CHAPTER THREE

Records from visual surveys, strandings and eDNA sampling reveal the regular use of Reunion waters by dwarf sperm whales

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Contents

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Abstract

The genus *Kogia* includes two extant species, the dwarf sperm whale (*Kogia sima)* and the pygmy sperm whales (*K. breviceps*). Due to their elusive behavior at the surface, which limits opportunities for observation, they are amongst the least known species of cetaceans and knowledge of their ecology mostly comes from stranded individuals. Although they have overlapping ranges, dwarf sperm whales seem to be distributed preferentially in warmer tropical and subtropical waters, while pygmy sperm whales tend to be associated with more temperate waters. Both species have previously been recorded in the western Indian Ocean, but little is known about their distribution patterns. Data from different sources, including vessel-based and aerial surveys, environmental DNA and strandings were compiled to report on the occurrence of *Kogia* around the remote oceanic island of Reunion. The combination of sightings data, eDNA detections and stranding events indicated that the dwarf sperm whale was more common than the pygmy sperm whale and seems to use the territorial waters of Reunion on a regular basis. The northern part of the island in particular might provide suitable habitats for the species. Groups of 1–5 individuals were sighted and occurred mainly over the insular slope, in 1310 m deep waters and 8.2 km from the shore on average; no clear seasonality pattern could be determined. Stranding data were consistent with a calving period during the austral summer and highlighted the vulnerability of these species to human activities.

1. Introduction

When first described, the dwarf sperm whale (*Kogia sima*, Owen, 1866) and the pygmy sperm whale (*Kogia breviceps*, Blainville, 1838) were recognized as types of sperm whale and included in the Physeteridae family, together with the sperm whale (*Physeter macrocephalus*). Their inclusion was based on several similar characteristics, such as the presence of a spermaceti organ, teeth in the lower jaw only and the asymmetrical alignment of the left blowhole. The genera *Kogia* and *Physeter* have since been revised into two distinct Kogiidae and Physeteridae families (Rice, 1998). Recent phylogenetic and morphological studies support the close relationship between *Kogia* and sperm whales, based on mitochondrial DNA and the anatomical structure of the head (Clarke, 2003; May-Collado and Agnarsson, 2006), further supporting their grouping within the sperm whale superfamily Physeteroidea.

The genus *Kogia* includes two extant species, the dwarf (*K. sima*) and the pygmy (*K. breviceps*) sperm whales, which were definitively identified as separate species in 1966 (Handley, 1966). The two species have overlapping range and occur in tropical and temperate waters of the Atlantic, Indian and Pacific Oceans (Rice, 1998), although dwarf sperm whales seem to prefer warmer tropical and subtropical waters and pygmy sperm whales are usually associated with higher latitudes (Bloodworth and Odell, 2008; Kiszka and Braulik 2020a,b; McAlpine, 2009; Moura et al., 2016). Recent genetic analyses suggested that dwarf sperm whales may actually consist of two parapatric species occupying the Atlantic and the Indo-Pacific Oceans, with the Cape of Good Hope in South Africa representing a barrier (Chivers et al., 2006; Plön et al., 2023a). However, recognition of a putative third *Kogia* species requires further supporting evidence (McAlpine, 2009).

Both the dwarf and pygmy sperm whales were recently listed as "Least Concern" on the IUCN Red List of Threatened Species based on their wide distribution, indications that they might not be as uncommon as initially thought, and the lack of evidence that they are facing major threats (Kiszka and Braulik 2020a,b). However, due to their elusive surface behavior, *Kogia* represents one of the least known of the nine extant families of toothed whales (Baird et al., 2021). They are typically motionless at the surface (logging behavior) or are slow moving (no splash), produce no visible blow and dive by sinking into the water, without showing their flukes, thus providing few cues for detection and making them relatively inconspicuous at the surface unless in very good sea conditions (Baird et al., 2021; Caldwell and Caldwell, 1989). As there are so few at-sea sightings, they are usually described as rare. However, it is not always clear if this is due to their low detectability or low densities. High numbers of strandings and relatively high sighting frequencies (compared to other cryptic species such as beaked whales) which have been reported around some oceanic islands, along continental slope margins and canyons in association with upwelling (Anderson, 2005; Baird et al., 2021; Bloodworth and Odell, 2008; Collins et al., 2002; Dunphy-Daly et al., 2008; Hodge et al., 2018; Plön 2004) suggest that certain areas might provide suitable habitats for populations to establish.

Very few at-sea studies focusing on *Kogia* have been conducted (Baird, 2005, Baird et al., 2021; Dunphy-Daly et al., 2008) and there are few local population estimates, most of which do not discriminate between the two species (Barlow, 2006; Garrison et al., 2010; Laran et al., 2017; Mullin and Fulling, 2004). Species abundance at a global scale is currently unknown and knowledge of population structure, seasonality, and movement patterns is also very limited. Most information on the biology and ecology of these species comes from the analysis of stranded animals (e.g., Plön, 2004; Ross, 1979). Analysis of stomach contents indicates that *Kogia* feed primarily on cephalopods and suggest that each species might use a different ecological niche, with dwarf sperm whales foraging on smaller squid inshore and in shallower depths than pygmy sperm whales (Ross, 1979; Willis and Baird, 1998). Social segregation might also occur, as analysis of prey indicates that females with their young, and immature individuals, might feed on smaller-sized cephalopods closer inshore, than mature males and non-reproductive mature females (Plön, 2004; Ross, 1984). The social structure of *Kogia* remains largely unknown.

Recently, environmental DNA techniques have been shown to be an effective means to assess the distribution of cryptic species such as *Kogia*, as they allow for detection of species presence without relying on direct observations of the animals at the surface (Hodge et al., 2018; Juhel et al., 2021). Kogia can also be detected using passive acoustic monitoring methods (Hildebrand et al., 2019; McCullough et al., 2021; Ridgway and Carder, 2001), although it is not yet possible to acoustically discriminate between the two species, both of which produce high frequency clicks with peak frequencies around 123–130 kHz (Madsen et al., 2005; Malinka et al., 2021; Marten, 2000; Merkens et al., 2018). Tonal calls have not been reported for this species.

In the south-western Indian Ocean (SWIO), the occurrence of both species of *Kogia* has been reported but little is known about their ecology. Historically, relatively high numbers of strandings of both species have been reported along the south-eastern coast of South Africa (106 *K. breviceps* and 85 *K. sima* between 1880 and 1995, Plön, 2004; Ross, 1979). Vessel-based surveys indicated that dwarf sperm whales appear to be relatively common north of the Seychelles (Ballance and Pitman, 1998) and around the Maldives (representing 4.2% of all on-effort sightings), while *K. breviceps* was not recorded (Anderson, 2005). Elsewhere in the region, sightings and strandings of both species of *Kogia* have been reported in lower numbers (Cerchio et al., 2022; Dulau-Drouot et al., 2008; Kiszka et al., 2010; Plön et al., 2023b). Large scale aerial surveys conducted in the SWIO have produced population estimates for both species combined (corrected for availability bias), and showed that although *Kogia* were among the least abundant cetacean taxa, higher densities were observed around the Seychelles (population estimates of 305 individuals), compared to other oceanic islands of the south-western Indian Ocean (Reunion, Mauritius, Mozambique Channel islands) and Madagascar (Laran et al., 2017).

This study aims to investigate the occurrence of *Kogia* around the island of Reunion in the south-western Indian Ocean. Reunion is located at a latitude of ~21°S, at the southern end of the Mascarene Plateau, which extends approximately ~2000 km from the Seychelles to the Mascarene islands (Reunion, Mauritius, Rodrigues). Unlike the Seychelles, which have a continental origin, the Mascarenes are volcanic islands originating from the still-active volcanic hotspot under Reunion. Being geologically young (~3 million years ago), the island of Reunion has a very narrow shelf and steep slope, with depths increasing rapidly to 3000 m. This topography brings oceanic cetacean species in close proximity to the shore, facilitating opportunities for studying deep-sea species that are typically difficult to access. In this study, the occurrence of *Kogia* around Reunion Island was investigated by collating data on the presence of these cryptic species from multiple sources, including visual surveys (both boat-based and aerial), stranding records, and environmental DNA. The objective was to provide new insights into the distribution and phenology of these poorly documented species and highlight potential threats that they may face in these waters.

