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Auckland UniServices Limited
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Albatross diet: Composition of natural prey versus fisheries bait/waste

Albatrosses scavenging bait discards from fishers (Bank Peninsula, New Zealand 2024). Photo credit: Aimee van der Reis.

Aimee van der Reis, Fang Fei Tham & Andrew Jeffs

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Executive Summary

Seabird injury or mortality caused by interactions with New Zealand commercial fishing activities is a major conservation concern with the majority of interactions occurring in the surface longline (SLL) and trawl (TWL) fisheries. Albatrosses (Diomedeidae) are among the most threatened and the majority of these long-lived, large seabirds have broad geographic ranges. Seabirds are attracted to fishing vessel activity as an additional food source and this puts them at risk of interacting with vessel structures and fishing gear. This includes incidental capture whilst feeding on bait and discards. It is not clear to what extent the diet of albatrosses consists of naturally foraged prey in comparison to fisheries bait/waste associated with fishing activity, and ultimately their reliance on commercial fisheries as a food source.

In this dietary study, scat from colony birds and stomach contents from necropsy samples (commercial fishing mortalities) were used to detect taxa consumed by 10 albatross species using DNA metabarcoding. Scat samples (n=86) were opportunistically collected from four subantarctic islands between January 2019 to April 2024. Albatross necropsies (n=72) took place from September 2022 to February 2024. Based on the frequency of occurrence, the diet among all albatross samples consisted largely of fishes (> 50% deep-sea and beyond known albatross diving depths) and to lesser extent cephalopods. Differences in prey diversity (higher in necropsy samples) were found to be significant between sample type, however, no specific prey species were found to be responsible for this difference. Observer and fisher reported bait and discard species were predominantly squid and mackerel.

Overall, the majority of fish and cephalopod species identified in both colony scat and necropsy samples overlapped extensively with species that were most likely to be made available through SLL and TWL fisheries activities, i.e., discard/species targeted/bait used. These results suggest that albatrosses are heavily reliant on fisheries as a food source whether they were sampled from fishing vessels (i.e., necropsy) or from nesting sites (i.e., scats).

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Introduction

Seabirds are recognised as one of the most vulnerable groups of birds globally, with 41% of species listed as 'Threatened' or 'Near Threatened' (BirdLife International, 2022). Albatrosses are among the most threatened bird families (i.e., Diomedeidae), with the population numbers decreasing for 11 of the 22 species (IUCN, 2024). The majority of these long-lived, large seabirds have broad geographic ranges, and they usually breed on remote islands (Phillips et al., 2016). These breeding sites typically gain the status of 'international importance' as significant sites for seabird conservation (ACAP, 2015; Phillips et al., 2016). During the breeding season, albatrosses are known to forage over both large distances and distinct foraging areas closer to colonies (Walker & Elliott, 2022; Weimerskirch et al., 1993). The latter particularly so during the guard stage, which is when chicks are more susceptible and require high levels of parental care (pers. comm. Department of Conservation; DOC). The threats to survival while albatrosses are foraging include interactions with human fishing activities both directly (e.g., incidental capture whilst feeding on bait and discards) and indirectly (e.g., reduction in the abundance of key prey species).

The injury or mortality of seabirds that is associated with fishing vessel interactions is a major seabird conservation concern, particularly in surface longline (SLL) and trawl (TWL) fisheries (Baker et al., 2002; Oliveira et al., 2022; Zhou & Brothers, 2021). Seabirds scavenging from baited longline hooks can become hooked causing injury or death, or when colliding or becoming entangled with trawl nets and warp cables (Phillips et al., 2016). They are also vulnerable to bycatch when aggregating around fishing vessels to scavenge discards and fish processing offal/discharge. Numbers of albatross vessel-related mortality are variable depending on life stage, season, fishing region and gear type (e.g., SLL versus TWL; Baker et al., 2007; Walker & Elliott, 2006), resulting in varied impacts on albatross populations. Fisheries New Zealand (FNZ) and the New Zealand commercial fishing industry utilise a number of mitigation methods to reduce seabird-vessel interactions, including procedures for offal and discard management contained in 'Vessel Management Plans'. Despite the risk of bycatch, fisheries discards and fish processing offal may contribute in a positive manner to albatrosses well-being, by contributing to their nutrition and potentially reducing energetic expenditure associated with foraging (James & Stahl, 2000; Rolland et al., 2008). Overall, these seabird-fisheries interactions highlight the need for a deeper understanding of albatross dietary dependencies and their links to fisheries to better inform DOC and FNZ conservation and management decisions.

Different techniques for dietary analysis of organisms vary in their advantages and disadvantages and most rely on the analyses of recovered gut or faecal material, or the analyses of chemical signatures in tissues. A large proportion of albatross dietary studies have used morphological techniques to identify prey items (James & Stahl, 2000; Xavier et

al., 2014), while a smaller proportion of studies have used biochemical techniques, such as stable isotope ratios (Cherel et al., 2017) and DNA based approaches, such as DNA metabarcoding (McInnes, Alderman, Deagle, et al., 2017). Morphological techniques for analysing digesta or faecal material can allow for the estimation of prey age, size-class or mass for individual prey items, and can also be used to identify numbers of prey consumed. However, differential rates of digestion mean that morphological techniques may have an underestimation of soft-bodied prey and overrepresentation of prey with hard parts, especially for digested samples such as regurgitates and scat (Oehm et al., 2017; Xavier et al., 2005). Importantly, identifying prey items at the species level requires sufficient morphological structure, which is variable with digested content and not always achievable (e.g., if using scat there would be a greater reliance on indigestible items). Stable isotopes are also a well-established tool for assessing diet and can provide a means for tracing dietary changes over time (e.g., early versus adult life stages), however, this method typically lacks species-level taxonomic resolution (Carreon-Martinez & Heath, 2010) that can be obtained from DNA analyses. DNA metabarcoding (Taberlet et al., 2012) is a molecular method that can be used on a variety of materials, such as digesta and faecal material, to provide reliable species-level resolution, and the method is typically less impacted by digestion in comparison to morphological analyses (Oehm et al., 2017). Therefore, using DNA metabarcoding in the current study was critical. This method has proven to be a time- and cost-effective method to detect the presence of different species in samples without any reliance on morphological structure of items, and thus has had increasing application in dietary studies (Ando et al., 2020; de Sousa et al., 2019; van der Reis & Jeffs, 2020, 2021). However, there are some limitations to this method, such as an inability to directly measure the abundance of prey items (Ando et al., 2020; Deagle et al., 2013; Lamb et al., 2019; Piñol et al., 2015).

In a review on methods used to analyse albatross diets, at least one dietary study has been conducted for each of the 22 currently recognised albatross species with nearly two-thirds utilising morphological techniques, while the remainder used biochemical techniques (McInnes et al., 2016). Since, only a few have utilised DNA metabarcoding for investigating albatross diet (McInnes, Alderman, Deagle, et al., 2017; McInnes et al., 2020), but there is an increase in the uptake of this method for dietary analysis in seabirds in general (e.g., king shag - *Kawau pāteketeke,* van der Reis & Jeffs, 2020, 2021; little auk - *Alle alle*, de Leeuw et al., 2024). Research indicates albatrosses are opportunistic surface feeders undertaking short dives that rarely exceed 5 to 10 m depth and mostly feeding during daylight (Guilford et al., 2022; Phalan et al., 2007; Prince et al., 1994). The diet of albatrosses consists predominantly of cephalopods and/or fishes (e.g., Arata & Xavier, 2003; Cherel et al., 2000; Cherel et al., 2017; Cooper et al., 1992; Granadeiro et al., 2014; Imber, 1999; James & Stahl, 2000; Rodhouse et al., 1987; Waugh et al., 1999; West & Imber, 1986; Xavier et al., 2014). Crustaceans form a secondary component, while carrion and gelatinous zooplankton contribute on a relatively small scale (McInnes, Alderman, Lea, et al., 2017). Stable isotope analyses indicates that prey found in

epipelagic, mesopelagic and benthic zones dominate the diet of albatrosses (Cherel & Klages, 1998; Granadeiro et al., 2014). Some albatross species-specific studies have concluded that a large proportion of the diet is likely due to scavenging commercial fishing vessel discards and have investigated the occurrence of this interaction (e.g., Granadeiro et al., 2014; Walker & Elliott, 2006; Waugh et al., 2005). It must also be noted that cephalopods have been the focus taxa of some albatross dietary studies (e.g., Imber, 1992; Queirós et al., 2021; Rodhouse et al., 1987; van den Hoff, 2001; Xavier et al., 2014), but research suggests that the sample type analysed influences which taxa are more readily detected. For example, stomach content is a better representative of taxa ingested for morphological analyses, as voluntary regurgitates from chicks tends to reflect the cephalopod component more than fishes and/or crustaceans as they are more easily digested (Cooper et al., 1992; Imber, 1999; Xavier et al., 2005). The use of DNA metabarcoding would help mitigate these biases.

Little is known about population impacts to albatrosses if adult birds that are incubating or feeding chicks were to become increasingly reliant on fishing vessels for foraging success. A modification of foraging behaviour that results in greater reliance on vessels will likely increase bycatch risk. This is based on the greater likelihood of interacting with fishing gear if seabird-vessel encounter rates increase. For breeding birds, the population impact of an egg or chick left alone in the nest is severe and exacerbated by the reproductive characteristics of albatross species. Therefore, it is important to monitor changes in foraging preference to better inform bycatch mitigation for albatross species. The use of DNA metabarcoding is a cost-effective method for continuously monitoring temporal changes in albatross species diet to support conservation efforts.

The aim of this pilot study was primarily to investigate albatross species reliance on fishing vessels for food by assessing the effectiveness of DNA metabarcoding as a monitoring tool. It was also hoped that we could establish the proportion of fisheries vs naturally foraged food, but as the project progressed it became clear that the available data was insufficient to support this approach. Relevant literature was reviewed and is not contained as a separate section in the report, but rather it was used to inform discussions. DNA metabarcoding was used to identify ingested species (focusing on fishes and cephalopods) from albatross scat opportunistically collected from various New Zealand subantarctic islands during breeding seasons between 2019-2024 and stomach contents collected from albatrosses caught by commercial fishers between 2022-2024. For the latter we also used morphological assessments during necropsy to identify the number of birds caught by vessels that were likely feeding chicks or incubating eggs. The species consumed by albatrosses identified from DNA in the scat and necropsy samples were then compared to those species recorded in FNZ data as target species and bycatch. Three primer pairs were used to target eukaryotes in general, fishes, and cephalopods.

<u>CSP Project Objectives:</u>

1. Identify food items (species-level) from existing albatross scat/stomach samples using

established DNA metabarcoding techniques for dietary analysis.

2. Obtain information on vessel bait/discard species in the SLL fishery from the FNZ Centralised Observer Database and compare with DNA results to identify proportion of naturally foraged vs fisheries related food species for albatrosses.
3. Conduct a literature review and use findings from the current study to inform current knowledge about the reliance of albatross on fishing vessels for foraging, especially during breeding season.
4. Develop recommendations for future work that could better inform seabird bycatch risk assessment and identify potential for improved mitigation efforts to reduce attractiveness of vessels to seabirds.

Materials and methods

Sample collection

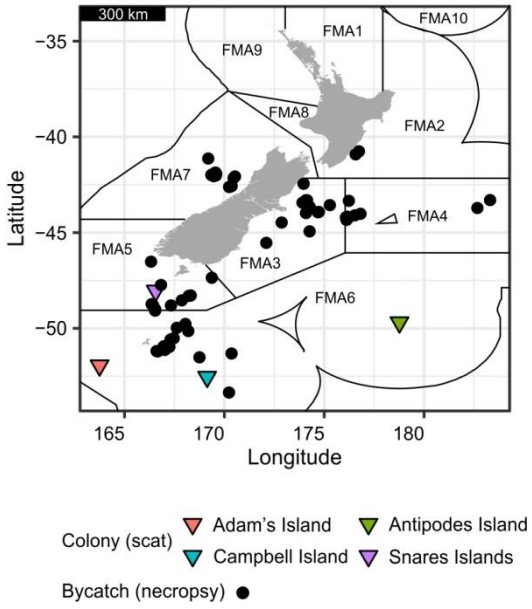

Figure 1. New Zealand albatross colony locations where scat samples were collected and locations of albatross bycatch mortalities. New Zealand fishery management areas (FMAs) are indicated.

Albatross scat samples (n=86) were opportunistically collected from breeding colonies on New Zealand's subantarctic islands, the Snares (n=41), Adam's (n=8), Antipodes (n=35), and Campbell (n=2) Islands, by researchers who were primarily conducting other seabird fieldwork (Figure 1; Table 1 and 2). Sample collection took place from January 2019 to April 2024. Albatross stomach samples were collected by DOC (K. Middlemiss) during necropsies (n=72; fishing vessel fatalities; Figure 1) that took place from September 2022 to February 2024 (with necropsies undertaken by Wildlife Management International Limited, Blenheim, New Zealand; Table 1 and 2). The majority of seabird fatalities were linked to trawl vessels (TWL: n=54; SLL: n=13; FNZ data for n=5 could not be linked to specific fishing events). Where possible the sex of the albatross was recorded either from data supplied by field work teams at each colony, or from morphological investigations during necropsy. In total, 33 necropsy samples were female, 38 male, and one unknown. For the scat samples, 14 were known to originate from females, 11 from males, and 61 were unknown. Samples were preserved in RNAlater, 70% ethanol, or DESS.

