Hammer Chyme: The Russian-Doll Effect Limits Reconstructing Marine Trophic Food Webs

Mark de Bruyn (mark.debruyn@sydney.edu.au)
The University of Sydney
Matteo Barbato
The University of Sydney
Joseph D. DiBattista
Australian Museum Research Institute
Matt K. Broadhurst
Southern Cross University

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Hammer chyme: the Russian-doll effect limits reconstructing marine trophic food webs

Mark de Bruyn[1,*], Matteo Barbato[1,2], Joseph D. DiBattista[3], and Matt K. Broadhurst[4,5,*]

Affiliations:

[1] *The University of Sydney, School of Life and Environmental Sciences, Sydney, NSW 2006, Australia.*

[2] *Department of Biology, University of Padova, Padova, Italy.*

[3] *Australian Museum Research Institute, Australian Museum, 1 William Street, Sydney, NSW 2010, Australia.*

[4] *NSW Department of Primary Industries, Fisheries Conservation Technology Unit, National Marine Science Centre, Southern Cross University, 2 Bay Drive, Coffs Harbour, NSW 2450, Australia.*

[5] *Marine and Estuarine Ecology Unit, School of Biological Sciences, University of Queensland, St Lucia, Australia*.

* Corresponding authors: mark.debruyn@sydney.edu.au & matt.broadhurst@dpi.nsw.gov.au

ORCID: de Bruyn (0000-0003-1528-9604); Barbato (0000-0001-5368-2090), DiBattista (0000-0002-5696-7574); and Broadhurst (0000-0003-0184-7249)

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Abstract

Increasing fishing effort, including bycatch and discard practices, are impacting marine biodiversity, particularly among slow-to-reproduce taxa such as elasmobranchs, and specifically sharks. While some fisheries involving sharks are sustainably managed, collateral mortalities continue, contributing towards >35% of species being threatened with extinction. To effectively manage shark stocks, life-history information, including resource use/feeding ecologies is pivotal, especially among those species with wide-ranging distributions and habitats. Two cosmopolitan sharks bycaught off eastern Australia are the common blacktip shark (*Carcharhinus limbatus*; globally classified as Near Threatened) and great hammerhead (*Sphyrna mokarran*; Critically Endangered). We opportunistically sampled the digestive tracts of these two species and also any whole prey; (termed the 'Russian-doll' approach) caught in bather-protection gillnets off northern New South Wales to investigate their regional feeding ecologies and the capacity for DNA metabarcoding to delineate trophic interactions. *Sphyrna mokkaran* fed predominantly on Myliobatiformes and Rajiformes, but also teleosts, while *C. limbatus* mostly consumed teleosts, with some inter-specific dietary overlap of prey items. Extensive cross-contamination of predator and prey digestive tracts, likely via the predator's stomach chyme, was evident from the metabarcoding assays limiting the opportunity to delineate trophic interactions from these data. This Russian-doll effect requires further investigation in DNA metabarcoding studies focused on dietary preferences, but implies any outcomes will need to be interpreted concomitant with traditional visual approaches.

Introduction

Global biodiversity is under threat, with accelerating losses of species and increasing concern about ecosystem changes (Diaz et al., 2020). In the oceans, escalating fishing effort and associated bycatches and discarding practices are impacting on marine biodiversity (Jones et al., 2020). Levels of extinction threat vary regionally and by taxonomic group, but owing to their low reproductive rates and late age-at-maturity, elasmobranchs, especially sharks, are among those taxa highly susceptible to increasing anthropogenic pressures (Dulvy et al., 2014). More specifically, among the total known species of 'ground sharks' or the Carcharhiniformes (n = 287), 36% are listed as threatened with extinction, and a further 18% are classified as 'Data Deficient' (IUCN, 2018).

In Australia, several shark species are targeted in commercial gillnet fisheries (e.g. gummy sharks *Mustelus antarcticus* and sandbar sharks *Carcharhinus plumbeus*; Walker et al., 2005; Braccini et al., 2012) and also in bather-protection programs using gillnets and/or baited hooks (e.g. white *Carcharodon carcharias*, tiger *Galeocerdo cuvier*, and bull sharks *Carcharhinus leucas*; Sumpton et al., 2011; Broadhurst and Cullis, 2020). Various ancillary species also incur collateral mortalities as bycatch from the above and other commercial fishing activities (Stevens and Wayte, 2009; Roff et al., 2016, 2018).

Two of the more abundant, cosmopolitan Carcharhiniformes found in Australia and well represented in bycatches of bather-protection fishing gears (Sumpton et al., 2011, Broadhurst and Cullis, 2020) are the common blacktip shark (*Carcharhinus limbatus*) and great hammerhead (*Sphyrna mokarran*), which are globally classified as Near Threatened and Endangered, respectively (IUCN, 2018). *Sphyrna mokarran* is also registered in Appendix II of the Convention on International Trade in Endangered Species (www.cites.org), which has

precipitated their legislated protection across many jurisdictions. Notwithstanding these classifications, the biology and ecology of *S. mokarran* remain poorly understood, particularly their diet, which limits effectively managing their remaining stocks (Dulvy et al., 2014; Raoult et al., 2019; 2020).

Sharks are characterized by complex and plastic ecological roles at the highest levels of the food web (Heithaus et al., 2008). Their ontogeny induces a dietary shift through their life stages and they can prey on communities across different marine ecosystems (e.g. coastal, demersal and pelagic) depending on resource availability (Bizzarro et al. 2017). Because of their feeding role, the ecological importance of sharks is considered a form of top-down control. These direct (prey consumption) and indirect (behavioural, interaction and community dynamics) effects shape lower trophic communities. In both temperate and tropical ecosystems, it is useful to differentiate larger sharks into apex (>3.0 m total length; TL) or mesopredators (~1.3 to 3.0 m TL) (Heupel et al., 2014).