2. Material and methods

2.1 Boat-based and aerial surveys

Sightings data of *Kogia* were compiled from different type of surveys, including boat-based surveys conducted around the island and aerial surveys conducted off the northern part of the island.

Different types of boat-based cetacean surveys were conducted around Reunion over a 13-year period (Table 1). During 2010–2023, one-day surveys were conducted on small vessels (5–6 m) that covered coastal waters to a distance of ~10 km from shore, without pre-defined transects. Surveys were conducted year-round, at an average of three surveys per week. Surveys were launched from different harbors located mainly on the western side of the island, where boats were more available and weather conditions more favorable. Survey tracks and sighting positions were recorded using a portable GPS unit. Sighting conditions were recorded every 15 min using a 5-level Visibility Index (V): 1: very poor (Beaufort > 4, dawn or dusk); 2: poor (Beaufort 4, numerous white caps and/or poor light), 3: moderate (Beaufort 2 to 3, scattered white-cap, moderate swell), 4: good (Beaufort 2, no or very long swell), 5: excellent (Beaufort 0–1, no swell). Upon sighting a group of cetaceans, the time, GPS position, species identification, group size estimate and behavior data were recorded on a standardized datasheet. Photographs were taken to confirm species identification using a digital SLR camera equipped with a

Table 1 Summary the survey effort per survey type: number of daily surveys, total survey effort (in km) and percentage of effort in good to excellent sighting conditions (visibility index 4 to 5), and number of sightings of dwarf sperm whales (*K. sima)*, pygmy sperm whale (*K. breviceps)* and *Kogia sp.* (not identified to species level) reported between 2010 and 2023.

Survey type	Methodology	Survey area	Period	N of daily surveys	Survey effort (km)	% in good to excellent conditions	N of *K. sima* sightings	N of *K. breviceps* sightings	N of *Kogia sp.* sightings
Boat-based	No pre-defined transects	Reunion's coastal waters (up to 10 km)	2010–2023	1915	74,974	78%	4	1	0
Boat-based	Line-transect	Reunion's territorial waters (up to 22 km)	2010–2020	42	4356	80%	1	0	0
Boat-based	Line-transect	Northern part of Reunion (up to 37 km)	2015–2022	379	21,873	75%	19	0	1
Aerial	Line-transect	Northern part of Reunion (up to 50 km)	2015–2022	262	87,588	80%	14	0	44
TOTAL				**2840**			**38**	**1**	**45**
eDNA		Reunion's territorial waters (up to 22 km)	**2018–2022**	33			**1**	**1**	**0**

For the eDNA study, effort and *Kogia detections are reported in numbers of sampling locations (off survey effort).*

150–300 mm or 150–600 mm lens. Identification to species level was based upon good quality photographs and sightings for which species identification was uncertain were assigned to *Kogia* sp.

Line-transect surveys were also conducted around the island once annually between 2010 and 2020, using a 11 m long fishing vessel with an observation platform located 4 m above the sea surface. Transects covered territorial waters up to 22 km offshore, with designs constrained by the locations of harbors. Each survey was conducted over a 4–5 day period allowing a complete circumnavigation of the island. Due to weather constraints, these 4–5 days surveys around the island could not be completed at the same time every year. Vessel tracks, survey effort and sighting data were recorded using the Logger2000 © interface developed by the International Fund for Animal Welfare (IFAW).

Increased survey effort was completed in the northern part of the island, linked to the construction of a new coastal road viaduct along 12 km of coastline. Both boat-based and aerial surveys were conducted in the area of the construction between January 2015 to June 2022, using pre-defined line-transects. Weather permitting, four boat-based surveys were conducted per month, with transects covering waters up to 37 km offshore, based on the same methodology described above on a 12 m vessel. In addition, two aerial surveys were conducted each month onboard an ultra-light aircraft flying at 300 m altitude that covered waters up to 50 km from shore. As for boat-based survey, the survey track, visibility index (recorded every 15 min using the same five categories as described above), and cetacean sighting positions were collected using a portable GPS and standardised datasheet. Upon sighting, the aircraft left the transect and circled-back to the group to facilitate photographs for species identification and collection of group size estimates, following which the transect was resumed.

Survey effort was reported in a 2 km x 2 km grid, as the cumulative distance (in km) searched in each cell, for boat-based and aerial surveys separately. Spatial distribution was assessed by conducting a kernel density estimation using the sightings data for all surveys combined (boat-based and aerial surveys), using the package adehabitatHR in Rstudio (v2022.07.2). Distribution maps, depth and distance to the nearest coast of each sighting were estimated in QGis (v3.24.3), using bathymetry data provided by the French Naval Hydrographic Services (SHOM).

2.2 Environmental DNA (eDNA)

Seawater samples were collected for eDNA analyses during dedicated surveys from June 2018 to May 2019 and from February 2021 to June 2022 at 34 locations around Reunion, within waters up to 10 km from shore (Fig. 5). Sampling occurred in different months, based on vessel availability and weather conditions and each location was sampled only once. Samples were collected by two trained operators wearing protective clothing and gloves to avoid contamination. At each sampling location, three replicates of 10 liters (L) of surface water were collected and filtered onboard using sterile filter capsules (Sterlitech 0.8 μm,), sterile and single-use tubes and a peristaltic pump. Filter capsules were preserved in RNAlater buffer (Qiagen©) and stored at −20 °C until they could be sent to a dedicated eDNA laboratory (Nature Metrics, UK) for genetic analysis. DNA from each filter was extracted using a DNA extraction kit from Nature Metrics Mammal. Specific primers (MiMammal-UF: 5′-GGG TTG GTA AAT TTC GTG CCA GC-3′, MiMammal-UR: 5′-CAT AGT GGG GTA TCT AAT CCC AGT TTG-3′) were used to amplify ~230 bp of the hypervariable region of the 12S rRNA gene (Ushio et al., 2017).

DNA amplifications were performed with 12 PCR replicates in a final volume of 10 μL (Nikolic et al., submitted). PCR Master Mix (Thermo Scientific) included 0.4 μM of each of the tailed primers, 2 μM of a human blocking primer, 0.8 μg/μL bovine serum albumin (BSA—Thermo Scientific), 3% of DMSO (Thermo Scientific), 1.5 mM of $MgCl_2$ (Invitrogen), and topped up with PCR grade water (Thermo Scientific). PCR conditions were as follows: an initial denaturation at 98 °C for 3 min, followed by 45 cycles of 20 s at 98 °C, 15 s at 69 °C, and 15 s at 72 °C, and a final elongation step at 72 °C for 5 min. Three negative extraction controls and three negative PCR controls (ultrapure water, 12 replicates) were used to assess potential contamination. Amplification success was determined by gel electrophoresis. DNA was purified to remove PCR inhibitors using a DNeasy PowerClean Pro Cleanup Kit (Qiagen). Purified DNA extracts were quantified using a Qubit dsDNA HS Assay Kit on a Qubit 3.0 fluorometer (Thermo Scientific). PCR replicates were pooled and sequencing adapters were added. The final library was sequenced using an Illumina MiSeq V2 kit at 15 pM (Illumina, San Diego, CA, USA) with a 10% PhiX spike.

Metabarcoding pipelines was processed using a NatureMetrics © custom bioinformatics pipeline including quality filtering, trimming, merging paired ends, removal of sequencing errors such as chimeras, clustering of similar sequences into molecular Operational Taxonomic Units (OTUs; each of which approximately represents a species), and matching one sequence from each cluster against the National Center for Biotechnology Information

(NCBI) nucleotide database (GenBank—https://www.ncbi.nlm.nih.gov), using the online nucleotide Basic Local alignment Search Tool (BLAST) and with the default algorithm parameters (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The NCBI database included one referenced sequence for the 12S rRNA region of interest (100% coverage) for *K. sima* (Accession number: NC_041303.1, Shan et al., 2019) and *K. breviceps* (Accession number: AJ554055.1, Arnason et al., 2004), so each *Kogia* species could theoretically be detected. Assignments were made to the lowest possible taxonomic level where there was consistency in the matches and a 100% similarity required for assignment at the species level. Results were presented in terms of total number of reads for each detected species in each sample. For each sampling site, the sample showing the highest number of cetacean reads (out of the three replicates) was reported.

