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Research Paper

## Metabarcoding fecal samples to investigate spatiotemporal variation in the diet of the endangered Westland Petrel (*Procellaria westlandica*)

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**ABSTRACT.** As top predators, seabirds can be indirectly impacted by climate variability and commercial fishing activities through changes in marine communities. However, high mobility and foraging behavior enable seabirds to exploit prey distributed patchily in time and space. Despite this environmental adaptability, seabirds are the world's most threatened birds and there is, therefore, an urge to acquire information about their ecological and foraging requirements through the study of their diet. Traditionally, the diet of seabirds is assessed through the morphological identification of prey remains in regurgitates. This sampling method is invasive for the bird and limited in terms of taxonomic resolution. However, the recent progress in DNA-based approaches is now providing a noninvasive means to more comprehensively and accurately characterize animal diets. We used a noninvasive metabarcoding approach to characterize the diet of the Westland Petrel (*Procellaria westlandica*), which is an endangered burrowing species, endemic to the South Island of New Zealand. We collected 99 fresh fecal samples in 2 different seasons and in 2 different subcolonies. Our aim was to describe the Westland Petrel's diet and to investigate seasonal and spatial variations to their diet to understand the feeding requirements of this species. We also specifically investigated potential links with the New Zealand fishery industry to inform efficient conservation practices. Our noninvasive dietary DNA (dDNA) approach also highlighted significant differences in diet between seasons (incubation vs. chick-rearing season) and between sampling sites (two subcolonies 1.5 km apart). This suggests plasticity in the foraging strategy of the Westland Petrel. We found that amphipods were the most common prey, followed by cephalopods, and fish. Our findings could be the result of natural foraging behavior but also suggest a close link between the composition of prey items and New Zealand's commercial fishing activities. In particular, the high abundance of amphipods could be the result of Westland Petrels feeding on discarded fisheries waste (fish guts). This close relationship to New Zealand's fisheries may put stress on the resilience of the Westland Petrel. This valuable knowledge about Westland Petrel foraging needs is key to design efficient conservation plans for this iconic species. We illustrate how dDNA can inform the conservation of endangered or at-risk species that have elusive foraging behaviors.

### Métacodage à barres d'échantillons fécaux pour étudier les variations spatio-temporelles du régime alimentaire du Puffin du Westland (*Procellaria westlandica*), espèce en voie de disparition

**RÉSUMÉ.** En tant que prédateurs supérieurs, les oiseaux marins peuvent être indirectement touchés par la variabilité du climat et les activités de pêche commerciale par l'intermédiaire de changements sur le plan des communautés marines. Cependant, leur grande mobilité et leur comportement de recherche de nourriture leur permettent d'exploiter des proies réparties dans le temps et l'espace. Malgré cette capacité d'adaptation à l'environnement, les oiseaux marins sont les oiseaux les plus menacés au monde et il est donc urgent d'acquérir des connaissances sur leurs besoins écologiques et de recherche de nourriture en étudiant leur régime alimentaire. Traditionnellement, on procède à la détermination du régime alimentaire d'oiseaux marins en identifiant la morphologie des restes de proies dans les régurgitations. Cette méthode d'échantillonnage est invasive pour l'oiseau et limitée en termes de résolution taxonomique. Or, des approches récentes basées sur l'ADN offrent désormais un moyen non invasif de caractériser de manière plus complète et précise le régime alimentaire des animaux. Nous avons utilisé une approche non invasive de métacodage à barres pour caractériser le régime alimentaire du Puffin du Westland (*Procellaria westlandica*), espèce fousseuse menacée, endémique de l'île du Sud de la Nouvelle-Zélande. Nous avons récolté 99 échantillons de fèces fraîches au cours de deux saisons différentes et dans deux sous-colonies. Notre objectif était de décrire le régime alimentaire du Puffin du Westland et d'étudier ses variations saisonnières et spatiales afin de comprendre les besoins alimentaires de l'espèce. Nous avons également examiné les liens possibles avec l'industrie de la pêche en Nouvelle-Zélande afin de recommander des pratiques de conservation efficaces. Notre approche non invasive de l'ADN alimentaire (ADNa) a également mis en évidence des différences significatives du régime alimentaire entre les saisons (saison d'incubation comparativement à saison d'élevage des poussins) et les sites d'échantillonnage (deux sous-colonies distantes de 1,5 km). Ce résultat révèle une plasticité dans la stratégie de recherche de nourriture du Puffin du Westland. Nous avons constaté que les amphipodes étaient les proies les plus communes, suivies par les céphalopodes et les poissons. Nos résultats pourraient être le fruit d'un comportement naturel de recherche de nourriture, mais laissent également entrevoir un lien étroit entre la composition des proies et les activités de pêche commerciale en Nouvelle-Zélande. En particulier, la forte abondance d'amphipodes pourrait être attribuable au fait que les Puffins du Westland se nourrissent de déchets de pêche rejetés (boyaux de poissons). Cette relation étroite avec les pêcheries néo-zélandaises peut mettre à l'épreuve la résilience de ce puffin. Ces connaissances précieuses en matière de besoins alimentaires du Puffin du Westland sont essentielles pour qu'on puisse concevoir des plans de conservation efficaces pour cette espèce emblématique. Nous décrivons comment l'ADNa peut contribuer à la conservation d'espèces en voie de disparition ou en péril qui ont des comportements de recherche de nourriture obscurs.

**Key Words:** biodiversity; conservation; dietary DNA; noninvasive DNA sampling; New Zealand; Procellariidae

## INTRODUCTION

The study of animal diets is a critical component in several aspects of ecology, including community ecology (Corse et al. 2010), population dynamics (Read and Bowen 2001, Morrison et al. 2014), and conservation biology (Xiang et al. 2012, Lyngdoh et al. 2014). In top predators, spatial and seasonal changes in diet composition may reflect a certain degree of flexibility in foraging behavior (Whelan et al. 2000) and habitat and ecosystem status (Horswill et al. 2018). This variation could be relevant for understanding trophic interactions and conserving endangered species (Vander Zanden et al. 2000, Davies et al. 2001, Farias and Kittlein 2008, Vinson and Angradi 2011).

The way generalist top predators exploit resources in the wild varies, not only between populations, but also among individuals of the same species and even within the same population (Hughes et al. 2008, Araújo et al. 2011, Bolnick et al. 2011, Horswill et al. 2016). Such intraspecific variations in the use of resources are frequent in predators and often closely related to ecological opportunity, which can be driven by internal and external factors. External factors include prey availability or climatic conditions (Evangelista et al. 2014, Horswill et al. 2016), although foraging range for instance, is an internal driver of ecological opportunity (Kernaléguen et al. 2015, Horswill et al. 2018). Hence, ecological opportunity can undergo spatiotemporal shifts, the drivers of which must be considered to fully understand the foraging behavior of generalist predators (Araújo et al. 2011). These ecological predator-prey interactions become of special interest in marine seabirds. First, because dietary item distribution often shows high variability in space and time due to physical processes such as infrequent sea currents originating from drastic changes in climatic conditions (Pace et al. 1999, Behrenfeld et al. 2006, Horswill et al. 2016) and second, because of the central place constraint of seabirds' foraging ranges during breeding seasons. Indeed, because they carry the prey back to the nesting sites, marine seabirds are central place foragers (CPFs; see Bell 1990). This feeding strategy restricts their foraging range, resulting in a potential restriction of access to certain prey. Therefore, diet switches in marine seabirds can provide an accurate signature of marine food webs and the status of the marine ecosystem (Parsons et al. 2008, Horswill et al. 2016, 2018).

Seabirds are known to adapt their feeding habits and their breeding sites depending on the time of year (Thompson et al. 1999, Harding et al. 2007, Kowalczyk et al. 2015, McInnes et al. 2017a). These birds spend most of their lives at sea. However, during breeding season, some remain in coastal areas because their foraging trips are restricted in number and length to allow regular feeding of their chicks in the nest. To achieve this, seabirds have adopted a variety of foraging strategies (Ydenberg et al. 1994, McInnes et al. 2017a), such as switching between short and long foraging trips to feed their chicks, while maintaining their body condition during the breeding season (Baduini 2003, Ropert-Coudert et al. 2004), or providing the chicks with highly nutritive processed stomach oil (Baduini 2003). Most studies that aim to describe the seabirds' diet have been carried out during the chick-rearing period only. Often, this is because data based on the morphological analysis of regurgitates are obtained from parents coming back to the nest to feed their chicks (Croxall et al. 1988, Klages and Cooper 1992, Calixto-Albarrán and Osorno 2000,

Suryan et al. 2002). Thus, this approach considers prey communities as a fixed parameter across time, instead of treating it as a dynamic variable (Barrett et al. 2007, Komura et al. 2018).

