	14	–	2 (13.3)	2 (13.3)	–	–	–	–	–	2 (13.3)
2006	27	25	–	–	7 (25.9)	2 (7.4)	2 (7.4)	1 (3.7)	1 (3.7)	1 (3.7)	7 (25.9)
2007	39	29	–	–	–	2 (5.1)	1 (2.6)	1 (2.6)	–	2 (5.1)	5 (12.8)
2008	25	21	–	–	–	–	3 (12.0)	2 (8.0)	1 (4.0)	1 (4.0)	5 (20.0)
2009	35	29	–	–	–	–	–	4 (11.4)	2 (5.7)	1 (2.8)	6 (17.1)
2010	33	25	–	–	–	–	–	–	3 (9.1)	3 (9.1)	3 (9.1)
2011	42	35	–	–	–	–	–	–	–	9 (21.4)	9 (21.4)
2012	77	66	–	–	–	–	–	–	–	–	–
Total	309	260	–	–	–	–	–	–	–	–	39 (12.6)

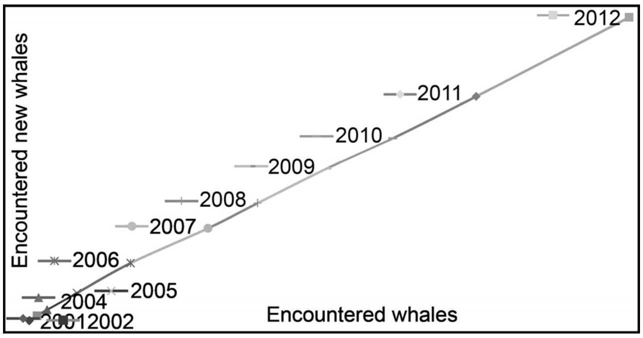

Fig. 5. Discovery curve of humpback whale individuals during the summer research season from 2001 to 2012. No data were available for 2003.

between 1986 and 2001. In West Greenland, the annual rate of increase in humpback whale sightings was 9.4% between 1984 and 2007 (Heide-Jørgensen *et al*., 2012). These results have been related to the recovery of depleted populations previously subject to whaling. However, changes in humpback whale abundance trends within the northwest Atlantic could also be related to prey distribution shifts, comparable to what has been observed in the Gulf of Maine, where humpback whales have been shifting their distribution dependent on herring (*Clupea* sp.) and sandeel (*Ammodytes* sp.) occurrence (Stevick *et al.*, 2006).

A clear increasing trend in sightings of humpback whales in Icelandic waters was recorded from 1969 to 1988 by Sigurjónsson and Gunnlaugsson. It is possible that increasing numbers of whales might reflect changing feeding conditions, due to shifts in the distribution of small fish, or the immigration of whales from different North Atlantic stocks. However, recently published data indicate that the increase in numbers in Icelandic waters levels off after the year 2000, and no relationship was found with prey abundance (Víkingsson *et al.*, 2015).

For Skjálfandi Bay, the percentage of humpback whale positive surveys shows an increasing trend throughout the years. Since 2011, the humpback whale has been the most common cetacean species recorded in Skjálfandi Bay (Klotz, 2014). Our results indicate that the percentage of positive surveys remained constant during the research time period for the harbour porpoise and that it has decreased in recent years for white beaked dolphins. These patterns may be due to interspecific competition. For example, MacLeod *et al.* (2008) reported interspecific competition between white-beaked dolphins and common dolphins (*Delphinus delphis*). White-beaked dolphins dominated in waters less than 13°C, whereas common dolphins preferred temperatures above 14°C. Lynch and Whitehead (1984) recorded spatial segregation between different cetacean species in Newfoundland and Labrador in relation to capelin (*Mallotus villosus*) abundance and density.

In the present study whalewatching vessels were used as research platforms and the comparison between the percentages of positive surveys among different species could be biased. Whalewatching operators mostly focus on the most popular cetacean species, meaning that most of the time whale species such as humpback whales or blue whales (*Balaenoptera musculus*) are the target of their interest. Thus, a decreasing percentage of positive surveys for white beaked dolphin might be attributed to a higher focus on humpback whales. However, the percentage of positive surveys of harbour porpoises did not change through time, suggesting that the registered increase of percentage of positive surveys of humpback whales is not due to bias. On the other hand, the number of tours per day varied throughout the season, and more trips with observers were carried out between June and August, which incorporates the main touristic season. Similarly, the number of research assistants was usually higher in mid-summer, when more volunteers worked for the Húsavík Whale Museum and the Húsavík Research Center. An increased number of tours per day in the peak season enabled a more intensive data collection as reflected in the fact that October (low season) had to be excluded from our GLM analyses due to lack of data.

The significantly lower SPUE recorded in September could be due to individual migration to southern latitudes, which starts in the autumn (Norris, 1977; Pomilla and Rosenbaum, 2005) and that could lead to decreasing encounter rates in Skjálfandi Bay.

Ranging patters of humpback whales in 2012 as explored though Kernel densities varied temporally within Skjálfandi Bay. Higher densities have been estimated in the western part of Skjálfandi Bay close to the coast. These are the deepest areas (down to 220m), with steep slopes. In such areas different processes, particularly upwelling, lead to increased nutrient concentrations in surface waters, promoting primary production and plankton growth (Allen *et al.*, 2001; Olson and Backus, 1985; Woodley and Gaskin, 1996). Besides environmental variables such as Sea Surface Temperature, distance to shore and sea-floor topography, previous studies revealed that dynamic mesoscale oceanographic processes such as thermal fronts, eddies and upwellings, can influence prey distribution, growth of plankton and thus the abundance of planktivorous fish (Olson and Backus, 1985; Woodley and Gaskin, 1996). Interestingly, the distribution of humpback whales varied between months in 2012. In the beginning of the feeding season (April–May) the humpback whales need to restock their energy stores after a period of minimal feeding and the long migration. They therefore seek rich feeding grounds to gain as much energy as possible for the lowest amount of effort (Doniol-Valcroze *et al.*, 2007; MacArthur and Pianka, 1966). During April, May and June whale densities were higher closer to the shore, especially in the southern and western part of the bay, where nutrient input from rivers is highest. Freshwater nutrient input enhances plankton growth and thereby food availability for baleen whales (Gíslason, 2004). Densities changed during July when humpback whales occurred in every part of Skjálfandi Bay except for its southern-most area. July also represents the period when most of the humpback whales have reached their feeding grounds and individuals could spread out in the bay to avoid competition. In addition, in July most individuals have already restocked their energy stores and will expend energy in other activities such as breaching. Breaching events were more frequent during July and August independently of sighting location (Klotz, 2014). From August to October, high densities were limited to the north or northwestern part of the bay. This distribution may be due to the existence of food-exhausted areas within the southern bay and the start of the migration of some individuals.

Since 2001 only a small number of the humpback whales identified in the research area have been re-sighted. The Discovery curve (see Fig. 5) shows a linear increase of newly identified animals over the study period indicating that the humpback whale stock in Skjálfandi Bay is not a closed population that is returning to the bay every year, but part of a larger population in the North Atlantic with different or broader feeding areas.

Compared to other humpback whale feeding grounds, the resighting rate in Skjálfandi Bay is low (< 27%). In contrast, in the Gulf of Maine for example, rates as high as 73.2% have been reported (Clapham *et al.*, 1993). Obtaining more information about humpback whale site fidelity and higher percentages of resightings in our research area would require covering a larger study area. However, Boye *et al.* (2010) and Weinrich (1998) found evidence for small-scale site fidelity for the majority of the whales within only a few squared kilometers in Godthaabsfjord, Greenland, and the Gulf of Maine, respectively. In different years, sightings of 16 individuals known from Skjálfandi Bay have been made in other coastal areas in Iceland such as Faxaflói, in southwest Iceland, or Eyjafjörður, further west of Skjálfandi Bay (Húsavík Humpback Whale Catalogue, unpublished). These resightings confirm that some individuals travel between different locations or move on to other regional feeding areas.

Fluctuations in SPUE and percentage of positive surveys may be due to varying environmental conditions within the bay, with potential implications for whalewatching operations. Given the scientific value of our conclusions, this study also supports the use of whalewatching vessels as opportunistic platforms for cetacean research.

Whalewatching operations in the bay are growing due to increasing cetacean sighting (of humpback whales, in particular) and of greatly increased tourism in general in Iceland. Although this business increases the public's awareness on whale conservation needs, strict guidelines for the vessels are needed to avoid disrupting the animals (Hoyt, 2001; Cunningham *et al.*, 2012). A recent study showed that whalewatching boats in Iceland can approach the whales at high speed and without keeping the suggested buffer distance of at least 50m (Martin, 2012). Our research, however, is an example of whalewatching best practice at sea and of a successful collaboration between the scientific community and the whalewatching industry.

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Marine traffic and potential impacts towards cetaceans within the Madeira EEZ

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ABSTRACT

Human population growth has resulted in an increase of marine traffic. This has been associated with wildlife disturbance and the effects are expected to increase with continued traffic expansion. A particularly impacted group is cetaceans, known to play an important role in the sustainability and regulation of marine ecosystems. An assessment of marine traffic can therefore contribute towards wildlife conservation measures, especially when evaluated in the context of important areas for cetaceans. The present study took place in Madeira's Exclusive Economic Zone (EEZ), an area hosting a high diversity of cetacean species as well as island-associated groups. Automatic Identification System (AIS) data were collected from a land station between 2008 and 2011 and marine traffic and cetacean visual data collected during shipboard surveys between 2001 and 2011. Results show that Madeira's offshore traffic (up to 12 n.miles from the shore) corresponds to approximately 12% and 22% of the traffic observed in the Baltic and North Sea, respectively. It is mostly composed of cargo ships navigating over fixed routes and using the area as a passage towards different destinations. Cruise ships intersect the area mainly to reach Funchal's port. The number of recreational boats in the area was found to be underestimated since many of them are not equipped with AIS devices. The level of Madeira inshore traffic is harder to evaluate since it is a small area encompassing a shipping route, yet it may represent 0.8% of the traffic recorded in the Strait of Gibraltar. According to the inshore shipboard survey data, coastal marine traffic is mainly composed of fishing boats (47%), recreational boats (24%), ships (17%), whalewatching boats (10%) and big game fishing boats (2%). Most inshore and offshore vessels were found to be navigating at over 10 knots. An inshore 'higher use corridor' common to both vessels and cetaceans was identified as a potential danger zone.

KEYWORDS: ATLANTIC OCEAN; SUSTAINABILITY; SURVEY – VESSEL; DISTRIBUTION; NORTHERN HEMISPHERE; FIN WHALE; SHORT-FINNED PILOT WHALE; BEAKED WHALES; SPERM WHALE; BOTTLENOSE DOLPHIN; ATLANTIC SPOTTED DOLPHIN; COMMON DOLPHIN

INTRODUCTION

Marine traffic has been associated with a high potential of disturbance towards marine species, such as whales and dolphins, and this negative effect is expected to continue increasing as a consequence of continued traffic expansion (Nowacek *et al.*, 2001).

Cetaceans manifest behavioural changes (Piwetz *et al.,* 2012) that could trigger shifts in habitat use, temporary displacement and an increase in energy consumption. When continuously exposing the animals to these pressures long term consequences, such as changes in survival rates or population size, might follow (Bejder *et al*., 2006; Constantine *et al*., 2004; Nowacek *et al.,* 2001).

Over recent decades, due to the vast expansion of marine traffic, cetaceans have also been victims of increased ship strikes all around the world (Carrillo and Ritter, 2008; Laist *et al.,* 2001; Silber *et al.,* 2012; Waerebeek *et al*., 2007). Marine traffic is acknowledged as a worldwide threat towards whale and dolphin populations and is being addressed by various mitigation strategies, some supported by the International Maritime Organization (IMO), especially in regions where there are overlapping areas of busy marine traffic and high cetacean density (IWC, 2011; Panigada *et al.*, 2006; Ritter, 2007; Silber *et al.*, 2012). The International Whaling Commission (IWC) is playing a lead role by proposing mitigation measures and legislation on ship strikes, creating a ship strikes database, working with other bodies both intergovernmental and non-governmental and supporting specialist workshops (IWC, 2016).

Moreover, cetaceans attract a significant interest from the general public, resulting in significant growth of the whalewatching industry (Jelinski *et al.*, 2002; Orams, 2000). This in turn raises the need to monitor the industry and adopt codes of conduct and regulations in order to minimise its impact on cetaceans and ensure its sustainability (Ritter, 2003).

Shipping traffic appears to be a significant fraction of the anthropogenic sound input into the marine environment (Southall, 2005; Weilgart, 2007) and the potential impact of this on cetaceans has been considered an important issue (Southall, 2005). Marine mammals rely on hearing as their main sense. Thus, they are vulnerable to ocean noise pollution that might be the cause of some strandings and mortality incidents, among other disturbances, or chronic effects such as 'masking', altered vocal behaviour, hearing damage, increase in stress levels, habitat displacement and alterations in migration routes (Weilgart, 2007).

Coastal cetaceans are even more exposed to anthropogenic disturbances, especially in highly populated areas, where the effects can be cumulative (Piwetz *et al.,* 2012).

The present study took place in Madeira's Exclusive Economic Zone (EEZ) and provides, for the first time, information regarding traffic distribution patterns within the study area. The geographical position of Madeira's

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archipelago near the shipping corridors connecting Europe to South America and Africa. Madeira is itself the destination of many cruise ships, cargo ships and leisure crafts. It is constantly surrounded and sought out by marine traffic. These islands, as other islands in the Atlantic, are feeding, reproductive and breeding grounds for several whale and dolphin species (Alves *et al.,* 2013; Dinis, 2014; Freitas *et al.*, 2004b).

In January 2005, the use of Automatic Identification System (AIS) devices became mandatory for ships with gross tonnage (GT) equal or superior to 300 and all passenger ships of every size, following the ruling of IMO's International Convention for the Safety Of Life At Sea (SOLAS). AIS is a ship-to-ship and ship-to-shore message system based on VHF signals, which provides static and dynamic data related with each vessel trip (Silber *et al*., 2012). Later the European Union, through the Directive 2009/17/EC, established the mandatory use of AIS devices in fishing vessels and other vessels over 15m (IWC, 2011), where the Madeira Autonomous Region is included (Ministério da Agricultura do Mar do Ambiente e do Ordenamento do Território, 2012). The previously mentioned ruling promoted the wide use of AIS that in turn became an important source of data on marine traffic worldwide.

The main goals of the present study are to: (1) assess the spatial and temporal distributions of the inshore and offshore marine traffic of the Madeira archipelago; (2) identify zones of higher and lower marine traffic within the study area according to the type of vessel; and (3) identify zones of overlap between higher marine traffic areas and higher occurrence of cetaceans.

METHODOLOGY

Study area

The research focused on the Madeira EEZ (Fig. 1), including the inshore waters around Madeira, Desertas and Porto Santo Islands. These volcanic islands are located in the Atlantic Ocean at an average latitude and longitude of 32° 46'N and 16° 46'W and 635km from Africa's West coast. Madeira stands isolated from the closest mainland and nearby archipelagos by depths greater than 4,000m. The archipelago main islands are surrounded by several steep submarine canyons, with a small continental shelf, and often influenced by the Gulf Stream current, thus presenting favourable conditions to hold a substantial level of marine biodiversity (Aguin-Pombo and Carvalho, 2009).

The offshore study area comprises the entire Madeira EEZ, an area of approximately 454,479km^2 (VLIZ, 2014). The inshore study area is about 4,500km^2, which includes the coastal waters from shore up to 12 n.miles, divided into eight survey sectors, covering depths from 0 to –2,000m (Fig. 1).

Data sources

In order to characterise the vessels' temporal and spatial distribution in the offshore and inshore waters of the Madeira Archipelago, two different types of data were used: (1) records collected during shipboard surveys carried out by the Madeira Whale Museum (MWM) research team from 2001–12, in the context of different projects (2001–02 – Project Cetáceos Madeira; 2007–08 – Project Emacetus; 2010–12 – Project Cetáceos Madeira II); (2) AIS data supplied by Administração dos Portos da Região Autónoma da Madeira (APRAM) recorded between 2008 and 2011.

The first type of data was used to build inshore 'traffic sighting rates distribution maps' to compare with 'cetacean sighting rate distribution maps' obtained from data gathered by the same platforms and within the same time period. This gives an overview of the regional marine traffic patterns, allowing identification of higher and lower inshore vessel traffic zones and the detection of possible areas of potential conflict between cetaceans and vessels.

The second type of data was used for: (1) preliminary characterisation of the Madeira offshore traffic; and (2) corroboration of the inshore traffic sighting rates distribution maps (previously described).

Both spatial and temporal analyses were run, pooling the data by boat type and season. The summer and winter

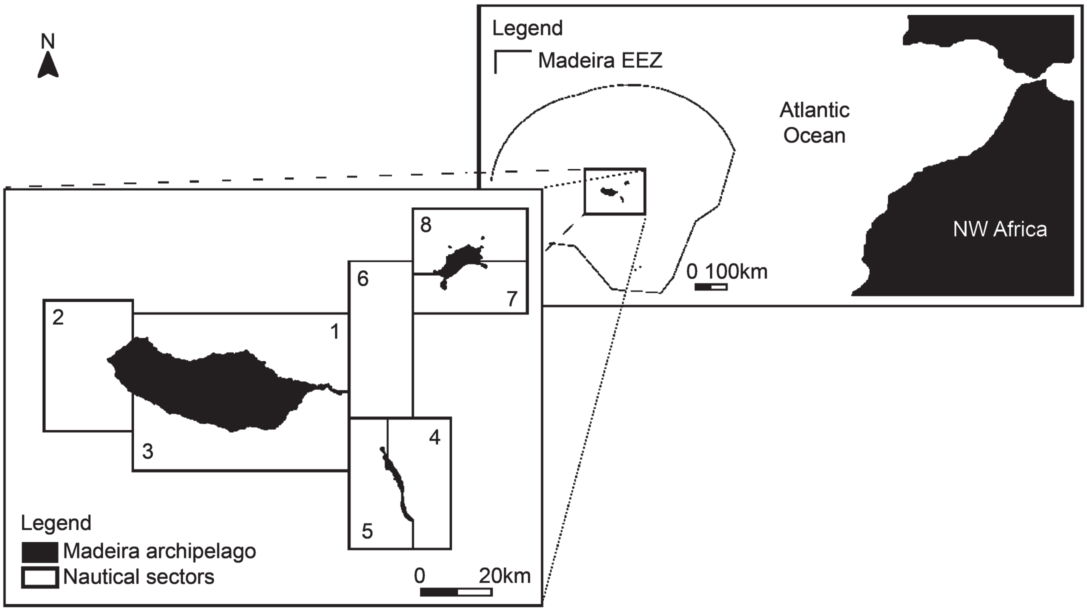


Fig. 1. Study area.

seasons were defined as the periods between June–October and November–May, respectively.

AIS data

Database management

Among the several AIS ship report parameters, only the equipment ID, position, date, Speed Over Ground (SOG) and type of boat attributes were used in this paper.

The data gathered were displayed and validated using ArcGIS 10.1 (ESRI, Redlands, CA, USA). The number of the vessels' position tracks was reduced to one point of coordinates (and related parameters) for each thousand degree cell within the polygon that limits Madeira EEZ, thus reducing the number of coordinates to be handled. This procedure enabled reconstruction of routes, although possibly with some sample bias in areas of heavy route crossing.

Mapping vessels positions tracks

In order to keep the maps presenting the vessels tracks perceptible, a seven day period of vessels' tracks was considered enough to illustrate the traffic scenario (Eiden and Martinsen, 2010). The first week from each month was chosen, except for March from which the last week was used (the only available data).

Vessel types from the AIS data files were organised into four categories: cargo ships (cargo vessels or cisterns); cruise ships (passenger ships); recreational boats (wing-in-ground-effect craft, high speed craft or practical dinghy); and other type of vessels (tugboat, vessel or no classification). When counting the number of vessels per week, each vessel was considered only once.

Speed grid maps

Vessel speed is one of the important factors which might determine the severity of a ship collision with a whale.

A raster file (in ASCII format) was generated, where the vessels medium SOG value was associated to each pixel, creating speed grid maps. The speed grid maps for the offshore traffic area are represented in a grid of 10 × 10 n.miles (18.52 ×18.52km), while the speed grid maps for the inshore traffic area are represented in a grid of 2 × 2 n.miles (3.704 × 3.704km). All vessel types were considered in the same text file.

The probability of a whale being lethally injured by a ship strike is > 50% if the vessel is navigating at speeds over 10 knots, but if the incident occurs while the ship is moving > 15 knots, the chances of a lethal injury increases from 80% to 100% (Vanderlaan and Taggart, 2007). For this study, vessels travelling at ≤ 10 knots were considered as 'low' speed while vessels > 10 knots were considered as 'high' speed.

Shipboard survey collected data

Field work methods

The sampling methods and protocol for cetacean sightings and traffic data collection remained unchanged in its critical aspects over the years.

The shipboard surveys were carried out in Beaufort Sea state ≤ 3. Two research vessels were used during the study period, R/V *Calcamar* for the 2001–02 surveys and the R/V *Ziphius* from 2004 onwards. The first vessel is a 12m open deck wooden fishing boat with an average surveying speed of 5.5 knots and with the observing points at an average height of 3m. The second vessel is a steel motor sail boat with an average survey speed of 6.5 knots and two dedicated observation platforms, one placed ahead midship (one observer) and the other astern (two observers), both at an average height of 4.5m. The field work aimed primarily at surveying cetaceans and was done according to distance sampling methodology (single platform). Each sector of the study area was sampled on average twice every three months, with randomly placed zig-zag transects.

A minimum of three observers scanned the sea continuously looking for animals and every hour all the marine traffic observed up to the horizon, 360° around the vessel, was recorded in a computer using Logger 2000 software, along with the observation effort.

Traffic data recorded

The traffic data recorded included: sighting time; observed vessel(s) type(s) (recreational boat – private vessel with less than 24m in length; fishing boat – commercial fishing boat of any size; big game fishing boat; ships – private or commercial vessel with more than 24m in length; whalewatching boats – vessels with less than 24m in length); number of boats of each type; estimated visibility recorded in classes (visibility ≤ 1 n.miles; 1 < visibility ≤ 3 n.miles; 3 < visibility ≤ 5 n.miles; visibility > 5 n.miles); and the identification of the data recorder.

Every observation was tagged with the research vessel's GPS position at the moment of the sighting, i.e. the longitude and latitude recorded do not correspond to the precise position of the observed vessels. Consequently the records of the vessel sightings have an associated position with an error up to 15 n.miles (≈ 22km) (roughly the maximum distance at which a vessel would be identified taking into account the observation platform height) and vary also according to the observed vessel's type/height (smaller boats are detected at much closer ranges and with a smaller associated position error in relation to the research vessel GPS position). One pair of coordinates might correspond to more than one vessel, according to the number of boats spotted at a particular time. The vessel estimated distance and direction was not taken into account. Furthermore (considering that the traffic data was collected every hour) it is possible that a few vessels might have been registered more than once, depending on their trajectory, change in trajectory and speed in relation to the research vessel and observation conditions.

The data collected were separated into two periods according to the vessels' classification: (1) 2001–09: vessels were classified only as ships, recreational boats or fishing boats; (2) 2010–12: whalewatching and big game fishing were added to the vessel classification list.

Cetacean information records

Data on cetacean sightings included: the date and initial time of the sighting; vessels position at the initial time of the sighting; estimated radial distance and angle to the bow at the initial sighting time; species; minimum, average and maximum group size; and the number of calves.

Mapping vessels estimated locations

The vessels' locations were plotted in a vector environment over a grid with resolution of 2 × 2 n.miles (3.704 × 3.704km), covering the inshore study area. The Madeira Archipelago coastlines and pre-defined inshore survey sectors were also overlaid within the same range.

The observation effort value of each grid cell was represented through a colour gradient in order to show the areas that were more intensely surveyed. The effort was measured as the sum of kilometres of the research vessel's trackline in each cell.

Subsequently, the results were represented on three types of maps: (1) types of vessels distribution maps – pie chart maps, where the proportion of every type of boat was represented per cell; (2) plot of research vessel's locations projected over the effort grid where the sightings points were represented with variable diameters according to the number of boat detections associated with each pair of coordinates; and (3) traffic sighting rates distribution maps – each grid cell's vessel sighting rate, calculated by dividing the number of boats sighted in each cell by the respective survey effort and represented through a colour gradient over the grid.

To avoid misleadingly high sighting rate values in surveyed cells with very low effort, any grid cell with survey effort less than 5km (grid cell diagonal length) (Fortuna, 2006) was filtered out and not quantified in either of the resulting GIS maps. This procedure was systematically applied to all the maps, according to vessel type and seasonality. A seasonal analysis was only possible for traffic sighting rate distributions for vessel types recorded during the whole sampling period (2001–12), i.e. ships, recreational boats and fishing boats.

Mapping cetacean estimated locations

Cetacean sightings data were displayed in the same vector environment as the traffic data.

Following the same procedure, two types of maps were created: (1) cetacean species' distribution maps: pie chart maps, where the proportion of the more relevant species of cetaceans was represented per cell, i.e. by dividing the estimated number of sighted animals of a certain species in an encounter by the total number of animals of all species sighted in the same cell; and (2) cetaceans' sighting rate distribution maps: each grid cell corresponds to the cetacean sighting rate, calculated by dividing the number of cetacean sightings for each cell by the respective survey effort, and represented through a colour gradient over the grid. An additional map was made showing the sighting rates of cetacean groups with calves, i.e. the number of cetacean groups sighted with calves in each cell (disregarding the number of calves) divided by the corresponding survey effort and represented through a colour gradient over the grid.

Likewise, for the marine traffic maps, only cells with a minimum value of 5km of survey effort were displayed.

Data analysis

The normality of the AIS traffic data, as well as the shipboard surveys traffic and cetaceans' data were tested using Shapiro-Wilk and Kolmogorov-Smirnov tests. A *t*-test was applied to the AIS data to check for the existence of a significant difference between winter and summer season distributions.

A Kruskal-Wallis test was run with both general and seasonal traffic sighting rates distributions from the shipboard surveys to check for the existence of significant differences between the sectors. If one distribution was found to be heterogeneously distributed across the area ($p < 0.05$), a Mann-Whitney test was applied to find out which sectors were statistically different from each other and to detect significant seasonal variations in the same sector. The same procedure was applied to the cetacean distributions.

The statistical tests were run in IBM SPSS Statistics 19 software.

RESULTS

AIS data

Offshore traffic distribution

The number of vessels crossing the Madeira EEZ varied between 100 and 300 vessels per week (Fig. 2a). The highest traffic peaks are in the summer months (August 2008 and September 2009) and the lowest in the winter season (February 2009 and May 2011). Nevertheless, no significant differences were found (t-test results $p > 0.05$) between seasons.

The Madeira EEZ was found to be intersected by an average of 188 vessels per week, i.e. 0.0004 vessels per km^2.

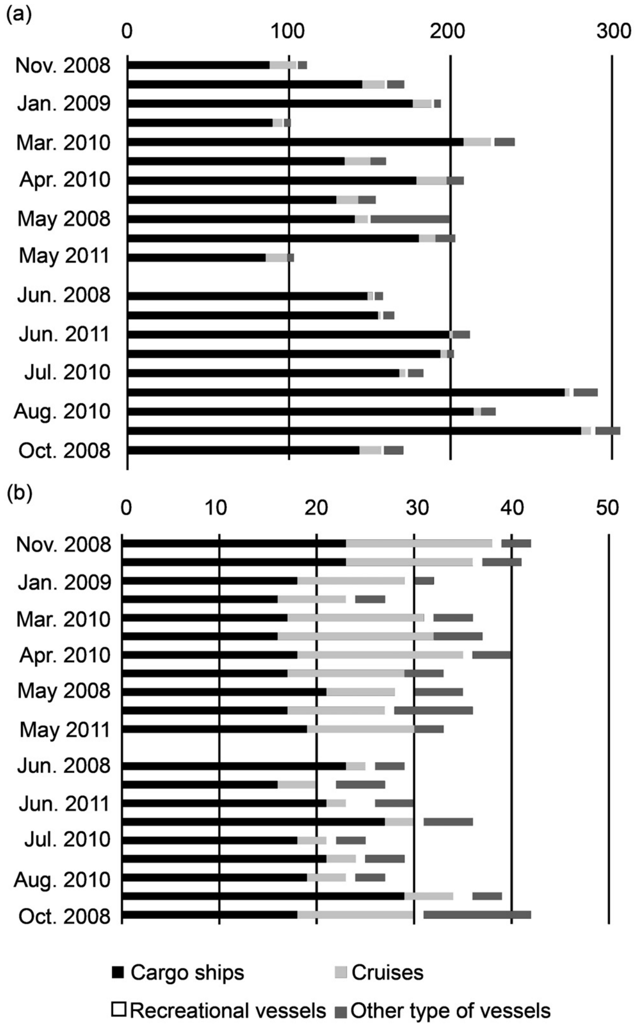


Fig. 2. Number of vessels per week and the percentage of vessel type incidence per each representative month during the Winter (November–May) and Summer (June–October), between 2008 and 2011: (a) offshore traffic; (b) inshore traffic.

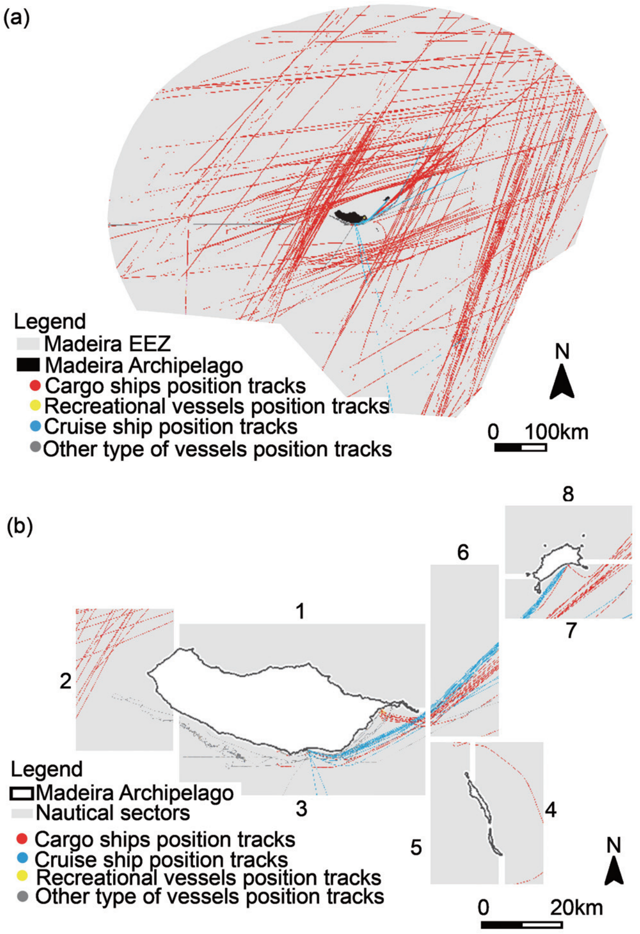


Fig. 3. Vessel position tracks projected over the Madeira EEZ area. Some areas show higher track density shipping lanes. Only one representative map (July 2008) is displayed among the 20 representative months used in the present study: (a) offshore traffic; (b) inshore traffic.

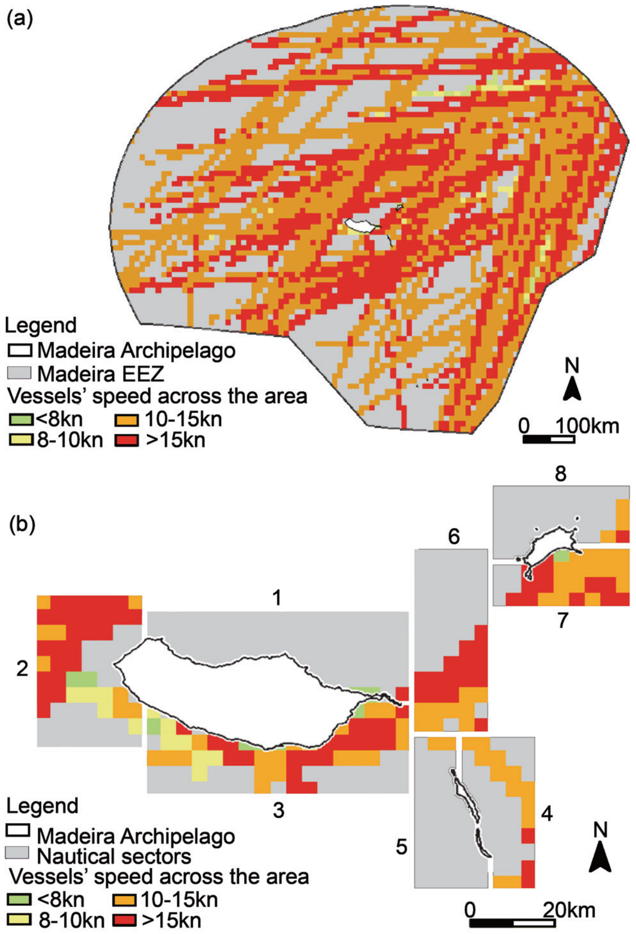


Fig. 4. Speed grid cell maps displayed within the Madeira ZEE area. Each pixel represents the vessels Speed Over Ground (SOG) average value. Only one representative map (July 2008) is displayed among the 20 representative months used in the present study. The 'low' speed cells are coloured in green and yellow and the 'high' speed cells are coloured in orange and red: (a) offshore traffic; (b) inshore traffic.

Traffic composition does not vary considerably, with cargo ships representing at least 70% of all traffic, followed by either unclassified vessels or cruises/ferries, in variable proportions (Fig. 2a). May 2008 stands out as an outlier from the remaining sampled months, with the 'other type of vessels' category representing a greater proportion of the traffic composition.

It was possible to identify five recurrent shipping lanes (see Fig. 3), common to all 20 AIS traffic monthly maps (only July is presented for 2008): three lanes presenting a NE–SW orientation, one at the West side and the other two at the East side of the islands; two orientated on an E–W axe: one to the South and the other to the North of the islands. The NE–SW orientated shipping lane, located further East from the Madeira Island seems to be more intensively used. All these shipping lanes are mostly used by cargo ships.

Inshore traffic distribution

The number of vessels registered by the AIS system varied between 27 and 42 boats per week, year round. This inshore area was also mainly intersected by cargo ships (Fig. 2b and Fig. 3b), many of them navigating closer to Madeira.

The inshore traffic area was intersected by an average of 34 vessels per week, i.e. 0.008 vessels per km^2.

Vessel speeds

Though all vessel types were considered, the speed values are mostly from ships (including cargos and cruises), accounting for at least 70% in both offshore and inshore traffic composition of one week of each sampled month (Fig. 4).

In general, both inshore and offshore cells present on average 'high' speed traffic.

Traffic data collected in inshore shipboard surveys

A total of 830 vessels were sighted, 401 between 2001 and 2009 (54% fishing boats, 27% recreational boats and 15% ships) and 429 between 2010 and 2012 (47% fishing boats, 24% recreational boats, 17% ships, 10% whalewatching boats and 2% big game fishing boats).

All types of vessels' sighting rates revealed a non-normal distribution ($p < 0.05$).

The descriptive statistics data (mean and standard deviation) for the general and seasonal vessels' sighting rate distribution, for each type of vessel and for all sectors, are presented in Table 1.

The Mann-Whitney test results are presented in Fig. 5, according to the type and season of vessels' sighting rate distributions.

All vessels

According to Fig. 5 and Figs 6a and 6b, Sector 3 is significantly different (Mann-Whitney test significant results for $p < 0.05$) from all the remaining sectors and holds the highest average

Table 1

Mean and standard deviation (SD) of the traffic sighting rates (number of vessels per 10km) distribution maps according to boat per vessel type and seasonality.

Vessel type	Season	Total area		Sector 1		Sector 2		Sector 3		Sector 4		Sector 5		Sector 6		Sector 7		Sector 8	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
AV	S0	0.66	1.22	0.42	0.69	0.41	0.86	1.59	2.16	0.30	0.57	0.41	0.57	0.56	0.72	0.31	0.34	0.66	0.95
	S1	0.69	1.53	0.53	1.36	0.62	1.29	1.46	2.59	0.49	1.23	0.48	0.88	0.50	0.76	0.32	0.49	0.55	0.90
	S2	0.58	1.42	0.34	0.76	0.32	1.01	1.35	2.43	0.26	1.01	0.39	0.60	0.67	1.23	0.27	0.45	0.53	1.40
S	S0	0.14	0.42	0.03	0.11	0.14	0.59	0.37	0.68	0.06	0.16	0.08	0.20	0.15	0.31	0.07	0.13	0.06	0.19
	S1	0.14	0.41	0.05	0.16	0.19	0.69	0.32	0.59	0.12	0.32	0.06	0.24	0.13	0.28	0.08	0.20	0.04	0.23
	S2	0.13	0.47	0.02	0.09	0.12	0.69	0.34	0.74	0.05	0.19	0.11	0.29	0.17	0.42	0.05	0.13	0.05	0.20
RB	S0	0.18	0.46	0.09	0.34	0.02	0.10	0.46	0.78	0.11	0.31	0.12	0.33	0.20	0.44	0.13	0.20	0.12	0.30
	S1	0.17	0.57	0.18	0.77	0.06	0.19	0.38	0.89	0.11	0.44	0.10	0.33	0.14	0.32	0.13	0.28	0.08	0.25
	S2	0.19	0.69	0.03	0.17	0.00	0.00	0.55	1.23	0.05	0.16	0.07	0.17	0.34	0.93	0.12	0.29	0.13	0.53
FB	S0	0.29	0.54	0.29	0.46	0.23	0.59	0.52	0.77	0.10	0.21	0.22	0.27	0.21	0.35	0.11	0.18	0.48	0.75
	S1	0.33	0.81	0.31	0.70	0.36	1.01	0.49	1.21	0.26	0.65	0.32	0.50	0.23	0.43	0.11	0.24	0.43	0.86
	S2	0.24	0.63	0.29	0.73	0.22	0.72	0.44	0.78	0.04	0.17	0.21	0.37	0.16	0.54	0.10	0.23	0.32	0.87
WWB	S0	0.04	0.30	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…
BGF	S0	0.007	0.70	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…

Key: Vessel type: AV = all vessels; S = ships; RB = recreational boats; FB = fishing boats; WWB = whalewatching boats; BGF = big game fishing boats. Season: S0 = general distribution; S1 = winter distribution; S2 = summer distribution.