2.3 Strandings

Stranding events and responses in Reunion have been coordinated by GLOBICE since 2006, under the authority of the French stranding network lead by Pelagis laboratory. Post-mortem investigation and tissue sampling were based on the French stranding network's protocols. Data were collected using a standardized form, to record at minimum: date, location, species, number of individuals, and state of decomposition. Five Decomposition Condition Categories (DCC) were used: 1. extremely fresh caracass (just dead); 2: fresh carcass; 3 moderate decomposition, 4: advanced decomposition; 5: mummified or skeletal remains (IJsseldijk et al., 2019). Whenever possible, the sex, dental formula (i.e. number of teeth on the lower and upper jaw) and different morphometrics measurements and tissue samples were collected and a necropsy was carried out by a veterinary from the stranding network (VE or others). *Kogia* species were distinguished based on physical characteristics and in particular on the number of teeth (*K. sima*:14–24 mandibular teeth, rarely 26 and 0–6 maxillary teeth; *K. breviceps*: 24–32 mandibular teeth, rarely 20–22 and no maxillary teeth) and the height of the dorsal fin (D) relative to body length (L) (D/L ratio > 5% in *K. sima*; <5% in *K. breviceps*; Ross, 1979). A confidence rating of "low", "medium" or "high" was assigned to the species identification (Plön et al., 2023b). When possible, identification was confirmed genetically. DNA was extracted from skin samples using DNeasy™ Tissue Kits (Qiagen) and the control region (~800 base pairs) of the mitochondrial DNA (mtDNA) was amplified by Polymerase Chain Reaction (PCR) using existing primers (Dlp-1.5 f from Baker et al., 1998 and MtCRr from Hoelzel and Green, 1998). PCR were carried out in a 25 μL reaction volume consisting of 5–50 nano grams (ng) of extracted DNA, 0.2 μM of each primer, 0.15 mM of mixed dNTPs, 1.5 mM $MgCL_2$, 2 units of SilverStar *Taq* DNA polymerase and 1x reaction buffer (Eurogentec® Inc). PCR amplifications comprised 6 min (min) at 94 °C followed by 39 cycles of 45 s (s) at 94 °C, 30 s at 50–56 °C and 50 s at 72° followed by 9 min at 72 °C. PCR amplification products were sequenced using BigDye (v. 3.1, ThermoFisher Inc.) and by electrophoresis using a 3730XL DNA Analyzer™ (ThermoFisher Inc.). Species identification was confirmed by matching the mtDNA sequences against the NCBI depository, based on 100% similarity with known *K. sima* and *K. breviceps* sequences. Strandings for which confidence in species identification was low and no genetic identification was available were referred as *Kogia sp.* For strandings reported in 2006 and before, species identification was confirmed based on photographs and by inspection of the specimens kept or reproduced to scale by the Natural History Museum of Reunion.

3. Results

3.1 Distribution surveys

A total of 2598 surveys were conducted around Reunion island over the 13 year study period representing a total distance of 101,203 km from boat-based surveys and 87,588 km from aerial surveys (Figs. 1 and 2). Surveys were generally conducted in good to excellent visibility conditions, with visibility index of 4 and 5 representing between 75% and 80% of the survey effort, respectively (Table 1, SM1). A total of 84 sightings of *Kogia* were made, which were all reported in good to excellent sea-state conditions (27% in visibility index 4 and 73% in visibility index 5). Of the 84 sightings of *Kogia*, one was a group of two pygmy sperm whales and 38 were confirmed dwarf sperm whales (*K. sima),* of which 24 were recorded during boat-based surveys (Fig. 1) and 14 during aerial surveys (Fig. 2), representing a cumulative number of 78 individuals. Forty-five sightings of *Kogia* could not be identified to species level (44 from aerial surveys and one from boat-based surveys).

Most of the sightings of *K. sima* were detected along the northern coasts of the island (50% kernel contour, Fig. 3), where systematic boat-based and aerial survey effort was completed year-round (Table 1). Three *K. sima* sightings occurred on the west coast of the island, and one in the south. No sightings

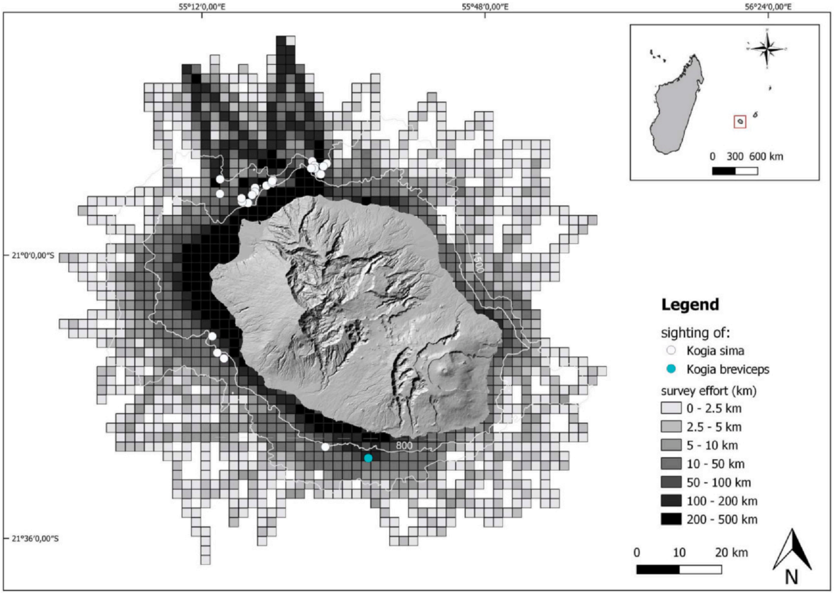

Fig. 1 Map showing boat-based survey effort conducted around Reunion between 2010 and 2023 and the location of the sightings of dwarf (*Kogia sima*) and pygmy (*Kogia breviceps*) sperm whales.

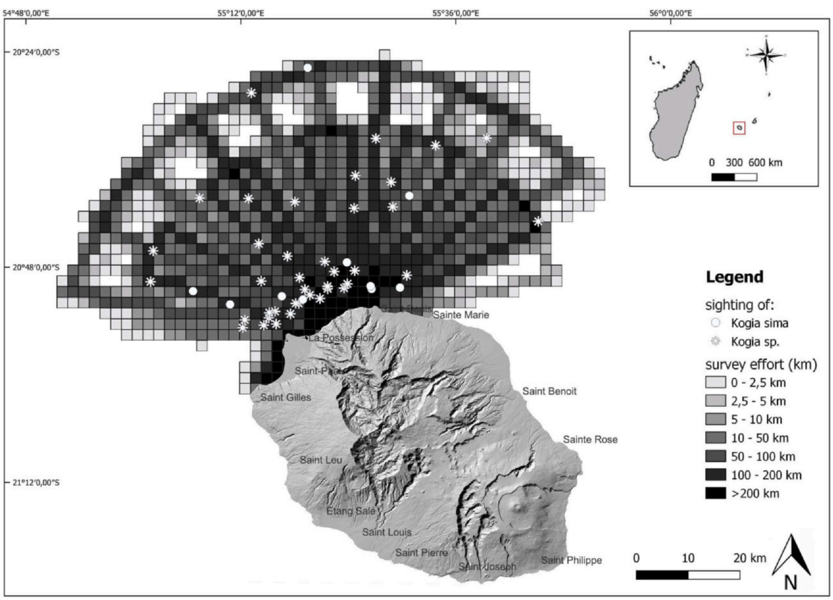

Fig. 2 Map showing aerial survey effort conducted in the northern part of Reunion between January 2015 and June 2022 and location of the sightings of dwarf sperm whales (*Kogia sima*) and *Kogia* sp.