Capturing the dynamic nature of the seabirds' diet is difficult, however, several long-term studies necessary to uncover seabirds' foraging patterns (Waluda et al. 2012, 2017, Howells et al. 2018, Wanless et al. 2018, Mills et al. 2021, Harris et al. 2022) and explore diet plasticity have been undertaken. The acquisition of such knowledge is essential because the seabirds' ability to switch to new prey represents a potential mechanism to avoid large population declines, potentially leading to local extinctions of threatened populations in birds (Marone et al. 2017). Moreover, these switches provide a potential mechanism to buffer large fluctuations in prey abundance that can impact local breeding investment (Horswill et al. 2017, Campbell et al. 2019) and subsequent demographic parameters (Horswill et al. 2014). In fact, several seabird populations have been decreasing rapidly in recent years (Grémillet et al. 2018, Thibault et al. 2019) and, therefore, detailed spatiotemporal knowledge of their diet preference is key to understanding and managing current and future threats, including commercial fishing activities or climate-driven changes to their ecosystem (Frainer et al. 2017).

For decades, the morphological identification of stomach contents or regurgitates has been widely used to identify the predators' prey items (Imber 1976, Freeman 1998, Carreon-Martinez and Heath 2010, Krüger et al. 2014, Egeter et al. 2015). However, these analyses usually require obtaining the gut content through stimulation of regurgitation after capturing individual birds, often using a technique called "lavage" (Wilson 1984, Ryan and Jackson 1986, Barrett et al. 2007), which is highly invasive (Lefort et al. 2022). Other studies rely on stable isotope or fatty-acid analyses to infer the trophic position of predators in the food web, as well as potential switches in feeding sites (Hobson and Clark 1992, MacNeil et al. 2005, Logan et al. 2006, Phillips and Eldridge 2006, Elsdon 2010, Taipale et al. 2011). However, these methods do not reach a fine-scale resolution, usually lacking genus or species-level identification, which may be critical for the planning of conservation management actions (Bocher et al. 2000, Chérel et al. 2000, Deagle et al. 2007, Guest et al. 2009). In the last decade, parallel to the development and optimization of genomic techniques, DNA metabarcoding approaches using fecal material as a source of dietary DNA (de Sousa et al. 2019) have allowed the accurate identification of prey species within the diet of a wide variety of taxa including invertebrates (Mollet et al. 2014, Piñol et al. 2014, Boyer et al. 2015, Valentini et al. 2016, Kerley et al. 2018) and vertebrates (Leray et al. 2015, Guilleraut et al. 2017, Kamenova et al. 2018, Sullins et al. 2018, Andriollo et al. 2019).

In recent years, dietary DNA (dDNA) has been a useful tool for studying seabirds' diet and food webs (Deagle et al. 2007, McInnes et al. 2017b, Kleinschmidt et al. 2019, Carreiro et al. 2020, Young et al. 2020, Nimz et al. 2022). Dietary DNA approaches using feces have helped, for instance, to unravel the dietary spectrum of Macaroni Penguins (*Eudyptes chrysolophus*; Deagle et al. 2007) and it also shed light onto the diet of the Black-browed Albatross (*Thalassarche melanophris*) and Campbell Albatross (*Thalassarche impavida*) in the Falkland Islands (McInnes et al. 2017b). Despite

the current importance of these molecular techniques, there are still several seabird species of great interest from a conservation point of view, such as the Westland Petrel (*Procellaria westlandica*), whose diet has never been studied using modern molecular tools.

The Westland Petrel is endemic to New Zealand and listed as an endangered species by the International Union for the Conservation of Nature (IUCN) Red List (BirdLife International 2020). It is one of the few burrowing birds breeding on the main islands of New Zealand. This iconic species was once widespread in New Zealand (Wood and Otley 2013, Waugh and Wilson 2017), but its breeding distribution is now restricted to the west coast of the South Island, within the Paparoa National Park and its surroundings (Jackson 1958, Waugh and Wilson 2017). Between May and June, females lay a single egg, which is incubated by both parents for 69 days (Warham 1991). Chick rearing is carried out by both parents between September and November and chicks are fed via regurgitation. After the breeding season, Westland Petrels fly east to south American waters (Baker and Coleman 1977), where they remain until late March (March to November; Landers et al. 2011). Regarding their foraging behavior, Westland Petrels are known to be nocturnal, but they occasionally feed during the daytime (Waugh et al. 2018). Moreover, Westland Petrels make short trips exclusively during chick rearing (Poupart et al. 2020) to provide parental care and avoid the starvation of the chicks due to long periods without food (Xavier et al. 2013). Previous studies based on morphological analysis of regurgitates found fish to be their most abundant prey item, followed by cephalopods, and crustaceans (Imber 1976, Freeman 1998). The Westland Petrel's diet is, therefore, assumed to be closely linked to fishing activity in New Zealand waters because they are known to feed on fish and molluscs and use offal for a significant part of their diet (Waugh et al. 2003, Wood and Otley 2013). Moreover, their foraging ranges overlap with several fisheries (Waugh et al. 2018). Even if the overall population has increased significantly since the 1970s (Waugh et al. 2003, Wood and Otley 2013), together with the rise of fishing activity, it remains unclear whether fishing has a net positive or negative impact on the Westland Petrel (Waugh et al. 2003, Wood and Otley 2013). In fact, the increase of intensive fishing has led to many Westland Petrels being trapped and killed in fishing nets and it is, therefore, one of the main threats of their populations together with mammal predation, degradation of habitat, and erosion of their nesting grounds (Taylor 2000, Waugh et al. 2008, Waugh and Wilson 2017).

The precise composition of the Westland Petrel's current diet is unknown, and potential temporal variations in diet throughout the breeding season have never been investigated. We present the first attempt to characterize the diet of this seabird through a DNA-based approach. To do this, we used a noninvasive DNA sampling (Lefort et al. 2022) by collecting fecal samples and carried out a DNA metabarcoding analysis using 16S rRNA gene to identify prey items within the diet of the Westland Petrel. This amplicon was chosen as a target for the study because it has shown to be effective for the characterization of the seabirds' diet (McInnes et al. 2017b, Komura et al. 2018, Young et al. 2020) and, moreover, it overcomes the limited detection until species level using a COI marker because of variable primer binding sites (Deagle et al. 2009, Olmos-Pérez et al. 2017). The birds' diet was

compared with two breeding subcolonies (1.5 km apart) at two different times (10 weeks apart) because another of our objectives was to describe potential differences between seasons and subcolonies. We hypothesized that there would be differences in diet, which would be consistent with switches in feeding and foraging behavior between the early phase of breeding season, corresponding to incubation (I) and the post-hatching phase corresponding to chick rearing (CR). These switches, reflected in composition and diversity of prey items, may be driven by ecological opportunity or external factors such as prey availability which, as aforementioned, is known to be highly variable within marine environments (Pace et al. 1999, Behrenfeld et al. 2006, Araújo et al. 2011, Horswill et al. 2017). However, we did not expect to find significant differences in the diet of the different subcolonies because of their relatively close proximity and given that during sea trips, foraging ranges varied on average from 147 to 284 km (Waugh et al. 2018). A secondary goal was to better understand the impact fishing activities have on Westland Petrels by more accurately describing the composition of their diet.

## METHODS

### Study area and sample collection

A total of 99 fecal samples were collected from 2 different sampling sites located on the west coast of the South Island of New Zealand, the Paparoa National Park (NP), -42° 8' 46.7412", 171° 20' 25.0548", (49 samples) and a private land (PL), -42° 9' 51.6888", 171° 20' 15.3708" (50 samples; Appendix 2, Table A2.1). The collected samples were fresh and usually line shaped, which could only correspond to feces produced by birds during landing on the previous day. Hence, each bird could only produce one of these faeces on a given day. Because feeding bouts generally last several days (Waugh et al. 2018), the same bird typically produces two consecutive line-shaped poos at several day intervals. However, very few older fecal samples were observed on the sites because these were probably rapidly washed away in this extremely rainy location. For all these reasons, it is very unlikely to find two consecutive line-shaped faeces from the same bird that both look fresh, cooccurring on the forest floor, on the same day. Our samples were therefore considered to be independent.

Forty-eight samples were collected during a short window within the phase of incubation (I) on the 9th and 10th of July 2015, and 51 samples were collected during another short window within the phase of chick rearing (CR) on the 22nd and 23rd of September 2015 (Appendix 2, Table A2.1). For each sampling campaign, the first day was dedicated to collecting samples from NP and the second day to collecting from PL. To avoid cross-contamination, each fresh fecal sample was collected using an individual sterile cotton swab and placed in a clean, single-use Ziplock bag. Samples were then placed in a cooled icebox for transportation to the laboratory (within the following two days), where they were stored at -80°C until DNA extraction. Leaf litter samples were also collected to serve as negative controls.

