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Metabarcoding gillnets to assess unaccounted catch depredation or escape

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Abstract

Gillnets are the world's most common net-based fishing gear, comprising walls of light mesh designed to entangle fish. Gillnets are often retrieved with holes in the netting, which means some animals escape or are depredated unseen, but with some mortality. To effectively manage fisheries around the world, information is required on not only the harvested and discarded mortalities, but also problematic interactions and mortalities caused by the fishing gear and especially those involving protected species. This study sought to assess a novel method for determining such interactions by sampling five adjacent pieces of netting around each of ten holes in two bather-protection polyethylene gillnets for environmental DNA or "eDNA". Here we show that eDNA correctly identified all previously entangled-and-landed species. Also, eDNA from three uncaptured taxa were recorded: bull shark, *Carcharhinus leucas*, white shark, *Carcharodon carcharias* and dolphins (Delphindae), illustrating the potential to reveal previously cryptic gillnet interactions. We propose that as scientific methods evolve and autonomous real-time DNA surveillance becomes routine, eDNA testing of fishing gears and vessels could provide a novel, complementary fishery-monitoring tool.

KEYWORDS
fisheries, forensics, illegal, metabarcoding, trace DNA, illegal, unreported and unregulated (IUU) fishing

1 | INTRODUCTION

No fishing gear is entirely selective for the targeted catches, which means that in addition to a global harvest from marine fisheries of ~80 million tons per annum, an additional ~10 million tons of non-target organisms (termed bycatch) is discarded, mostly dead (Zeller et al., 2018). These collateral mortalities have raised concerns over deleterious cascading effects on ecosystems, especially among priority stocks which include endangered, threatened, and protected (ETP) species (Gray & Kennelly, 2018; Hall, 1996; Pacoureau et al., 2021; Zeller et al., 2018). Recognition of these issues has supported ongoing global bycatch resolution efforts, typically via technical modifications to fishing gears, but also spatial and temporal fishing closures (reviewed by Broadhurst, 2000; Gilman et al., 2006; Hamilton & Baker, 2019; Uhlmann & Broadhurst, 2015).

Beyond bycatch mortality are other important, less-studied impacts of fishing gears involving cryptic injuries or deaths of organisms after escaping or being depredated (Broadhurst et al., 2006;

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Uhlmann & Broadhurst, 2015; Warden & Murray, 2011). The extent of collateral impacts is likely gear-specific, but some fishing methods, and especially gillnetting, probably evoke extensive cryptic mortalities (Gray & Kennelly, 2018). In many cases, animals are meshed and then either depredated or drop out of gillnets, without being recorded (Uhlmann & Broadhurst, 2015). Cryptic encounters need to be quantified to understand fishing-gear impacts and to identify priorities for conservation efforts, particularly for ETP species (Pacoureau et al., 2021; Warden & Murray, 2011).

Surveillance systems are ever evolving to provide new options for monitoring fishing gears, and traditionally include hydroacoustics (Flowers & Hightower, 2013) and camera systems (Underwood et al., 2012), which have expanded to encompass drones (Toonen & Bush, 2020). Another possible option for assessing broader impacts of a fishing gear is residual DNA metabarcoding. Recently, there have been considerable advancements in using DNA to determine species identification, genetic diversity, dispersal, and relative population abundances—even from trace levels of material via metabarcoding (Bakker et al., 2017; Stat et al., 2017; Yates et al., 2019). The sensitivity of new approaches has burgeoned into the research field of "environmental DNA" (eDNA) to evaluate patterns and processes in biodiversity science (Barnes & Turner, 2016; Bohmann et al., 2014; Deiner et al., 2017; Rourke et al., 2021).

Conceivably, during capture, organisms embed tissue within mesh twine structures, and DNA metabarcoding could be used to identify these taxa. Here, we propose a new application of DNA metabarcoding to sections of netting from new and previously unused gillnets targeting sharks (mostly carcharhinids) as part of two consecutive trials during the austral autumn/summer to improve bather protection off New South Wales (NSW), Australia. We compared these data against recorded species entanglements (i.e., known history of the gillnets), including those listed as ETP (Broadhurst & Cullis, 2020). We sought to test whether those species that remained trapped, or escaped or were depredated from the gillnets, left detectable traces of their DNA in the netting strands.

2 | MATERIALS AND METHODS

2.1 | Fishing gear and sampling

The methods were performed in accordance with relevant guidelines and regulations and approved by the NSW Department of Primary Industries. Samples were collected from two identical bather-protection gillnets (termed "treatments" and labeled as nos. one and two) that were singularly and alternately deployed (i.e., only one gillnet was in the water at any one time) along with four other nonsampled gillnets (not considered further) in 5–8 m of water ~500 m off Evans Head, NSW (29.11° S, 153.44° E) between December 30, 2016 and May 10, 2017 (trial no. one) and then between November 23, 2017 and May 2, 2018 (trial no. two) (Figure 1; Broadhurst & Cullis, 2020, for specific technical details). Both treatment gillnets (and all other gillnets) were brand new and purpose-built for the trials (i.e., not previously fished anywhere else). Attempts were made to always continuously fish a single gillnet during the trials, and to check and clear it every 12–24 h and replace it as required (typically every 2 weeks or sooner if excessively damaged; see below). All gillnet checks/replacements were done with an onboard scientific observer.