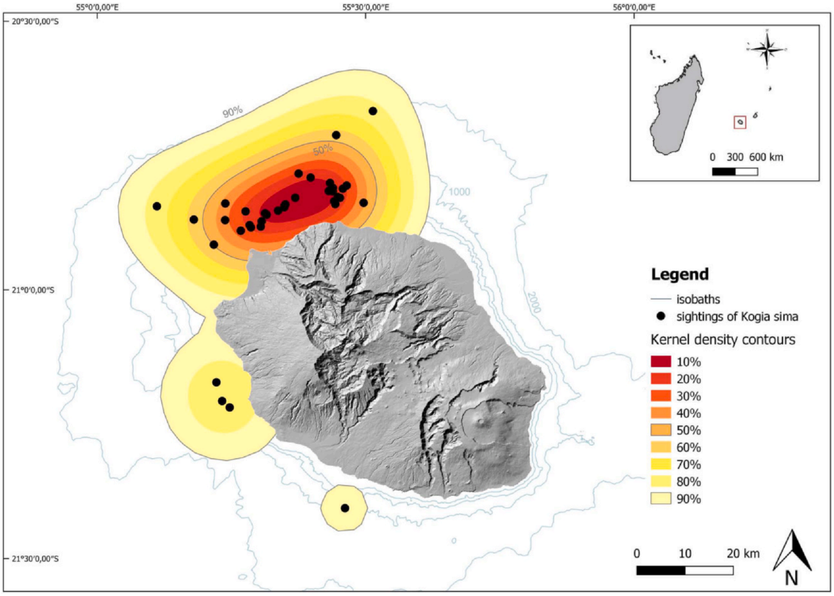

Fig. 3 Map showing the distribution of the sightings of dwarf sperm whales (*K. sima*) from boat-based and aerial surveys, and kernel density contours.

were reported from the east coast of the island, where survey effort was lower. The single sighting of *K. breviceps* occurred in the south, during boat-based survey, and kernel analysis could not be performed for this species.

Sightings of confirmed *K. sima* were mostly of single individuals (45%, n = 17), but groups of 2 to 5 individuals were also reported (Table 2). Out of the 38 sightings of *K. sima*, 7 included a mother with a calf or a juvenile. Mean group size for *K. sima* was 2.0 (SD = 1.2, min = 1, max = 5). Other sightings of *Kogia sp.*, that could not be confirmed to species, had similar group sizes (1.8, SD = 1.4, min = 1, max = 7, Kruskall–Wallis Chi-squared H = 1.4826, p = 0.223).

The majority of *K. sima* sightings occurred in waters between 800 m and 1500 m of depth (n = 28, 74%) and at a distance of 3–10 km from the coast (n = 33, 87%). The mean depth of *K. sima* sightings was 1310 m (SD = 518, min = 653, max = 2500) and mean distance to the shore was 8.2 km (SD =7.3, min = 3.2, max = 45.5, Table 2). There was no difference in depth between sightings of *K. sima* and unidentified *Kogia sp.* (Kruskall–Wallis Chi-squared H = 3.818, p = 0.051), although the latter occurred at greater distance to shore, being mainly reported from aerial surveys (Kruskall–Wallis Chi-squared H = 9.601, p = 0.002).

Table 2 Mean group size, depth and distance to the coastline of dwarf sperm whales (*K. sima*) and *Kogia sp*. sighted in January 2010–June 2022 around Reunion (all surveys combined), and values for the single sighting of pygmy sperm whale (*K. breviceps).*

	***K. sima* (n = 38)**			***K. breviceps* (n = 1)**	***Kogia* sp. (n = 45)**		
	Mean	**SD**	**Range**		**Mean**	**SD**	**Range**
Group size	2.0	1.2	1–5	2	1.8	1.4	1–7
Bottom depth (m)	1331	518	653–2500	1000	1786	858	408–3700
Distance to shore (km)	8.2	7.3	3.2–45.5	5	15.9	11.9	2.9–48,5

Sightings of *Kogia* occurred throughout the year, with slightly higher number of *K. sima* sightings reported in October-November (n = 20) (Fig. 4). Calves were also sighted throughout the year (Fig. 4). Depth and distance to the nearest coast of *K. sima* sightings were not significantly different between months (Kruskall-Wallis Chi-squared H = 1.568, p = 0.979 and H = 6.368, p = 0.497 respectively), which was also true when combining all sightings of *Kogia* (H = 11.895, p = 0.72 and H = 13.086, p = 0.289 respectively), and no seasonal pattern was observed (Fig. 5).

3.2 Environmental DNA

The filters yielded an average of 8.15 ng/μL of DNA and no contamination was detected in the negative controls. Among the high-quality sequences produced with the MiMammal primers, a total of 459,813 reads were assigned to cetacean taxa. Detection of cetaceans occurred at 29 of the 33 sampling sites and resulted in the identification of a total of 8 species, based on a 100% similarity with referenced sequences from NCBI along the full length of the 12S rRNA amplified sequence (171 bp). Among the 8 cetacean species detected were the two species of *Kogia*. The dwarf sperm whale (*K. sima*) was identified at two sampling locations, with 8188 and 25,598 reads, respectively, matching at 100% similarity to the referenced *K. sima* sequence from the NCBI database (Accession number: NC_041303.1). The pygmy sperm whale (*K. breviceps*) was detected at one sampling location with 11,200 reads matching at 100% similarity the referenced *K. breviceps* sequence from the NCBI database (Accession number: AJ554055.1). Reads that matched at 100% similarity with *K. sima* matched at only 94.1% similarity with *K. breviceps* (and vice-versa). *K. sima* and *K. breviceps* had 10 nucleotide differences along the 171 bp sequence (94.1% similarity), supporting the discrimination at the species level. *Kogia* were not reported in the BLAST hit results from other locations.

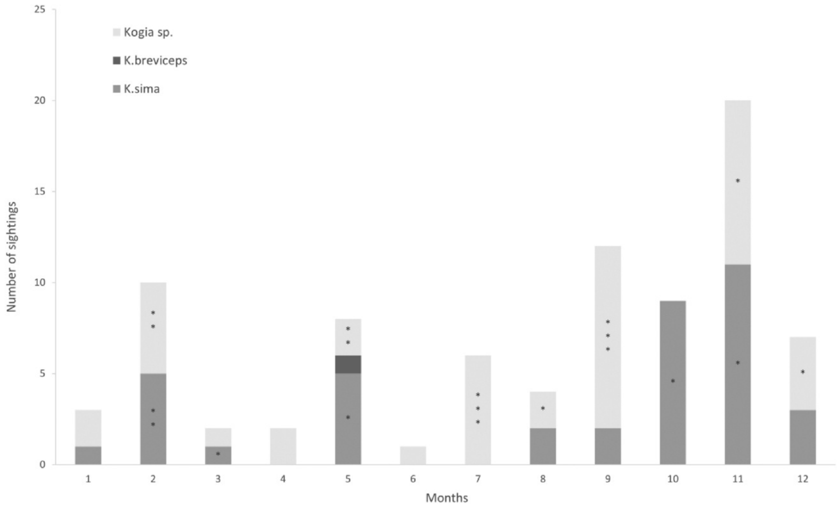

Fig. 4 Seasonal distribution of dwarf sperm whales *(K. sima)*, pygmy sperm whales *(K. breviceps)* and Kogia sp. (unidentified Kogia), recorded around Reunion in 2010–2023 (all surveys combined), with *representing the number of calves.

Detections of *Kogia* from eDNA were located in the northern part of the island (Fig. 5), in waters of 800–1800 m of depth for *K. sima* and 1600 m for *K. breviceps*. The detection of *K. breviceps* occurred in February 2019, while *K. sima* was detected in samples collected in February and March 2022 (Table 3).

The other cetacean species identified with 100% similarity were the humpback whale (*Megaptera novaeangliae*), the bottlenose dolphin (*Turisops truncatus*), the Indo-Pacific bottlenose dolphin (*T.aduncus*), the pantropical spotted dolphin (*Stenella attenuata*), the melon-headed whale (*Peponocephala electra*) and Fraser's dolphin (*Lagenodelphis hosei*), with the number of reads per sample ranging between 124 and 57,914 (Table SM2.1 and Figure SM2.1 in Supp. Mat 2).

