Investigating spatiotemporal variation in the diet of Westland Petrel through metabarcoding, a non-invasive technique

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Abstract

As top predators, seabirds are directly impacted by any changes in marine communities, whether they are linked to climate change or caused by commercial fishing activities. However, their high mobility allows them to adapt to changing conditions. For example, seabirds can adapt their foraging behaviour according to the resources available at different seasons. This capacity of adaptation comes to light through the study of their diet. Traditionally, the diet of seabirds is assessed through the morphological identification of prey remains in regurgitates, a method that is invasive for the bird and limited in terms of

resolution. However, the recent optimization of DNA-based approaches is now providing a non-invasive and more comprehensive and accurate characterization of animals' diet. Here, we used a non-invasive metabarcoding approach to characterize the diet of the Westland petrel (*Procellaria westlandica*), an endangered burrowing species, endemic to the South Island of New Zealand. We collected 99 fresh faecal samples at two different seasons and in two different sub-colonies. Besides from describing the diet of the Westland petrel, our aim was to account for seasonal and geographical variations in the diet of the petrel and assess potential links with the fishery industry in New Zealand.

We found that amphipods were the most common prey, or secondary prey, followed by cephalopods and fish, suggesting a close link between the composition of prey items and New Zealand's commercial fishing activities but, also, some level of natural predation.

Our results show significant differences in diet between seasons (before hatching vs chick rearing season) and between sampling sites (two sub-colonies 1.5 km apart), which suggests variability and adaptability in the foraging strategy of the Westland petrel.

Due to its non-invasive nature, the method used here can be applied on a great number of samples to draw a comprehensive picture of the diet dynamic in seabirds and unravel their adaptability or ecological requirements. This work demonstrates how environmental DNA can inform the conservation of an endangered species with elusive foraging behaviour, providing, in this case, valuable information regarding the diet preferences of an iconic species within New Zealand's biodiversity.

Introduction

The study of animal diets is a critical component in many aspects of ecology, including community ecology (Corse et al., 2010), population dynamics (Morrison et al., 2014; Read and Bowen, 2001) and conservation biology (Lyngdoh et al., 2014; Xiang et al., 2012). In predators in particular, spatial and seasonal variations in diet composition may reflect a certain degree of flexibility in foraging behaviour (Whelan et al., 2000), that could be relevant for understanding trophic interactions and, also, for conserving endangered species (Davies et al., 2001; Farias and Kittlein, 2008; Vander Zanden et al., 2000; Vinson and Angradi, 2011). Shedding light onto these patterns is essential in the case of seabirds, which are top predators within marine ecosystems. Seabirds are known to modify their feeding habits depending on the time of the year (Harding et al., 2007; Kowalczyk et al., 2015) and their breeding site (McInnes et al., 2017a; Thompson et al., 1999). These birds spend most of their lives at sea but during the breeding season, some remain in coastal areas as their foraging trips are restricted in number and length to allow them to regularly feed their chicks in the nest. To achieve this, seabirds have adopted a variety of foraging strategies (McInnes et al., 2017a; Ydenberg et al., 1994), 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). The majority of studies that aim at describing the diet of seabirds have been carried out during the chick rearing period only. Often, this is because data are collected based on the morphological analysis of regurgitates obtained from parents coming back to the nest to feed their chicks (Calixto-Albarrán and Osorno, 2000; Croxall et al., 1988; Klages and Cooper, 1992; Suryan et al., 2002). This approach however, leads to consider prey communities as a fixed parameter across time, instead of treating it as a dynamic pattern (Barrett et al., 2007; Komura et al., 2018). Consequently, many studies do not explore switches in diet, although it is known that the ability to switch to new prey may potentially represent a driver to escape from striking population declines and, even, from local extinctions of threatened populations (Marone et al., 2017). Many seabird populations have been decreasing rapidly in recent years (Grémillet et al., 2018; Thibault et al., 2019) and detailed knowledge of their diet preferences through space and time is key to understand and better manage current and future threats,

including commercial fishing activities or climate-driven changes to their ecosystem (Frainer et al., 2017).

Selecting the correct experimental design and the most efficient methodological approach for the accurate characterization of seabird diet is essential, but also challenging (Ocké, 2013), mainly because direct observations of elusive seabirds (e.g. nocturnal) are difficult and rare. For decades, the morphological identification of stomach contents or regurgitates has been widely used to identify prey items of predators (Carreon-Martinez and Heath, 2010; Egeter et al., 2015; Freeman, 1998; Imber, 1976; Krüger et al., 2014). However, this methodology usually requires that gut content is obtained by stimulating regurgitation after capturing individual birds through a technique that has been called “lavage” (Barrett et al., 2007; Ryan and Jackson, 1986; Wilson, 1984). Such an invasive sampling method (Lefort et al., 2019) is not only unethical, but also potentially dangerous for the birds. Furthermore, the efficiency of this method is usually limited because many individuals would have empty stomachs at the moment of sampling, and highly digested prey items may not be identifiable to genus or species level. The ability to identify prey remains from stomach content also varies in relation to prey species, because some species (in particular soft-bodied prey) are digested faster than others, leading to potential biases in the characterization of the diet (Boyer et al., 2015; Deagle et al., 2007; Gales, 1988). More recent approaches, such as fatty-acid or stable-isotope analyses, can be used to infer the trophic position of predators in the food web, as well as potential switches in feeding sites. Although they provide valuable information about trophic interactions, 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; Cherel et al., 2000; Deagle et al., 2007; Guest et al., 2009; Guillerault et al., 2017). In the last decade, parallel to the development and optimization of genomic techniques, DNA metabarcoding approaches have allowed the accurate identification of prey species within the diet of a high variety of taxa including invertebrates (Kerley et al., 2018; Mollot et al., 2014; Pinol et al., 2014; Valentini et al., 2016) and vertebrates (Andriollo et al., 2019; Guillerault et al., 2017; Kamenova et al., 2018; Leray et al., 2015; Sullins et al., 2018).