### DNA extraction, polymerase chain reaction (PCR) amplification, and sequencing

For each fecal sample, we performed a DNA extraction on one small subsample of the cotton swab used for field sampling. Because the gastrointestinal tracts of most animals are excellent

mixers, no further homogenization was required (Lear et al. 2018). We used the QIAamp DNA stool mini kit (Trevelline et al. 2016, 2018) for which we followed the manufacturer's protocol (Handbook from 03/2014, reference: 1081060\_HB\_LS) with few modifications. In brief, half volumes of all reagents were used, and the extraction was carried out in 1.5 ml tubes, instead of 2 ml tubes. In addition, after adding half an InhibitEx Tablet, we performed only one centrifugation, rather than two (steps 6 and 7 of the protocol were joined). Later, at step 13, we mixed 200  $\mu$ l of ethanol by pipetting and 600  $\mu$ l of the mix was added to the column. At step 14, volumes recommended by the manufacturer's protocol were used. Finally, samples were eluted in 100  $\mu$ l of elution buffer (AE) and DNA extracts were stored at  $-20^{\circ}\text{C}$ .

Two different PCR amplifications were performed from each DNA extract. First, we used a pair of primers specific for Malacostraca, crustaceans (16S1F, 16S2R), which amplified 155 bp of the 16S rRNA gene (Deagle et al. 2005, 2009). Second, we used a pair of primers originally designed for Chordata, fish (Chord\_16S\_F\_TagA, Chord\_16S\_R\_Short; Deagle et al. 2009) but also known to amplify cephalopods DNA in an efficient manner (Olmos-Pérez et al. 2017). This second pair of primers targets a 205 bp region of the 16S rRNA gene (Deagle et al. 2009). These two pairs of primers were tagged with sequence fragments, which are complementary to the Illumina ligation adaptor. These primers were chosen to allow the detection of a wide range of potential dietary items, including the main taxa identified morphologically in previous studies, namely fish, cephalopods, and crustaceans (Imber 1976, Freeman 1998). Polymerase chain reaction conditions for both primer pairs were the same as in Olmos-Pérez et al. 2017, with the exception of the Taq polymerase. Here, the FirePOLE® Taq polymerase was used for all amplifications following the manufacturer's protocol (Solis BioDyne). Negative controls containing DNA-free water were added to each PCR run. After checking the results in a 1.5% agarose gel, PCR products were purified using AMPURE magnetic beads, following the manufacturer's standard protocol (1.8  $\mu$ l AMPure XP per 1.0  $\mu$ l of sample) and, finally, for each sample, both PCR products from the same sample were equalized at 2 ng/ $\mu$ l and pooled. Second stage PCR amplifications were then conducted, and PCR products indexed with Nextera adapters (Index Set C) in unique combinations were arranged in two plates. Subsequent sequencing steps were carried out by New Zealand Genomics Limited (NZGL, University of Otago) according to the Illumina "16S Metagenomic Sequencing Library Preparation Manual" Rev B.

DNA sequencing was performed by NZGL on Illumina MiSeq 2x300bp reads (Illumina MiSeq v3 8 reagent kit).

### Bioinformatic library filtering

Metabarcoding library filtering was performed using a toolbox of software. First, we trimmed the two pairs of primers separately using "cutadapt" (Martin 2011), leaving the maximum error rate as default ( $e = 0.1$ ). At this point, we had two sets of trimmed sequences. The following filtering steps were done twice for each pair of primers. Pair-end reads were merged using "PEAR" (Zhang et al. 2014), setting the minimum quality threshold (Phred score) at 30 ( $-q 30$ ). After merging, all sequences were combined into a single "fastq" file using the "sed" command and all the

subsequent steps were performed using "vsearch" v2.8.1 (Rognes et al. 2016). This step was followed by the quality rate filtering to remove sequences with sequencing errors, using the "fastx\_filter" command (`fastq_maxee = 1`). The library was dereplicated using the "derep\_fulllength" command and, after, frequency errors were detected and deleted using again the "fastx\_filter" command (`minsize = 2`) to delete the singletons, because such low frequency variants are likely to be PCR errors. The next step filtered sequences by length (indel filtering) with the command "fastx\_filter" (`fastq_minlen = 50, fastq_maxlen = 325`). At this stage, merged sequences that were shorter than 50 bp or longer than 325 bp were discarded. This step was followed by the filtering "de novo" of potential chimeras using the "uchime\_denovo" command. After this step, we obtained a "fasta" file, with amplicon single variants (ASVs). Next, we performed the operational taxonomic unit (OTU) clustering (which could be considered as a proxy of species), applying the centroid-based greedy clustering algorithm with a cut-off threshold of 97% (Xiong and Zhan 2018) using the "cluster\_size" command (`id 0.97`) and obtained a "fasta" file with all the OTUs present in the sampling. Finally, we mapped the reads in each sample to OTUs to obtain an OTU table, using the "search\_exact" command. Thus, at this point of the pipeline, we obtained two output files, an OTU table and a "fasta" file with the subsequent sequence of all the OTU sequences.

All OTUs were compared to the National Center for Biotechnology Information (NCBI) database using the NCBI BLAST web interface (Johnson et al. 2008) and the pertinent multiple-file JSON was downloaded from this web interface. We then used a customized R script, based on the functions "fromJSON" and "classification," from R packages "rjson" v.2.5.7 (Couture-Beil and Couture-Beil 2018) and "taxize" v.0.9.99 (Chamberlain and Szöcs 2013), respectively, to retrieve the best hit from the taxonomic classification of each clustered OTU from the NCBI Genbank nr/nt database. Moreover, we performed a SINTAX classification against the MIDORI database (Leray et al. 2018). For that purpose, we used the "vsearch" commands "makeudb\_search" to convert the database, which was downloaded from the MIDORI website in a "fasta" file, into database format and "sintax" to retrieve the taxonomic classification.

Regarding the taxonomic assignment, we discarded OTUs with BLAST query coverage under 60% or BLAST identities lower than 75%. The number of reads for each OTU present, both in the negative control and samples, were subtracted from each OTU because they were considered potential contaminations. In addition, singletons among samples and OTUs were also considered potential contamination or artifacts and removed from the dataset (Gobet et al. 2010, Shade et al. 2012, Lindahl et al. 2013, Brown et al. 2015, Majaneva et al. 2015). We also filtered the taxonomic assignment table, discarding every OTU that was classified as prokaryotes, fungi, insects, mammals, and the Westland Petrel itself because they could not be potential dietary items for biological reasons. Potential dietary items' OTUs within the phyla Arthropoda, Chordata, and the Mollusca families *Octopodidae* and *Histioteutidae* were assigned using the following criteria to taxonomical categories: OTUs with identity higher than 97% were determined at species level, OTUs between

93% and 97% were assigned to genus level, and OTUs with identity below 93% were assigned to family level. In the case of the Mollusca family, *Loliginidae*, we obtained a taxonomic assignment corresponding to species not present in New Zealand's waters. Therefore, OTUs were aligned with 100 *Loliginidae* sequences retrieved from NCBI GenBank nr/nt (Benson et al. 2012). This alignment revealed that the 16S rRNA gene fragment was exactly the same for several genus of this family, meaning that this amplicon fragment does not have sufficient resolution to resolve genus and species identity within this family. Thus, OTUs matching the *Loliginidae* family were only assigned to family level, regardless of the percentage of identity retrieved from the BLASTn taxonomic assignment. Similarly, when both percentage identity and query cover were relatively low (< 90%), OTUs could not confidently be assigned below family level. These OTUs were assigned to the super family level and all fell within the *Talitroidea*.

## Biodiversity analyses

To evaluate the impact of commercial marine species on the Westland Petrel's diet, we collected ecological information from "FishBase" (Froese 1990), "SeaLifeBase" (Palomares and Pauly 2010), and previous studies (Appendix 3, Table A3.1) to determine the distribution of juvenile and adult for each prey taxa. Considering that the Westland Petrel can dive up to 15 m for fishing (Waugh et al. 2018), we specifically looked for information about the depth at which the prey species are usually present (shallow versus deep sea) and whether they were naturally reachable for the Westland Petrel. We also checked whether those prey species had been detected in previous publications based on stomach contents (Appendix 3, Table A3.1). To measure the completeness of our sampling, we evaluated the total richness of prey in the diet of the Westland Petrel using a rarefaction curve and a bootstrap estimator with the function "specpool" in the "vegan" v.2.5.7 R package (Oksanen et al. 2013). Moreover, as a measure of the quality of our sequencing, we plotted the number of sequence reads per OTU detected (Appendix 4, Table A4.1) and the cumulative frequency of OTU detected in relation to the number of sequence reads produced (Appendix 5, Table A5.1). The Westland Petrel's diet was described using two different metrics. First, we calculated the frequency of occurrence (FOO), which gives information about the number of samples in which an OTU is present. This was calculated by transforming the number of reads to presence/absence (1-0) and, subsequently, summing the counts of samples that contain a given prey item (OTU) expressed as a proportion of all samples (Deagle et al. 2019). Second, we calculated the relative read abundance (RRA), which is the proportion of reads obtained for each prey item (OTU) in each sample (Deagle et al. 2019). Relative read abundance was calculated using the OTU table of read abundances. Both metrics were computed with customized scripts using the R package "dplyr" v.1.09 (Wickham et al. 2021; available in figshare <https://figshare.com/s/82d428efa7ccdc43b78>). The overall FOO and RRA were calculated to describe the Westland Petrel's diet as a species, and also compared between seasons: incubation (I) versus chick rearing (CR); and between subcolonies: natural park (NP) versus private land (PL).