3.3 Strandings

A total of 10 Kogia were reported dead in Reunion between 1993 and 2023 (Table 4). Among them, nine were found stranded on the coast, and one was reported drifting at sea and brought back to shore in February 2022. For convenience, all events are referred to as "strandings" in the manuscript. Photographs and measurements were not available from the specimen reported in 1993 in the west (Saint Paul) so the identification

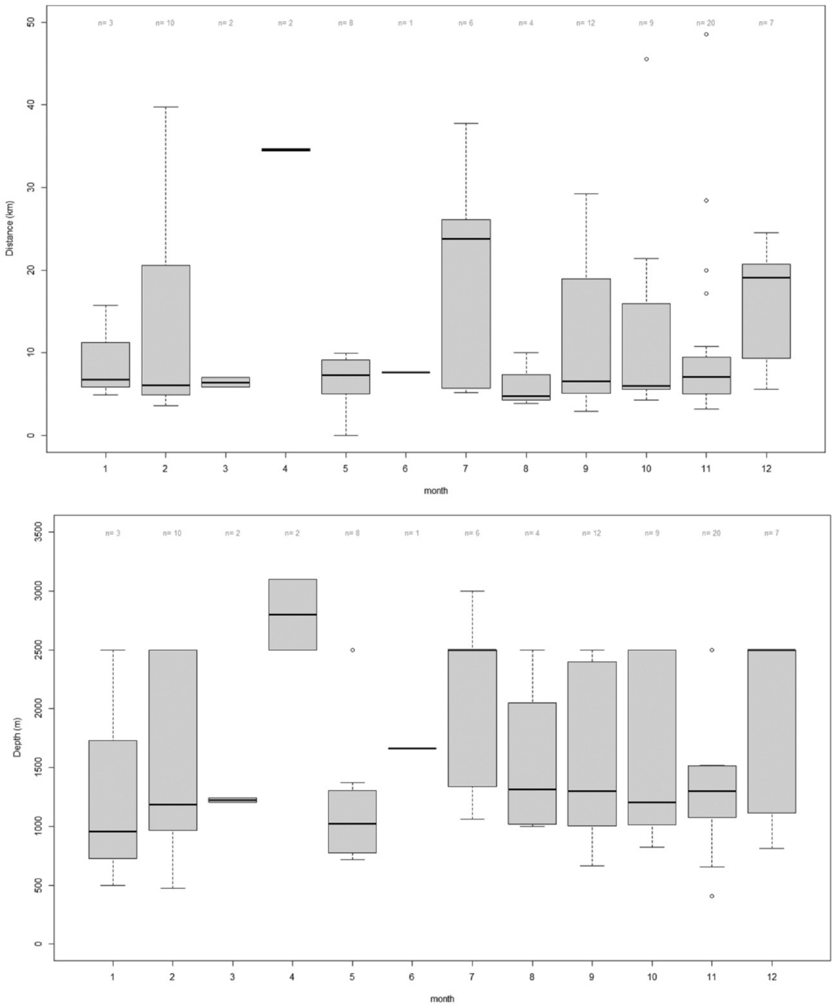

Fig. 5 Box plot of the distance to the shore and depth of *Kogia* sightings (including *K. sima*, *K. breviceps* and *Kogia sp.*) across months. Median values are represented by the middle horizontal line, with upper and lower box lines representing the 75th and 25th quartile respectively (50% of sightings are within the box), vertical lines represent 1.5 times the interquartile range, circles represent outliers, and sample size is indicated at the top.

could not be confirmed to species level. An animal that stranded in 1998 in the South of the island (Saint-Pierre) was kept at the National History Museum of Reunion and was identified *a posteriori* as a *K. breviceps*, although the specimen was in an advanced state of decomposition when

Table 3 Summary of the eDNA results presenting sampling location where *Kogia* were detected total number of reads (Nr) attributed to cetacean taxa in the sample and number of reads attributed to dwarf (*K. sima)* and pygmy (*K. breviceps)* sperm whales (and % of the number of cetacean reads) and bottom depth (in m) at sampling location.

Sampling site	Date	Latitude	Longitude	N reads cetaceans	N reads K. sima (%)	N reads *K. breviceps* (%)	Depth (m)
14	06/02/2019	−20.78868	55.44835	15,315	0	11,200 (73.13%)	1600
22	27/02/2022	−20.77166	55.36057	25,598	25,598 (100%)	0	1800
32	15/03/2022	−20.85961	55.65447	8188	8188 (100%)	0	800

examined (most teeth missing and decomposed dorsal fin). Individuals stranded between 2003 and 2022 (n = 7) were identified as *K. sima* with a high level of confidence after examination of the specimens on site. For three of these animals, identification was confirmed genetically (Table 4). One juvenile animal (155 cm) that stranded in 2023 was identified as a pygmy sperm whale (*K. breviceps*) with a medium level of confidence (26 teeth on the lower mandibular, dorsal fin to total length ratio of 5% (Ross, 1979), while body proportions might be biased in young individuals). Skin samples were taken for further genetic identification.

Strandings of *Kogia* were distributed all around the island (Fig. 6). One stranding of *K. sima* occurred in the south (Saint-Pierre), one in the east (Sainte-Anne), two in the west (Saint-Paul), including one collected at sea, and three in the north (Sainte-Marie). The latter strandings all occurred at the same location behind the port, within a few meters of one another, with one each in February 2006 (adult), in May 2020 (calf) and in November 2020 (pregnant female).

Necropsies were conducted on four fresh *K. sima* individuals. The individual that stranded in December 2003 in Saint Paul was a young, emaciated male, with no teeth, indicating that the animal was not yet weaned. A gross examination was carried out on the animal. About 600 mL of sero-hemorrhagic effusion was present in the abdominal cavity. Unfortunately, this fluid was not analyzed, and the heart was not dissected to look for a heart disease. The tail fluke was missing with a clear cut of the caudal peduncle. The necropsy revealed that the fluke was cut after the death of the animal.

The necropsy of the individual stranded in March 2017 in the south (Saint-Pierre) revealed that this mature male was in a good body condition with a stomach full of otoliths, fish bones and cephalopods beaks (analysis yet to be undertaken). A high level of parasitism by *Anisakis simplex* was found in the stomach and the small intestine of the animal. An abundant tracheo-bronchial foam was also present. The tympanic bullas were analyzed and did not reveal any lesions associated with exposure to underwater noise.

The individual that stranded in November 2020 in the north (Saint Marie) was a pregnant female, with a 58 cm long foetus. This animal was in a good body condition and the specimen was fresh (dermal abrasions revealed that the animal had stranded alive). A diffuse hematoma was present along the left side. The forestomach was opened and showed the presence of otoliths, providing evidence that the animal had fed shortly before its death. The thoracic tissues examination showed a pulmonary

Table 4 Details of *Kogia* strandings recorded around Reunion from 1993 to 2023.

Date	Location	Latitude	Longitude	Species	Confidence	Photo	Genetic confirmation	DDC	Sex	Age class	L	D	D/L ratio (%)	S	S/L ratio (%)	Nb of teeth	Necropsy/ Histopathologica analysis	Possible cause of death
1993	St Paul	−21.01849	55.23850	*Kogia sp.*				U	U	Adult							None	—
1998	St Pierre	−21.33495	55.45412	*K. breviceps*	Medium	Yes	Sample to be analysed	2[a]	U	Adult	203						None	—
30/12/2003	St Paul	−21.00309	55.27217	*K. sima*	High	Yes	Yes	2	M	Calf	118[b]	5.6	4.75	6	5.08	0/0	-Emaciated -Fluke missing (post mortem cut) −600 mL of sero-hemorrhagic effusion in the abdominal cavity	Bycatch Pathology -probable heart failure
04/02/2006	Ste Marie	−20.89393	55.54003	*K. sima*	High	Yes	No sample	2	U	Adult	230	14.4	6.26	14.5	6.30		None	—
08/05/2009	Ste Anne	−21.06894	55.73655	*K. sima*	High	Yes	Yes	4	U	Adult	231	—	—	—	—		None	—
23/03/2017	St Pierre	−21.35111	55.48559	*K. sima*	High	Yes	Yes	1	M	Adult	225	18	8.00	15	6.67		-High level of parasitism (*Anisakis simplex*) in the stomach and small intestine - Good nutritional state - Full stomach -Abundant tracheobronchial foam	Parasitic infestation

