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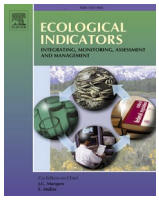

Environmental DNA helps reveal reef shark distribution across a remote archipelago

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ABSTRACT

Environmental DNA (eDNA) methods are being increasingly used in proof-of-concept studies to detect shark species, many populations of which are experiencing severe declines. These methods are widely seen as the future of biodiversity monitoring, but they have yet to become established as routine monitoring techniques for elasmobranch species. Here, we developed species-specific quantitative PCR assays for the detection of grey reef shark (*Carcharhinus amblyrhynchos*) and silvertip shark (*Carcharhinus albimarginatus*). We assessed whether species-specific eDNA methods could infer the distribution of the two species around the atolls of the Chagos Archipelago, which, despite being surrounded by a large marine protected area, experience contrasting levels of illegal fishing leading to heterogeneity in shark population densities. We found that eDNA detections were significantly reduced and sporadic around the northern atolls, which are under high pressure from illegal fishing. By contrast eDNA detections of both species were ubiquitous and consistent around the highly protected atoll Diego Garcia. We postulate that current levels of illegal, unreported and unregulated (IUU) fishing is having a significant impact on the shark community in the northern atolls and suppressing local reef shark populations. In the northern atolls we also employed visual and acoustic telemetry techniques to reveal the distribution of reef sharks. We found that despite eDNA samples being taken directly after visual surveys, detection results did not correlate, suggesting a need for further optimisation of eDNA methods for detecting sharks. However, both species were detected by eDNA in sites where they were not observed, highlighting that the scale of the sampling environment must be considered when inferring eDNA results and showing that eDNA methods can be used to fill gaps in data from more established monitoring techniques. We conclude that eDNA methods should be used in combination with other techniques to provide a complete picture of shark distribution so that threatened species can be better protected.

1. Introduction

Approximately one-third of elasmobranchs (sharks, skates and rays) are threatened with extinction as a result of overfishing and overexploitation (Dulvy et al., 2021). Reef sharks are functionally important mesopredators (Roff et al., 2016) that have experienced population declines across the world (Robbins et al., 2006; Graham et al., 2010) and may now be functionally extinct in the waters of at least

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eight nations (MacNeil et al., 2020). As many elasmobranch species are highly mobile and elusive, having a versatile methodological toolkit for species monitoring has become increasingly important in the face of these declines.

Established elasmobranch monitoring techniques to identify the presence and abundance of species include analyses of fishery-dependent data (Nakano and Clarke, 2006) and scientific fishing surveys (Simpfendorfer et al., 2002), baited remote underwater video systems (BRUVS) (MacNeil et al., 2020; Sherman et al., 2020) and visual censuses by divers (Robbins et al., 2006; Graham et al., 2010; Rizzari et al., 2014). Additionally, movement and behavioural patterns can be revealed through electronic tagging (Andrzejaczek et al., 2022; Espinoza et al., 2011; Jewell et al., 2013). However, the effectiveness and logistics of applying these approaches for monitoring rare species in marine habitats, including marine protected areas (MPAs), can be limited. Subsequently, species-specific amplification of environmental DNA (eDNA) collected in water samples is being increasingly applied to detect elasmobranchs (Sigsgaard et al., 2016; Simpfendorfer et al., 2016; Gargan et al., 2017; Weltz et al., 2017; Lafferty et al., 2018; Lehman et al., 2020; Postaire et al., 2020; Schweiss et al., 2020; Budd et al., 2021). By identifying species from these samples, researchers can gather information on their presence without the need for direct observation. Although the majority of eDNA studies focus on species presence (Rourke et al., 2021), recently, eDNA data have been used to produce estimates of fish species abundances in certain areas (Doi et al., 2015; Lacoursière-Roussel et al., 2016; Spear et al., 2021).