The Westland petrel (*Procellaria westlandica*) is endemic to New Zealand and listed as an endangered species on the IUCN red list (BirdLife International, 2020). It is one of the few burrowing birds breeding on the main islands. This iconic species was once widespread in New Zealand (Waugh and Wilson, 2017; Wood and Otley, 2013), 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) (March to November) (Landers et al., 2011). Between May and June, females lay a single egg, which is incubated by both parents during 69 days (Warham, 1990). Chick rearing is also carried out by both parents between September and November. After the breeding season, Westland petrels travel to South American waters (Baker and Coleman, 1977) where they remain until late March (Landers et al., 2011). Regarding their foraging behaviour, Westland petrels are known to be nocturnal, but they occasionally feed during daytime (Waugh et al., 2018). Previous studies based on morphological analysis of regurgitates found that their most abundant prey items were fish, followed by cephalopods and crustaceans (Freeman, 1998; Imber, 1976). The diet of Westland petrels is therefore closely linked to fishing activity in New Zealand waters. However, it remains unclear whether fishing has a net positive or negative impact on *P. westlandica*. The overall population has increased significantly since the 70's (Wood and Davis, 2003; Wood and Otley, 2013), together with the rise of fishing activity, potentially because of increase feeding on bycatch and other fishing waste. However, being trapped and killed in fishing nets is one of the main threats of *P. westlandica*, together with mammal predation, degradation of habitat and erosion of their nesting grounds (Taylor, 2000; Waugh et al., 2008; Waugh and Wilson, 2017).

Although the diet of the Westland petrel has been assessed before (Freeman, 1998; Imber, 1976), the precise composition of their current diet is unknown, as is potential temporal variations in diet throughout the breeding season. In this work, we present the first attempt to characterize the diet of this seabird through a DNA-based approach. To do this, we used a non-invasive DNA sampling approach (Lefort et al., 2019) by collecting faecal samples, and carried out a DNA metabarcoding analysis using the 16S gene to identify prey items within the diet of the Westland petrel. Prey identity was used to establish potential interactions with the fishing activity in New Zealand. The birds' diet was compared between two breeding sub-colonies (1.5 km apart), and two different times (10 weeks apart). We expected to find differences in diet between early breeding season (before hatching) and

146 late breeding season (after hatching or chick rearing), which would be consistent with
147 switches in feeding and foraging behaviour. We expected to find no significant differences in
148 the diet of the different sub-colonies owing to their relatively close proximity. Moreover,
149 this study also aims to better understand the impact that fishing activities could have on
150 Westland petrels.
151

152 ## Material and Methods

153 *Study area and sample collection*

154 A total of 99 fresh faecal samples were collected from two different sampling sites located
155 in the West Coast of the South Island of New Zealand, the Paparoa National Park (NP) (-
156 42.146317, 171.340293) (49 samples) and a private land (PL) (-42.164358, 171.337603) (50
157 samples) (Table S1). Forty-eight samples were collected before hatching (BH) on the 9th and
158 10th of July 2015, and 51 samples were collected during chick rearing (CR) on the 22nd and
159 23rd of September 2015 (Table 1). To avoid cross-contamination, each fresh faecal sample
160 was collected using an individual sterile cotton swab and placed in a clean, single-use
161 Ziplock bag. Samples were then placed in a cooled icebox for transportation to the
162 laboratory (within the following two days), where they were stored at -80°C until DNA
163 extraction. Leaf litter samples were also collected to serve as negative controls.
164

165 *DNA extraction, PCR amplification and sequencing*

166 We performed a DNA extraction on one small subsample of each faecal sample, using the
167 QIAamp DNA Stool Mini Kit for which we followed the manufacturer's protocol with few
168 modifications. In brief, half volumes of all reagents were used and the extraction was
169 carried out in 1.5 ml tubes, instead of 2 ml tubes. Also, after adding half an InhibitEx Tablet,
170 we performed one centrifugation, rather than 2 (Steps 6 and 7 in the protocol were joined).
171 Later, on step 13, we mixed 200 µl of ethanol by pipetting and 600 µl of the mix were added
172 to the column. From step 14, volumes recommended by the manufacturer's protocol were
173 used. Finally, samples were eluted in 100 µl of elution buffer (AE) and DNA extracts stored
174 at -20°C.
175

176 Later, two different PCR amplifications were performed from each DNA extract. First, we
177 used a pair of primers specific for Chordata (16S1F, 16S2R), which amplifies 155 bp of the
178 16S gene (Deagle et al., 2009, 2005). Second, we used a pair of primers specific to
179 Malacostraca (Chord_16S_F_TagA, Chord_16S_R_Short), which amplifies 205 bp of the 16S
180 gene (Deagle et al., 2009). These two pairs of primers were chosen to allow the detection of
181 a wide range of potential prey, including the main taxa identified morphologically in
182 previous studies (Freeman, 1998; Imber, 1976). PCR conditions for both primer pairs were
183 the same as in (Olmos-Perez et al., 2017), with the exception of the Taq polymerase. Here,
184 the FirePOLE® Taq polymerase was used for all amplifications, following manufacturer's
185 protocol (Solis BioDyne). After checking the results in a 1.5% agarose gel, PCR products were
186 purified using magnetic beads, and for each sample, both PCR products were pooled.
187 Second stage PCR amplifications and subsequent sequencing steps were carried out by New
188 Zealand Genomics Limited (NZGL, University of Otago). The resulting amplicons were
189 arranged in two plates and indexed with Nextera adapters (Index Set C) in unique
190 combinations. Each plate included a 16S mock community sample using Index Set D.
191 Sequencing was performed by NZGL on Illumina MiSeq 2x300bp reads (600 cycles) (Illumina
192 MiSeq v3 reagent kit).
193
194 *Bioinformatic library filtering*
195 Demultiplexing and adapter trimming was carried out by NZGL. The metabarcoding library
196 filtering was performed using the software *vsearch v2.8.1* (Rognes et al., 2016). First, we
197 used the *fastq_mergepairs* command to align the forward and reverse read pairs, allowing a
198 maximum of 25 differences between both pairs. At this stage, merged sequences that were
199 shorter than 100bp or longer that 300bp were discarded. This step was followed by the
200 calculation of the quality statistics for each read pair retained, using the *fastq_eestats*.
201 Paired reads were then filtered by quality using the *fastq_filter* command
202 (*fastq_maxee*=0.5) and fastq files converted to fasta. The library was dereplicated at sample
203 level using the *derep_fulllength* command leading to unique sequences in each individual
204 sample, at this point one fasta file was produced per sample. All reads were then
205 aggregated in one single fasta file. Reads were then dereplicated across all samples, again
206 with *derep_fulllength* command, and singletons were removed (*minuniquesize* = 2). This
207 step was followed by a pre-clustering step before chimera detection at 98% using the