Sphyrna mokarran can reach a maximum size of 6.0 m total length (TL) and in situ observations imply it predominantly feeds on rays (Chapman and Gruber, 2002; Cliff, 1995; Strong et al., 1990) but, like most of its apex congeners also consumes teleosts and other sharks (Mourier et al., 2013; Roemer et al., 2016). Nevertheless, data are limited and in a recent review Gallagher and Klimley (2018) stated that *S. mokarran* feeding ecology requires further assessment. Relatively more is known about the smaller, mesopredator *C. limbatus* (maximum TL of ~2.6 TL), which typically feeds on teleosts throughout their cosmopolitan distributions (Barry et al., 2008; Tavares, 2008; Plumlee and Wells, 2016). Nevertheless, much of the research describing *C. limbatus* is restricted to the Atlantic Ocean and less is known about their foraging ecology off Australia.

Shark feeding ecology has been quantified mainly by visual identification of stomach contents, biochemical techniques (e.g. stable isotope, lipid and amino acid signatures) or telemetry (e.g. satellite and acoustic tagging) via habitat association (Young et al., 2015). Each of these methods has inherent strengths and weaknesses (reviewed in Leigh et al., 2017; Amundsen and Sánchez-Hernández, 2019). More recent innovations in genetic techniques, particularly high-throughput metabarcoding approaches, are now routinely contributing to our understanding of predator-prey relationships, and in general, providing improved taxonomic resolution of prey versus traditional methods (Alberdi et al., 2019; Nielsen et al., 2018; Pompanon et al., 2012). The DNA metabarcoding of digestive-tract contents is now commonplace in studies on terrestrial taxa (reviewed in Alberdi et al., 2019; Deagle et al., 2019; Taberlet et al., 2018) and recently has also been applied to marine taxa, mostly teleosts and elasmobranchs (e.g. Barbato et al., 2019; Berry et al., 2015; Bessey et al., 2019; Clarke et al., 2020; Sousa et al. 2016; Takahashi et al., 2020; Yoon et al. 2017).

A promising application of DNA metabarcoding for digestive-tract content analyses is in the reconstruction of trophic food webs (Nielsen et al., 2018). Where prey items remain relatively intact in a predator's digestive-tract, it should be possible to sequence the digestive-tract contents of both the predator and its prey to determine multiple levels of trophic interactions (Clare et al., 2014, Nielsen et al., 2018). However, one aspect of this so-called 'Russian-doll' approach (Varennes et al., 2014) that deserves further attention is the extent to which DNA cross-contamination occurs between predator and (whole) prey digestive-tracts, via the predator's stomach chyme. Considering the above, our objectives here were to use opportunistically sampled *S. mokarran* and *C. limbatus* caught in bather-protection nets off eastern Australia to not only (1) contribute towards better understanding their poorly studied

122 regional diets, but also (2) further investigate the Russian-doll DNA metabarcoding
123 application to trace trophic interactions in marine apex- and mesopredators.
124
125 **Material and Methods**
126 *Ethics declaration*
127 All experimental protocols were approved by New South Wales Fisheries, Australian
128 Government Department of Primary Industries, and carried out in accordance with relevant
129 guidelines and regulations. All methods reported are in accordance with ARRIVE guidelines
130 (https://arriveguidelines.org).
131
132 *Sample collection*
133 The study was done using seven *S. mokarran* (three females and four males) and four *C.*
134 *limbatus* (two of each sex) that died in bather-protection gillnets deployed off Lennox Head,
135 Ballina, and Evans Head, NSW, Australia (28.77° S, 153.60° E to 29.10° S, 153.44° E)
136 between 7 February and 13 March, 2018 (Fig. 1). Each gillnet measured 150 m long × 4 or 6
137 m deep and comprised 600- or 800-mm mesh made from either 1.8- or 2.1-mm diameter
138 twisted polyethylene, or 2.5-mm diameter polyamide twine (see Broadhurst and Cullis, 2020
139 for details of the fishing gear).
140
141 Whole sharks were removed from the gillnets and stored at -20°C (within 3 hours) until being
142 necropsied in May 2018. During the necropsy process, specimens were defrosted for 12 hours
143 and measured for TL before the stomach cavity was opened in a sterile field laboratory with
144 bleach sterilized tools. All digestive contents were removed, with any animal matter
145 identified (where possible), preserved in 100% ethanol, and stored at -20°C until further
146 analyses. Extraction controls for DNA (ultrapure water samples, Invitrogen, Waltham, USA)

were collected in sterile 1.5 mL microcentrifuge tubes alongside the stomach-content samples and subjected to the same workflow described below.

Stomach-content analyses and DNA extraction

Whole prey removed from *S. mokkaran* and *C. limbatus* were identified by visual inspection, mostly to the species level. Some of the incomplete specimens, typically fish jawbones (Fig. 2) or scales, were identified to genus only. For each *S. mokkaran* and *C. limbatus*, the remainder of its unidentifiable stomach contents (both liquids and solids) were homogenised using a previously sterilised (washed with detergent, followed by a 10% bleach wash, and rinsed thoroughly in MilliQ water) commercial food blender. The digestive-tract contents of any whole prey were separately homogenised as above.

For all samples, DNA was extracted from approximately 5 mL of the homogenate using a QIAamp PowerFecal DNA kit (Qiagen, Sydney, Australia) according to manufacturer's instructions. This kit effectively removes PCR inhibitors from fecal and stomach content samples. The DNA extraction was carried out in a pre-PCR laboratory to minimise contamination, and clean-room protocols were followed with extensive bleaching and UV treatment of the area and equipment for all laboratory steps. Filter pipette tips were used in all instances and gloves were frequently changed, particularly between handling specimens or plasticware.