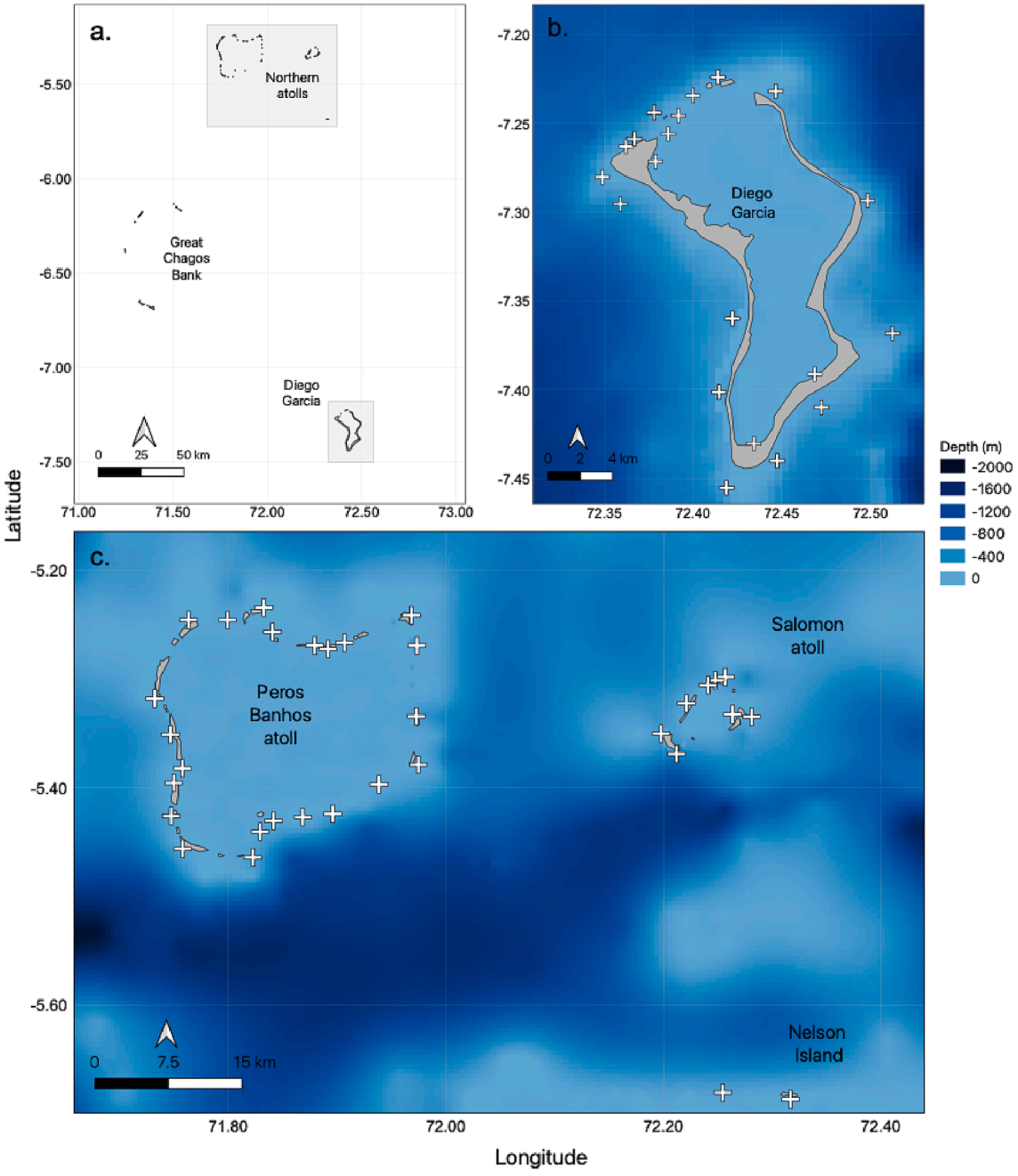

Fig. 1. Sampling site for the current study. Map (a) shows the islands within the Chagos Archipelago MPA and the location of each region surveyed in this study are highlighted in grey. The eDNA sampling sites are shown as white crosses for the 2019 eDNA survey around Diego Garcia (b) and for the 2021 survey in the northern atolls (c) where the crosses also represent receiver locations for the acoustic array. The bathymetry data displayed on the main map was downloaded from GEBCO (gebco.net).

As eDNA methods have developed, researchers have found them to be more sensitive, more effective and cheaper than 'traditional' methods for species detection (Fediajevaite et al., 2021). Species-specific eDNA methods have been shown to be more effective than established catch-based monitoring techniques for both marine (Schmelzle and Kinziger, 2016; Zhu et al. 2023) and freshwater (Pawlowski et al., 2018) fish species. For shark species, eDNA metabarcoding has been shown to detect a number of species that were not detected by conventional surveys in the south-western Pacific (Boussarie et al., 2018) and in Singapore (Ip et al. 2021). However, the eDNA method also missed species that were detected with the other techniques (Boussarie et al., 2018). The sensitivity of species-specific eDNA methods for monitoring sharks compared to more established monitoring techniques has not yet been assessed.

The Chagos Archipelago in the central Indian Ocean is encompassed by a large no-take MPA (Sheppard et al., 2012), but continued Illegal, Unreported and Unregulated (IUU) fishing activity (Collins et al., 2021; Ferretti et al., 2018; Jacoby et al., 2020) has coincided with populations of reef sharks experiencing declines (Graham et al., 2010). Presently, IUU activity is focused around the northern atolls of the MPA (Collins et al., 2021; Ferretti et al., 2018; Hays et al., 2020; Jacoby et al., 2020; Tickler et al., 2019), as the presence of a military base on Diego Garcia atoll (ca. 200 km from the northern atolls, Fig. 1), effectively deters IUU activity in the local vicinity (Collins et al., 2021; Ferretti et al., 2018; Jacoby et al., 2020). As a result of long-term exploitation, populations of grey reef shark (*Carcharhinus amblyrhynchos*) and silvertip shark (*Carcharhinus albimarginatus*) around the archipelago are significantly below historical baselines (Ferretti et al., 2018). To gain a better understanding of their spatial and temporal activity in the archipelago, individuals from each species have recently been the subject of a multi-year acoustic tracking programme (Andrzejaczek et al., 2020; Carlisle et al., 2019; Curnick et al., 2020; Jacoby et al., 2020; Tickler et al., 2019; Williamson et al., 2021), which has yielded fine-scale insights into how they move around the atolls (Carlisle et al., 2019; Jacoby et al., 2020; Williamson et al., 2021). This research has estimated that the activity spaces of *C. amblyrhynchos* and *C. albimarginatus* individuals from the northern atolls do not extend to Diego Garcia (Jacoby et al., 2020), suggesting that there is little movement between the two areas.

Although the majority of shark research in the MPA has been carried out in the northern region (Jacoby et al., 2020; Williamson et al., 2021) anecdotal reports and accounts suggest that, compared to the more protected waters around Diego Garcia, IUU activity is suppressing reef shark densities in the area. In this study, we undertook a comprehensive eDNA survey around the unfished site of Diego Garcia as well as illegally fished sites in the northern atolls of the archipelago, focusing on the presence of *C. amblyrhynchos* and *C. albimarginatus*. Using the fished and unfished sites as reference locations which likely reflect heterogeneity in reef shark population densities, we evaluated the likelihood of detecting each species using species-specific eDNA methods and compared results with concurrent visual survey results and distribution patterns revealed through a long-term acoustic telemetry study.