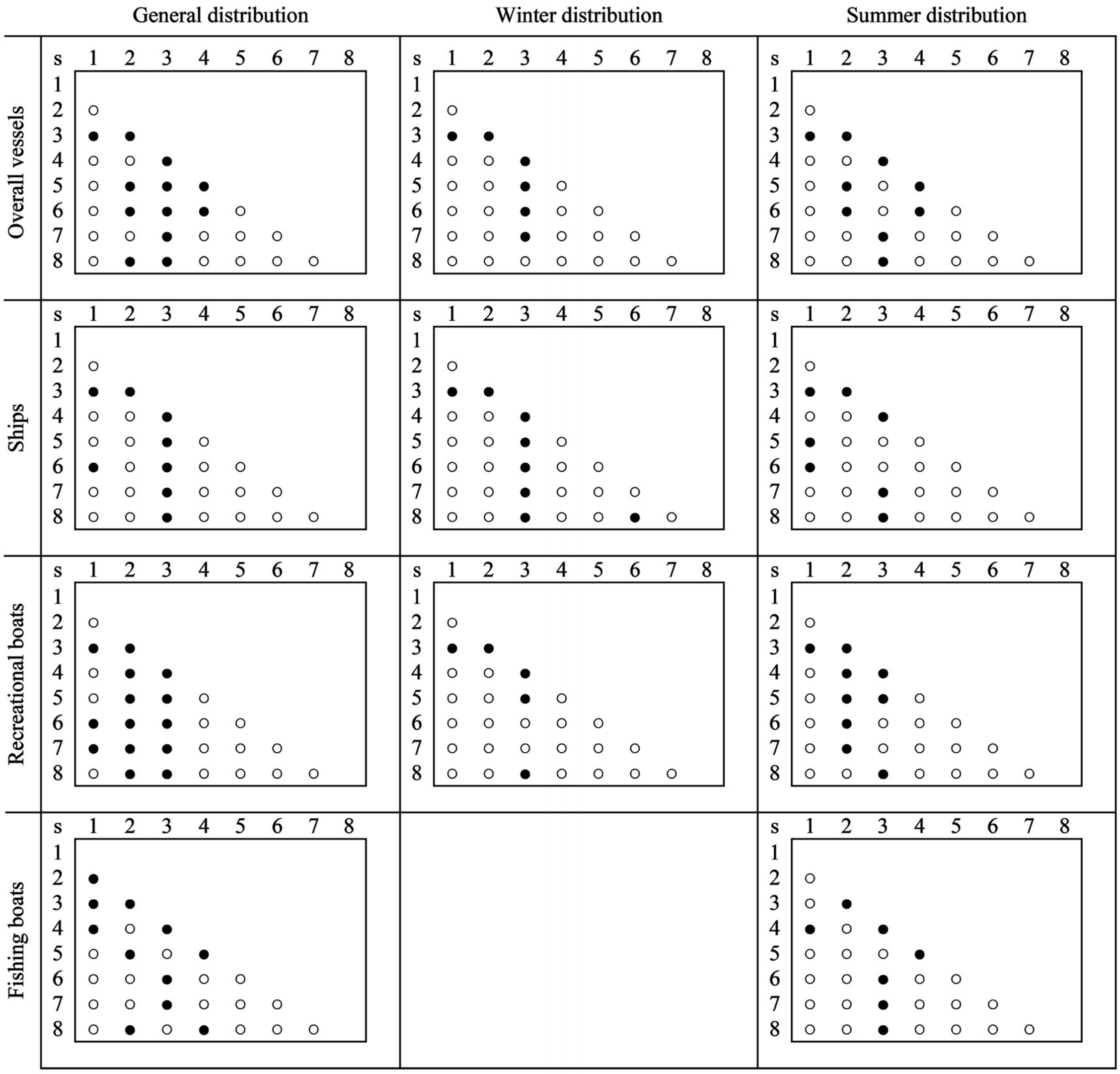


Fig. 5. Results of the Mann-Whitney tests for the different types of vessels and seasonality (winter and summer). Matrix comparing vessel sighting rates distribution across the sectors of the study area. Key: Black circles P ≤ 0.05; White circles P > 0.05.

vessels' sighting rates, standing out as the inshore traffic 'busy zone'. Sectors 2 and 4 are the less intensive traffic areas. Sector 2 is significantly different from every other sector except 4 and 7. However, apart from Sector 3, only Sectors 5 and 6 are also significantly different from Sector 4. Thus, Sectors 3, 5 and 6 present higher traffic activity. During the winter, Sector 3 is the more intensively used sector, significantly different from all the remaining sectors, except Sector 8. During the summer season, Sector 3 is significantly different from all sectors except 5 and 6, indicating a higher traffic activity in those areas. However, no significant seasonal variations were found in any of the sectors.

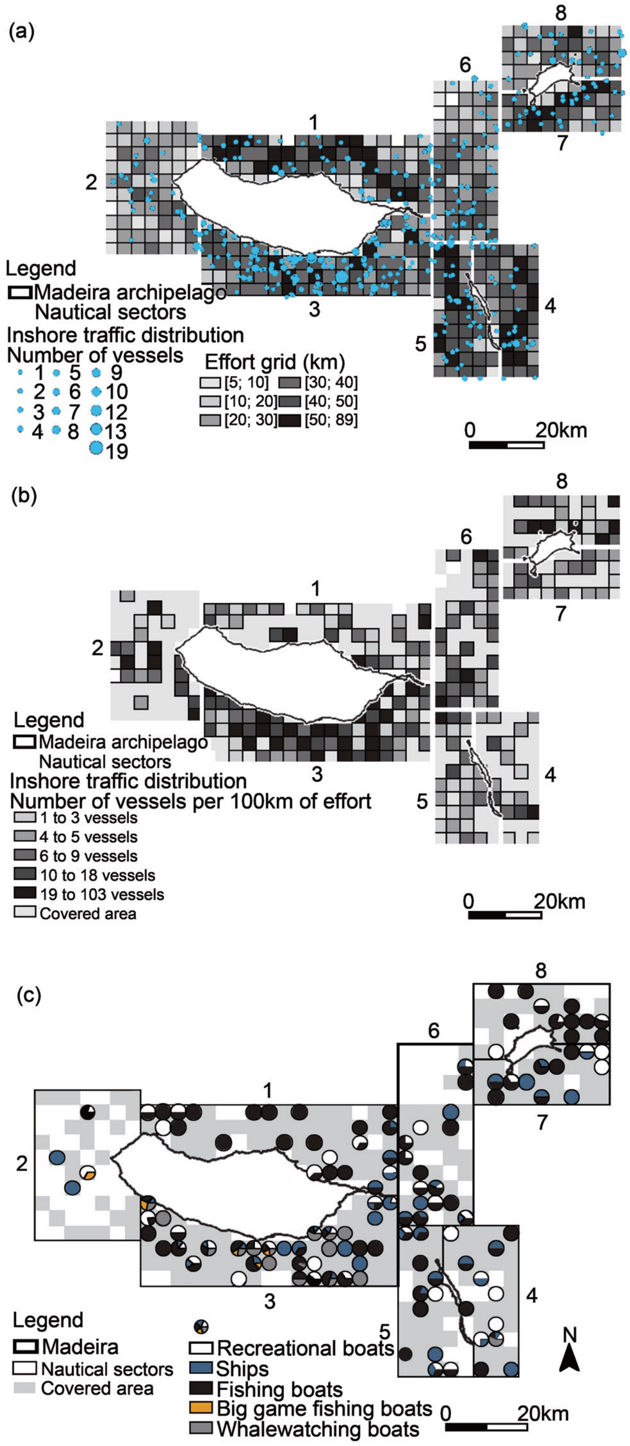


Fig. 6. Inshore traffic distribution maps: (a) plot of all vessel locations over the effort grid. Each coordinate point may correspond to more than one vessel sighting, represented through a variable circle diameter. Each effort grid cell presents a different gradient depending how much it was surveyed; (b) general traffic sightings rates distribution map (each cell value corresponds to the number of vessels per 100km of effort); (c) inshore vessel type distribution represented through a pie chart per grid cell representing the proportion of each type of vessel sighted in that location for the period 2010–12.

Ships

In both general and winter ship traffic distributions, Sector 3 had the higher activity and is significantly different from the remaining sectors. Sector 6 has a higher level of ship traffic, being also significantly different from the Sectors 1 and 8, with the lowest general and winter ship traffic distributions, respectively. During the summer season, Sector 3 is no longer significantly different from Sectors 5 and 6, indicating an increase in ship traffic in those areas. However, no significant seasonal variations were found in any of the sectors.

Recreational boats, whalewatching boats and big game fishing boats

Though recreational boats are spread throughout all sectors, Sectors 3, 6 and 7 present a higher activity (Table 1 and Fig. 5), particularly in Sector 3 that is significantly different from all sectors, except 6 and 7, for both winter and summer seasons. Sector 2 is more active during the winter (Mann-Whitney test significant results for $p < 0.05$).

All the whalewatching boats' sightings positions are located in Sector 3, except for one sighting location in Sector 4.

Big game fishing boats use mainly Sector 3.

Fishings boats

Considering the fishing boats' general distribution, Sector 3 is the most used, followed by Sectors 5 and 8. Sector 2 is the least used sector, followed by Sector 1 (Table 1 and Fig. 5). There were found no significant differences among the sectors during the winter season. Traffic activity seems to drop in Sector 4 during the summer season (Mann-Whitney test significant results for $p < 0.05$).

Based on these results it is possible to identify a 'higher use corridor' composed by Sectors 3 and 6, both sectors of higher activity in most traffic distributions across the inshore study area, as further described below.

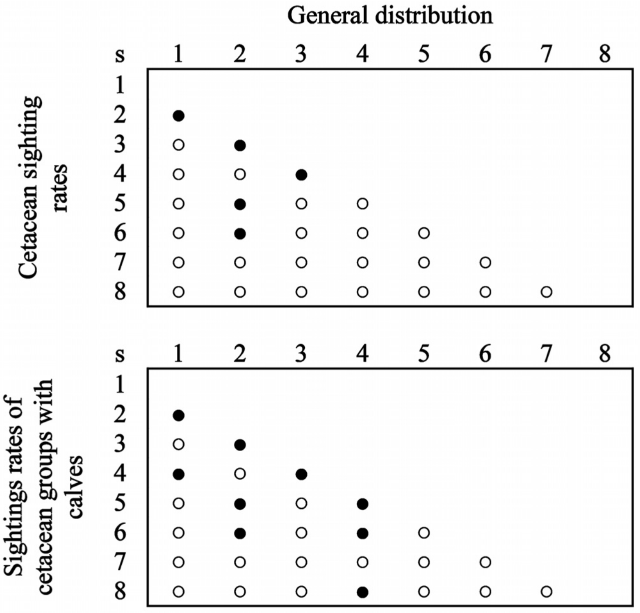
Cetaceans data collected during shipboard surveys

The descriptive statistics data (mean and standard deviation) for the general sighting rate distribution, for all cetaceans and cetaceans groups with calves, for all sectors, are presented in Table 2.

Table 2

Mean and standard deviation (SD) of the general sighting rates distribution maps, for overall cetaceans and cetacean groups with calves, according to sectors.

	Cetaceans		Cetacean groups with calves	
	Mean	SD	Mean	SD
Sector 1	0.39	0.40	0.07	0.15
Sector 2	0.25	0.45	0.07	0.26
Sector 3	0.46	0.47	0.12	0.19
Sector 4	0.26	0.29	0.02	0.08
Sector 5	0.32	0.33	0.08	0.15
Sector 6	0.36	0.38	0.09	0.18
Sector 7	0.31	0.46	0.06	0.13
Sector 8	0.34	0.38	0.09	0.20



General distribution — Cetacean sighting rates

s	1	2	3	4	5	6	7	8
1								
2	●							
3	○	●						
4	○	○	●					
5	○	●	○	○				
6	○	●	○	○	○			
7	○	○	○	○	○	○		
8	○	○	○	○	○	○	○	

Sightings rates of cetacean groups with calves

s	1	2	3	4	5	6	7	8
1								
2	●							
3	○	●						
4	●	○	●					
5	○	●	○	●				
6	○	●	○	●	○			
7	○	○	○	○	○	○		
8	○	○	○	●	○	○	○	

Fig. 7. Results of the Mann-Whitney tests for the cetacean distributions. Matrix comparing cetacean sighting rates distributions (overall and of groups with calves) across the sectors of the study area. Key: Black circles P ≤ 0.05; White circles P > 0.05.

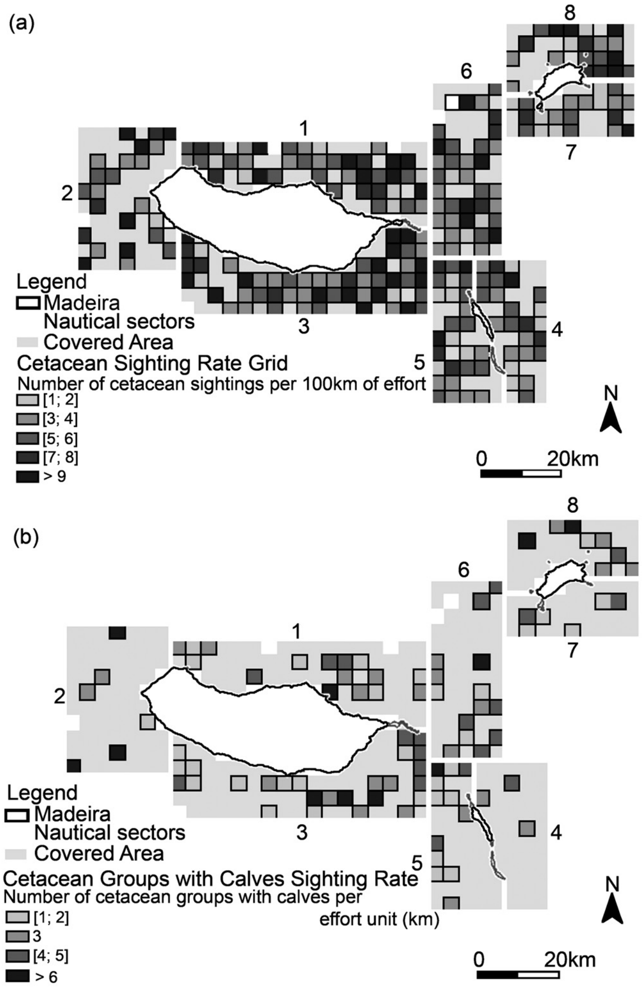


Fig. 8. Inshore cetacean sighting rates distribution maps: (a) general cetacean sighting rates distribution map (each cell value corresponds to the number of sightings per 100km of effort); (b) cetacean groups with calves sighting rates distribution map (each cell value corresponds to the number of sightings where calves were present per 100km of effort).

All cetaceans

According to the Mann-Whitney test results (Table 4), Sectors 2 and 4 presented the lowest cetacean presence. Sector 2 is significantly different ($p < 0.05$) from all sectors, except Sectors 4, 7 and 8. Sector 3 has the highest cetacean presence, being significantly different from Sectors 2 and 4, followed by Sectors 1, 6 and 5.

Cetacean groups with calves

According to the Mann-Whitney test results (Fig. 7), Sectors 2 and 4 have the lowest presence of groups with calves, while Sectors 3, 5 and 1 have the highest, followed by Sector 6. In Fig. 8b it is possible to see that most cells in Sector 5 marked with cetacean presence are in the upper part of that sector, between Sectors 3 and 6.

DISCUSSION

AIS Data

Traffic distribution

Even though some cargo ships are headed to Caniçal or Funchal, the study area seems to be mostly crossed by cargo ships heading to different destinations such as the North Sea, Middle East, the North and South America or the Mediterranean region. Cruises/ferries on the other hand cross the area usually to reach Funchal Port, one of the traditional cruise ship stops in this region of the Atlantic.

An exceptional event or combination of events, that the authors could not identify may have led to a much higher than normal traffic of vessels of the ‘other type of vessels’ category in May 2008.

Madeira EEZ maritime traffic level, though less intensive, is still considerable when compared with some of the busiest waterways in the world. The Baltic Sea, with a surface area of approximately 392,978km^2 (Lepparanta and Myrberg, 2009), corresponding to approximately 86% of Madeira EEZ, is crossed by an average of 1,319 vessels per seven day period (0.0034vessels km^{-2}) (Eiden and Martinsen, 2010) and the Madeira EEZ corresponds to approximately 12% of its traffic. The North Sea, with approximately 750,000km^2 (Lepparanta and Myrberg, 2009), is crossed by an average of 1,335 per seven days period (0.0018vessels/km^2) (Eiden and Martinsen, 2010). Madeira EEZ corresponds to 61% of the North Sea area and to 22% of its traffic. However, Eiden and Martinsen (2010) reported that the presented number of vessels crossing the North Sea is underestimated.

To put this into perspective, the inshore study area should be compared with other coastal areas with similar surface areas. The Strait of Gibraltar is located between the southern coast of Spain and the northern coast of Morocco (58km long and approximately 13km at shorter distance between shores) and is an important shipping route since it is the only connection between the Atlantic Ocean and the Mediterranean Sea. Its surface area is roughly 1,914km^2 (considering the strait’s western extreme 43km wide, between Barbate and Tanger and the eastern extreme 23km wide, between Rock of Gibraltar and Ceuta Canyon) corresponding approximately to 43% of the Madeira inshore study area and is crossed by an average of 1,975 vessels per week (IWC, 2011), i.e. approximately 1.03 vessels km^{-2}. The inshore traffic of the study area is about 0.8 % of the Strait of Gibraltar’s traffic.

Vessel speeds

A closer look at the average traffic speed in inshore cells indicates that 'low' speeds are common (green and yellow coloured cells) near Madeira and Porto Santo Islands ports (Funchal, Caniçal and Porto Santo ports), which are mostly associated with vessels approaching and leaving harbours or mooring places (Sectors 3 and 7). However, 'high' speeds cells are the most common in these two sectors (Fig. 4b). In Sectors 4 and 5 (East and West Desertas) the 'low' speed cells closest to the shore are from small vessels, as ships would not be able to moor there. Desertas Islands are frequently sought out for touristic purposes, by local recreational and commercial boats and foreign recreational boats. Cargo and cruise ships also use these sectors, passing farther away from the coast to different destinations at cruising speed. The same happens in Sector 6 (the passageway between Madeira and Porto Santo island) and Sector 2 (West of Madeira), where 'low' speeds are rare (Fig. 4b).

AIS data limitations

Though this kind of data has revealed itself to be very useful and accurate, it has some limitations. The AIS transmission range is limited and can vary depending on the transmitting and receiving aerial heights as well as meteorological conditions that can affect the spatial coverage (dependent on the VHF signal range from the coast). This is particularly true for the greater distances from Funchal (Eiden and Martinsen, 2010; Mou *et al.,* 2010), as shown in Fig. 3 where large gaps can be found between points from the same route.

The original database files were large and hard to manage, so file converters were used to reduce their sizes. The discontinuity and heterogeneity of AIS data available for each of the sampling units throughout the study period was also a problem. Data was unavailable for some months of a particular year or some months were integrally represented while others only had data covering a few days.

Even though AIS covers a great variety of vessel types, smaller recreational boats may not have such devices (Eiden and Martinsen, 2010; Evans *et al.,* 2011; IWC, 2011; Mou *et al.,* 2010). Nevertheless, the percentage of smaller recreational boats should still be smaller than cargo or cruise ships, as they tend to be either local boats navigating mostly inshore waters or they are sailing boats crossing the Atlantic or passing through Madeira on their way to the Canary Islands or the Caribbean. This type of traffic is more frequent during the autumn season (October to December), when they can take advantage of the trade winds.

The number of vessels given here is also certainly underestimated as no AIS data on fishing vessels was available.

Traffic data collected in inshore shipboard surveys

Considering the inshore traffic composition (Fig. 6c), Sector 3 stands out as the zone with the higher traffic level, used by every type of vessel, as expected. The south of Madeira Island, with calmer waters sheltered from the trade winds (NE), is the most populated coast and is where most of the small harbours and main ports are located in the archipelago. These characteristics justify why this is the sector with higher traffic both in summer and winter. Sector 6 follows, with an important amount of movement between the two main islands, frequently done by recreational boats, fishing boats and ships, especially in summer time.

Though Sectors 3, 5 and 6 appear to be the most frequently used sectors, when crossing these data with the available AIS data for the same area (Fig. 3b), mainly composed of ships, Sector 5 is rarely crossed. This may be justified by the associated discrepancy of some recorded positions of observed bigger vessels, as explained in the methodology section.

The Madeira fishing fleet is 89% composed of vessels less than 12m in length (Direcção-Geral das Pescas e Aquacultura, 2007), carrying out demersal fishing and operating near the harbours. The most profitable are the tuna and black scabbard fishing fleets, which together accounted for 84% of the total landings and 87% of 2012 economic revenue of the fishing activity in the region (Instituto Nacional de Estatistica, 2013), also operate offshore, away from the area covered by the inshore shipboard surveys. These fleets may be underrepresented in the traffic sighting rates maps, especially during the summer period. The black scabbard fishery usually runs May–December and the tuna fishery usually runs between April and October.

Potential impact towards cetaceans

Comparing the distributions of traffic and cetaceans (all groups and groups with calves) with the inshore study area it can be seen that the traffic 'higher use corridor' (including Sectors 3 and 6) overlaps a substantial part of the cetaceans' preferential distribution area, where the encounter rates are higher. Therefore, this corridor can be considered a 'potential danger zone'. According to previous studies, Sectors 3 and 6 include a critical area for cetaceans in general, where these are more frequently sighted (Freitas *et al,* 2014).

Cetaceans may be disturbed by vessels in different ways, such as (eco)tourism, ship strikes or water noise and pollution produced by boats (Bejder and Samuels, 2003; Laist *et al.*, 2001; Weilgart, 2007). However, some types of vessels are associated with specific cetacean interactions that should be considered independently. Likewise, some species are more prone to traffic interactions, than others.

The interactions between cetaceans and whalewatching vessels have been previously investigated in the study area when short term effects were observed among the Delphinidae (Ferreira, 2007). Stress responses have also been reported for short-finned pilot whales when followed by whalewatching boats, especially when these encounters were not conducted following the voluntary guidelines (Freitas *et al.,* 2004a).

Unfortunately, there are no data available on underwater noise or water pollution in the Madeira archipelago. Therefore, the following discussion will be mainly focused on ship strikes.

Potential ship strike risk in inshore waters

The incidences of ship strikes in a certain area are not easy to quantify. Their probability depends on different variables such as the level of traffic activity, the number of cetaceans and their behaviour within that area. The amount of time whales spend underwater away from watercraft and their ability to detect and consequently avoid them are related to the probability of ship strikes within a certain area.

Every type and size of vessel can strike whales, but the more serious or lethal cases registered occurred with ships with a length of 80m or more, travelling at speeds over 14 knots (Evans *et al.*, 2011; Laist *et al.*, 2001), usually ferries, cargo and cruise ships. In previous studies focused on the collisions between vessels and Mediterranean fin whales (Panigada *et al.,* 2006), ferries and cargo ships were the type of vessels with the highest number of strikes, accounting for 62.5% and 16.7% of cases, respectively.

The probability of a ship strike being fatal increases from 20% to 80% as ship speed increases from 8.6 to 15knots. At speeds below 11.8 knots, the likelihood of lethal injury is less than 50%, while at speeds over 15 knots, the probability rises from 80% to 100% (Vanderlaan and Taggart, 2007).

Even though in Sector 3 ships tend to reduce speed or slowly pick up speed as they get close to or away from the ports, temporarily giving time and space for whales to avoid them, cells with average speed over 15 knots are still present (Fig. 4), specially away from the shore and at higher depths where the presence of whales is more likely. In Sector 6, also part of the 'higher use corridor', speeds over 10 knots are the most frequent. This means that if a ship strike takes place within this area there is a high probability it will be fatal.

Fin whales (*Balaenoptera physalus*), short-finned pilot whales (*Globicephala macrorhynchus*), Cuvier's beaked whales (*Ziphius cavirostris*) and sperm whales (*Physeter macrocephalus*) are the species present in the study area (Fig. 9) and are among the species known to be more frequently involved in ship strikes (Carrillo and Ritter, 2008; Laist *et al.*, 2001; Panigada *et al.*, 2006).

All the species mentioned above are present in Sectors 3 and 6 (Figs. 9 and 10). The short-finned pilot whale stands out from the remaining species due to its localised distribution (adults and calves), which overlaps the 'higher use corridor', making it potentially more vulnerable to ship strikes (Fig. 8). Baleen whales, sperm whales and beaked whales were sighted in the same area, especially across Sector 6 (Freitas *et al.*, 2004b; Freitas *et al.*, 2014).

Vessel strikes involving small cetaceans are more frequently associated with small vessels and in many cases the animals show evidence of vessel propeller cuts (Waerebeek *et al.,* 2007). 'Small vessels are here defined as fast small to medium size planing craft powered by inboard or outboard engines, where most of the recreational, whalewatching and big game fishing boats are included. Considering these three vessel types all together, they represent almost 40% of the Madeira inshore traffic fleet. Recent reports also refer to cases of vessel collisions with cetaceans caused by sailing boats (Ritter, 2012).

Bottlenose dolphins (*Tursiops truncatus*), Atlantic spotted dolphins (*Stenella frontalis*) and common dolphins (*Delphinus delphis*) are some of the species globally reported

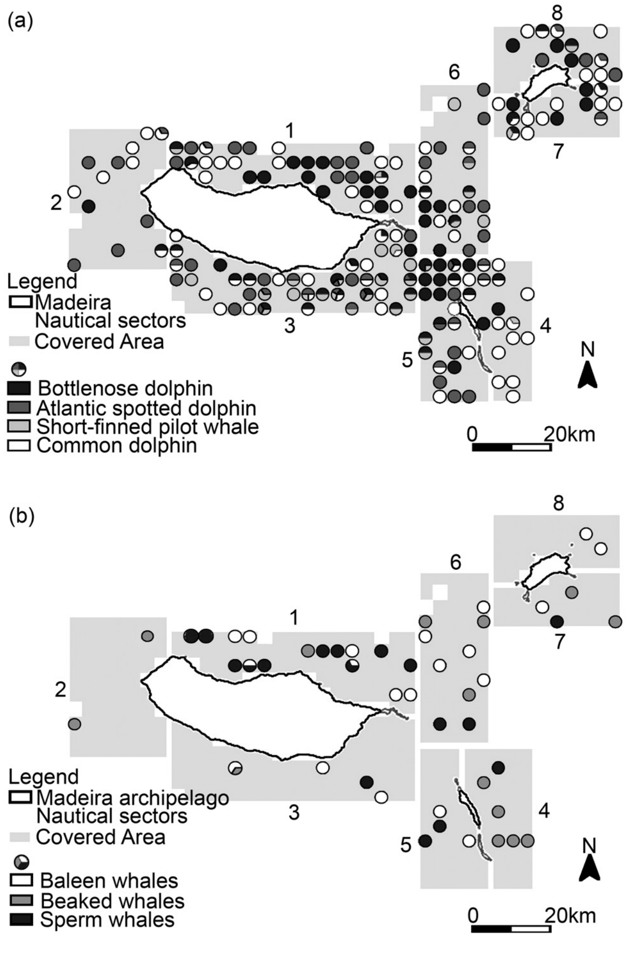


Fig. 9. Distribution of cetacean species presence represented through a pie chart where each pie represents the proportion of each species sighted within each grid cell through the period 2001 until 2012: (a) dolphin presence distribution pattern; (b) whale presence distribution pattern.

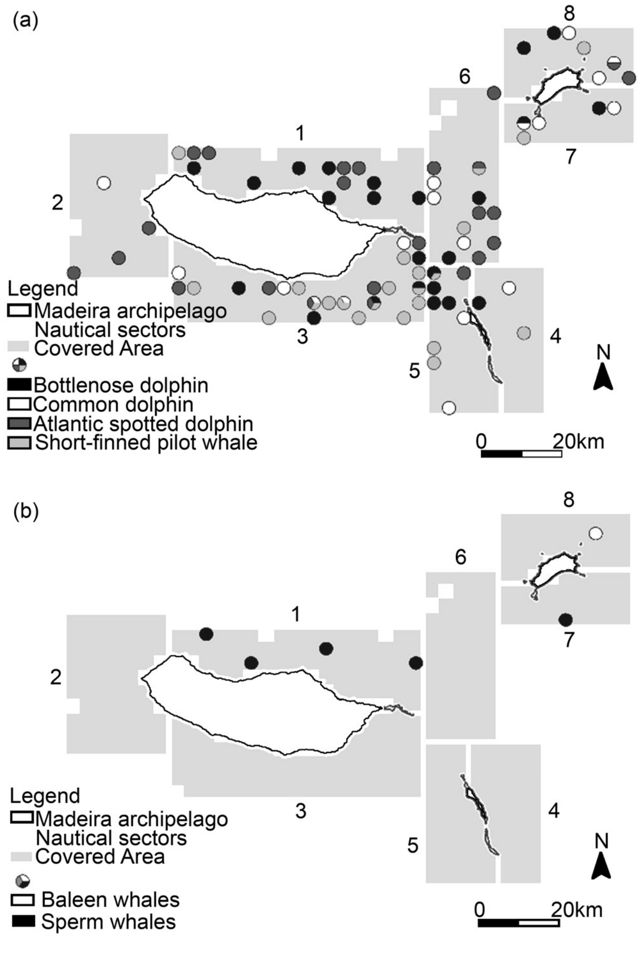


Fig. 10. Distribution of cetacean species presence represented through a pie chart where each pie represents the proportion of each species sightings with calves within each grid cell through the period 2001 until 2012: (a) dolphin presence distribution pattern; (b) whale presence distribution pattern.

as casualties of small vessel strikes. Both adults and calves are present in the sectors most intensively used by recreational boats (Sectors 3, 6 and 7), whalewatching boats (Sector 3) and big game fishing boats (Sector 3), and thus subject to its potential impact.

In the MWM strandings database (1986–2012) there are three deaths associated with ships strikes out of the 136 stranded animals recorded: a possible ship strike with Gervais' beaked whale (*Mesoplodon europaeus*) and two confirmed ship strikes, one with a Cuvier's beaked whale (one out of five recorded standings of this species) and another with a common dolphin. These species were previously reported as more vulnerable to traffic incidents (Laist *et al.*, 2001; Waerebeek *et. al.*, 2007).

Although not many strandings associated with vessel strikes have been reported so far in the Madeira islands, these might have been overlooked, since most carcasses usually sink to deep waters before stranding or refloating due to decomposition. Some animals may be hit in the open ocean or may drift away from the islands' coast never to be detected (Laist *et al.*, 2001; Silber *et al.*, 2012; Weilgart, 2007). Also, carcasses in advanced states of decomposition may mask signs of possible causes of death (Laist *et al.*, 2001; Silber *et al.*, 2012) and blunt trauma impacts may not show any external signs (Evans *et al.,* 2011; Silber *et al.,* 2012).

Madeira is expected to have far less ship strikes than continental coastal areas due to the oceanic nature of most marine traffic (lower cetacean densities in open ocean) in the archipelago and the relatively small coastal traffic, namely ferries, when compared with, for example, the Canary Islands. Madeira has one ferry (20 knots) connecting the two main Islands travelling at most twice a day, while the Canary Islands have several fast ferries (≥30 knots) and regular ferries connecting all the islands with several trips per day.

The 'higher used corridor' thus stands as a potential vessel strike risk area (Fig. 11) in the context of the Madeira archipelago marine traffic.

Potential ship strike risk in offshore waters

Unfortunately, there are no data on cetacean distribution (e.g. sightings rates per cell) in the offshore waters of the Madeira EEZ making it impossible to compare both cetaceans' and vessels' distribution patterns to identify overlapping areas of higher cetacean numbers and traffic presence. Nevertheless, cetaceans' densities are expected to be lower in oceanic open waters, both because of the expected lower food availability and the huge areas involved. However, the months with higher level of traffic activity correspond to the summer period, where the presence of calves is more likely, increasing the possibility then of being hit by a ship.

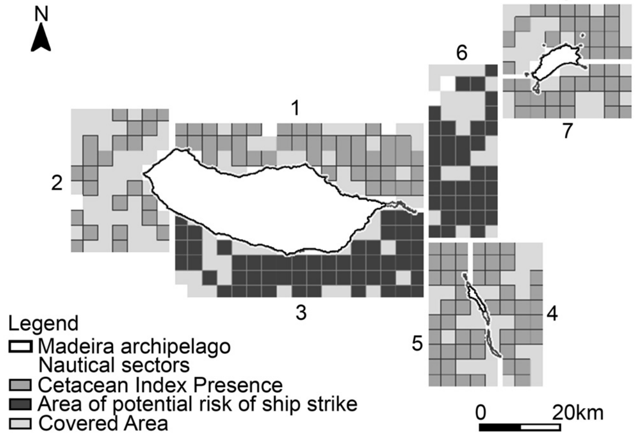


Fig. 11. The 'potential danger zone'. The darker cells represent the cetacean index presence that intersected Sectors 3 and 6, the 'higher use corridor', a preferential area for both cetaceans and ships, where the latter often cross the area moving at speeds over 10 knots , i.e. a potential ship strike area.

As expected, there is very little evidence of ship strikes in Madeira offshore waters, mainly because it is a large area with little human presence and a relatively small nearby coast line where carcasses may come ashore. Difficulties in gathering ship strike evidence have been reported in most other related studies (IWC, 2011; Laist *et al.,* 2001; Waerebeek *et al.,* 2007), even in areas where ship strikes are a serious concern (Carrillo and Ritter, 2008). Some of the most intensive studies on the subject, have focused not only on stranding archives but also on historical and anecdotal records, and still, only a few of the total number of ship strikes were revealed (IWC, 2011; Laist *et al.*, 2001). This type of archival data was not collected in the present study.

CONCLUSION

The marine traffic in the Madeira EEZ, while not so alarming as in other areas of higher traffic level, is still a concern and may have an important impact in the surrounding environment that should not be ignored.

A 'higher use corridor' in Madeira inshore waters used by both vessels and cetaceans was identified, standing as a potential ship strike risk zone. Even so, based on the available evidence, the marine traffic impacts are not apparently high and the animals continue to use the area, indicating that at the present impact level it is, at least, tolerable.

It is important that studies of the spatial and temporal characterisation of the maritime traffic in Madeira EEZ continue, in order to identify specific routes and produce traffic density maps for this area. To obtain real positions of sighted vessels a radar should be used during the inshore shipboard surveys run by the MWM around Madeira inshore waters.

The potential impact regarding ship strikes and water noise on cetaceans should be quantified for the present study area.

In order to infer the probability of a ship strike in the study area, some of the ASCOBANS (IWC, 2011) recommendations on the subject could be followed. Among other measures, a dedicated trained observer should be placed on board cargo and cruise ships to register cetacean presence and interactions/behaviour towards marine traffic in the vicinity (Correia *et al.,* 2015). The available species photo-identification catalogues should also be used to detect possible signs of blunt trauma, such as propeller cuts, in either adults or young cetaceans.

It is recommended that fast ferries in the Madeira EEZ should not be permitted as it has already been proven that these are responsible for several ship strike incidents with cetaceans elsewhere (Carillo and Ritter, 2008).

It is too soon to understand the real impact of marine traffic in the Madeira EEZ based on these initial results. It is important to keep track of the traffic expansion and ascertain how it is impacting cetacean populations so that, if required, mitigation measures may be implemented in time.

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Mitigation of harm during a novel behavioural response study involving active sonar and wild cetaceans

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ABSTRACT

Some studies of how human activities can affect wild free-ranging animals may be considered to have potential negative outcomes too severe to be ethically studied. This creates a societal dilemma involving choices between continuing risky activities with high uncertainty about their potential effects on wildlife, often with considerable associated precaution or undertaking focused research to reduce uncertainty, but with some risk of harm from either strong response leading to potential stranding or direct physical injury from sound exposure. Recent and ongoing field experiments have measured the conditions in which wild cetaceans respond to military sonar, and provided insight into the nature of responses. Here mitigation measures are reported for one of the first such experiments designed to measure fine-scale behavioural responses to controlled exposures of mid-frequency (3–4 kHz) active sonar. The objective was to do so without causing the kinds of physical harm that have been previously observed (e.g. stranding events) and that motivated the study. A critical goal of this experimental study was to identify a response that was safe but that could be used as an indicator of the probability of risk from more extreme or sustained exposure from real military operations. A monitoring and mitigation protocol was developed using a feedback control procedure for real-time mitigation of potential harm. Experimental protocols were modulated relative to indicators of potential risk with the explicit objective of detecting potentially harmful consequences of sound exposure and taking appropriate corrective action. Three categories of mitigation methods were developed and integrated within the experimental protocol incorporating designed, engineered, and operational mitigation measures. Controlled exposure experiments involving free-ranging animals were conducted without any evident harm to the experimental subjects, while successfully eliciting behavioural responses that provided meaningful results to inform management decisions. This approach demonstrates the importance of careful design of protocols in exposure-response experiments, particularly in pioneering studies assessing response where both the potential for harm and level of uncertainty may be high.

KEYWORDS: ACOUSTICS; CONSERVATION; BEHAVIOUR; MANAGEMENT PROCEDURE; SHORT-TERM CHANGE; BEAKED WHALES; DELPHINIDS; NORTHERN HEMISPHERE

INTRODUCTION

Scientific research plays a key role in understanding the effects of human activities on wildlife and ecosystems. An ethical approach to the management of protected species requires those who undertake the experimental studies involving potential or actual harm to animals to implement best practices in assessing potential trade-offs associated with their work (Farnsworth and Rosovsky, 1993; Gales *et al*., 2010). Careful and deliberate measures must be taken to reduce the number of animals that will be disturbed and to minimise the amount of pain and suffering required to obtain scientific results. If specific research procedures pose a risk of harm to individuals, it may only be justifiable when there are sufficient, identifiable benefits for effective conservation and management (Boyd, 2002). This study looks at ethical issues of research designed to protect wild animals from poorly understood human risks. They were assessed in a situation where it was difficult to guarantee protection of subjects in the wild. It was also impossible to accurately estimate (in advance) the number of whales required to guide management decisions.