PCR amplification and Illumina sequencing

The DNA extracts from each stomach sample were amplified, tagged separately, and then pooled for sequencing. Two group-specific mini-barcode primers were selected for the amplification of teleost and crustacean DNA, targeting 12S (MiFish; Miya et al., 2015) and

16S (Crust16S short: Berry et al., 2017) mitochondrial DNA genes, respectively. We also used a universal 18S primer set (Zhan et al., 2013) targeting the hypervariable V4 region of the nuclear small subunit ribosomal DNA to amplify templates from a broader fraction of marine metazoans. 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, 51 and 50^{o}C for MiFish, Crust16S, and Uni18S primer sets, respectively, and products were run on a 1% agarose gel to confirm amplification of the correct target size (MiFish = ±170 bp; Crust16S = ±170 bp, Uni18S = ±220 bp). A second round of PCR was undertaken with the cleaned PCR products using unique dual-indexed primers for each sample, which included the Illumina-specific sequencing adaptors. PCR products were sent to the Ramaciotti Centre for Genomics at the University of NSW for cleaning, normalising, and pooling prior to paired-end sequencing, which was performed using a 500 cycle MiSeq V3 Reagent Kit on an Illumina MiSeq platform (Illumina, San Diego, CA, USA). Sample demultiplexing based on the incorporated indexes was conducted by the sequencing centre.

Bioinformatic pipeline

All sequence data were quality filtered prior to taxonomic assignment using the following tools: 1) Geneious v10.1.3 (https://www.geneious.com) for stitching R1 and R2 reads using default settings, trimming low quality reads from the 5'/3' end (quality score = 30), removing adapters, and filtering out reads below a minimum threshold length (150 bp for MiFish, 80 bp for Crust16S, and 250 bp for Uni18S), 2) USEARCH (v11.0.667; Edgar, 2010) for renaming files and file format conversion (.fastq to .fasta), dereplication (identifying unique sequences), removing singletons, removing chimeric sequences, and generating a zero-radius operational taxonomic unit) (ZOTU) table with the UNOISE algorithm.

The ZOTUs were queried against the National Centre for Biotechnology Information's (NCBI) GenBank nucleotide database (accessed in 2020) using BLASTn with the following settings: percentage identity = 97, query coverage = 100, best hit score edge of 0.05, best hit overhang of 0.25, and an E-value of 1e-3. An additional, less-stringent data set was generated for each assay using the following BLASTn settings: percentage identity = 90, query coverage = 95, best hit score edge of 0.05, best hit overhang of 0.25, and an E-value of 1e-3. The LULU algorithm (Frøslev et al., 2017) was then run to curate the assignments assessing sequence similarity and their co-occurrence patterns with the default parameters: minimum_ratio_type = min, minimum_ratio = 1, minimum_match = 84, minimum_relative_cooccurence = 0.95. This entire process was completed on the Zeus SGI cluster based at the Pawsey Supercomputing Centre in Kensington, Western Australia using an in-house script developed by Mousavi et al., (2020). All raw sequencing data needed to replicate the study are available from Dryad Digital Repository XX.

Results

Four of the *S. mokarran* individuals had identifiable whole or partial prey in their stomachs (Table 1, Supplementary Table 1). These prey taxa included stingarees (*Urolophus* sp.) (Fig. 2), eastern shovelnose rays (*Aptychotrema rostrata*), thornback cowfish (*Lactoria fornasini*), eastern smooth boxfish (*Anoplocapros inermis*), threebar porcupinefish (*Dicotylichthys punctulatus*), reef ocean perch (*Helicolenus percoides*) and a whiting (*Sillago* sp.). Notably, one *S. mokarran* stomach contained nine whole *Urolophus* sp., with a total stomach weight of 6.26 kg, and a second *S. mokarran* stomach contained three whole *Urolophus* sp. and three whole *A. rostrata* (6.15 kg) (Fig. 2; Table 1).

The stomach contents of seven of the *Urolophus* sp. and two of the *A. rostrata* rays predated by the two *S. mokarran* individuals were included in the metabarcoding analysis (Table 1). In contrast, none of the *C. limbatus* stomachs contained recognisable prey, and maximum stomach weights were generally much lighter (range 0.19–0.71 kg) than those of *S. mokarran* (0.38–6.26 kg) (Table 1).

Metabarcoding assays

After quality filtering, 1,264,497 Mifish (12S) reads, 1,157,551 Crust16Sshort reads, and 237,745 Uni18S reads were retained for analyses (Supplementary Table 1). The PCR negative controls showed low levels of human contamination for the 18S assay only (Table 1), whereas the control water sample collected alongside the *C. limbatus* stomach samples demonstrated contamination from Carcharhinidae DNA (most likely from *C. limbatus*). Carcharhinidae and/or Chondrichthyes reads were ubiquitous across all three genetic assays, including the *Urolophus* sp. and *A. rostrata* rays removed from the *S. mokarran* stomachs, and most likely represent the host predator's DNA.

Taxa identified in the *S. mokarran* metabarcoding assays included crustaceans, cartilaginous fish and teleosts. Crustaceans and teleosts were identified in the *C. limbatus* stomach contents (Table 1). In most cases, stomach-content analyses on the *Urolophus* sp. and *A. rostrata* removed from the *S. mokarran* stomachs displayed high taxon similarity to that of the apex predators' stomachs. For example, a comparison of 4H (*S. mokarran*) and 4SR (*Urolophus* sp.) revealed *Gnathophis* spp., *Lepidotrigla* spp., *Pseudorhombus* spp., and *Anoplocapros* spp. in common.