DNA extraction and amplification

A homogenous subsample was taken from the scat and necropsy stomach content samples which varied in size based on the size of the recovered sample from the albatross. The subsamples were extracted using the Nucleospin Tissue DNA extraction kit (Macherey-Nagel). The manufacturer's protocol was followed, including implementing an overnight lysis. The elution procedure followed the manufacturer's suggested modifications; elution buffer was preheated to 70 °C before use, 70 µl was used for the elution to increase the concentration and two elution steps were performed to obtain a high concentration and yield. Where possible, steps were conducted in a UV-sterilized laminar flow cabinet to mitigate possible contamination. The DNA quality and quantity was assessed visually by running 2 µl DNA run on a 0.8 % agarose gel, and visualized using Gel Red (Biotium), in a Gel Doc XR+ (Bio-Rad).

Table 1. The number of albatross scat (n=86) and necropsy (n=72) samples collected for dietary analyses. All samples were opportunistically collected from January 2019 to February 2024. For the necropsy samples, the number of albatross that were recorded during morphological examination as being with chick or egg is given in parentheses (58%). It was not possible to identify failed breeders. IUCN status of albatross species investigated in this study and geographical range (IUCN, 2024). * denotes an internationally important breeding site, constituting >1% of the global population. The Department of Conservation, New Zealand recognises the wandering albatross as two subspecies and they are treated as such in this study (*Diomedea antipodensis antipodensis* and *Diomedea antipodensis gibsoni*; Robertson et al., 2021). However, the IUCN recognises them as one species, *Diomedea antipodensis* (Burg & Croxall, 2004) and thus their known territories are the same. Population status is categorised as stable (=), decreasing (-), increasing (+), or not available (NA).

Code	Common name	Scientific name	Status	Known territory	Necropsy	Scat
ANT	Antipodean albatross	*Diomedea antipodensis antipodensis*	-	Breeding on Antipodes*, Adams*, Disappointment*, Auckland*, Campbell and Pitt Islands	0	35
BUL	Buller's albatross	*Thalassarche bulleri bulleri*	=	Breeding on Snares, Solander, Big* and Little Sister*, Rosemary Rock and Three Kings Islands	17 (10)	41
CAM	Campbell albatross	*Thalassarche impavida*	+	Breeding on Campbell Island*	1 (1)	0
CHA	Chatham albatross	*Thalassarche eremita*	=	Breeding on Chatham Island*	1	0
GIB	Gibson's albatross	*Diomedea antipodensis gibsoni*	-	Breeding on Antipodes*, Adams*, Disappointment*, Auckland*, Campbell and Pitt Islands	0	8
WHI	White-capped albatross	*Thalassarche steadi*	-	Breeding on Disappointment, Auckland, and Adams Islands	20 (10)	0
SAL	Salvin's albatross	*Thalassarche salvini*	NA	Breeding on Bounty*, Western Chain islets, The Pyramid and The Forty-Fours Islands	28 (21)	0
BLA	Black-browed albatross	*Thalassarche melanophris*	+	Breeding on Campbell and Antipodes Islands	1	0
ROY	Southern royal albatross	*Diomedea epomophora*	=	Breeding on Campbell*, Adams, Enderby, and Auckland Islands, and Taiaroa Head	3	2
WAN	Wandering albatross	*Diomedea exulans*	-	Non-breeding birds frequent New Zealand waters around the Tasman Sea, and from subantarctic waters to East Cape	1	0
		Total			**72**	**86**

Table 2. Albatross scat and necropsy samples month-year of collections/mortalities. For the necropsy samples, five samples were data deficient and could not be linked to a fishing event and thus month-year is unknown. Albatross mortalities have been linked to the type of fishing method, 'T' for trawl and 'S' for surface longline. The albatross samples were grouped by species; Antipodean albatross=ANT, Buller's albatross=BUL, Campbell albatross=CAM, Chatham albatross=CHA, Gibson's albatross=GIB, White-capped albatross=WHI, Salvin's albatross=SAL, Black-browed albatross=BLA, Southern royal albatross=ROY, and Wandering albatross=WAN.

	Year	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec
Necropsy	**2022**	-	-	-	-	-	BUL_T=1	BUL_T=1 WHI_T=1	BUL_T=3 CAM_T=1 WHI_T=1	BUL_T=1 CHA_T=1 ROY_T=1 SAL_T=3	SAL_T=1	BUL_T=1 SAL_T=3	SAL_T=5 WHI_T=1
	2023	SAL_T=2	SAL_T=3 WHI_T=2	BUL_T=1 SAL_T=2	BUL_T=1 ROY_S=1 WAN_T=1 WHI_T=2	BUL_T=2 WHI_T=1	BUL_T=1 WHI_T=1	BUL_T=1 SAL_T=1 WHI_S=4	BLA_S=1 BUL_S=2 WHI_S=4	ROY_S=1	-	SAL_T=1	SAL_T=3
	2024	BUL_T=2 WHI_T=1	-	-	-	WHI_T=1	-	-	-	-	-	-	-
	Subtotal	5	5	3	5	4	3	8	12	7	1	5	9
Scat	**2019**	ANT=4	-	-	-	-	-	-	-	-	-	-	-
	2020	-	-	ANT=1	-	-	-	-	-	-	-	-	ANT=4
	2022	ANT=6	-	-	BUL=10	-	-	-	-	-	-	-	GIB=4
	2023	ANT=3	ANT=8	BUL=11	-	-	-	-	-	-	-	-	ANT=2 GIB=4 ROY=1
	2024	ANT=6 ROY=1	ANT=1	-	BUL=20	-	-	-	-	-	-	-	-
	Subtotal	20	9	12	30	-	-	-	-	-	-	-	15
	Total	***25***	***14***	***15***	***35***	***4***	***3***	***8***	***12***	***7***	***1***	***5***	***24***

Albatross sex identification was attempted using molecular markers, as applied in van der Reis and Jeffs (2020). To confirm sex identification, and to resolve the sex of 'unidentified' samples, the chromodomain helicase DNA binding (CHD) gene was used. The CHD gene is a universal molecular marker for sexing birds. The different lengths in Z and W chromosomes make it an ideal marker, females generally displaying two bands of different sizes (CHD-Z and CHD-W gene fragments) and males have two identical- sized CHD-Z copies (Çakmak et al., 2017). CHD1F/CHD1R primer set (Çakmak et al., 2017; Lee et al., 2010) was used for sexing samples (Table 3). The primer set 2250F/2718R was also tested (Fridolfsson & Ellegren, 1999), but it was found that CHD1F/CHD1R amplified more readily and thus CHD1F/CHD1R was used for all further sex identifications.

The ingested taxa within the samples were targeted by using universal and taxa-specific primers (Table 3). To amplify a broad range of taxa, a ~300 bp region of COI (mitochondrial DNA) was targeted for amplification (Geller et al., 2013; Leray et al., 2013). It is a conserved gene region but has enough variation to discriminate among closely related taxa and typically provides resolution to species-level. Two other taxa-specific primer sets were also used (Table 3). These primer sets target a ~200 bp portion of the 16S gene region (mitochondrial DNA) and were designed for amplification of fish (Berry et al., 2017; Deagle et al., 2007) and cephalopods (Berry et al., 2017; Peters et al., 2015). All primers were ordered with the Illumina Nextera adapters (Illumina, 2013).

Polymerase chain reactions (PCRs) were done in triplicate using the MyTaq Red Mix (Bioline; Meridian Bioscience) master mix; 12.5 µl MyTaq Red Mix, 0.5 µl of each primer (10 µM), 11.5 µl UltraPure DNase/RNase-Free Distilled Water (Invitrogen; Thermo Fisher Scientific), and 1 µl DNA. BSA (2 µl; 1%) was added when necessary and water volume was decreased proportionally. If poor amplification was seen, the DNA volume was doubled in an attempt to increase amplification by increasing the amount of template DNA in the PCR reaction. Furthermore, the addition of BSA was found to only be advantageous for sex identification PCRs, with no substantial increase in amplification seen for the gut content PCRs when incorporated into the PCR protocol. Negative controls were included in the DNA extractions (extraction blank - no tissue added) and subsequently in the PCRs, to check for possible contamination. Possible contamination was also monitored by including a PCR blank (no DNA added) in every PCR run. The PCR products were run on a 1.6% agarose gel, and visualized using Gel Red (Biotium), in a Gel Doc XR+ (Bio-Rad).

Table 3. Primer sets used to amplify gut content taxa in albatross scat and necropsy samples. Nextra adapters were added to all primers at the 5' end when ordering (F: TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG; R: GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG).

Gene	Sequence 5'-3'	PCR profile
CHD	CHD1F (Lee et al., 2010): TATCGTCAGTTTCCTTTTCAGGT CHD1R (Çakmak et al., 2017): CCTTTTATTGATCCATCAAGCCT	94 °C 4 min Touchdown: [94 °C 30 sec, 57-50 °C 45 sec, 72 °C 45 sec] 30 x [94 °C 30 sec, 50 °C 45 sec, 72 °C 45 sec] 74°C 5 min
COI	M1COIintF (Leray et al., 2013): GGWACWGGWTGAACWGTWTAYCCYCC jgHCO2198 (Geller et al., 2013): TANACYTCNIGRTGNCCRAARAAYCA	94 °C 1 min 30 x [94 °C 30 sec, 54 °C 90 sec, 72 °C 1 min] 72 °C 5 min
16S fish	Fish16sF/D (Berry et al., 2017): GACCCTATGGAGCTTTAGAC 16s2R (Deagle et al., 2007): CGCTGTTATCCCTADRGTAACT	95 °C 5 min 30 x [95 °C 30 sec, 54 °C 30 sec, 72 °C 45 sec] 72 °C 10 min
16S cephalopod (16S ceph)	Ceph16S1_F (Peters et al., 2015): GACGAGAAGACCCTADTGAGC Ceph16SR_Short (Berry et al., 2017): CCAACATCGAGGTCGCAATC	95 °C 5 min 30 x [95 °C 30 sec, 54 °C 30 sec, 72 °C 45 sec] 72 °C 10 min

PCR products were pooled by sample per gene region and cleaned up to remove primer dimers following the Illumina protocol (Illumina, 2013). The concentration of the PCR products was determined using Qubit HS (Invitrogen). Equal molarity was achieved by diluting (where possible) the PCR products; the robotic liquid handler (Eppendorf epMotion 5075) aliquoted calculated water volumes per sample per gene and then a standardized volume of PCR product was added. The PCR products underwent indexing before sequencing occurred using an Illumina MiSeq system (2×300 paired-end) by Auckland Genomics. The raw sequencing data was returned demultiplexed.

Bioinformatics and data quality control

Raw demultiplexed sequencing data underwent primer removal (Cutadapt v4.1; Martin, 2011) and initial quality assessment (Qiime 2 v2023.5; Bolyen et al., 2019). The raw reads were visually assessed for quality (i.e., quality scores) and the optimal length identified for truncation to remove successive poor-quality bases. DADA2 (within Qiime 2; Callahan et al., 2016) was used to implement truncation and for further quality control; filtering to retain only high-quality sequences that passed denoising, merging, and chimera formation assessment. This produced sequences that were then clustered at 100% identity (known as amplicon sequence variants – ASVs; Callahan et al., 2017). The ASVs were then assigned taxonomy using GenBank's nucleotide database (v2024- 04) and megaBLAST (BLAST v2.13.0; Morgulis et al., 2008).

The resulting sequence data were process in RStudio (v1.4.1106; R base v4.1.0; R Core Team, 2021). As the albatross samples had been extracted in batches (max n=30) and PCR'd in plates (n=96; 1×scat and 1×necropsy), the DNA and PCR negative controls were treated in different ways. The DNA negative ASVs were assigned to the respective samples in their extraction batch by ASV (unique identifier), whereas the PCR negatives

were assigned to the entire plate of samples. The number of control reads (PCR and DNA negative) per ASV per sample were added. The total control reads were doubled and then subtracted from the sample's reads for that ASV. All ASVs that were ≤ 0 reads were removed. This ensured ASVs were not omitted prematurely based purely on being present in the controls when it was obvious it was not contamination. For example, ASV a31f805b2d9a68b40ed3cd48767f171a (*Batoteuthis skolops*; bush-club squid) for sample AA7 had 107,952 reads and the control reads were 243. Furthermore, only ASVs (per sample) with > 10 reads post subtraction were retained to further mitigate possible cross-contamination and PCR artefacts. The parameters set for an ASV assignment was qcovhsp ≥ 95%, an e-value ≤ 0.001, and pident ≥ 90% (≥ 97% set as the threshold for genus-level).

Data analyses

Prior to filtering ASVs based on BLAST taxonomic assignments parameter thresholds, ASV assignments were investigated (COI dataset used as an exemplar). If the assignment met the genus-level threshold, it was included as part of the assignment label, otherwise a generic ASV number was assigned. ASVs that had a relative read abundance (RRA) > 5% were retained for sample clustering purposes. The RRA values were then converted to a distance matrix (function: *dist*, method="euclidean", package: stats v4.1.0) and hierarchical cluster on dissimilarities was subsequently performed (function: *hclust*, method="complete", stats). The hierarchical clustering for ASVs and samples was then visualized as a dendogram (function: *as.dendrogram*, stats) using ggtree (v3.9.1; Yu et al., 2017), and further annotated. A RRA heatmap was created for the ASVs with RRAs > 5% using ggplot2 (v3.4.3; Wickham, 2016). The final figure was compiled in CoralDRAW Essentials X5 (v15.2.0.686). This was done separately for both necropsy and scat samples.