To estimate the effects of seasonality and subcolony location on diet diversity and composition, we computed a negative binomial generalized linear model (GLM; McCullagh and Nelder 1989)

with a log-link function, applying the function "manyglm" from the R package "mvabund" v.4.1.3 (Wang et al. 2017). Two different GLM analyses were performed, one with read abundance as the dependant variable and one with occurrences as the dependant variable. For both GLM analyses, the predictor variables were season (two factor levels: I and CR) and site (two factor levels: NP and PL) as well as the interaction between these variables. An analysis of deviance (Dev) was performed to test the statistical significance of the model, with 999 bootstraps iterations as a resampling method (Davison and Hinkley 1997) using the function "anova.manyglm" from the package "mvabund" v.4.1.3 (Wang et al. 2017). Moreover, a canonical correspondence analysis (CCA) ordination to visualize the differences in community composition between the two seasons (BH and CR) and the two subcolonies or sites (NP and PL) was computed and plotted using the "ordinate" function from the R package "phyloseq" v.1.40.0 (McMurdie and Holmes 2012).

Finally, we estimated and plotted the standard alpha diversity (inverse Simpson index) as a proxy for prey species richness by comparing the two factors studied, season and site. For that purpose, we used the functions "diversity" and "plot\_richness" from R packages "vegan" v.2.5.7 and "phyloseq" v.1.40.0 (McMurdie and Holmes 2012, Oksanen et al. 2013), respectively. In addition, we computed pairwise comparisons between the alpha diversity values (inverse Simpson index) of the group levels through the pairwise Wilcoxon rank sum test (Gehan 1965) using the function "pairwise.wilcox.test" from the R package "stats" v.3.6.2 (R Core Team 2018).

## RESULTS

### Amplification success and library quality

All 98 samples were successfully amplified with both pairs of primers and sequenced with Illumina MiSeq to produce 9,847,628 raw reads. After all filtering steps, we obtained 31,691 ASVs, which were clustered in 1147 OTUs (Appendix 2, Table A2.1). In the case of the Chordata pair of primers, after trimming, we only obtained 321,240 reads corresponding to 20 OTUs of which only 1 OTU with 3 reads was different from the OTU set obtained with the Malacostraca pair of primers. Thus, the sequences from the Chordata primers were discarded, and only the reads obtained with the Malacostraca pair of primers were retained for subsequent analyses. The 1147 OTUs comprised 2,567,254 reads, from which 127,088 (243 OTUs) were considered contaminants (not potential dietary items), 560,586 reads (102 OTUs) were considered low-quality assignment (query cover < 60% and percentage of identity < 75%), 1,371,994 reads (723 OTUs) did not match against GenBank, and 507,231 reads (20% of reads) were considered potential prey of the Westland Petrels. These potential prey reads belonged to 79 OTUs (Table 1; Appendix 3, Table A3.1), and 17 samples had only unassigned or undetermined OTUs and, hence, were not used in the subsequent analyses. We were not able to recover any additional assignment from MIDORI compared to those obtained from GenBank. Thus, this information was discarded.

### Characterization of the Westland Petrel's diet

Species richness estimation (based on a bootstrap analysis) suggested that our sampling captured 88.6% of the total diversity

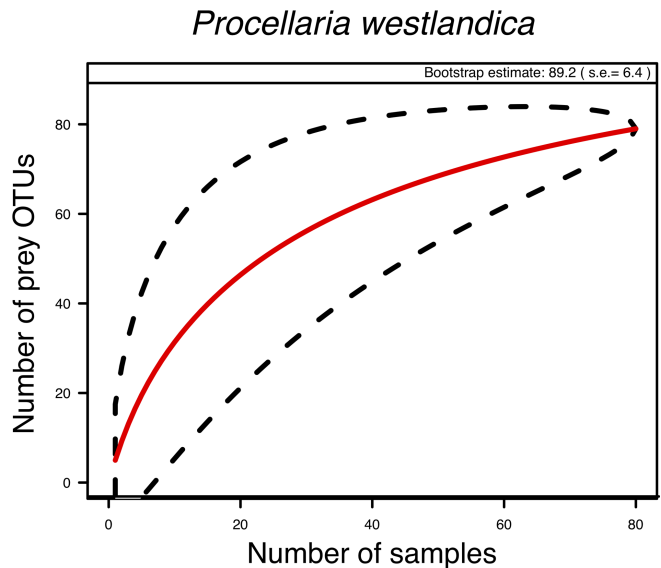
**Table 1.** Summary table with the number of operational taxonomic units (OTUs) per family of dietary item detected.

Phylum	Family	Number of Operational Taxonomic Units (OTUs)
Arthropoda	<i>Artemiidae</i>	1
Arthropoda	<i>Candaciidae</i>	1
Chordata	<i>Cyttidae</i>	1
Chordata	<i>Euclichthyidae</i>	1
Chordata	<i>Gempylidae</i>	1
Mollusca	<i>Histioteuthidae</i>	1
Mollusca	<i>Loliginidae</i>	5
Chordata	<i>Macrouridae</i>	4
Chordata	<i>Merlucciidae</i>	5
Chordata	<i>Moridae</i>	1
Chordata	<i>Nettastomatidae</i>	1
Mollusca	<i>Octopodidae</i>	2
Chordata	<i>Ophidiidae</i>	2
Arthropoda	<i>Penaecidae</i>	1
Arthropoda	<i>Pilumnidae</i>	1
Arthropoda	<i>Talitridae</i>	48
Chordata	<i>Trichiuridae</i>	1
Chordata	<i>Triglidae</i>	1
Chordata	<i>Zenionidae</i>	1

of prey items within the Westland Petrel's diet (Fig. 1; Appendix 3, Table A3.1). The number of sequence reads per OTUs detected (Fig. not shown but available on request) and the cumulative frequency of the OTUs detected (Fig. not shown but available on request), which are both measures of sequencing depth, were sufficient to characterize the Westland Petrel's diet. Out of the 79 OTUs recovered by metabarcoding, 24.02% (19 OTUs, 195,358 reads) were identified at species level, 5.06% (4 OTUs, 29,089 reads) were identified at genus level, and 70.89% (56 OTUs, 316,587 reads) were identified at family level (Table 1; Appendix 3, Table A3.1).

Arthropods (crustaceans in this case) were the most common dietary items in the Westland Petrel's diet, being present in 62.03% of the samples (FOO), represented 45.57% of the sequences (RRA), and 65.82% of the OTUs. Actinopterygii (bony fish) were next, being present in 59.49% of the samples, comprising 42.13% of all sequences and 24.05% of all OTUs. Finally, cephalopods were present in 53.16% of the samples, made up 12.29% of the sequences, and 10.12% of the OTUs (Fig. 2). Within arthropods, *Talitridae* (landhoppers and sandhoppers) were by far the most abundant taxa. Although there are marine talitrids (Fenwick 2001, Lowry and Bopiah 2012), there is insufficient information in the databases and possible faulty matches because amphipodan taxonomy is challenging and under continuous change. That is the reason why we used the higher-level taxonomic assignment until superfamily *Talitroidea*. They were present in 58.23% of the samples and made up 44.35% of the sequences. Other minor arthropod taxa were identified, such as the families *Pilumnidae* (pilumnid crabs) and *Penaecidae* (penaeid shrimps), among others (< 1% total reads; Table 2; Appendix 4, Table A4.1). Except for four OTUs, which were identified to species level, arthropods were identified to family level.

**Fig. 1.** Accumulation curve representing the cumulative number of dietary item operational taxonomic units (OTUs) detected against the number of fecal samples analyzed (n = 87). Horizontal solid line represents the number of prey OTUs expected with limitless sampling, based on bootstrapped estimates.