Discussion

247 The data collected here represent the first efforts at metabarcoding the stomach contents of *S.*
248 *mokarran* and *C. limbatus* off eastern Australia. Our results support the general trends in the
249 literature describing the diet of *S. mokarran* as being dominated by rays (Chapman and
250 Gruber, 2002; Cliff, 1995; Strong et al., 1990), but also including teleosts and other sharks
251 (Mourier et al., 2013; Roemer et al., 2016), and reports of *C. limbatus* predominantly feeding
252 on teleosts (Barry et al., 2008; Tavares, 2008; Plumlee and Wells, 2016). By 'Russian-
253 dolling' (Varennes et al., 2014) the stomach contents of these marine apex- and
254 mesopredators and their prey, we have also quantified some of the issues encountered when
255 attempting to reconstruct trophic interactions from metabarcoding stomach contents. These
256 two themes are discussed separately below.
257
258 Prior to considering the dietary preferences of the two species, it is important to acknowledge
259 sampling constraints imposed by the selectivity characteristics of the fishing gear (Broadhurst
260 et al., 2007). While some sharks are known to depredate catches from gillnets and might
261 therefore be caught via attraction, in many cases animals are simply entangled as the move
262 through the fished area. This is an important distinction from baited hook-and-line
263 techniques, where captured animals are actively feeding. Such gear-specific catching
264 mechanisms could explain the absence of larger prey items in some of the *S. mokarran* and
265 all of the *C. limbatus* stomachs: these specimens might not have been foraging and certainly
266 had not fed immediately prior to capture. An alternative hypothesis for the absence of whole
267 prey in *C. limbatus* is that because these sharks were smaller, any ingested prey, which in
268 turn would also be smaller, might be more rapidly digested than the relatively large
269 *Aptychotrema rostrata* and *Urolophus* sp. consumed by *S. mokarran* (Wetherbee and Cortes,
270 2012).
271

Irrespective of causes for the absence of whole prey in several sharks, clearly, some *S. mokarran* had recently fed in the general area (considering the regional abundances of some whole prey items) and so, of the two species, their dietary preferences were the most clearly discernible. Based on whole prey items, *S. mokarran* consumed Myliobatiformes and Rajiformes, but also teleosts including both slow- (*Anoplocapros inermis*) and fast-moving species (*Sillago* sp.). Observations of prey handling by *S. mokarran* are limited, however the species has been observed to use its laterally expanded head (cephalofoil) to immobilise prey on the ocean floor during both the pursuit of bottom-dwelling rays (*Dasyatis americana*) (Strong, 1990), and the post-capture manipulation of pelagic rays (*Aetobatus narinari*) (Chapman and Gruber, 2002). Most of the prey items of the *S. mokarran* individuals identified in our study were bottom-dwellers (e.g. *Urolophus* sp., *A. rostrata* and *H. percoides*), which supports the above mechanisms of prey-capture and/or -manipulation (e.g. Compagno, 1988; Johnsen and Teeter, 1985; Nakaya, 1995).

Unlike for *S. mokarran*, we found no whole fish in the digestive-tracts of the four *C. limbatus*. Nevertheless, metabarcoding indicated the presence of large species including *Platycephalus* spp. Several platycephalids occur nearshore off northern NSW, especially eastern bluespotted flathead, (*P. caeruleopunctatus* up to 0.6 m TL) and, to a lesser extent, dusky flathead (*P. fuscus* up to 1.2 m TL). Large individuals of these species were unlikely to be prey of other animals in the stomachs of *C. limbatus*, but in the absence of whole specimens any conclusions based on the exact platycephalid species are speculative.

Other primary target species identified by metabarcoding in both *S. mokarran* and *C. limbatus* included *Dexillus* spp., probably tufted sole, *Dexillus muelleri*; the only known species from this genus, occurring off more tropical areas of Australia. This might imply

these sampled sharks (5H, 6H, 1BT, and 4BT) moved southwards from a more northern, tropical foraging area (Raoult et al., 2020), or less likely that an as yet unidentifed species of *Dexillus* occurs in northern NSW waters. Five of the *S. mokarran* and two of the *C. limbatus* also had *Anoplocapros* spp. metabarcoding reads sequenced from their stomach contents, and we observed *A. inermis* scales in the stomach of one *S. mokarran*, suggesting some dietary overlap with *C. limbatus*. Indeed, *A. inermis* is the only species in this genus (of three total species, including *A. amygdaloides* and *A. lenticularis*) found off eastern Australia.

While some larger primary prey items of *S. mokarran* and to a lesser extent *C. limbatus* could be identified, it was difficult to ascribe predation sources to the smaller taxa, such as the sandy sprat, *Hyperlophus vittatus* or biddies *Gerres* sp. (likely silver biddy *G. subfasciatus*, although this species overlaps with at least two other congeners in northern NSW) detected in the 12S assay. These species could either be primary prey of the sharks or secondary prey consumed by the platycephalids. The Russian-doll effect (Varennes et al., 2014) is exemplified well by comparing predator-prey stomach content metabarcoding reads (Table 1). For those *S. mokarran* that contained whole prey items (e.g. Myliobatiformes), DNA metabarcoding assays yielded close matches between predator and prey stomach contents. It is therefore difficult to conclude whether the *S. mokkoran* (less likely) or *Urolophus* sp. or *Aptychotrema rostrata* (most likely) were feeding on smaller prey items, such as decapod crustaceans and smaller fish species. It is likely that the chyme of both predator and (whole) prey cross-contaminated the other.

Similarly, our metabarcoding approach could not determine whether smaller sharks had been consumed by *S. mokarran* (as expected from the literature), due to the fact that all *S. mokarran* and their whole prey stomach samples included an abundance of 12S

Carcharhinidae reads. However, the 16S assay did suggest these reads likely reflected the host predator's DNA, considering *S. mokarran* reads were identified both from *S. mokarran* and *Urolophus* sp. stomach contents, but at very low abundances. Blocking primers designed to exclude the host predator's DNA would be a worthwhile inclusion in future metabarcoding dietary studies (e.g. Leray et al., 2013, 2015; Takahashi et al., 2020), while shark-specific primers could also prove more informative on this topic (Taberlet et al., 2018). Alternatively, increasing the depth of sequencing may facilitate picking up the low template (prey) fraction of the metabarcoding chyme despite the presence of host DNA that would otherwise swamp the PCR amplification process.