2. Materials and methods

2.1. Field sampling

Water samples were collected across two research expeditions to the Chagos Archipelago in September 2019 and May 2021. In 2019, a total of 32 samples were taken from 20 sites in a systematic survey around Diego Garcia atoll (Fig. 1) at the surface and a depth of 40 m where possible on the outside of the atoll, this was to target sampling above and below the estimated thermocline for the region for a metabarcoding study (Dunn et al., 2022). The sampling sites in 2021 corresponded with 33 receiver locations in an established acoustic array around Peros Banhos Atoll, Salomon Atoll, and Nelson Island, deployed around the archipelago between 2013 and 2021 (Carlisle et al., 2019; Jacoby et al., 2020) (hereafter 'the northern atolls', Fig. 1). As Dunn et al. (2022) found little difference in metabarcoding detection rates for the two target species between the surface and 40 m samples, we chose to sample around the northern atolls at 15 m depth to match the depth of the acoustic array receivers. At each sample location across both surveys, a 5-litre (L) Niskin bottle was rinsed with surface water and then deployed from the side of a small boat to sample at the desired depth. To minimise the effects of temporal degradation, each sample was then decanted into 5L sterile bottles stored on ice for transport back to the research vessel, and filtered within three hours of collection. To avoid contamination, gloves were worn when setting the Niskin bottle and decanting into the 5 L bottles and samples. Filtration on the research vessel was performed in three 1L subsamples using vacuum filtration through 0.22 μm Sterivex^{TM} (Merck-Millipore) filter capsules. The 5L bottle was inverted 10 times to mix the water within and tubing connected to the Sterivex^{TM} capsule was placed in the bottle, once one litre had been filtered, the filter was run dry and then removed from the vacuum tubing. The Sterivex^{TM} filter capsule was then filled with 2.5 mL of Longmire's buffer (Renshaw et al., 2015). Capsules were capped at both ends and the three subsamples were placed in 100 mL Whirlpak^{TM} bags for storage at room temperature and transport. After sample processing, bottles and vacuum tubing were soaked in 20% bleach for 20 min and rinsed thoroughly to remove any residue and surfaces were wiped with 20% bleach followed by absolute ethanol. A negative control of 500 mL sterile water was filtered after every wash cycle to test for any cross-contamination and gloves were worn throughout the filtration to minimise the potential of sample contamination.

2.2. DNA extraction

Before the commencement of any lab work, surfaces were wiped with 20% bleach followed by absolute ethanol and gloves were worn throughout. Extraction of DNA from tissue for assay testing was done using the DNeasy Blood & Tissue Kit (Qiagen) following the manufacturer's protocol and eluting the DNA in 100 μL nuclease-free water. DNA extraction from the filters followed the protocol described by Spens et al. (2017) using the DNeasy Blood & Tissue Kit (Qiagen). The lysis solutions from the capsule and buffer components were combined before the addition of buffer AL and the continuation of the Blood & Tissue Kit protocol. Final DNA elution occurred with 100 μL nuclease-free water and negative extraction controls were performed with distilled water in place of the buffer. Extracted DNA was quantified with a Qubit 4.0 fluorometer (Life Technologies) and stored at −20 °C.

2.3. Quantitative PCR assays

Species-specific primer regions for the target species were identified using mitogenome sequences from 182 elasmobranch species and new mitogenome sequences for *C. amblyrhynchos* and *C. albimarginatus* (Dunn et al., 2020; Johri et al., 2020), aligned using the MUSCLE (Edgar, 2004) alignment tool in Geneious Prime (2021.1). The resulting searches identified regions within the NAD5 gene that contained sufficient variation for the design of species-specific primers and hydrolysis probe sequences for our target species. Assays for the detection of each species from eDNA samples were first tested for specificity *in silico* in Geneious Prime and then in quantitative PCR (qPCR) reactions using non-target DNA.

Non-target tissue samples were obtained from various sources, including fin clips stored in absolute ethanol taken during previous expeditions to the archipelago and from seized catches from IUU fishing activity around the Chagos Archipelago, additional non-target species tissue stored in absolute ethanol was obtained from the sample sharing site Otlet (otlet.io). DNA was extracted from the tissue using the Qiagen Blood & Tissue Kit, following the manufacturer's protocol and eluting with 100 μL PCR-grade water. To confirm their taxonomy, DNA extracts from samples taken from animals within seized IUU catches were

amplified using the protocol outlined by Holmes et al. (2009) with the FishF1, FishF2, FishR1, FishR2 (Ward et al., 2005) and HCO2198 (Folmer et al., 1994) primers. The resulting PCR products were sequenced using Sanger sequencing, performed by GENEWIZ (UK). The species was confirmed from the returned sequences by assigning the top hit from the web-based BLASTn in the NCBI nucleotide database with a > 97% sequence identity match and 100% query coverage. The non-target species used in the specificity testing were sandbar shark (*Carcharhinus plumbeus*), silky shark (*Carcharhinus falciformis*), bull shark (*Carcharhinus leucas*), spinner shark (*Carcharhinus brevipinna*), spot-tail shark (*Carcharhinus sorrah*), whitetip reef shark (*Triaenodon obesus*), great hammerhead shark (*Sphyrna mokorran*), scalloped hammerhead shark (*Sphyrna lewini*), tiger shark (*Galeocerdo cuiver*) and blue shark (*Prionace glauca*). *C. albimarginatus* was also tested as a non-target species in the *C. amblyrhynchos* assay and vice versa.

In qPCR, a gradient of annealing temperatures between 57 and 62 °C was run to determine the optimum temperature for the assay with target DNA. The amplification of DNA from each non-target species (ca. 10 ng/μL per species) was then tested at this temperature. If there was no non-target amplification, the assay was accepted as species-specific. Each assay was optimized for primer and probe concentration for 15 μL reactions by performing concentration gradient experiments. For both assays, the final volumes of each element of the qPCR were as follows: 7.5 μL Taqman environmental master mix 2.0 (Thermo Fisher), 0.5 μL of each primer (10 μM) (final concentration 333 nM), 0.5 μL hydrolysis probe 2.5 μM (final concentration 83.3 nM), 1 μL PCR grade water and 5 μL template DNA and the thermal profile of the qPCR assays were: 95 °C for ten minutes followed by 45 cycles of 95 °C for 15 s followed by 60 °C for one minute. All reactions were run on a Roche LightCycler 96 (Roche).