208 *cluster_size* command. After pre-clustering, we only retained unique sequences using *grep*
209 command and we detected chimeras *de novo* using the *uchime_denovo* command, again
210 only retaining the unique sequences after this step. All non-chimeric and non-singleton
211 sequences from each sample were then retained in a single fasta file, using a customized
212 Perl script, *map.pl*, from the *vsearch* pipeline available at this Github repository:
213 https://github.com/torognes/vsearch (last accessed on April 2020). Finally, we performed
214 the Operational Taxonomic Unit (OTU) picking, with a cut-off threshold of 97% (Xiong and
215 Zhan, 2018), using the *cluster_size* command, which produced an OTU table and fasta file
216 with all the sequences belonging to each OTU.
217
218 All OTUs were compared to the NCBI database using BLASTn (Johnson et al., 2008) and the
219 pertinent multiple-file JSON was downloaded from the web interface. We then used a
220 customized R script, based on the functions *fromJSON* and *classification* from R packages
221 *rjson* (Couture-Beil and Couture-Beil, 2018) and *taxize* (Chamberlain and Szöcs, 2013),
222 respectively, to assign a taxonomic classification to each clustered OTU. Regarding
223 taxonomic assignment, OTUs with BLAST query coverage under 60% or BLAST identities
224 lower than 74% were discarded. Read abundances equal to 1 (singletons among samples
225 and OTUS) were considered as potential contamination and removed from the dataset as
226 were any OTUs matching to Westland Petrel. Every other OTUs were retained for
227 subsequent statistical analyses. Potential prey (or secondary prey) OTUs within the phyla
228 Arthropoda and Chordata were assigned using the following criteria to taxonomical
229 categories: OTUs with identity higher than 97% were determined at species level, OTUs
230 between 93 and 97% were assigned to genus level, and OTUs with identity below 93% were
231 assigned to family level. In the case of the phylum Mollusca, we determined the assignment
232 threshold from the distance matrix of the alignment of the sequences. To do that, we first
233 computed a “raw” pairwise genetic distance matrix within the alignment of 411
234 Cephalopoda homologous retrieved from Genbank sequences using the function *dist.dna*
235 from R package *ape* (Paradis et al., 2005), we then calculated the threshold value applying
236 the function *localMinima* from R package *spider* (Brown et al., 2012). Based on this analysis,
237 we used a threshold of 98.6% to assign OTUs to species level within Mollusca.
238
239

Biodiversity analyses

In order to evaluate the impact of commercial marine species on the diet of *P. westlandica*, we collected ecological information from Fishbase (Froese and Pauly, 2010) and Sealifebase (Palomares and Pauly, 2010) to determine the distribution of each prey taxa. Considering that *P. westlandica* is able to dive up to 15 m for fishing (Waugh et al., 2018), we specifically looked for information about the depth at which the prey species is usually present (shallow versus deep sea) and therefore naturally reachable for the Westland petrel. We also checked whether those prey species had been detected in previous publications (Table 1). To measure the completeness of our sampling, we evaluated the total richness of prey in the diet of *P. westlandica*, using a rarefaction curve and a bootstrap estimator using the function *specpool* in the R package *vegan* (Oksanen et al., 2013). The diet of Westland petrels was described using two different metrics. First we calculated the Frequency of Occurrence (FOO) 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 of each prey item (OTU) in a given sample (Deagle et al., 2019). These two metrics are different proxies, FOO is a proxy for occurrence and RRA is a proxy for relative food biomass (Cavallo et al., 2018; Deagle et al., 2019). FOO and RRA were calculated overall and also compared between seasons: before hatching (BH) versus chick rearing (CR); and between sub-colonies: natural park (NP) versus private land (PL).

To estimate the effects of seasonality and sub-colony 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* (Wang et al., 2017). Two different GLM analyses were performed, one with abundance as the dependant variable and one with occurrences (presence/absence) as the dependant variable. For both GLM analyses, the predictor variables were season (two factor levels: BH 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 fitness of the model, with 999 bootstraps iterations as a resampling method (Davison and Hinkley, 1997), using the function *anova.manyglm* from the package *mvabund* (Wang et al., 2017).

Finally, we estimated and plotted the standard alpha diversity, as a proxy for prey species richness, comparing the two factors studied, season and site. For that purpose, we used the functions *estimate_richness* and *plot_richness* from R package *phyloseq* (McMurdie and Holmes, 2012). In addition, we computed pairwise comparisons between the alpha diversity values (Shannon) of the group levels through the pairwise Wilcoxon rank sum test (Gehan, 1965), using the function *pairwise.wilcox.text* from the R package *stats* (Team and others, 2013).