In this study, metabarcoding assays provided some novel insights into the dietary preferences of *S. mokarran* and *C. limbatus* off eastern Australia. These species appear to have some dietary overlap, but with consistency among prey species identified in studies of their feeding ecology from across their broader ranges (Barry et al., 2008; Chapman and Gruber, 2002; Cliff, 1995; Mourier et al., 2013; Roemer et al., 2016; Strong et al., 1990; Tavares, 2008; Plumlee and Wells, 2016). *Sphyrna mokkaran* fed predominantly on Myliobatiformes and Rajiformes, but also teleosts, whereas *C. limbatus* fed predominantly on teleosts, which is consistent with its smaller body size and lack of cephalofoil that allows specialized feeding of benthic prey.

Nevertheless, the 'Russian-doll effect' made the reconstruction of trophic interactions from stomach metabarcoding data problematic. Extensive cross contamination in situ between predator-prey digestive tracts was evident, which also limited the ability to discriminate between first-order predators of smaller teleosts and crustacean taxa. The literature on the Russian-doll effect in metabarcoding dietary studies is limited (Varennes et al., 2014;

reviewed in Clare, 2014; Nielsen et al., 2018) but certainly deserves further attention. We suggest that while the approach offers some utility in identifying unseen taxa, confirmation via visual identification and across sufficient replication (ideally from various sampling methods) is still required to comprehensively understand diet preferences.

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Data Archiving Statement: The datasets generated and analysed during the current study will be archived in Dryad

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Author Contributions

M.K.B. and M.B. collected the samples. M.B., M.K.B., and M.d.B. designed and conducted the experiments. J.D.D. analyzed the metabarcoding data. M.d.B. and M.K.B. wrote the paper. All authors contributed intellectually to the interpretation of the results and writing of the manuscript.

Table 1. Sample identification, species and sex (where identifiable) and the contents of their stomachs ('gut') determined via metabarcoding (to the closest taxonomic level) and through visual inspection. Prey items identified by metabarcoding assays are listed for each of two filtering stringencies per amplicon (see Methods). Values in parentheses after species designation show the number of reads observed for that taxon (values listed in red reflect ≤10 reads; 'n/a' indicates that the sample was not sequenced, while '0' indicates no amplification of relevant taxa). Species IDs shown in bold are those taxa identified in both the predator and the prey's digestive-tract via metabarcoding, and likely reflect cross-contamination via the predator's stomach 'soup'.

Sample ID	**Species (sex)**	**Gut 12S reads:** ≥95% query coverage, ≥90% identity match	**Gut 12S reads:** 100% query coverage, ≥97% identity match	**Gut 16S reads** ≥95% query coverage, ≥90% identity match	**Gut 16S reads:** 100% query coverage, ≥97% identity match	**Gut 18S reads:** ≥95% query coverage, ≥90% identity match	**Gut 18S reads:** 100% query coverage, ≥97% identity match	**Stomach contents weight (g)**	**Whole or partial prey items in gut** (sample ID in bold if sequenced)
1H	*S. mokarran* (M)	n/a	n/a	*Portunus* spp. (142,213)	*Portunus* spp. (142,213)	Carcharhinidae (1,977); Chondrichthyes (4,662)	Carcharhinidae (2,364); Chondrichthyes (3,444); Myliobatiformes (74)	100	0
2H	*S. mokarran* (F)	n/a	n/a	*Sphyrna mokarran* (19)	*Sphyrna mokarran* (19)	Carcharhinidae (4,354); Chondrichthyes (8)	Carcharhinidae (4,331); Chondrichthyes (2)	789	
3H	*S. mokarran* (F)	**Carcharhinidae** (76,525)	**Carcharhinidae** (76,525)	***Callianassa spp.*** (140)	***Callianassa spp.*** (140)	**Actinopteri** (1); **Carcharhinidae** (11,656); **Chondrichthyes** (2,738)	**Carcharhinidae** (12,456); **Chondrichthyes** (1,503); Myliobatiformes (4)	6264	9 whole *Urolopus* sp. (**3A-3D**)

4H	*S. mokarran* (F)	***Gnathophis* spp.** (332); *Trachurus* spp. (231); ***Lepidotrigla* spp.** (5,607); ***Pseudorhombus* spp.** (10); ***Anoplocapros* spp.** (61,831); **Carcharhinidae** (2,555)	***Gnathophis* spp.** (332); *Trachurus* spp. (231); ***Lepidotrigla* spp.** (5,607); ***Anoplocapros* spp.** (61,831); **Carcharhinidae** (2,555)	***Portunus* spp.** (18,030); ***Ranina spp.*** (36,346)*;* ***Penaeoidea* spp.** (1,347); *Sphyrna mokarran* (2)	***Portunus* spp.** (18,030); ***Penaeoidea spp.*** (1,347); *Sphyrna mokarran* (2)	Cichlidae (17); **Actinopteri** (46); **Carcharhinidae** (7,230); **Chondrichthyes** (5,179)	Anguilliformes (3); Cichlidae (10); **Actinopteri** (21); **Carcharhinidae** (7,935); **Chondrichthyes** (3,945)	6149	3 *Aptychotrema rostrata* (**4SN, 4SN2**); 3 *Urolopus* sp. (**4SR, 4***)
5H	*S. mokarran* (M)	*Platycephalus* spp. (24,007); *Dexillus* spp. (35,992); *Anoplocapros* spp. (57,956); Carcharhinidae (4)	*Anoplocapros* spp. (57,956); Carcharhinidae (4)	*Callianassa* spp. (365)*; Trachysalambria* spp. (87,918)	*Callianassa* spp. (365)	Cichlidae (470); Actinopteri (611); Carcharhinidae (4,228); Chondrichthyes (5,200)	Anguilliformes (21); Cichlidae (447); Actinopteri (314); Carcharhinidae (4,798); Chondrichthyes (4,350); Myliobatiformes (2)	3319	*Lactoria fornasini*; 3+ *Urolopus* sp.; 2 *Sillago* sp.; *Anoplocapros inermis* (2 scales)
6H	*S. mokarran* (M)	*Dexillus* spp. (506); *Anoplocapros* spp. (1,428); Carcharhinidae (63,304)	*Anoplocapros* spp. (1,428); Carcharhinidae (63,304)	*Sphyrna mokarran* (103)	*Sphyrna mokarran* (103)	Carcharhinidae (5,619); Chondrichthyes (5,200)	Carcharhinidae (5,541)	384	0