The particular case discussed here involves several species of beaked whales (*Mesoplodon* sp. or *Ziphius cavirostris*) for which lethal strandings have been reported to coincide with naval sonar exercises (D'Amico *et al*., 2009). Cox *et al*. (2006) reviewed potential mechanisms by which sonar might harm these whales. They conclude that physical effects of sound on tissue, which could be studied in tissue *in vitro*, requires sound levels so high that it is unlikely to initiate strandings. They conclude that anthropogenic noise may in some conditions elicit a behavioural reaction that may disrupt diving physiology and lead to strandings. These behavioural reactions can realistically only be studied with beaked whales at sea. The challenging goal for this study was to identify a response to sonar that was safe for the subject, but could also indicate risk of stranding if exposure were longer and/or more intense, and to quantify the exposure conditions required to elicit the response.

This study is the first to directly examine the behavioural mechanisms underlying these adverse effects of the specific types of mid-frequency active (MFA) military sonars involved in previous stranding events with cetaceans, especially beaked whales. The experiment was conducted on a Navy training range in the Bahamas and involved a controlled exposure experiment (CEE) paradigm (see Tyack *et al*., 2011 for full details of the playback stimuli). This experimental approach can test which sound exposures actually cause behavioural effects, a test that may not be possible in observational research. Opportunistic observations during actual (uncontrolled, non-experimental)

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sonar events provide some general insight into understanding of behavioural responses. However, CEEs occur within an experimental paradigm to allow the collection of adequate pre-exposure behaviour measurements, the ability to carefully control sound source output characteristics and location relative to experimental subjects in order to achieve a desired range of sound exposures. For the collection of post-exposure behaviour in particular, a dose-escalation protocol was used, which can identify the lowest sound exposure level that elicits a particular behavioural response.

Before this Behavioural Response Study (BRS) took place, the type and magnitude of potential responses of individual whales to exposure of simulated sonar, especially MFA, and other sounds were largely unknown. Thus, a highly precautionary approach was required to evaluate and mitigate harmful impacts from the experiment by using an adaptive design to enable rapid response to negative indicators. This study agrees with Farnsworth and Rosovsky (1993) that the scientific community should incorporate more explicit discussion and evaluation of ethical issues associated with field ecology experiments, but this study also required evaluation of ethical issues by outside bodies as well. This study involved marine mammals, so the planning for the study required evaluation of these issues in applications for approval by bodies external to the study team, as required by combinations of the funding organisations, federal or local regulatory requirements, and the requirements of participating organisations. These included the Office of Protected Resources of the US National Marine Fisheries Service, which issues permits for scientific research on marine mammals, a US Institutional Animal Care and Use Committee (Woods Hole Oceanographic Institution), and a UK Animal Welfare and Ethics Committee.

The present analysis considers the effectiveness of an operational control procedure employed in Tyack *et al.* (2011) involving the playback of three different sound stimuli: (i) a simulated mid-frequency naval sonar signal (MFA) with both constant frequency and frequency modulated tonal components in the 3–4kHz band; (ii) a pseudo-random noise signal (PRN) with overall bandwidth and timing similar to simulated MFA; and (iii) killer whale (ORCA) sounds from wild marine mammal eating (transient) killer whales (*Orcinus orca*). Blainville's beaked whales (*Mesoplodon densirostris*) and several species of small cetaceans (short-finned pilot whales*, Globicephala macrorhynchus*; false killer whales, *Pseudorca crassidens*; and melon-headed whales, *Peponocephala electra*) were the subjects of these experimental exposures. The beaked whales were selected as the primary species identified as sensitive to sonar. Delphinids were included as a series of comparison species with differing social structures to test their relative sensitivity to the beaked whales, and whether differential social responses to potential threats might affect the probability of flight reactions and potential associated risk of stranding.

Given the objectives for studying these aspects of behaviour in an experimental context, but recognising the potential for responses that could result in harm to these species, some of which had been involved in previous stranding events involving actual MFA sources, the integrated and adaptive mitigation strategy described here was designed. This strategy included specific integrated and adaptive elements both in the planning, implementation, and evaluation of noise exposure and response. Mitigation measures were included in the overall experimental design (e.g. site selection, testing conditions), engineering of experimental protocols (e.g. source ramp-up), and operational implementation of mitigation in different experimental modes (e.g. source shut-down, post-hoc visual surveys of the study area). Particularly the operational measures are integrated to provide multi-variable data (e.g. visual surveys, real-time passive acoustics) on the distribution and behaviour of experimental and other subjects in order to effectively monitor the experiment to ensure successful testing of responses while mitigating any potential harm. While they may have some broader implications, the resulting protocols and data from this study are particularly relevant to the informed and adaptive development of experimental design and potential real-time mitigation for studies of the effects of real sonar operations on cetaceans.

METHODS

The Tyack *et al.* (2011) study took place in July–September 2007 and 2008 in the Bahamas. The study was conducted under marine mammal research permits issued by the US National Marine Fisheries Service to John Boreman (Permit No.1121-1900; B. Southall was the designated principal investigator) and to Peter Tyack (Permit No.981-1578), and issued by the Government of the Bahamas to the Bahamas Marine Mammal Research Organization (Bahamas permit No.01/09) and Ian Boyd (Bahamas permit No.02/07 and No.02/08). The study was carried out in strict accordance with the conditions of these permits and the US Animal Welfare Act following the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (Clark *et al*., 1996); protocols were also approved by the Institutional Animal Care and Use Committees of the main participating institutions.

A strategically integrated, multi-faceted monitoring and mitigation protocol was developed with the explicit objective of detecting potentially harmful consequences of experimental trials and taking appropriate corrective action in an informed and adaptive manner before, during, and following experiments. To meet this objective, three categories of mitigation methods were developed and integrated within the experimental protocol, incorporating designed, engineered and operational mitigation measures. Research took place within an operational control procedure with clearly specified lines of communication and responsibility (Fig. 1).

Designed (pre-experimental) mitigation

Site selection

The field site was to the east of Andros Island, Bahamas in the Tongue of the Ocean (24.0903°N, 77.2350°W), a deep water basin surrounded by islands and sand banks. This site was selected because of the presence of the study species and the demonstrated capability to detect and locate beaked whales acoustically using the US Navy's Atlantic Undersea Test and Evaluation Centre (AUTEC), an underwater acoustic range (DiMarzio *et al*., 2008). AUTEC had 82

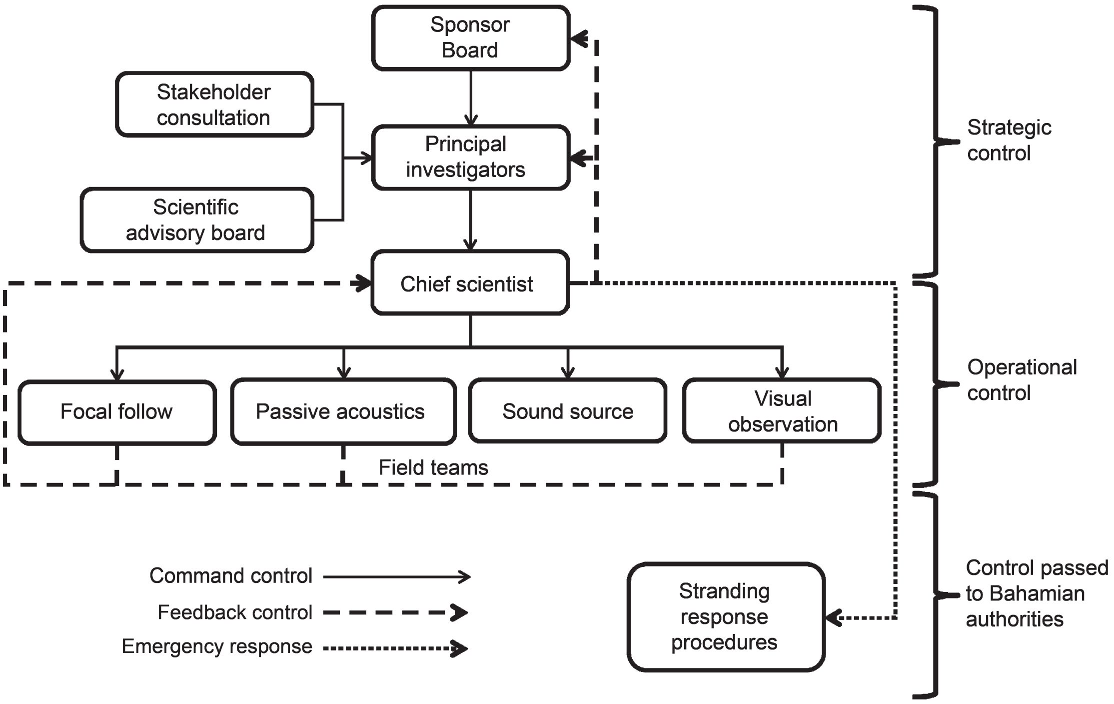

Fig. 1. Flow of communication and control in the strategic and operational management of the experimental design. This process involves the command control process as well as the flow of information that provides feedback control to the management team from those gathering information in real-time. Not shown here are the real-time data audit procedures that were designed to allow a *post-hoc* detailed analysis of the activities that could have resulted in the death or stranding of animals.

hydrophones mounted in a grid at roughly 4km spacing on the seafloor at depths of ≤2,000m that were cabled back to shore. All playbacks took place within the boundaries of the underwater range to allow continual real time acoustic tracking and mitigation, as has been demonstrated during military training exercises (McCarthy *et al*., 2011).

Observation time and space scales

Three time- and space-scales were used for obtaining observations that allowed the assessment of the effects of experiments (Table 1).

Selection of environmental conditions

Since locating, observing and tagging focal animals was not possible in high sea states, sound playbacks were not conducted if Beaufort Sea state was > 3. Similarly, hours of darkness (or periods of low visibility), were avoided as much as possible by not conducting playbacks after midday.

Engineered (experimental sound source) mitigation

Engineered measures primarily focussed on the sound source output. The signal used by Tyack *et al*. (2011) was a simulation of a typical operational US Navy mid-frequency sonar signal, which had an initial 0.5s linear frequency-modulated upsweep from 3.5–3.6kHz, followed by a 0.5s constant frequency tone at 3.75kHz, a 0.1s silent period, and then a 0.5s constant frequency tone at 4.05kHz. The total duration was thus 1.6s, with a repetition rate of every 25s from the onset of one signal to the onset of the next. The custom source used had a maximum source level of 211–212dB re 1µPa@1m. The distance between the source and focal animal was adjusted to ensure the received level did not exceed 160dB re 1µPa@1m. This was a level that was not expected to cause any temporary or permanent hearing threshold shifts based on a very conservative interpretation of the available data on auditory impacts of noise exposure available at that time (Southall *et al.,* 2007). The acoustic engineers who utilised the AUTEC range for marine mammal monitoring had experience with the propagation of mid-frequency sonar signals in the study area. They used standard parabolic equation and Bellhop sound propagation models with the sound source output and known features of the AUTEC range (e.g. bottom type) to model the range of predicted received levels for multiple depths in the water

Table 1

Representation of the three time and space scales covered by different platforms using specific modes of observation that provided feedback of information used in mitigation.

Scale	Space	Time	Mode	Platform
Large	10–> 100km	Days–weeks	1. Aerial survey 2. Acoustic array	1. Twin engine aircraft 2. AUTEC hydrophones
Medium	0–10km	Hours–< 1 day	1. Ship-based elevated platform using big-eye binoculars 2. Acoustic array	1. > 30m vessel 2. AUTEC hydrophones
Small	10m–1km	Seconds–hours	Focal follow of exposed animals	< 6m vessel

column around possible positions of the sound source. It was assumed that animals could be at any reasonable depth based on their species-typical dive behaviour that corresponded with the highest received levels. This information was used to determine appropriate source-animal ranges to meet the experimental goals without exceeding this target received level. Propagation modelling was conducted *a priori* for possible areas where experiments could occur to identify any potential site-specific differences. In addition, a ramp up of the amplitude of the sound was performed as part of the dose-escalation protocol, and as required under conditions of the research permit (see Tyack *et al.,* 2011 for details).

Operational (experimental and post-experimental) mitigation

The actual conduct and post-exposure monitoring of the experiment took place under five integrated and adaptive operational modes, each defining different sets of activities. The explicit distinction of these modes and the mitigation measures being employed ensured clear communication and a coordinated approach between the Chief Scientist and all teams (Fig. 1). The overall adaptive approach involved the use and integration of information from all of the available information from the various field teams (e.g. tagging, visual survey, passive acoustic) by the Chief Scientist in order to ensure the experiment met the mitigation goals and requirements. While elements of the operational mitigation used here were not novel (e.g. source shut-down), the integration of various data streams and the dynamic nature of visual data for surface animals transitioning to acoustic data in real time for diving animals with a seamless, real-time transition between these tools based on the behaviour of experimental subjects was a unique development in this study. The operational procedure progressed sequentially from one mode to the next, with each mode having associated and adaptive operational mitigation measures:

(1) *Search and assessment* involved the localisation of candidate whales for a CEE. Initial identification was carried out either acoustically using the AUTEC hydrophone sensors or by visual observers with subsequent photo-identification of individual animals. Mitigation measures aimed to identify all animals prior to the CEE, to ensure that no single animal was exposed more than once, and that a suitable age class animal was chosen (Table 2);

(2) *Tagging* involved attachment of acoustic and movement loggers (DTAG – see Johnson and Tyack 2003, Tyack *et al.*, 2011) on focal animals. Mitigation measures ensured that tagging was completed by experienced personnel to minimise approach attempts and ensure good tag attachment (Table 3);

(3) *Playback* involved sound source operation. Mitigation measures were implemented to ensure that maximum source levels were not exceeded and ramp up protocols were followed (Table 2). Shut-down mitigation (all sound transmission immediately ceased) was applied if any animals were observed within 200m of the source vessel (a required permit condition to prevent any potential physical injury or animals coming very close to the sound source). This was determined through range finding binoculars or estimated by the naked eye. Source

Table 2

Actions in the sequence carried out during playback experiments in 2007.

Stage	Action
Start:	
Pre-start preparation	Cast XBT if required to measure sound speed profile Transmit XBT data to modellers Run sound propagation loss model Decide on source depth based upon thermocline depth
Focal follow group of whales	Determine whether a juvenile is in the group Photo-identification of each whale in the group to determine whether any have been exposed before
Initiate post-exposure monitoring and mitigation procedure	Alert aircraft for deployment for aerial survey
Spatial disposition of platforms	Ensure vessel carrying the sound source is 1,000–2,000m from whales Ensure no other whales are within 200m of the vessel using 360° sweep with bigeye binoculars
Playback shutdown:	
Passive Acoustic Monitoring (PAM)	Cessation of clicking in beaked whales determined by the AUTEC array Unusually rapid movement or vocalisations
Visual observers	Strong and abnormal directed swimming (at surface) Increased and abnormal surfacing rate and respiration rate Animal surfacing with pattern(s) of directed movement, especially toward shore Unusual and abnormal surface/subsurface behaviour involving apparent disorientation and confusion or loss of group cohesion Animal defaecation on an unusual scale during or immediately after playback transmission Focal follow cannot be maintain because of weather Whale sited within 200m zone around the source
Post-exposure monitoring:	
	Track the tagged whale and its associated group with the assistance of M3R until nightfall Survey a region covering a 5km radius around the exposure site Conduct a regional aerial survey including the coastlines

Table 3

Summary of monitoring and mitigation effort during BRS divided down by each team and activity.

Team	Personnel	Location	Main activity	Region of operation	Monitoring and mitigation responsibility	Communication
Visual observers	Two teams of four, on a 30 minute rotation, each with an experienced team leader, 2 individuals scanning with 25 × 150 big-eye binoculars, 1 with 7 × 50 binoculars and 1 was in charge of communications	On main support vessel that also contained the sound source	Animal detection, tracking and behavioural observation pre-tagging and during and post the CEE. Observations ceased when conditions prevented effective monitoring, at which time radio direction finding on the VHF signals of the Dtag was used to track individuals.	10km radius of ship using 25 × 150 big-eye binoculars	Report unusual behaviour or dangerous occurrences	Team leader in constant communication with own team members via a duplexed 2-way communication system, the Chief scientist and focal follow team.
Focal follow	A team of five individuals under the direction of an experienced skipper	On a small boat	Close up animal detection, tracking, photo-identification and behavioural observation. Follows occurred before, during and after the CEE, until conditions required return to base at which time, radio direction finding on the tag was used to track individuals.	Up to 15km radius of main ship often guided by the Visual observation and the M3R teams	Report unusual behaviour or dangerous occurrences	The skipper was in constant communication with the Chief Scientist and was directed on to the whales by the team leader of the visual observers.
Marine mammal acoustic monitoring on the AUTEC array	A team of experienced acoustic personnel	At the AUTEC base	Animal detection, tracking and acoustic behaviour observation in real-time, before, during and after the CEEs, using detection and localisation (Moretti *et al.*, 2002; DiMarzio *et al.*, 2008; Tyack *et al.*, 2011).	Across the whole AUTEC array using the acoustic monitoring capability of the range	Report changes in behaviour in terms of vocalisations and/or animal movement	Directly with the Chief Scientist during the CEE.
Tagging	A team of one tagger and one boat driver	On a small boat	Deployment of tags, quick look analysis on retrieved tags, in case of requirement to adapt protocol to reduce the potential for harmful impacts on individuals.	Within a few 10s of metres of the focal group	Report unusual behaviour or dangerous occurrences	Directly with the focal follow skipper and Chief Scientist.
Aerial survey	A team of three observers and a pilot	Flights took off and landed at AUTEC	Systematic survey of coastline and appropriate sea areas, along the central section of TOTO, primarily down-current and down-wind of the playback location. Surveys were carried out if abnormal behaviour was observed or if normal baseline behaviour was not observed post-playback, A final aerial survey was conducted on when all CEE trials were complete.	Up to 100km around region of operation	Report dead or moribund animals either floating or stranded	Observations were reported to the Chief Scientist and communicated with all team leaders.

transmissions were sustained if cessation of clicking observed on the AUTEC hydrophones and held through ascent and first shallow dive. These adaptive measures included both visual and acoustic monitoring that allowed information on the location and behaviour of subjects in real time based on where they were in the water column and their behaviour and potential response. These were used to different degrees based on the situation at any one time, with information from both teams provided to the Chief Scientist;

(4) *Post playback* involved visual and acoustic monitoring of the playback area and surveys for stranded marine mammals. Mitigation measures ensured all protocols were rigorously followed (Table 3); and

(5) *Stand-down* was when no CEEs were conducted. Mitigation measures ensured all post CEE monitoring had taken place in accordance with the strategic and operational management design (Fig. 1)

Communication was via an open VHF radio circuit to ensure all teams at all times were in contact with the Chief Scientist. The actions undertaken to ensure mitigation of negative effects of the CEEs are listed in Table 2. Visual and acoustic monitoring by trained personnel (on the sound source vessel, small tagging and focal follow RHIBs, and ashore listening to the acoustic sensors) under the direct communication from an experienced team leader was implemented during all operational modes except 'stand-down'. Operational safety to people and animals was the primary consideration. Protocols to terminate sound transmissions were enacted (Table 2) if animals occurred within a specified physical range of the active sound source or if a potentially harmful response was observed. The NMFS permit (No.1121-1900) governing this research specified that, 'a playback episode must be discontinued if an animal exhibits a strong adverse reaction to the playback activity or the vessel (e.g. breaching, tail lobbing, underwater exhalation, or disassociation from the group).' This requirement was integrated into the mitigation protocols, but additional measures capturing other means of detecting and responding to potential responses were added above and beyond these requirements (see Table 2).

While it was highly unlikely that the limited duration and much lower power (than actual MFA sonar) sound sources, during the first year (2007) of this BRS would cause any direct injury or result in strandings, seven aerial surveys were flown in different contexts to survey for any potentially stranded animals. Two of the flights took place prior to any CEEs, to ensure no existing stranded or dead animals were in the vicinity of the field site. Four were mitigation flights to search the shoreline along the eastern coast of Andros Island, the small islands in North and Middle Bight, the bank edge and cays along the east side of Tongue of the Ocean (TOTO), and the coastal areas of New Providence Island. The final flight was the post BRS monitoring flight. Flights were flown at an altitude of 500ft and at 90 knots. A total of 16.4 hours of flight time was cumulated covering 1,476 n.miles (2,731km). There were three marine mammal sightings; two of bottlenose dolphins (*Tursiops truncatus*) and one of rough-toothed dolphins (*Steno bredanensis*). No sightings of beaked whales were recorded during any flight, based on which what was determined from this form of monitoring to survey for stranded marine mammals was not necessary for the 2008 study. However, as an adaptive approach, when a single flight was called for in 2008 in order to locate the VHF signal of a tag that had detached from a research subject, additional survey effort was added to search nearby beaches for any stranded animals (none were detected – see Table 4).

All operational measures were designed to be adaptive; any information received through feedback control from monitoring and mitigation activities was used to inform decisions (see Fig. 1) on when or whether it was appropriate to proceed in the sequence. In the event of any negative reactions being observed, assessment against the permitted level of disturbance and/or injury was made and reported against the conditions of the issued permits, both in terms of the authorised number and nature of sound exposures relative to predicted sound exposures and in terms of compliance with the required shut-down conditions.

The operational components of the experiment involved teams of researchers under the direction of a Chief Scientist (Fig. 1). The Chief Scientist role was filled by several different individuals at different intervals, but effective communication ensured consistency in decision-making. In 2007, the Chief Scientist was stationed on-shore at a console showing the acoustic data from across the AUTEC range, including the disposition of different vessels involved in the study; in 2008 he was located on the visual observation research vessel (R/V *Roger Revelle*). To achieve the objectives of the monitoring and mitigation strategy, teams were required to work together in a coordinated manner, with each team leading specific activities of key importance to the study (Table 3). Daily team leader meetings were led by the Chief Scientist to discuss any operational changes and the plan for the day.

RESULTS

Seven CEE sequences were conducted during the study. Two of these, one in each of 2007 and 2008, involved beaked whales. Of the remainder, one in 2007 and four in 2008, involved delphinids. In 2007, a playback involving simulated mid-frequency sonar and social calls of transient killer whales was conducted on a tagged female Blainville's beaked whale. In 2008, a group of three Blainville's beaked whales, one of which was tagged, were involved in a CEE, with exposure stimuli of simulated MFA sonar and PRN (for further details see Tyack *et al*., 2011). In 2007, a CEE was also conducted on a group of short-finned pilot whales containing two tagged individuals. During this CEE, playback transmission was temporarily stopped because a group of short-finned pilot whales, not containing the tagged animals entered the 200m shut-down zone around the sound source. In 2008, the first CEE was conducted with a group of 15–20 short-finned pilot whales containing one tagged individual, but the tag was not recovered. The second and third CEEs in 2008 were on two groups of 12 false killer whales, each containing one tagged individual. The fourth was on a group of 12 short-finned pilot whales and approximately 100 melon-headed whales, during which one

Table 4

Details of the operational mitigation measures carried out during the sound exposures in 2007 and 2008, species include short finned pilot whales (Gm), Blainville's beaked whales (Md), false killer whales (Pc), and melon headed whales (Pe).

Date	Species	Exposure shutdown	Post exposure focal follow	Post exposure Dtag data review	Post exposure mitigation flight (time)
2007					
17 Aug.	Gm	Initial exposure terminated due to animals within 200m of source	Immediately post exposure, the 2 whale groups had joined and were moving towards the source vessel; they were subsequently tracked for 12 hours.	No overt or potentially injurious behaviours were observed on the dive records.	18 Aug (03:55 hrs): 0 animals sighted, 0 stranded animals reported.
17 Aug.	Gm		As above.	No overt or potentially injurious behaviours were observed on the dive records.	As above.
02 Sep.	Md		Visual observers sighted the whale five hours after exposure. The ship tracked the tag by tag radio transmissions until it released 14 hours after exposure.	Maximum received level was 150dB re 1µPa. No potentially injurious behaviours were observed on the dive records.	04 Sep. (02:10 hrs): 2 *Tursiops truncatus* sighted, 0 stranded animals reported; 06 Sep. (05:01 hrs): 3 *Tursiops truncatus*, 0 stranded animals reported; 27 Sep. (02:45 hrs): Undetermined number of *Tursiops truncatus*, 0 stranded animals sighted.
2008					
22 Sep.	Gm		One hour. The two groups of whales joined and continued on a southeast direction into the southern part of the range. However, tag failure and poor weather resulted in loss of contact with the individual.	No Dtag data were analysed as the tag was not retrieved. However, the tagged animal was sighted (verified through photo-ID) on the 26 Sep.	Based on 2007 mitigation results, 2008 risk assessments were adapted to reduce the need for mitigation flights after every exposure. However, due to loss of contact with the individual in this case, flights were carried out as a precautionary measure: 0 animals sighted, 0 stranded animals reported.
26 Sep.	Pc		One hour. Whales slowed their speed but remained grouped together. The whales were then observed swimming at speed away from the source vessel.	Maximum received level was less than 160dB re1µPa (RSM). No overt or potentially injurious behaviours were observed on the dive records.	As above; no mitigation flights carried out.
27 Sep.	Md		One hour. Whales continued to show typical behaviour at the surface.	Maximum received level was less than 150dB re1µPa (RSM). No overt or potentially injurious behaviours were observed on the dive records.	As above; no mitigation flights carried out.
28 Sep.	Pc		One hour. Whales continued on their original course at the same speed.	Maximum received level was less than 160dB re1µPa (RSM). No overt or potentially injurious behaviours were observed on the dive records.	As above; no mitigation flights carried out.
29 Sep.	Gm/Pe		Over one hour. Whales continued to travel slowly and exhibited social behaviour at the surface such as lob-tailing and spy-hopping.	Maximum received level was less than 150dB re1µPa (RSM). No overt or potentially injurious behaviours were observed on the dive records.	As above; no mitigation flights carried out.

pilot whale and one melon-headed whale were tagged at the time of exposure. As in the beaked whale CEEs, exposure stimuli consisted of simulated military sonar, killer whale calls and band-limited noise (for further details see Tyack *et al*., 2011).

From all the information available to us, the mitigation measures implemented in the adaptive approach described above were effective. During relatively good environmental conditions, visual observers from elevated platforms on the sound source vessel were able to monitor marine mammals at the surface in the nearby vicinity of the sound source. In one instance, observers implemented a CEE shut-down as specified in the operational protocols when any marine mammal came inside a 200m radius of the active sound source. Visual observers from small boats were also able to monitor animals within the focal group containing the tagged whale. While it is not possible to ensure that some behavioural responses occurred that were not detected, no observations of the kinds of very strong, overt responses identified as shut-down requirements were observed. Finally, no stranding of cetaceans was observed from the aerial surveys of large areas of the Tongue of the Ocean after either individual playbacks or the experiment as a whole. Although reactions to the sonar were observed in beaked whales (Tyack *et al*., 2011), reactions on the part of the delphinids were more difficult to distinguish from normal variability in behaviour. There were no indications from any of the playbacks that whales were injured or otherwise harmed by the signals played to them (Table 4). The beaked whale that showed the strongest reaction was the one tagged and exposed to playback of sonar and then killer whale sounds in 2007. This whale had an unusually long ascent after exposure, an unusually long interval between deep foraging dives after the killer whale playback, and a prolonged avoidance reaction (Tyack *et al*., 2011). Models of diving physiology suggest that none of the changes in dive profile in response to this sonar playback posed a risk to the subject (Kvadsheim *et al*., 2012). The tag monitoring the response fell off 10 hours after playback, while the whale was still engaged in an avoidance response (Allen *et al*., 2013), so it is not known when its behaviour ceased being disturbed by the playback. This kind of strong directed avoidance response may be used as an indicator of risk of stranding, but this whale was positively identified from photos when re-sighted in apparently good health in 2008, 2009, 2011, 2012 and 2013 (Bahamas Marine Mammal Organization, unpublished data).

DISCUSSION

The evidence presented here suggests that the controlled exposure experiment achieved its objectives of providing novel empirical information to inform management decisions without causing injury, harmful or permanent changes in behaviour to experimental subjects. The effects of acoustic exposure may depend on various contextual factors including source operation, deployment environment, and individual characteristics, such as age, sex, behaviour, social and motivational state of the exposed animals (Southall *et al*., 2007; Ellison *et al*., 2012). Given the available information at the time about these kinds of acoustic signals and the focal species, there was no *a priori* reason to assume at the start of this experiment that it could not have caused harm for some individuals. The historical stranding record reveals MFA sonar exercises using these specific kinds of signals that coincide with cetacean strandings, (Cox *et al*., 2006; Brownell *et al*., 2009; D'Amico *et al*., 2009). The evidence for a link between sonar exposure and stranding is strongest for atypical mass strandings of beaked whales (D'Amico *et al*., 2009, Filadelfo *et al*., 2009). However, there was insufficient information about what components of the sonar exposure led to these strandings. This study was designed to measure the parameters of sonar exposure required to elicit behavioural responses that were safe for the subjects but that could be used as indicators of risk. However, it was difficult to be completely certain that these experiments would not injure or strand cetaceans. The results of the study, both in the types of behavioural responses observed (directed, sustained avoidance – see Tyack *et al*., 2011) and in the lack of any observed extreme short-term responses that might pose a risk to diving physiology or long-term responses that might pose a risk of stranding suggest that it may require specific conditions (e.g. sustained transmissions following initial responses, multiple sound sources, particularly reverberant environments, high sound exposure levels) to elicit extreme responses that could lead to stranding.

It remains possible, although very unlikely, that the experiment led to some level of harm that remained undetected. The scale-based approach adopted to detect negative consequences was as comprehensive as resources and technology would allow. Animals that had been exposed were followed, to the extent possible, until their behaviour returned to normal. Behavioural responses were generally too subtle for significant changes to be observed based upon surface visual monitoring alone, even though they did occur (Tyack *et al*., 2011). It is also important to note that the observation effort for detecting and mitigating harm, even when supported by considerable technological capability from the AUTEC range, was considerably greater than the effort that was required as mitigation as a condition of authorisation of the research or that would be required during potentially damaging use of high intensity sound sources such as sonars, pile driving and seismic air guns. Consequently, this study also raises questions about the utility of current mitigation of the effects of high intensity sound sources in the ocean that rely exclusively on visual observers, especially to monitor for highly cryptic animals such as beaked whales. Rather, an integration of visual and acoustic monitoring approaches with a priori acoustic modelling and explicit mitigation and shut-down protocols is a more effective and responsible mitigation approach, especially for particularly sensitive species or important habitat areas (e.g. Nowacek *et al*., 2013).

Tyack *et al*. (2011) combined the results of experimental exposures to two beaked whale subjects with acoustic and satellite tag monitoring of responses of beaked whales to actual sonar exercises. A key approach was to use sophisticated tags to extract the maximum amount of acoustic dosage and behavioural response information from a small number of experiments in order to inform less detailed opportunistic observations. The dose-escalation protocol was designed to detect the minimum exposure required to elicit a response. The duration and level of exposure during experiments was reduced to the minimum required to elicit a silencing response in beaked whales that were using echolocation to forage. The duration and source level of exposure were markedly lower than those used during sonar exercises; the sound exposure protocol was designed specifically to minimise exposure to the minimum required to obtain the required scientific results. Combining results from experimental and opportunistic studies made it possible to reduce the number of animals exposed to sound that was transmitted as part of an experiment as opposed to ongoing sonar training. This kind of reduction in number of animals exposed and reduction in intensity of exposure forms a key component of animal welfare regulations and was endorsed by Huntingford (1984), and that for experimental exposures that could pose a risk to the subject.

This research was designed to help guide management of the risks from sonar exposure. The results from Tyack *et al.* (2011) suggested that responses consistent with elevating risk occurred at sound exposures of about 140dB, well under the previous regulatory thresholds. The threshold used by the US regulator to predict disturbance was subsequently changed to a 140dB step function threshold within several years of the publication of the Tyack *et al.* (2011) study (NMFS, 2013). If this change in threshold provides greater and more realistic protection of beaked whales from risks of exposure to levels that heretofore were thought to be safe, then the small costs to the experimental subjects needs to be weighed against the benefit to beaked whale populations worldwide.

The experiment complied with the guidelines suggested by Gales *et al.* (2009) and Huntingford (1984), overall, achieved an appropriate balance between the costs to the animals involved and the benefits in terms of novel data on reactions of animals to sonars. The results of this experiment and the mitigation measures used have directly served to inform subsequent research efforts involving sonar and marine mammals (e.g. Southall *et al.,* 2012; DeRuiter *et al.,* 2012; Goldbogen *et al.,* 2013; Miller *et al.,* 2012). None of these later studies exactly replicated the mitigation measures used here, because the situations and subjects involved were different and because results are increasingly showing that responses are likely to be subtler behavioural changes rather than physical injury or responses likely to lead to a stranding event. However, the basic approaches of an integrated system of visual and acoustic monitoring of the survey area (as well as use of sound propagation modelling in real time to visualise ranges of potential impacts) were derived to some extent from the Tyack *et al.* (2011) study. As described by Nowacek *et al.* (2013) for a multi-faceted monitoring and mitigation approach for a seismic survey conducted in critical feeding habitat for endangered western grey whales, the relatively costly and time-consuming mitigation process outlined here was beyond what was required and is likely beyond what may be possible in all subsequent studies. There is likely a justifiable reduction in certain elements from the broad approach described here, particularly in studies where such sounds are relatively common and species being tested have some baseline information on their basic behaviour and typical kinds of behavioural responses. A logical progression may be to retain certain fundamental protocols while relaxing others. Key elements that would logically be retained include sound propagation modelling to inform appropriate selection of exposure location, shut-down for animals within close proximity to loud sources and more protective protocols for particularly sensitive or endangered species. Other precautionary requirements such as aerial surveys for stranded animals following every sound transmission or shut-down of sound sources immediately based on cessation of sound production in animals in the vicinity could and have been have been relaxed based on scientific results occurring in subsequent studies. This is especially the case when considering best practices for smaller scale behavioural response studies, where budget limitations may in part dictate realistic protocols. Priority should be given to integrated mitigation measures for the specific circumstance of the study that ensure robust metrics to determine appropriate behavioural response data are collected while achieving mitigation goals.

In conclusion, this study found that a robust and open discussion of ethical issues associated with field experiments led to a mitigation protocol that allowed the meeting of scientific and applied objectives while minimising adverse impacts to the subjects of the study and animals nearby. As scientists develop more experience with novel kinds of study, their increased experience may support reduction of precautionary mitigation and monitoring measures, but each stage of the process requires careful and open evaluation of benefits and risks. This study agreed with Farnsworth and Rosovsky (1993) that scientists should include an explicit consideration of ethical issues in their peer-reviewed scientific publications, especially when there is uncertainty as to the impact of new study designs or when studies may have adverse or large scale impacts.

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Development of an abundance estimate for the eastern Bering Sea stock of beluga[1] whales (*Delphinapterus leucas*)

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ABSTRACT

The first dedicated aerial surveys for beluga whales in the Norton Sound/Yukon Delta region of Alaska were flown during May, June and September 1992. During May 1992 surveys, all of the survey area was covered with pack ice and only a few belugas were seen. In June 1992, many whales were seen in the region of Pastol Bay and the Yukon River Delta, with a few animals seen in eastern Norton Sound. In September 1992, whales were more dispersed and occurred both off the Yukon Delta and in coastal waters of northern Norton Sound. Based on those results, subsequent surveys were flown in June 1993–95 and 1999–2000. In all years except 1999 when there was extensive sea ice in the area, belugas were common off the Yukon Delta and in southern Norton Sound. In most years they were also seen in central Norton Sound. Density and abundance were estimated from the 2000 survey as it represented the most recent data and had the most complete and systematic coverage of the area. In June 2000, belugas were rare in the northern portion of Norton Sound, so the study area was reduced to central and southern Norton Sound and the Yukon Delta, which was divided into four strata by latitude. The density that was estimated with the model that received most Akaike Information Criterion support was 0.121 belugas km^{-2} and the number of belugas at the surface in the study area was estimated to be 3,497 (CV = 0.37). A generally accepted correction factor for availability of 2.0 was applied, resulting in an abundance estimate for the eastern Bering Sea beluga stock in June 2000 of 6,994 (95% confidence interval 3,162–15,472). This estimate is likely to be conservative. There are no previous abundance estimates for this region, so a population trend cannot be determined. The available evidence suggests that the current Alaska Native subsistence harvest from this stock is sustainable. Beluga consumption of prey populations is likely significant in the regional ecosystem and may have a particular impact on some stocks of Pacific salmon.

KEYWORDS: WHITE WHALE; ARCTIC; BERING SEA; ABUNDANCE ESTIMATE; SURVEY-AERIAL; WHALING-ABORIGINAL; CONSERVATION; NORTHERN HEMISPHERE

INTRODUCTION

During the ice-free season along the western coast of Alaska, annual concentrations of beluga whales (*Delphinapterus leucas*; also called white whale) predictably occur in Bristol Bay, the Norton Sound/Yukon Delta region, Kotzebue Sound and at Kasegaluk Lagoon. This distribution pattern was used to identify three provisional management stocks (Frost and Lowry, 1990). Studies of mitochondrial DNA have confirmed the existence of three beluga stocks that occur in western Alaska during summer months (O'Corry-Crowe *et al*., 1997, 2002). These are referred to as the Bristol Bay stock, the eastern Bering Sea (EBS) stock and the eastern Chukchi Sea stock (Fig. 1). Studies of the distribution and abundance of belugas in Bristol Bay began in the 1950s (Brooks, 1955; Frost *et al*., 1984, 1985) and the eastern Chukchi Sea in the 1970s (Seaman *et al*., 1988; Frost *et al*., 1993). However, prior to 1992 there had been no dedicated surveys of beluga whales in the EBS region.