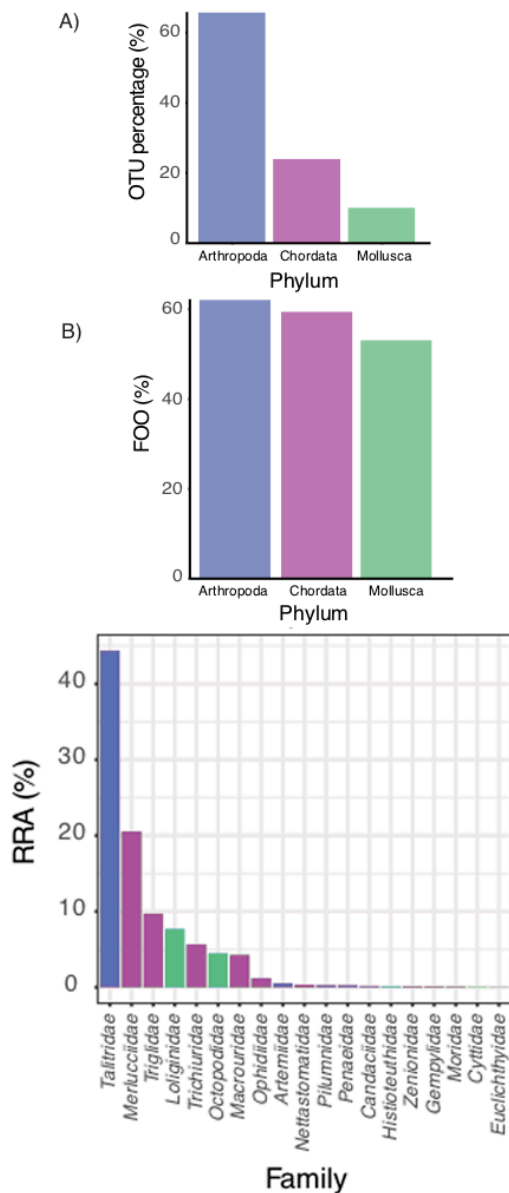
Within Chordata (ray-finned fish in this case), hoki (*Macruronus novaezelandiae*) was the most common species because it was present in 26.58% of the samples and represented 10.5% of all sequences. The cocky gurnard (*Lepidotrigla modesta*) and the southern hake (*Merluccius australis*) were also important dietary items, being present in 18.99% and 17.72% of the samples and comprising 9.69% and 10.01% of all sequences, respectively. Next were cutlassfish, identified to family level (*Trichiuridae*), and the thornthroat grenadier (*Lepidorhynchus denticulatus*) both present in 11.39% of the samples and comprising 5.77% and 3.8% of all sequences, respectively. As in the case of arthropods, we detected a few other minor taxa, such as the pink cusk-eel (*Genypterus blacodes*) or the hawknose grenadier (*Coelorinchus oliverianus*), among others (around 1% of the reads; Table 2; Appendix 4, Table A4.1). Out of 19 OTUs of Actinopterygii, 3 OTUs were identified to genus level, 3 OTUs were identified to family, and the remaining 13 OTUs were identified to species level (Table 1; Appendix 3, Table A3.1).

According to our results, within cephalopods, eight different OTUs were identified as dietary items, six of which were assigned to family level, one to genus level, and one to species level (Table 1; Appendix 3, Table A3.1). The most common cephalopod prey item was the common octopus (*Octopus vulgaris*), which was present in 32.91% of the samples, followed by the pencil squids (family *Loliginidae*), present in 31.65%. However, in terms of number of reads, pencil squids comprised 7.68% of all reads and octopodids only 4.46%. Finally, oegopsida squids (Family *Histioteuthidae*) were present in 2.53% of the samples but comprised less than 1% of the reads (Table 2, Fig. 2C; Appendix 4, Table A4.1).

**Table 2.** Summary table with the total by season and by site, relative read abundance (RRA), and frequency of occurrence (FOO) classified by phylum. Note: I = incubation, CR = chick rearing, NP = Paparoa National Park, and PL = private land.

Phylum	Total FOO (%)	Total RRA (%)	FOO I (%)	FOO CR (%)	FOO NP (%)	FOO PL (%)	RRA I (%)	RRA CR (%)	RRA NP (%)	RRA PL (%)
Arthropoda	62.03	45.57	17.72	43.04	35.44	25.32	4.72	78.07	68.87	30.73
Chordata	59.49	42.14	32.91	25.32	22.78	35.44	81.09	11.12	17.32	57.90
Mollusca	53.16	12.29	22.78	29.11	27.85	24.05	14.20	10.81	13.80	11.37

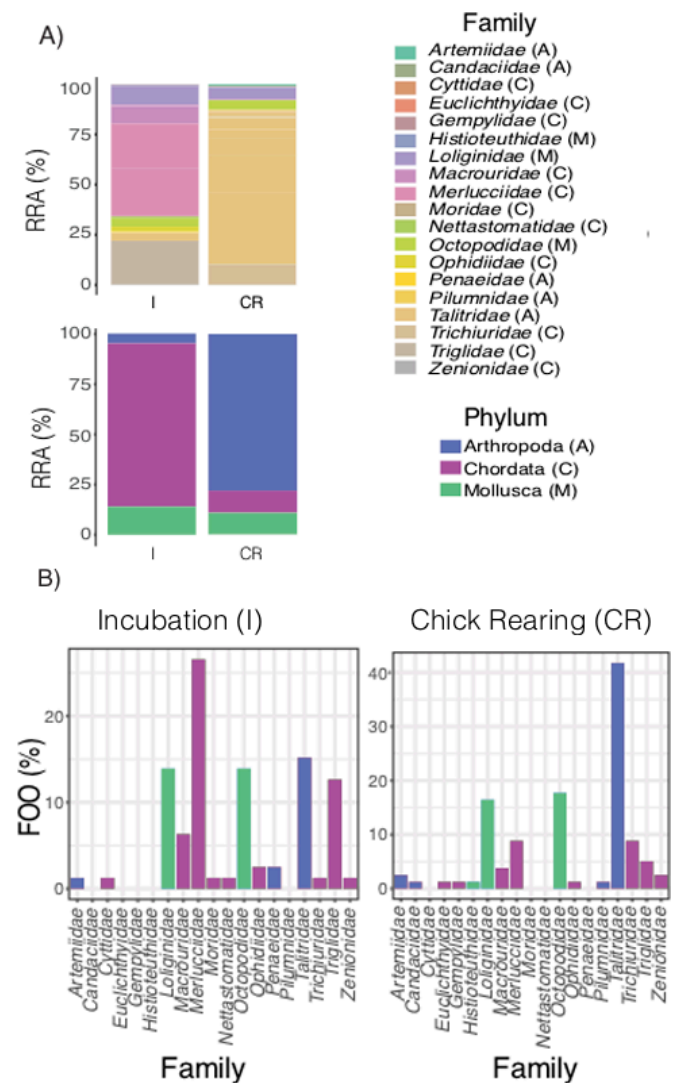
**Fig. 2.** Dietary items identified using different biodiversity metrics: A) number of operational taxonomic units (OTUs) per phylum, B) frequency of occurrence (FOO) per phylum refers to the percentage of samples in which the prey item is present, and C) relative read abundance (RRA) per family colored by phyla as a proxy of biomass.



### Seasonal variation in the Westland Petrel's diet

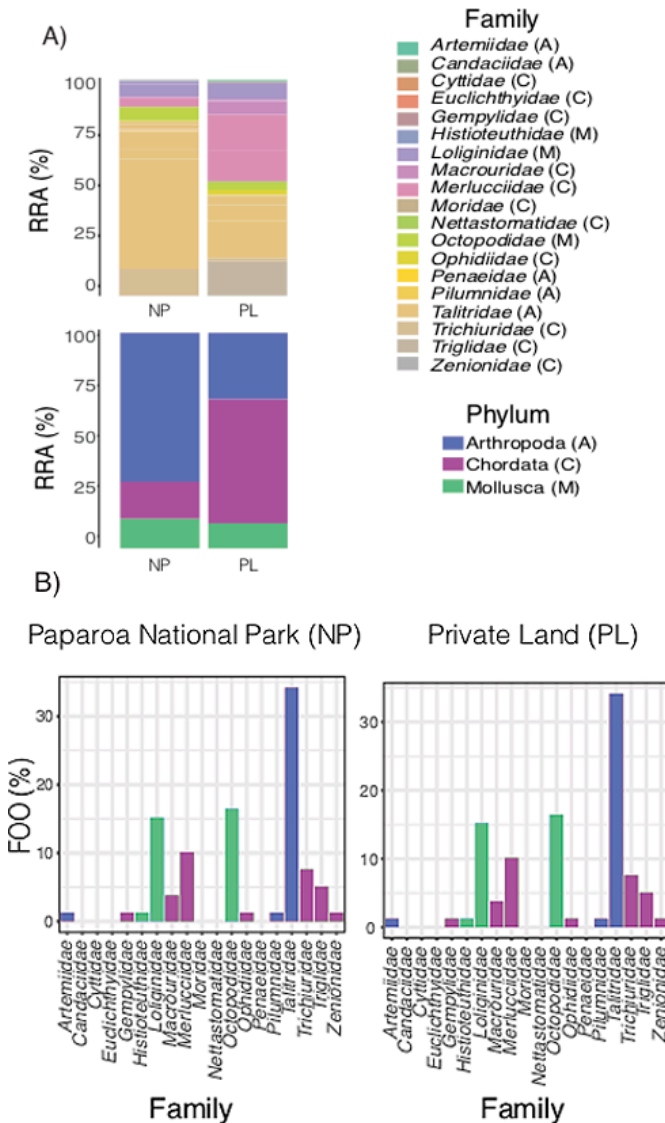
According to the frequency of occurrence (FOO) and the relative read abundance (RRA), our results show differences between seasons (Fig. 3) and between sampling sites (Fig. 4).

**Fig. 3.** Seasonal variations at family level between the phase of incubation or during incubation (I) and the phase of breeding or chick rearing (CR), according to two biodiversity metrics: A) relative read abundance (RRA) and B) frequency of occurrence (FOO). Taxa with less than 1% of FOO or RRA were not included in the plots.