G-blocks of each respective amplicon were obtained from Integrated DNA Technologies (IDT) so that accurate copy number calculations using qPCR could be produced. These were resuspended following the manufacturer's protocol and their concentration was confirmed using a Qubit 4.0 fluorometer. The copy number of each G-block was calculated using the ThermoFisher Scientific online calculator. A dilution series standard curve consisting of ten replicates per dilution was run for each assay to determine the limit of detection (LOD) and the limit of quantification (LOQ) in copies of amplicon per reaction. We defined the LOD for each reaction as the lowest value with at least one positive replicate being amplified before 45-cycles following Hobbs *et al.* (Hobbs et al., 2019) and the LOQ values were calculated statistically using the eLowQuant script (Lesperance et al., 2021).

Amplification of extracted DNA from the three field subsamples was run in triplicate, therefore resulting in nine PCR reactions per site. Reactions were run on 96-well plates with a standard curve of a 10-fold dilution series ranging from 100 copies/reaction to 1 copy/reaction to act as a positive control, and three non-template negative controls (NTC) on each plate. Only values above the LOD for their respective assay were retained for further analysis and only detections above the LOQ were used for quantitative analysis. A linear model of logged copy number and cycle-threshold value (Cq) was produced for all standard curve values above the LOQ and these models were used to calculate the quantity of DNA in the field samples from their Cq values, allowing concentrations to be standardised across reaction plates.

There was no amplification in any negative control from the 2021 samples (n = 9) but there was amplification in three of the negative controls taken during the 2019 samples (n = 8) (range 0.2 – 1.4 copies/reaction). Subsequently, the calculated concentration in the negative was subtracted from the concentration of the affected samples to result in a corrected concentration for that sample, if the corrected concentration for the sample was lower than the LOD for the reaction, it was considered negative. This resulted in the removal of five 'detections' in the *C. amblyrhynchos* samples and two in the *C. albimarginatus* samples. Non-parametric Mann-Whitney U tests were then run in R version 4.0.3 (R Core Development team, 2020) to test for significant differences between the average concentration of detections above the LOQ between northern atolls and Diego Garcia and z-tests were used to test for statistical significance in the differences in probabilities of detection. As the samples were taken across two expeditions in different years and there is little evidence to show that sharks move from one region to the other (Jacoby et al., 2020), we believe that the assumptions of independence for these statistical tests are met in our data.

2.4. Acoustic receiver data and visual survey

During the 2021 survey of the northern atolls, immediately prior to water sampling, divers on SCUBA collected each receiver from its mooring (15–25 m depth) and performed a stationary-point-count (SPC) survey for 10 min recording the number of individuals of any shark species. This order of sampling and surveys allowed water samples to be taken and processed as quickly as possible to avoid degradation. *C. amblyrhynchos* individuals were identified through distinctive black markings along the caudal fin and *C. albimarginatus* individuals were identified by white tips to their dorsal and pelvic fins. It was not possible to carry out visual surveys during the 2019 survey of Diego Garcia.

The receiver array dataset from the northern atolls consisted of records from 33 VEMCO VR2W receivers and all acoustic tags deployed were VEMCO V16 4H coded transmitters (69 kHz) with tagged individuals from *C. amblyrhynchos* (n = 88) and *C. albimarginatus* (n = 103). Data was manipulated using "dplyr" (Wickham et al., 2021) and visualised with "ggplot2" (Wickham, 2016) in R v4.0.3 to investigate the historical hotspots of *C. amblyrhynchos* and *C. albimarginatus* activity in previous years. To avoid the potential of false detections (Simpfendorfer et al., 2015), only detections from animals with known ID codes were used for the analyses following Williamson et al. (2021) and any repeat detections from the same ID code at the same receiver within the minimum repeat time (30–125 s) were removed from the dataset. To remove any seasonal artefacts in the data, we removed detections from outside March, April and May to reflect activity during our eDNA sampling period and standardised these detections by the number of days across all years that receivers were active in these months. The remaining data were then taken forward for statistical analyses and for visualising shark hotspots in the northern atolls. We used generalised linear models in R v4.0.3 to investigate the relationships between eDNA detection and non-detection and either visual detection or historical receiver pings at a site using a binomial error distribution. Finally, the relationship between visual detections and historical receiver pings was investigated using a similar approach, but with a Gaussian error distribution.

3. Results

3.1. qPCR assays

Primers and probes for each species were designed to amplify specific regions of the NAD5 gene (Table 1). The assays were found to be specific to the target species (Fig. S1) and both showed optimal amplification with a 60 °C annealing/extension temperature in qPCR. The LOD was calculated to be 0.1 copies per reaction for both assays. This sensitivity suggests that if the target is present at a concentration of one copy per 50 μL of extracted DNA, there will be amplification in one out of ten PCR reactions. We therefore defined detection at a site as positive if one of the nine PCR replicates per site (total 45 μL DNA template across nine PCR reactions) achieved amplification above the cycle-threshold value of the LOD. The LOQ was 0.8 copies per reaction for the *C. amblyrhynchos* assay (Fig. S2) and 3.1 copies per reaction for the *C. albimarginatus* assay (Fig. S3), similar to those calculated with the eLowQuant code previously (e.g. Matthias et al., 2021).

3.2. Differences in eDNA detection across atolls

Around Diego Garcia, eDNA from *C. amblyrhynchos* was detected in

Table 1
Sequence and melting temperature for each oligonucleotide used in the qPCR assays for grey reef shark (*Carcharinus amblyrhynchos*) and silvertip shark (*C. albimarginatus*) detection.