Prior to the surveys described in this paper there was little information on the distribution of EBS belugas beyond the knowledge of the traditional Alaska Native hunting areas, and places where whales were seen on an opportunistic basis. A compilation of all available observations showed that belugas occurred throughout the coastal zone of the northeastern Bering Sea, particularly from the mouth of the

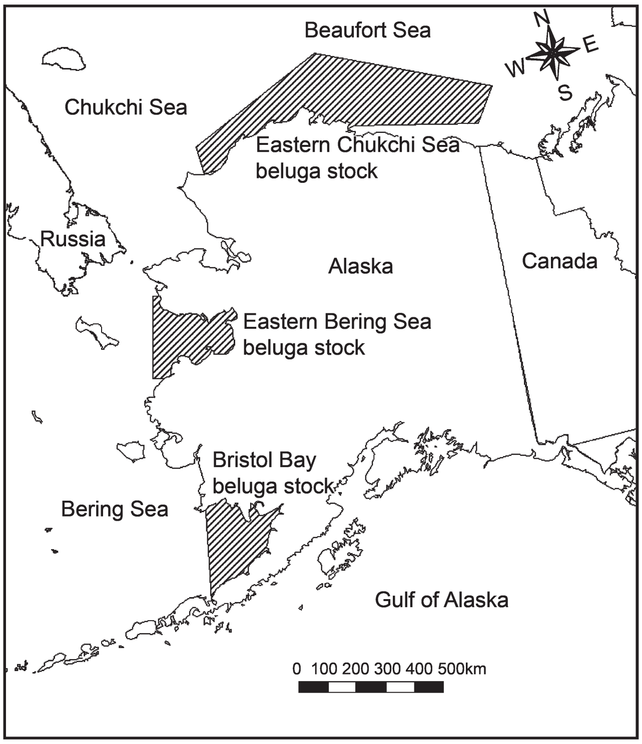

Fig. 1. Map showing the summer concentration areas of beluga whales in western Alaska (cross-hatching).

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[1] The agreed common name for *Delphinapterus leucas* by the Scientific Committee of the International Whaling Commission is 'white whale.' However, 'beluga' is commonly used in several parts of the world, including Alaska, and is used in this paper.

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Yukon River to northern Norton Sound near Nome. Relatively few sightings were reported far away from the shoreline (Frost and Lowry, 1990). Belugas were seen predominantly during ice-free months. This was from just after the breakup of the ice (usually mid-May) until freeze-up (usually November), and whales were harvested during spring, summer and autumn at villages in southern, eastern and northern Norton Sound (Lowry *et al*., 1989; Frost and Suydam, 2010). Traditional knowledge of hunters in the region indicated that the belugas arrive in the area at spring time and stay through to late autumn (Huntington, 1999).

Since 1992, the US Government has provided funds for the Alaska Beluga Whale Committee (ABWC) to conduct studies of beluga whales in Alaska. Part of the ABWC research program has consisted of aerial surveys to estimate the abundance and trends of western Alaska beluga stocks. This paper describes the results of ABWC surveys flown in the EBS over six years, 1992–95 and 1999–2000.

METHODS

Survey design and field methods

In 1992, several aerial surveys were conducted during three periods in May, June and September to assess the distribution of beluga whales during those periods (Lowry *et al*., 1999; DeMaster *et al*. 2001). The surveys found relatively few belugas in May and September, but a large number of whales in June. Based on those results, surveys in subsequent years were only conducted in June.

The survey was designed to cover coastal and offshore waters of Norton Sound and the Yukon Delta. Coastal transects were parallel to the shoreline with the centerline of the aircraft approximately 0.9km offshore. Offshore transects were flown east–west along lines of latitude, north–south along lines of longitude, or on diagonals when travelling to and from airports. An adaptive sampling design was used in 1992–95 to increase survey effort in areas where belugas had been sighted. When the whales were seen on an offshore transect, additional parallel transects were flown at a 3.6–9.3km spacing on both sides of the original line. Parallel transects were continued as long as whales were seen, and usually stopped after two transects if there were no sightings (Fig. 2). In 1999 and 2000, the survey was designed to cover all of Norton Sound and the Yukon River mouth with east–west transects regularly spaced at 9.3km intervals (Fig. 3).

The total length of survey transects flown during each survey period was limited by the aircraft time available. The completion of transects was sometimes limited by weather conditions, particularly fog or high winds, and in June 1999 ice cover was a factor.

The survey aircraft was a high-wing, twin-engine Aero Commander equipped with bubble windows, based in Nome. The crew included the pilot, a data recorder in the right front

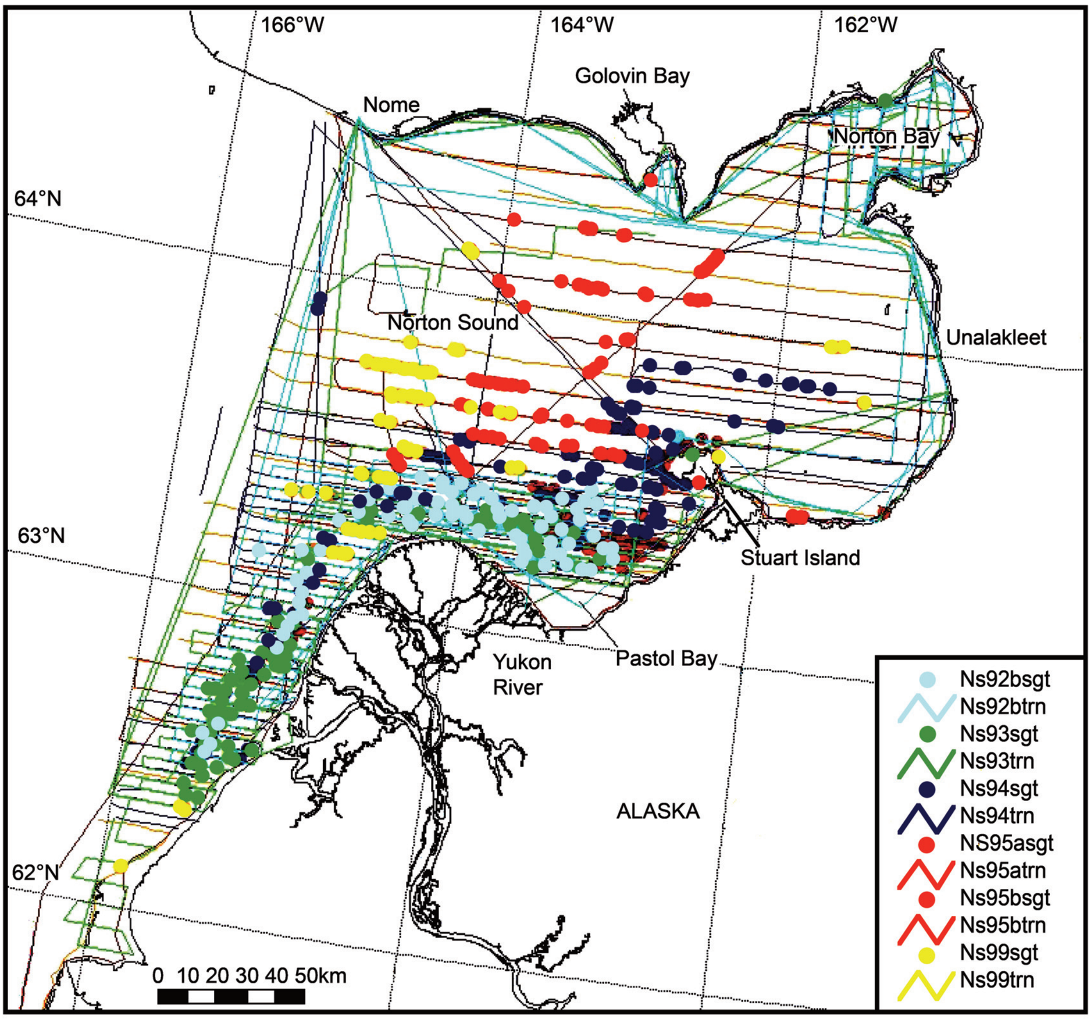

Fig. 2. Transects flown and beluga sightings made during ABWC beluga whale surveys in the eastern Bering Sea, June 1992–99.

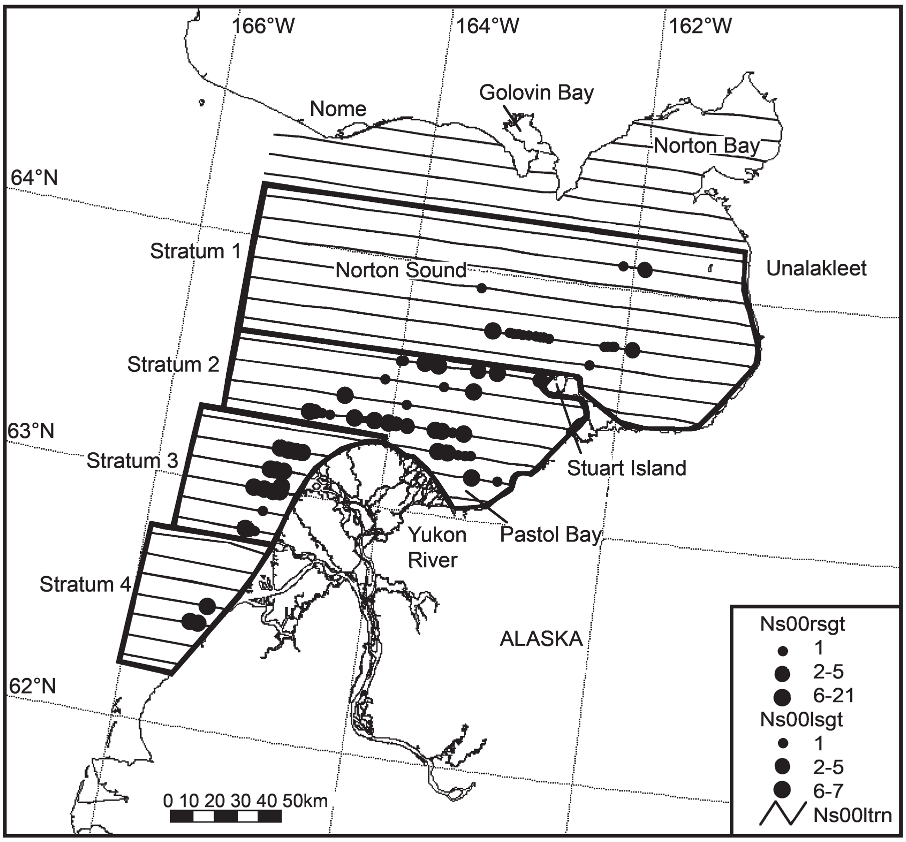

Fig. 3. Transects flown, strata used in the analysis, and beluga sightings made during ABWC beluga whale surveys in the eastern Bering Sea, June 2000.

seat and two observers seated behind the pilot on the left and right sides of the aircraft. Survey altitude was usually 305m, and ground speed was 274km/hr in 1992 and 220km/hr in all other years. Navigation was done by reference to landmarks and with a Global Positioning System. The survey was done in a passing mode, where whales were counted while staying on effort on the trackline. On the coastal transects, all beluga whales visible along the survey track were counted. For the 1992 offshore transects, observers counted whales within 915m wide strips on each side of the aircraft. The strips were offset 305m from the centerline to eliminate the blind spot under the plane. In 1993–95 and 1999–2000, the offshore transects on each side of the aircraft were divided into seven zones and each whale sighting was recorded in one of the zones. Inclinometers were used to delineate the inner and outer bounds of zones as follows: zone 1, 45°–51°; zone 2, 40°–45°; zone 3, 33°–40°; zone 4, 27°–33°; zone 5, 21°–27°; zone 6, 14°–21°; and zone 7, < 14°. In 1992 and 1993, sightings and other data were recorded on datasheets by observers in one-minute intervals and were then entered into a computer database. In 1994–95 and 1999–2000, a computer-based data entry program was used, logging the locations and times for the beginning and end of transects, the position on transect every 1 minute, and the exact time and position of each sighting. Wind speed (from the aircraft navigation system), cloud cover (%), ice coverage (%) when present, sea state (using the Beaufort scale), glare (present or absent) and overall sighting conditions (excellent, good, fair, poor) were reported by observers and any changes were recorded. The overall sighting conditions were characterised as follows:

- Excellent-ocean conditions, calm or very small waves; ability of observers to discriminate objects on the water not impeded by waves, whitecaps, fog, haze, low ceiling, glare, or precipitation;

- Good-ocean conditions, small waves with few or no whitecaps; ability of observers to discriminate objects on the water only slightly impeded by waves, whitecaps, fog, haze, low ceiling, glare, or precipitation;

- Fair-ocean conditions, small to medium waves with frequent whitecaps; ability of observers to discriminate objects on the water moderately impeded by waves, whitecaps, fog, haze, low ceiling, glare, or precipitation; and

- Poor-ocean conditions, medium to large waves with constant whitecaps; ability of observers to discriminate objects on the water substantially impeded by waves, whitecaps, fog, haze, low ceiling, glare, or precipitation.

Data analyses for the 2000 survey

Uncorrected density and abundance estimates were only obtained from the 2000 dataset because it was the most recent and included the most complete and systematic coverage of the EBS study area (see Table 1 and Fig. 3). Beluga sightings and transect data were entered into a geographic information system (ArcView), they were then plotted and visually inspected. During the surveys, there were no belugas seen on the seven northernmost transect lines, indicating extremely low densities within that area. That part of northern Norton Sound was excluded from

Table 1

Survey lengths and areas, counts and encounter rates for beluga whales in the eastern Bering Sea region, based on aerial surveys conducted in June 1992–95 and 1999–2000.

Survey dates	Trackline flown (km)	No. belugas counted	Encounter rate, belugas per km	Study area (km^2)
17–21 June 1992	7,278	1,625	0.223	6,145
14–18 June 1993	5,539	374	0.068	10,975
11–16 June 1994	5,746	370	0.064	13,965
5–8 June 1995	4,450	750	0.169	19,983
20–22 June 1995	1,776	456	0.257	3,352
15–17 June 1999	3,366	589	0.175	15,794
17–20 June 2000	4,226	428	0.101	38,104

further analysis, and the study area used for density calculations was limited to the area in central and southern Norton Sound and off the Yukon River Delta. The study area was post-stratified into four strata by latitude. Stratifying by latitude places similar survey tracklines together and reduces the considerable variability of habitat coverage among the survey lines.

Sightings data were truncated by subtracting 305m from the perpendicular distances of all sightings (equivalent to the blind spot under the plane). Truncation was also applied by excluding all sightings at distances greater than 1,000m from the centerline (the inner bound of the last zone). Detection probability was estimated with Conventional (CDS) and Multiple Covariate Distance Sampling (MCDS) methods. CDS and MCDS analyses included the half normal and the hazard rate functions with no series expansions. MCDS models also included covariates individually (Table 2). Model selection was performed according to the Akaike Information Criterion (AIC, see Burnham and Anderson, 2002).

Encounter rates and group sizes were computed separately for each stratum. An exploratory analysis indicated that cluster size did not correlate with the detection distance and therefore expected group sizes were calculated as simple means (Buckland *et al*., 2001). Model parameter estimates were computed with program DISTANCE 6, Release 2 (Thomas *et al*., 2010). Variances for all model parameters, density and abundance were empirically estimated as specified by Buckland *et al*. (2001). Uncorrected density and abundance were computed for the model most supported according to AIC (see results below).

Correction factor for availability bias

The DISTANCE program used in the analysis estimated the density and abundance of belugas visible at the surface from the plane. When available, data on dive behaviour are generally used to estimate the number of additional whales that would have been submerged when the survey aircraft passed. Telemetry data on beluga diving behaviour were not available for the EBS region, but in other regions where belugas have been tagged with satellite-linked dive recorders, it has been found that they spend half or less of their time at or near the surface (Heide-Jorgensen *et al*., 1998; Lerczak *et al*., 2000; Kingsley *et al*., 2001; Citta *et al*., 2013). Several studies have concluded that aerial counts should be multiplied by two or more to account for animals diving (Sergeant, 1973; Frost *et al*., 1985; Kingsley *et al*., 2001). In this study the uncorrected abundance was multiplied by 2.0 to estimate the total abundance.

Table 2

Covariates included in conventional and multiple covariate distance sampling analyses for eastern Bering Sea beluga survey data.

Covariate	Type	Range or levels
Glare	Factor	Present or absent
Group size	Numerical	1–21
Observer	Factor	LL and RA
Sea state (Beaufort scale)	Factor and numerical	1–3
Sighting conditions	Factor	Excellent, good, fair, poor

RESULTS

Beluga surveys and sightings

Transect lines and beluga sightings for surveys conducted in June 1992–95 and 1999 are shown in Fig. 2 and summarised in Table 1. During May 1992 surveys, all of the survey area was covered with pack ice and only a few belugas were seen. In June 1992, many whales were seen in the region of Pastol Bay and the Yukon River Delta, with only a few animals seen in eastern Norton Sound. In September 1992, whales were more dispersed and occurred both off the Yukon Delta and in coastal waters of northern Norton Sound. Based on these results, in subsequent years the surveys were only conducted in June and our efforts focused on the region off the mouths of the Yukon River and Pastol Bay. Belugas were commonly sighted within the region in every subsequent survey. The overall size of the study area expanded over the years as the area of beluga occurrence increased with coverage added by our adaptive sampling and sightings of belugas in other regions during transit lines. In 1995, a more comprehensive coverage was attempted of the entire Norton Sound-Yukon Delta region but persistent fog prevented us from surveying off the Yukon River. We returned later in June and were able to survey the Yukon mouth, but without a better understanding of beluga behaviour and movements we were not comfortable with combining the data for the surveys, as they were conducted about two weeks apart (Lowry *et al*., 1999). June 1999 was unlike previous years when the survey area had been virtually ice free and pack ice covered much of Norton Sound. Beluga distribution was also unusual with relatively few whales seen in open water off the Yukon Delta and most sightings in pack ice in the southwestern Sound. Nearly all sightings were in ice coverage of 10%–50% and very few belugas were seen in 60% or greater ice coverage. Because of the anomalous conditions, the 1999 survey was terminated earlier than planned. During 17–20 June 2000, the survey covered the entire study area with east–west transects spaced at 9.3km intervals; 428 belugas were counted in 297 sightings, on 4,226km of surveys (Fig. 3). Most of the beluga whales were seen off the Yukon Delta and in Pastol Bay, but a number of sightings were made in central Norton Sound west and north of Stuart Island.

Density and abundance estimates for the 2000 survey

As described in the methods section, the northernmost part of Norton Sound (where no belugas were sighted) was excluded from the analysis. This reduced the survey effort to 3,052km of trackline and the study area to 28,936km^2. Truncation of the inner and outer boundaries of the survey strip reduced the number of groups in the dataset to 232, and the number of individuals counted to 366.

Detection probability models considered in the study are listed in Table 3. Models with group size were not included because their results were inconsistent with the hypothesis that detection probability increases with group size. The model that received most support from the data was a half normal without covariates (AIC = 746.17). The estimated average detection probability ranged from 0.48 to 0.56, which translates into effective strip half-widths (ESW) of 493–576m.

Estimates of encounter rate, group sizes, density and abundance for each stratum for the most supported model are presented in Table 4. All proposed models provided similar estimates irrespective of their AIC score. Overall, uncorrected density and abundance were estimated at 0.121 whales/km^{-2} and 3,497 individuals (CV = 0.37, 95% CI = 1,581–7,736). Estimates corrected for availability bias were 0.242 whales/km^{-2} and 6,994 individuals.

Table 4

Encounter rate, group size, density, and abundance estimates for eastern Bering Sea belugas in 2000. (*N* = number of sightings; *CV* = coefficient of variation) computed with the most-supported detection probability model (model 1).

	Estimate	*CV*
Stratum 1 (16,128km^2)		
N	23	
Encounter rate	0.014	0.79
Mean group size	1.04	0.04
Uncorrected density (individuals/km^2)	0.015	0.79
Uncorrected abundance	233	0.79
Corrected abundance	466	
Stratum 2 (6,894km^2)		
N	133	
Encounter rate	0.181	0.58
Mean group size	1.52	0.11
Uncorrected density (individuals/km^2)	0.280	0.60
Uncorrected abundance	1,933	0.60
Corrected abundance	3,866	
Stratum 3 (3,171km^2)		
N	65	
Encounter rate	0.191	0.38
Mean group size	1.95	0.09
Uncorrected density (individuals/km^2)	0.380	0.40
Uncorrected abundance	1,206	0.40
Corrected abundance	2,412	
Stratum 4 (2,743km^2)		
N	11	
Encounter rate	0.038	1.03
Mean group size	1.18	0.10
Uncorrected density (individuals/km^2)	0.045	1.03
Uncorrected abundance	124	1.03
Corrected abundance	248	
TOTAL (28,936km^2)		
Uncorrected density (individuals/km^2)	0.121	0.37
Uncorrected abundance	3,497	0.37
Corrected abundance	6,994	

DISCUSSION

Beluga distribution in the Eastern Bering Sea region

Based on the information available prior to our surveys belugas were expected to be found mostly near the coast during May–June. Contrary to this, with the exception of around Stuart Island, very few whales were sighted on transects that covered the strip within 1.8km of the coast, or in areas such as Golovin Bay or Norton Bay (Figs 2 and 3). Instead, the most predictable region in which to find belugas was from the south mouth of the Yukon River to Stuart Island. West of the Yukon Delta whales were seen every year in a narrow band approximately 10km wide located 9–18km offshore. North and east of the Yukon Delta belugas were more broadly distributed in Pastol Bay. In essence, each year belugas were distributed in a continuous band around the Yukon Delta that was approximately 200km long. This band was centered around the 5m isobath and largely corresponded to the sediment plume discharged by the Yukon River (Fig. 4). In several years whales were seen in central Norton Sound and in 1995 the distribution of belugas extended well into the northern half of Norton Sound.

The distribution of belugas observed during the surveys was consistent with observations made more than 100 years ago. Zagoskin (1967) described the occurrence of belugas in Norton Sound in the 1840s, and noted that beginning in July

Table 3

Conventional and multiple covariate distance sampling detection probability models for eastern Bering Sea beluga survey data. (hr = hazard rate; hn half normal; f() = covariate included in the model as a factor; ESW = effective strip width, *N* = total estimated number of belugas; *CV* = coefficient of variation).

Model no.	Model name	No. of parameters	Delta AIC	*P*	*CV(P)*	ESW	*N*	*CV(N)*
1	hn	1	0.00	0.49	0.06	498	3,497	0.37
2	hr	2	0.56	0.49	0.08	500	3,484	0.37
3	hn + f(sighting conditions)	4	1.46	0.48	0.05	493	3,535	0.36
4	hn + f(sea state)	2	1.72	0.49	0.05	498	3,501	0.36
5	hn + f(glare)	2	1.80	0.49	0.05	498	3,499	0.36
6	hn + f(observer)	2	1.85	0.49	0.05	498	3,499	0.36
7	hn + f(sea state)	3	2.64	0.49	0.05	497	3,508	0.36
8	hr + f(sea state)	4	4.46	0.49	0.05	498	3,498	0.36
9	hr + f(sighting conditions)	5	5.02	0.51	0.05	519	3,360	0.36
10	hr + f(sea state)	3	5.87	0.56	0.04	576	3,028	0.36
11	hr + f(glare)	3	5.87	0.56	0.04	576	3,027	0.36
12	hr + f(observer)	3	5.90	0.56	0.04	576	3,025	0.36

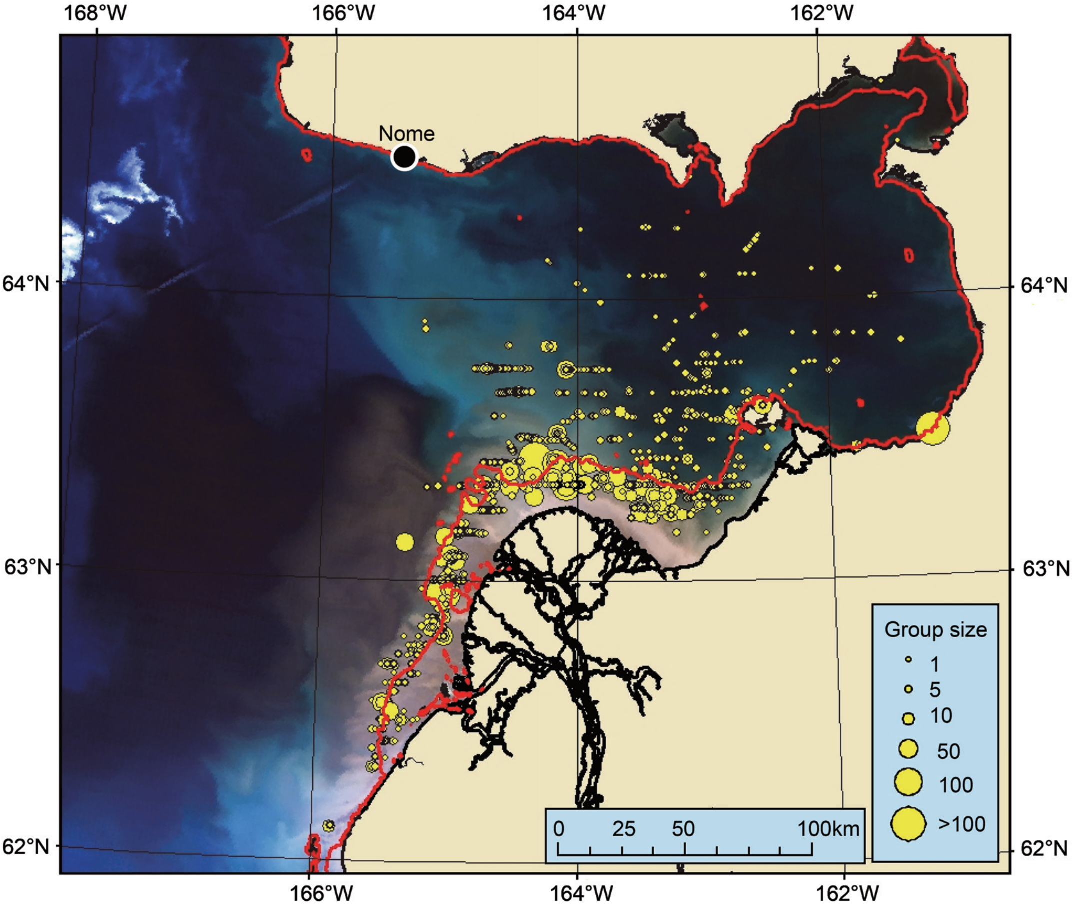

Fig. 4. MODIS image of Norton Sound and the Yukon River Delta taken from the Terra satellite on 17 June 2002. Yellow dots are sightings of beluga whales made during aerial surveys 1995–2000. Red line indicates the 5m isobath. The discharge plume of the Yukon River shows as gray/brown.

'the beluga appear in great numbers with their young as they follow the fish outside the mouths of the Yukon.' He described large organised hunts that occurred in mid–July in Pastol Bay, where as many as 100 animals were taken in a single drive. According to Nelson (1887), belugas usually appeared at Stuart Island between 5 June and 10 June and schools of 20 to over 100 animals were frequently seen in the bay nearby. He documented the summer occurrence of belugas at the mouth of the Yukon River, and as much as 800km upstream.

Limited observations from aerial surveys in the 1970s and 1980s also indicated that belugas frequented the waters off the Yukon Delta. Harrison and Hall (1978) flew bird and mammal surveys in this region and made five sightings of belugas in southern and eastern Norton Sound in late August 1976. During 1981, Ljungblad *et al*. (1982) flew whale surveys in the northern Bering Sea and saw belugas in Norton Sound on 22 June (12 animals), 6 July (10 animals) and 12 July (137 animals). Sightings made by Ljungblad *et al*. (1982) were all in southern Norton Sound in the region between Stuart Island and the north mouth of the Yukon River. They noted that on 12 July a sonobuoy recorded a variety of calls from more than 100 belugas 'vigorously feeding in shallow, muddy water near the Yukon River delta.' Each year during 1976–88, Alaska Department of Fish and Game biologists flew aerial surveys to assess herring (*Clupea pallasii*) stocks in Norton Sound shortly after ice breakup (late May and early June). Those surveys provided numerous sightings of beluga whales throughout Norton Sound and off the Yukon River delta (Frost and Lowry, 1990).

As survey effort was concentrated in June, little information on seasonal distribution patterns was obtained. However, results of the surveys in May and September 1992 confirm observations of local residents (Huntington, 1999) that belugas arrive in the Sound in the spring while it is still covered with ice and they are more common in the northeast part of the Sound in the autumn than in the summer.

Population abundance

The surveys conducted for the ABWC in 1992–95 and 1999–2000 have provided the first systematic information on the distribution and abundance of beluga whales in the Norton Sound/Yukon Delta region. In June 2000, systematic survey lines were flown over the entire region. Using distance sampling models an uncorrected estimate of 3,497 belugas at the surface in the study area was calculated. To estimate the true abundance it is necessary to account for any whales that were diving and not available to count when the survey aircraft passed (availability bias) and whales that were at the surface in the study area but were not recorded by observers (perception bias). Off the mouth of the Yukon River water is shallow and beluga dives must have been also, but the water was very turbid and whales could only be seen when part of their back was above the surface. Further offshore water was clearer and deeper, and while whales were easier to see they also could make deeper and longer dives. Because there are no data available on beluga diving behaviour in the EBS region, a commonly accepted correction factor of 2.0 was used to correct for this availability bias (e.g. Reeves *et al*., 2011) and the estimate of the total population size was then 6,994. This estimate is likely to be conservative for two reasons. Firstly, the analysis did not account for perception bias. By comparing observer counts of belugas in Cook Inlet, Alaska with videotapes, Hobbs *et al*. (2000) concluded that observers missed a significant number of animals. Photographic studies using models have shown that turbidity and rough water affect beluga visibility, especially for the younger animals that are grey (Kingsley and Gauthier, 2002). Secondly, the survey area focused on the main concentration of belugas in the EBS during June, however it is possible that some whales were elsewhere. For example, during the summer months some belugas move into and up the Yukon River (Nelson, 1887; Lensink, 1961; Frost and Lowry, 1990), and the surveys presented here did not include the river system itself.

There are no historical data available that can be used for comparison with this studies' abundance estimate. Results from this study indicate that the estimate of 1,000–2,000 whales for the EBS stock suggested by Seaman *et al*. (1988) based on local reports was too low by a substantial amount.

Survey methods and adequacy

This survey effort demonstrates that an adequate population assessment of EBS belugas can be done using line-transect surveys flown in June provided that: (1) surveys of the Yukon Delta and Norton Sound areas can be done during the same range of dates; and (2) survey transects cover all of Norton Sound and the Yukon Delta. However, it should be noted that that future survey efforts may well be complicated by sea ice that sometimes persists into the survey period, heavy fog that often develops off the Yukon Delta and simply the size of the area that must be surveyed.

The survey efforts in this study were restricted to Norton Sound and nearshore waters off the Yukon River Delta. Belugas are commonly seen in the Bering Sea to the west of Norton Sound during April–May when they are migrating northward through sea ice (Moore *et al*., 1993). However, aerial surveys that were conducted during summer in the northern Bering Sea in 1975–1977 (Harrison and Hall, 1978) and 1981–1983 (Ljungblad *et al*., 1984; Moore *et al*., 1993) did not detect any belugas west of our survey area. The lack of sightings at the western ends of our transect lines also indicates that our study area covered most of the summer concentration area used by the EBS beluga stock.

The density of belugas along tracklines varied from a few sightings near shore to high densities and then to very low densities at the offshore extremity. This gave us confidence that the concentration of belugas along each trackline was fully sampled. The observed density on each trackline was determined by the relative lengths of high and low density segments. Thus, it is likely that the *CV* for this abundance estimate could be reduced significantly if the transect lines were stratified by water depth or distance from shore as well as latitude. However, the mechanisms of choice of water depth and/or distance from shore that result in the observed distribution are not understood and consequently a stratification could not be devised *a priori* to the survey, or necessarily replicated in future surveys. By contrast the stratification by latitude allows greater flexibility for future surveys since a northward or southward shift of the population can be accommodated by adjusting the effort in the survey strata.

For survey counts to be useful for monitoring population trend they should be made in similar circumstances on a regular basis (e.g. annually). In addition, factors that affect the counts should be recorded and accounted for in the analysis (e.g. Frost *et al*., 1999). Using our EBS beluga survey data from 1993–95, DeMaster *et al*. (2001) showed that sightings were much more common in Beaufort state 1 than in state 2, 3 or 4 and they recommended that future data analyses incorporate sea state effects. This was done by using MCDS methods that took into account Beaufort state, glare, sighting conditions and observer. However, for these analyses using both half normal and hazard rate functions the most supported model was the one without covariates. The next two best supported models were half normal with sighting conditions as a covariate and half normal with sea state as a covariate. This apparent contradiction with DeMaster *et al*. (2001) may be due to the generally good sighting conditions encountered in 2000. In that year only 12% of sightings were made in poor or fair sighting conditions and only 9% were in Beaufort states greater than 2. Palka (1996) showed similar effects of Beaufort state on aerial survey counts of harbour porpoises (*Phocoena phocoena*). We continue to believe that sea state, glare and sighting conditions may seriously impact observers' ability to detect belugas and that those parameters should be recorded during surveys and considered as covariates during analyses.

Other factors such as the timing of environmental and biological events (e.g. sea ice breakup, discharge from the Yukon River and the appearance of migratory fishes) may also affect beluga distribution and movements, and therefore counts. Clearly, the biology of belugas in this region is not yet fully understood and more studies will be needed before a satisfactory population assessment and monitoring program can be developed.

Management considerations

Management of subsistence hunting

The ABWC was formed in 1988 to coordinate efforts of Alaska Native hunters, scientists and managers in the conservation and management of western Alaska beluga whale stocks (Adams *et al*., 1993). The Committee is a co-manager of these stocks under an agreement with the US National Marine Fisheries Service and it undertakes a number of research and management activities to fulfil its co-management obligations[6].

One of the first research programs supported by the ABWC was the collection and analysis of genetics samples to determine whether summer concentration areas in the Bering and Chukchi seas comprise separate management units. Results showed that belugas harvested in Norton Sound and the Yukon Delta do comprise a stock that is separate from animals that summer in Bristol Bay and the Chukchi Sea (O'Corry-Crowe *et al*., 1997; 2002). This led

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[6] *http://www.north-slope.org/departments/wildlife-management/co-management-organizations/alaska-beluga-whale-committee.*

the Committee to support aerial surveys to develop a more realistic estimate of abundance for the EBS stock.

Another early effort by the ABWC was to systematically collect information on the Alaska Native subsistence harvest of belugas. Results have shown that belugas are a very important resource for people living in the Norton Sound/Yukon Delta area with whales being harvested in at least 20 communities. From 1987 through 2006, the estimated annual harvest from the EBS stock was 191 belugas (range 103–309; Frost and Suydam, 2010).

The only identified human-caused mortality in this population is Alaska Native subsistence hunting (Allen and Angliss, 2013). Using the estimate of 7,000 belugas from this study (which is believed to be conservative), this harvest in recent years has been about 2.7% of the population. Considering that studies in nearby Bristol Bay have shown that Alaska beluga populations can increase by more than 4% per year (Lowry *et al*., 2008), it is likely that this harvest is sustainable. While written records are sparse, those that are available, combined with the local and traditional knowledge of current beluga whale hunters, suggest that there has been a large, healthy, beluga whale population in the Norton Sound/Yukon Delta region since at least the mid 1800s.

Management as a component of the Norton Sound ecosystem

Beluga whales prey on Pacific salmon (*Oncorhynchus* spp.) throughout much of Alaska. In Bristol Bay and Cook Inlet where annual runs of several species of salmon occur, belugas feed on outmigrating smolt in spring and on adult salmon returning to spawn in the summer (Frost *et al*., 1984; Moore *et al*., 2000; Quakenbush *et al*., 2015). In Norton Sound and off the Yukon River, belugas have also been reported to feed on salmon in July and August, although herring and saffron cod (*Eleginus gracilis*) are more commonly found in stomachs examined at other times of year (Nelson, 1887; Seaman *et al*., 1982). Alaska Native subsistence hunters from Norton Sound and Yukon River villages report that belugas arrive during the herring runs and remain throughout the summer feeding on adult salmon (ABWC, unpublished). Because belugas are generally hunted before and after the salmon season (when hunters are engaged in commercial salmon fishing), few summer beluga stomachs have been examined.

Five species of salmon occur off the mouth of the Yukon River and in Norton Sound. These salmon, particularly chinook (*O. tshawytscha*) and chum (*O. keta*) are harvested in commercial, sport and subsistence fisheries. Sockeye (*O. nerka*), pink (*O. gorbuscha*) and coho salmon (*O. kisutch*) are also present, and although they may be quite abundant, they are of less commercial importance. During June when the beluga aerial surveys were conducted, summer-run chum and chinook salmon are the main species present. The average run size for summer chums is about 1.8 million fish (range 0.55–4.0 million), and for chinook about 100,000 (Bergstrom *et al*., 2009; Bue *et al.,* 2009; Evenson *et al*., 2009). The 'run size' is estimated from counting stations in the Yukon River after most predation has occurred and therefore true run sizes for ocean fish would be larger than the estimates made for fishery management purposes. The average annual commercial harvest of summer chums is about 630,000 and there is currently little or no harvest of chinook. In 2012, the commercial harvest of all salmon species for all of Norton Sound and the Yukon was 989,000 salmon (Eggers *et al*., 2013). Frost *et al*. (1984) estimated the consumption of sockeye salmon by belugas in Bristol Bay by using estimates of average beluga body weight (350kg), daily consumption (5% of body weight) and the percentage of salmon in their stomach during the period of interest (70%). Although such estimates are imprecise, particularly without detailed information about diet, they can be useful for identifying the general magnitude of salmon consumption. Data from captive belugas indicates that consumption rate varies by size/age and may range from 4.5% for younger animals to < 2% for larger/older belugas, and about 3% for an average 350kg beluga (Sergeant, 1969; Kastelein *et al*., 1994). Using these figures the daily salmon consumption of a single beluga is estimated to be 7.35 kg (350kg*0.03*0.7). Multiplying that times the abundance estimate developed in this study (6,994 belugas) indicates that eastern Bering Sea belugas could consume about 51,470 kg of salmon per day, or about 1,500,000 kg of salmon in a month. If an 'average' salmon weighs 3.2kg (the average weight of chum and coho salmon in this region), belugas would consume about 16,000 salmon per day, or about 500,000 salmon in a month. Thus, in a single month belugas may eat about half the number of salmon that were harvested in all Yukon and Norton Sound commercial fisheries during the entire 2012 fishing season. This impact could be greater if whales feed predominantly on particular species or stocks. Belugas occur in this region throughout the summer (Frost and Lowry, 1990) and almost certainly eat salmon in other months as well. Considerable quantities of non-salmonid prey are also being taken, especially during spring and fall. While there are several uncertainties in the estimates above, it is clear that beluga whales are very important in the trophic ecology of the Norton Sound/Yukon Delta region.