Results

Amplification success and library quality

All 99 samples were successfully amplified with both pairs of primers and sequenced with Illumina MiSeq. We obtained a total of 9,847,628 raw reads, which resulted in 2,061,401 merged reads. After quality filtering, dereplication and removal of chimeras and singletons, 1,844,930 reads remained. These included only 494 (0.027%) singletons, which were removed as they were considered as potential contaminants. The remaining reads clustered into 395 OTUs. After assigning a taxonomy to each OTU, 532,228 reads had no hit against the BLAST database, 147,134 reads could not be confidently identified (low percentage of identity and/or low query coverage), 223,239 reads were considered as contaminants. The remaining 942,329 reads were considered as prey or secondary prey and, hence, were included in the subsequent analyses (Fig.S1). These prey reads belonged to 78 OTUs (Table 1), and were detected in 87 out of 99 samples.

Characterization of the diet of P. westlandica

Species richness estimation (using bootstrap) suggested that our sampling captured 83.75% of the total diversity of prey items within the diet of *P. westlandica* (Fig.S2). Out of the 78 OTUs recovered by metabarcoding, 13.69% (15 OTUs, 129,038 reads) were identified to species level, 13.77% (17 OTUs, 129,802 reads) were identified to genus level and 86.22% (61 OTUs, 812,527 reads) were identified to family level (Table 1).

Arthropods (crustaceans in this case) were the most common prey or secondary prey phylum across both seasons and sites. In total, they were present in 86.21% of the samples

303 (FOO) and represented 73.79% of the sequences (RRA) and 71.8% of the OTUs.
304 Actinopterygii (bony fish) were next, being present in 37.93% of the samples and comprising
305 15.02% of all sequences and 17.95% of all OTUs. Finally, cephalopods were present in
306 56.32% of the samples and made up 11.17% of the sequences and 6.41% of the OTUs
307 (Fig.1). Within arthropods, talitrids (landhoppers and sandhoppers) were by far the most
308 abundant taxa. They were present in 85.1% of the samples and made up 73.34% of the
309 sequences. Other minor arthropod taxa were identified, such as the families Pilumnidae
310 (pilumnid crabs) and Penaeidae (penaeid shrimps), among others (<1% total reads; Fig.S3;
311 Table 2). With the exception of four OTUs, which were identified to species level,
312 arthropods were identified to family level.
313
314 Within Chordata (fish in this case), Hoki (*Macruronus novaezelandiae*) was the most
315 common species as it was present in 14.94% of the samples and represented 3.87% of all
316 sequences. The Southern hake (*Merluccius australis*) and the Cocky gunard (*Lepidotrigla*
317 *modesta*) were also important prey items, being present in 10.34% and 9. % of the samples
318 and comprising 3.6% and 3.53% of all sequences, respectively. Next were cutlassfish,
319 identified to family level (Trichiuridae), and present in 5.75% of the samples and comprising
320 1.67% of all sequences. Finally, the Thorntooth grenadier (*Lepidorhynchus denticulatus*) was
321 present in 6.9% of the samples and comprised 1.62% of all sequences. As in the case of
322 arthropods, we detected few other minor taxa, such as the Pink cusk-eel (*Genypterus*
323 *blacodes*) or the Hawknose grenadier (*Coelorinchus oliverianus*), among others (<1% reads:
324 Table 2; Fig.2). Out of 14 OTUs of Actinopterygii, one OTU was identified to genus level, two
325 OTUs were identified to family and the remaining 11 OTUs were identified to species level
326 (Table 1).
327
328 According to our results, within cephalopods, five different OTUs were identified as prey
329 items and were all assigned to family level (Table 1). The most common cephalopod prey
330 item were pencil squids (family Loliginidae), which were present in 31.03% of the samples
331 and comprised 9.39% of all sequences. Octopodids (family Octopodidae) were present in
332 28.74% of the samples and made up 1.73% of all sequences. Finally, Oegopsida squids
333 (Family Histiotheutidae) were present in 1.15% of the samples but comprised less than 1%
334 of the reads (Table 2; Fig. 2).