The ASV dataset, where pident ≥ 90% and inclusive of all primers, was converted to a binomial matrix. Alpha diversity (function: *diversity*, index="shannon", vegan v2.5-7; Oksanen et al., 2019) values were calculated. Diversity differences among necropsy and scat samples (sample type) were tested using an analysis of variance (ANOVA; function: *aov*, stats). A permutational multivariate analysis of variance (PERMANOVA) was then used to test for possible difference between sample type, albatross species, month of collection/mortality occurrence, and sex (independent variables). The data set was first converted to a distance matrix (function: *vegdist*, method="jaccard", vegan) and then the PERMANOVA (function: *adonis2*, vegan) was run. If a significant difference existed, a generalised linear model (GLM; function: *glm*, stats) was run to investigate the independent variable(s) using the binomial dataset to identify which ASV may be driving the difference(s) (function: *emmeans*, method="response", emmeans v1.6.1 - Lenth, 2019; within the function: *pairs*, graphics v4.1.0). The results were then filtered to retain only those results with a p-value < 0.05 and a standard error < 5. A non-metric multidimensional scaling (NMDS) was run for visualization purposes (function: *metaMDS*,

vegan).

Bait, discard and target species data for bycatch events were obtained from FNZ (Centralised Observer database and fisher reported) to compare with species identified in scat and necropsy samples. Data extracts were requested for TWL and SLL events for the albatross scat and necropsy sampling duration (2019-2024), and then filtered to retain data recorded below 40° S to align with known location of seabird mortalities. From these data, the recorded target and non-target fish species were categorized into broad groups. If a target species, i.e., category label 'target', of one fishing event was also caught when targeting a different species, it was recategorized as 'discard/target'. Non-target species were categorized as 'discard'. No bait species were recorded for the SLL sub-dataset (i.e., no data recorded for fishing events), but other records indicated that longline bait species currently used in the SLL fishery primarily consist of squid (predominantly arrow squid), pilchard, and saury (Hickcox et al., 2024). Thus, these taxa were categorized as 'bait'.

To combine the fisheries information with the fish and cephalopod genus-level dataset (pident ≥ 97%), the taxa identified from the necropsy and scat samples were assigned into the above-mentioned FNZ board group categories at genus-level. For the necropsy samples, if the taxa overlapped with the fishing event where the fatality occurred it was recorded as a 'catch match'. Fishbase (rfishbase v4.1.2; Boettiger et al., 2012) was used to download fish species (function: *species*) information that included depth range and population range (function: *country*). Any discrepancies identified among primers for fish species (further investigated on GenBank) or missing data from rfishbase (looked in published records) was resolved as best possible. Fish taxa were placed in groups with respect to minimum depth they have been recorded at (i.e., smallest value of the depth range extracted from Fishbase). Most albatrosses are unlikely to dive beyond 20 m, and typically do shallow dives, thus fishes with a minimum depth ≤ 20 m were separated from those whose minimum depth was > 20 m. This separation provided an estimate of fishes consumed within or outside albatrosses diving range. This was only done for fishes as they were the predominant prey item and the majority of scat samples did not contain cephalopod species and thus limited comparison to necropsy samples.

Results

Ingested taxa identified from albatrosses

At a gross level, two main groups of taxa were identified to have the most frequent ingestion for both the scat and necropsy samples: fishes (ray-finned and cartilaginous) and cephalopods. The frequency of occurrence for fishes was 94% (n=64) and 92% (n=44), and 44% (n=30) and 17% (n=8) for cephalopods for necropsy and scat samples, respectively. Other taxa groups identified, but occurring in three or less samples were Cnidaria, Porifera, Tunicata, Echinodermata, Hexanauplia, and Malacostraca (Figure 2).

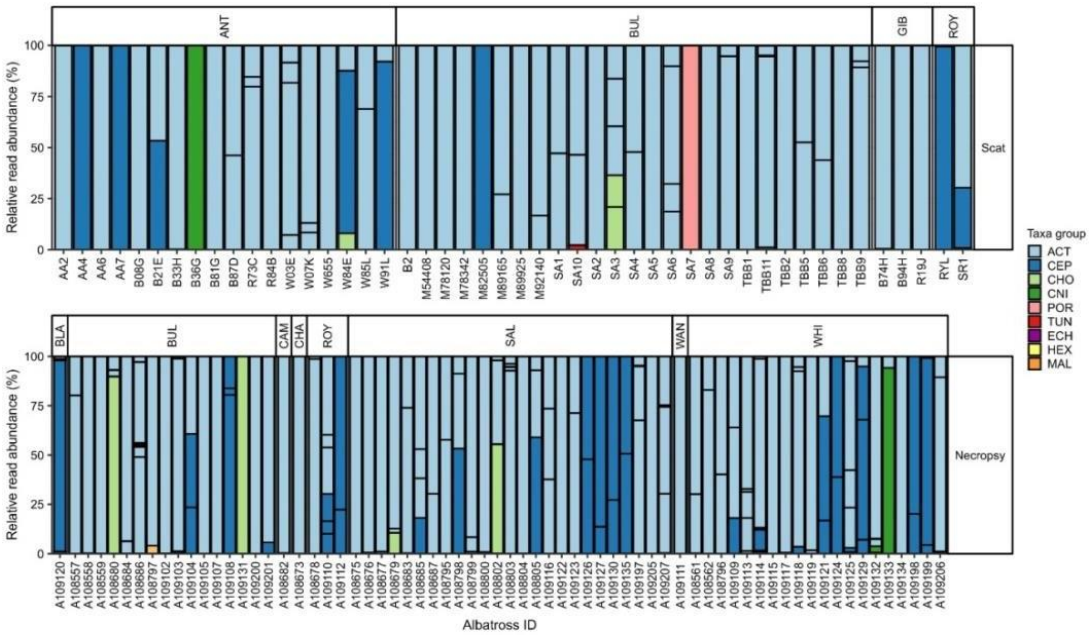

Figure 2. Taxa identified from scat and necropsy samples grouped for 10 albatross species; Antipodean albatross=ANT, Buller's albatross=BUL, Campbell albatross=CAM, Chatham albatross=CHA, Gibson's albatross=GIB, White-capped albatross=WHI, Salvin's albatross=SAL, Black-browed albatross=BLA, Southern royal albatross=ROY, and Wandering albatross=WAN. Taxa are colour coded by gross taxonomic level; Actinopteri/Actinopterygii=ACT, Cephalopoda=CEP, Chondrichthyes=CHO, Cnidaria=CNI, Porifera=POR, Tunicata=TUN, Echinodermata=ECH, Hexanauplia=HEX, and Malacostraca=MAL.

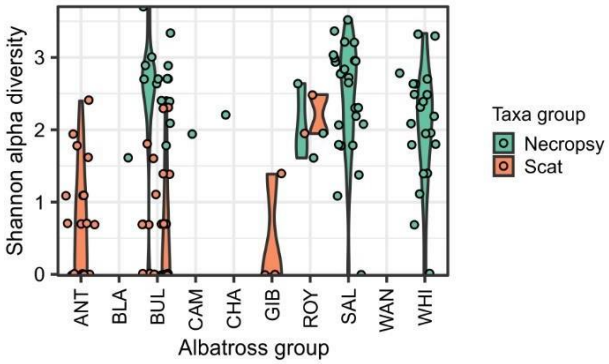

Figure 3. Alpha diversity (Shannon index) of taxa identified from scat and necropsy samples for 10 albatross species.

The alpha diversity analysis (using ASVs) indicated a difference between the necropsy and scat samples ($p < 0.05$; Figure 3). The mean diversity for the scat albatross groups was typically less than one, except ROY that was 2.2. The necropsy albatross groups were all above a mean alpha diversity of 1.5 (Table S1).

A PERMANOVA indicated differences for sample type (necropsy versus scat; R^2 =0.02, F=2.31, p=0.001) and month of collection/bycatch (R^2=0.25, F=1.13,

p=0.001). No differences were identified among albatross species or between sexes, nor for any of the pairwise interaction combinations. Due to opportunistic sampling of albatross species and limited replication per month, only sample type was further investigated using a GLM, however, no significant differences between necropsy and scat (per ASV) could be found. No visualisation of potential differences was possible as the NMDS failed to converge.

Fishes (≥68 spp.) and cephalopod (9 spp.), assigned at a minimum of genus-level, were identified as the main dietary items across all albatross species and sample type (i.e., necropsy versus scat). The majority of taxa identified in these groups were associated with fisheries discard/target/bait categories based on FNZ observer and fisher reported data (fishes=57; cephalopods=9; Figure 4 and 5). Taxonomic assignments not linked to vessel data as possible discard/target/bait (n=11) were lanternfishes/lightfishes (*Bolinichthys supralateralis*, *Lampadena notialis*, *Lampanyctodes hectoris*, *Gymnoscopelus piabilis*, *G. microlampas*, *Phosichthys argenteus*, and *Protomyctophum bolini*), bluebottle-fish (*Nomeus gronovii*), smallscale waryfish (*Scopelosaurus hamiltoni*), giant oarfish (*Regalecus glesne*), ox-eyed oreo (*Oreosoma atlanticum*). Each of these fish species were found to occur in ≤ 2 individual birds (Figure 6). More than 50% of species identified were known to reside at depths greater than 20 m (Figure 6; Table 4 and S2). The most commonly ingested genera (> 10% frequency of occurrence; FOO) identified in the necropsy samples were *Nototodarus sloanii* (squid; 31.8%), *Macruronus novaezelandiae* (28.8%), *Seriolella brama* (18.2%), *Lepidorhynchus denticulatus* (15.2%), *Coelorinchus oliverianus* (10.6%), and *Merluccius australis* (10.6%; Figure 6). In comparison, there were two genera identified from the scat samples (> 10% FOO); *Coelorinchus fasciatus* (11.9%), and *Macruronus novaezelandiae* (11.9%; Figure 6).

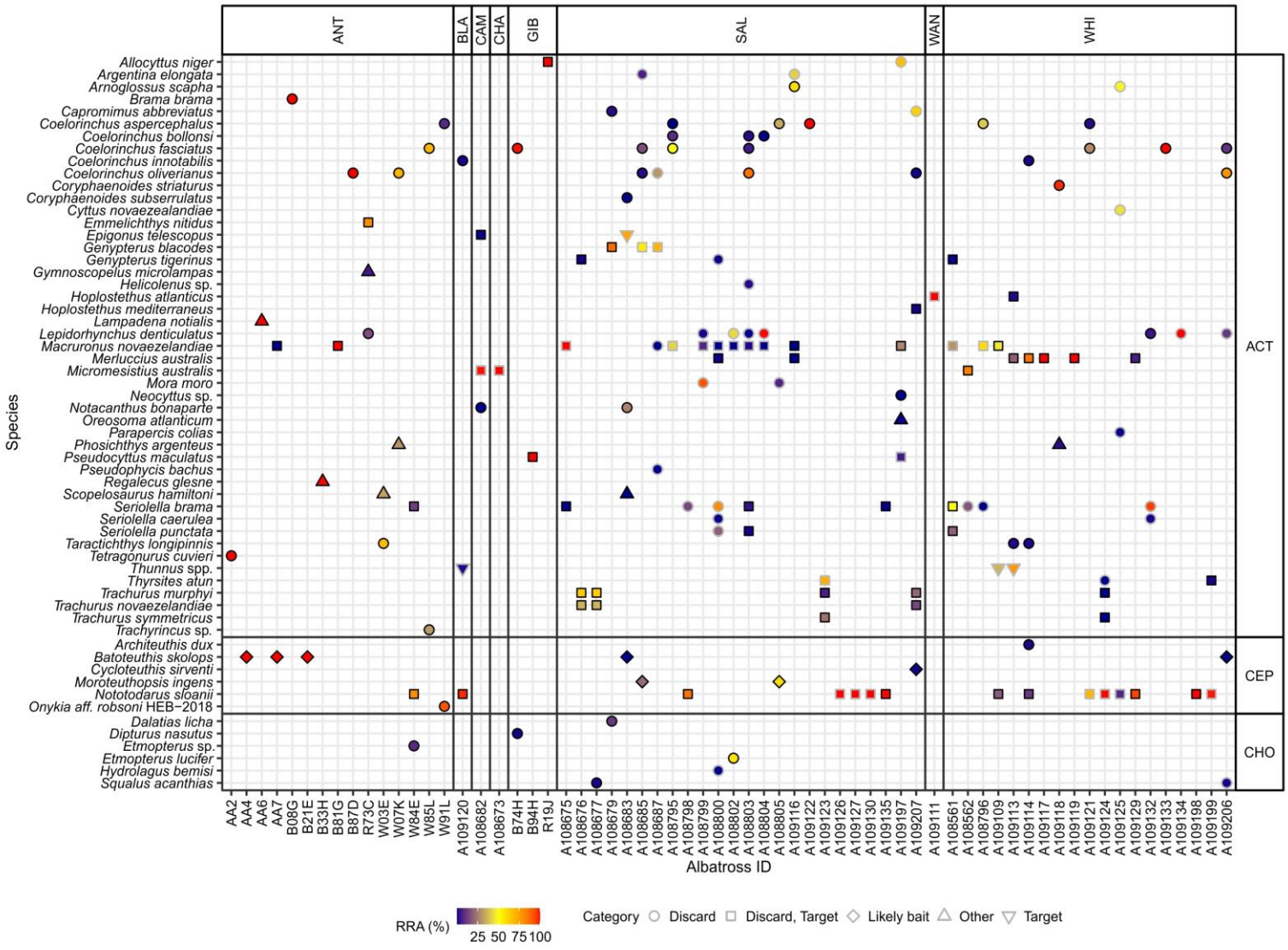

Figure 4. Taxa identified from albatross scat and necropsy samples (A10xxxx; WMIL unique IDs). The shape indicates the category the taxa is assigned to; discard, discard/target, target, likely bait, or other. For the necropsy samples, if the taxa identified were also reported by an observer during the fishing event when the albatross was caught (catch match), the outline colour is gray. Colour of the shape indicates the relative read abundance (RRA) percentage. Samples are grouped by albatross species on the x-axis; Antipodean albatross=ANT, Campbell albatross=CAM, Chatham albatross=CHA, Gibson's albatross=GIB, White-capped albatross=WHI, Salvin's albatross=SAL, Black-browed albatross=BLA, and Wandering albatross=WAN. Taxa identified are grouped on the y-axis; Actinopteri/Actinopterygii=ACT, Cephalopoda=CEP, and Chondrichthyes=CHO.