During each gillnet check, all entangled animals were removed, identified, sexed, and measured. Any damage to the two treatment gillnets was recorded and contact and escape/depredation were considered to have occurred when there was no animal entangled, but there were two or more broken adjacent mesh bars (Figure 1; creating a hole at least 600 × 600 mm). Most small-sized holes (<~five mesh bars) were repaired prior to redeployment of the same gillnet on the same day, but where there was considerable damage, the affected gillnet was replaced, stored on shore and then eventually repaired (and prior to redeployment at a later date).

Sampling of the twines (1.8-mm diameter braided polyethylene) from the two treatment gillnets was done during trial no. two only. In each case, the treatment gillnets were sampled for pieces of twine before their deployment (post-storage) and after being stored in air for 27–323 days (including between fishing trials) and then again (fishing) during a check when there was damage as defined above (Figure 1). On one occasion, twine samples were taken (on board the boat) around an entangled common blacktip shark, *Carcharhinus limbatus* (224 cm total length; TL) in gillnet no. one as a positive control. During twine sampling, a researcher wearing sterile gloves cut (using sterilized scissors) five adjacent twine pieces (~2 cm in length) from around randomly selected small to medium holes in the bagged (and dry-stored) gillnet, or around the perimeters of large holes identified (and repaired) in the field, or around the captured *C. limbatus* (positive control), and placed these into sealed 1.5 ml microcentrifuge tubes containing ethanol. Extraction controls for DNA (ultrapure water samples, Invitrogen, Waltham, USA) were collected in sterile 1.5 ml microcentrifuge tubes alongside the net samples and subjected to the same workflow described below.

2.2 | DNA extraction and amplification

For each sample, the five twine pieces were independently extracted using a Qiagen DNeasy Blood and Tissue kit following manufacturer's instructions. Ethanol precipitation was also used to isolate DNA from the twine-storage ethanol from each of the five twine pieces (per sample) and then extracted using a Qiagen DNeasy Blood and Tissue kit following manufacturer's instructions (Barbato et al., 2019). Within samples, the extracts from each of the five twine and twine-storage ethanol extractions were then combined to maximize DNA yield. The DNA extraction was carried out in a pre-PCR laboratory to minimize contamination, and clean-room protocols were followed with extensive bleaching (10-min rinse with 3% hypochlorite) and UV treatment of the area and equipment. Filter pipette tips were used, and gloves were frequently changed. Negative controls for extraction and PCR