29/05/2020	Ste Marie	−20.89352	55.53850	*K. sima*	HIgh	Yes	Sample to be analysed	3	U	Calf	111	5.9	5.32	8	7.21	0/10	None	—
21/11/2020	Ste Marie	−20.89349	55.53850	*K. sima*	High	Yes	Sample to be analysed	2	F	Adult	222	14	6.31	—	—		- Good nutritional state - Pregnant, foetus = 58 cm -diffuse hematoma on the left side -presence of otoliths in the forestomach -Pulmonary edema -abundant serohemorrhagic foam on the section of lungs and trachea -Disseminated petechiae into the melon	Ship-strike/ Bycatch
16/02/2022	St Paul	−20.97223	55.2500	*K. sima*	HIgh	Yes	Sample to be analysed	2	M	Calf	98	6	6.12	5	5.10	0/14	-Neonate -Good nutritional state -digested milk in the stomach and intestines -Numerous subcutaneous diffuse hematomas -Pulmonary	Chronic pathology Possible peri-mortem collision

(continued)

Table 4 Details of *Kogia* strandings recorded around Reunion from 1993 to 2023. (*cont'd*)

Date	Location	Latitude	Longitude	Species	Confide-nce	Photo	Genetic confirma-tion	DDC	Sex	Age class	L	D	D/L ratio (%)	S	S/L ratio (%)	Nb of teeth	Necropsy/ Histopat-hologica analysis	Possible cause of death
																	edema -Abundant sero-hemorrhagic tracheobron-chial foam -Histopathol-ogica analysis: prominent alveolar histiocytosis suggesting a chronical process consistent with heart or respiratory failure	
0/09/2023	Trou d'eau	−21.10168	55.24499	*K. breviceps*	Medium	Yes	Sample to be analysed	4	F	Juvenile	155	7	4.52	16.5	10.65	0/26	None	—

DCC: Decomposition condition Category (1: very fresh; 2: fresh carcass; 3: moderate decomposition, 4: advanced decomposition, U: unknown), sex (Male/Female/Unknown), Age class, total length (L, in cm), dorsal fin height (D, in cm), D/L ratio, Snout-to-blow hole distance (S, in cm), S/L ratio, necropsy comments, and possible cause of death.

[a]DCC: 2 when stranded, DCC:4 when examined;

[b]Based on the reconstruction of the individual by the Natural History Museum.

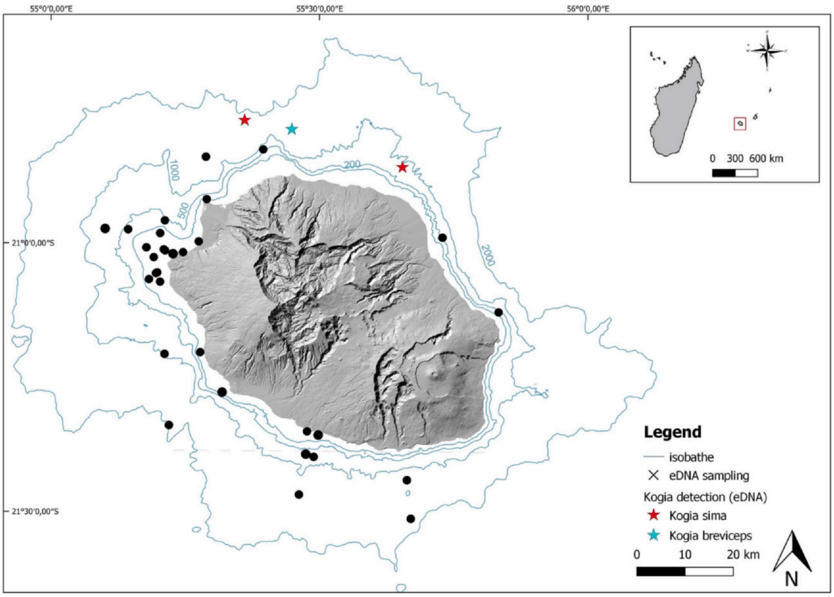

Fig. 6 Map showing the distribution of the eDNA sampling locations and the detection of *K. sima* and *K.breviceps* around Reunion Island.

edema, with an abundant sero-haemorrhagic foam. The sagittal section of the melon revealed disseminated petechiae.

The individual examined in 2022 was collected at sea off Saint-Paul and frozen until the necropsy could be carried out. The necropsy revealed that it was a neonate, in good body condition. Numerous subcutaneous diffuse hematomas were present (in the ventral part and around the head on the right and left side of the body). Sections of these hematomas showed that they were made before or at the time of the death. Inspection of the digestive tract showed the presence of digested milk in the stomach and the intestines. The lungs were enlarged, with hemorrhagic foci disseminated along the tissues and pulmonary edema. An abundant sero-haemorrhagic tracheobronchial foam was present when trachea and lungs were cross-sectioned.

4. Discussion

The combination of sighting data, eDNA detections and stranding events confirmed that both species of *Kogia* are present within the territorial waters (<22 km from shore) of Reunion and might possibly be sympatric. The dwarf sperm whale, *K. sima,* appeared to be more common than the pygmy sperm whale, *K. breviceps*. The northern part of the island might provide suitable habitats for the dwarf sperm whale relatively close to shore while the examination of stranded individuals highlighted potential threats to the species arising from human activities.

4.1 Species occurrence

Most *Kogia* detected during boat-based surveys were identified as dwarf sperm whales (24 sightings of *K. sima*, and one sighting of undetermined *Kogia* sp.). Only one confirmed sighting of pygmy sperm whales was reported during the boat-based surveys and none from the aerial surveys. Strandings data also mirror this trend, with seven strandings of dwarf sperm whales recorded over the last decade, compared to two strandings of pygmy sperm whales in 1998 and 2023.

Both dwarf and pygmy sperm whales were detected in the eDNA sequences, confirming the use of Reunion waters by both species. Detecting the two species in the relatively low number of sampling stations ($n = 33$) at the surface was particularly remarkable, and further emphasizes the efficiency of this emergent technique to detect cryptic, deep-diving species like the dwarf and pygmy sperm whales, which have a low probability of detection in traditional visual surveys (Juhel et al., 2021).

The combination of records from different sources indicates that the dwarf sperm whale is more common in the territorial waters of Reunion than initially reported, while also confirming the presence of the pygmy sperm whale (Dulau-Drouot et al., 2008). These results are consistent with dwarf sperm whales being more common in tropical habitats than pygmy sperm whales, which prefer more temperate waters (Bloodworth and Odell, 2008; Caldwell and Caldwell, 1989; Moura et al., 2016). In the western tropical Indian Ocean, the dwarf sperm whales appears to be relatively common in the Maldives, a chain of island located close to the equator (0–7°N of latitude), with 74 sightings reported (representing 4.2% of all on-effort sightings), while the pygmy sperm whale has not been recorded (Anderson, 2005). This is consistent with the results of a wide-ranging vessel-based survey conducted in the western Indian Ocean, which indicated that dwarf sperm whale was relatively frequent north of the Seychelles, in waters between 5°S and 7°N of latitude (Ballance and Pitman, 1998). Large-scale line-transect aerial surveys also confirmed a high density of *Kogia* around the Seychelles (Laran et al., 2017), but identification to species level could not be undertaken. In more temperate waters, such as in South Africa, where the highest numbers of *Kogia* strandings are reported from the region, both species are recorded evenly, with 106 strandings of *K. breviceps* and 85 of *K. sima* reported over a 15-year period (1880–1995) along an approximate 3000 km of coastline (Plön, 2004; Ross, 1979).