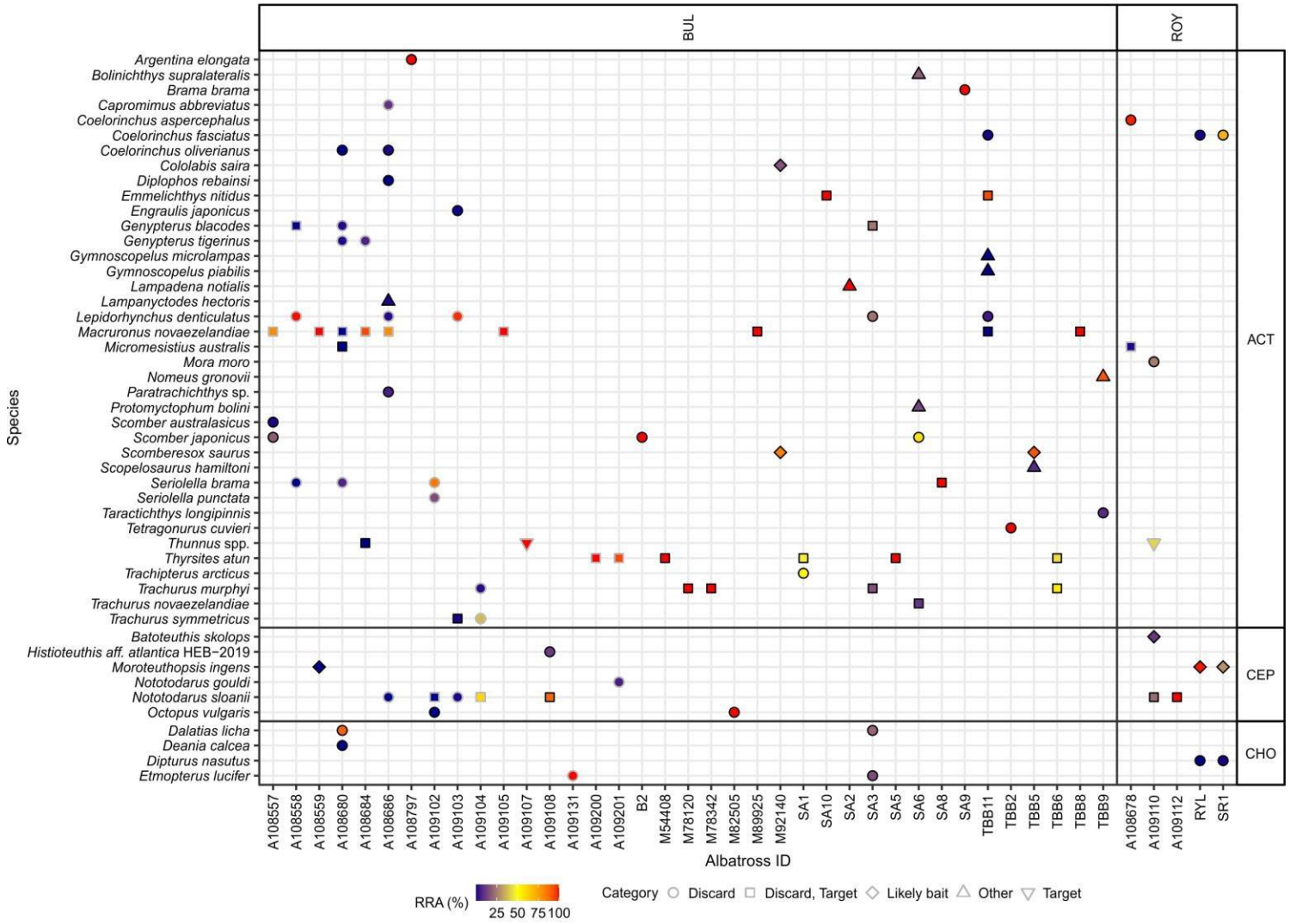

Figure 5. Taxa identified from albatross species for which there are scat and necropsy (A10xxxx; WMIL unique IDs) stomach samples. The shape indicates the category the taxa is assigned to; discard, discard/target, target, likely bait, or other. For the necropsy samples, if the taxa identified were also observed during the fishing event the albatross was caught (catch match), the outline colour is gray. Colour of the shape indicates the relative read abundance (RRA) percentage. Samples are grouped by albatross species on the x-axis; Buller's albatross=BUL and Southern royal albatross=ROY. Taxa identified are grouped on the y-axis; Actinopteri/Actinopterygii=ACT, Cephalopoda=CEP, and Chondrichthyes=CHO.

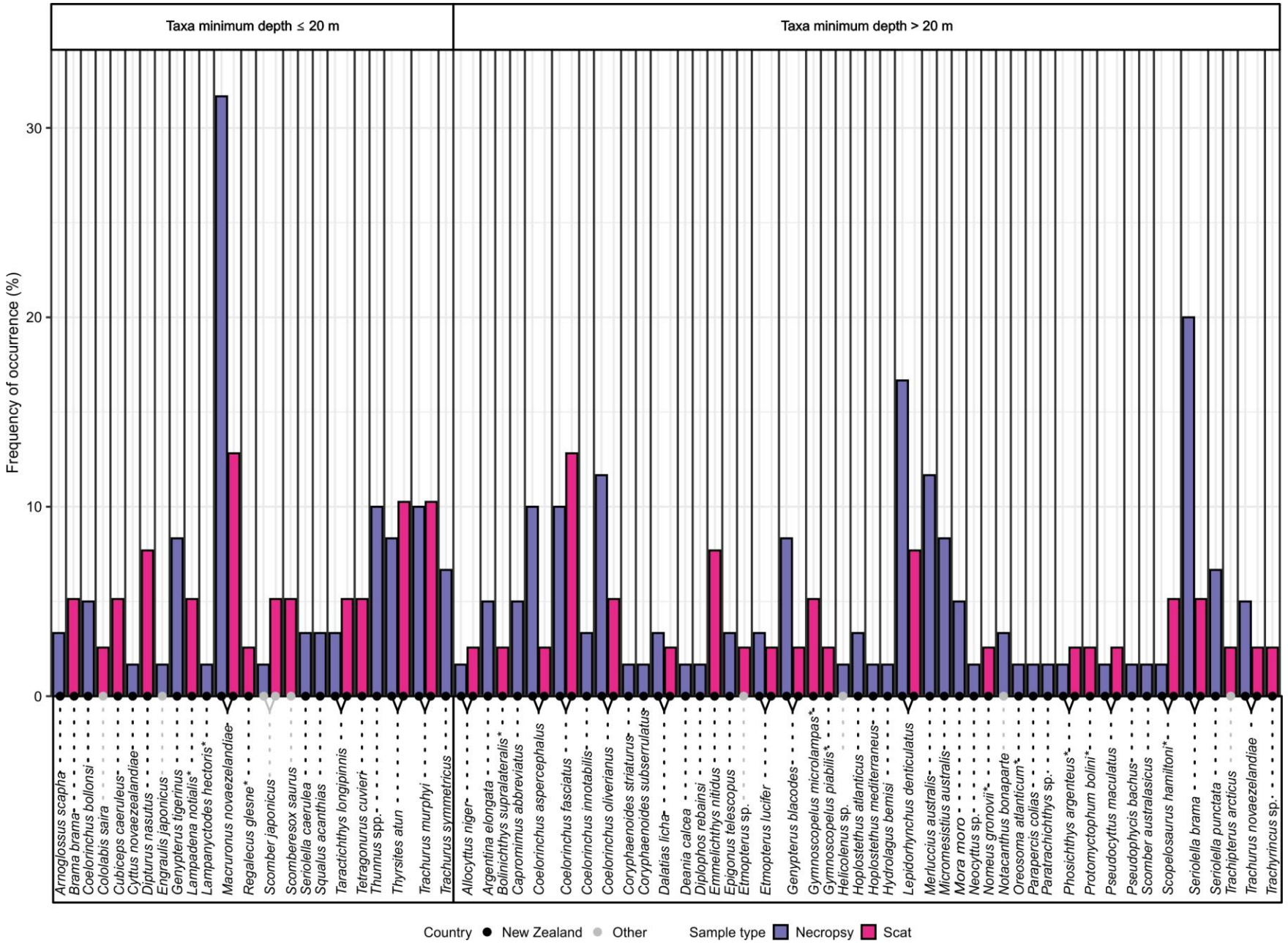

Figure 6. The frequency of occurrence of the fish taxa identified in a total of 39 scat and 60 necropsy samples (genus-level dataset) from 10 albatross species. Taxa are grouped by a minimum depth ≤ 20 m or >20 m. Eleven taxa could not be categorised as fisheries discard/target/bait (*).

Table 4. Fishes detected in albatross necropsy (nec) stomach and scat samples from colonies. Fishes minimum depth was sourced from Fishbase and fishes were grouped if minimum depth was recorded as being above/equal to or below 20 m. Samples are grouped by albatross species; Antipodean albatross=ANT, Campbell albatross=CAM, Chatham albatross=CHA, Gibson's albatross=GIB, White-capped albatross=WHI, Salvin's albatross=SAL, Black-browed albatross=BLA, and Wandering albatross=WAN. Taxa are grouped into Actinopteri/Actinopterygii=ACT and Chondrichthyes=CHO. Total number of albatross per group that had fishes detected was calculated (in parentheses in header). Eleven taxa could not be categorised as fisheries discard/target/bait (*). Values in the table indicate the number of albatross per species for which the fish was identified in, and the percentage is provided in parentheses.

Depth (m)	Taxa	Species	ANT Scat (14)	BLA Nec (1)	BUL Nec (15)	BUL Scat (20)	CAM Nec (1)	CHA Nec (1)	GIB Scat (3)	ROY Nec (2)	ROY Scat (2)	SAL Nec (21)	WAN Nec (1)	WHI Nec (18)
≤ 20	ACT	*Arnoglossus scapha*	-	-	-	-	-	-	-	-	-	1 (4.76)	-	1 (5.56)
		Brama brama	1 (7.14)	-	-	1 (5)	-	-	-	-	-	-	-	-
		Coelorinchus bollonsi	-	-	-	-	-	-	-	-	-	3 (14.29)	-	-
		Cololabis saira	-	-	-	1 (5)	-	-	-	-	-	-	-	-
		Cubiceps caeruleus	2 (14.29)	-	-	-	-	-	-	-	-	-	-	-
		Cyttus novaezealandiae	-	-	-	-	-	-	-	-	-	-	-	1 (5.56)
		Engraulis japonicus	-	-	1 (6.67)	-	-	-	-	-	-	-	-	-
		Genypterus tigerinus	-	-	2 (13.33)	-	-	-	-	-	-	2 (9.52)	-	1 (5.56)
		*Lampadena notialis**	1 (7.14)	-	-	1 (5)	-	-	-	-	-	-	-	-
		*Lampanyctodes hectoris**	-	-	1 (6.67)	-	-	-	-	-	-	-	-	-
		Macruronus novaezelandiae	2 (14.29)	-	6 (40)	3 (15)	-	-	-	-	-	10 (47.62)	-	3 (16.67)
		*Regalecus glesne**	1 (7.14)	-	-	-	-	-	-	-	-	-	-	-
		Scomber japonicus	-	-	1 (6.67)	2 (10)	-	-	-	-	-	-	-	-
		Scomberesox saurus	-	-	-	2 (10)	-	-	-	-	-	-	-	-
		Seriolella caerulea	-	-	-	-	-	-	-	-	-	1 (4.76)	-	1 (5.56)
		Taractichthys longipinnis	1 (7.14)	-	-	1 (5)	-	-	-	-	-	-	-	2 (11.11)
		Tetragonurus cuvieri	1 (7.14)	-	-	1 (5)	-	-	-	-	-	-	-	-
		Thunnus spp.	-	1 (100)	2 (13.33)	-	-	-	-	1 (50)	-	-	-	2 (11.11)
		Thyrsites atun	-	-	2 (13.33)	4 (20)	-	-	-	-	-	1 (4.76)	-	2 (11.11)
		Trachurus murphyi	-	-	1 (6.67)	4 (20)	-	-	-	-	-	4 (19.05)	-	1 (5.56)
		Trachurus symmetricus	-	-	2 (13.33)	-	-	-	-	-	-	1 (4.76)	-	1 (5.56)
	CHO	*Dipturus nasutus*	-	-	-	-	-	-	1 (33.33)	-	2 (100)	-	-	-
		Squalus acanthias	-	-	-	-	-	-	-	-	-	1 (4.76)	-	1 (5.56)
> 20	ACT	*Allocyttus niger*	-	-	-	-	-	-	1 (33.33)	-	-	1 (4.76)	-	-
		Argentina elongata	-	-	1 (6.67)	-	-	-	-	-	-	2 (9.52)	-	-
		*Bolinichthys supralateralis**	-	-	-	1 (5)	-	-	-	-	-	-	-	-
		Capromimus abbreviatus	-	-	1 (6.67)	-	-	-	-	-	-	2 (9.52)	-	-
		Coelorinchus aspercephalus	1 (7.14)	-	-	-	-	-	-	1 (50)	-	3 (14.29)	-	2 (11.11)
		Coelorinchus fasciatus	1 (7.14)	-	-	1 (5)	-	-	1 (33.33)	-	2 (100)	3 (14.29)	-	3 (16.67)
		Coelorinchus innotabilis	-	1 (100)	-	-	-	-	-	-	-	-	-	1 (5.56)
		Coelorinchus oliverianus	2 (14.29)	-	2 (13.33)	-	-	-	-	-	-	4 (19.05)	-	1 (5.56)
		Coryphaenoides striaturus	-	-	-	-	-	-	-	-	-	-	-	1 (5.56)
		Coryphaenoides subserrulatus	-	-	-	-	-	-	-	-	-	1 (4.76)	-	-
		Diplophos rebainsi	-	-	1 (6.67)	-	-	-	-	-	-	-	-	-