Species	Oligo type	Sequence 5′-3′	Melting temperature (°C)
C. amblyrhynchos	Forward primer	CACCAACTCTCACTTCAAAATTAATCCTAT	55.5
	Reverse primer	CAGATCGATTAGATGTGTTGAGACATG	55.4
	Probe	[6FAM]-TAGTTACAATCATAGGTCTTCTCCTAGCT-[BHQ]	56.3
C. albimarginatus	Forward primer	ATTGCCGGCCTAATCATTACTCTC	56.9
	Reverse primer	GTGAGGATGGAGTGTGGGG	57.6
	Probe	[6FAM]-AGCCCTCTTAGTCACAATTTCAGGCCTCCT-[BHQ]	64

24 of the 32 samples, showing widespread distribution around the atoll (Fig. 2). There were 102 positive replicates in 276 reactions (37.0%) and there was just one site where all PCR replicates amplified (see Table S1 for full table of detections by site). There were 56 reactions that exceeded the LOQ for the assay, the highest recorded concentration was 84.9 copies per reaction and the mean of the quantifiable reactions was 10.4 (standard error ± 2.9) copies per reaction. There was no significant difference between the proportion of positive replicates and sampling depth. In the northern atolls, *C. amblyrhynchos* was detected in 5 of the 33 water samples with no sample achieving consistent amplification across all nine PCR replicates. There were seven positive reactions out of a total of 297 (2.4%), five exceeded the LOQ for the reaction and the highest concentration of 3.11 copies per reaction was detected in site SA03, the mean concentration for the quantifiable reactions was 1.76 (±0.4) copies per reaction. The proportion of detections around Diego Garcia was significantly greater than the proportion of detections in the northern atolls ($\chi^2 = 108.6$, $p < 0.001$) but the difference in average concentration between the detections above the LOQ between the two locations was not found to be significantly different.

C. albimarginatus was detected in 29 of the eDNA samples taken around Diego Garcia (Fig. 2) with 100% of the technical replicates amplifying in six samples and no significant difference between the proportion of positive replicates and sampling depth. There were 147 positive replicates in 276 reactions (53.3%) and 95 of these exceeded the LOQ for the assay. The highest recorded concentration was 248.3 copies per reaction with a mean of 34.6 (±6.7) copies per reaction. In the northern atoll samples, *C. albimarginatus* was detected at five sites, in just six reactions (2.0%). No samples achieved 100% amplification in the PCR replicates and two reactions exceeded the LOQ for the assay, with a mean of 3.36 (±0.2) copies per reaction. The proportion of detections around Diego Garcia was significantly greater than the proportion of detections in the northern atolls ($\chi^2 = 188.7$, $p < 0.001$) and the average concentration was significantly higher in the samples from Diego Garcia compared to the northern atolls ($W = 181.5$, $p < 0.05$).

3.3. Telemetry

In the northern atolls of the archipelago, from February 2013 to March 2021, the number of acoustic detections for *C. amblyrhynchos* was 942,692, of which 171,178 were from March, April and May. The maximum number of detections from a single individual was 13,657, the minimum was one and the median was 323 (Q1: 37, Q3: 1889). There was a total of 1,519,050 detections from the tagged cohort of *C. albimarginatus*, of which 40,188 were from March, April and May. The maximum number of detections from a single individual was 6,087, the minimum was one and the median was 197 (Q1: 53, Q3: 562.25). There were clear hotspots for acoustic detections in the northwest of Peros Banhos atoll for tagged *C. amblyrhynchos* (Fig. 3a) which has remained

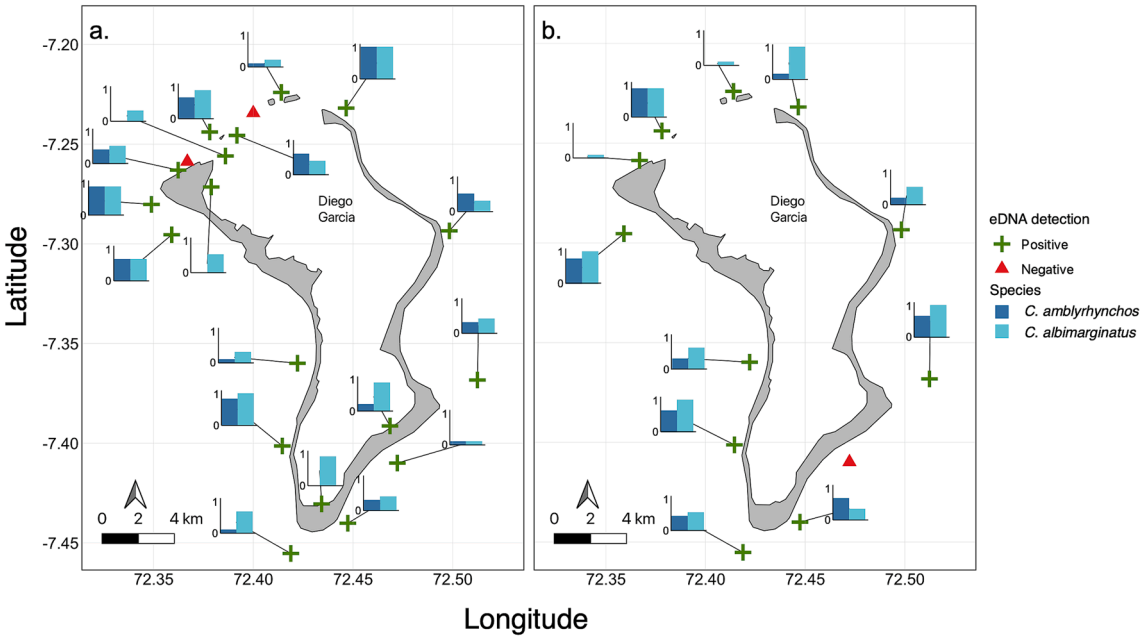

Fig. 2. Detection of each species around Diego Garcia from eDNA samples. Samples with detection of at least one species are denoted by green crosses and samples with no detection are shown as red triangles. The proportion of positive technical replicates for *Carcharhinus amblyrhynchos* (dark blue) and *Carcharhinus albimarginatus* (light blue) in each eDNA sample taken around Diego Garcia atoll in surface water (A) and at 40 m depth (B). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