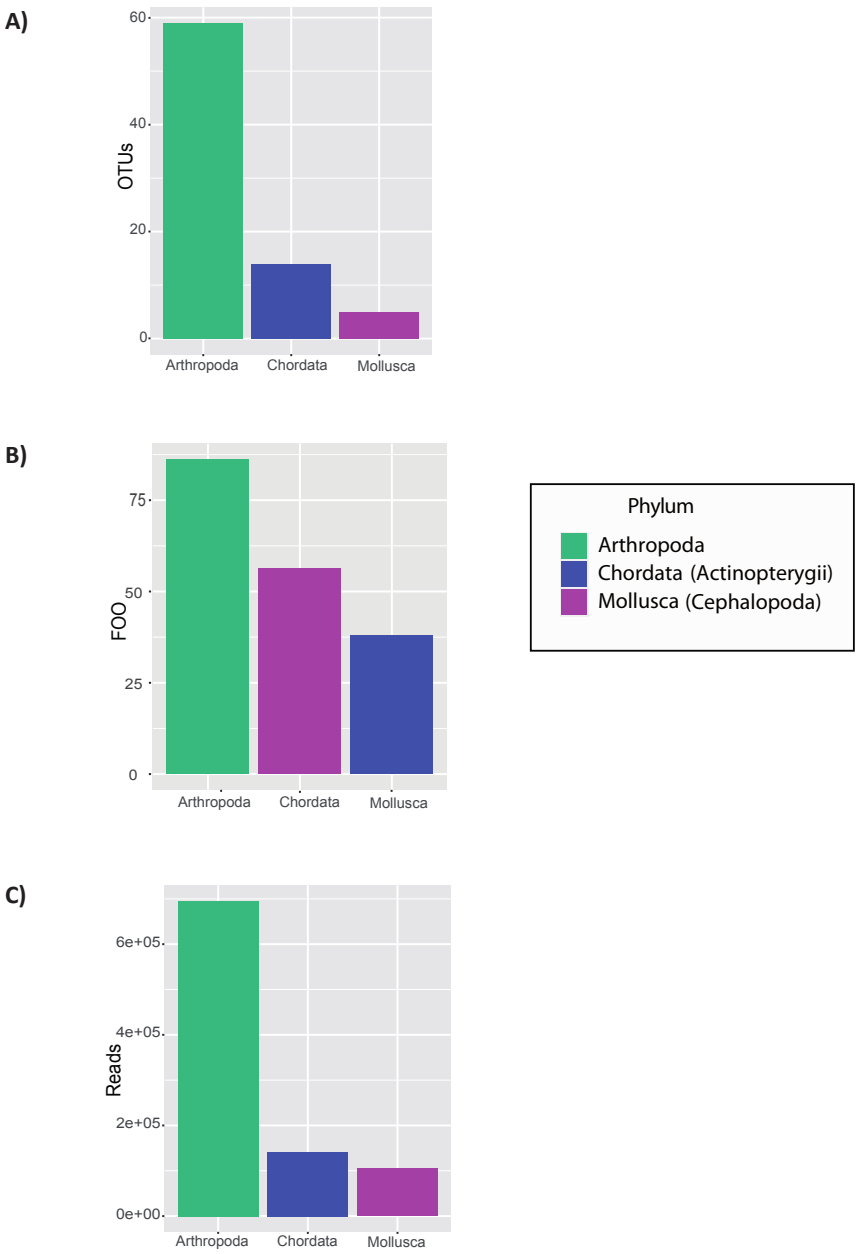
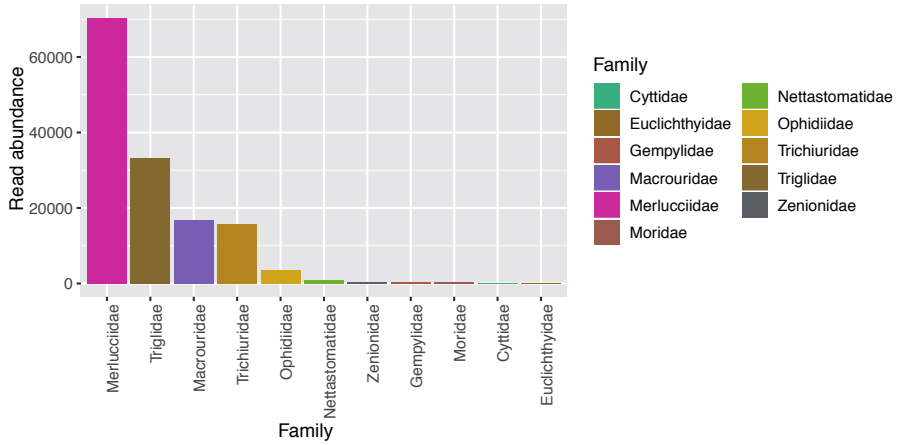


Figure 1. Phyla identified using three different biodiversity metrics: A) Number of OTUs as a proxy of diversity, B) Frequency of occurrence (FOO) refers to the percentage of samples in which the prey item is present and C) Read abundance as a proxy of relative food biomass.

A)



B)

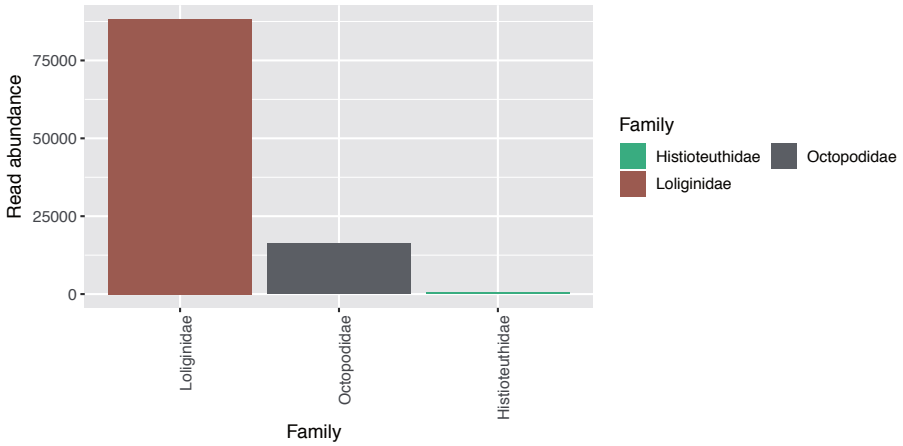


Figure 2. Read abundance classified by family for A) Fish prey items B) Cephalopod prey items

Seasonal variation in the diet of P.westlandica

According to Frequency of Occurrence (FOO) and Relative Read Abundance (RRA), our results show important differences between seasons (Figs 3) and between sampling sites (Fig.4).

Prey community composition varied significantly between the two different seasons both in terms of read abundance (Analysis of Deviance: $Dev_{1,87} = 205.3$, $p = 0.002$) and prey occurrence (Analysis of Deviance: $Dev_{1,87} = 181.1$, $p = 0.001$) (Fig. 3).

When looking at frequency of occurrence, in both seasons, crustaceans (mostly talitrids, amphipods) were the most common prey, followed by cephalopods and then by Actinopterygii fish (Table 2; Fig.). The same pattern was observed for relative read abundance, except that during the early breading season (before hatching), fish reads were more important than those of cephalopods (Table 2; Fig.3B).