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Photo-identification of franciscanas (*Pontoporia blainvillei*) in Babitonga Bay, Santa Catarina State, Brazil

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ABSTRACT

The franciscana (*Pontoporia blainvillei*) is a small cetacean endemic to the South Atlantic distributed in coastal waters from Espírito Santo State in Brazil to Chubut Province in Argentina. Babitonga Bay, on the northern coast of Santa Catarina State, Brazil, is home to the only known franciscana population that resides throughout the year in an estuary. Photo-identification is a technique that serves to identify individuals in their natural environment through photographs of natural or artificial body marks. The objective of this paper was to assess the feasibility of identifying individuals from this population from marks present on the dorsal fin and the body. From February 2011 to August 2013, 172 boat surveys were carried out in Babitonga Bay. Groups of franciscanas were recorded on 576 occasions and on 542 of these (94.09%) were photographed. A total of 6,953 (11.89%) from a total of 58,471 photographs were considered of high enough quality to distinguish the features used to identify individuals. Throughout the sampling period, 23 franciscanas were identified. Most of the animals exhibited nicks on the trailing edge of the dorsal fin (82.6%), a mark of long-term duration. Scratches were recorded on only one animal on a single occasion. The rate of resightings ranged from 5.26% to 78.95%, with 39.13% of the individuals showing a rate higher than 50%. A total of 41.8% of the Babitonga Bay population was identified by the presence of marks on the dorsal fin. The study indicates that photo-identification can be applied to franciscanas, which may allow the realisation of various future studies. Because of this species threatened status, the use of this technique may become particularly important for monitoring franciscanas in Babitonga Bay and perhaps in other regions.

KEYWORDS: PHOTO-ID; BRAZIL; SURVEY–VESSEL; ATLANTIC OCEAN

INTRODUCTION

The franciscana[1] (*Pontoporia blainvillei*, Gervais and d'Orbigny, 1844, *Cetartiodactyla-Pontoporiidae*) is a small cetacean endemic to the Southwest Atlantic. This species inhabits coastal waters from Espírito Santo State in Brazil (Siciliano, 1994) to the Chubut Province in Argentina (Crespo *et al.,* 1998). It is one of the smallest and most endangered dolphins in the South Atlantic (Pinedo *et al.,* 1989; Reeves *et al.,* 2008), with incidental capture in fishing nets being the main threat to its long-term survival (Siciliano, 1994; Bertozzi and Zerbini, 2002; Kinas, 2002; Secchi *et al.,* 2003). Studies of franciscanas in their natural habitat are uncommon due to the great difficulty in observing these animals in the wild (Bordino *et al.,* 1999; Cremer and Simões-Lopes, 2005). Their small size, discrete surface behavior and the brownish grey coloration limit field observations (Cremer and Simões-Lopes, 2005).

In southern Brazil, data systematically collected over the last ten years indicate that the franciscana population that inhabits Babitonga Bay is resident throughout the year. Genetic analysis comparing samples from animals inside and outside the Bay indicated that the population of Babitonga has lower genetic diversity, reinforcing the residence hypothesis of the animals (Dias *et al.,* 2013). A study with satellite-linked telemetry and visual monitoring of tagged franciscanas was conducted in the area, and the results indicated that the animals remained in the same region where they were instrumented, in the inner Bay (Cremer *et al*., 2012b). Data indicates that the abundance of this population remains stable over a period of ten years (Cremer and Simões-Lopes, 2008; Zerbini *et al.,* 2011). Moreover, dolphins in Babitonga Bay are typically concentrated in an area where weather conditions are relatively good compared to the open coast, making them easier to observe and study (Cremer and Simões-Lopes, 2005).

Photo-identification is a non-invasive method that consists of identifying animals individually by visible marks, either natural or artificial (e.g. Hammond *et al*., 1990). In cases where a substantial portion of a population can be individually identified, photo-identification data can be used to investigate various aspects of their ecology, including social structure, life history, presence of skin disease, among others (Wells *et al.,* 1987; Hammond *et al.,* 1990; Wursig and Jefferson, 1990). For many cetaceans, the marks used for individual recognition consist of scars, scratches, nicks and mutilations present on the dorsal fin and dorsal surface of the animals (Wursig and Jefferson, 1990). The origin of these marks may vary according to the behaviour of each species. Nicks and scratches are caused mainly by bites from conspecifics or abrasions from the ground (Wursig and Jefferson, 1990; Dufault and Whitehead, 1998). Marks can also be caused by collisions with vessels and non-fatal fishery interactions. In general, only a proportion of the individuals in a population have marks that allow photo-identification (Gowans and Whitehead, 2001).

Photo-identification studies of franciscanas have been conducted in two locations other than Babitonga Bay. In Paranaguá Bay (Brazil) five individuals presenting distinguishable natural marks on the dorsal fin have been observed, but only one individual was resighted, seen twice in 2008 in the Summer and Winter (Santos *et al*., 2009). In Anegada Bay (Argentina), a total of 27 resightings of a single marked individual was documented. In this latter location, three different dorsal fin shapes were distinguished (Thompson, 2000).

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[1] Since 2008 listed as Vulnerable, IUCN Red List of Threatened Species (*http://iucnredlist.org*).

The aim of this paper was to evaluate the feasibility of using photo-identification techniques to study the ecology of franciscanas. In particular, photo-identification was employed to characterise natural marks in franciscanas from Babitonga Bay, to estimate the percentage of dolphins with recognisable marks, and to investigate the resighting rates of marked individuals.

METHODS

Study area

Babitonga Bay (26°16'S, 48°42'W) is an estuary located on the northern coast of Santa Catarina State in Southern Brazil (Fig. 1). It has an area of approximately 160km², an average depth of 6m, but depths of up to 28m are found in the main canal. In addition, the bay presents shallow areas that may become exposed during low tide. Babitonga Bay receives freshwater inputs from rivers flowing through surrounding cities, mainly those located in the northern portion of the Bay. The area is also affected by anthropogenic activity, e.g. boat traffic and net fishing (Vieira *et al.,* 2008).

Data collection

Franciscana dolphins were searched for with the aid of 7×50 binoculars. Photographs were collected from two vessels, an aluminium boat (5.5m in length, 60hp outboard motor) and a rigid-hull inflatable boat (6.2m in length, 200hp outboard motor), with two digital cameras (Canon EOS 7D), one with a 100–300mm zoom lens and the other with 100–400mm zoom lens.

Sampling strategies varied over the years. The search for franciscanas focused on areas where the species is known to occur (Cremer and Simões-Lopes, 2008). The photographs were collected occasionally between February and May 2011, and fortnightly between September 2011 and August 2013. Between May 2012 and August 2013 data collection was carried out through scans, covering pre-established routes at regular intervals of at maximum fifteen days. Two routes (Fig. 1) were simultaneously covered, and the boats started the routes in opposite directions. During the scans, the boats maintained a constant speed of about 20km/h. The photographs were taken only in calm sea conditions (Beaufort 0 and 1), and without rain.

When a group of franciscanas was sighted, the boat approached at low speed. The time spent with the dolphins, the number of individuals, and the presence of calves were recorded for each group. Care was exercised to ensure that all individuals in the group were photographed without favouring any animal, and to minimise disturbance and a consequent change in the behaviour of the dolphins in the group (Wursig and Jefferson, 1990). Whenever possible, the photographer was positioned at an approximate 90° angle relative to the animals and in favourable light conditions to ensure good contrast and quality of the photographs. Each group was followed until all individuals were photographed, or for a

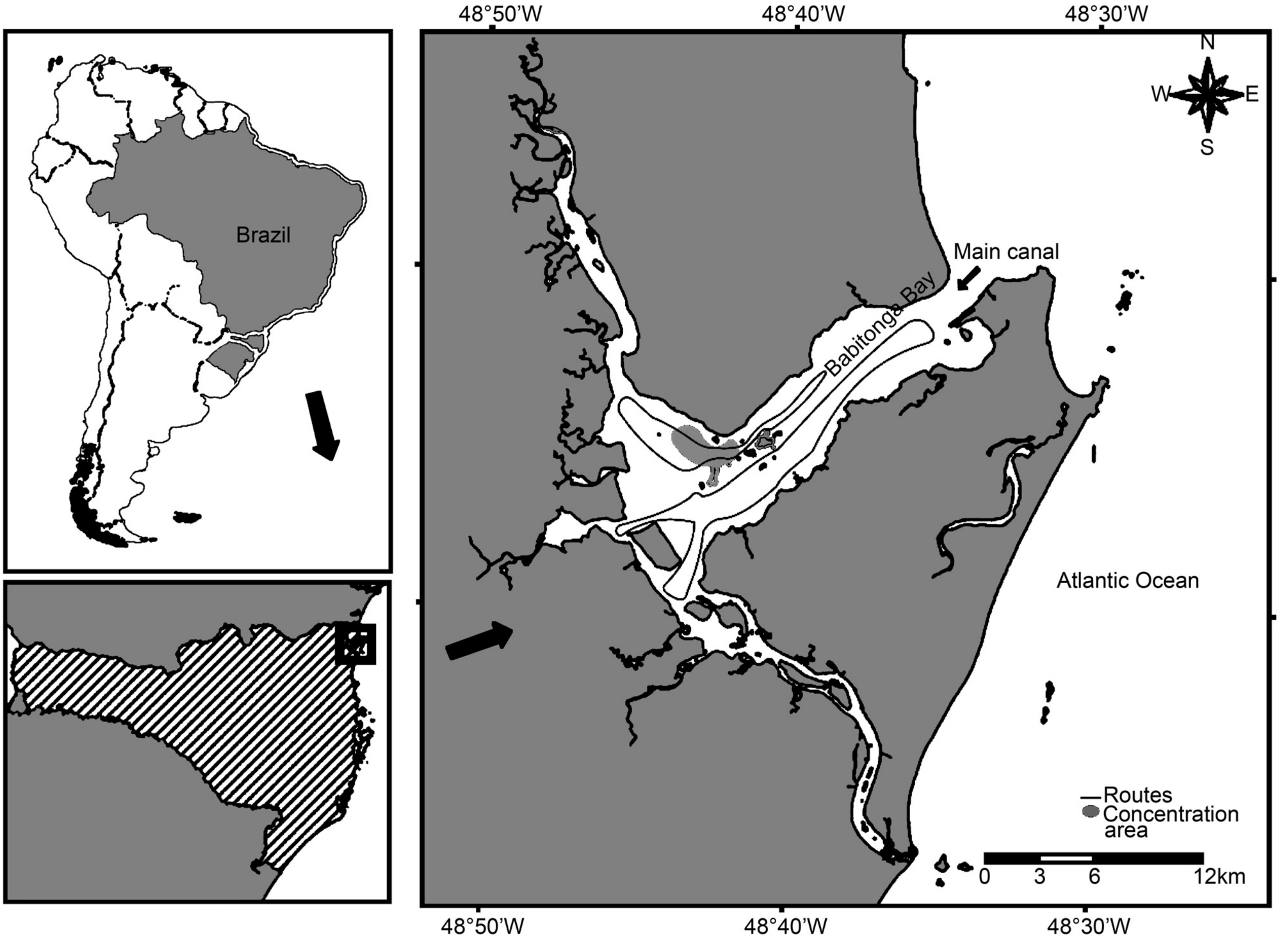

Fig. 1. Babitonga Bay, located on the northern coast of Santa Catarina State, where photo-identification studies for franciscanas have been conducted. The solid tracks and the grey polygons on the panel on the right correspond, respectively, to predetermined routes taken to perform scans and to the area where animals are typically concentrated (Cremer and Simões-Lopes, 2008).

maximum of 20 minutes, whichever occurred first. That time was stipulated in search of a balance between the need to photograph all the animals and the concern not to stress them. This decision was based on prior knowledge about this population, knowing that for large groups this time could not be sufficient to photograph all individuals in the group.

Data analyses

Franciscana photographs were separated into two categories: high quality and low quality. Images with focus, sharpness and at an approximate angle of 90° to the photographer were considered of high quality and, therefore, suitable for analysis (Wursig and Jefferson, 1990). High quality images were then divided into two sub-categories: animals with marks on the dorsal fin and animals without marks.

Visual analyses of individuals with marks were performed by two independent researchers. In addition to the presence of marks, the shape of the dorsal fin was also examined (Wursig and Jefferson, 1990; Gomez-Salazar *et al,*. 2011) by the two researchers who made a comparison of the fins analysed. In instances where there was no consensus on whether an individual could be identified, the image was included in a sub-category 'without marks', meaning in this case that the animal did not have prominent enough marks to ensure identification.

To estimate the percentage of the population with marks, we considered the abundance of franciscanas in Babitonga Bay (55 individuals in 2011) as estimated by Zerbini *et al.* (2011). The formula used was: number of individuals identified/55×100.

To evaluate sampling sufficiency, collector and rarefaction curves were produced (following Magurram, 1988, op. cit. Colwell and Coddington, 1994) which evaluated the necessity of additional sampling effort to identify individuals in the population with marks. The collector curve shows the accumulated value (the individuals, in this case) and the rarefaction curve shows a statistical proportion of this value.

To calculate the resighting rate we adapted the method based on the Jolly-Seber model proposed by Simões-Lopes and Fabian (1999) for residence patterns. In particular, the term 'resighting rate' used in this paper is based on the premise that the data do not reflect residence patterns but rather the susceptibility of each individual to being photographed. The resighting rate of the individuals was calculated as the total number of months in which the identified animal was sighted/the total number of sampled months×100, expressed as a percentage (%).

RESULTS

From February 2011 to August 2013, a total of 172 surveys were carried out in 19 sampling months. Of these, 162 were made at regular intervals (fortnightly), 114 in the area with the highest concentration of animals, and 48 along the predetermined scanning routes. Sampling effort totaled 458 hours and 22 minutes. A total of 576 groups of franciscana were recorded, 542 of which (94.09%) were photographed. A total of 58,471 images were taken, 6,953 of which were classified as high quality images (11.89% of photos used). Throughout the period, 23 franciscanas (Fig. 2) were identified by marks in the dorsal fin.

Twenty-two franciscanas were identified by the presence of nicks on the dorsal fin and another individual was identified based on the unusual shape of the dorsal fin (PbB-20 in Fig. 2). Thirteen franciscanas had only one nick (56.5%), 7 had 2 nicks (30.4%) and 2 had 3 nicks (8.6%). The disposition of the nicks in the dorsal fin, and the percentage of individuals identified are listed in Table 1. One individual (PbB-12) acquired a new mark throughout the study. It remained for 25 months with one nick and was then seen with two nicks on two separate occasions.

The resighting rate of the animals ranged from 5.2% (animals sighted in only one month) to 78.9% (animals sighted in 15 months) (Fig. 2). A total of 39.1% of the individuals showed a resighting rate higher than 50%. Individuals PbB-13 and PbB-15 showed the highest resighting rate (78.9%). Individual PbB-23 was not re-sighted possibly because it was registered only in the last month of sampling.

Four shapes of dorsal fin were observed: falcate (with more rounded upper part and the rear concave), rounded (with rounded upper end forming a larger angle, ~60°), triangular (with pointed upper end forming a sharp angle, 40°, and straight sides), and an anomalous shape (with the silhouette turn to the opposite side) (Thompson, 2000). For some individuals whose marks were similar (PbB-03 and PbB-13; PbB-10 and PbB-14), identity was confirmed by dorsal fin shape. The anomalous shape of the dorsal of individual PbB-20 was confirmed by inspecting sequences of images of the animal while surfacing, leaving no doubt of its identity (Fig. 3). This dolphin had no dorsal fin marks.

Scratches were rarely observed, being recorded near the top of the dorsal fin on one individual on one occasion.

The data indicated that 41.8% of the population showed features that allowed individual identification. However, the asymptotic curve was not reached (Fig. 4), suggesting that the number of individuals in the population having marks may be higher.

DISCUSSION

The results of this study indicate that franciscanas have long-term marks on the dorsal fin that are useful for photo-identification, making this technique a potentially useful tool for studying franciscana ecology. The relatively calm weather conditions found within the enclosed environment of Babitonga Bay contributed to the successful application of this method.

Nicks on the dorsal fin were practically the only type of mark observed in this species. Nicks are considered long-term and the most common mark used for individual identification of small cetaceans. This type of mark allows recognition independent of body side (Wursig and Jefferson, 1990). These marks likely originated from social interactions between individuals of the same species, for example during mating or feeding, as reported for boto (*Inia geoffrensis*) (Martin and da Silva, 2006) and Risso's dolphin (*Grampus griseus*) (Kruse *et al.,* 1999). Mutilations and skin spots were not recorded during this study. Mutilations are usually associated with interactions with human activities, such as collision with boats or accidental interaction with fishing activities, or as a consequence of predator attacks (Wood

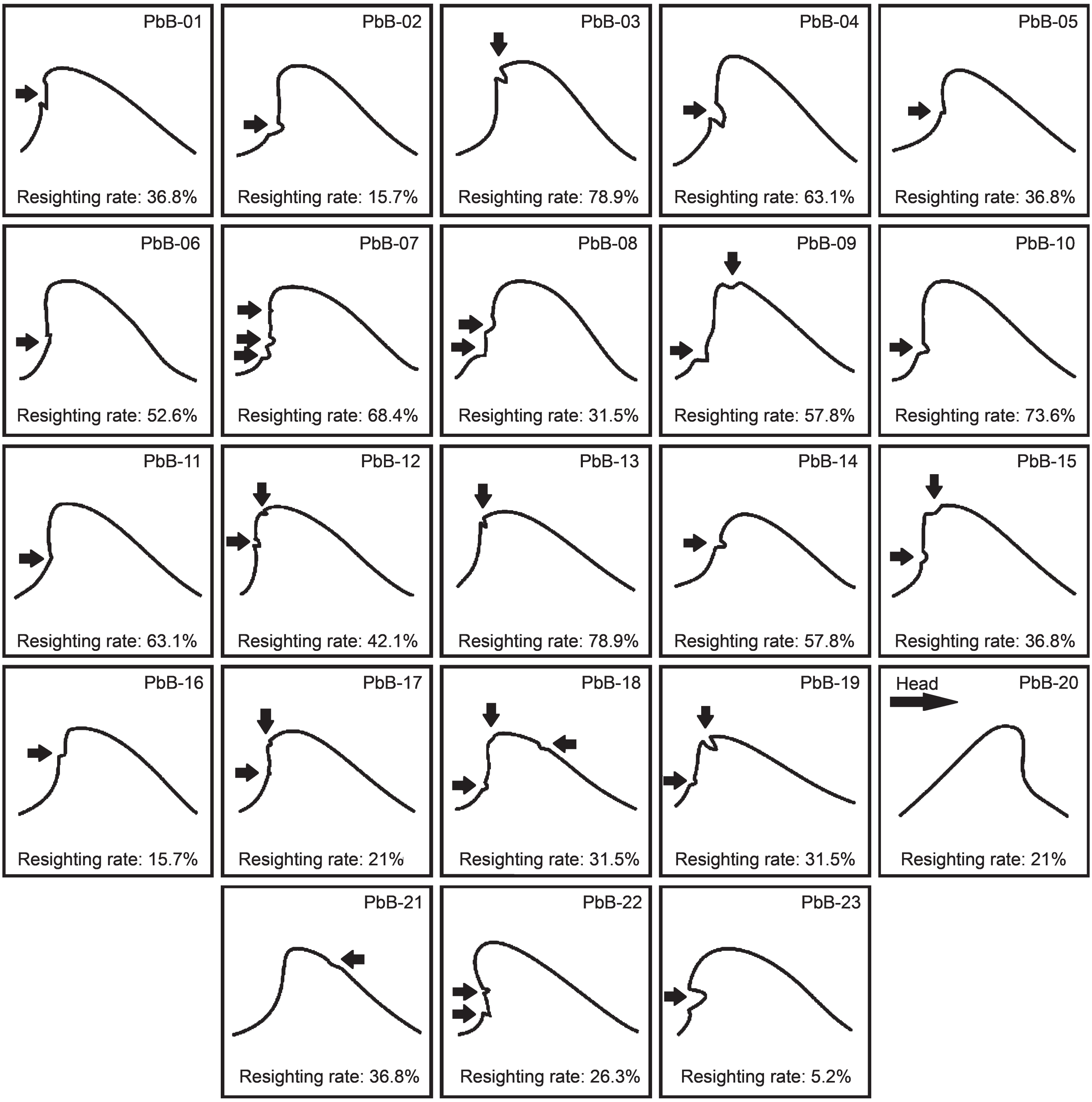

Fig. 2. Schematics of the dorsal fin shape, and disposition of marks of 23 franciscanas (*Pontoporia blainvillei*) individually identified in Babitonga Bay. The resighting rate of each individual (number of months each individual was sighted divided by the total number of sampling months).

Table 1

Position of the nicks on the dorsal fin of the franciscanas, the percentage of individuals with nicks on the indicated part of the dorsal fin, and the identity of individuals who have nicks on the indicated part of the dorsal fin.

Position of nicks on the dorsal fin	% of individuals that have nicks on the indicated part of the dorsal fin	Individuals who have nicks on the indicated part of the dorsal fin
Posterior edge	56.52%	PbB-01; PbB-02; PbB-04; PbB-05; PbB-06; PbB-07; PbB-08; PbB-10; PbB-11; PbB-14; PbB-16; PbB-22; PbB-23
Top	8,69%	PbB-03;PbB-13
Anterior edge	4.34%	PbB-21
Different parts of the dorsal fin	26.08%	PbB-09; PbB-12; PbB-15; PbB-17; PbB-18; PbB-19
No nick	4,34%	PbB-22

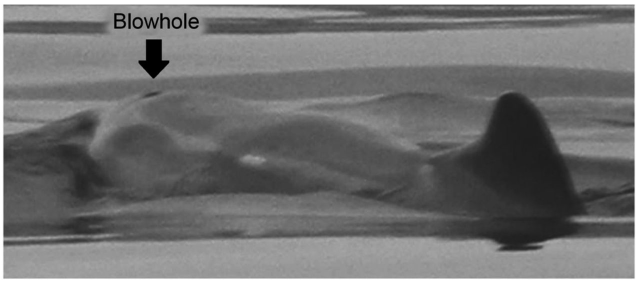

Fig. 3. Dorsum of individual PbB-20 showing the anomalous (inverted) shape of the dorsal fin. The blow whole is indicated for reference.

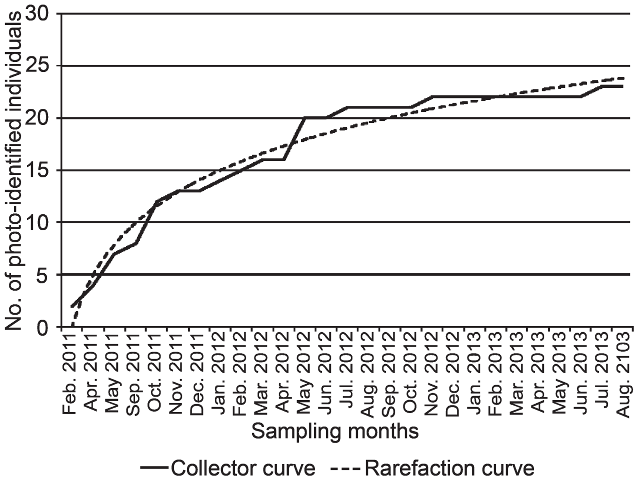

Fig. 4. Collector curve and rarefaction curve with the number and percentage of franciscanas (*Pontoporia blainvillei*) identified in each sampling month in Babitonga Bay, respectively.

et al., 1970; Corkeron *et al.,* 1987; Wells *et al.,* 1987; Heithaus, 2001). However, the small size of franciscanas likely reduces the probability of survival after boat collisions or predator attacks (Brownell, 1975; Praderi, 1985). Observations in the field indicated that individuals with marks in the top of dorsal fin are easier to recognise, which could influence in the resighting rate. However, because only photographs that showed the entire dorsal fin (bottom to top) were used in this study, it is believed that the likelihood of resighting franciscanas in this study to be similar across individuals.

Only one individual was observed with a visible scratch. This scratch, characterised by a sequence of parallel lines, was probably produced by the teeth of another franciscana. However, because this individual had no other identifiable mark which could allow for recognition during multiple resightings, it was not possible to determine the duration of this type of mark. Scratches are considered low-duration marks in other cetacean species (Auger-Méthé and Whitehead, 2007; Gomez-Salazar *et al.,* 2011) and possibly not a useful feature to individually identify franciscana dolphins. Scratches are usually caused by intra or interspecific contact, such as bites, but can also be caused by contact with abrasive materials (Wursig and Wursig, 1977; 1980; Wursig and Jefferson, 1990; Gonzalez, 1994; Dufault and Whitehead, 1998). Di Beneditto *et al.* (2001) reported scratches on 26.6% of franciscanas accidentally caught in fishing nets in southeastern Brazil, and proposed that they are probably caused during rescue attempts when an animal becomes entangled in a gillnet. Pilleri (1971) reported three individuals caught on fishing nets, and one of them was a female calf which had teeth marks on the tail and body. The distance between the scratches corresponded to the distance between the teeth of an adult female. Cremer *et al.* (2006) reported a dead franciscana calf with several scratches and net marks on the rostrum, suggesting the occurrence of epimeletic behaviour.

The low occurrence of body scratches on franciscanas reinforces the hypothesis that male disputes are rare or nonexistent for this species (Danilewicz *et al.,* 2004). Analysis of the gonads suggests that franciscanas have a monogamous mating system (Rosas and Monteiro-Filho, 2001), a feature also supported by genetic studies (Mendez *et al.,* 2008). This is different from the boto (*Inia geoffrensis*), which has a promiscuous mating system, and scars and scratches are common and intensify with the age; marks are particularly common in sexually active males, which compete for females (Martin and da Silva, 2006). Risso's dolphin (*Grampus griseus*) is a species that is often heavily scratched too. These marks can be produced by individuals of the same species, mainly by males competing for females or individuals feeding on squid, their main prey (Kruse *et al.,* 1999).

The four dorsal fin shape patterns identified in franciscanas were similar to the shapes described by Thompson (2000). The existence of different dorsal fin shapes suggests the possibility of a relationship between these shapes and some biological characteristics of the species, such as age, gender and sexual maturity (Jefferson *et al.,* 2008). In killer whales (*Orcinus orca*) the dorsal fin of females and juvenile males are falcate and measure maximally 0.9 meters in height, while adult male dorsal fins are triangular and can be as tall as 1.8 meters (Heyning and Dahlheim, 1988). For spinner dolphins (*Stenella longirostris*) the shape of the dorsal fin varies from triangular to falcate, being predominantly triangular in adult males (Perrin, 2002), like males of *Stenella longirostris orientali,* which have the dorsal fin forward-canted (Ralls and Mesnick, 2009). However, a comparative analysis between the dorsal fin shape and gender in Babitonga Bay franciscanas has not yet been performed. In every case but one, dorsal fin shape was not used for identification alone, but rather as an additional feature.

The unusual shape of the dorsal fin of the individual PbB-20 (which was identified by shape alone) is an uncommon pattern in cetaceans. This feature has been recorded for sexually active males of some species of Delphinidae, killer whale and spinner dolphin (Jefferson *et al.,* 2009). This anomaly could be a consequence of reproductive problems related to contamination (Couch *et al.,* 1972; Haskins and Robinson, 2007). Pollution has been shown to have various effects on the health of cetaceans, such as reduction in reproductive potential, immunosuppression, endocrine disruption and cancer (Borrel and Aguilar, 1994; Martineau *et al.,* 1999; Schecter *et al.,* 2006). The franciscanas of Babitonga Bay showed high liver concentration of difenilpolibromado ether (PBDE) and polybrominated biphenyl (PBB) (Alonso *et al.,* 2012). These agents are highly persistent in the environment, are lipophilic and have a high potential for bioaccumulation (Ghiselli and Garden, 2007). Another hypothesis to explain the inverted dorsal fin is inbreeding depression, a result of mating between genetically close individuals which increase the chances of offspring being affected by deleterious recessive genes (Jiménez *et al.,* 1994). The pectoral fin malformations registered in six franciscanas from Cananéia, São Paulo State, were shown to be related to inbreeding (Rodrigues and Monteiro-Filho, 2012). The Babitonga Bay population is small, estimated at nearly 50 individuals (Cremer and Simões-Lopes, 2008; Zerbini *et al*., 2011), is resident of the Babitonga Bay estuary (Cremer *et al.,* 2012a) and has low genetic diversity (Dias *et al.,* 2013), reinforcing the hypothesis that inbreeding could be happening.

The data indicate that 41.8% of the Babitonga Bay franciscanas have features allowing identification, and the resighting rate was greater than 50% for 39.13% of the animals. In Argentina, Thompson (2000) estimated that less than 20% of the franciscanas in Anegada Bay were identifiable by natural marks on the dorsal fin. Differences in the number of individuals with marks between populations were registered for short-finned pilot whale (*Globicephala macrorhynchus*), but the causes were not identified (Shane and McSweeney, 1990). For the boto, 55% of the population in the Colombian Amazon and Orinoco rivers was individually identified (Gomez-Salazar *et al.,* 2011), while for narwhal (*Monodon monocerus*)*,* 84% of the Koluktoo, Canada population included identified individuals (Auger-Methe *et al.,* 2010). For the sympatric Guiana dolphin (*Sotalia guianensis*) population in Babitonga Bay, 37% of the individuals were identified (Schulze, 2012).

The collector and rarefaction curves suggest that the number of individuals in the population having marks may be higher. Although this population is relatively small, we probably did not identify all the individuals. A high number of dolphins were identified when photo-identification efforts were first initiated. However, later in the study mainly previously identified individuals were being sighted with just a few newly identified dolphins being added to the catalogue at that stage. The main problem with photo-identification of franciscanas is the difficulty in observing the species and not the absence of defining marks on individuals. Even under favourable conditions such as in Babitonga Bay, cryptic colouration, small size and discrete surface behaviour in this species make good quality dorsal fin photographs difficult to obtain. These characteristics may also hinder use of the technique with other species, as in the case of the vaquita (*Phocoena sinus*) (Jefferson *et al.,* 2009), the Dall's porpoise (*Phocoenoides dalli*) (Jefferson, 1991) and the baiji (*Lipotes vexillifer*) (Yuanyu *et al.,* 1990). However, other locations along the distributional range of the franciscana could present conditions favourable enough for the application of this technique. For example, studies performed in Cananéia (Santos *et al.,* 2009) and Anegada Bay (Thompson, 2000) indicated individual identification of franciscanas was possible in these locations.

Photo-identification is a powerful tool to improve knowledge about the life history of small cetaceans. In the case of franciscanas, this technique can potentially provide important information about the home range, residence patterns, habitat use, life history and behaviour, which are still largely unknown for this species. At the local level, this information can contribute greatly to the conservation of fransciscanas in Babitonga Bay, especially considering the threats resulting from increasing anthropogenic activities in this area. Therefore, continued photo-identification and monitoring of this population are strongly recommended.

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Individual gray whale use of coastal waters off northwest Washington during the feeding season 1984–2011: Implications for management

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ABSTRACT

Gray whales (*Eschrichtius robustus*) in northwest Washington were studied, with the aims to: (1) increase understanding of gray whale use of the study area; (2) document the annual and seasonal fluctuations in the numbers of whales utilising the area; and (3) assess the fidelity of whales to the study area within and between years. Together these goals establish a baseline of gray whale behaviour during summer and autumn in the region of the Makah Tribe's proposed whale hunt. From 1984 to 2011, a total of 225 unique gray whales were observed, with 49% being observed again in a future year. There was significant variability in observation rates of gray whales by month and year. During the feeding season, the observation rate increased to a peak in August in the north research segment in the Pacific Ocean and to a peak in October in research segments in the Strait of Juan de Fuca and in the southern research segment in the Pacific Ocean. Gray whales were most commonly observed at depths of 5–15m over rocky substrates and often near kelp forests, although the locations where they fed were dynamic by both month and year. Some whales habitually returned to northwest Washington, however the average whale in the study area was observed in only 31.6% (SE = 1.6%) of the possible years in which they could have been observed. Gray whales in the study area had an average minimum tenure (residency time) of 24.8 days out of a possible 183 days of the feeding season. A discovery curve analysis did not reach an asymptote over the 27 years of this study showing that there is no population closure to the research area. Based on these findings, it can be concluded that even though northwest Washington is an important feeding area, most Pacific Coast Feeding Group (PCFG) gray whales do not have strong fidelity to this one region within the IWC defined PCFG range. The findings presented in this paper provide a baseline for evaluating the impact of Makah hunting activities on the behaviour of PCFG whales that utilise the Makah's traditional hunting area once hunting activities resume.

KEYWORDS: GRAY WHALE; PACIFIC OCEAN; FEEDING GROUND; MOVEMENTS; SITE FIDELITY; NORTHERN HEMISPHERE; SURVEY–VESSEL

INTRODUCTION

Most Eastern North Pacific (ENP) gray whales (*Eschrichtius robustus*) migrate from wintering grounds in Baja California, Mexico, to feeding grounds in the Bering, Chukchi and Beaufort seas. A small subset of the ENP gray whale population does not complete the migration to arctic feeding grounds and instead spends the summer and autumn at feeding grounds along the coast of the Pacific Ocean from California through Southeast Alaska (Calambokidis *et al.*, 2002). This group of whales has been referred to by many names since it was first studied in the 1970s and is currently recognised as the Pacific Coast Feeding Group (PCFG) by the International Whaling Commission (IWC, 2011) and the US Government (Carretta *et al.*, 2013). The IWC defines the PCFG as gray whales seen in more than one year in the months of June to November within the range of northern California to northern British Columbia (41°N–52°N), excluding gray whale sightings in Puget Sound, Washington (IWC, 2012). The range is restricted to 52°N even though PCFG whales are known to frequently occur as far north as Kodiak Island, Alaska (Gosho *et al.*, 2011) and have been observed in the Beaufort Sea (Calambokidis *et al.*, 2014). The IWC-defined range of the PCFG is narrower than previous definitions of this group. This is primarily because most photo-identification surveys have been focused on 41–52°N. Population estimates are therefore more reliable for this range. There are few historic or projected future catches of gray whales north of 52°N and south of the Bering Sea, making the more narrowly defined range more applicable to management (IWC, 2012). The abundance estimate for the PCFG in 2012 was 209 whales (Calambokidis *et al.*, 2014).

Recent genetic studies have found small but statistically significant differences in frequencies of mtDNA haplotypes between samples collected from PCFG whales and other ENP whales in other portions of their range (Frasier *et al.*, 2011; Lang *et al.*, 2014). No statistically significant differences have been found in the frequencies of nuclear DNA (D'Intino *et al.*, 2013; Lang *et al.*, 2014). Despite the significant difference in mtDNA haplotype frequency, PCFG and ENP whales had similar haplotype diversity which suggests that immigration into the PCFG could be occurring (Lang *et al.*, 2014). The results of a genetics simulations study (Lang *et al.*, 2012) and photo-identification work (Calambokidis *et al.*, 2014) were consistent with immigration from other portions of the ENP range into the PCFG having a significant role in the

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population dynamics of the group. Given that there is evidence both for the PCFG having open population dynamics and evidence for matrilineal recruitment, there is currently debate on whether or not the PCFG is a stock. NOAA Fisheries used a panel of experts to evaluate whether the PCFG is a stock; the panel could not agree whether the PCFG is a stock for US domestic purposes but did agree that more research is needed (Weller *et al.*, 2013).

Interest in PCFG whales has been inspired by concern regarding the possible impacts on the PCFG of the Makah Tribe resuming their treaty protected right to hunt whales. In 1855, the Makah Tribe protected its whaling rights in the Treaty of Neah Bay. In the 1920s, the Tribe voluntarily suspended whale hunting due to the impacts of commercial whaling on gray and humpback whale populations (Renker, 2012; Thompson, 2006). In 1994, when the gray whale was removed from the US Endangered Species List, the Makah Tribe informed the US Government of its intentions to resume traditional whale hunting. The US Government has obtained aboriginal whaling catch limits for the harvest of gray whales from the IWC to be used by the Makah Tribe since 1997. However, since that time the Makah Tribe has only landed one gray whale due to domestic court cases and regulatory processes suspending the hunt in 2000. The Tribe has submitted a proposed management plan to the US Government and the IWC for review. The management plan restricts the hunt to the migratory season in the Pacific Ocean portion of the Makah Usual and Accustomed (U&A) fishing grounds to minimise the risk that a hunt takes a PCFG whale. Nonetheless, it is recognised that the hunt may still take PCFG whales, so the management plan also has a provision to limit the number of PCFG whales landed through a conservative calculation based on the abundance of PCFG whales (IWC, 2013). The IWC evaluated the impact of Makah hunting on PCFG population dynamics and found that the Tribe's proposed management plan meets the conservation goals of the IWC of ensuring the PCFG will remain above 60% of its carrying capacity over a 100-year simulation (IWC, 2013).

Past studies have documented the behaviour of PCFG whales throughout their entire range (Calambokidis *et al.*, 2002; 2010; 2012; 2014). This paper reports on the behaviour of gray whales in the coastal waters of northwest Washington during the summer and autumn feeding season. Data were collected from 1984–2011 with the goals of: (1) increasing our understanding of gray whale use of the study area; (2) documenting the annual and seasonal fluctuations in the numbers of whales utilising the area; and (3) assessing the fidelity of whales observed within the study area within and between years. Together these three goals establish a baseline of gray whale behaviour in the region of the Makah Tribe's proposed whale hunt to evaluate (once the hunt is approved) whether the hunt impacts gray whale behaviour in the northwest Washington.

METHODS

Study area

Research effort was conducted along the northwest tip of Washington State, USA (Fig. 1). Northwest Washington is bounded by two bodies of water: the Strait of Juan de Fuca to the north and the Pacific Ocean to the west. The rocky shorelines are interspersed with sandy beaches, and rocky underwater habitats dominated by forests of bull kelp (*Nereocystis luetkeana*) and giant kelp (*Macrocystis spp.*) in waters 5–15m deep. The waters of northwest Washington have high biological productivity due to the confluence of currents from the California Current and the drainage of Puget Sound through the Strait of Juan de Fuca, and seasonal winds causing upwellings (Marchetti *et al.*, 2004). The study area encompasses most of the nearshore habitat of the Makah U&A and the entire area in which the Makah Tribe has proposed for hunting gray whales (Makah Tribal Council, 2011).