**Fig. 4.** Geographical variations at family level between the two subcolonies: the Paparoa National Park (NP) and the private land (PL), according to two biodiversity metrics: A) relative read abundance (RRA) and B) frequency of occurrence (FOO). Taxa with less than 1% of FOO or RRA were not included in the plots.



Prey community composition varied significantly between the two different seasons both in terms of read abundance ( $Dev_{1,79} = 232.5$ ,  $p = 0.004$ ) and prey occurrence ( $Dev_{1,79} = 189.2$ ,  $p = 0.004$ ; Fig. 3). These differences are clearly visible on the graphical ordination through CCA (Fig. 5).

When looking at FOO, during the phase of breeding season (incubation), merlucciids were the most common dietary item, followed by *Talitroidea*, and then by cephalopods (pencil squids and octopuses showing the same value of FOO). In contrast, during the late breeding season (chick rearing), *Talitroidea* were the most

common dietary item followed by octopodids and pencil squids (Table 2, Fig. 3A; Appendix 4, Table A4.1). A similar pattern was observed for relative read abundance, although with greater differences in the metric values (Table 2, Fig. 3B; Appendix 4, Table A4.1).

*Talitroidea* were the most common dietary item group overall and during chick rearing (CR), representing more than 99% of all arthropods identified in this study. Although a minor dietary item, the banana shrimp (*Penaeus merguensis*) was present in 2.53% of samples during incubation, but it was absent during the chick-rearing phase. In the same way, the bristly crab (*Pilumnus hirtellus*) and *Candacia armata* comprised both 1.27% of samples during incubation and were absent during the chick-rearing phase (Appendix 4, Table A4.1).

Fifteen OTUs of Actinopterygii fish were identified in the samples collected during incubation (13 identified at species level and 2 at family level), compared to 9 OTUs (corresponding to 8 species) during the chick-rearing phase. Hoki was the most common fish species detected during incubation, followed by cocky gurnard and southern hake. During the chick-rearing season, *Trichiuridae* fish were the most common followed by southern hake and cocky gurnard (both showed the same FOO value).

With regard to cephalopods, pencil squids (*Loliginidae*) and octopodids (*Octopodidae*) were present in the same number of samples, while, during the chick-rearing season, octopodids were more common than pencil squids. Interestingly, an oegopsida squid (*Histioteuthidae*) was also detected during the chick-rearing phase of the season, although it was completely absent during incubation (Table 2, Fig. 3A, B; Appendix 4, Table A4.1).

Regarding species richness, the values of alpha diversity (Simpson) were not significantly different between seasons, with during incubation  $\alpha = 0.31 \pm 0.05$  [mean  $\pm$  SE] chick-rearing phase of the season  $\alpha = 0.28 \pm 0.04$  [mean  $\pm$  SE]. See Figure 5.

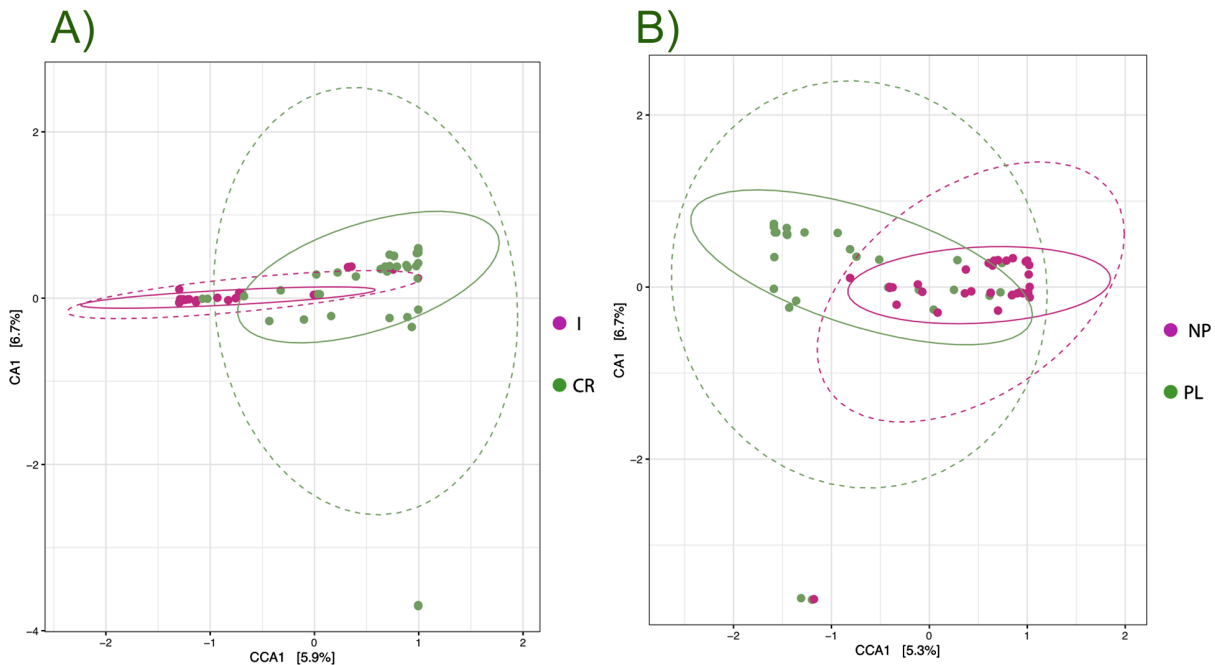
### Geographical variation in the Westland Petrel's diet

Significant differences in prey community were observed between the two subcolonies, both in terms of read abundance ( $Dev_{1,79} = 203$ ,  $p = 0.002$ ) and occurrence of dietary items ( $Dev_{1,79} = 172.6$ ,  $p = 0.003$ ; Fig. 4). Differences in prey community composition between subcolonies are visible on the canonical correspondence analysis (CCA) ordination biplot (Fig. 5).

Arthropods (*Talitroidea*) were found to be by far the most commonly detected dietary item group in the subcolony located within the Paparoa National Park (NP), followed by octopodids and pencil squids. In contrast, in the private land (PL), merlucciids were the most common group of dietary items, followed by *Talitroidea* and pencil squids.

Eleven OTUs of Actinopterygii were identified in samples collected in PL (10 identified at species level and 1 at family level), while 17 OTUs (15 identified at species level and 2 at family level) were found in NP. Cutlassfish (family *Trichiuridae*) were the most common prey within NP, followed by hoki, southern hake, and cocky gurnard. In PL samples, however, hoki was the most common fish taxon, followed, in this case, by cocky gurnard and southern hake (Appendix 4, Table A4.1).

**Fig. 5.** Ordination of Westland Petrel (*Procellaria westlandica*) diet composition through a canonical correspondence analysis (CCA) visualization by A) seasonal differences, phase of incubation or during incubation (I), and the phase of breeding or chick rearing (CR), and B) geographical differences, subcolony from the Paparoa National Park (NP) and the subcolony from the private land (PL) at operational taxonomic unit (OTU) level based on Bray-Curtis distance.



With regard to cephalopods, common octopuses were the most common group followed by pencil squids in NP, and both were present in the same number of samples in PL. In terms of read abundance, pencil squids were slightly more abundant than common octopuses in NP and in PL. (Fig. 4A, B; Appendix 4, Table A4.1).

Similar to seasonal variation, no significant differences in species richness (alpha diversity) were observed in prey diversity when comparing the two subcolonies NP ( $??$  [mean  $\pm$  SE] =  $0.31 \pm 0.05$ ) and PL ( $??$  [mean  $\pm$  SE] =  $0.28 \pm 0.04$ ).

## DISCUSSION

Our study is the first attempt to characterize the diet of the New Zealand endemic Westland Petrel using dDNA metabarcoding. By using DNA sourced from fecal material, we were able to demonstrate that a noninvasive dDNA approach can be an efficient tool to describe the diet of this endangered species. We found that amphipods were the most common dietary item detected, followed by cephalopods, and fish. These results could correspond to natural foraging behavior but also support close links between Westland Petrel diets and New Zealand's commercial fishing activities. The high abundance of amphipods could be due to petrels feeding on discarded fisheries waste (fish guts), which has been suggested previously as the reason why their population has increased in recent decades (Waugh et al. 2003, Wood and Otley 2013). We also showed significant differences in diet between phases of the season (incubation vs. chick-rearing season) and, against all odds, between sampling sites (two subcolonies 1.5 km apart), indicating that the Westland Petrel's

foraging strategies can be highly flexible. Our dDNA approach has the potential to contribute to the conservation of seabirds by noninvasively describing their diet.