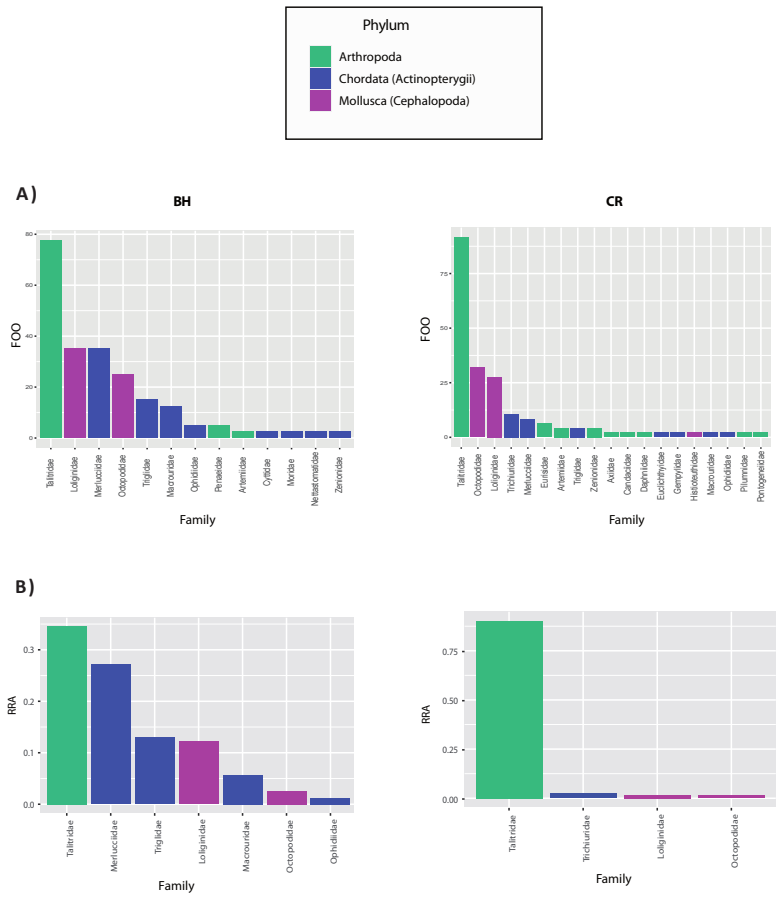


Figure 3. Seasonal variations at family level among the early breeding season or before hatching (BH) and the late breeding season or chick rearing (CR), according to two biodiversity metrics: A) Frequency of Occurrence (FOO) and B) Relative Read Abundance (RRA). Taxa with less than 1% of FOO or RRA were not included in the plots.

Talitrids were by far the most common prey group overall and represented more than 99% of all arthropods identified in this study. Although a minor prey, the Banana shrimp (*Penaeus merguiensis*), was present in 5% of samples before hatching but it was absent during the chick rearing season (Table 2).

Eleven species of Actinopterygii fish were identified in the samples collected before hatching, compared to ten species during the chick rearing season. Hoki was the most common fish species detected before hatching, followed by Southern Hake and Cocky gunard. During the chick rearing season, Trichiuridae fish were the most common followed by Southern hake and Cocky gunard.

With regards to cephalopods, Pencil squids (Loliginidae) were the most common taxa before hatching followed by octopodids (Octopodidae), while it was the other way around, during the chick rearing season. Interestingly, an Oegopsida squid (Histioteuthidae) was also detected during the chick rearing season while it was completely absent before hatching (Table 2; Fig.3A and B).

Regarding species richness, the values of alpha diversity (Shannon) were significantly different between seasons, with lower diversity observed before hatching (α [mean ± SE] = 0.31 ± 0.06) compared to the chick rearing season (α [mean ± SE] = 0.58 ± 0.07) (Fig.5).

Geographical variation in the diet of P.westlandica

Significant differences in prey community were observed between the two sub-colonies, both in terms of read abundance (Analysis of Deviance: $Dev_{1,87} = 152.6$, $p = 0.015$) and occurrence of prey items (Analysis of Deviance: $Dev_{1,87} = 139.5$, $p = 0.017$) (Fig. 4).

Arthropods were found to be by far the most commonly detected prey group in both sub-colonies. However, in NP, cephalopods were more common and produced more reads than Actinopterygii fish, while in PL, these two groups had the same occurrence and Actinopterygii fish produced more reads than cephalopods.

Thirteen species of Actinopteriigy were identified in samples collected in the PL, while nine were found in the NP. Hoki and fish from the Trichiuridae family were the most common fish detected in NP samples, followed by Cocky gunard. In PL samples, Hoki was also the most common fish item taxa, followed, in this case, by Southern hake and Cocky gunard (Table 2).

With regards to cephalopods, pencil squids were the most common group followed by octopodids. However, these values did not differ as much as in the case of seasonal variation (Table 2; Fig.4A and B).

In contrast to seasonal variation, no significant differences in species richness (alpha diversity) were observed in prey diversity when comparing the two sub-colonies NP (**α** [mean ± SE] = 0.48 ± 0.07) and PL (**α** [mean ± SE] = 0.58 ± 0.07) (Fig.5).