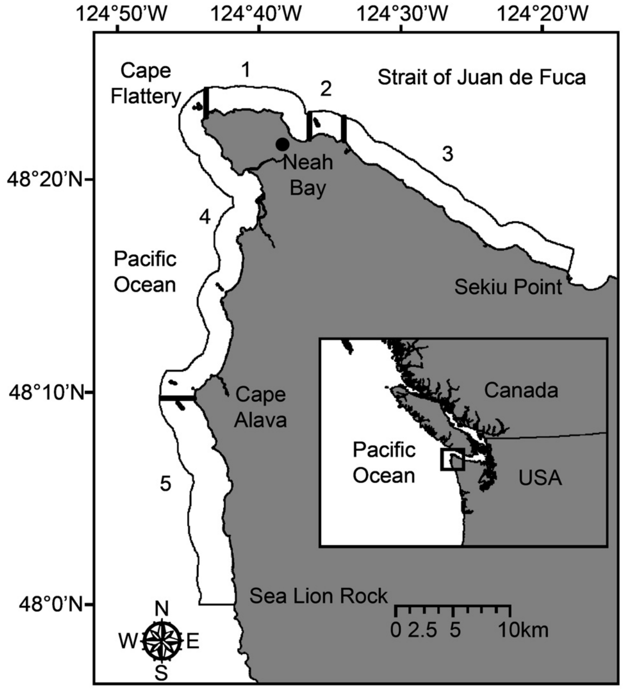

Fig. 1. Map of the gray whale survey region in northwest Washington with the focal survey area shown enclosed with a line. The numbered survey segments are: (1) West Strait; (2) Neah Bay Entrance; (3) East Strait; (4) North Ocean; and (5) South Ocean.

Survey methodology

The northwest Washington survey area is too large to be surveyed effectively in one day. One day of survey effort covered the area to the east of Neah Bay along the shores of the Strait of Juan de Fuca to Sekiu Point, approximately 25km from Neah Bay. The other survey day covered the area west along the shores of the Strait of Juan de Fuca to Cape Flattery and then south following the shoreline of the Pacific Ocean to Sea Lion Rock (47°59.58'N, 124°43.45'W). The total distance covered in the southbound leg is approximately 60km. Surveys for gray whales were generally conducted within 1–2km of shore because gray whales feeding in northwest Washington primarily congregate near shore. Portions of the survey in the Pacific Ocean, particularly south of Cape Alava, were conducted further from shore due to poorly charted submerged rocks.

Survey effort was variable by year. The early years of survey effort in northwest Washington were conducted opportunistically with three years of surveys in the 1980s (1984, 1986 and 1989) by Cascadia Research Collective

(CRC). Starting in 1992 surveys were conducted annually by the National Marine Mammal Laboratory (NMML) and CRC but effort was low and opportunistically conducted during studies of other marine mammal species. After 1996, surveys were standardised and were generally conducted on a bi-weekly basis from June through November as weather and ocean conditions allowed with NMML and the Makah Tribe as the primary research groups. The objective was to collect photo-identification of whales. Thus, if the researchers had good reason to suspect that survey effort in the Strait of Juan de Fuca would result in limited or no photographs of gray whales, then effort was focused on the Pacific Ocean and vice versa. The Pacific Ocean survey area was generally surveyed monthly regardless of anticipated opportunities to photograph gray whales because the surveys were also used for monthly California and Steller sea lion research. All research effort was conducted from small vessels of 6–9m in length.

During surveys, observers periodically recorded time and location and variables that could have influenced the probability of sighting a whale such as cloud cover and Beaufort sea state. When gray whales were sighted, their location, depth and activities were recorded. Observers then attempted to take photographs of the dorsal ridge along both flanks as well as the flukes. Photographs were taken using digital SLR cameras with a 70–300mm lens (35mm film cameras were used prior to 2004). The lens magnification allowed photo-documentation of unique colouration patterns on the lateral sides and flukes of the whales (Darling, 1984). The frame numbers from the photographs were recorded on the field data sheet with the sighting information.

Photo-identification methodology

All gray whale photographs of suitable quality were compared to a catalogue of gray whales previously seen in the PCFG as described in Calambokidis *et al.* (2012) by CRC. If a photographed whale was matched to a catalogued whale then the catalogue number of the whale was recorded. If a match could not be made, and the photograph was of sufficient quality, then the photographed whale was assigned a new catalogue number. All catalogue numbers of sighted gray whales were recorded in a database along with attributes of the sighting such as date, time, water depth, location and whale behaviour.

Data exploration

The three primary goals of this research were: (1) to increase understanding of gray whale use of the study area; (2) to document seasonal and annual fluctuations in the numbers of whales using the study area; and (3) to assess fidelity of whales to the study area. The analyses conducted could be interpreted as achieving one or more of these goals but for the purpose of explaining each method, and why it was conducted, each method is listed by research goal. For all analyses observations of uniquely identified whales were used instead of all gray whale observations to prevent pseudo-replication. Research effort and data collection was not consistent in all years (as described above) and as a result some analyses could not use all collected data whereas others could (Table 1).

To address the goal of increasing understanding of gray whale use of the study area four analyses were conducted. The first analysis was to characterise the depth range and habitat types where gray whales were observed. The second analysis was to document the occurrence of new whales in the study area. The purpose of this analysis was to determine the turnover of individuals in the study area. New whales were simply defined as whales not previously observed in the study area although they may have been observed within the PCFG in the past. For each year the number of new whales observed and the proportion of those that were observed to 'recruit' into the study area and be observed again in a subsequent year were determined. The third analysis documented how many calves were observed and calculated an estimate of proportion of newly observed whales that were calves (see Calf Analysis below). The last analysis determined if there is population closure to the study area. Calambokidis *et al.* (2010) concluded that gray whales who utilise northwest Washington have fidelity to a region at least as large as Oregon to Southern Vancouver Island. Despite the findings of Calambokidis *et al.* (2010), domestic processes for evaluating the impact of the proposed Makah whale hunt still question what the local area should be for analysis. To evaluate closure discovery curves were constructed both for all whales observed and for whales that were observed to have some fidelity to the area and were observed in more than one year.

Two analyses were used to document seasonal and annual fluctuations of whales in the study area. In the first analysis

Table 1

Years of data used and justification for each analysis.

Analysis	Years of data used	Justification
Depth	1984–2011	All depths recorded were used for the analysis.
Temporal and spatial distribution of sightings	1996–2011	Data prior to 1996 was not used for analysis because effort was opportunistic in nature and could not be quantified to research segment.
Mapping	2004–2011	We used 2004–11 only because during prior years whale locations were not recorded precisely leading to challenges in interpreting maps.
Fidelity to research area	1984–2011	All data was used.
Minimum tenure	1996–2011	Survey effort was standardised for 1996–2011 in all years but 2004 with effort throughout the summer and fall feeding season.
Occurrence of new whales	1996–2011	All years were used in the analysis. Some of the analysis focused on 1996–2011 to ensure that new whales were not whales that commonly use the study area but had not been 'discovered' yet.
Photo analysis of new whales	2004–2011	The analysis was performed at Makah Fisheries and only photographs after 2004 were available for analysis.
Population closure in study area	1984–2011	All data was used.

all sightings were divided into five research segments (Fig. 1). The five research segments were: (1) East Strait (Sekiu Point to Third Beach); (2) Neah Bay Entrance (Third Beach to Waadah Island); (3) West Strait (Waadah Island to Tatoosh Island); (4) North Ocean (Tatoosh Island to Cape Alava); and (5) South Ocean (Cape Alava to Sea Lion Rock). The number of sightings were divided by the number of surveys in the research segment and the length of the research segment in km to standardise the number whales observed per segment for comparison purposes, hereafter this standardised sighting rate will be referred to as 'observation rate'. Observation rates were compared by month and year within each research segment using ANOVA. The second analysis used was mapping and is described in more detail below. The purpose of these analyses was to provide a baseline of habitat use behaviour in the area.

To evaluate gray whale fidelity to the study area, two analyses on different temporal scales were used. Fidelity was evaluated on an annual basis by analysing sighting histories of individual whales to determine the proportion of individuals that were observed in a subsequent year after being first observed. The average percent of years whales were observed in the study area was determined by dividing the number of years each whale was seen in the study area by the number of possible years it could have been observed in the study area. Fidelity was also evaluated within each feeding season by calculating the average 'minimum residency time' for each identified individual by year. For this analysis, minimum residency time was defined as the number of days between the first and last day a whale was seen during the June through November survey time period. The residency time estimate is a minimum because it was possible that a whale was present before the first day (or after the last day) it was sighted during a given year. This estimate may also overestimate residency time because whales could have left the survey area for some unknown length of time between the first and last sighting of the year. Minimum residency time calculations are sensitive to the number of days of survey effort within a year and the temporal distribution of surveys within the survey season. Calambokidis *et al.* (2014) noted that whales observed in the PCFG range during the summer can generally be described as 'transient' whales who are only observed in one year and then not observed in the future and 'PCFG whales' who show some level of fidelity to the IWC defined PCFG range. Fidelity analyses were conducted both for all whales including transients and for whales that have been seen in more than one year. This analysis was conducted to determine a baseline of gray whale fidelity to the area where hunts were planned.

Mapping

To analyse trends in monthly and annual gray whale use of northwest Washington coastal water, the number of photo-identifications made during a whale survey were mapped onto a grid of 1km^2 cells that were aggregated into one of five regions: (1) East Strait; (2) Neah Bay Entrance; (3) West Strait; (4) North Ocean; and (5) South Ocean. Each of these regions extended 2km offshore except the South Ocean which extended 3km, and according to the survey protocol, any survey effort in one of these regions was counted as a full day of effort.

To develop spatial statistics for the survey effort, latitude/longitude coordinates from whale sightings were spatially joined to the 1km^2 grid in ArcGIS 10.1 and exported to MS Excel where total whale counts per 1km^2 grid cell were divided by the survey effort from the same monthly or yearly period to determine sighting density of whales corrected for effort. The sighting densities for each grid cell were re-imported to ArcGIS and plotted as estimates of areal use by gray whales. The grid cells with whale sighting density less than 0.1 were ranked as 'Rare'; cells with sighting density greater than 0.1 but less than 0.3 were ranked as 'Seldom'; cells with sighting densities greater than 0.3 but less than 0.6 were 'Common'; and cells with sighting density greater than 0.6 were ranked as 'Very Common'. This coding was standardised for monthly and annual maps.

The objective of mapping was to document what areas within the larger study area were most important to gray whales and to document how use of those sites changed by month and year.

Calf analysis

During the surveys a whale was recorded as a calf if it was in close association with a much larger individual and appeared to be less than 8m in length. It is possible that calves weaned prior to when they were first observed in the study area as cow-calf pairs in the PCFG have been observed separated as early as the beginning of July (Calambokidis *et al.*, 2012). To make an estimate of what proportion of new whales observed in the study area are calves, photographs were analysed following methods developed by Bradford *et al.* (2011). The analysis was limited to new whales in the study area that were also seen in the PCFG for the first time in that year. Only whales with suitable photo-quality of the

Table 2

Number of gray whale dedicated surveys tallied by year for each segment of research area and total opportunistic surveys by year.

	East Strait	West Strait	North Ocean	South Ocean	Neah Bay entrance	Opportunistic surveys
1984	–	–	–	–	–	3
1986	–	–	–	–	–	10
1989	–	–	–	–	–	2
1992	–	–	–	–	–	2
1993	–	–	–	–	–	5
1994	–	–	–	–	–	7
1995	–	–	–	–	–	5
1996	13	32	23	7	40	5
1997*	22	54	38	14	63	6
1998	28	37	29	13	55	4
1999	14	23	17	15	30	1
2000	13	19	13	8	26	4
2001	12	15	15	10	28	1
2002	10	12	8	6	21	0
2003	15	19	15	8	27	0
2004	4	2	1	1	6	0
2005	11	17	14	6	21	1
2006	15	22	15	9	30	0
2007	13	19	11	8	27	1
2008	25	19	10	5	35	3
2009	23	22	12	7	32	0
2010	18	28	22	14	40	0
2011	11	29	24	18	35	1
Total	**247**	**369**	**267**	**149**	**516**	**81**

*20 surveys were conducted during effort to monitor the Makah setnet fishery. All of these surveys transited the West Strait and into the Northern Ocean research segment.

head and postcranial region were used for the analysis. Whales with evidence of only recently attached barnacles, no old barnacle scars, and white pigmentation mottling the postcranial region were recorded as calves (Bradford *et al.*, 2011). The goal of this analysis was to determine how important northwest Washington was as a site for cow-calf pairs and for recently weaned calves.

RESULTS

Effort to photographically identify gray whales in northwest Washington was conducted between 1984 and 2011. From 1996–2011, surveys were conducted on a more dedicated and rigorous basis resulting in 516 surveys in the research area. Survey effort was greatest from 1996–1998 and 2008–11 (Table 2). By month, effort during dedicated surveys was greatest in the late summer and early autumn (Table 3). The majority of field effort during the autumn was conducted within the Strait of Juan de Fuca due to weather conditions in the Pacific Ocean and the distribution of gray whales. Research effort resulted in the collection of photographs from 225 gray whales that could be identified as unique individuals during the months of June through November from 1984 through 2011.

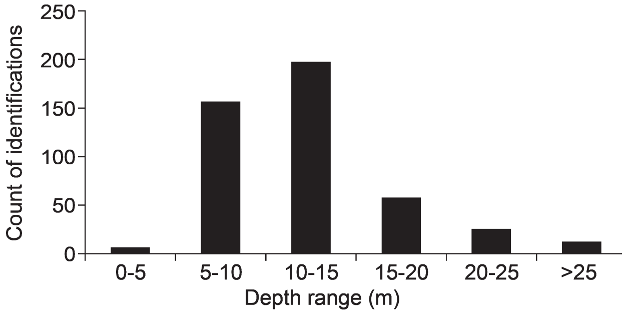

Fig. 2. Histogram of the count of gray whale identifications by depth binned in 5m increments.

Table 3

Number of surveys tallied by month for each segment of research area during gray whale dedicated survey effort from 1996 through 2011.

	East Strait	West Strait	North Ocean	South Ocean	Neah Bay entrance
Jun.	29	50	40	26	64
Jul.	43	78	59	34	99
Aug.	40	98	69	31	120
Sep.	56	79	57	31	114
Oct.	51	41	27	19	78
Nov.	28	23	15	8	41
Total	**247**	**369**	**267**	**149**	**516**

Gray whales were most often observed in water 5–15m deep, often associated with either kelp forests or emergent offshore rocks (Fig. 2). Sightings of gray whales in waters greater than 20m or less than 5m were rare and were not associated with any obvious habitat type (Fig. 2).

Temporal and spatial distributions of sightings

Gray whale distribution in the Strait of Juan de Fuca (hereafter Strait) varied widely by month and year. Gray whale use of feeding sites in the West Strait and East Strait

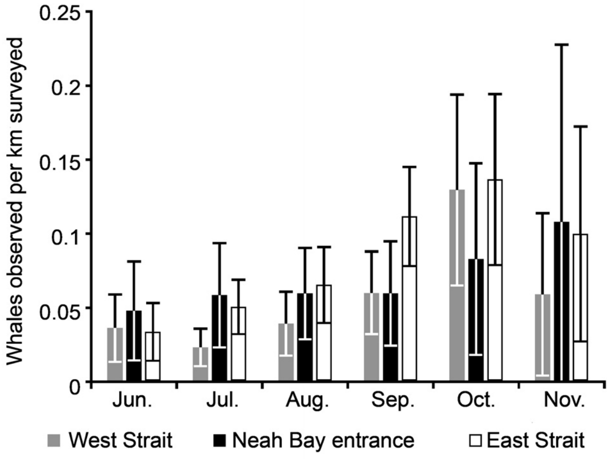

Fig. 3. Average observation rates in the three research segments in the Strait of Juan de Fuca by month for the years 1996 to 2011. Error bars are two times the SE.

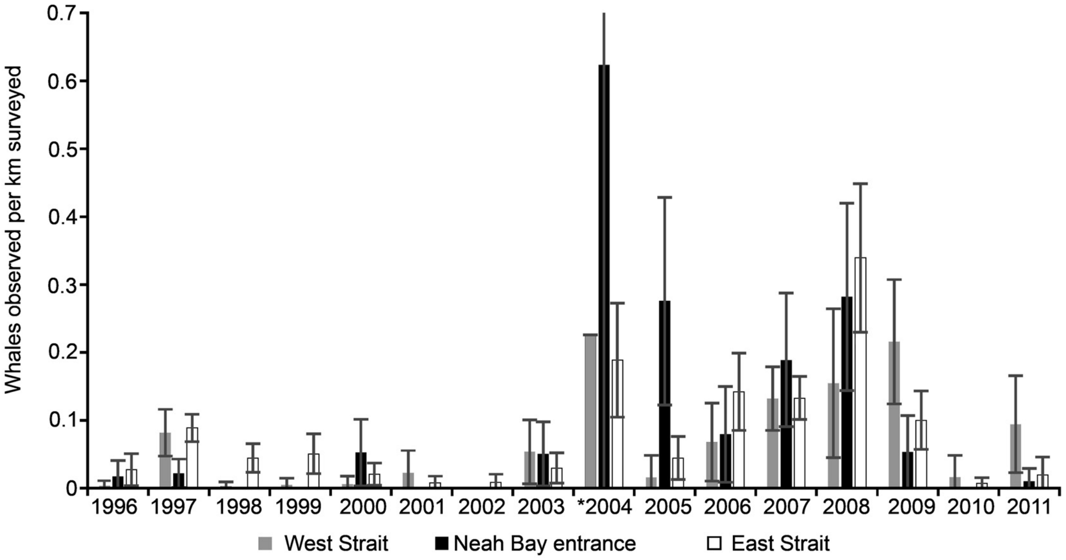

Fig. 4. Average observation rates in the three research segments of the Strait of Juan de Fuca by year with months of the feeding season, June to November, pooled. Error bars are 2 times standard error. * 2004 had much lower effort than other years of the study.

research segments increased through the summer and early autumn until use peaked in October (Fig. 3). The average observation rate varied significantly between months in both the West Strait (ANOVA, df = 368, $p < 0.001$) and the East Strait (ANOVA, df = 246, $p = 0.004$) as the observation rate increased from June to a peak in October. At the entrance to Neah Bay, no significant differences in observation rate by month were detected (ANOVA, df = 515, p = 0.73).

Significant differences in observation rate by year were observed in the Strait of Juan de Fuca in all three research segments (ANOVA: West Strait, df = 325, $p < 0.001$; Neah Bay, df = 514, $p < 0.001$; East Strait, df = 249, $p < 0.001$) (Fig. 4). From 1996 to 2003 (particularly 2000–03) and from 2010 through 2011, there were low observation rates in all three of the research segments (Fig. 4). In contrast, the time period 2004–09 had higher observation rates (Fig. 4).

Gray whale distribution in the Pacific Ocean (hereafter Ocean) also varied by month and year. Within the North Ocean survey area (Cape Flattery to Cape Alava), the observation rate varied significantly by month (ANOVA, df = 266, $p = 0.001$), peaking in August and with lows in June and November (Fig. 5). In the South Ocean research segment (Cape Alava to Sea Lion Rock), there were no significant differences in observation rate by month (ANOVA, df = 148, $p = 0.34$).

Similar to the Strait, significant year to year variability in observation rate was observed in both ocean survey segments (ANOVA: North Ocean, df = 266, $p < 0.001$; South Ocean, df = 148, $p < 0.001$) (Fig. 6). Years of high and low observation rates were not the same years as observed for the Strait (Fig. 4, Fig. 6). Like the Strait survey areas, the Ocean research segments had low observation rates during the early years of the time series from 1996 to 2001. Opposite the Strait, the observation rate increased in 2001 through 2003 and was also high in 2010 and 2011. The years with greatest observation rates were 2005–11. The South Ocean showed more year to year variability than the North Ocean.

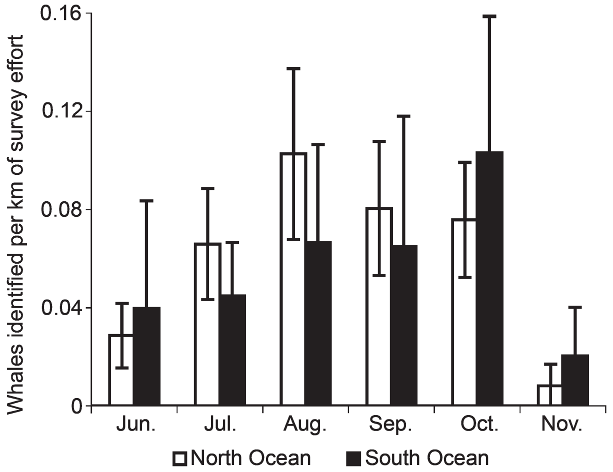

Fig. 5. Average observation rates in the two research segments of the Pacific Ocean by month for the years 1996 to 2011, error bars are two times standard error.

Maps were made using the average number of whales identified per km^2 of research area to examine finer scale trends in gray whale distributions in northwest Washington by month and year. Trends observed in whale densities by month reaffirm our findings that the number of gray whales identified per survey increased to greatest densities and greatest spatial coverage in September and October in the Strait and in August and September in the North Ocean (Fig. 7). Some sites were consistently used both in the Strait and in the Ocean each month; whale densities at these sites increased through the summer and into autumn in the Strait

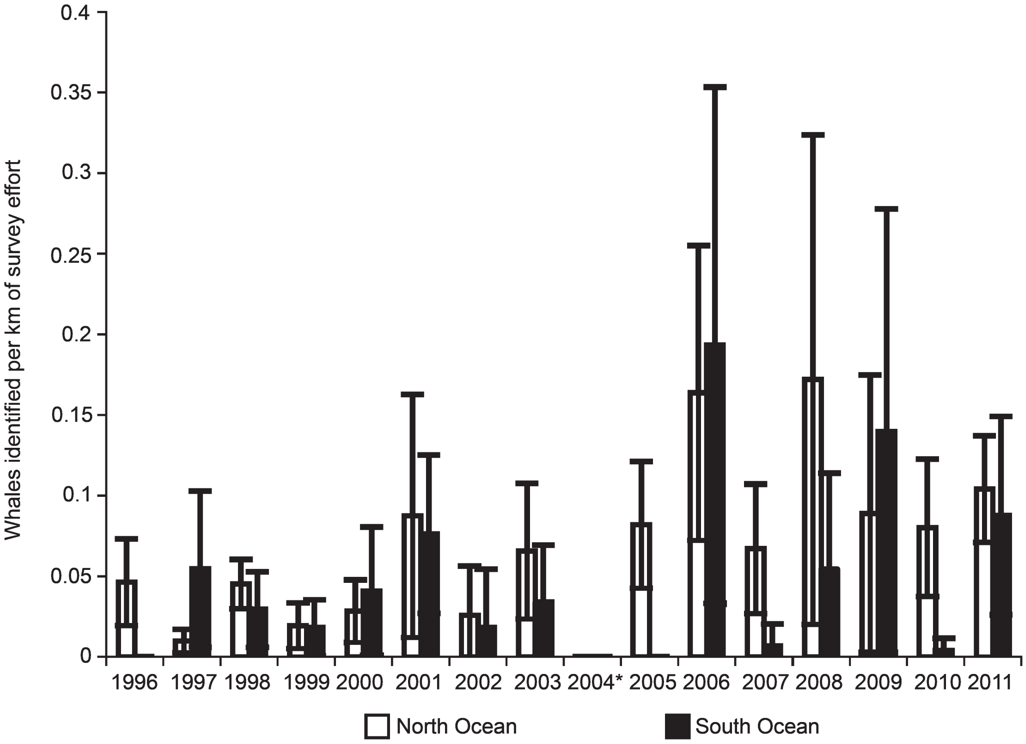

Fig. 6. Average observation rates in the two research segments of the Pacific Ocean by year for the months of the feeding season, June to November. Error bars are two times standard error. *No surveys were conducted in the ocean in 2004.

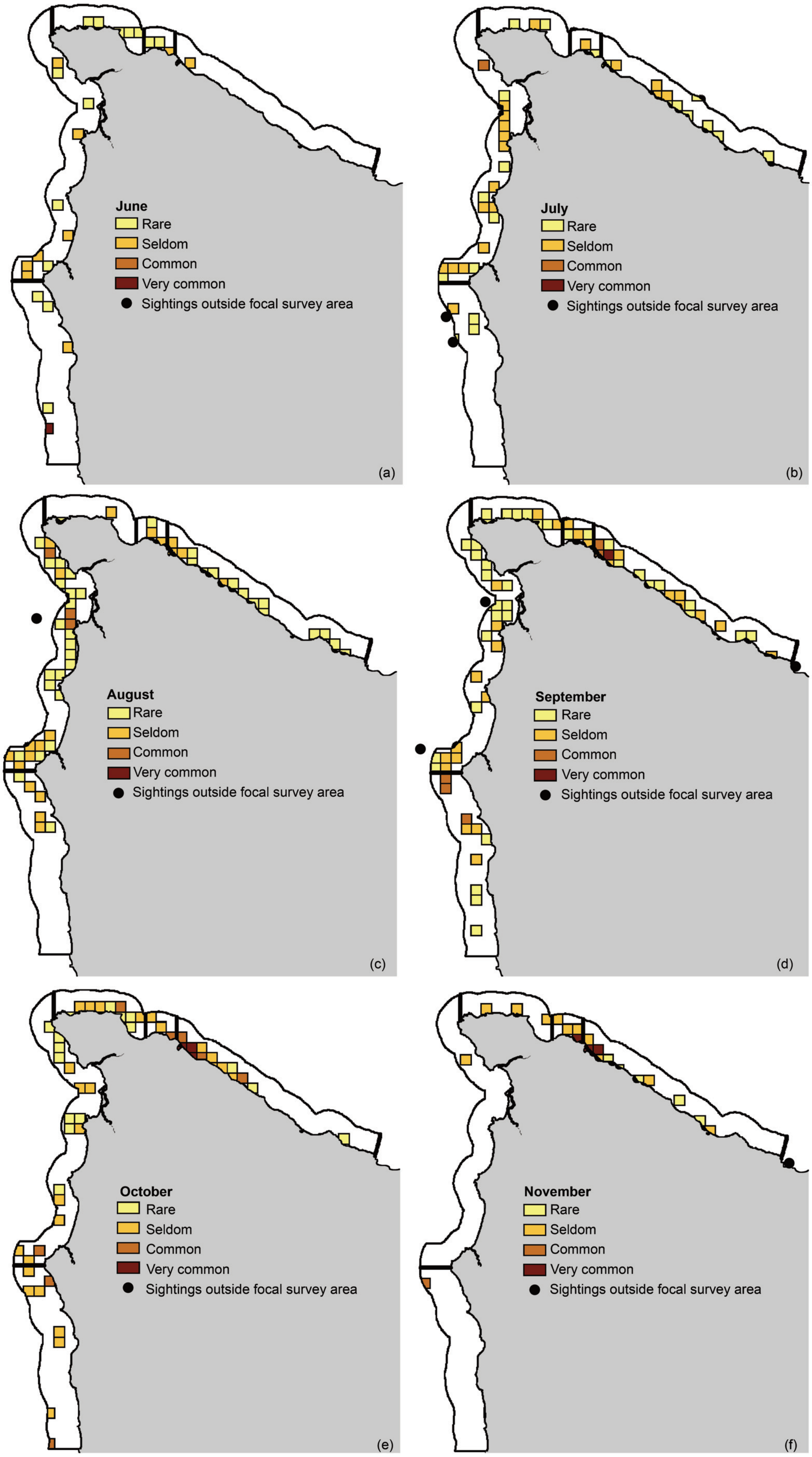

Fig. 7. Sighting density of gray whales identified per km^2 in northwest Washington per day of research effort in 2004 to 2011 by month: (a) June; (b) July; (c) August; (d) September; (e) October; and (f) November. Grid cells with sighting densities of less than 0.1 whales were ranked as 'Rare', cells with sighting density greater than 0.1 and less than 0.3 whales were ranked as 'Seldom', cells with sighting densities greater than 0.3 and less than 0.6 whales were ranked as "Common" and cells with sighting densities greater than 0.6 whales were ranked as 'Very Common'.

and increased until late summer/early autumn in the Ocean (Fig. 7). A review of nautical charts and knowledge of the area show that sites with high use were generally characterised by rocky bottoms and large kelp forests, whereas sites with low use were characterised by sandy bottoms. The maps do show sightings of whales in areas of sandy bottoms, however these sightings were primarily of whales that were presumed to be travelling or resting. The greater distance from shore of gray whale distributions in the ocean as compared to the Strait was likely due to the gradual slope of the bottom in the ocean as compared to the steep drop off in the Strait.

Maps of the yearly distribution of whales display greater variability in gray whale site use, where whales appeared to use some areas frequently for a number of years and then subsequently either abandon those areas or use them intermittently (Fig. 8). This phenomenon can be observed by examining the area just east of the Neah Bay research segment. From 2006 to 2009, high densities of whales were observed in this area and then were not observed using the site at all in 2010 and only rarely in 2011. Other areas appeared to be used intensively for one year and then not used again. This can be seen most easily by looking at the southern border of the South Ocean research segment and noting the changes in gray whale sighting density through the years.

Fidelity to the research area

Fidelity to the research area was examined by comparing the number of individual whales that returned to the northwest Washington research area after the first year observed and estimating how long individual whales used the research area within a given year. Some gray whales were observed to use the waters of northwest Washington consistently after they were first observed. Sixteen percent of whales were observed in six or more years in the study area, although not necessarily in consecutive years. Roughly half (51%) of the whales identified in this study were only observed in the area during one year (Fig. 9). The average whale was observed in 2.48 years (SE = 0.14). Removing the individuals that were only observed in one year, the average whale was seen in 4.01 years (SE = 0.20). Whales first observed in 2010 or earlier were observed in an average of 31.6% (SE = 1.6%) of possible years after they were first observed (number of years observed divided by total number of possible years to be observed for each whale); removing whales only seen in one year increased the average percentage to 38.7% (SE = 1.9%) of possible years. Among the whales that were first identified prior to 2010 and therefore have more than one year in which they could have been resighted, only two whales were seen in all possible years after the first observation; these whales were seen in every year after being first observed in 2004 and 2006, respectively.

The length of time a whale used the study area during the feeding season was estimated by calculating minimum tenure, in this case the minimum number of days an individual whale resided in the research area assumed to be equal to the difference in time between the date of first and last observation. The average minimum tenure calculated for whales observed in the northwest Washington research area

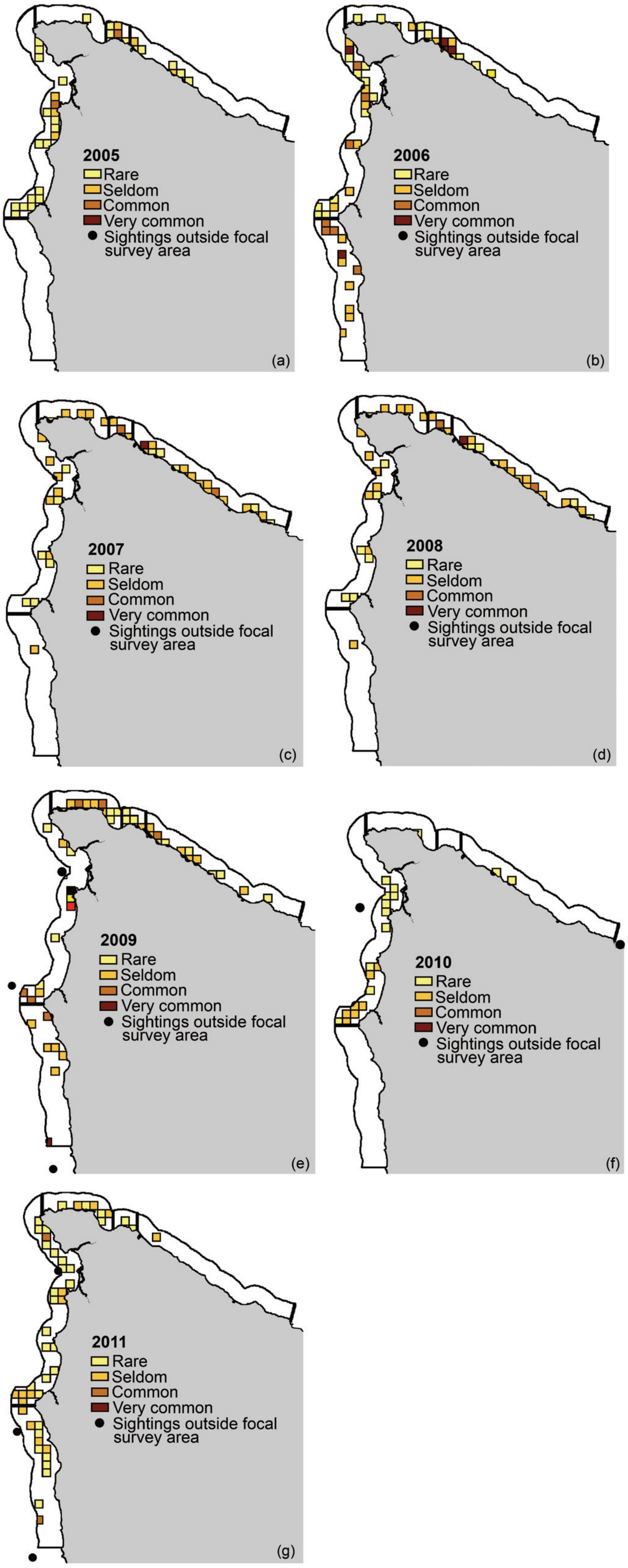

Fig. 8. Sighting density of gray whales identified per km^2 in northwest Washington per day of research effort in the feeding season, June through November by year: (a) 2005; (b) 2006; (c) 2007; (d) 2008; (e) 2009; (f) 2010; and (g) 2011. Grid cells with densities of less than 0.1 whales were ranked as 'Rare', cells with sighting density greater than 0.1 and less than 0.3 whales were ranked as 'Seldom', cells with sighting densities greater than 0.3 and less than 0.6 whales were ranked as 'Common' and cells with sighting densities greater than 0.6 whales were ranked as 'Very Common'. No map was provided for 2004 because data collection lacked spatial and temporal resolution.

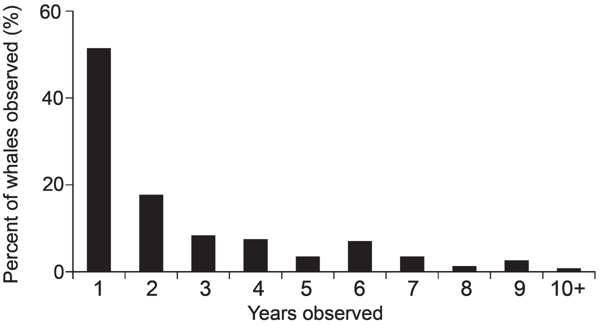

Fig. 9. Count of unique whales observed by the number of years a whale was observed.

was 24.8 days (range 1 to 151 days) out of a possible 183 days in the June to November feeding season. A large degree of variability in minimum tenure by year was observed in the research area (ANOVA, df = 493, $p < 0.01$) (Fig. 10).

No evidence was found that the number of years a whale has been observed in northwest Washington affected average minimum tenure during the study (ANOVA, df = 202, $p = 0.62$) (Fig. 11). However, it was found that average minimum tenure was a good predictor of whether a whale would be seen in the following year. Whales seen in year *Y* and in the following year (*Y*+1) had an average minimum tenure of 28.3 days, which was significantly greater than whales seen in year *Y* but not year *Y*+1 (19 days; Two-sample *t*-test, df = 506, $p = 0.002$).

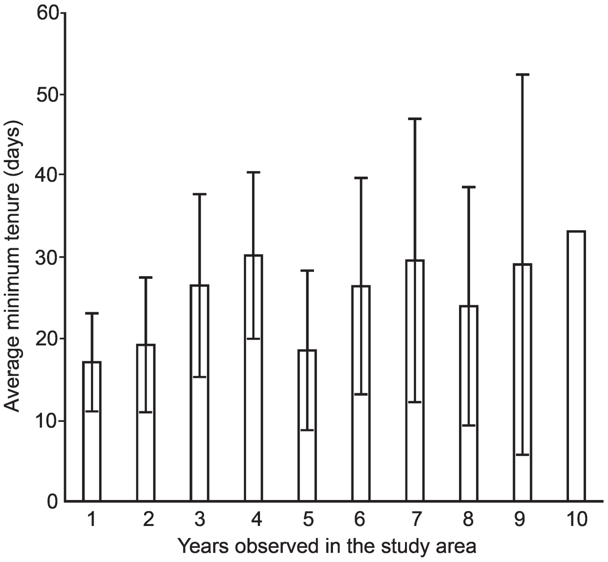

Fig. 11. Average minimum tenure of whales in days compared to the number of years they have been observed in northwest Washington.

Occurrence of new whales

From 1996 through to 2011, an average of 10.8 new whales were observed per year (SE = 1.8) in the northwest Washington study area. From 1996 through 2010 (excluding 2011 to allow a year for recruitment), an average of 5.6 new whales per year (SE = 1.1) were observed again in a future year. The number of new whales observed was not consistent between years. High numbers of new whales (> 15) were observed in 1993, 1995, 1998, 2001, 2006 and 2008 (Table 4). It is possible that the high numbers of new whales observed in 1993 and 1995 were not actually new whales to the research area; rather it is likely that some of these whales regularly used the area but had not been seen previously due to low research effort in the early years of the study. In a time series of population estimates, Calambokidis *et al.* (2014) found a large increase in PCFG gray whale abundance in the late 1990s and early 2000s that they postulated was caused, at least in part, by immigration from

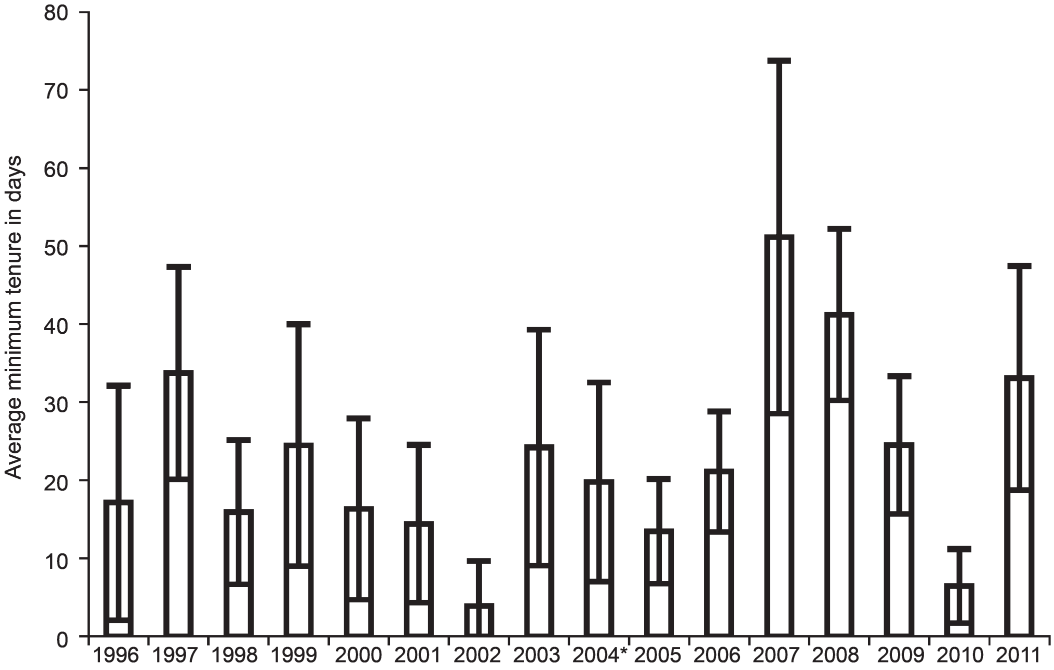

Fig. 10. Average minimum tenure (residency time) computed as the number of days between the first and last sighting of an individual in a given year. *2004 had lower total survey effort and lower temporal coverage of survey effort than other years, and the estimate of minimum tenure is likely underestimated.