7H	*S. mokarran* (M)	***Gnathanacanthus* spp.** (57,053); *Anoplocapros* spp. (2,318); Diodontidae (35,675); *Monocentris* spp. (2,740); **Carcharhinidae** (4,016)	*Anoplocapros* spp. (2,318); Diodontidae (35,675); *Monocentris* spp. (2,740); **Carcharhinidae** (4,016)	0	0	Cichlidae (10); **Actinopteri** (36); **Carcharhinidae** (4,188); **Chondrichthyes** (402)	Actinopteri (29); **Carcharhinidae** (3,559); **Chondrichthyes** (239); Myliobatiformes (12)	5300	1 *Helicolenus percoides*.; 1 *Urolopus* sp. (**7SR**); 1+ *Dicotylichthys punctulatus* (jawbone)
CH	Sample control – *S. mokarran*	0	0	0	0	0	0	n/a	n/a
PCRneg1	PCR negative 1	0	0	0	0	*Homo sapiens* (8,126)	*Homo sapiens* (8,126)	n/a	n/a
1BT	*C. limbatus* (M)	*Hyperlophus vittatus* (19,547); *Gerres* spp. (25,466); *Dexillus* spp. (378); *Anoplocapros* spp. (933); Carcharhinidae (72);	*Hyperlophus vittatus* (19,547); *Anoplocapros* spp. (933); Carcharhinidae (72)	Carcharhinidae (16)	Carcharhinidae (16)	Cichlidae (19); Actinopteri (6); Carcharhinidae (1,739); Chondrichthyes (5)	Actinopteri (25); Carcharhinidae (1); Chondrichthyes (4)	708	0
2BT	*C.s limbatus* (F)	Carcharhinidae (63,975)	Carcharhinidae (63,975)	*Chrysomya* spp. (373);	*Chrysomya* spp. (373);	Carcharhinidae (5,223)	0	232	0

				Carcharhinidae (30)	Carcharhinidae (30)				
3BT	*C. limbatus* (M)	Carcharhinidae (10,460)	Carcharhinidae (10,460)	0	0	Carcharhinidae (6,201); Chondrichthyes (9)	Carcharhinidae (591); Chondrichthyes (1)	195	0
4BT	*C. limbatus* (F)	*Platycephalus* spp. (1,636); *Dexillus* spp. (2,011); *Anoplocapros* spp. (4,461); Carcharhinidae (24,679)	*Anoplocapros* spp. (4,461); Carcharhinidae (24,679)	*Lucilia* spp. (2)	*Lucilia* spp. (2)	Carcharhinidae (4,008); Chondrichthyes (9)	0	297	0
CBT	Sample Control – *C. limbatus*	Carcharhinidae (1,145)	Carcharhinidae (1,145)	0	0	*Homo sapiens* (575)	*Homo sapiens* (575)	n/a	n/a
PCRneg2	PCR negative 2	0	0	0	0	*Homo sapiens* (1,679)	*Homo sapiens* (1,679)	n/a	n/a
3A	*Urolopus* sp.	*Homo sapiens* (3); **Carcharhinidae** (4,643)	*Homo sapiens* (3); **Carcharhinidae** (4,643)	0	0	Chondrichthyes (9,489)	Carcharhinidae (11); Chondrichthyes (9,476)	unknown	0
3B	*Urolopus* sp.	**Carcharhinidae** (18,590)	**Carcharhinidae** (18,590)	***Callianassa* spp.** (143,578); *Matuta* spp. (71)	***Callianassa* spp.** (143,578)	Callianassidae (10); **Actinopteri** (1); **Carcharhinidae** (43); **Chondrichthyes** (7,934)	Callianassidae (10); **Carcharhinidae** (128); **Chondrichthyes** (7,857)	unknown	0

3C	*Urolopus* sp.	**Carcharhinidae** (5,135)	**Carcharhinidae** (5,135)	***Callianassa* spp.** (66,115)	***Callianassa* spp.** (66,115)	0	0	unknown	0
3D	*Urolopus* sp.	**Carcharhinidae** (3,923)	**Carcharhinidae** (3,923)	0	0	**Actinopteri** (3); **Carcharhinidae** (3,208); **Chondrichthyes** (14,433)	**Carcharhinidae** (40); **Chondrichthyes** (14,403)	unknown	0
4SR	*Urolopus* sp.	***Gnathophis* spp.** (69); ***Lepidotrigla* spp.** (958); ***Pseudorhombus* spp.** (50); ***Anoplocapros* spp.** (29,358); Rhinobatidae (2); **Carcharhinidae** (41,434)	***Gnathophis* spp.** (69); ***Lepidotrigla* spp.** (958); ***Anoplocapros* spp.** (29,358); **Carcharhinidae** (41,434)	***Sphyrna mokarran*** (2)	***Sphyrna mokarran*** (2)	**Actinopteri** (9); **Carcharhinidae** (3,208); **Chondrichthyes** (9,806)	**Carcharhinidae** (5,506); **Chondrichthyes** (7,071)	unknown	0
4SN	*Aptychotrema rostrata*	***Lepidotrigla* spp.** (11); ***Pseudorhombus* spp.** (57,577); ***Anoplocapros* spp.** (6,329); Rhinobatidae (9,815); **Carcharhinidae** (2,335)	***Lepidotrigla* spp.** (11); ***Anoplocapros* spp.** (6,329); **Carcharhinidae** (2,335)	***Portunus* spp.** (67,051); ***Ranina* spp.** (18,815)	***Portunus* spp.** (67,051)	Decapoda (3); **Actinopteri** (3); **Carcharhinidae** (74); **Chondrichthyes** (16,656)	Decapoda (3); **Actinopteri** (3); **Carcharhinidae** (230); **Chondrichthyes** (16,462)	unknown	0