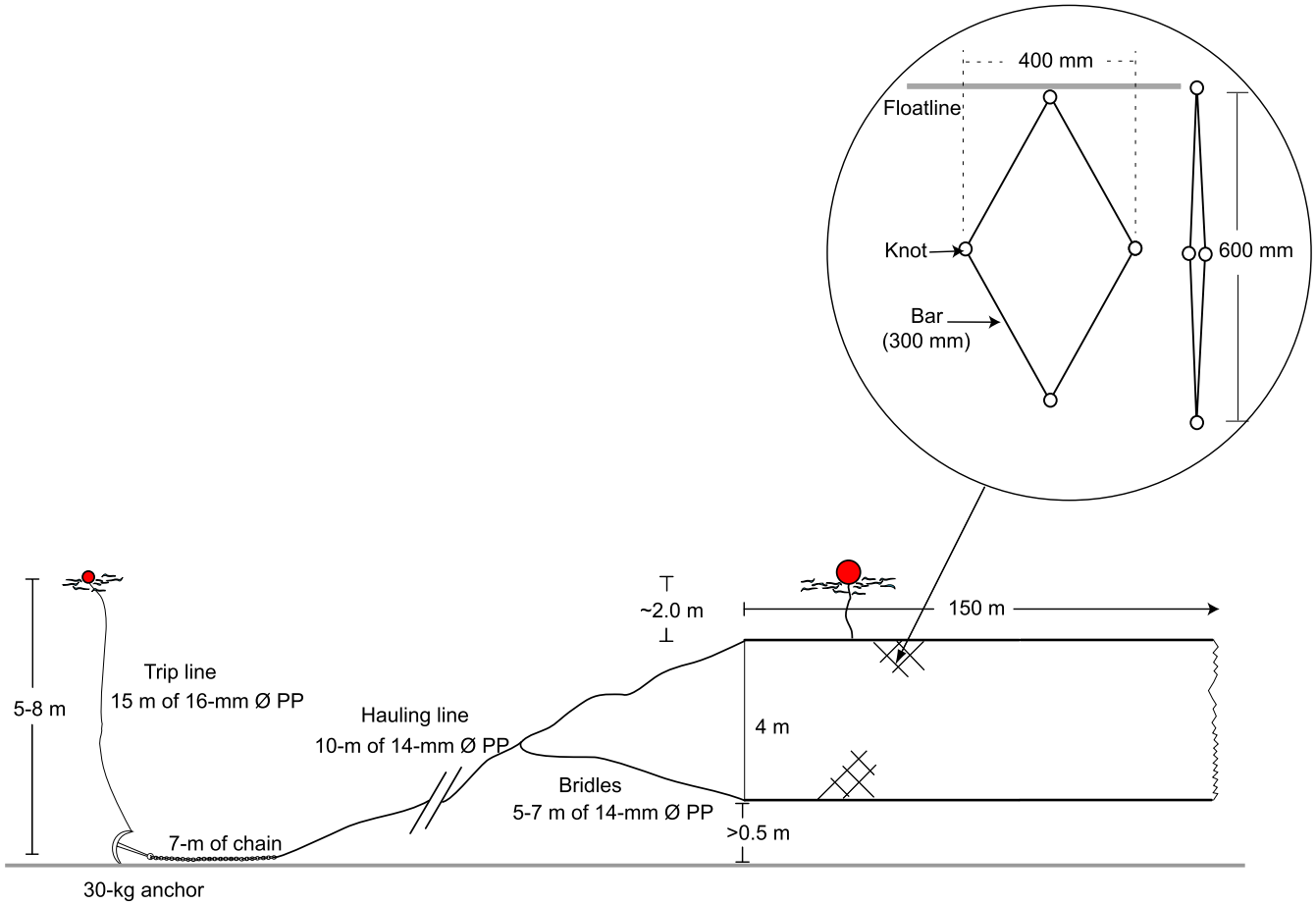


FIGURE 1 Schematic diagram of the bather-protection gillnets fished off Evans Head, Australia

(ultrapure water samples, Invitrogen, Waltham, USA) were included for all stages of the work.

2.3 | Metabarcoding assay

Two group-specific minibarcode primers were selected for teleosts and elasmobranchs, targeting 12S mitochondrial DNA (MiFish (Miya et al., 2015) and Elasmo02 (Taberlet et al., 2018)). Polymerase chain reaction (PCR) was performed using the AmpliTaq Gold 360 protocol and thermocycling conditions recommended in Taberlet et al. (2018). The PCR hybridization temperatures were 50 and 59°C for MiFish and Elasmo02 primers, respectively, and products were run on a 1% agarose gel to confirm amplification of the correct target size. A second round of PCR was undertaken on the cleaned PCR products using unique dual-indexed primers on each sample, that included the Illumina adaptors (as detailed in Holman et al., 2019). The PCR products were sent to the Ramaciotti Centre for Genomics at the University of NSW for cleaning, normalizing, and pooling before paired-end sequencing, which was performed using a 500-cycle MiSeq V3 Reagent Kit on an Illumina MiSeq platform (Illumina, San Diego, CA, USA). Demultiplexing was conducted by the sequencing center.

2.4 | Bioinformatic pipeline

Demultiplexed Illumina reads were first processed using Geneious software for pairing, merging and trimming the forward/reverse primers (Kearse et al., 2012). Reads were discarded in the absence of a 100% match to primers, adapter, and barcodes sequences. USEARCH was used to conduct the operational taxonomic unit (OTU) analysis (Edgar, 2010). Following USEARCH guidelines for OTU creation, reads were additionally quality filtered according to ambiguities ($N = 0$), length (minimum length 150 bp) and maximum error rate (error <0.5).

Reads were then dereplicated into unique sequences and finally the UPARSE algorithm *cluster_otus* (97% similarity) was applied to define OTUs. This algorithm facilitated removing possible sequencing errors, PCR artefacts, chimeras, and low-abundance clusters <0.75% from the total number of unique sequences identified within the sample. The USEARCH command *otutab* enabled mapping the relative abundance of each OTU within the sample of filtered reads. Read counts were assumed to approximate the biomass of tissue left embedded in the twine, and so those taxa with <1% of total filtered reads per sample were deemed unlikely to be responsible for damage to gillnets.

The basic local alignment search tool (BLASTn) at the National Center for Biotechnology Information's (NCBI) GenBank nucleotide database (Altschul et al., 1990) was executed for each samples' OTUs to assess taxonomic diversity. The BLASTn outputs were visualized using MEGAN6 (MEtaGenome ANalyzer) (Huson et al., 2016) to inspect taxonomic identification using the LCA parameter set as a minimum bit score of 150.0 and the top 5% of matches. Taxa other than marine megafauna were excluded from downstream analyses, but are listed in Table S1. These species comprised standard laboratory metabarcoding contaminants (e.g., humans, cows, pigs, and chickens), but also marine taxa including crustaceans and teleosts (mostly Clupeocephala).

3 | RESULTS

In total (during both trials), treatment gillnets nos. one and two were cumulatively fished for 100 and 63 days, catching 65 and 26 animals, comprising ten species and incurring 22 and 14 holes (4–1000 broken bars), respectively (Figures 1–3). The gillnets were temporally sampled for twines six and four times each, which encompassed post-storage samples after 27, 34, 192, and 323 days storage in air, and then fishing samples of holes (including the positive control) following deployment (but with repeated checking) after 4, 6 or 10 days (Figures 2 and 3).