Depth (m)	Taxa	Species	ANT Scat (14)	BLA Nec (1)	BUL Nec (15)	BUL Scat (20)	CAM Nec (1)	CHA Nec (1)	GIB Scat (3)	ROY Nec (2)	ROY Scat (2)	SAL Nec (21)	WAN Nec (1)	WHI Nec (18)
		Emmelichthys nitidus	1 (7.14)	-	-	2 (10)	-	-	-	-	-	-	-	-
		Epigonus telescopus	-	-	-	-	1 (100)	-	-	-	-	1 (4.76)	-	-
		Genypterus blacodes	-	-	2 (13.33)	1 (5)	-	-	-	-	-	3 (14.29)	-	-
		*Gymnoscopelus microlampas**	1 (7.14)	-	-	1 (5)	-	-	-	-	-	-	-	-
		*Gymnoscopelus piabilis**	-	-	-	1 (5)	-	-	-	-	-	-	-	-
		Helicolenus sp.	-	-	-	-	-	-	-	-	-	1 (4.76)	-	-
		Hoplostethus atlanticus	-	-	-	-	-	-	-	-	-	-	1 (100)	1 (5.56)
		Hoplostethus mediterraneus	-	-	-	-	-	-	-	-	-	1 (4.76)	-	-
		Lepidorhynchus denticulatus	1 (7.14)	-	3 (20)	2 (10)	-	-	-	-	-	4 (19.05)	-	3 (16.67)
		Merluccius australis	-	-	-	-	-	-	-	-	-	2 (9.52)	-	5 (27.78)
		Micromesistius australis	-	-	1 (6.67)	-	1 (100)	1 (100)	-	1 (50)	-	-	-	1 (5.56)
		Mora moro	-	-	-	-	-	-	-	1 (50)	-	2 (9.52)	-	-
		Neocyttus sp.	-	-	-	-	-	-	-	-	-	1 (4.76)	-	-
		*Nomeus gronovii**	-	-	-	1 (5)	-	-	-	-	-	-	-	-
		Notacanthus bonaparte	-	-	-	-	1 (100)	-	-	-	-	1 (4.76)	-	-
		*Oreosoma atlanticum**	-	-	-	-	-	-	-	-	-	1 (4.76)	-	-
		Parapercis colias	-	-	-	-	-	-	-	-	-	-	-	1 (5.56)
		Paratrachichthys sp.	-	-	1 (6.67)	-	-	-	-	-	-	-	-	-
		*Phosichthys argenteus**	1 (7.14)	-	-	-	-	-	-	-	-	-	-	1 (5.56)
		*Protomyctophum bolini**	-	-	-	1 (5)	-	-	-	-	-	-	-	-
		Pseudocyttus maculatus	-	-	-	-	-	-	1 (33.33)	-	-	1 (4.76)	-	-
		Pseudophycis bachus	-	-	-	-	-	-	-	-	-	1 (4.76)	-	-
		Scomber australasicus	-	-	1 (6.67)	-	-	-	-	-	-	-	-	-
		*Scopelosaurus hamiltoni**	1 (7.14)	-	-	1 (5)	-	-	-	-	-	1 (4.76)	-	-
		Seriolella brama	1 (7.14)	-	3 (20)	1 (5)	-	-	-	-	-	5 (23.81)	-	4 (22.22)
		Seriolella punctata	-	-	1 (6.67)	-	-	-	-	-	-	2 (9.52)	-	1 (5.56)
		Trachipterus arcticus	-	-	-	1 (5)	-	-	-	-	-	-	-	-
		Trachurus novaezelandiae	-	-	-	1 (5)	-	-	-	-	-	3 (14.29)	-	-
		Trachyrincus sp.	1 (7.14)	-	-	-	-	-	-	-	-	-	-	-
	CHO	*Dalatias licha*	-	-	1 (6.67)	1 (5)	-	-	-	-	-	1 (4.76)	-	-
		Deania calcea	-	-	1 (6.67)	-	-	-	-	-	-	-	-	-
		Etmopterus sp.	1 (7.14)	-	-	-	-	-	-	-	-	-	-	-
		Etmopterus lucifer	-				-	-	-	-				
		Hydrolagus bemisi	-	-	-	-	-	-	-	-	-	1 (4.76)	-	-

Sex identification

There was 79.2% amplification success for the CHD gene used for sex identification in necropsy samples and 93.1% for scat. There were some differences in assigned sex between the necropsy samples for the morphological (a means of control for DNA sex identification as only one necropsy sample could not be identified) and DNA results (Table 5). For example, 33 females out of a total of 72 birds were identified morphologically from the necropsy samples, however, DNA testing of those same samples identified 21 as females, three males, and nine remained unidentifiable (no DNA amplification). For statistical analyses that incorporated sex as a variable, morphological identification was used, but for any samples where the morphological sex determination was not available, then the DNA identification was incorporated.

Table 5. Results of sex identification from albatross scat and necropsy samples using morphological (morph) and DNA methods. To note, DNA sex identification was undertaken from DNA extracted from stomach content (necropsy) and scat samples. The morphological results have been broken down to show a comparison with the matching DNA results for the same set of individuals, as presented in parentheses.

Sample	Female (F)	Male (M)	Unidentified (U)
Necropsy - morph	33 (F21; M3; U9)	38 (F5; M27; U6)	1 (U1)
Necropsy - DNA	26	30	16
Scat – morph	14 (F5; M2; U7)	11 (F1; M6; U4)	61 (F24; M29; U8)
Scat - DNA	30	37	19

Primer choice

In general, there was poorer DNA amplification of the scat samples when compared to the necropsy samples; despite there being high molecular weight DNA in the samples (DNA gel visualization). This was likely due to consumed items have undergone the entire digestion process resulting in the DNA being degraded; as a test bacterial primers amplified readily (likely reason for the high molecular weight DNA seen on the gel). PCR protocols were optimized as best as possible to increase amplification (increasing DNA volume or adding BSA), only achieving a slight improvement. PCR success, based on gel visualization, indicated COI had 95.8% and 80.6% success, 16S cephalopod had 54.2% and 15.3%, and 16S fish had 36.1% and 9.7%, for necropsy and scat samples respectively. Cephalopods were detected only by the COI and 16S cephalopod primer sets. The design of the 16S cephalopod primer set also proved to be well-suited to identify fishes.

Sequence quality control

The majority of samples were retained post-DADA2 chimeric sequence removal. One necropsy sample was removed in the COI dataset, four necropsy and 25 scat in 16S fish, and three necropsy and 22 scat in 16S ceph (Table S4). DNA and PCR negative controls that were retained, varied per primer set, but typically had low reads. For the necropsy samples, COI dataset retained five DNA (1—15 reads) and zero PCR negatives, 16S fish

dataset retained one DNA (20 reads) and two PCR negatives (1—4 reads), and 16S ceph dataset retained four DNA (1—4 reads) and one PCR (1 read) negatives (Table S4). For the scat samples, COI dataset retained two DNA (6—19) and zero PCR negatives, 16S fish dataset retained four DNA (1—14 reads) and two PCR (5—23) negatives, and 16S ceph dataset retained four DNA (11—531 reads) and three PCR (40—2,547) negatives (Table S4). The 16S ceph scat dataset accumulated the most reads from the negative controls. Upon further investigation, read numbers decreased substantially as ASVs in the negatives did not match those linked to the samples; two DNA (130—175 reads) and three PCR (10—251) negatives. Overall, proportional subtraction and filtering for a minimum read number was found to be satisfactory for controlling cross-contamination and PCR artefacts (Table 6). The number of ASVs per primer dataset substantially decreased for the scat samples (COI: 63%, 16S ceph: 54%, 16S fish: 38%), and the number of samples retained decreased by > 20 for the 16S ceph and 16S fish datasets (Table 6). This decrease was not seen for the necropsy samples (COI: 39%, 16S ceph: 26%, 16S fish: 13%; Table 6). Regardless of primer, the results from 71 necropsy and 84 scat samples were retained.

Table 6. Quality control processing of samples, and the associated decrease of amplicon sequence variants (ASVs) and reads. The sequence of numbers separated by a semi colon is (1) the number of samples, (2) the number of ASVs, and (3) the number of reads.

Dataset	DADA2	Negative controls	Assigned ASVs	Unassigned ASVs
Nec-COI	71; 579; 2,358,716	71; 356; 2,357,555	71; 259; 2,086,415	47; 97; 271,140
Nec-ceph	69; 309; 686,024	68; 228; 685,298	68; 186; 681,593	28; 42; 3,705
Nec- fish	68; 267; 1,227,615	64; 233; 1,227,230	64; 220; 1,158,422	8; 13; 68,808
Scat-COI	86; 5,279; 1,219,712	81; 1,949; 1,197,812	81; 317; 1,023,139	60; 1,632; 174,673
Scat-ceph	64; 224; 262,376	38; 102; 259,972	34; 55; 249,547	23; 47; 10,425
Scat-fish	61; 117; 132,343	40; 72; 131,944	36; 68; 131,712	8; 4; 232

Taxonomic assignments

In general, more ASVs had taxonomy assigned for the necropsy versus the scat samples. The scat COI dataset had the greatest proportion of unassigned ASVs (83%; Table 6; Figures S1 and S2). The 16S fish dataset had the least proportion of unassigned ASVs (~5%). Removing ASVs assigned as Aves decreased the number of COI reads by > 900,000 for both the necropsy and scat samples, with the number of 16S ceph ASVs decreasing by 2,711 and 439 for the necropsy and scat samples, respectively. Aves were not assigned to any ASVs in the 16S fish dataset. It is known that albatrosses do prey on other birds (e.g., Cherel et al., 2000), but there was only one scat sample that had a high RRA for the common diving petrel (*Pelecanoides urinatrix*) that would suggest it may have been consumed, alternatively there may have been cross-contamination when collecting the scat. Notably, out of the ten albatross species only four matched their ASV assignment namely, *Diomedea epomophora*, *D. exulans*, *Thalassarche bulleri bulleri* (although matched to *T. bulleri*), and *T. melanophris*.

Diomedea antipodensis antipodensis and *D*. *antipodensis gibsoni* matched *D. exulans. Thalassarche steadi* and *T. salvini* matched *T*. *cauta*, and *T*. *impavida* and *T*. *eremita* matched *T*. *melanophris*.

Further filtering based on assignments removed ASVs that were not considered to be informative to the diet. This included microscopic organisms (Amoebozoa, Apicomplexa, Bacteria, Fungi, Rotifera), marine worms, parasites, insects, algae, 'uncultured/environmental', and human (occurred in three scat samples). Post taxonomic assignment filtering, 16S ceph dataset had 150 (589,876 reads) and 27 (228,846) ASVs, 16S fish had 208 (1,084,728) and 64 (131,548), and COI had 146 (1,060,840) and 35 (84,405) for the necropsy and scat samples, respectively. In total, 68 necropsy and 48 scat samples were retained. Generating a genus-level subset of the data, regardless of primer set used, the necropsy dataset retained 66 samples, 454 ASVs, and 2,597,494 reads, and the scat dataset retained 43 samples, 106 ASVs, and 382,875 reads.

Discussion

In this study, taxa from scat (colony samples) and necropsy stomach samples of ten albatross species were identified using DNA metabarcoding methods. Three primer sets were used to target a broad range of taxa. Based on the FOO, the diet among all albatross samples consisted largely of fishes and to lesser extent cephalopods. Differences in prey diversity (higher in necropsy samples) were found to be significant between sample type, however, no specific prey species were found to be responsible for this difference. Overall, the majority of fish and cephalopod species identified in both scat and necropsy samples overlapped extensively with species that were most likely to be made available through fisheries activities, i.e., discard/species targeted/bait used. These results suggest that albatrosses are heavily reliant on fisheries as a food source whether they were sampled from fishing vessels (i.e., necropsy) or from colonies (i.e., scats).

Fishing vessels as a food source for albatrosses

Seabirds are notably vulnerable to marine ecosystem changes due to their high trophic level in the food web (Pardo et al., 2017), with fluctuations in prey availability having potentially significant impact on their survival and breeding success (Mills et al., 2020). Understanding and monitoring the dietary composition and source for albatrosses is a key tool in their conservation and management. Whilst there are many published diet studies for albatrosses, there remain significant knowledge gaps that are important, especially in terms of identifying and addressing anthropogenic factors influencing their diet that have the potential to impact the success of populations. This study revealed that among all ten species of albatrosses sampled, albatross diet is comprised mostly of fishes and to a lesser extent cephalopods, which is consistent with some previous studies of the diets of albatrosses (e.g., Arata & Xavier, 2003; Cherel et al., 2000). A larger sample size is required to give greater confidence around this.