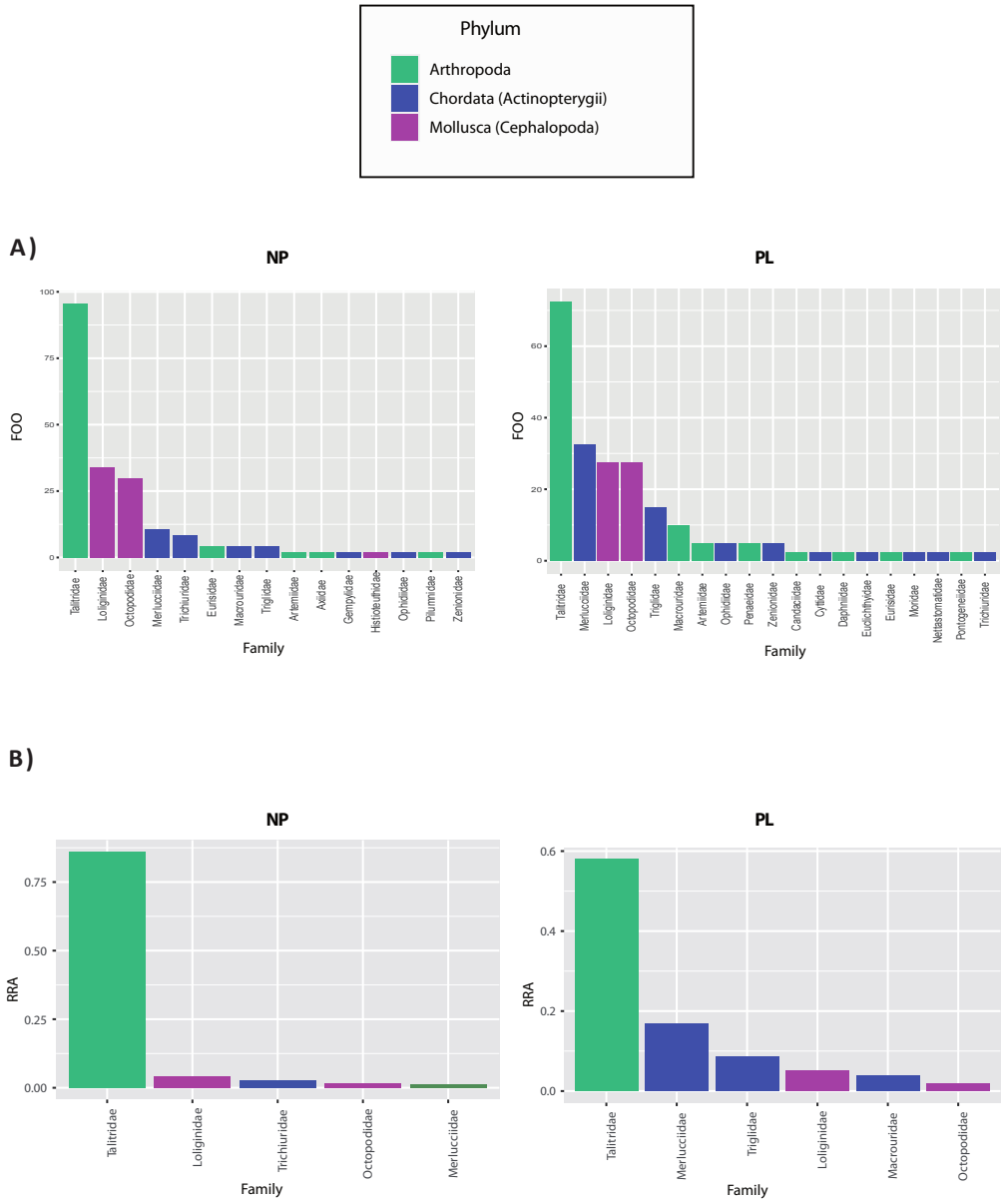


Figure 4. Geographical variations at family level among the two sub-colonies: the Paparoa National Park (NP) and the private land (PL), according to two biodiversity metrics: A) Frequency of Occurrence (FOO) and B) Relative Read Abundance (RRA). Taxa with less than 1% of FOO or RRA were not included in the plots.

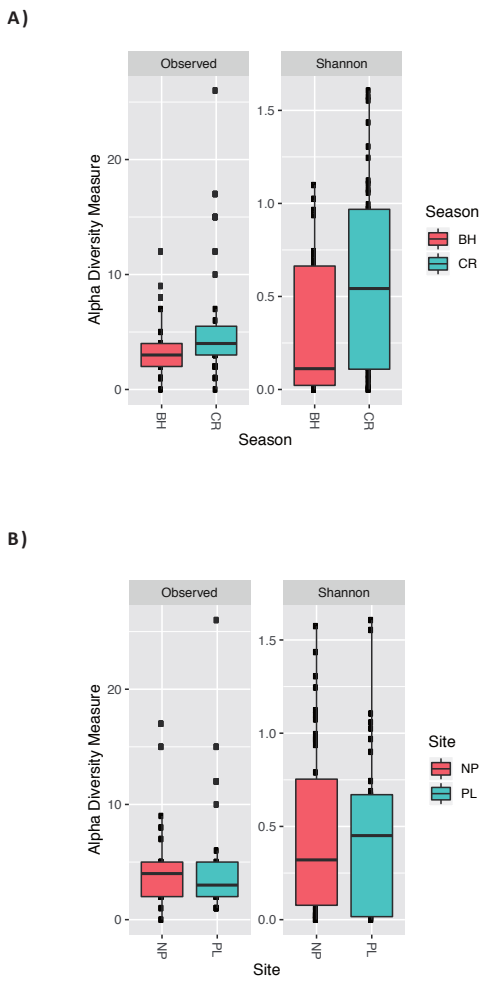


Figure 5. **A)** Seasonal and **B)** geographical differences in prey items according to alpha diversity measures.

Discussion

This is the first attempt to characterize the diet of the New Zealand endemic Westland petrel using a DNA metabarcoding approach. Aside from the molecular non-invasive approach, the novelty of this study lies in the analysis of samples from multiple seasons and sub-colonies. The observed seasonal and geographical variations in the diet of *P. westlandica* provide a broad picture of the feeding requirements and foraging ecology of this species. Previous works on the diet of *P. westlandica* were based on morphological identification of prey remains and carried out exclusively during the breeding or chick

rearing season (Freeman, 1998; Imber, 1976). Our study shows the presence of fish, cephalopods and amphipods (crustaceans) in the diet of *P. westlandica*, confirming the results of previous approaches (Freeman, 1998; Imber, 1976). However, the relative importance of each type of prey differs considerably between these studies and the current work, where we identified a number of taxa undetected before in such high percentage.