Table 4

This table shows the sighting history of whales by the first year they were observed (row). Column totals report the number of uniquely identified whales from each cohort in each feeding season. The first value in each row is the number of new whales observed for that year.

Year	1984	1986	1989	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
1984	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1986		4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
1989			4	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0
1992				2	0	1	1	2	2	0	1	0	0	0	0	1	1	1	0	0	0	0	1
1993					21	4	4	4	4	10	4	1	3	1	3	1	4	4	2	6	5	1	2
1994						5	2	0	1	1	0	0	1	1	1	0	0	1	1	1	0	1	0
1995							15	5	7	2	1	0	2	0	0	0	3	2	2	3	1	0	0
1996								8	4	3	2	1	1	0	1	1	1	4	0	3	4	1	2
1997									8	1	1	0	1	0	1	1	2	1	1	1	1	0	0
1998										17	1	1	1	0	0	0	0	2	1	2	0	1	0
1999											1	0	0	0	0	0	0	0	0	0	0	0	0
2000												11	6	3	2	0	2	5	1	5	3	4	3
2001													16	2	2	1	0	1	0	1	1	0	0
2002														1	1	1	1	1	0	1	1	1	1
2003															11	3	2	3	0	1	1	2	1
2004																12	7	7	3	7	5	3	5
2005																	10	4	2	3	2	1	1
2006																		20	5	10	7	4	6
2007																			2	1	2	0	1
2008																				29	11	3	3
2009																					11	1	1
2010																						4	1
2011																							11
Total	**2**	**4**	**4**	**2**	**21**	**10**	**22**	**19**	**27**	**35**	**11**	**14**	**32**	**8**	**22**	**21**	**33**	**56**	**20**	**74**	**56**	**27**	**39**

northern feeding grounds during the 1999/2000 mortality event (Gulland *et al.*, 2005). Based on the findings of Calambokidis *et al.* (2014) a large increase in the number of new whales observed and of new whales observed in a future year during the time period of 1998–2002 was to be expected. Instead, the average number of new whales observed from 1998–2002 was lower than the 1996–2010 average, with 9.2 new whales (SE = 4.3) of which 4.3 whales (SE = 1.5) were seen in a future year. The percentage on average of new whales observed from 1998–2002 that were seen in a future year (44.3%, SE = 18.4%) was also lower than the 1996–2010 average.

Calf analysis

There were seven mother-calf pairs observed during surveys (Table 5), showing that some of the new whales observed in this study were internally recruited. One mother, CRC 67, was observed with three calves: a suspected calf (CRC 169) in 1995 and a confirmed calf in both 2004 (CRC 819) and 2011 (CRC 1350). Four other females were each observed with one calf (Table 5).

Some new whales were first observed later in the year (i.e. autumn) than when calves become independent of their mothers (Bradford *et al.*, 2011; Calambokidis *et al.*, 2012). To determine the proportion of new whales which are actually calves digital photographs taken between 2004 and 2011 were analysed. Only new whales for which photographs had already been obtained from the first year they were seen in the entire PCFG (i.e. not just the first year seen in northwest Washington) were analysed. Twenty one photographs of new whales for which the first year they were sighted in northwest Washington was also the first year they were sighted in the PCFG were available. Of those, 18 photographs showed the head and post-cranial region clearly in order to be able assess if they were calves. Of the 18 whales evaluated, 4 (22%) were either confirmed calves (CRC 819 and CRC 1350) or were most likely calves (CRC 1047 and CRC 1054) and the other 14, based primarily on observation of old barnacle scars, were not calves of that year. CRC 1047 and CRC 1054 were both first observed in 2008.

Table 5

All known mother-calf pairs observed in northwest Washington from 1984–2011 with whales only suspected to be calves noted with an asterisk.

Mother	Calf	Dates observed together
105	104	09/07/94
43	107	09/07/94 to 04/08/94
67	169*	19/07/95 to 23/07/95
596	595	26/06/01
216	860*	26/07/03 to 28/07/03
67	819	27/08/04
67	1350	23/06/11 to 01/09/11

The occurrence of calves in northwest Washington shows that the site is used by cow-calf pairs and recently weaned calves. The number of calves observed during the study were low suggesting that the site is not a very important for cow-calf pairs for the PCFG as a whole although it does appear important for CRC 67.

Population closure in the study area

If population closure exists within the study area (no immigration or emigration), one would expect that over the 17 years of research effort that all of the whales in the 'population' would have been photographed and identified and the best fit line would approach a horizontal asymptote. To test if there is closure a discovery curve was plotted with the number of new whales observed for 1984 through 2011 and the number of whales observed in more than one year for 1984 through 2010 (Fig. 12). The function best fitting the discovery curve was linear for all new whales ($y = 9.15x–$

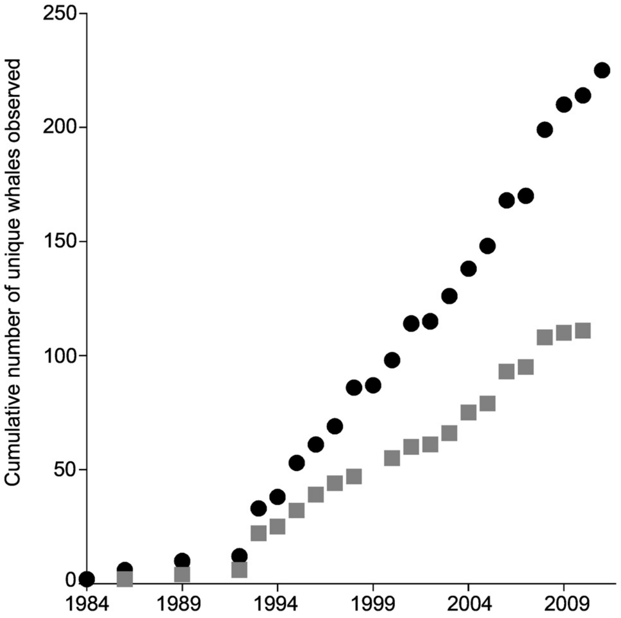

Fig. 12. Plot of the cumulative number of whales observed during the duration of this project for all whales (black dots) and whales observed in greater than one year (grey squares).

18,193, $r^2 = 0.95$) and whales observed in more than one year ($y = 5.07x–10,076$, $r^2 = 0.97$), suggesting that closure is not occurring for the northwest Washington survey area.

DISCUSSION

Temporal and spatial distribution of whales

There was large annual variability in the numbers of whales identified per survey in all research segments and large amounts of inter-year and intra-year variability in where whales were observed. Observation of variability is similar to Darling *et al.* (1998) who concluded that year-to-year variability in timing, prey type and feeding location is the key feature of gray whale observations from the central coast of Vancouver Island. Gray whale researchers of the PCFG have noted that the whales are commonly observed to exhibit benthic feeding behaviours (Avery and Hawkinson, 1992; Darling *et al.*, 1998; Dunham and Duffas, 2001; Kvitek and Oliver, 1986; Oliver *et al.*, 1984). However, in the present study mud plumes were rarely observed, suggesting that benthic feeding is uncommon in the northwest Washington area. Within the dynamic nature of site use it was found that more whales were observed per day of survey effort in the autumn in both the Strait of Juan de Fuca and the South Ocean research segment, whereas in the North Ocean research segment peak use was late summer. Also, the vast majority of gray whales were observed in waters between 5 and 15m of depth. This depth range coincides with the primary depth range of the mysid shrimp (small epibenthic and planktonic crustaceans of the family *mysidae*, suborder *pericarida*) (Nelson *et al.*, 2009). The primary mysid species consumed by gray whales off Vancouver Island were *Holmesimysis sculpta, Acanthomysis pseudomaropsis* and *A. anassa californiensis* (Murrison *et al.*, 1984; Darling *et al.*, 1998; Dunham and Duffus, 2002; Feyrer and Duffas, 2011) and they are also likely to be the primary prey species in northwest Washington. Feyrer and Duffus (2011) found that average mysid density was significantly correlated with the average number of whales in the survey area near Vancouver Island. We hypothesise that shifting mysid density and fluctuations in abundance caused the observed variability in gray whale counts in northwest Washington since most of the gray whale sightings occurred in optimal mysid habitat. Systematically monitoring prey at sites commonly used in northwest Washington would allow testing of this hypotheses on prey preference and specifically the influence of mysid abundance on whale distributions.

A consistent pattern observed through the years was lower observation rates in June compared to later in summer and autumn. This fits with the movements of migrating gray whales which generally reach Arctic feeding grounds from May to June (Swartz *et al.*, 2006). To date, there have been three publications on the movements of six satellite tagged PCFG whales, each of which had active tags between April and June; of these six whales, four were observed to migrate steadily north into southeast Alaska before their transmitters stopped transmitting (Calambokidis *et al.*, 2014; Ford *et al.*, 2013; Mate *et al.*, 2010). Given that 66% (4 out of 6) of the PCFG whales with documented spring movement patterns travelled north of the PCFG area, it is quite possible that other whales that feed in the PCFG also feed further north in the spring and early summer before returning south to the PCFG area later in the summer and autumn. It should be noted that the migratory behaviour of four of the six individuals may not be representative of all PCFG whales, as the three tags applied by Ford *et al.* (2013) targeted whales presumed to be migrating past Vancouver Island and one tag applied to a PCFG whale by Calambokidis *et al.* (2014) targeted a feeding whale.

Occurrence of new whales in northwest Washington

From 1996 to 2011, an average of 10.8 new whales were observed each year, of which 5.6 were observed in a future year. Many of the whales that were new to the northwest Washington study area had been seen previously in another research area of the PCFG. For whales that were photographed in northwest Washington during the first year they were seen in the PCFG, analysis of photographs using techniques described by Bradford *et al.* (2011) found that 22% of the whales were calves. Thus 78% of the new whales observed in our research area and to the PCFG were either born in a previous year in the PCFG and were not observed, or were non-calves who emigrated from another feeding area into the PCFG.

An analysis of the time series of population estimates of PCFG whales shows a large increase in the number of whales in the PCFG from 1998 through 2002 concurrent with the timing of the 1999 gray whale mortality event (Calambokidis *et al.*, 2014). Somewhat surprisingly, a smaller average of new whales (9.2) was observed from 1998 to 2002. The lower number of new whales observed in that time period could have been a result of poorer feeding conditions in Washington compared to later years in the data series. Of the new whales observed during those five years, a smaller portion was observed again in a subsequent year (44.3%) than the average for the whole data series. Based on the calculated population increase of the overall PCFG, we would have expected the average proportion of new whales and new whales seen in more than one year to be much greater from 1998 to 2002 than was observed in this study.

CONCLUSION

Northwest Washington is a small but important region within the summer and autumn feeding range of PCFG gray whales. Individual gray whale use of this region is variable, with some individuals observed regularly whereas most do not show strong site fidelity to this region. This study allowed examination of trends in site use over multiple decades within northwest Washington and it was found that rocky habitat in the 5–15m depth range is very important to gray whales and that gray whale use of these habitats is dynamic by year. The impacts of the Makah gray whale hunt are a debated issue, thus it is hoped that the baseline of gray whale behaviour provided here can be used to help evaluate if there are discernible effects on PCFG whale behaviour in the proposed hunt area when hunting resumes.

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The effects of seismic operations in UK waters: analysis of Marine Mammal Observer data

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ABSTRACT

Sixteen years of information from observers on board seismic survey vessels in UK and adjacent waters have demonstrated the varied responses of marine mammals to seismic surveys. Most species showed some response to firing with 'large arrays' (airgun volume 500 cubic inches or more), but responses were less evident when 'small arrays' (less than 500 cubic inches) were active. Several species/species groups responded proactively to the soft start procedure (where the level of firing is increased gradually) indicating that this can be an effective mitigation measure in reducing the risk of physiological damage. Despite the challenge in assigning ecological significance to the varied observed effects, the analyses in this study confirm that marine mammals are sensitive to noise from seismic surveys and therefore mitigation measures should continue to be applied to all seismic operations and such measures should cover all species.

KEYWORDS: SHORT-TERM CHANGE; BEHAVIOUR; NOISE; MONITORING; CONSERVATION; EUROPE; SURVEY – VESSEL

INTRODUCTION

Over the past few decades, concern has developed over the potentially negative impact of anthropogenic noise on marine mammals, including that from marine seismic surveys used to explore oil and gas reserves. Sound produced by airguns is directed at the seabed with the resultant reflections being analysed to map the geological structures below the sea floor. The airguns produce high levels of impulsive low frequency sound with an inherent risk of disturbance and possibly acoustic trauma (e.g. auditory injury) to marine mammals and other marine organisms. Although no direct evidence exists for a causal link between airgun sound and injury to marine mammals, data on auditory sensitivities and comparisons with human and other terrestrial mammal data (Southall *et al.,* 2007), together with propagation modelling, suggest that hearing could be damaged by the sound levels emitted by airguns if the animals are very close to the guns. There is also evidence for short-term behavioural responses of marine mammals to seismic surveys such as avoidance of the area shown by some species of mysticete (e.g. McCauley *et al.,* 1998; 2000; Richardson *et al.,* 1986; 1999) and some small odontocetes (Barkaszi *et al.,* 2012; Stone and Tasker 2006; Thompson *et al.,* 2013; Weir, 2008a). However, investigating biologically significant effects at the population level has proven very challenging (e.g. Gordon *et al.,* 2004; NRC, 2005; Thomsen *et al.*, 2011).

To address conservation concerns in relation to seismic surveys, in 1995 the UK government and the Joint Nature Conservation Committee (JNCC) issued guidelines for seismic operations taking place on the UK continental shelf (latest version: JNCC, 2017). The guidelines aim to reduce the risk of causing injury, and may assist with reducing potential disturbance to marine European Protected Species as part of measures related to Article 12 of the EC Habitats Directive (92/43/EEC) and the Directive's transposition into UK legislation. All applications to conduct seismic surveys for oil and gas exploration within the UK continental shelf require consent from the Department for Business, Energy and Industrial Strategy (BEIS, formerly known as the Department of Energy and Climate Change), with the JNCC guidelines informing the consent conditions for such surveys. Amongst the provisions of the guidelines is a requirement for having marine mammal observers (MMOs) on board to monitor for the presence of marine mammals prior to commencing firing the airguns, with the commencement of firing being delayed if marine mammals are detected within a defined mitigation zone. For some surveys, there are also provisions for passive acoustic monitoring (PAM) to be employed during conditions that are not conducive to visual observations (e.g. darkness). The primary role of the MMO or PAM operator, therefore, is to provide advice to enable the crew to comply with the guidelines and hence mitigate potential negative impacts of seismic operations on marine mammals. In the course of this work, MMOs and PAM operators collect data on the seismic operations, the watches and any marine mammals detected. In addition to monitoring for the presence of marine mammals and delaying the commencement of airgun firing if marine mammals are detected within the defined mitigation zone, the guidelines also require that when airguns do commence firing, the level of firing must increase gradually by using a soft start/ramp up procedure. The assumption is that animals will show an avoidance response to lower levels of sound enabling them to leave the area where they could potentially be injured before sound levels reach certain thresholds. However, there is a need for evidence on the effectiveness of this method (Barlow and Gisiner, 2006).

All data from seismic surveys in UK waters are returned to JNCC where, after quality checks, they are included in a database; over the years a large amount of data has accrued. This paper presents the results of analyses of MMO data from UK and adjacent waters, including all data from 1994, just prior to the introduction of the JNCC guidelines, until the end of 2010. Previous analyses have used subsets (up to four years) of these data (e.g. Stone and Tasker, 2006). Analysis of this longer dataset with increased sample sizes has permitted further statistical testing of a larger number of species. The aim of the analyses was to examine whether there were any detectable effects of seismic operations on marine mammals, with a focus on the responses of marine mammals to the soft start procedure to provide some insight as to its effectiveness.

METHODS

Data collection and quality control

MMOs working on seismic surveys operating in UK and sometimes adjacent waters (Norway, Ireland, Faroes, the Netherlands, Denmark, Germany and France) between 1994 and 2010 submitted records of their monitoring effort and the marine mammals observed during daylight hours using standardised recording forms. Observers ranged from biologists experienced in marine mammal surveys to non-scientific personnel, most of whom had undergone basic JNCC-recognised MMO training. In addition, PAM was utilised on some surveys during night-time operations and sometimes also during the day. Information on the watch/acoustic monitoring period included the time, location, source activity and weather conditions. Sea state was categorised as 'glassy' (equivalent to Beaufort sea states of 0–1), 'slight' (Beaufort sea states 2–3), 'choppy' (Beaufort sea states 4–5) and 'rough' (Beaufort sea states > = 6); swell was categorised as 0–2m, 2–4m or > 4m; visibility was categorised as < 1km, 1–5km or > 5km; sun glare was categorised as 'none', 'weak', 'strong' or 'variable'. Information on marine mammal sightings/acoustic detections included species, number of animals, behaviour, closest surface distance of approach to the airguns and the airgun activity at the time of the encounter. Observers most commonly used a rangefinder stick (Heinemann, 1981) to estimate the range to animals but other methods were also used (e.g. reticle binoculars or by relating to an object at a known distance).

All data extracted from MMO reports were subjected to rigorous quality checks (including checks on species identification, source activity corresponding between the different recording forms and consecutive positions being credible given the time interval and speed of the vessel) and only those considered to be of acceptable quality were used in the analysis. Where species descriptions were missing or inadequate or did not correspond with the identification given, identifications were usually down-graded from a single species to a group of similar-looking species, based on the description given. Photographs, where available, were used to confirm identification. Some sighting records did not have associated effort data; approximately 15% of surveys had effort or operational data (the latter were not required for this analysis) that were either missing or discarded due to errors. Sightings without associated effort records were not used when calculating detection rates but were used for other aspects of the analysis. Data of acceptable quality that were used in the analysis corresponded to 1,196 seismic surveys, 91% of which were entirely in UK waters.

Size of airgun arrays

Airgun array volume ranged from 6 cubic inches (in^3) to 10,170 in^3 (only nine surveys used volumes exceeding 5,500 in^3), although precise airgun volumes were not always recorded. Where airgun volume was known, 'small arrays' (total airgun volume less than 500 in^3, e.g. site surveys) were used on 678 surveys (15.9% of monitoring effort) and 'large arrays' (total volume 500 in^3 or more) were used on 500 surveys (84.1% of monitoring effort). Reports from 18 surveys did not provide sufficient information to assign them to either category.

Data analysis

It was considered that if the operation of airguns during seismic surveys had no effect on marine mammals (the null hypothesis) then there would be no difference in the occurrence or behaviour of animals regardless of source activity. Occurrence might be reflected by detection rates per hour (although detection rates may also be influenced by behaviour, e.g. dive duration). Behavioural response was examined by considering clearly defined behaviours and analysing the closest distance of surface approach to the source. The null hypothesis (i.e. airgun operation had no effect) would be rejected if a statistically significant difference in response was found.

As the characteristics of airgun arrays may influence the degree of any response of marine mammals to the sound produced, surveys with 'small arrays' were analysed separately from those with 'large arrays' where possible. Results are presented for individual species where sample size permitted. When this was not possible, groups of combined species were used (e.g. all mysticetes) comprising all identified and unidentified animals within that taxonomic grouping. Combined species groups were more often used for surveys with 'small arrays' than those with 'large arrays', as surveys with 'small arrays' tended to be of short duration so sample sizes were lower. Non-parametric statistical tests were used throughout. In some analyses only small sample sizes were available but, despite this, significant results were still able to be detected with the non-parametric analysis techniques used (Siegel and Castellan, 1988).

Marine mammal responses to the soft start

As soft starts are of relatively short duration (recommended minimum 20 minutes), only a minority (2.6%) of encounters occurred during the soft start, therefore the response to the soft start could only be examined for a few species or species groups. Detection of marine mammals that are present may be influenced by weather (e.g. Hammond *et al.,* 2013), location, season, observer ability and monitoring method (visual or acoustic). Matched samples were used to compare detection rates (number of encounters per hour, an encounter being one or more animals occurring together) at three different source activities (not firing, full power or soft start). Each matched sample comprised three detection rates (one for each of the three source activities) where date, survey, monitoring method, sea state, swell, visibility and sun glare were the same, thus controlling to the extent possible for these variables. Comparing detection rates on the same day of the same survey for each matched sample also controlled for location. PAM data were included but each matched sample contained either only PAM data or only visual data and not both (i.e. a matched sample compared either three acoustic detection rates or three sighting rates). Only survey days when there was effort at all three source activities and where the other variables matched were used; effort during the soft start was only recorded as distinct from effort at full power from July 2009 onwards, therefore only surveys between July 2009 and December 2010 were used to calculate detection rates per hour. As this was a relatively short time period, sample sizes during the soft start were too small to analyse either 'large arrays' or 'small arrays' separately, therefore arrays of both sizes were analysed together. Small sample sizes also precluded any control for inter-observer variation in ability to detect, although comparing within the same day of the same survey for each matched sample limited the influence of inter-observer variation as on 67% of survey days only one observer was used.

The closest surface distance that marine mammals approached the airguns during the soft start was compared to that at other times. Data from all years (1994–2010) were used as all sightings records distinguished between firing at full power and firing during the soft start. 'Large arrays' were analysed separately from 'small arrays'. Range estimation with PAM can be subject to errors due to factors such as the position of the hydrophone array and the angle of the animal with respect to the array (Von Benda-Beckmann *et al.,* 2013); therefore, only sightings were used (although of course it is recognised that there are also errors in visual range detection). As noted above, weather conditions affect the ability of animals to be detected at distance so only sightings during 'good' weather conditions (defined as 'glassy' or 'slight' sea states, equivalent to Beaufort sea state three or less, swell <2m and visibility >5km) were used. Small sample sizes during the soft start meant that there was no allowance for inter-observer variation in ability to detect animals at distance.

Behaviour was compared using sightings (from all years) where source activity did not change during the course of the encounter (i.e. not firing, full power or soft start). PAM data were not used as behaviour cannot be determined from acoustic detections. 'Large arrays' were analysed separately from 'small arrays'. Only clearly defined behaviours were considered; behaviours more prone to individual interpretation by observers (e.g. fast/slow swimming, frequent/infrequent surfacing, etc.) were not included. Similar behaviours (e.g. avoiding the ship or swimming quickly away) were combined to allow for inter-observer variation in terminology. Confirmation of feeding is difficult but during training, MMOs are taught that behaviours indicative of feeding might include cetaceans being observed with a fish, lunge-feeding in rorqual whales and erratic, fast swimming in dolphins with frequent changes of course and birds diving alongside, etc. Behaviour was also compared between encounters where the soft start commenced while the animals were still visible and those where the airguns were not firing throughout or were performing a soft start throughout; the sample size of encounters where the soft start commenced during the course of the encounter was insufficient to analyse 'large arrays' and 'small arrays' separately so arrays of both sizes were analysed together.

Marine mammal responses to airguns in general

When no distinction was made between firing during the soft start and firing at full power, larger sample sizes permitted examination of a wider range of species and for 'large arrays' and 'small arrays' always to be analysed separately. Larger sample sizes also allowed for control of the influence of inter-observer variation in ability to detect. Matched pairs were used throughout the whole dataset (1994–2010) to compare detection rates per hour at different source activities (airguns firing versus not firing). For each matched pair (detection rate when firing and detection rate when not firing) the date, survey, monitoring method, observer, sea state, swell and visibility were the same, thereby controlling for these variables. PAM data were included but each matched pair contained either only PAM data or only visual data and not both (i.e. a matched pair compared either two acoustic detection rates or two sighting rates).

Changes in detection rates over time were examined using surveys with 'small arrays', as these corresponded mostly to site surveys where firing occurred within a small area (surveys with 'large arrays' often covered a wide area with temporal variation in the precise location of firing throughout the survey). Only sightings were used as PAM was employed less often on surveys with 'small arrays'. Matched pairs were used to compare detection rates at different stages during each survey; a matched pair comprised a detection rate during the first week of a survey and a detection rate throughout later weeks of the same survey. Only surveys lasting three weeks or longer where firing commenced during the first week were used. The influence of weather was controlled by using only periods of good weather conditions (as defined above).

The closest surface distance that marine mammals approached the airguns was compared between periods when the airguns were firing (at any level) and when they were not firing. Only sightings during good weather conditions (as defined above) were used; acoustic detections were not used due to difficulty in range estimation using PAM. Potential inter-observer variation was controlled for by using sightings by observers with a demonstrated ability to detect marine mammals at distance. An initial examination of data from a small subset of known experienced observers found that a minimum of 20% of detections were more than 1km away. This was applied as a criterion for selecting observers with good detection skills throughout the database, selecting from only those observers who had at least 20 sightings.

Recorded behaviours were compared between periods of firing (at any level) and not firing. Only sightings were used and similar behaviours (e.g. logging or resting at the surface) were combined to allow for inter-observer variation in terminology. PAM data were not used as behaviour was not apparent from acoustic detections.

RESULTS

Survey effort

A total of 190,728 hours were recorded as monitoring for marine mammals (95% visual monitoring and 5% acoustic monitoring), with the airguns firing for 38.8% of the total time spent monitoring. Observations covered 199 quadrants (1° rectangles), with survey effort not evenly distributed either spatially or temporally (Fig. 1). Most effort was in the central and northern North Sea, reflecting the location of geology of interest to the oil and gas industry, and between April and September. There were 9,073 sightings or acoustic detections of marine mammals (Table 1).

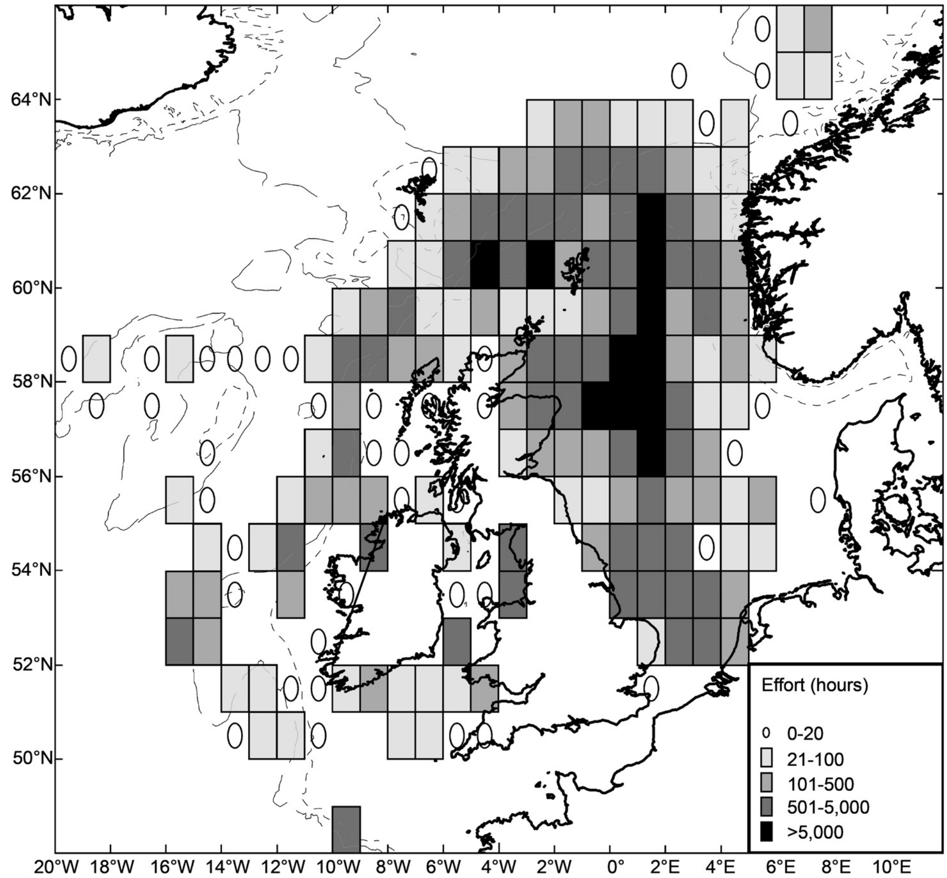

Fig. 1. Effort (hours of visual and acoustic monitoring) during seismic surveys with data available to JNCC between 1994 and 2010 (short dashed line = 200m isobath; long dashed line = 1,000m isobath).

Table 1

Marine mammal encounters during seismic surveys in UK and adjacent waters from 1994–2010 and estimated number of individuals (where number of individuals could not be determined with PAM a minimum number of one was assigned). Encounters with mixed species groups are listed under each species but are only counted once in the totals for each column (though may be included in more than one column if the different species were detected by different means).

Species	No. sightings (and no. of individuals)		No. acoustic detections (and no. of individuals)		No. detections both visual and acoustic (and no. of individuals)	
Seal sp.	92	(122)				
Grey seal (*Halichoerus grypus*)	108	(113)				
Harbour seal (*Phoca vitulina*)	23	(24)				
Cetacean sp.	496	(4,107)	40	(40)	5	(34)
Whale sp.	610	(1,265)			1	(1)
Mysticete sp.	410	(843)			1	(1)
North Atlantic right whale (probable) (*Eubalaena glacialis*)	1	(1)				
Humpback whale (*Megaptera novaeangliae*)	22	(48)				
Blue whale (*Balaenoptera musculus*)	13	(14)				
Fin whale (*Balaenoptera physalus*)	342	(789)				
Sei whale (*Balaenoptera borealis*)	23	(34)				
Minke whale (*Balaenoptera acutorostrata*)	724	(854)				
Sperm whale (*Physeter macrocephalus*)	392	(588)	136	(137)	19	(33)
Beaked whale sp. (*Hyperoodon*/*Mesoplodon*/*Ziphius*)	9	(21)				
Northern bottlenose whale (*Hyperoodon ampullatus*)	10	(44)				
Sowerby's beaked whale (*Mesoplodon bidens*)	6	(14)				
Long-finned pilot whale (*Globicephala melas*)	471	(9,104)			14	(217)
Killer whale (*Orcinus orca*)	331	(2,227)			1	(2)
False killer whale (*Pseudorca crassidens*)	1	(7)				
Delphinid sp.	376	(7,210)	9	(9)	6	(1,755)
Dolphin sp. or porpoise	1,305	(19,109)	276	(579)	33	(763)
Risso's dolphin (*Grampus griseus*)	77	(661)			4	(55)
Bottlenose dolphin (*Tursiops truncatus*)	100	(1,329)			1	(20)
White-beaked dolphin (*Lagenorhynchus albirostris*)	1,146	(15,847)			20	(322)
Atlantic white-sided dolphin (*Lagenorhynchus acutus*)	670	(39,801)	4	(4)	53	(6,121)
Common dolphin (*Delphinus delphis*)	304	(7,635)			11	(570)
Striped dolphin (*Stenella coeruleoalba*)	10	(427)				
Harbour porpoise (*Phocoena phocoena*)	396	(969)	138	(144)	5	(10)
Total	**8,302**	**(113,207)**	**603**	**(913)**	**169**	**(9,904)**

Table 2

Marine mammal detection rates in relation to airgun activity (not firing or soft start or full power) for the period July 2009 to December 2010 for all array types. Differences in detection rates were tested using the Friedman two-way analysis of variance by ranks (F_r = Friedman statistic; n = number of three-way matched samples for detection rates at the different source activities where other conditions were the same). The null hypothesis is that there is no difference in detection rates at different source activities. Significant differences are shown in bold.

	Median detection rate per hour *(+ 1st and 3rd quartiles)*					
Species	Not firing	Soft start	Full power	F_r	n	p-value
All cetaceans combined	*0.00* 0.22 *0.39*	*0.00* 0.00 *0.00*	*0.00* 0.00 *0.28*	36.873	723	**< 0.001**
All mysticetes combined	*0.00* 0.26 *0.61*	*0.00* 0.00 *0.00*	*0.00* 0.07 *0.52*	11.438	723	**< 0.01**
Minke whale	*0.00* 0.37 *0.71*	*0.00* 0.00 *0.00*	*0.00* 0.20 *0.42*	6.077	723	**< 0.05**
All delphinids combined	*0.00* 0.20 *0.31*	*0.00* 0.00 *0.00*	*0.00* 0.00 *0.25*	18.919	723	**< 0.001**
White-beaked dolphin	*0.13* 0.21 *0.43*	*0.00* 0.00 *0.00*	*0.00* 0.00 *0.00*	20.000	723	**< 0.001**
Atlantic white-sided dolphin	*0.07* 0.22 *0.26*	*0.00* 0.00 *0.00*	*0.00* 0.00 *0.12*	10.500	723	**< 0.01**

Table 3

Multiple comparisons of treatments comparing marine mammal detection rates during the soft start with those at other times for the period July 2009 to December 2010 for all array types. Differences in detection rates were tested using the Wilcoxon signed ranks test (T^+ = sum of ranks of matched pairs where detection rate when not firing/at full power exceeded detection rate during the soft start [T^+ increases as detection rates when not firing/at full power exceed detection rates during the soft start more often and/or by a greater amount]; z = statistic for large samples; n = number of matched pairs of detection rates at different source activities when other conditions were the same). The null hypothesis is that there is no difference in detection rates at different source activities. Significant differences are shown in bold.

Species	Median detection rate per hour *(+ 1st and 3rd quartiles)*					
Not firing *vs* soft start	Not firing	Soft start	T^+	z	n	p-value
All cetaceans combined	*0.19* 0.27 *0.49*	*0.00* 0.00 *0.00*	1,289	3.675	57	**< 0.001**
All mysticetes combined	*0.26* 0.57 *0.85*	*0.00* 0.00 *0.00*	55	–	10	**0.001**
Minke whale	*0.37* 0.58 *1.22*	*0.00* 0.00 *0.00*	15	–	5	**0.031**
All delphinids combined	*0.16* 0.24 *0.35*	*0.00* 0.00 *0.00*	635	2.651	41	**0.004**
White-beaked dolphin	*0.13* 0.21 *0.43*	*0.00* 0.00 *0.00*	55	–	10	**0.001**
Atlantic white-sided dolphin	*0.20* 0.24 *0.27*	*0.00* 0.00 *0.00*	45	–	9	**0.002**
Full power *vs* soft start	Full power	Soft start				
All cetaceans combined	*0.13* 0.32 *0.57*	*0.00* 0.00 *0.00*	373.5	–1.301	34	0.097
All mysticetes combined	*0.20* 0.42 *0.56*	*0.00* 0.00 *0.00*	36	–	8	**0.004**
Minke whale	*0.20* 0.32 *0.57*	*0.00* 0.00 *0.00*	15	–	5	**0.031**
All delphinids combined	*0.06* 0.33 *0.57*	*0.00* 0.00 *0.56*	168.5	–0.530	24	0.298
White-beaked dolphin	–	–	–	–	0	–
Atlantic white-sided dolphin	*0.23* 0.57 *0.64*	*0.00* 0.00 *0.00*	6	–	3	0.125

Marine mammal responses to the soft start

All species/species groups able to be tested showed that detection rates differed significantly with source activity (Table 2). Subsequent multiple comparisons of treatments showed that for all species/species groups, detection rates were significantly lower during the soft start than when the airguns were not firing (Table 3). Detection rates were also significantly lower during the soft start than when the airguns were firing at full power for all mysticetes combined and the common minke whale (*Balaenoptera acutorostrata*) (Table 3).

The closest surface distance that marine mammals approached the airguns differed significantly with source activity on surveys with 'large arrays' for the majority of species or species groups tested with the exception of the common minke whale (Table 4; Fig. 2). Multiple comparisons of treatments showed that for all species or species groups where there was a significant difference, animals were significantly further from the airguns when they were firing at full power than when they were not firing but the closest surface distance during the soft start did not differ significantly from the closest surface distance at other times. There was no significant difference in the closest surface distance of approach of cetaceans (all species combined) with source activity for 'small arrays' (Table 4; Fig. 2).

With both 'large arrays' and 'small arrays', all species and species groups tested showed a reduced tendency to engage in positive interactions with the survey vessel or its equipment (e.g. bow-riding, approaching close to the vessel) or to travel towards the vessel during the soft start compared with when the airguns were not firing; a further reduction was detected when the airguns were firing at full power (Table 5). All species groups that could be tested showed an increased tendency to avoid or travel away from the vessel during the soft start of 'large arrays' than at any other time (Table 5). A reduction in observed feeding in all cetaceans combined was apparent when 'large arrays' were firing at full power but there was little difference in observed feeding between periods when 'large arrays' were not firing and the soft start (Table 5).

There were 84 encounters when marine mammals were present both prior to and during the soft start. On 15 of those encounters (18%), responses were observed concurrent with the soft start commencing that could constitute a startle response (e.g. alteration of course to avoid the vessel, a relative increase in swimming speed, diving, resurfacing, leaping, porpoising, spy-hopping and raising tail flukes). There was only one occasion where animals (*Lagenorhynchus sp.*) that initially moved away at the onset of the soft start subsequently re-approached. There was variation in observed

Table 4

Closest distance of approach of marine mammals to the airguns during the soft start compared to at other times. Differences were tested using the Kruskal-Wallis one-way analysis of variance by ranks (KW = Kruskal-Wallis statistic; degrees of freedom = 2 in all cases; *n* = number of sightings where closest distance was recorded). The null hypothesis is that there is no difference in how close animals approach the airguns at different source activities. Significant differences are shown in bold.