There was extensive diversity identified in the diet of the ten albatross species using DNA metabarcoding. The composition of ingested species, mostly fishes and cephalopods, was highly variable among individuals with up to eight (median=3) and six (median=1) species from a total of ≥77 detected species occurring in any necropsy and scat sample, respectively. The most commonly occurring ingested species for necropsy samples was *Nototodarus sloanii* (n=21 versus n=1 for scat; squid) and for scat *Coelorinchus fasciatus* (n=5 versus n=6 for necropsy; deep-sea rattail unlikely to be landed whole; Stevens et al., 2010). *Nototodarus sloanii* is a targeted species of New Zealand fisheries (Fisheries New Zealand, 2022) and *C. fasciatus* is a known bycatch species (e.g., scampi trawls; Anderson et al., 2023), indicating increased availability of these food items. The majority of fish species consumed by the albatrosses reside at depths beyond 20 m (deepest dive recorded for albatrosses). Specifically, 41 out of a total of 60 New Zealand fish species identified reside beyond 20 m (i.e., 68%). This

result increases the likelihood that a significant proportion of the food items consumed by the sampled albatross were obtained through commercial fishing as non-target fish (discards), processing waste, and/or bait. Seabirds could potentially have also sourced these taxa from recreational fishers, but is highly unlikely for this study given the remoteness of the islands (scat samples) and the fishing locations (necropsy samples) being in deep offshore waters. Furthermore, SLL samples had a greater FOO for squid, tuna and hake (*Merluccius australis*), which mirrors the fishing as squid are used as bait, tuna are the target catch and hake are caught as bycatch (Griggs et al., 2024). The TWL necropsy samples had a greater FOO for hoki (*Macruronus novaezelandiae*) which is one of New Zealand's largest fisheries with most trawl fishing events taking place from 200 m depth (McKenzie, 2018). Despite FishBase records of this fish being present in the upper 20 m, they are more commonly found at depths ≥ 50 m. It must be noted that the 20 m depth to group fishes is based on the maximum diving depth on the black-browed albatross (Guilford et al., 2022) and New Zealand SLL fisheries having hookpods opening between 10–20 m (pers. comms., DOC; Goad & Sullivan, 2015). Literature prior to this study suggested this albatross species naturally forages on benthic and semipelagic fishes, but at the time it remained unclear how this was possible and emphasized the ecology of the different consumed taxa needed to be better understood (e.g., ontogenic migration, unknown diurnal patterns, feeding behaviours, dead floating taxa; Cherel et al., 2000). Understanding the ecology of the different prey taxa still holds true as this explains natural prey availability, but insight is also needed into the depths albatrosses can dive and if it differs across species as a potential niche partitioning mechanism (food resource derived from fisheries likely relaxes niche partitioning, increasing trophic niche overlap; Bugoni et al., 2010; Jiménez et al., 2017).

It is possible that some of the fish and cephalopod taxa detected are from secondary predation. For example, hoki (*Macruronus novaezelandiae*) are known to prey on lanternfish (Horn et al., 2013). In a TWL necropsy sample (A108686) hoki and lanternfish (*Lampanyctodes hectoris*) were detected. The diet of southern bluefin tuna is diverse and includes, squid, lanternfish, and Big-scale pomfret (*Taractichthys longipinnis*; Horn et al., 2013; Itoh et al., 2011); all of which were identified in a SLL necropsy sample (A109113). Thus, distinguishing sources as primary from secondary is difficult to resolve with certainty given the food-web overlap among dietary species.

Overall, the variety seen in the diet is consistent with opportunistic foraging by the albatross species across highly diverse food sources, such as the wide diversity of species made available from fishing vessels (i.e., bait, waste from processing targeted fish, and discards). This diverse dietary pattern among all 10 albatross species that were sampled is also consistent with previous research that shows albatross species co-occurring when pursuing food material made available by fishing vessels further explaining why it was not possible to detect differences in the composition of diet detected among the different albatross species in this current study (Basham et al., 2023; Cherel et al., 2000; Conners et al., 2018; Dellacasa et al., 2022; Jiménez et al.,

2017; Kuepfer et al., 2022; McInnes, Jarman, et al., 2017). In addition, taxa identified in the scat samples clearly confirm feeding interactions with fisheries such as the identification of bait species that do not occur naturally in New Zealand's waters (i.e., sauries - *Scomberesox saurus* and *Cololabis saira*).

While this study undertook statistical analyses to look if differences in the diet that may depend on a number of variables (i.e., albatross species, sex, sample type – necropsy versus scat, and month of collection/mortality), the sample size regarding replication within albatross species and across month-year heeds caution of the result's interpretation. Differences were only identified when testing sample type and month of collection/mortality. The R^2 value for sample type was low and only explained 2% of the variance. Conversely, the R^2 value for month of sample collection/mortality 'explained' 25%. This does not necessarily mean different seasons are responsible for differences to the diet, as the presence of relatively few data points representing some months versus more in others make it difficult for this test to make reliable statistical contrasts (e.g., n=35 samples in April and n=1 in October; Table 2). Similarly, small comparative sample sizes also probably limited the ability to detect any differences in composition of food items between what might be natural foraging (i.e., scat samples) versus foraging from around fishing vessels (i.e., necropsy samples). Buller's albatrosses were the only species to have larger sample sizes (n=17 necropsy; n=41 scat) sufficient to be able to detect differences if they were present, however, all scat samples were collected in March and April and only one necropsy sample in each of these two months. Regardless, it was not possible to distinguish differences in the composition to diet among the ten albatross species in this study or between scat and necropsy samples.

Molecular considerations

Only three of the ten albatross (sub)species in this study had a COI sequence present on Genbank; *Diomedea epomophora, D*. *exulans*, and *Thalassarche melanophris*. The lack of public data for albatross species hinders confirming their species identification; this confirmation is especially applicable for scat samples. It is possible that the shorter COI marker (313bp) used in this study may also lack sufficient variation to have the resolution required to correctly distinguish between closely related species and subspecies (e.g., Burg & Croxall, 2004), but this cannot be confirmed. Cytochrome *b* has been used in albatross phylogenetic studies (Chambers et al., 2009), but is not as frequently used in DNA metabarcoding studies as COI is.

The discrepancies in genetic sex identification by the CHD gene may be a result of allelic dropout of CHD-W (Toouli et al., 2000). This may result in a false identification of true females (ZW) as males (ZZ). Furthermore, a preferential amplification of the smaller CHD-Z fragment and a fainter CHD-W band (Dawson et al., 2001) may also result in the misidentification of true females as males. Another potential source of error in assigning sex on the basis of the DNA results may be a result of Z-polymorphism, which occurs in some male birds, having two variable-sized Z alleles (Dawson et al., 2001). Thus, it is

possible to observe two different-sized amplicons on a gel after PCR resulting in inaccurate identification of a true male as a female.

In general, the scat samples had poorer DNA amplification which directly influenced sex identification. DNA in scat is often degraded as the ingested material has undergone full digestion, and the quality of DNA extracted from the resulting scat is variable. As scat collection was opportunistic, it is likely the scat was not collected and preserved immediately after defecation, which would also influence DNA quality. This was expected given the opportunistic nature of sample collection from the colonies for this pilot study and is something that would need to be addressed in any future study design. Quickly preserved scat has been shown to have a higher quantity DNA and results in better PCR amplification due to better quality DNA template (McInnes, Alderman, Deagle, et al., 2017; Vynne et al., 2012). Regardless, sex identification from samples where sex is unknown provides a very useful metric for subsequent statistical analyses that would have not been captured otherwise (van der Reis & Jeffs, 2020, 2021). However, in this current study differences in ingested food did not differ between the sexes, so reliable sexing of birds in this context would appear of less importance.

Both a universal primer (COI) and group-specific markers (16S fish and 16S cephalopod) were utilised in this study. This was to gain a robust understanding of albatross diet, whilst also increasing the probability of amplifying the DNA from known prey groups (i.e., fishes and cephalopods; e.g., Arata & Xavier, 2003; James & Stahl, 2000; Xavier et al., 2014). The degenerate universal (COI) marker allowed for a broad coverage of potential dietary taxa (Geller et al., 2013; Leray et al., 2013). However, it also targets DNA from all eukaryotes, including those not of interest in the investigation of the albatross diet, such as parasites, fungi, and plants. Primer pairs targeting fishes and cephalopods were incorporated to mitigate the effects of non-target taxa (e.g., fungi associated with scat) that may compete with target taxa during DNA amplification due to potentially having higher quantity and quality DNA in comparison. In addition, having a more targeted approach can reduce host DNA amplification and increase sequencing efficacy, as found in this study. Overall, using three primer sets provided robust results for the identification of albatross diet species and helped to mitigate disparity in DNA reference database entries for different gene regions. A few fish and cephalopod species identified were also noted as not falling within New Zealand's geographic range, even after trying to resolve any issues with incorrect or missing data. For example, for *Notacanthus bonaparte* had matches for all gene regions and even though, for example, *N*. *sexspinis* and *N*. *chemnitzii* (New Zealand species) were in the database, they did not match. A likely explanation is the genetic differences among New Zealand populations for the same species are great enough that it is a closer match to *N*. *bonaparte*, or perhaps it is an unidentified *Notacanthus* species. It was noted that some species identified are likely bait species (e.g., *Scomber japonicas*; Biosecurity New Zealand, 2020).

Conclusion

Overall, our results suggest that fishery discards are a major contribution to the diet of albatrosses and raises the question if some of these birds have somewhat abandoned foraging. Albatross fisheries interactions may be due to vessel location coinciding with the natural use of habitat by albatrosses that may stem from competing for the same resource (Cherel et al., 2000; McInnes, Jarman, et al., 2017; Torres et al., 2013) or a learned behaviour for energy-reduced foraging (Conners et al., 2018). There is contrasting results from studies in the literature about what having the discards as the primary food may mean in the long term for the populations. Environmental conditions often dictate breeding success (Phillips et al., 2016; Thorne et al., 2015) and prey-switching (e.g., natural diet to discards) to potentially suboptimal food resources (Grémillet et al., 2008; Rosen & Trites, 2000) not only increases the mortality risk but in turn can also have negative impacts on long-term population stability (Kuepfer et al., 2022). Prey-switching can also be a short-term gain for population stability, or even help increase the population, due to increased availability of supplementary food source (Bugoni et al., 2010; McInnes, Alderman, Deagle, et al., 2017; Sherley et al., 2020). However, the underlying reason for prey-switching needs to be investigated thoroughly. For example, natural food sources may no longer exist in the quantities or quality needed to sustain the population relying on it as a food source (McInnes, Alderman, Deagle, et al., 2017). Regardless, the results from this study indicate fisheries discards are interfering with natural behaviour, feeding and diet, and fisheries are directly impacting the populations through seabird mortalities.

Recommendations

1. Bait used in fisheries overlapping with albatross foraging should be recorded at a species-level. Bait species used should be recorded for New Zealand Fisheries Observer data.
2. Future dedicated species-specific albatross studies would need to increase scat sampling replication at targeted sites/islands during the breeding season. Increased sample size from different colonies within the same month-year will allow for albatross species dietary differences to be investigated more thoroughly. GPS tracking when used on breeding pairs, and in conjunction with diet analyses of scat, could be invaluable for linking diet with foraging locations. In addition, linking GPS positions with corresponding GPS positions of fishing vessels, will help to determine what proportion of the diet is coming from fishing vessels.
3. Results show that fishing activities are influencing the diet and the feeding behaviour of albatrosses. The extent could not be precisely determined from the current pilot study, but the number of deep-sea dietary items and foreign bait species identified by DNA methods indicates that albatrosses are making extensive use of food derived from fishing activities. It is unclear whether this results in a net positive or negative effect on these birds. This can only be determined through detailed studies of the nutritional composition of the wild versus fisheries derived diets and would require a polyphasic approach. For example, we would recommend using biochemical analyses (i.e. fatty acid and stable isotope analyses), morphological content analyses and genetic methods (e.g., DNA metabarcoding or metagenomics methods) to investigate the diet and its nutritional value for albatrosses. The extent to which albatrosses are attuned to feeding on fisheries material has the potential to alter their behaviour for being able to successfully forage in the wild. It is not clear whether altering the availability of food material from fisheries, in an effort to reduce interactions/attractiveness with seabirds, would result in a negative or positive impact on the current diet of albatrosses, through reducing the extent and availability of the food material derived from fisheries.
4. Currently, only the first whole specimen of deceased white-capped albatross, sooty shearwater and white chinned petrel bycatch are retained by the deepwater trawl fishery for necropsy (all other fisheries retain all specimens). Feathers are collected from the remaining specimens for genetic analyses. We recommend retaining all albatross bycatch (and others as required) from deepwater trawl fishing events to increase sample sizes for future dietary studies. Increased sample size will improve comparisons among times of year, for individual albatross species, and with samples from breeding colonies (i.e., scat samples), as well as among fisheries and fishing techniques, and providing for risk assessment metrics (e.g., life cycle stage) for improved future albatross studies. Increasing the number of necropsy samples returned by fishers would be particularly helpful for improving dietary comparisons between breeding and non-breeding seasons.