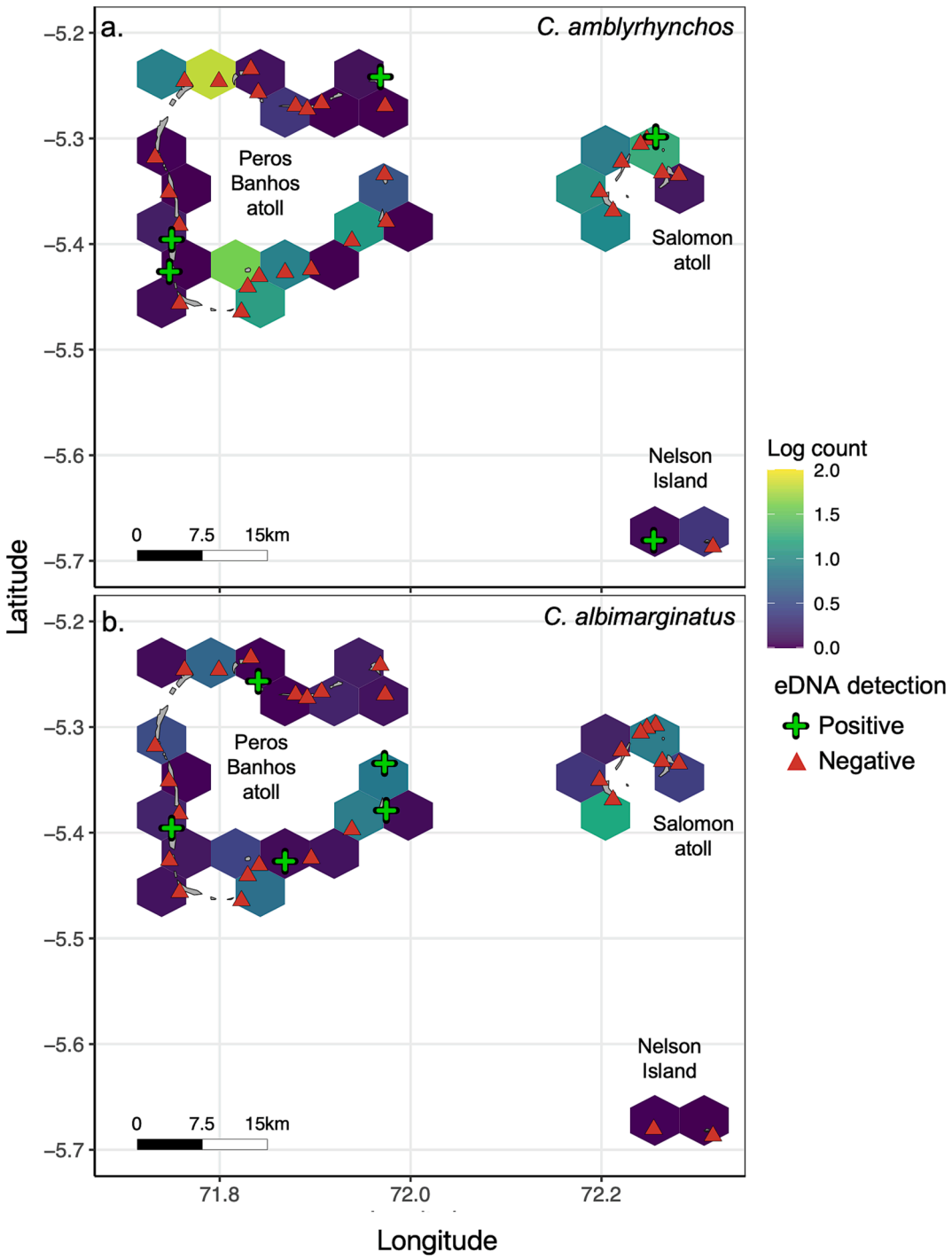

Fig. 3. Activity hotspots from acoustic telemetry. The log mean number of acoustic detections per day in March, April and May for all active years of the acoustic array around the northern atolls of the Chagos Archipelago MPA for (a) *Carcharhinus amblyrhynchos* and (b) *Carcharhinus albimarginatus* and the position of each receiver shown by the detection (green cross) or non-detection (red triangle) of eDNA for the species in the 2021 survey. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

consistent across years (Fig. S4), and in the southwest of Salomon Atoll for *C. albimarginatus* (Fig. 3b) which is also consistently identified across years (Fig. S5). No eDNA for these species was detected in these areas but there was detection around high-activity receivers in the northwest of Salomon Atoll for *C. amblyrhynchos* (Fig. 3a) and the west of Peros Banhos for *C. albimarginatus* (Fig. 3b). However, there were no significant relationships between the average daily acoustic detections and eDNA detection at a receiver site (Fig. S6). Across all active years, the number of acoustic detections of *C. albimarginatus* at each receiver shows a general trend of peaking in June, particularly around Salomon Atoll (Fig. S8). For *C. amblyrhynchos*, this trend was less evident (Fig. S9).

3.4. Visual surveys

In the visual surveys around the northern atolls, a total of 46 *C. amblyrhynchos* individuals were observed in the visual surveys, with the species detected in 13 of the 33 sites. Ten *C. albimarginatus* individuals were seen across two sites but the species was not seen in the

remaining 31 sites. There were sightings of *C. amblyrhynchos* in the northwest Peros Banhos acoustic telemetry activity hotspot (Fig. 4a), but there were no observations of *C. albimarginatus* around the telemetry hotspots around Salomon Atoll (Fig. 4b). There was just one occasion where a given species was observed and that same species was detected in the eDNA sample at that site (Fig. 4a), there was no relationship between the number of individuals seen in a visual survey and eDNA detection at that site for either species (Figs. S6 and S7).