A total of 2,104,024 raw reads were produced for the Elasmo02 data set, and 1,275,623 for MiFish. After filtering, 2,016,447 Elasmo02 reads and 1,164,033 MiFish reads were retained for analyses. Negative controls showed extremely low levels of possible cross-contamination for the MiFish amplicons (Gnathostomata), but some cross-contaminating taxa were evident for Elasmo02 (*Mobula* spp., *Aetobatus* spp., sandy sprat, *Hyperlophus vittatus*, and *Trachurus* spp.; Table S1), and were subsequently excluded from downstream analyses of the Elasmo02 data set (Table 1).

The Elasmo02 amplicons correctly identified all elasmobranchs previously caught in the gillnets to genus, with relatively high numbers of reads in all cases (Table 1). The MiFish amplicons were no less accurate in identifying taxa, but recovered only a subset of the taxa identified in the Elasmo02 amplicons (Table 1). The MiFish data set was dominated by human and teleost (Clupheocephala) reads, accounting for around 90% of the reads per sample (Table S1).

In most cases for sharks, assignments were only possible to *Carcharhinus* spp. or *Sphyrna* spp., but species-level assignments were achieved for grey nurse shark, *Carcharias taurus* (post-storage sample 3, and a species identified as previously being caught in the gillnet), and bull, *Carcharhinus leucas* (fishing sample 4) and white sharks, *Carcharodon carcharias* (fishing sample 10) (neither of which were previously caught in the gillnets, nor handled onboard the vessel used during sampling or in the laboratories; Table 1). Another identified group that was not previously caught in these gillnets or at the fishing location was Delphinidae (fishing sample 10, in both amplicon data sets) (Table 1, Table S1).