4SN2	*Aptychotrema rostrata*	***Lepidotrigla* spp.** (4); ***Anoplocapros* spp.** (1,190)	***Lepidotrigla* spp.** (4); ***Anoplocapros* spp.** (1,190)	n/a	n/a	**Actinopteri** (7,360); **Carcharhinidae** (533); **Chondrichthyes** (6,474)	**Actinopteri** (7,176); **Carcharhinidae** (625); **Chondrichthyes** (6,465)	unknown	0
4	*Urolopus* sp.	***Anoplocapros* spp.** (51); Rhinobatidae (2); **Carcharhinidae** (2)	***Anoplocapros* spp.** (51); **Carcharhinidae** (2)	*Callianassa* spp. (1); ***Penaeoidea* spp.** (68,616)	*Callianassa* spp. (1); ***Penaeoidea* spp.** (68,616)	**Actinopteri** (271); **Carcharhinidae** (1); **Chondrichthyes** (13,045)	**Actinopteri** (271); **Carcharhinidae** (35); **Chondrichthyes** (13,020)	unknown	0
7SR	*Urolopus* sp.	***Gnathanacanthus* spp.** (22,770); **Carcharhinidae** (84)	**Carcharhinidae** (84)	*Callianassa* spp. (25,765); *Portunus* spp. (273)	*Callianassa* spp. (25,765); *Portunus* spp. (273)	Callianassidae (29); Decapoda (81); **Actinopteri** (48); **Carcharhinidae** (43); **Chondrichthyes** (6,888)	Callianassidae (29); Decapoda (81); **Carcharhinidae** (193); **Chondrichthyes** (6,726)	unknown	0

550

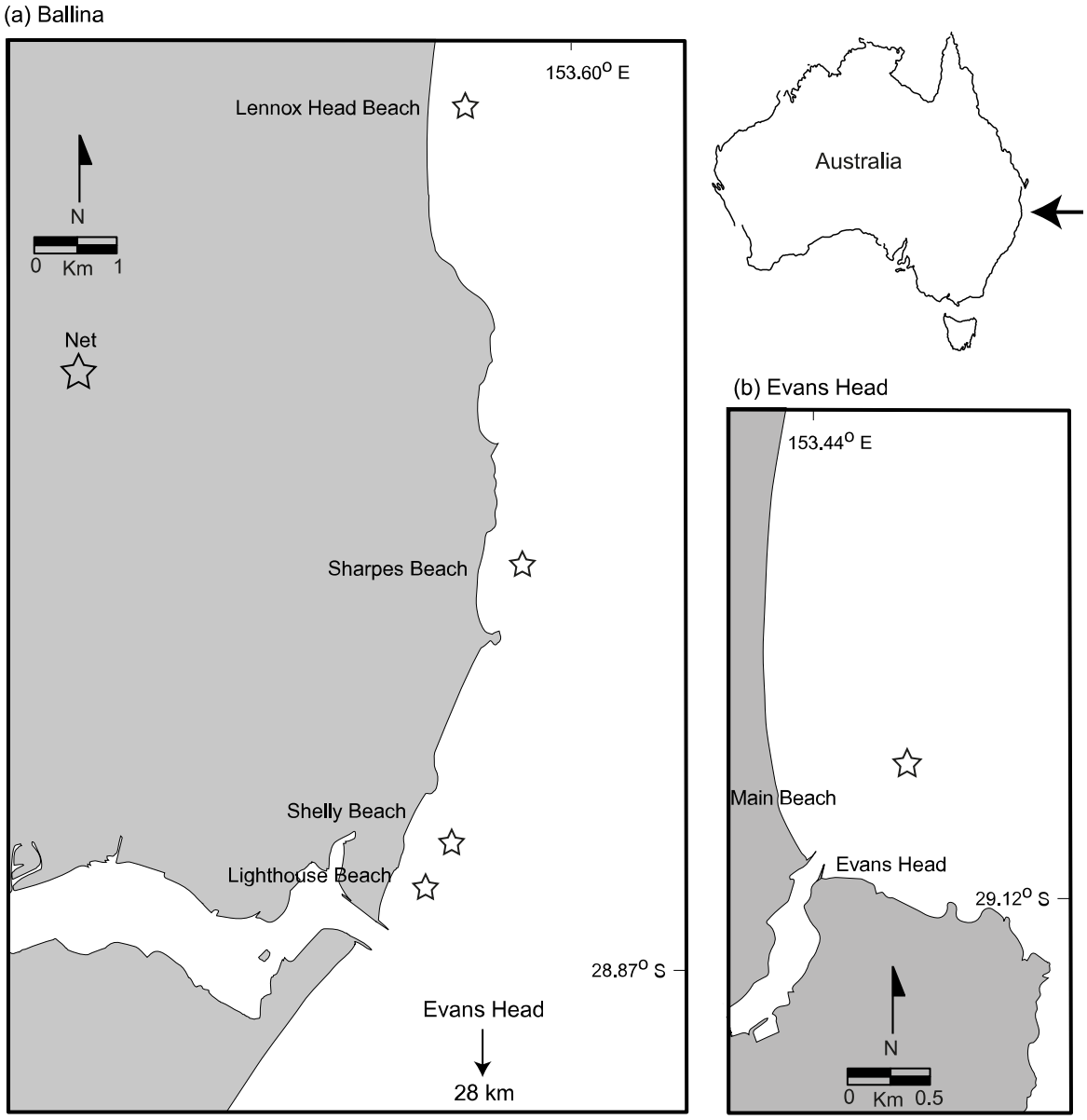
551 **Figure Legends**

552 **Figure 1.** Location of bather-protection gillnets deployed off (a) Ballina and (b) Evans Head

553 in New South Wales, Australia from which sharks were sampled between 7 February and 13