4. Discussion

As many shark populations experience declines that threaten their existence (Dulvy et al., 2021), improved management and conservation practices are required (Birkmanis et al., 2020). The use of eDNA methods for species detection has the potential to revolutionise biodiversity monitoring by providing vast amounts of data from sampling techniques that can be replicated across the globe. Here, highly sensitive and specific assays were developed for the detection of two ecologically important reef-associated shark species from eDNA. The assays were used to assess distributions across the Chagos Archipelago by sampling eDNA in the upper water column in areas of high and low instances of IUU fishing. Around Diego Garcia, where there is no documented evidence of IUU fishing for sharks, probabilities of eDNA detection were significantly higher for both species compared to the northern region of Peros Banhos, Salomon Atoll, and Nelson Island, where the species are under greater threat from IUU fishing (Jacoby et al., 2020) which is potentially suppressing populations (Graham et al., 2010). There was little correlation between eDNA detections and concurrent visual

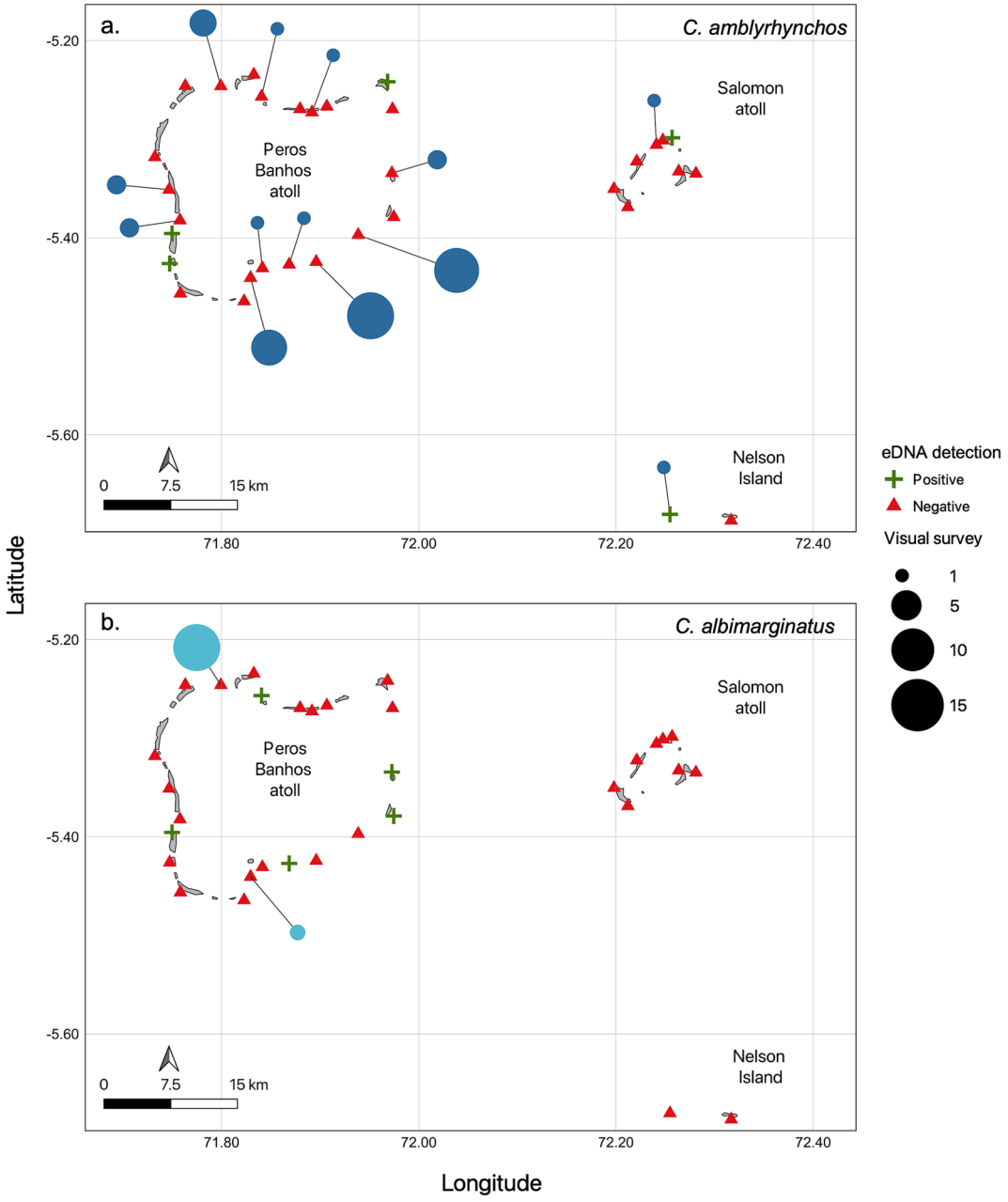

Fig. 4. Visual survey results. The number of individuals seen in the 10-minute stationary point count visual surveys in the northern atolls of the Chagos Archipelago for (a) *Carcharhinus amblyrhynchos* and (b) *Carcharhinus albimarginatus*. The area of the circle relates to the number of individuals seen and the position of each sampling site is shown by the detection (green cross) or non-detection (red triangle) of eDNA for the species in the 2021 survey. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

surveys or historical acoustic telemetry detection hotspots in the northern atoll region. Overall, these results indicate that species-specific eDNA methods can be used to assess broad-scale patterns of shark distributions and that they should be utilised in combination with more established monitoring methods to produce a complete picture of shark distributions in an area.

In our comparison of eDNA detection between Diego Garcia and the northern atolls region, we found significant differences in detection probability for both *C. amblyrhynchos* and *C. albimarginatus*. Whilst eDNA snapshots such as these cannot be used to quantify population abundances, it is likely that the continued IUU fishing is driving differences in population densities across the two regions and that this is being reflected in our eDNA results. Hydrodynamic modelling in the region suggests that wave energy around Peros Banhos is similar to that around Diego Garcia (Wu et al., 2021), suggesting that the observed pattern is not caused by differential current speeds driving differences in the dispersion of eDNA. This indicates that eDNA methods have the potential to assess broad-scale patterns of shark distributions. Repeated site visits should be considered so that robust occupancy estimates can be generated for each species across sites and this study should be followed up with a systematic, standardised, and multidisciplinary survey to further investigate shark populations across the archipelago. As there was a significant interval between the two surveys (September 2019 for Diego Garcia and March 2021 for the northern atolls), the differences could be due to interannual variation in shark distributions in the archipelago. However, this seems unlikely as the estimated activity spaces of the cohort of sharks tagged in the northern atolls do not extend to Diego Garcia (Jacoby et al., 2020). Differences in oceanographic conditions and water currents across the atolls may result in variability in localised eDNA retention. Yet, the fine-scale hydrodynamic data needed to investigate this is not currently available for the area, as the required oceanographic analysis has not yet been undertaken in the archipelago.