5. International genetic sequence database (e.g., GenBank) needs to be provisioned with verified COI sequence data for all albatross species to improve the ability to reliably identify species with DNA samples. Cytochrome b sequence entries exist, but COI is more readily used in DNA metabarcoding studies and in general for species identification. This could be achieved from DNA analyses of morphologically identified albatross species, such as the extraction of pure DNA samples from necropsy tissues of albatrosses. Likewise, feather samples from birds at known breeding colonies could also be used for this purpose. At a minimum the full COI sequences of albatrosses should be published in GenBank and other complementary gene regions should also be considered for guaranteed species resolution for genetic studies.
6. Genetic sex identification methodology in albatrosses should be further investigated to better understand reasons for differing results in comparison to identification via morphological analyses done in the field or in the lab. Necropsy samples would provide an ideal source from which to extract pure albatross DNA to be used to confirm sex identification methods.
7. Vessel specific management plans contain non-regulatory mitigation measures for discard and fish waste management, including avoidance of continuous discharge while towing, batch discharges, no discharge while shooting and hauling and minimising spillage on decks. Observers carry out audits on offal and whole fish and record information on a tow-by-tow basis. Since 2021 this has also been required of fishers in addition to reporting whole vessel fish discards on disposal reports. The two main reports that include information on discard/waste management are the Seabird Annual Reports and the Deepwater Annual Review Reports. Observer coverage on >28m vessels in the deepwater trawl fleet is around 50% (FNZ). Other fisheries such as scampi and ling longline have much lower coverage. In an effort to understand where improvements can be made to further reduce vessel attraction to seabirds it is recommended that consideration be given to how fisheries with low observer coverage could provide greater visibility on the application of discard / fish waste management practices. A review could also include whether electronic monitoring may be a suitable tool for assessing mitigation use. It is also recommended that for vessels without meal plants, consideration be given to recording data on the amount of discarded processing waste. This could potentially be calculated from difference between the recorded landed greenweight and product weight (FNZ).

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References

ACAP. (2015). Report on progress with the implementation of the agreement 2013–2015. Report MoP5 Doc 11. Agreement on the Conservation of Albatrosses and Petrels. https://www.acap.aq/meeting-of-the-parties/mop5/mop5-meeting-documents/2477-mop5-doc-11-report-on-progress-with-the-implementation-of-the-agreement-2013-2015/file

Ando, H., Mukai, H., Komura, T., Dewi, T., Ando, M., & Isagi, Y. (2020). Methodological trends and perspectives of animal dietary studies by noninvasive fecal DNA metabarcoding. *Environmental DNA, 2*(4), 391-406. https://doi.org/10.1002/edn3.117

Arata, J., & Xavier, J. C. (2003). The diet of black-browed albatrosses at the Diego Ramirez Islands, Chile. *Polar Biology, 26*(10), 638-647. https://doi.org/10.1007/s00300-003-0530-z

Baker, G., Gales, R., Hamilton, S., & Wilkinson, V. (2002). Albatrosses and petrels in Australia: A review of their conservation and management. *Emu-Austral Ornithology, 102*(1), 71-97. https://doi.org/10.1071/MU01036

Baker, G. B., Double, M. C., Gales, R., Tuck, G. N., Abbott, C. L., Ryan, P. G., Petersen, S. L., Robertson, C. J. R., & Alderman, R. (2007). A global assessment of the impact of fisheries-related mortality on shy and white-capped albatrosses: Conservation implications. *Biological Conservation, 137*(3), 319-333. https://doi.org/10.1016/j.biocon.2007.02.012

Basham, E., Briskie, J. V., & Martin, P. (2023). Variation in foraging strategies of New Zealand albatross species within a dominance hierarchy. *New Zealand journal of zoology, 50*(4), 461-477. https://doi.org/10.1080/03014223.2022.2137534

Berry, T., Osterrieder, S., Murray, D., Coghlan, M., Richardson, A., Grealy, A., Stat, M., Bejder, L., & Bunce, M. (2017). DNA metabarcoding for diet analysis and biodiversity: A case study using the endangered Australian sea lion (*Neophoca cinerea*). *Ecology and Evolution, 7*(14), 5435-5453. https://doi.org/10.1002/ece3.3123

Biosecurity New Zealand. (2020). Supplementary Import Risk Analysis: Frozen fish and cephalopod molluscs for fish bait. Version 4.0. Ministry for Primary Industries.

BirdLife International. (2022). State of the World's Birds 2022: Insights and solutions for the biodiversity crisis. BirdLife International. https://www.birdlife.org/wp-content/uploads/2022/09/SOWB2022_EN_compressed.pdf

Boettiger, C., Lang, D. T., & Wainwright, P. C. (2012). rfishbase: exploring, manipulating and visualizing FishBase data from R. *Journal of Fish Biology, 81*(6), 2030-2039. https://doi.org/https://doi.org/10.1111/j.1095-8649.2012.03464.x

Bolyen, E. et al. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology, 37*, 852–857. https://doi.org/10.1038/s41587-019-0209-9

Bugoni, L., McGill, R. A. R., & Furness, R. W. (2010). The importance of pelagic longline fishery discards for a seabird community determined through stable isotope analysis. *Journal of Experimental Marine Biology and Ecology, 391*(1), 190-200. https://doi.org/10.1016/j.jembe.2010.06.027

Burg, T. M., & Croxall, J. P. (2004). Global population structure and taxonomy of the wandering albatross species complex. *Molecular Ecology, 13*(8), 2345-2355. https://doi.org/10.1111/j.1365-294X.2004.02232.x

Çakmak, E., Akın Pekşen, Ç., & Bilgin, C. C. (2017). Comparison of three different primer sets for sexing birds. *Journal of Veterinary Diagnostic Investigation, 29*(1), 59-63. https://doi.org/10.1177/1040638716675197

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods, 13*(7), 581-583. https://doi.org/10.1038/nmeth.3869

Callahan, B. J., McMurdie, P. J., & Holmes, S. P. (2017). Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. *ISME Journal, 11*, 2639. https://doi.org/10.1038/ismej.2017.119

Carreon-Martinez, L., & Heath, D. D. (2010). Revolution in food web analysis and trophic ecology: Diet analysis by DNA and stable isotope analysis. https://doi.org/10.1111/j.1365-294X.2009.04412.x

Chambers, G., Moeke, C., Steel, R., & Trueman, J. W. H. (2009). Phylogenetic analysis of the 24 named albatross taxa based on full mitochondrial cytochrome *b* DNA sequences. *Notornis, 56*, 82-94.

Cherel, Y., & Klages, N. (1998). A review of the food of albatrosses. In G. Robertson & G. Gales (Eds.), *Albatross biology and conservation* (pp. 113-136).

Cherel, Y., Weimerskirch, H., & Trouvé, C. (2000). Food and feeding ecology of the neritic-slope forager black-browed albatross and its relationships with commercial fisheries in Kerguelen waters. *Marine Ecology Progress Series, 207*, 183-199. https://doi.org/10.3354/meps207183

Cherel, Y., Xavier, J. C., De Grissac, S., Trouvé, C., & Weimerskirch, H. (2017). Feeding ecology, isotopic niche, and ingestion of fishery-related items of the wandering albatross *Diomedea exulans* at Kerguelen and Crozet Islands. *Marine Ecology Progress Series, 565*, 197-215. https://doi.org/10.3354/meps11994

Conners, M. G., Goetsch, C., Budge, S. M., Walker, W. A., Mitani, Y., Costa, D. P., &

Shaffer, S. A. (2018). Fisheries exploitation by albatross quantified with lipid analysis. *Frontiers in Marine Science, 5*. https://doi.org/10.3389/fmars.2018.00113

Cooper, J., Henley, S., & Klages, N. (1992). The diet of the wandering albatross *Diomedea exulans* at subantarctic Marion Island. *Polar Biology, 12*, 477-484. https://doi.org/10.1007/BF00238186

Dawson, D. A., Darby, S., Hunter, F. M., Krupa, A. P., Jones, I. L., & Burke, T. (2001). A critique of avian CHD-based molecular sexing protocols illustrated by a Z-chromosome polymorphism detected in auklets. *Molecular Ecology Notes, 1*(3), 201-204. https://doi.org/10.1046/j.1471-8278.2001.00060.x

de Leeuw, J. J., van den Brink, X., Gabrielsen, G. W., & Nijland, R. (2024). DNA metabarcoding reveals high diversity of fish and macrofaunal species in diets of little auks and other Arctic seabird species in Svalbard. *Polar Biology, 47*(10), 1013-1023. https://doi.org/10.1007/s00300-024-03276-3

de Sousa, L. L., Silva, S. M., & Xavier, R. (2019). DNA metabarcoding in diet studies: Unveiling ecological aspects in aquatic and terrestrial ecosystems. *Environmental DNA, 1*, 199-214. https://doi.org/10.1002/edn3.27

Deagle, B. E., Gales, N. J., Evans, K., Jarman, S. N., Robinson, S., Trebilco, R., & Hindell, M. A. (2007). Studying seabird diet through genetic analysis of faeces: A case study on macaroni penguins (*Eudyptes chrysolophus*). *PLoS ONE, 2*(9), e831-e831. https://doi.org/10.1371/journal.pone.0000831

Deagle, B. E., Thomas, A. C., Shaffer, A. K., Trites, A. W., & Jarman, S. N. (2013). Quantifying sequence proportions in a DNA-based diet study using Ion Torrent amplicon sequencing: Which counts count? *Molecular Ecology Resources, 13*(4), 620-633. https://doi.org/10.1111/1755-0998.12103

Dellacasa, R. F., Chavez, L. N., & Tamini, L. L. (2022). Salvin's Albatross *Thalassarche salvini* interacting with freezer trawlers in waters of the Patagonian Shelf. *Marine ornithology, 50*, 99-102. https://doi.org/10.5038/2074-1235.50.1.1465

Fisheries New Zealand. (2022). Arrow squid (SQU). Ministry for Primary Industries. https://fs.fish.govt.nz/Doc/25157/04%20SQU%202022.pdf.ashx

Fridolfsson, A.-K., & Ellegren, H. (1999). A simple and universal method for molecular sexing of non-ratite birds. *Journal of Avian Biology, 30*(1), 116-121. https://doi.org/10.2307/3677252

Geller, J., Meyer, C., Parker, M., & Hawk, H. (2013). Redesign of PCR primers for mitochondrial cytochrome c oxidase subunit I for marine invertebrates and application in all-taxa biotic surveys. *Molecular Ecology Resources, 13*(5), 851-861. https://doi.org/10.1111/1755-0998.12138

Goad, D., & Sullivan, B. (2015). Testing the Hookpod-mini in the New Zealand pelagic

longline fishery. Report mit2015-02. Conservation Services Programme, Department of Conservation. https://www.doc.govt.nz/globalassets/documents/conservation/marine-and-coastal/marine-conservation-services/reports/pre-2019-annual-plans/mit2015-02-hookpod-trials-final-report.pdf

Granadeiro, J. P., Brickle, P., & Catry, P. (2014). Do individual seabirds specialize in fisheries' waste? The case of black-browed albatrosses foraging over the Patagonian Shelf. *Animal Conservation, 17*(1), 19-26. https://doi.org/10.1111/acv.12050

Grémillet, D., Pichegru, L., Kuntz, G., Woakes, A. G., Wilkinson, S., Crawford, R. J. M., & Ryan, P. G. (2008). A junk-food hypothesis for gannets feeding on fishery waste. *Proceedings of the Royal Society B: Biological Sciences, 275*(1639), 1149-1156. https://doi.org/10.1098/rspb.2007.1763

Griggs, L., Datta, S., & Finucci, B. (2024). Fish bycatch in New Zealand tuna longline fisheries 2018–19 to 2020–21. New Zealand Fisheries Assessment Report 2024/59. Fisheries New Zealand. https://fs.fish.govt.nz/Doc/25793/FAR-2024-59-Fish-bycatch-New-Zealand-tuna-longline-fisheries-2018-19-to-2020-21.pdf.ashx

Guilford, T., Padget, O., Maurice, L., & Catry, P. (2022). Unexpectedly deep diving in an albatross. *Current Biology, 32*(1), R26-R28. https://doi.org/10.1016/j.cub.2021.11.036

Horn, P., Ballara, S., Sutton, P., & Griggs, L. (2013). Evaluation of the diets of highly migratory species in New Zealand waters. New Zealand Aquatic Environment and Biodiversity Report No. 116. Ministry for Primary Industries. https://docs.niwa.co.nz/library/public/AEBR_116.pdf

Illumina. (2013). *16S metagenomic sequencing library preparation: Preparing 16S ribosomal RNA gene amplicons for the Illumina MiSeq system*. (Report no. 15044223 Rev. B). https://support.illumina.com/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf

Imber, M. (1999). Diet and feeding ecology of the Royal Albatross *Diomedea epomophora* - King of the shelf break and inner slope. *Emu, 99*(3), 200-211. https://doi.org/10.1071/MU99023

Imber, M. J. (1992). Cephalopods eaten by wandering albatrosses (*Diomedea exulans* L.) breeding at six circumpolar localities. *Journal of the Royal Society of New Zealand, 22*(4), 243-263. https://doi.org/10.1080/03036758.1992.10420819

Itoh, T., Kemps, H., & Totterdell, J. (2011). Diet of young southern bluefin tuna Thunnus maccoyii in the southwestern coastal waters of Australia in summer. *Fisheries Science, 77*(3), 337-344. https://doi.org/10.1007/s12562-011-0340-0

IUCN. (2024). The IUCN Red List of Threatened Species. Retrieved from https://www.iucnredlist.org/

James, G. D., & Stahl, J. C. (2000). Diet of southern Buller's albatross (*Diomedea bulleri bulleri*) and the importance of fishery discards during chick rearing. *New Zealand Journal of Marine and Freshwater Research, 34*(3), 435-454. https://doi.org/10.1080/00288330.2000.9516946

Jiménez, S., Xavier, J. C., Domingo, A., Brazeiro, A., Defeo, O., Viera, M., Lorenzo, M. I., & Phillips, R. A. (2017). Inter-specific niche partitioning and overlap in albatrosses and petrels: Dietary divergence and the role of fishing discards. *Marine Biology, 164*(8), 174. https://doi.org/10.1007/s00227-017-3205-y