Elsewhere in the western Indian Ocean, confirmed records of both species exist, but are too sporadic to further assess any latitudinal gradient in species distribution. A recent compilation of strandings data collected over the past 20 years (2000–2020) in the south-western Indian Ocean reported 24 *Kogia* stranding events, including 11 dwarf and 9 pygmy sperm whales (Plön et al., 2023b). From these data, the highest occurrence of dwarf sperm whale strandings were reported for Reunion (n = 6), with stranding events also reported for South Africa (n = 3), Mozambique (n = 1) and Seychelles (n = 1) during that period. Pygmy sperm whale strandings were recorded along the eastern coast of mainland Africa, in South Africa (n = 4), Mozambique (n = 1) and Kenya (n = 3) and in the continental island of Madagascar (n = 1). One at-sea sighting of *K. sima* was recorded off the south-west coast of Madagascar (Cerchio et al., 2022) and sightings of both species of *Kogia* (n = 2 *K. sima* and n = 1 *K. breviceps*) have also been reported around Mayotte, in the northern Mozambique channel (Kiszka et al., 2010).

In the northern part of the western Indian Ocean, sightings, strandings and osteological records have confirmed the presence of dwarf sperm whales off Eritrea, Oman, Pakistan and India (Baldwin et al., 1999; Collins et al., 2002; Gore et al., 2012; Kumaran, 2002; Notarbartolo di Sciara et al., 2017), while the pygmy sperm whale has only been reported off Pakistan (Gore et al., 2012).

4.2 Spatial distribution around Reunion

Several datasets provide evidence of the use of Reunion territorial waters (<22 km) by the dwarf sperm whale, especially off the northern coast, where systematic line-transect surveys (boat-based and aerial) were conducted over an 8-year period. Because effort was not homogeneously distributed around the island, it is still unclear if this area represents a preferred habitat for the species, compared to other areas around the island. The western side of the island was covered relatively intensively, but effort was lower in the species preferred depth range (800–1500 m) as deeper waters occur further off-shore in this area. The few *Kogia* sightings recorded around the island were located in areas where the 800 m depth contour runs closer to the coast. Increasing effort in the 800–1500 m depth waters (>10 km off-shore) might result in more detections around the island. Furthermore, given the elusive behavior of the species at the surface, the number of sightings is likely influenced by sea state and the type of survey vessel, with observers being more likely to detect *Kogia* from a higher survey platform (as used during the systematic line-transect surveys), than from smaller boats. Line-transect surveys on a larger boat were also conducted around the island, but at a lower frequency (one four-day survey per year on average) than in the north (three one-day surveys a month) and resulted in only one sighting of dwarf sperm whale in the north. Barlow (2015) reported that in Beaufort 2 sea conditions the probability of sighting *Kogia* during large vessel surveys drops to less than 10% of their sighting probability in Beaufort 0 conditions. In this study, visibility was generally good to excellent during surveys (representing from 75% to 90% of the survey effort) and sightings of *Kogia* were only made in visibility index of 4 and 5. Future studies for the species should consider the effect of survey effort (including sighting conditions and platform type) and environmental variables, such as depth, to further assess spatial distribution around the island and identify preferred habitats.

Results from eDNA sampling and stranding events also indicate more detections in the north, although the numbers were too low to be conclusive. Out of the 33 sampling sites and eight cetacean species detected around the island, *Kogia* were only detected in the north, further supporting the use of this area by both species. *Kogia* strandings occurred all around the island, but three of them (i.e., 33%) were found in the same location in the north, behind the port of Sainte-Marie, within a few meters of one another. The carcasses of two adults were fresh (in February 2006 and in November 2020), while the calf showed advanced decomposition (May 2020). Given that the access to the beach was closed due to the renovation of the port in early 2020, the discovery of the latter animal might have been delayed. The higher occurrence of strandings in the north, with most individuals discovered in a fresh decomposition state, is suggestive of individuals that were inhabiting adjacent waters.

The high number of sightings, stranding events and eDNA detections indicates that at least the northern sector of Reunion Island provides a suitable habitat for *Kogia*, and the dwarf sperm whale in particular. Evidence of site fidelity has been reported off Hawai'i, an island of similar size based on multi-year resightings and individual spatial distribution

(Baird et al., 2021). Further photo-identification work should be undertaken to assess the level of residency and the spatial ranges of individuals sighted off the northern coast of Reunion.

4.3 Habitat use

Although *Kogia* were sighted up to 48 km offshore, most sightings were distributed along the insular slope, in water depths from 800 m to 1500 m. Dwarf sperm whales occurred on average in 1310 m deep waters (SD = 518, min = 653, max = 2500) and unidentified *Kogia sp.* were reported in similar water depths (1786 m on average, SD = 858, min = 408, max = 3700). Most sightings of dwarf sperm whales off Reunion were located relatively close to the coast, at a mean distance of 8.2 km (SD = 7.3, min = 3.2, max = 45.5), and this is likely linked to the narrow shelf and steep relief around the island. It is however likely that they also use waters further off-shore as indicated by the occurrence of unidentified *Kogia sp.* from aerial surveys (mean distance from shore = 15.9 km, SD = 11.9, min = 2.9, max = 48.5). This spatial distribution is consistent with described habitat preferences for this species in other areas, although dwarf sperm whales were also recorded from oceanic waters elsewhere in the western Indian Ocean (Ballance and Pitman, 1998). Within the archipelago of Hawai'i, the mean depth of dwarf sperm whale sightings was 1425 m (SD = 954 m), with a range from 450 to 3200 m (Baird, 2005), and off the island of Hawai'i, sighting rates were highest in depths between 500 and 1000 m, and were lower than expected in shallower and deeper waters outside of this range (Baird et al., 2021). Around the Maldives, the species observed just outside the reef (Anderson, 2005). Off Great Abaco Island, in the Bahamas, dwarf sperm whales were always found in waters deeper than 300 m and were primarily distributed along the upper slope during winter, suggesting seasonal variability in their habitat preferences (Dunphy-Daly et al., 2008).

In this study, sightings of *Kogia* seemed to increase from September to November, during the austral summer. Although distribution surveys (boat-based and aerial) off the northern part of the island were conducted year-round in a systematic manner, further analyses accounting for effort and sea state would be needed to confirm seasonal trends. It would be reasonable to assume that for such a cryptic species the increased number of sightings in this period could reflect seasonal changes in weather and sea-state conditions. From eDNA sampling, *Kogia* were also detected during the austral summer, in the months of February and March. However, the sampling design did not allow for spatio-temporal comparison, which would require re-sampling each sampling site on a seasonal basis. No seasonal trends could be observed from the stranding events, which occurred throughout the year. Therefore, although the distribution data would tend to suggest an increased occurrence of *Kogia* during the austral summer, eDNA and stranding data were insufficient to confirm patterns of seasonality. Some evidence of seasonal movement has been documented off the Bahamas, with dwarf sperm whales mainly sighted during winter over slope habitats, while in summer, sighting rates decrease significantly in this stratum, together with group size (Dunphy-Daly et al., 2008). The authors suggest that this might reflect inshore-offshore movement, although it could also indicate a seasonal shift in distribution. Elsewhere, there is little evidence of seasonality, although seasonal differences in dwarf sperm whale stranding records have been observed in some areas that could reflect seasonal movements (McAlpine, 2009; Moura et al., 2016).

4.4 Group size and biology

Group sizes of dwarf sperm whales observed around Reunion ranged from 1 to 5 (mean = 2.0, SD = 1.2), with 46% of the sightings being of single individuals and the only sighting of pygmy sperm whales included 2 individuals. These results are consistent with the relatively small group sizes reported from other areas, with solitary animals or cow/calf pairs most commonly reported (Anderson, 2005; Baird et al., 2021; Ballance and Pitman, 1998; Dunphy-Daly et al., 2008; Ross, 1984). Around Reunion, sightings of calves were relatively common (n = 17) and occurred in groups of 3–5 individuals (except for a single mother-calf pair), suggesting some level of social grouping. However it remains to be investigated whether their social organization mirrors that of the common sperm whales, with females with their young forming social units (Best, 1979; Whitehead, 2003). Strandings data from South Africa tend to indicate that some age/sex segregation may occur, with groups consisting of solitary adult animals of both sexes, cow/calf pairs and small groups of immature animals (Plön and Baird, 2022; Ross, 1984).