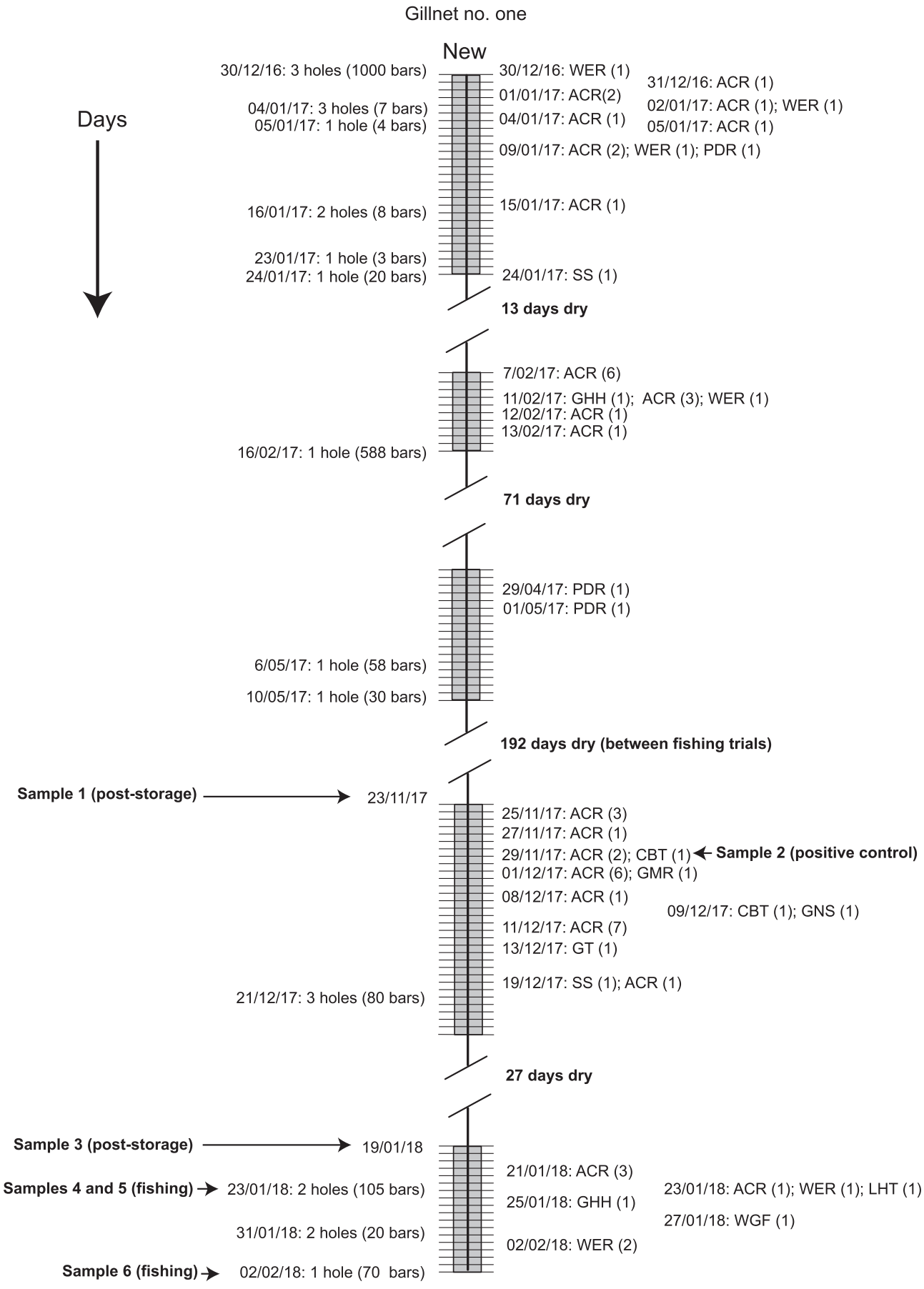
4 | DISCUSSION

This study demonstrates the utility of eDNA for detecting species interactions with PE twine used in gillnets. By sampling netting before deployment (after protracted storage and known fishing history since new) and from holes during fishing, we have not only resolved several cryptic apex–mesopredator interactions, but also demonstrated the longevity of elasmobranch DNA in stored PE twine used for netting. This information, along with consideration of contamination issues, can be used to postulate the future potential, and current limitations, of eDNA as a novel surveillance system for monitoring fishing gears.

All post-storage samples comprised the DNA of species previously identified as catches (during both trials) in the gillnets, and with percentage reads that were somewhat proportional to earlier abundances based on actual catch data (Broadhurst & Cullis, 2020). Specifically, both *Sphyrna* spp. and *Rhinoptera* spp. contributed large reads, which probably reflected recorded previous entanglements of great hammerheads, *Sphyrna mokkaran* and especially Australian cownose rays, *Rhinoptera neglecta*. While not as numerically abundant as *R. neglecta*, all *S. mokkaran* were very large (mean size of ~3 m TL) and frequently tangled very large sections of netting (Broadhurst & Cullis, 2020). Conceivably, these animals would shed DNA across considerable areas, which might be expected to remain in broken or damaged twines, especially when gillnets were packed into bags and stored soon after specimens were caught. The presence of these species' DNA after less than a year (320 days) is well within the timeframe from human forensics, whereby DNA can apparently remain viable on polypropylene twine for periods up to 23 years (Morris, 2019).

In support of a supposition that the abundance of reads reflected the historical amount of gillnet interactions, there was a high number of relevant reads in both Elasmo02 and MiFish datasets for the positive control (sample 2; *C. limbatus*). However, this information does not distinguish temporal abundances. For example, in sample 2, the relatively high number of *Rhinoptera* spp. reads might have reflected

FIGURE 2 Chronology of the entire fishing history (since new, with damage as holes and broken bars; and catches, *n* = number) and sampling (eDNA collected) for gillnet no. one fished off Evans Head, Australia, with horizontal bars representing days in each of two fishing trials, and shaded histograms are continuous replicate fishing periods (in water) while all other days involve the gillnet bagged in air (days dry). ACR, Australian cownose ray, *Rhinoptera neglecta*, WER, White spotted eagle ray, *Aetobatus ocellatus*; PDR, pygmy devilray, *Mobula eregoodoo*; WGF, white spotted guitarfish, *Rhynchobatus australiae*; SS, spinner shark, *Carcharhinus brevipinna*; GHH, great hammerhead¸ *Sphyrna mokarran*; CBT, common blacktip shark, *Carcharhinus limbatus*; GNS, grey nurse shark, *Carcharias taurus*; GT, green turtle, *Chelonia mydas*; LHT, loggerhead turtle, *Caretta caretta*


Gillnet no. one
New
Days
30/12/16: 3 holes (1000 bars)
04/01/17: 3 holes (7 bars)
05/01/17: 1 hole (4 bars)
16/01/17: 2 holes (8 bars)
23/01/17: 1 hole (3 bars)
24/01/17: 1 hole (20 bars)
16/02/17: 1 hole (588 bars)
6/05/17: 1 hole (58 bars)
10/05/17: 1 hole (30 bars)
21/12/17: 3 holes (80 bars)
31/01/18: 2 holes (20 bars)
02/02/18: 1 hole (70 bars)
30/12/16: WER (1)
31/12/16: ACR (1)
01/01/17: ACR(2)
02/01/17: ACR (1); WER (1)
04/01/17: ACR (1)
05/01/17: ACR (1)
09/01/17: ACR (2); WER (1); PDR (1)
15/01/17: ACR (1)
24/01/17: SS (1)
13 days dry
7/02/17: ACR (6)
11/02/17: GHH (1); ACR (3); WER (1)
12/02/17: ACR (1)
13/02/17: ACR (1)
71 days dry
29/04/17: PDR (1)
01/05/17: PDR (1)
192 days dry (between fishing trials)
Sample 1 (post-storage)
23/11/17
25/11/17: ACR (3)
27/11/17: ACR (1)
29/11/17: ACR (2); CBT (1)
Sample 2 (positive control)
01/12/17: ACR (6); GMR (1)
08/12/17: ACR (1)
09/12/17: CBT (1); GNS (1)
11/12/17: ACR (7)
13/12/17: GT (1)
19/12/17: SS (1); ACR (1)
27 days dry
Sample 3 (post-storage)
19/01/18
21/01/18: ACR (3)
Samples 4 and 5 (fishing)
23/01/18: 2 holes (105 bars)
23/01/18: ACR (1); WER (1); LHT (1)
25/01/18: GHH (1)
27/01/18: WGF (1)
02/02/18: WER (2)
Sample 6 (fishing)