Kuepfer, A., Votier, S. C., Sherley, R. B., Ventura, F., Matias, R., Anderson, O., Brickle, P., Arkhipkin, A., & Catry, P. (2022). Prey-switching to fishery discards does not compensate for poor natural foraging conditions in breeding albatross. *ICES Journal of Marine Science, 80*(9), 2414-2426. https://doi.org/10.1093/icesjms/fsac069

Lamb, P. D., Hunter, E., Pinnegar, J. K., Creer, S., Davies, R. G., & Taylor, M. I. (2019). How quantitative is metabarcoding: A meta-analytical approach. *Molecular Ecology, 28*(2), 420-430. https://doi.org/10.1111/mec.14920

Lee, J. C. et al. (2010). A novel strategy for avian species and gender identification using the CHD gene. *Mol Cell Probes, 24*(1), 27-31. https://doi.org/10.1016/j.mcp.2009.08.003

Lenth, R. (2019). *emmeans: Estimated marginal means, aka least-squares means* (Version 1.3.4). Comprehensive R Archive Network (CRAN). Retrieved from https://CRAN.R-project.org/package=emmeans

Leray, M., Yang, J. Y., Meyer, C. P., Mills, S. C., Agudelo, N., Ranwez, V., Boehm, J. T., & Machida, R. J. (2013). A new versatile primer set targeting a short fragment of the mitochondrial COI region for metabarcoding metazoan diversity: Application for characterizing coral reef fish gut contents. *Frontiers in Zoology, 10*, 34. https://doi.org/10.1186/1742-9994-10-34

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.journal, 17*(1), 3. https://doi.org/10.14806/ej.17.1.200

McInnes, J., Raymond, B., Phillips, R., Jarman, S., Lea, M.-A., & Alderman, R. (2016). A review of methods used to analyse albatross diets—assessing priorities across their range. *ICES Journal of Marine Science, 73*(9), 2125-2137. https://doi.org/10.1093/icesjms/fsw105

McInnes, J., Alderman, R., Deagle, B., Lea, M.-A., Raymond, B., & Jarman, S. (2017). Optimised scat collection protocols for dietary DNA metabarcoding in vertebrates. *Methods in Ecology and Evolution, 8*(2), 192-202. https://doi.org/10.1111/2041-

210X.12677

McInnes, J. et al. (2017). High occurrence of jellyfish predation by black-browed and Campbell albatross identified by DNA metabarcoding. *Molecular Ecology, 26*(18), 4831-4845. https://doi.org/10.1111/mec.14245

McInnes, J. et al. (2017). DNA metabarcoding as a marine conservation and management tool: A circumpolar examination of fishery discards in the diet of threatened albatrosses. *Frontiers in Marine Science, 4*, 277. https://doi.org/10.3389/fmars.2017.00277

McInnes, J., Tuck, G., & Alderman, R. (2020). *Using scat DNA to inform sustainable fisheries management and Ecological Risk Assessments: A Shy Albatross case study*. (Report no. P. Department of Primary Industries, Water and Environment. www.frdc.com.au/sites/default/files/products/2016-118-DLD.pdf

McKenzie, A. (2018). Assessment of hoki (Macruronus novaezelandiae) in 2018. New Zealand Fisheries Assessment Report 2019/22. Fisheries New Zealand. https://www.mpi.govt.nz/dmsdocument/35535-FAR-201922-Assessment-of-hoki-Macruronus-novaezelandiae-in-2018

Mills, W. F., Xavier, J. C., Bearhop, S., Cherel, Y., Votier, S. C., Waluda, C. M., & Phillips, R. A. (2020). Long-term trends in albatross diets in relation to prey availability and breeding success. *Marine Biology, 167*, 1-12. https://doi.org/10.1007/s00227-019-3630-1

Morgulis, A., Coulouris, G., Raytselis, Y., Madden, T. L., Agarwala, R., & Schaffer, A. A. (2008). Database indexing for production MegaBLAST searches. *Bioinformatics, 24*(16), 1757-1764. https://doi.org/10.1093/bioinformatics/btn322

Oehm, J., Thalinger, B., Eisenkölbl, S., & Traugott, M. (2017). Diet analysis in piscivorous birds: What can the addition of molecular tools offer? *Ecology and Evolution, 7*(6), 1984-1995. https://doi.org/10.1002/ece3.2790

Oksanen, J. et al. (2019). *vegan: Community ecology package* (Version 2.5-6). Comprehensive R Archive Network (CRAN). Retrieved from https://CRAN.R-project.org/package=vegan

Oliveira, N., Ramos, J. A., Calado, J. G., & Arcos, J. M. (2022). Seabird and fisheries interactions. In *Volume 1: Seabird Biodiversity and Human Activities* (pp. 77-89). CRC Press.

Pardo, D., Forcada, J., Wood, A. G., Tuck, G. N., Ireland, L., Pradel, R., Croxall, J. P., & Phillips, R. A. (2017). Additive effects of climate and fisheries drive ongoing declines in multiple albatross species. *Proceedings of the National Academy of Sciences, 114*(50), E10829-E10837. https://doi.org/10.1073/pnas.1618819114

Peters, K. J., Ophelkeller, K., Bott, N. J., Deagle, B. E., Jarman, S. N., & Goldsworthy, S.

D. (2015). Fine-scale diet of the Australian sea lion (*Neophoca cinerea*) using DNA-based analysis of faeces. *Marine Ecology, 36*(3), 347-367. https://doi.org/10.1111/maec.12145

Phalan, B., Phillips, R., A. , Silk, J., Afanasyev, V., Fukuda, A., Fox, J., Catry, P., Higuchi, H., & Croxall, J. (2007). Foraging behaviour of four albatross species by night and day. *Marine Ecology Progress Series, 340*, 271-286. https://doi.org/10.3354/meps340271

Phillips, R. A. et al. (2016). The conservation status and priorities for albatrosses and large petrels. *Biological Conservation, 201*, 169-183. https://doi.org/10.1016/j.biocon.2016.06.017

Piñol, J., Mir, G., Gomez-Polo, P., & Agustí, N. (2015). Universal and blocking primer mismatches limit the use of high-throughput DNA sequencing for the quantitative metabarcoding of arthropods. *Molecular Ecology Resources, 15*(4), 819-830. https://doi.org/10.1111/1755-0998.12355

Prince, P., Huin, N., & Weimerskirch, H. (1994). Diving depths of albatrosses. *Antarctic Science, 6*(3), 353-354. https://doi.org/10.1017/S0954102094000532

Queirós, J. P., Hilário, A., Thompson, D. R., Ceia, F. R., Elliott, G., Walker, K., Cherel, Y., & Xavier, J. C. (2021). From warm to cold waters: new insights into the habitat and trophic ecology of Southern Ocean squids throughout their life cycle. *Marine Ecology Progress Series, 659*, 113-126. https://doi.org/10.3354/meps13551

R Core Team. (2021). *R: A language and environment for statistical computing* (Version 4.1.0; R Studio Version 1.4.1106). R Foundation for Statistical Computing. Retrieved from https://www.R-project.org/

Robertson, H. et al. (2021). Conservation status of birds in Aotearoa New Zealand. *New Zealand Threat Classification Series 36*. Department of Conservation, New Zealand. https://www.doc.govt.nz/globalassets/documents/science-and-technical/nztcs36entire.pdf

Rodhouse, P. G., Clarke, M., & Murray, A. (1987). Cephalopod prey of the wandering albatross *Diomedea exulans*. *Marine Biology, 96*, 1-10. https://doi.org/10.1007/BF00394833

Rolland, V., Barbraud, C., & Weimerskirch, H. (2008). Combined effects of fisheries and climate on a migratory long-lived marine predator. *Journal of Applied Ecology, 45*(1), 4-13. https://doi.org/10.1111/j.1365-2664.2007.01360.x

Rosen, D., & Trites, A. (2000). Pollock and the decline of Steller sea lions: Testing the junk-food hypothesis. *Canadian Journal of Zoology, 78*(7), 1243-1250. https://doi.org/10.1139/z00-060

Sherley, R. B., Ladd-Jones, H., Garthe, S., Stevenson, O., & Votier, S. C. (2020).

Scavenger communities and fisheries waste: North Sea discards support 3 million seabirds, 2 million fewer than in 1990. *Fish and Fisheries, 21*(1), 132-145. https://doi.org/https://doi.org/10.1111/faf.12422

Taberlet, P., Coissac, E., Pompanon, F., Brochmann, C., & Willerslev, E. (2012). Towards next-generation biodiversity assessment using DNA metabarcoding. *Molecular Ecology, 21*(8), 2045-2050. https://doi.org/10.1111/j.1365-294X.2012.05470.x

Thorne, L. H. et al. (2015). Foraging behavior links climate variability and reproduction in North Pacific albatrosses. *Mov Ecol, 3*, 27. https://doi.org/10.1186/s40462-015-0050-9

Toouli, C. D., Turner, D. R., Grist, S. A., & Morley, A. A. (2000). The effect of cycle number and target size on polymerase chain reaction amplification of polymorphic repetitive sequences. *Analytical Biochemistry, 280*(2), 324-326. https://doi.org/10.1006/abio.2000.4547

Torres, L. G., Sagar, P. M., Thompson, D. R., & Phillips, R. A. (2013). Scaling down the analysis of seabird-fishery interactions. *Marine Ecology Progress Series, 473*, 275-289. https://doi.org/10.3354/meps10071

van den Hoff, J. (2001). Further observations on the cephalopod diet of Wandering Albatrosses (*Diomedea exulans* L.) at Macquarie Island. *Emu - Austral Ornithology, 101*(2), 169-172. https://doi.org/10.1071/MU00017

van der Reis, A., & Jeffs, A. (2020). Determining the diet of New Zealand king shag using DNA metabarcoding. Report BCBC2019-05. University of Auckland for the Conservation Services Programme, Department of Conservation. https://www.doc.govt.nz/globalassets/documents/conservation/marine-and-coastal/marine-conservation-services/reports/201920-annual-plan/bcbc2019-05-king-shag-dna-final-report.pdf

van der Reis, A., & Jeffs, A. (2021). Spatial and temporal patterns in the diet of New Zealand king shag. Report BCBC2020-05b. University of Auckland for the Conservation Services Programme, Department of Conservation. https://www.doc.govt.nz/globalassets/documents/conservation/marine-and-coastal/marine-conservation-services/reports/202021-annual-plan/bcbc20202-05-king-shag-dna-draft-final-report.pdf

Vynne, C., Baker, M., Breuer, Z., & Wasser, S. (2012). Factors influencing degradation of DNA and hormones in maned wolf scat. *Animal Conservation, 15*(2), 184-194. https://doi.org/10.1111/j.1469-1795.2011.00503.x

Walker, K., & Elliott, G. (2006). At-sea distribution of Gibson's and Antipodean wandering albatrosses, and relationships with longline fisheries. *Notornis, 53*, 265-290.

Walker, K., & Elliott, G. (2022). Antipodean wandering albatross satellite tracking and population study on Antipodes Island in 2021 and 2022.

https://www.doc.govt.nz/globalassets/documents/conservation/marine-and-coastal/marine-conservation-services/reports/non-csp-reports/antipodean-wandering-albatross-satellite-tracking-and-population-study-on-antipodes-island-in-2021-and-2022-final-report.pdf

Waugh, S., Weimerskirch, H., Cherel, Y., Shankar, U., Prince, P., & Sagar, P. (1999). Exploitation of the marine environment by two sympatric albatrosses in the Pacific Southern Ocean. *Marine Ecology Progress Series, 177*, 243-254. https://doi.org/10.3354/meps177243

Waugh, S., Filippi, D., Fukuda, A., Suzuki, M., Higuchi, H., Setiawan, A., & Davis, L. (2005). Foraging of royal albatrosses, *Diomedea epomophora*, from the Otago Peninsula and its relationships to fisheries. *Canadian Journal of Fisheries and Aquatic Sciences, 62*(6), 1410-1421. https://doi.org/10.1139/f05-001

Weimerskirch, H., Salamolard, M., Sarrazin, F., & Jouventin, P. (1993). Foraging strategy of wandering albatrosses through the breeding season: A study using satellite telemetry. *The Auk, 110*(2), 325-342. https://doi.org/10.1093/auk/110.2.325

West, J. A., & Imber, M. (1986). Some foods of Buller's mollymawk *Diomedea bulleri*. *New Zealand journal of zoology, 13*(2), 169-174. https://doi.org/10.1080/03014223.1986.10422659

Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis* (Vol. 2). Springer-Verlag. New York.

Xavier, J. C., Croxall, J. P., & Cresswell, K. A. (2005). Boluses: An effective method for assessing the proportions of cephalopods in the diet of albatrosses. *The Auk, 122*(4), 1182-1190. https://doi.org/10.1093/auk/122.4.1182

Xavier, J. C., Walker, K., Elliott, G., Cherel, Y., & Thompson, D. (2014). Cephalopod fauna of South Pacific waters: New information from breeding New Zealand wandering albatrosses. *Marine Ecology Progress Series, 513*, 131-142. https://www.jstor.org/stable/24894761

Yu, G., Smith, D. K., Zhu, H., Guan, Y., & Lam, T. T.-Y. (2017). ggtree: An R package for visualization and annotation of phylogenetic trees with their covariates and other associated data. *Methods in Ecology and Evolution, 8*(1), 28-36. https://doi.org/10.1111/2041-210X.12628

Zhou, C., & Brothers, N. (2021). Interaction frequency of seabirds with longline fisheries: Risk factors and implications for management. *ICES Journal of Marine Science, 78*(4), 1278-1287. https://doi.org/10.1093/icesjms/fsab014