However, the metabarcoding analysis of fecal samples still has a number of limitations that need to be resolved. Although we were able to infer 88.6% of the dietary item species within the Westland Petrel's diet, the resolution of the amplicon was insufficient for assigning *Talitroidea* to family, genus, or species level. This limitation may be because of the short size of the amplicon and/or the incompleteness of existing genetic databases (Pompanon et al. 2012, Wangenstein et al. 2018, Liu et al. 2020, Gold et al. 2021, Hleap et al. 2021). For this reason, we cannot confirm whether *Talitroidea* is a primary prey. It could be thought that the high prevalence of arthropods is because our primer has a higher affinity for arthropods or even for amphipods (Elbrecht and Leese 2015), making *Talitroidea* overrepresented in the characterization of the Westland Petrel's diet. However, this is highly improbable because the Malacostraca pair of primers efficiently amplified Mollusca DNA and produced sequences in other studies (Olmos-Pérez et al. 2017) without showing a higher affinity to arthropods or Amphipoda. From all the aforementioned, one can conclude that amphipods detected in this study are a dietary item of the Westland Petrel and that we were able to infer the diet of this endangered seabird. This provides an insight into its ecological network and identifies key dietary item species essential for its survival.

Previous works on the Westland Petrel's diet were based on morphological identification of prey remains and were carried out exclusively during the breeding or chick-rearing season (Imber

1976, Freeman 1998). The observed seasonal and geographical variations in the Westland Petrel's diet provide a broad picture of the feeding requirements and foraging ecology of this species. Our findings show the presence of fish, cephalopods, and amphipods (crustaceans) in the Westland Petrel's diet, confirming the results of previous approaches (Imber 1976, Freeman 1998). Notwithstanding, the relative importance of each type of prey differs considerably between these studies and the current work because we identified a number of taxa undetected before in such high proportions, as is the case of *Talitroidea*.

The phylum showing the highest percentage of dietary item reads was Arthropoda (45.57% of the reads, compared to 42.14% of the reads for fish). Arthropoda reads were mainly represented by *Talitroidea* (landhoppers or sandhoppers), order Amphipoda. With this approach, we cannot guarantee that these animals, ranging from 1 mm to 340 mm in size (Horton et al. 2017), are primary or secondary prey of the Westland Petrel but, as stated above, we can assure that it is an abundant prey item. Most *Talitroidea* species are microscopical benthic zooplankton and are known to be common prey of many cephalopods (Villanueva et al. 2017) and fish, including hoki (Livingston and Rutherford 1988, Connell et al. 2010) and hake (Dunn et al. 2010). Therefore, amphipods detected in this study could potentially be secondary dietary items. On the other hand, it is known that several Procellariiformes feed within coastal areas, which is the environment where amphipods are more present and reachable for seabirds (Warham 1991, Thomas et al. 2006). Moreover, several seabirds, such as penguins, feed regularly on amphipods (Knox 2006, Jarman et al. 2013), and large amphipods could potentially represent a fundamental food source for Antarctic seabirds (Navarro 2017) in which they play a similar role to the krill (*Euphausiacea*) in the water column. Amphipods are also found in the stomachs of other Procellariiformes, such as the Providence Petrel (*Pterodroma solandri*; Lock et al. 1992, Bester et al. 2011), the Blue Petrel (*Halobaena caerulea*; Croxall 1988), and the Wilson's Storm-Petrel (*Oceanites oceanicus*). These birds are known to feed on amphipods when krill is not available (Quillfeldt et al. 2000, 2001, 2005, 2019). Imber (1976) found no planktonic crustacean in the Westland Petrel's stomach and Freeman (1998) only detected a small percentage of taxa belonging to three different families: *Euphausiidae* or krill (*Nyctiphanes australis* and *Thysanoessa gregaria*), *Caridea* or caridean shrimps (*Notostomus auriculatus* and an unidentified species), and *Cymothoidae* (unidentified species). Another possible explanation lies in the geographic distribution of the Arctic benthos, including amphipods, which is now displaying a hotspot in the south of New Zealand due to the climate change (Barnes et al. 2009). This potential increase in abundance could have increased the availability of amphipods for the petrel. In short, it remains unclear whether amphipods are primary prey, secondary dietary item (Sheppard et al. 2005), or both, but we can confirm that these taxa play a major role in the flow of energy through the food web. Further research, potentially using a food web approach in which diets from each of the components of the network are characterized, would be useful to fill this knowledge gap.

Fish are major prey items of Procellariiformes (Imber 1976, Prince and Morgan 1987, Freeman 1998, Stewart et al. 1999, Bocher et al. 2000, da Silva Fonseca and Petry 2007, Spear et al.

2007, Bester et al. 2011), and the Westland Petrel is no exception. According to our results, fish (all belonging to the order Actinopteri) represent 15.03% of the prey reads, and they are the second most important dietary item phylum, in terms of RRA. In addition, fish DNA was detected in 37.93% of the samples. The fish species identified by our approach are consistent with previous studies (Imber 1976, Freeman 1998) but also include new species. In concordance with previous knowledge, the hoki was identified as the most abundant fish prey item. However, we also found hake, another *Merlucciidae*, and cocky gurnard (which was not identified by previous approaches), followed by *Trichiuridae* family in abundance and occurrence.

Hoki and hake live between 28 m and 1000 m below sea level (Appendix 3, Table A3.1), which makes these fish rarely naturally catchable for Westland Petrels because the birds can only dive down to 15 m below the surface (Freeman 1998). However, these species, especially hoki, are some of the main fishery species caught in New Zealand waters (Livingston and Rutherford 1988). The fishing season for Merlucciids occurs mainly between June and September, thereby encompassing most of the Westland Petrel's breeding season (Waugh and Wilson 2017, Waugh et al. 2018), including both of our sampling events. Thus, the Westland Petrels could scavenge these fish species by following fishing vessels. In numerous cases, what is available to seabirds in the wake of the fishing boats are the leftovers from the fish, such as stomachs. These stomachs may contain amphipod prey items, which could explain the high abundance of *Talitroidea* in our results.

The same conclusion could apply to a number of other fish species with deep depth ranges, which are naturally unreachable for the petrel, but are important fishery species (Froese 1990, Freeman 1998). These include rattails (*Macrouridae*), such as the thornmouth grenadier as well as two newly identified prey items, namely the hacknose grenadier and the banded whiptail, among other fish species living in deep-sea waters (Appendix 3, Table A3.1). It is worth noting that prey items, which were not previously identified, may not be part of the Westland Petrel's essential diet but, on the contrary, caught by chance during one fishing trip. In the case of hoki, however, natural predation may also be possible at night because this fish species is known to migrate to surface waters to feed during the night (McClatchie and Dunford 2003, O'Driscoll et al. 2009) when the Westland Petrel forages more actively (Waugh et al. 2018).

Cocky gurnard and several species from the family *Trichiuridae*, which can sometimes be found in shallow waters (Froese 1990), could be caught naturally by the petrel. However, as stated before, it is also a known fishery species that could have been scavenged from the fishing waste. Besides, juveniles of several fish taxa, such as those from the *Ophidiidae* family, the common mora, *Mora moro*, (Santos et al. 2021) or the king dory, *Cyttus traversi*, (Palomares and Pauly 2010) are known to be present in water surfaces and, therefore, they would be naturally available for the Westland Petrel if they fed on juveniles, as is the case of other Procellariiformes like the Leach's Storm Petrel (*Oceanodroma leucorhoa*; Watanuki 1985). It is as well worth remarking that Myctophid fishes, which were reported to be natural prey of the Westland Petrel (Imber 1976, Freeman 1998), were not identified in our sampling. It is possible that these species are no longer

available or no longer selected by the Westland Petrel because previous studies were conducted more than 20 years ago for Freeman (1998) and more than 45 years ago for Imber (1976). Another possibility is that our primers were not able to amplify this group of fishes.

In any of the potential fishing scenarios (natural fishing or scavenging), our findings confirm the importance of fish prey items in the petrel's diet, which could extensively use fish waste from the hoki fishery and other inshore small fisheries, at least in the winter season (Freeman 1998), but they could also catch some fish species naturally in certain situations. It is common for opportunistic seabirds to feed on fishery waste, however, if the dependence on this food source is very high, changes and new regulations in fishing activity could modify the bird's behavior and potentially impact their survival and population size (Abrams 1983, Oro et al. 1995, 1996, Freeman 1998). We identified hoki, southern hake, and cocky gurnard as key dietary item species for the Westland Petrel. Thus, wild populations of these fish species and fishing activities should be managed in a way that maintain these resources for the petrel.

Cephalopods are also a key component of the Westland Petrel's diet. They comprised 12.29% of prey reads, and these taxa were detected in 53.16% of the samples. Six out of eight cephalopod OTUs could only be assigned to family level. Only the common octopus was assigned to species level, a taxon already found in previous studies (Imber 1976, Freeman 1998). Our results are consistent with Freeman (1998), who states that fish prey items are followed by cephalopods within the Westland Petrel's diet. In the case of *Histioteuthis* sp., they are deep-sea squid (Voss et al. 1998) but migrate to surface waters at night by vertical migration (Roper and Young 1975), which makes them catchable by the Westland Petrel. The other two families, *Loliginidae* and *Octopodidae* (common octopus), which were also identified in previous studies, are present from surface waters down to 500 m deep, and thus naturally available for the Westland Petrel. Nevertheless, these families also include several commercial species as well as species commonly reported as bycatch (Davies et al. 2009, Pierce et al. 2010). Therefore, we cannot discard the hypothesis that petrels fed on some cephalopods through fishery waste.