Calves were observed in different months but the number of sightings were too few to provide any insights on breeding seasonality. However, the size of the foetus (58 cm) of the lone pregnant female examined during this study was consistent with the growth curve (fetal and calf body lengths plotted against date) established from stranding records of the dwarf sperm whale from the southern hemisphere (Pinedo, 1987) and indicated that birth would have occurred during the austral summer (Fig. 6). This estimate was also consistent with stranding data from South Africa, that indicates that births (and conception) occur between December and March (Plön, 2004). The month of stranding versus body size of calves reported in Reunion were also consistent with the southern hemisphere growth curve of *K. sima* from Pinedo (1987) and support a birthing period during the austral summer (Fig. 7). That this is not reflected in the sightings data might be linked to the difficulty of discriminating calves from juveniles at sea. The three *K. sima* calves that stranded in Reunion ranged from 98 to 118 cm in length which is consistent with the estimated body length at birth of approximately 1 m (Ross, 1979; Pinedo, 1987), although the 98 cm calf stranded in Reunion seem to be the smallest newborn individual recorded from the region (Plön, 2004; Ross, 1979). The necropsy indicated the presence of milk in the stomach, indicating that it was a neonate and not stillborn. The young *K. breviceps* stranded in September 2023 was 115 cm long, while length at birth has been estimated to be approximately 120 cm for this species (Ross, 1979). The species identification was based on the D/L ratio (4.5) and the number of teeth (26), but should be further confirmed genetically Fig. 8.

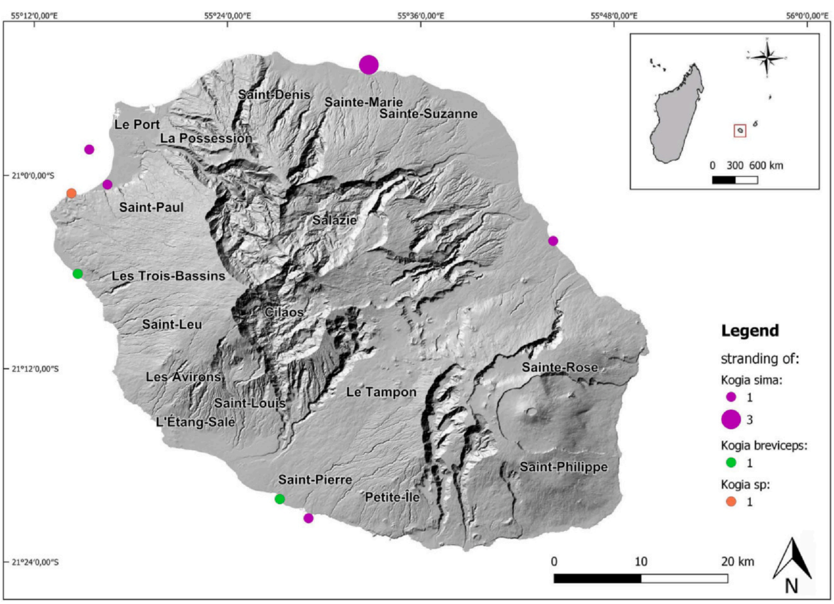

Fig. 7 Map showing the location of the 9 strandings of *K. sima*, *K. breviceps* and *Kogia sp*. around Reunion in 1993-2022.

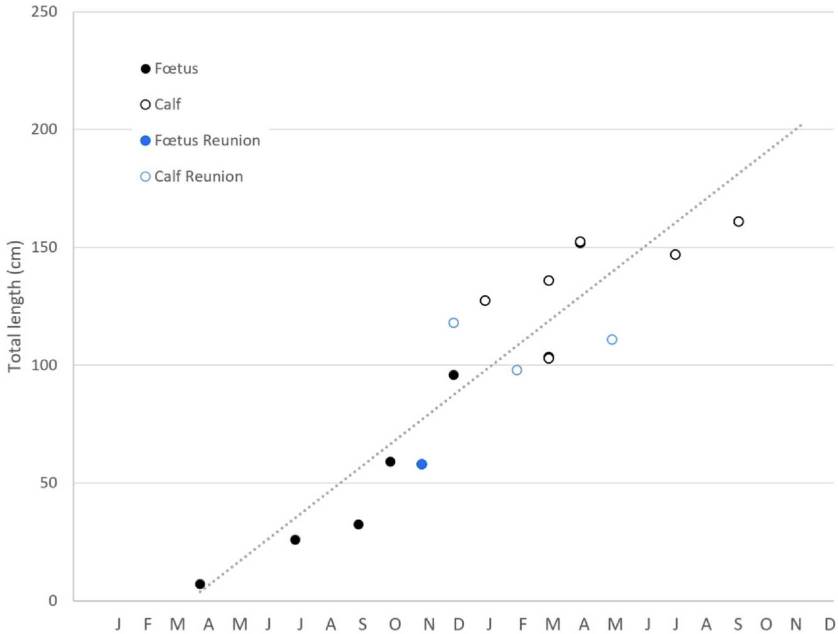

Fig. 8 Growth curve previously established by Pindeo (1987) for southern hemisphere *K. sima*, reporting the total length (in cm) of foetus versus month of foetus and calves, with added values for Reunion strandings.

4.5 Threats

Of the four fresh animals that were necropsied, three were in good body condition/nutritional state, with evidence of recent feeding, indicating that these animals were healthy enough to catch prey or to suckle milk shortly before they died. These findings, together with the results of the necropsies, ruled out any severe chronic causes of death, and are rather in favor of an acute event. The presence of an abundant sero-hemorrhagic tracheo-bronchial foam demonstrated that three animals died from hypoxia, inducing death. Indeed, hypoxia induces a loss of membrane integrity in the lung tissue, allowing fluid to leak into the airways. The fluid is then combined to residual air and forms the foam (Davis and Bowerman, 1990). For two animals, the thoracic tissues examination showed a pulmonary edema and foam was present in the lungs, indicating a possible drowning. Moreover, the presence of diffuse subcutaneous hematomas on the side or the ventral part of the body of these two animals is consistent with a mechanical trauma. For the female stranded in November 2020, disseminated petechiae were present in the melon, that would corroborate the trauma. All these findings (i.e., exclusion of other causes of death, persistent froth, oedematous lungs and bruises) have been described in cases of bycatch or ship strike (Kuiken, 1994). Hence, even if the exact cause of death for these two animals could not be ascertained, they are likely linked to anthropogenic activities. Reunion is located on one of the major shipping lanes of the south-western Indian Ocean, within which most of the marine traffic transiting between South Africa and Asia is concentrated (Tournadre, 2014) and the main commercial port is located in the north of the island, where relatively high concentrations of *Kogia* were reported.

5. Conclusions

In conclusion, this study utilized data from a variety of research approaches, including stranding reports, visual aerial and boat-based surveys, and environmental DNA techniques in order to provide new insights on the occurrence and distribution of *Kogia* in the waters of Reunion. The dwarf sperm whale, *K. sima*, was the most frequent species identified at sea and reported in stranding data. Comparatively, the pygmy sperm whale, *K. breviceps,* seemed to be less common, although uncertainty remains for some sightings which could not be identified to species level. Both species were detected in the eDNA water samples collected in the north of the island, confirming that dwarf and pygmy sperm whales are likely sympatric in Reunion waters, although habitat preferences could not be further clarified. Moreover, the reported strandings highlighted the importance of considering the vulnerability of these species to human activities. The confirmed high use of at least the northern part of the island serves as a scientific baseline for future research aimed at further investigating habitat use and suggests the need for further investigations into potential threats and conservation measures.

Furthermore, this multi-disciplinary study on *Kogia* highlighted the complementarity and limits of the different research approaches. The study confirmed the efficiency of environmental DNA in detecting and discriminating between the two *Kogia* species. This technique could be used in future surveys to further investigate seasonality. Visual surveys, while requiring substantial effort, were deemed effective under optimal sighting conditions, which should be considered when comparing levels of occurrence with other parts of the world where *Kogia* are reported to be relatively common. Aerial surveys were efficient for covering large areas but posed challenges in species identification. While strandings provided the first records of Kogia in Reunion, and are the only source of available information in some areas, the necropsies of stranded animals represented highly valuable resources for inferring threats to these cryptic species. Acoustic data were not investigated, but hydrophones were deployed around the island as a continuation of this study to enhance knowledge of *Kogia* occurrence and seasonality patterns.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at https://doi.org/10.1016/bs.amb.2024.08.003.

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