There is evidence to suggest that species monitoring using eDNA can be used to provide managers with localised distributions of target species (Jeunen et al., 2019; Lacoursière-Roussel et al., 2016; Larson et al., 2022; Yamamoto et al., 2016), enabling targeted and effective conservation measures to be put in place. In this study, individuals of *C. amblyrhynchos* were detected at 13 sites in the northern atolls during the visual surveys and five sites in the eDNA survey, and there was just one site where the species was detected by both methods. It is important to note that due to the logistics of sampling on a multidisciplinary expedition, only one sample per site was taken to provide a snapshot of shark distribution through both visual surveys and eDNA. Repeated sampling may have resulted in more agreement between the methods. However, there are a number of reasons that could have caused the mismatch, including stochasticity of sampling a large expanse of a heterogeneous environment with relatively small water volumes and potential disparity between individual presence and eDNA presence in an area, related to the shedding rates of the individuals and degradation rates of eDNA, which would be expected to degrade rapidly in the study area where surface water is around 30 °C (Sigsgaard et al., 2016; Jo et al., 2019). On the other hand, *C. albimarginatus* was detected in more sites from eDNA (five) than in visual surveys (two). This difference may be due to the species' tendency to inhabit pelagic waters and spend more time away from the reef (Curnick et al., 2019; Williamson et al., 2021) resulting in few sightings, which highlights that visual surveys can also be subject to biases. There is also potential that these detections were due to eDNA being transported to the site artificially by boats and divers. However, we believe that this is unlikely, as such a contamination event would likely have resulted in far more than the five positive detections for the species. As eDNA can move with water current, the eDNA particles we detected may have been advected to the site from their origin off the reef. Taken together, the inconsistency between the two methods emphasises the potential for eDNA methods to complement visual surveys for reef shark detection but highlights that further methodological optimisation may be required for consistent detection of sharks in eDNA studies.

When producing telemetry hotspots to compare eDNA and visual detections against, we did not have acoustic telemetry data from the time of sampling available. Instead, we used detection data from the historical dataset to identify hotspots of activity for the populations. Tagging locations were spread widely across the northern atolls (see Jacoby et al., 2020 Fig. 1) and analysis has shown that individuals move frequently between receiver sites (Jacoby et al., 2020) with no evidence of site residency. The mean number of detections per day at each receiver was used as a proxy for activity and it was hypothesised that if these hotspots remained stable across years, the mean number of detections at each site would directly correlate with eDNA concentration at that site. This was not the case. For example, there was a stable hotspot for *C. amblyrhynchos* detections in the northwest of Peros Banhos atoll, a location where we also observed individuals of the species but there was no eDNA detection at the site. As these two data series were not taken at the same time, there are many factors that could have resulted in the mismatch between detection results. For example, the behavioural (Tickler et al., 2017) and biological (Heupel and Simpfendorfer, 2014) factors may have influenced the distribution of sharks at receiver sites during the sampling period in a contrasting manner to previous years. The ephemeral nature of eDNA would also have contributed to a mismatch if eDNA had been shed and then degraded or advected away from the site before we sampled. We also do not know how the ongoing IUU fishing activity has impacted the behaviour of sharks in the region and how many individuals from the tagged cohort remain in the area (Tickler et al., 2019). However, the comparison of acoustic data and eDNA detection has great potential for developing our understanding of the sensitivity and reliability of eDNA in the field and should be further explored.

The ability to monitor fine-scale ecological patterns of species distributions is essential for the development of effective conservation strategies and when assessing and monitoring biodiversity, the scale of inference from the sampling method being employed is an essential consideration. Acoustic telemetry is often highly valuable for assessing fine-scale distribution and movement patterns of sharks (Jacoby et al., 2020; Williamson et al., 2021) and visual surveys can be performed consistently over long timeframes to build pictures of how population densities change over time (Robbins et al., 2006). In this study, eDNA methods revealed the presence of highly mobile and elusive reef shark species. Importantly, shark eDNA detection rates were also congruent with postulated reef shark population densities driven by well documented illegal fishing activity. Thus, this paper provides the first quantifiable evidence of the negative impact of IUU fishing on reef sharks around the Chagos Archipelago and its associated MPA. Therefore, monitoring via eDNA offers a simple and effective route towards long-term monitoring of species presence and population dynamics which can reinforce outputs from established monitoring techniques and allow researchers to gain a greater understanding of the distribution of sharks in an area.

CRediT authorship contribution statement

Nicholas Dunn: Conceptualization, Methodology, Investigation, Visualization, Writing – original draft, Writing – review & editing. **David J. Curnick:** Conceptualization, Methodology, Supervision, Writing – review & editing. **Chris Carbone:** Conceptualization, Supervision, Writing – review & editing. **Aaron B. Carlisle:** Methodology, Writing – review & editing. **Taylor K. Chapple:** Methodology, Writing – review & editing. **Rosalie Dowell:** Methodology, Writing – review & editing. **Francesco Ferretti:** Methodology, Writing – review & editing. **David M.P. Jacoby:** Methodology, Writing – review & editing. **Robert J. Schallert:** Methodology. **Margaux Steyaert:** Methodology, Writing – review & editing. **David M. Tickler:** Methodology. **Michael J. Williamson:** Writing – review & editing. **Barbara A. Block:** Methodology, Writing – review & editing. **Vincent Savolainen:** Conceptualization,

Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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