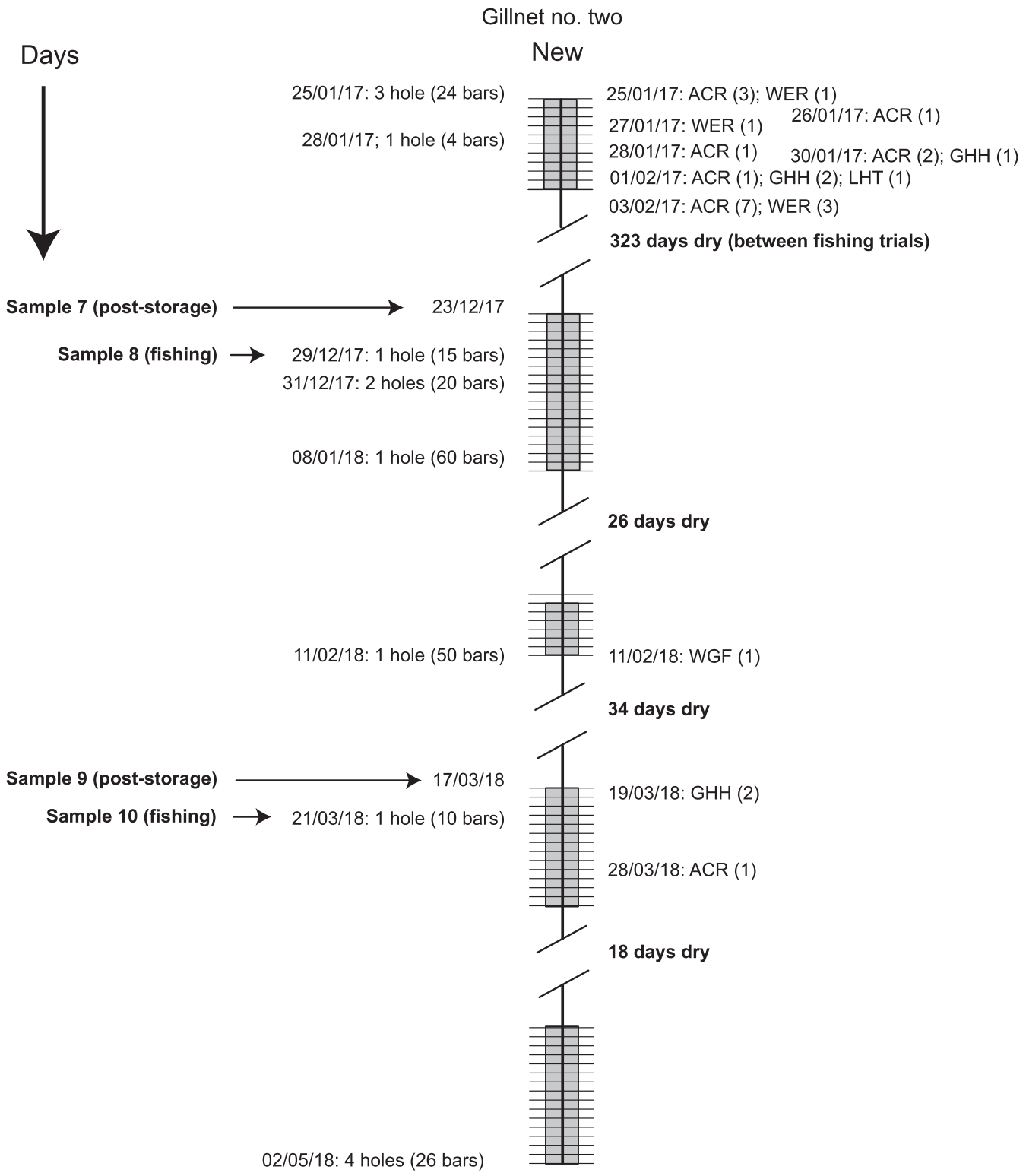


FIGURE 3 Chronology of the entire fishing history (since new, with damage as holes and broken bars; and catches, *n* = number) and sampling (eDNA collected) for gillnet no. two fished off Evans Head, Australia, with horizontal bars representing days in each of two fishing trials, and shaded histograms are continuous replicate fishing periods (in water) while all other days involve the gillnet bagged in air (days dry). ACR, Australian cownose ray, *Rhinoptera neglecta*, WER, White spotted eagle ray, *Aetobatus ocellatus*; GHH, great hammerhead¸ *Sphyrna mokarran*; LHT, loggerhead turtle, *Caretta caretta*

six *R. neglecta* caught in the previous 4 days, or 23 individuals caught over the previous 11 months during trial no. one. Haplotype analysis of metabarcoding reads could be informative in future for determining the minimum number of individuals interacting with a gillnet, and to avoid repeat recording of the same interaction (Adams et al., 2019).