	Median closest distance (m)					
Species	Not firing	Soft start	Full power	KW	*n*	p-value
'Large arrays'						
All cetaceans combined	900	1,000	1,500	82.183	2,927	**< 0.001**
All mysticetes combined	800	800	1,500	20.898	613	**< 0.001**
Minke whale	700	625	1,000	5.965	342	< 0.100
All delphinids combined	800	1,200	1,400	42.615	1,682	**< 0.001**
Lagenorhynchus spp.	500	700	1,000	62.672	721	**< 0.001**
White-beaked dolphin	450	600	1,500	44.825	391	**< 0.001**
Atlantic white-sided dolphin	500	750	800	18.045	263	**< 0.001**
'Small arrays'						
All cetaceans combined	600	1,100	500	4.061	296	< 0.200

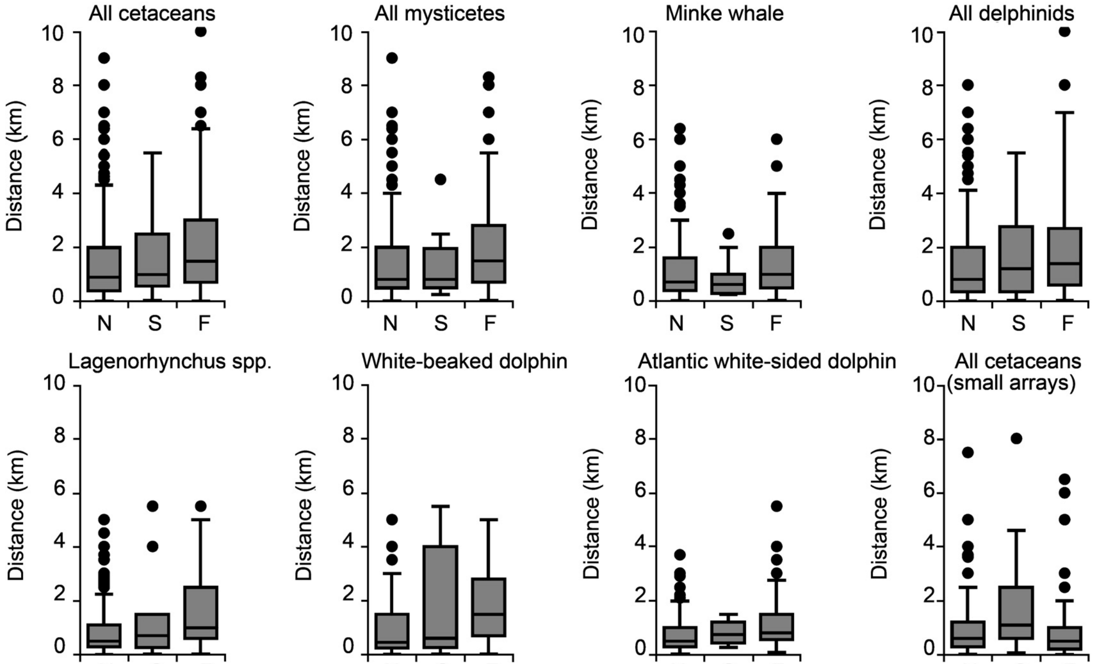

Fig. 2. Box-and-whisker plots of closest distance of approach to the airguns ('large arrays' unless otherwise stated) during the soft start compared to at other times (N = not firing; S = soft start; F = full power). Boxes show median, 1st and 3rd quartiles, whiskers denote range excepting outliers and dots show outliers (> 1.5 × interquartile range outside the 1st or 3rd quartile).

responses between individuals of the same species, e.g. one sperm whale (*Physeter macrocephalus*) dived when the soft start commenced while on another occasion a sperm whale that had recently dived resurfaced and proceeded to swim at speed along the surface. Diving was the only behaviour where sample sizes enabled comparison between encounters where (1) the airguns were not firing throughout (2) were performing a soft start throughout or (3) where the soft start commenced during the course of the encounter. More cetaceans were observed to dive if the soft start commenced during the encounter (Table 6).

Marine mammal responses to airguns in general

A significant reduction in detection rates was evident (Table 7) when 'large arrays' were firing for the grey seal (*Halichoerus grypus*), common minke whale, all beaked whales combined, killer whale (*Orcinus orca*), white-beaked dolphin (*Lagenorhynchus albirostris*), Atlantic white-sided dolphin (*Lagenorhynchus acutus*) and harbour porpoise (*Phocoena phocoena*). When 'small arrays' were firing detection rates of sperm whales and harbour porpoises were significantly lower (Table 7). Most surveys with 'small arrays' were of short duration, but on those that were prolonged (three weeks or more) detection rates of delphinids decreased significantly after the first week (Table 8).

The approach to 'large arrays' was significantly closer when the airguns were not firing for all mysticetes combined although not for fin (*Balaenoptera physalus*) or common minke whales individually, as well as for killer whales, bottlenose dolphins (*Tursiops truncatus*), white-beaked dolphins, Atlantic white-sided dolphins and the harbour porpoise (Table 9; Fig. 3). The difference in the median closest estimated distance of surface approach between firing

Table 5

Behaviour of marine mammals during the soft start compared to at other times. Differences were tested using the chi-squared test (degrees of freedom = 2 in all cases; *n* = number of sightings where the behaviour was exhibited). The null hypothesis is that there is no difference in behaviour at different source activities. Significant differences are shown in bold.

	% encounters when behaviour exhibited					
Behaviour and species	Not firing	Soft start	Full power	χ^2	*n*	p-value
'Large arrays'						
Avoidance or travel away from vessel						
All cetaceans combined	10.0	20.5	17.9	88.25	975	**< 0.001**
All delphinids combined	8.3	18.5	16.4	61.72	484	**< 0.001**
Lagenorhynchus spp.	7.7	24.5	16.9	35.68	186	**< 0.001**
Dived						
All cetaceans combined	5.3	8.5	6.0	3.82	432	< 0.20
Feeding						
All cetaceans combined	9.4	9.1	7.1	9.89	669	**< 0.01**
All delphinids combined	13.1	11.1	11.1	2.82	555	< 0.30
Lagenorhynchus spp.	14.4	12.2	15.1	0.28	257	< 0.90
Positive interactions or travel towards the vessel						
All cetaceans combined	13.5	10.2	6.7	66.92	873	**< 0.001**
All delphinids combined	18.9	12.0	9.2	54.51	710	**< 0.001**
Lagenorhynchus spp.	27.3	18.4	9.2	39.71	404	**< 0.001**
White-beaked dolphin	36.9	26.9	13.3	32.65	314	**< 0.001**
'Small arrays'						
Positive interactions or travel towards the vessel						
All cetaceans combined	25.4	12.0	9.0	18.93	227	**< 0.001**

Table 6

Behaviour of marine mammals in relation to whether the soft start commenced during the encounter or not (all array types). Differences were tested using the chi-squared test (degrees of freedom = 2; *n* = number of sightings where animals dived). The null hypothesis is that there is no difference in behaviour if the soft start commences during the encounter compared to at other times. Significant differences are shown in bold.

	% encounters when behaviour exhibited					
Behaviour and species	Encounters while not firing	Encounters during which the soft start commenced	Encounters wholly during the soft start	χ^2	*n*	p-value
Dived						
All cetaceans combined	5.7	10.3	9.0	6.67	358	**< 0.05**

and not firing ranged between 300m (Atlantic white-sided dolphin) and 1,500m (bottlenose dolphin). Conversely, mysticetes (all species combined) approached significantly closer to 'small arrays' when they were firing (Table 9; Fig. 3), with the median distance being over 1km further away when the airguns were not firing.

Firing of 'large arrays' affected the movement of cetaceans around the vessel (Table 10). Long-finned pilot whales (*Globicephala melas*), white-beaked dolphins and the combined group of all delphinids engaged in positive interactions with the vessel or its equipment (e.g. bow-riding etc.) or travelled towards the vessel more often when the airguns were silent. On surveys with 'large arrays', significantly more pods of fin whales, common minke whales, long-finned pilot whales, white-beaked dolphins, Atlantic white-sided dolphins and harbour porpoises avoided or travelled away from the vessel during periods when the airguns were firing compared to when they were not firing.

Effects on surfacing/diving behaviours were also apparent, with some cetacean groups remaining close to the water surface when 'large arrays' were active (Table 10). For example, during periods of firing a greater proportion of cetaceans (all species combined) were logging or apparently resting at the surface, whilst milling, where animals continue to surface in the same general vicinity, was more prevalent in mysticetes (all species combined). Delphinids (all species combined) were more often recorded both as diving and logging/ resting at the surface during periods of firing. However, there were no significant differences in surfacing/ diving behaviour for individual species.

Several cetacean species were observed feeding less often when 'large arrays' were firing. Whilst the difference was not statistically significant for individual species, where sample sizes were relatively small, it was significant when all cetaceans were combined (Table 10).

Fewer effects on behaviour were evident with 'small arrays'. When species were combined, it was apparent that positive interactions with the vessel or its equipment or travel towards the vessel occurred more often when the airguns were not firing, while avoidance or travel away was more prevalent when the airguns were firing (Table 10).

DISCUSSION

Use of one of the largest existing datasets of MMO observations of marine mammals during seismic surveys allowed a more thorough examination of the response of marine mammals to seismic surveys in UK waters than has previously been possible (Stone, 2006; Stone and Tasker, 2006). However, it is difficult to infer with certainty the mechanism underlying the observed responses. While

Table 7

Marine mammal detection rates in relation to airgun activity (firing or not firing). Differences in detection rates were tested using the Wilcoxon signed ranks test (T^+ = sum of ranks of matched pairs where detection rate when not firing exceeded detection rate when firing [T^+ increases as detection rates when not firing exceed detection rates when firing more often and/ or by a greater amount]; *z* = statistic for large samples; *n* = number of matched pairs of detection rates for active and inactive airguns where other conditions were the same). The null hypothesis is that there is no difference in detection rates whether the source is active or not. Significant differences are shown in bold.

	Median detection rate per hour *(+ 1st and 3rd quartiles)*									
Species	Not firing			Firing			T^+	*z*	*n*	p-value
'Large arrays'										
Grey seal	*0.10*	0.16	*0.34*	*0.00*	0.00	*0.05*	521	2.956	36	**0.002**
Harbour seal	*0.00*	0.07	*0.25*	*0.00*	0.07	*0.10*	25	–	9	0.410
Humpback whale	*0.00*	0.00	*0.22*	*0.00*	0.09	*0.11*	16	–	7	0.406
Fin whale	*0.00*	0.06	*0.19*	*0.00*	0.09	*0.25*	2,543	–0.444	103	0.330
Sei whale	*0.00*	0.03	*0.18*	*0.00*	0.03	*0.17*	39	–	12	0.515
Minke whale	*0.00*	0.13	*0.25*	*0.00*	0.00	*0.19*	24,027.5	3.093	281	**0.001**
Sperm whale	*0.00*	0.11	*0.29*	*0.00*	0.08	*0.18*	3,947.5	1.528	116	0.063
All beaked whales	*0.21*	0.25	*0.28*	*0.00*	0.00	*0.00*	27	–	7	**0.016**
Long-finned pilot whale	*0.00*	0.00	*0.21*	*0.00*	0.08	*0.16*	4,329.5	0.639	127	0.261
Killer whale	*0.00*	0.15	*0.24*	*0.00*	0.00	*0.13*	3,531.5	2.808	103	**0.003**
Risso's dolphin	*0.00*	0.14	*0.36*	*0.00*	0.00	*0.28*	301	1.039	31	0.149
Bottlenose dolphin	*0.00*	0.09	*0.24*	*0.00*	0.08	*0.15*	308	1.176	31	0.119
White-beaked dolphin	*0.00*	0.19	*0.34*	*0.00*	0.00	*0.16*	57,223	7.061	403	**< 0.001**
Atlantic white-sided dolphin	*0.00*	0.13	*0.25*	*0.00*	0.00	*0.20*	26,533.5	3.208	295	**< 0.001**
Common dolphin	*0.00*	0.14	*0.22*	*0.00*	0.00	*0.17*	484	1.312	39	0.152
Harbour porpoise	*0.16*	0.27	*0.43*	*0.00*	0.00	*0.00*	4,278	8.330	92	**< 0.001**
'Small arrays'										
All seals combined	*0.00*	0.00	*0.11*	*0.00*	0.18	*0.37*	5	–	7	0.078
All mysticetes combined	*0.00*	0.00	*0.16*	*0.00*	0.13	*0.24*	196	–1.272	32	0.102
Minke whale	*0.00*	0.00	*0.21*	*0.00*	0.06	*0.19*	103	0.322	19	0.375
Sperm whale	*0.14*	0.17	*0.31*	*0.00*	0.00	*0.00*	114	–	15	**< 0.001**
All delphinids combined	*0.00*	0.15	*0.33*	*0.00*	0.07	*0.23*	3,810	1.419	116	0.078
Long-finned pilot whale	*0.12*	0.26	*0.55*	*0.00*	0.00	*0.31*	29	–	9	0.248
White-beaked dolphin	*0.00*	0.13	*0.28*	*0.00*	0.00	*0.23*	93	0.327	18	0.371
Atlantic white-sided dolphin	*0.00*	0.14	*0.24*	*0.00*	0.00	*0.17*	397	0.687	37	0.245
Common dolphin	*0.00*	0.23	*0.32*	*0.00*	0.21	*0.55*	10	–	6	0.500
Harbour porpoise	*0.18*	0.44	*0.78*	*0.00*	0.00	*0.00*	21	–	6	**0.016**

Table 8

Marine mammal detection rates during the first and later weeks of seismic surveys with 'small arrays'. Differences in detection rates were tested using the Wilcoxon signed ranks test (T^+ = sum of ranks of matched pairs where detection rate during week one exceeded that of later weeks [T^+ increases as detection rates during week one exceed detection rates during later weeks more often and/or by a greater amount]; *n* = number of matched pairs of detection rates for the first and later weeks of each survey). The null hypothesis is that there is no difference in detection rates as surveys progress. Significant differences are shown in bold.

	Median detection rate per hour *(+ 1st and 3rd quartiles)*								
Species	Week 1			Later weeks			T^+	*n*	p-value
All cetaceans combined	*0.02*	0.11	*0.29*	*0.00*	0.02	*0.16*	39	10	0.138
All mysticetes combined	*0.01*	0.02	*0.10*	*0.00*	0.00	*0.03*	11	5	0.219
All delphinids combined	*0.03*	0.10	*0.15*	*0.00*	0.00	*0.05*	31	8	**0.039**
Harbour porpoise	*0.00*	0.01	*0.09*	*0.00*	0.00	*0.00*	11	5	0.219

displacement of animals might be the most likely explanation for reduced detection rates (particularly when combined with increased travel directed away from the vessel), other explanations are also possible. A reduced sighting rate could be a result of longer dive durations, while a reduced acoustic detection rate could result from a reduction in vocalisations. Nevertheless, whatever the underlying mechanism, the results provide clear evidence of responses to the operation of seismic airguns.

Whether the soft start is an effective mitigation measure has been long identified as a key question of interest (e.g. Barton *et al.,* 2008). All species or species groups tested had reduced detection rates during the soft start compared to when the airguns were not firing. All also showed an increased tendency to avoid or travel away from the vessel during soft starts of 'large arrays'. These responses suggest that the soft start can be a useful mitigation tool, causing some marine mammals to move away from the immediate vicinity of airguns before full power is reached, helping to reduce exposure to high levels of sound. Movement directed away from the source can only reduce exposure levels if the avoidance speed of the animal is much greater than the approach speed of the source (Von Benda-Beckmann *et al.,* 2014); seismic survey vessels typically travel at relatively

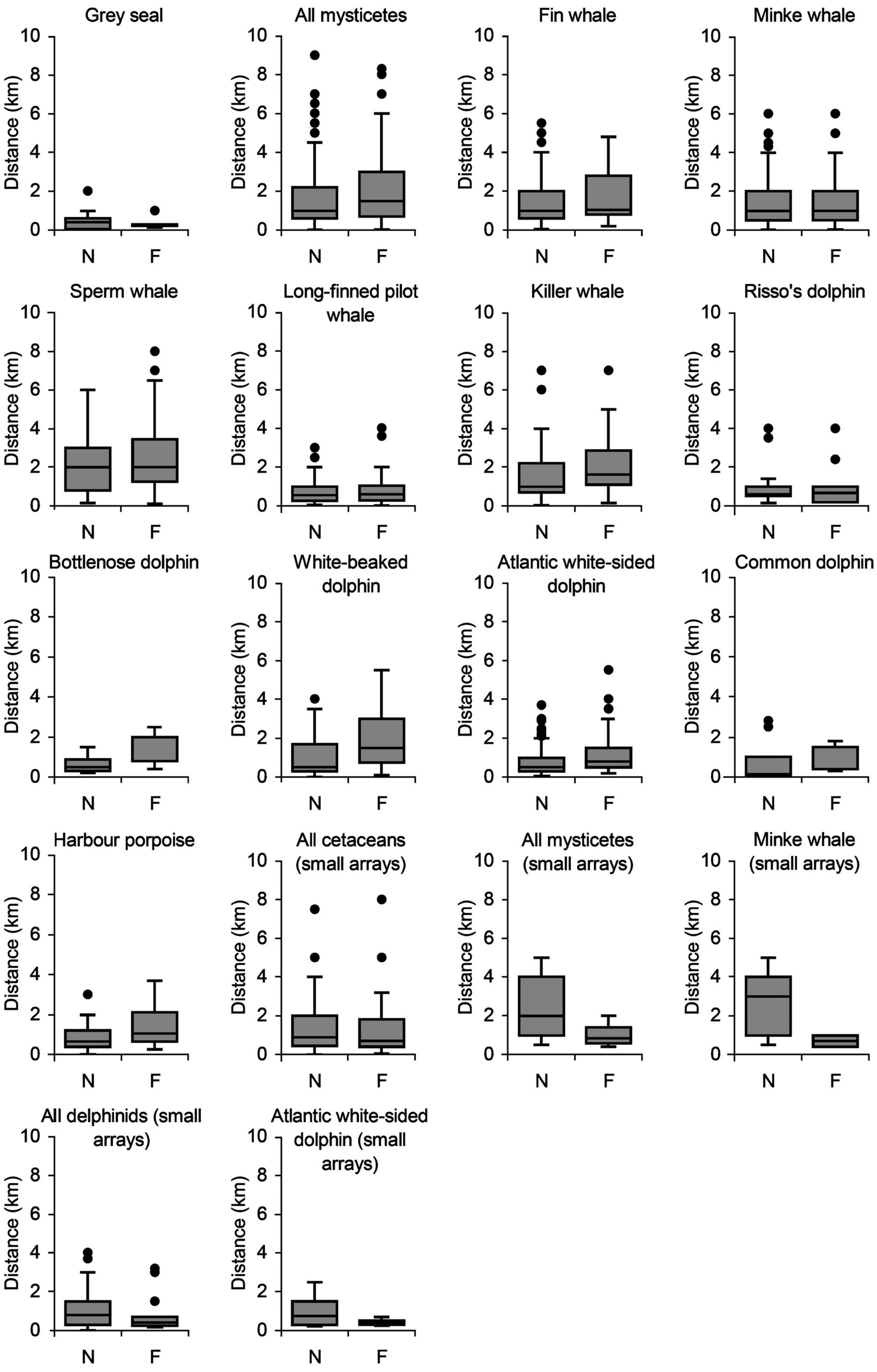

Fig. 3. Box-and-whisker plots of closest distance of approach to the airguns ('large arrays' unless otherwise stated) relative to airgun activity (N = not firing; F = firing). Boxes show median, 1st and 3rd quartiles, whiskers denote range excepting outliers and dots show outliers (> 1.5 × interquartile range outside the 1st or 3rd quartile).

Table 9

Closest distance of approach of marine mammals to the airguns in relation to airgun activity (firing or not firing). Differences were tested using the Wilcoxon-Mann-Whitney test (*n* = number of sightings where closest distance was recorded). The null hypothesis is that there is no difference in how close animals approach the airguns whether the source is active or not. Significant differences are shown in bold.

	Median closest distance (m)				
Species	Not firing	Firing	*z*	*n*	p-value
'Large arrays'					
Grey seal	400	275	0.000	27	0.500
All mysticetes combined	1,000	1,500	9.283	477	**< 0.001**
Fin whale	1,000	1,225	1.382	107	0.084
Minke whale	1,000	1,000	0.813	248	0.209
Sperm whale	2,000	2,000	0.953	111	0.171
Long-finned pilot whale	550	600	0.439	79	0.330
Killer whale	1,000	1,625	2.099	81	**0.018**
Risso's dolphin	600	675	–0.281	23	0.390
Bottlenose dolphin	500	2,000	–1.799	12	**0.036**
White-beaked dolphin	500	1,500	6.075	302	**< 0.001**
Atlantic white-sided dolphin	500	800	3.133	213	**< 0.001**
Common dolphin	150	1,500	1.420	16	0.078
Harbour porpoise	650	1,050	3.065	126	**0.001**
'Small arrays'					
All cetaceans combined	900	700	–0.953	136	0.171
All mysticetes combined	2,000	850	–2.311	25	**0.010**
Minke whale	3,000	700	–0.187	14	0.425
All delphinids combined	700	400	–0.428	66	0.334
Atlantic white-sided dolphin	750	400	–0.147	18	0.440

low speeds (around 4–5 knots) therefore movement away from the source may be effective at reducing exposure to sound from seismic airguns. However, not all individuals travelled away from the vessel during the soft start procedure, highlighting the need to continue to monitor for marine mammals prior to commencing firing airguns, with subsequent delay of firing if marine mammals are detected within the defined mitigation zone. When animals are undetected, the soft start may offer protection to some by causing them to move out of the vicinity of the airguns before full power is reached.

Although the results showed a high level of agreement in the response to the soft start between the species and species groups tested, only a few individual species were tested due to data availability. Caution should thus be exercised as sample sizes were small and another species may respond differently. Nothing is known, for example, about the effectiveness of the soft start for sensitive species such as beaked whales (Barlow and Gisiner, 2006). Moulton and Holst (2010) suggested that the effectiveness of the soft start varies with species and probably circumstances; in the north-west Atlantic they found mysticetes responded to the soft start but, in contrast to the present study, found no response in delphinids or toothed whales. Weir (2008b) observed that a single pod of short-finned pilot whales (*Globicephala macrorhynchus*) initially moved away during a soft start but then changed to milling at the surface, although it was noted that this might represent vertical avoidance. Whilst much can be gained from noise modelling set in the context of assumed response thresholds (Hannay *et al.,* 2011; Von Benda-Beckmann *et al.,* 2014), this needs to be supported by detailed studies on the actual response of marine mammals to the soft start procedure. Von Benda-Beckmann *et al.* (2014) noted that critical research questions that need addressing are documentation of avoidance strategies, behavioural context and estimates of sound dosage that predicts the onset of an avoidance response for different sound types.

The 16-year dataset allowed the response of beaked whales in UK waters to be examined for the first time, although sample size was low (*n* = 7). For all but one pair of observations, detection rates were lower when 'large arrays' were active, whereas previously there has been little evidence that beaked whales respond overtly to the noise from seismic airguns (Moulton and Holst, 2010). Beaked whales are known to be sensitive to other anthropogenic noise, with cases of mass strandings related to the use of military mid-range frequency sonar (Balcomb and Claridge, 2001; Cox *et al.,* 2006; Evans and England, 2001; Fernández *et al.,* 2005). Southall *et al.* (2007) suggested adopting provisional injury criteria for beaked whales exposed to military sonar at lower levels than for other mid frequency cetaceans. Mid-range frequency sonar uses frequencies of around 3–8kHz (Evans and England, 2001; Tyack *et al.,* 2011), higher than those predominantly produced by airguns (up to about 200Hz: Gausland, 2001; Gulland and Walker, 2001), and has a very long signal duration compared with seismic shots, so it is not necessarily directly comparable. More research is needed to understand the effects of seismic surveys on all species but especially beaked whale species.

Greater responses were observed in mysticetes than had been noted previously in UK waters. Previously, only localised avoidance was evident for all mysticetes combined (Stone and Tasker, 2006), with no significant effects of airgun activity observed for any individual species. The present study revealed responses of common minke whales and fin whales when 'large arrays' were active. The frequency and sound source level of the airguns used on seismic surveys in UK waters were often not recorded, but from available information 'large arrays' produce

Table 10

Behaviour of marine mammals in relation to airgun activity (firing or not firing). Differences were tested using the chi-squared test (*n* = number of sightings where the behaviour was exhibited). The null hypothesis is that there is no difference in behaviour whether the source is active or not. Significant differences are shown in bold.

Behaviour	% encounters when behaviour exhibited				
Species	Not firing	Firing	χ^2	*n*	p-value
'Large arrays'					
Avoidance or travel away from vessel/equipment					
Fin whale	14.6	24.3	3.95	61	**< 0.05**
Minke whale	8.2	16.3	8.44	70	**< 0.01**
Sperm whale	18.7	19.7	0.05	68	< 0.90
Long-finned pilot whale	5.1	13.9	9.49	41	**< 0.01**
Killer whale	11.9	18.1	1.70	41	< 0.20
White-beaked dolphin	8.2	19.2	22.24	115	**< 0.001**
Atlantic white-sided dolphin	6.3	12.2	5.80	51	**< 0.05**
Harbour porpoise	20.0	37.5	7.78	82	**< 0.01**
Diving					
Fin whale	11.2	12.5	0.11	38	< 0.80
Minke whale	10.4	7.0	1.77	59	< 0.20
Sperm whale	50.0	43.0	0.90	168	< 0.50
All delphinids combined	1.4	2.2	4.10	75	**< 0.05**
Feeding					
All cetaceans combined	10.3	8.2	7.85	706	**< 0.01**
Fin whale	12.9	9.7	0.71	37	< 0.50
Long-finned pilot whale	8.0	6.3	0.48	32	< 0.50
Killer whale	26.5	16.9	2.34	72	< 0.20
White-beaked dolphin	11.1	12.3	0.28	118	< 0.70
Atlantic white-sided dolphin	23.4	19.9	0.73	139	< 0.50
Logging/resting at surface					
All cetaceans combined	2.6	3.7	6.81	216	**< 0.01**
Sperm whale	29.9	35.9	0.95	115	< 0.50
All delphinids combined	1.3	2.1	4.18	72	**< 0.05**
Long-finned pilot whale	8.9	8.7	0.01	39	< 0.95
Milling					
All mysticetes combined	0.7	3.0	10.16	22	**< 0.001**
Lagenorhynchus spp.	3.1	2.5	0.43	53	< 0.70
White-beaked dolphin	3.2	2.5	0.26	31	< 0.70
Positive interactions or travel towards vessel/equipment					
All mysticetes combined	6.1	3.9	3.22	72	< 0.10
Minke whale	6.8	6.1	0.13	42	< 0.80
All delphinids combined	18.1	9.5	47.60	725	**< 0.001**
Long-finned pilot whale	27.0	15.4	6.93	96	**< 0.01**
White-beaked dolphin	37.1	15.2	31.02	324	**< 0.001**
Atlantic white-sided dolphin	13.1	7.7	3.48	71	< 0.10
Common dolphin	25.7	23.3	0.06	63	< 0.90
'Small arrays'					
Avoidance or travel away from vessel/equipment					
All cetaceans combined	8.7	18.0	12.42	103	**< 0.001**
All delphinids combined	6.7	17.3	11.27	51	**< 0.001**
Diving					
All cetaceans combined	7.2	6.6	0.09	70	< 0.80
Feeding					
All cetaceans combined	9.2	8.2	0.16	89	< 0.70
All delphinids combined	9.4	12.5	0.86	59	< 0.50
Positive interactions or travel towards vessel/equipment					
All cetaceans combined	26.2	11.5	13.84	232	**< 0.001**
All delphinids combined	36.1	11.6	16.35	190	**< 0.001**
Lagenorhynchus spp.	49.5	16.3	8.94	105	**< 0.01**
White-beaked dolphin	67.0	46.7	0.84	84	< 0.50

frequencies predominantly up to around 200Hz with a source level of around 262dBpk–pk re. 1µPa @ 1m and 'small arrays' produce frequencies predominantly up to around 250Hz with a source level of around 241dBpk–pk re. 1µPa @ 1m. Mysticetes are in a low frequency hearing group, estimated to have functional hearing within the range 7Hz to 22kHz (Southall *et al.,* 2007), so airgun sound would be clearly audible to them. The present results correspond more closely to studies elsewhere, where seismic operations have resulted in localised avoidance by common minke whales in the northwest Atlantic (Moulton and Holst, 2010) and a change in vocalisations and some evidence of displacement of fin whales in the western Mediterranean Sea and adjacent waters (Castellote *et al.,* 2012). In the present study, no responses were observed in either humpback whales (*Megaptera novaeangliae*) or sei whales (*Balaenoptera borealis*), although sample sizes were low. Elsewhere avoidance of seismic survey vessels has been demonstrated for humpback whales (McCauley *et al.,* 1998, 2000; Moulton and Holst, 2010) as well as other mysticetes (e.g. Ljungblad *et al.,* 1988; Richardson and Greene 1993; Richardson *et al.,* 1986, 1999; Yazvenko *et al.,* 2007).

Odontocetes hear best at frequencies above those at which the peak energy from seismic airguns is produced, although seismic airguns also emit higher frequency sounds that would be audible to odontocetes (De Ruiter *et al.,* 2006; Goold and Fish 1998; Madsen *et al.,* 2006; Potter *et al.,* 2007). Most odontocetes belong to a mid-frequency hearing group with functional hearing from about 150Hz to 160kHz, while porpoises belong to a high frequency hearing group with functional hearing between 200Hz and 180kHz (Southall *et al.,* 2007). As most of the energy from seismic airguns is at lower frequencies it is often assumed that mysticetes would be the cetaceans most vulnerable to disturbance from the sound of seismic airguns. Although some odontocetes showed no response, such as the common dolphin (*Delphinus delphis*) and the Risso's dolphin (*Grampus griseus*), in the present study, most mid frequency odontocetes tested and harbour porpoises showed some response to 'large arrays'. In some cases, the response was greater than found previously; killer whales and harbour porpoises had previously been found not to approach so close to airguns when they were active (Stone and Tasker, 2006), but in the present study reduced detection rates were also found. In other cases (white-beaked dolphins, Atlantic white-sided dolphins, bottlenose dolphins and long-finned pilot whales) the present results confirmed previous results (Stone and Tasker, 2006). Similar responses of small or medium-sized odontocetes to noise from seismic airguns have also been observed elsewhere (Barkaszi *et al.,* 2012; Weir 2008a). The present results therefore confirm previous studies showing that the response of cetacean species to noise from seismic airguns does not necessarily correlate with what might be expected based solely on their hearing abilities. It could be that the responses are driven not only by the ability to hear the sound but also by how the sound is perceived; for example, animals may avoid sounds that they might interpret as indicating the presence of predators, to which smaller species may be more vulnerable.

Previous analysis of UK MMO data was limited to cetaceans (Stone and Tasker, 2006) but the larger dataset also allowed investigation of the responses of pinnipeds. Detection rates of grey seals were significantly reduced when 'large arrays' were active. In Alaska, pinnipeds such as ringed seals (*Pusa hispida*) have shown minor avoidance during seismic operations (Harris *et al.,* 2001).

The larger dataset also allowed the effects of 'small arrays' to be tested on more species. Previously a change in orientation had been noted for Atlantic white-sided dolphins and reduced detection rates found for all small odontocetes combined (Stone and Tasker, 2006). In the present study, detection rates of sperm whales (n = 15) and harbour porpoises (n = 6) were reduced when 'small arrays' were active, while mysticetes did not show any negative response. Although responses were fewer than with 'large arrays', it is appropriate that some form of mitigation continues to be applied to surveys using 'small arrays'. Sub-bottom profilers (e.g. boomers, pingers and sparkers, with frequencies ranging from 700Hz to 12kHz) are used on some surveys with 'small arrays', but not at the same time as airguns. Any response to the use of sub-bottom profilers during some periods when airguns were not firing could have reduced the statistical significance of any response during periods when the airguns were firing. Detailed records of the operation of sub-bottom profilers were not kept, but they were only used during some of the times when the airguns were not firing. Recording the operation of such equipment would enable future studies to examine any response of marine mammals to their use.

The harbour porpoise was the only species with lower detection rates for both 'large arrays' and 'small arrays', suggesting an increased sensitivity to airgun noise compared to other species. Previous results found only that harbour porpoises tended not to approach so close to 'large arrays' when they were active (Stone and Tasker, 2006). However, the current results are in line with other studies, both field and experimental observations, which all seem to suggest that this species is highly sensitive to underwater noise. For example, a single captive harbour porpoise exposed to noise from a seismic airgun exhibited aversive behavioural responses at received sound pressure levels above 174dBpk–pk re. 1μPa and a masked temporary threshold shift level of 199.7dBpk–pk re. 1μPa, lower than for other odontocetes (Lucke *et al.,* 2009). It seems likely that received levels of sound from the arrays of airguns used in surveys, even 'small' arrays, would be sufficient to elicit a response in harbour porpoises within the vicinity of arrays. Seismic operations within the Moray Firth (UK) using a relatively small array (470in^3) resulted in short-term avoidance by harbour porpoises at received sound pressure levels of 165–172dBpk–pk re. 1μPa, these levels being found at 5–10km from the source, although animals were typically detected again within a few hours and there were indications of possible habituation or tolerance as the survey progressed (Thompson *et al.,* 2013). Nevertheless, those porpoises remaining within the Moray Firth area did reduce their buzzing activity that relates to prey capture or social communication (Pirotta *et al.,* 2014).

On surveys with 'small arrays', where operations were typically concentrated within a small area, detection rates of delphinids decreased significantly after the first week of operations, although sample sizes were low (n = 8). Although the amount of time spent firing in each week of a seismic survey varies hugely, depending on factors such as weather and technical problems, increased habituation or increased sensitisation may occur with repeated exposure to sound (Richardson *et al.,* 1995). An initial tolerance of 'small arrays' by delphinids might give way to increasing sensitisation as surveys progress; alternatively, there could be some other explanation for the later decrease in sighting rates, such as a delayed response due to prey moving out of the area or natural variations in abundance. A reduction in rates of delays in firing (required when marine mammals are within 500m of the airguns) after the initial use of airguns on surveys (Stone, 2015) might point to an adaptive response, with animals 'warned' by previous firing perhaps being less likely to approach close to the vessel.

Although many of the observed responses suggest avoidance, marine mammals may respond to seismic operations in other ways. Some behavioural responses were evident and although not all members of a group may have exhibited the behaviour, in assessing response to noise not all group members need to be observed (Southall *et al.,* 2007). Although feeding is not always apparent from surface observations, when all cetaceans were combined significantly fewer animals were recorded as feeding when 'large arrays' were active; a reduction in foraging effort may have significant consequences for individuals and populations. In the Gulf of Mexico, sperm whales did not avoid seismic operations but may have decreased their foraging effort (Jochens *et al.,* 2008; Miller *et al.,* 2009). Although in the present study there were no observed effects of noise from 'large arrays' on sperm whales, the results are limited to those observations detectable by MMOs; as sperm whales forage at depth, a reduction in foraging would not be readily apparent.

When 'large arrays' were active there were indications that some cetaceans may remain close to the surface (e.g. logging), where noise levels may be lower due to the Lloyd's mirror effect (Richardson *et al.,* 1995; Urick, 1983). Other studies have also observed changes in the surfacing behaviour of cetaceans in response to noise from seismic operations, with some reporting a reduction in time at the surface (Gailey *et al.,* 2007; Robertson *et al.,* 2013) but most reporting an increase in surfacing behaviour (Barkaszi *et al.,* 2012; Jochens *et al.,* 2008; McCauley *et al.,* 1998, 2000; Miller *et al.,* 2009). Changes in surfacing, respiration and dive behaviours of cetaceans exposed to seismic operations may have implications for the ability to detect animals (Robertson *et al.,* 2013). As most of the effort in the present study was visual, any behaviours which may have influenced sighting rates could potentially mask any changes in numbers of animals in the vicinity. Therefore, a lack of any significant difference in detection rates for some species does not necessarily rule out avoidance. Changes in surfacing, respiration and dive behaviours can be context-dependent, depending on the circumstance and the activity of the animal, with greater responses to noise when animals are travelling than when socialising or feeding (e.g. Robertson *et al.,* 2013). The response of marine mammals to airgun activity is likely to be complex, involving many variables that may contribute to results such as those for sperm whales in the present study, where detection rates were reduced when 'small arrays' were active, but no response to 'large arrays' was observed.

MMO observations of cetacean behaviour have the potential to be biased given the difficulty in observing cetaceans and measuring/estimating distances, the subjective nature of interpreting behaviour and the possibility that the MMO may have an expectation, even if subconsciously, that animals will respond differentially between when seismic airguns are firing and when they are not firing. Blind field trials cannot be achieved on board seismic surveys thus the data collected by MMOs currently provide the only data resource with which to test the hypotheses put forward in this paper. Every attempt has been made to limit potential bias where possible, for example by using matched pairs in the statistical analyses. While behavioural response studies with controlled exposures are the best tool to determine whether responses such as reduced detection rates are due to displacement of animals or a change in behaviour, experimental set ups (e.g. Cato *et al.,* 2013) are complex and costly and would not be feasible over the same spatial and temporal scales and range of species as this study. MMO data provide the potential for examining behavioural responses with larger sample sizes across a range of species and geographical areas. Continued collation and use of MMO data to address questions such as the effectiveness of the soft start is therefore encouraged.

The National Research Council (NRC, 2005) encouraged the examination of the wealth of marine mammal data collected in compliance with regulatory requirements in order to increase understanding and management. The present study increases our knowledge of the effects of seismic airguns on marine mammal species found in UK waters, demonstrating previously unknown responses in beaked whales, common minke whales, fin whales and grey seals and emphasising the sensitivity of the harbour porpoise. Previously observed effects on some other small and medium sized odontocete species were confirmed. Although effects were more evident for 'large arrays', there were also some effects noted of 'small arrays' on sperm whales, harbour porpoises and all delphinids combined. Despite the variation in effects observed the results thus far confirm that mitigation measures should continue to apply to all types of seismic surveys and cover the risk to all marine mammal species. Despite the difficulties in collecting sufficient data on the effects of seismic surveys on marine mammals to allow for a thorough species by species analysis, some progress has been made with the current study. Understanding the ecological significance of those observable effects to individuals and populations remains however, a much greater challenge.

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