554 March, 2018.

555



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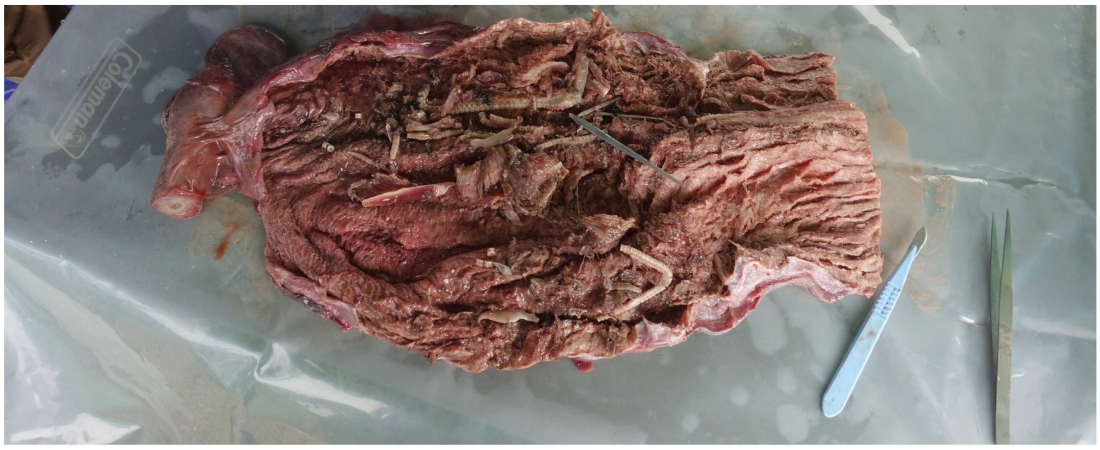
558 **Figure 2.** Photos of (a) jaw bones and (b) *Urolophus* sp. in the stomachs of two *Sphyrna*

559 *mokarran.*

(a)



(b)

560

Figures

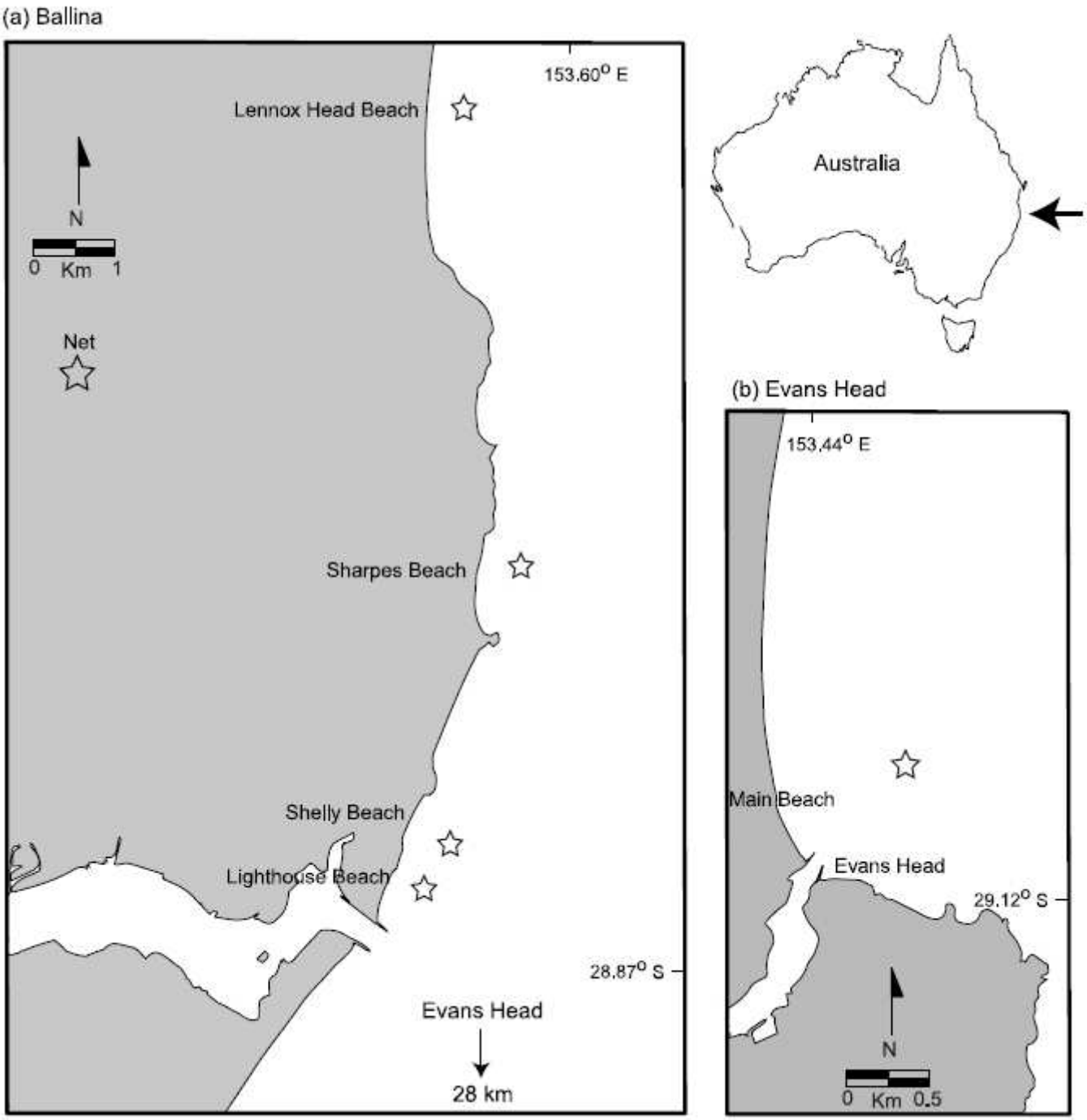


Figure 1

Location of bather-protection gillnets deployed off (a) Ballina and (b) Evans Head in New South Wales, Australia from which sharks were sampled between 7 February and 13 March, 2018. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country,

territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

Figure 2

Photos of (a) jaw bones and (b) Urolophus sp. in the stomachs of two Sphyrna mokarran.

Supplementary Files

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