TABLE 1 Summary of filtered metabarcoding reads from consecutive (paired) samples for two primer sets targeting 12S mitochondrial DNA (Elasmo02 and MiFish)

Sample type, number, and date	Elasmo02	Reads (%)	Prior catch?	MiFish	Reads (%)	Prior catch?
Post-storage sample 1	*Sphyrna* spp.	114789 (45.74)	Yes	*Carcharhinus* spp.	1657 (1.35)	Yes
23/11/17	*Rhinoptera* spp.	46840 (18.66)	Yes	*Mobula* spp.	1537 (1.25)	Yes
	Carcharhinus spp.	35114 (13.99)	Yes			
	Mobula spp.[a]	23476 (9.35)	Yes			
	Dasyatoidea spp.	19216 (7.66)	Yes			
Positive control sample 2	*Carcharhinus* spp.	20861 (16.17)	Yes	*Mobula* spp.	4177 (4.50)	Yes
29/11/17	*Rhinoptera* spp.	12283 (9.52)	Yes	*Carcharhinus* spp.	3254 (3.50)	Yes
	Sphyrna spp.	3300 (2.56)	Yes			
	Mobula spp.[a]	4025 (3.12)	Yes			
Post-storage sample 3	*Aetobatus* spp.[a]	47762 (15.89)	Yes			
19/01/18	*Rhinoptera* spp.	37374 (12.28)	Yes			
	Pristiformes/ Rhiniformes group	26088 (8.57)	Yes			
	Greynurse shark, *Carcharias taurus*	8719 (2.87)	Yes			
Fishing sample 4	*Sphyrna* spp.	88450 (41.81)	Yes	*Carcharhinus* spp.	1934 (1.19)	Yes
23/01/18	*Rhinoptera* spp.	45945 (21.72)	Yes	Bull shark, *Carcharhinus leucas*	1807 (1.12)	No
	Carcharhinus spp.	42923 (20.29)	Yes			
	Carcharhinus spp.	18297 (8.65)	Yes			
Fishing sample 5	Aetobatus spp.[a]	90917 (32.35)	Yes			
23/01/18	*Rhinoptera* spp.	42343 (15.07)	Yes			
	Sphyrna spp.	5107 (1.82)	Yes			
Fishing sample 6	*Aetobatus* spp.[a]	74912 (85.48)	Yes	*Aetobatus* spp.	10234 (7.65)	Yes
02/02/18	*Rhinoptera* spp.	5259 (6.00)	Yes			
	Sphyrna spp.	6918 (7.89)	Yes			
Post-storage sample 7	*Mobula* spp.[a]	77708 (42.40)	Yes	*Rhinoptera* spp.	3963 (3.11)	Yes
23/12/17	*Aetobatus* spp.[a]	71254 (38.87)	Yes			
	Sphyrna spp.	17463 (9.63)	Yes			
	Rhinoptera spp.	11401 (6.22)	Yes			
Fishing sample 8	*Aetobatus* spp.[a]	77997 (51.76)	Yes	*Aetobatus* spp.	12932 (14.20)	Yes
29/12/17	*Mobula* spp.[a]	72686 (48.23)	Yes			
Post-storage sample 9	*Aetobatus* spp.[a]	58494 (26.46)	Yes			
17/03/18	*Mobula* spp.[a]	44840 (20.28)	Yes			
	Sphyrna spp.	33011 (14.93)	Yes			
	Pristiformes/ Rhiniformes	7100 (3.21)	Yes			

(Continues)

TABLE 1 (Continued)

Sample type, number, and date	Elasmo02	Reads (%)	Prior catch?	MiFish	Reads (%)	Prior catch?
Fishing sample 10	*Aetobatus* spp.[a]	28449 (14.45)	Yes	Delphinidae	3571 (2.91)	No
21/3/18	*Mobula* spp.[a]	20911 (10.62)	Yes			
	Sphyrna spp.	20130 (10.23)	Yes			
	White shark, *Carcharodon carcharias*	7312 (3.71)	No			
	Carcharhinus spp.	3003 (1.53)	No			

Note: Only reads >1% of total filtered reads per sample are shown.

[a]Possible contamination (see Section 3).

While pygmy devilrays, *Mobula eregoodoo* and white spotted eagle rays, *Aetobatus ocellatus* were also caught in one or both treatment gillnets, respectively, and manifested as high DNA reads, negative controls revealed these taxa as sources of contamination in the Elasmo02 data set. The MiFish data set showed no such contamination issues, and identified *Mobula* and *Aetobatus* spp. only in those samples following capture. This outcome underscores the requirement for negative controls throughout the sampling and lab-work processes, and inclusion of multiple gene-target regions.