A number of other Mollusca prey species were listed in previous studies (Imber 1976, Freeman 1998) but not detected in our approach. These include cephalopods belonging to the orders Sepioidea or Vampyromorpha, among others. It is unclear whether their absence in our analysis is because of the fact that the primer was not able to amplify these taxa or a change in the feeding habits of the birds in the past 20 years. Further research focusing on Mollusca would be required to solve this knowledge gap.

Marked dietary switches between breeding and nonbreeding seasons have been documented for several seabirds (Howells et al. 2018) and are considered a sign of plasticity in behavior (Quillfeldt et al. 2019). These switches may reflect variation in external factors, such as prey availability, a change of strategy between seasons related to ecological opportunity, or a combination of both (Paleczny et al. 2015, Sydeman et al. 2015, Howells et al. 2018). Because these variations can severely affect populations of top marine predators (Cury et al. 2000, Reid and Croxall 2001),

it is essential to understand their drivers to ensure the conservation of the Westland Petrel. Adaptability to different temperatures and availability of resources would be a sign of resilience of the petrel's population to different environments and can greatly inform the design of conservation plans (Yellen 1977, Berkes and Jolly 2002, McDonnell and Hahs 2015, Jones et al. 2020).

As hypothesized, we found a clear seasonal variation in the Westland Petrel's diet, both in terms of read abundance and the occurrence of prey species, meaning that the composition of the diet changes in a substantial way between incubation and chick-rearing season. This change is particularly visible for fish (specifically merluccids) and *Talitroidea*, with fish being the most abundant prey during incubation although *Talitroidea* are by far the most common dietary item during the chick-rearing season. Even if it is speculative, the most plausible explanation of these dissimilarities is the fact that, as previously stated, observational studies confirmed that Westland Petrels make exclusively short trips near the shore (Poupart et al. 2020) during chick rearing to avoid chick starvation (Xavier et al. 2013). And, near the shore, amphipods would be more abundant and easily available than fish or cephalopods. However, these shifts of abundance of dietary items between short windows, during seasons, could also be a consequence of stochastic events or particular climatic conditions of the sampling seasons. In this case, the choice of prey items by adults may be influenced by the nutritional stage and the needs of the chicks. Despite these seasonal differences in prey preferences, prey species richness remains similar between seasons.

Our results suggest that the Westland Petrel's diet could be altered by seasonal changes in foraging strategy and in prey availability. Indeed, the peak of the hoki fishery in New Zealand encompasses both July (during incubation period) and September (chick-rearing period), which means, fishery waste would be equally available during both seasons. These results should, however, be taken with caution because our sampling window in each phase was relatively short (two days), which could bias the outcome because of external factors such as climatic conditions or prey availability on the petrel's foraging behavior. There is therefore the possibility that if the sampling was carried out in another window of time, the composition of the diet would be different. Be that as it may, our results are coherent with the behavior singularities of the Westland Petrel in the phases studied.

Regarding subcolonies and contrary to our expectation, we found significant differences in prey composition between both subcolonies, which were close enough for their foraging ranges to overlap (Waugh et al. 2018). A possible explanation for these differences could be that seabirds from nearby subcolonies forage in different locations (Grémillet et al. 2004, Wakefield et al. 2013, Cecere et al. 2015). Birds' diet could also change every day depending on resource availability, and prey resources may have been very different in the two consecutive days used for collecting samples in both subcolonies because of short-term variations in temperature and/or resource availability.

Sustainable management of worldwide fishing activity needs information regarding the overlap of marine organisms, such as seabirds, with the fishing industry (Frederiksen et al. 2004, Okes et al. 2009, McInnes et al. 2017a). Seabirds scavenge food from fishery waste, which results in a high number of incidental kills

through bycatch, potentially disturbing population dynamics (Brothers 1999, Sullivan et al. 2006, Watkins et al. 2008, Waugh et al. 2008, Tuck et al. 2011, McInnes et al. 2017a, Waugh and Wilson 2017). However, the seabirds diets rely on this commercial activity because fishery waste represents a nutritious food source, naturally unreachable for seabirds. Therefore, understanding these interactions is essential for seabird conservation and efficient ecosystem-based fishing regulations (Freeman 1998, Phillips et al. 1999, Furness 2003, Becker and Beissinger 2006, Waugh et al. 2008, McInnes et al. 2017a). In this context, noninvasive dietary studies can provide knowledge to assess risks as well as detect the needs of these species that may rely heavily on commercial fishing activity (McInnes et al. 2017a, b, Gaglio et al. 2018). This issue is particularly urgent in the case of endangered species, such as the Westland Petrel. Our findings show a probable link between fisheries in New Zealand and the petrel's diet that should be considered in management and conservation strategies.

Our results show the potential of noninvasive dietary studies in highlighting the reliance of endangered seabirds on commercial fishing activity (McInnes et al. 2017a, b, Gaglio et al. 2018). This study should draw attention to the complexity that lies in the implementation of fishing regulations and the associated risks for the conservation of endangered species. In the case of Westland Petrel, these regulations should consider the close link between commercial fishing and the diet preferences of the birds regarding fish and cephalopods. Several mitigation solutions have been suggested by practitioners or already included in conservation reports (OpenSeas 2019) to limit the number of accidental kills in seabirds and to find a sustainable equilibrium between fishing activities and threatened species. Hence, knowledge on how seabirds in general, and Westland Petrel in particular, interact with fishing vessels and fishing gear is necessary to develop bycatch reduction techniques and using or developing gear less dangerous to the seabirds.

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#### Author Contributions:

*Designed the study: S. B. Obtained funding: S. B. Collected samples: S. B. Performed laboratory analyses: M. C. L., and S. B. Analyzed the data and prepared the figures: M. Q. Wrote the first draft of the manuscript M. Q., and S. B. All authors contributed to the writing of the final manuscript.*

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#### Data Availability:

*Data and R code are publicly available at the following repository: <https://figshare.com/ls182d428efa7ccdc43b78>*

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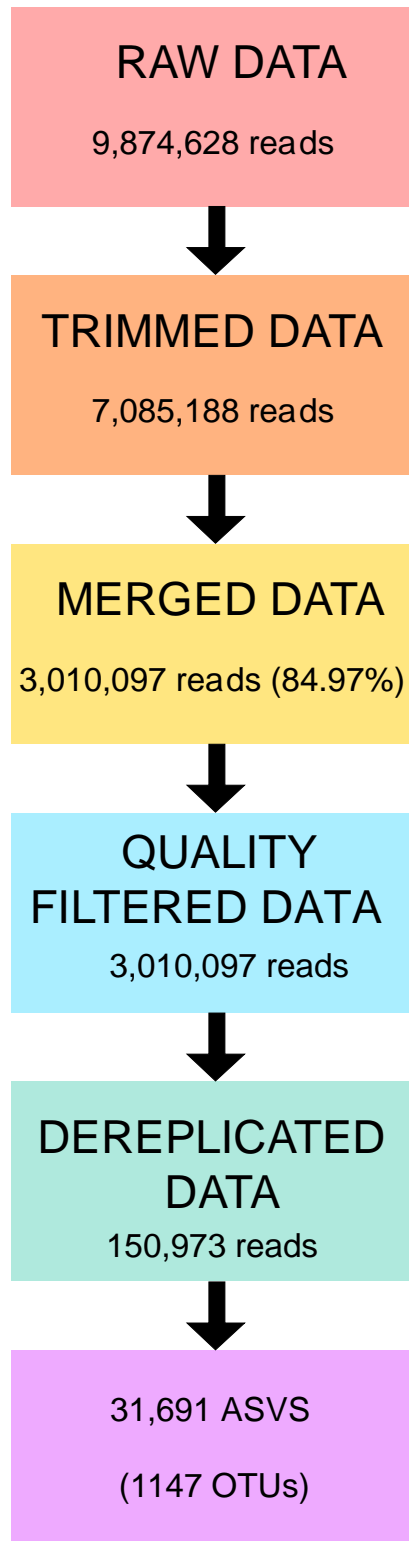
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**APPENDIX 1. Figure A1.1** Bioinformatic results from the 16S dietary metabarcoding approach showing the number of reads at each step of the filtering process.

# BIOINFORMATIC PIPELINE



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**Appendix 2.** Sample list showing the sample identification code (ID), the season when it was collected, the exact date, as well as the site where it was collected from and whether it was included or not in the biodiversity analysis.

*Please click [here](#) to download file 'appendix2.xlsx'.*

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**Appendix 3.** Taxonomy table classified by operational taxonomic unit (OTU) ID.

*Please click here to download file 'appendix3.xlsx'.*

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**Appendix 4.** Taxonomical classification of the prey items of the Westland Petrel (*Procellaria westlandica*) until family level with its corresponding relative read abundance (RRA) and frequency of occurrence (FOO) values for the whole sampling and showing the differences among: **A**) the two different phases, incubation (BH) and breeding or chick rearing (CR) and **B**) the two different sites, the Paparoa Natural Park (NP) and the private land (PL) in the surroundings.

*Please click here to download file 'appendix4.xlsx'.*

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