Other contamination included common laboratory metabarcoding sources, but there was also DNA from teleosts not recorded as caught (or observed) in the gillnets, but conceivably possible for the study area (Broadhurst & Cullis, 2020). These species might have contacted the vessel, but more likely they contacted meshes (e.g., teleosts were herded in by predators that were then entangled), or were present in the mouths of sharks and deposited on netting around the holes during escape. A similar hypothesis might support the observation of the Delphinidae DNA in gillnet no. two alongside that of *C. carcharias*. Neither animal was previously caught in the treatment gillnets or at the fishing site, nor handled onboard the gillnet-sampling vessel or by the crew. Potentially, the *C. carcharias* had already consumed a dolphin, or chased one into gillnet no. two and was then entangled, leaving a mixture of predator/prey DNA in the hole. Alternatively, the *C. carcharias* depredated an entangled dolphin. Broadhurst and Cullis (2020) recorded only 7% of trapped animals as being depredated, but these might represent unsuccessful removals, considering the numbers of holes in the two treatment gillnets (36 holes vs 91 animals here). The same interactions might explain the high number of sequencing reads for *C. leucas* (which was also unique to an eDNA sample, and not captured in the net).

The possibility that the above reads reflected contamination by passing eDNA in the water column is unlikely, especially for the sharks, given the high number of reads for *C. carcharias*, and *C. leucas*. The holes in the treatment gillnets were most probably made well before (up to 2 days) they were removed from the water. The metabarcoding assay pattern observed for these samples reflected that of the known positive *C. limbatus* control (a high number of reads left on the surrounding twine) most likely due to direct contact with meshes after the shark became entangled. In addition, the rarity of *C. carcharias* (2500–6750 individuals along the entire eastern Australian coastline; Hillary et al., 2018) strongly suggests a direct (rare) gillnet interaction, rather than that the water column contained an abundance of free-floating *C. carcharias* eDNA available to bind to rigid twine fibers in such high quantities (>7000 reads). The location of the gillnets in the surf zone, exposed to strong currents and tidal flux, also argues against an abundance of free-floating *C. carcharias* DNA remaining in place to contaminate the gillnet twine long after the animal had moved away.

Notwithstanding the likely limitations for free-floating DNA to bind to the polyethylene twine across such dynamic environments in sufficient quantities, future research would benefit from assessing any such effects among the various different materials commonly used in fishing gear and key temporal or extrinsic affecting factors. Such work would also require concomitant sampling of the adjacent water column and, if possible, during known periods of high abundances of various schooling species.

Irrespective of the species, it is clear that following mechanical interactions with elasmobranchs and polyethylene gillnets and then their subsequent storage, DNA appears to bind and remain viable in twine fibers for a considerable amount of time. This characteristic could potentially be useful as a long-term archive of collective catch, with applications for regulating illegal, unreported, and unregulated fishing. For example, fishery observers could collect samples from fishing gear (or vessels) to determine if longer-term interactions with ETP species had occurred. Over time, such data could be used to infer problematic spatio-temporal fishing effort, and ultimately be used to manage negative impacts (Pacoureau et al., 2021). Notwithstanding the above, one shortfall of the DNA longevity signal is potential cross-contamination; both on board fishing vessels, and post-sample collection. Further, the longevity of DNA on other materials commonly used in gillnets, and especially monofilament polyamide, remains unknown. These issues require careful consideration and assessment.

Environmental DNA is a rapidly evolving research area, with many possible applications for providing accurate spatio-temporal information on fishery interactions. On-board DNA processing devices, such as Oxford Nanopore Technologies' microfluidic device, the MinION, would provide capacity for near real-time monitoring. Decreasing costs of DNA analyses and sequencing will facilitate greater sampling effort, while increasing automation associated

with these devices reduces the need for skills in molecular biology. Other technical developments required for broadscale uptake of DNA analyses into fisheries science include completed reference databases (e.g., GenBank), and further understanding of whether we can assess biomass and/or temporal effects from DNA data (e.g., do number of sequencing reads reflect many individuals, or more recent interactions?). In this study, we utilized an OTU threshold "lumping" approach, not an unique amplicon sequence variant (ASV) counting approach, which may have limited the species resolution achievable from our data. Additionally, not all species captured in the nets had local reference database sequences, which could also have led to genus-level assignments only in several cases (e.g., *Aetobatus* and *Rhinoptera* spp.). Notwithstanding such caveats, based on our results here, we propose eDNA will facilitate the future surveillance of problematic fishing gears.

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CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

AUTHOR CONTRIBUTIONS

MKB conceived the study. MB and MKB collected the data. MdB and MB conducted molecular lab work. MB analyzed the data. MdB, MB, and MKB wrote the paper.

DATA AVAILABILITY STATEMENT

The data sets generated and analyzed during the current study are available at Dryad: https://doi.org/10.5061/dryad.6q573n5zw.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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