The phylum showing the highest percentage of prey items is Arthropoda (71.8% of the OTUs), mainly represented by the order Amphipoda. These animals can range from 1 mm to 34 cm in size. However, most species are microscopical benthic zooplankton and are known to be common prey of many cephalopods (Villanueva et al., 2017) and fish, including Hoki (Connell et al., 2010; Livingston and Rutherford, 1988) and Hake (Dunn et al., 2010). Therefore, Amphipods detected in this study could potentially be secondary prey. However, several seabirds such as penguins feed regularly on amphipods (Jarman et al., 2013; Knox, 2006), and large amphipods could potentially represent a fundamental food source for Antarctic seabirds (Centro de Investigacion Dinamica de Ecosistemas Marinos de Altas Latitudes, 2017), where they play a similar role as the krill (Euphausiacea) in the water column. Moreover, amphipods are found in the stomachs of other Procellariiformes, such as the Providence petrel (*Pterodroma solandri*) (Bester et al., 2011; Lock et al., 1992), the Blue petrel (*Halobaena caerulea*) (Croxall, 1987) and the Wilson's storm petrel (*Oceanites oceanicus*). These birds are known to feed on amphipods when krill is not available (Quillfeldt et al., 2019, 2005, 2001, 2000). Imber (1976) found no planktonic crustacean in the stomach of *P. westlandica* and Freeman (1998) only detected 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). Although it remains unclear whether Amphipods are primary or secondary prey (Sheppard et al., 2005), we can confirm that these taxa play a major role in the flow of energy through the food web.

Fish are major prey items of Procellariiformes (Bester et al., 2011; Bocher et al., 2000; da Silva Fonseca and Petry, 2007; Freeman, 1998; Imber, 1976; Prince and Morgan, 1987; Spear et al., 2007; Stewart et al., 1999), and the Westland petrel is not an exception. According to our results, fish (all belonging to the order Actinopteriigy) represent 15.03% of the prey

reads, and are the second most abundant phylum. In addition, fish DNA was detected in 37.93% of the samples. The fish species identified by our approach are consistent with previous studies (Freeman, 1998; Imber, 1976) but also include new species. In coherence with previous knowledge, the Hoki was identified as the most abundant fish prey item. We also found that Hake, another Merlucciidae not previously identified in the diet of the Westland petrel, is the second most predated fish species. Merlucciids, especially Hoki but also Hake, are some of the main fishery species caught in New Zealand waters (Livingston and Rutherford, 1988). The fishing season for Merlucciids spans mainly between June and September, thereby encompassing most of the Westland petrel's breeding season (Waugh et al., 2018; Waugh and Wilson, 2017), and including both sampling events of this study.

Hoki and Hake live between 28 and 1,000 m below sea level (Table 1), which makes these fish not naturally catchable for Westland petrels, who only dive down to 15 m below the surface (Freeman, 1998). Therefore, it is likely that these species are scavenged from fishing vessels. The same conclusion could apply to a number of other fish species with deep depth ranges, that are naturally unreachable to the petrel, but are important fishery species (Freeman, 1998; Froese and Pauly, 2010). These include rattails (Macrouridae), such as the Thorntooth 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 (Table 1). In the case of Hoki, however, natural predation may be possible at night, as 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 *P. westlandica* forages more actively (Waugh et al., 2018).

Another species that could potentially have been predated naturally by the Westland petrel is the Cocky gurnard, a Perciforme belonging to the family Triglidae, which can be found sometimes in shallow waters (Froese and Pauly, 2010). However, this gurnard is also a known fishery species that could have been scavenged from the fishing waste. Also, many fish species belonging to the family Trichiuridae can live close to the surface. Myctophid fishes, which were reported to be natural prey of the Westland petrel (Freeman, 1998; Imber, 1976), were not identified in our sampling. It is possible that these species are no

longer selected by the Westland petrel, as previous studies were conducted more than 20 years ago for Freeman (Freeman, 1998) and more than 40 years ago for (Imber, 1976).

In conclusion, our study confirms that Westland petrel 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 birds' behaviour and potentially impact their survival and population size (Abrams, 1983; Freeman, 1998; Oro et al., 1996, 1995).

According to our results, 11.18% of prey reads, belonged to cephalopods, and these taxa were detected in 56.32% of the samples (compared to 37.93% for fish). Four out of five cephalopod OTUs could only be assigned to family level. Only *Histioteuthis sp.* was assigned to genus level, a taxon already found in previous studies (Freeman, 1998; Imber, 1976). Our results are consistent with Freeman (1998), which states that fish prey items are followed by (Davies et al., 2009; Pierce et al., 2010) cephalopods within the Westland petrel's diet. *Histioteuthis sp.* are deep-sea squid (Voss et al., 1998), but migrate to surface water at night by vertical migration (Roper and Young, 1975), thus they become catchable by Westland petrel. The other two families, Loliginidae and Octopodidae, which were also identified in previous studies, are present from surface waters down to 500 m deep, and thus naturally catchable 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, it is possible that petrels fed on some cephalopods through fishery waste.

A number of other Mollusca prey species were, listed in previous studies (Freeman, 1998; Imber, 1976), 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 due to the lack of genetic sequences in the NCBI database or a change in the feeding habits of the birds in the past 20 years.

Marked dietary switches between breeding and non-breeding seasons have been documented for several seabirds (Howells et al., 2018), and are considered a sign of plasticity in behaviour (Quillfeldt et al., 2019). These switches may reflect variation in prey availability, a change of strategy between seasons, or a combination of both (Howells et al., 2018; Paleczny et al., 2015; Sydeman et al., 2015). Because these variations can severely affect marine top predator's populations (Cury et al., 2000; Reid and Croxall, 2001) it is essential to understand their drivers to ensure the conservation of the Westland petrel.
As hypothesized before, there is a clear seasonal variation in the diet of *P. westlandica*, both in terms of read abundance (food biomass) 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, but also for cephalopods, which are both more abundant before hatching than during the chick rearing season. One explanation could be that adult petrels feed their chicks with highly nutritive fish and cephalopods, while they feed themselves mainly with crustaceans (and some cephalopods). This hypothesis is highly consistent with the significant loss of weight in adult seabirds during the breeding season, while their chicks experience rapid growth (Ainley, 1990; Barrett et al., 1985; Leal et al., 2017). In this case, the choice of prey items by adults may be influenced by the developmental stage and the needs of the chicks.

Our results suggest that these changes would be more influenced by a change of foraging strategy, rather than a change in prey availability, as the peak of the Hoki fishery in New Zealand encompasses both July (before hatching period) and September (chick rearing period), which means, fishery waste would be equally available during both seasons.
Our results show significant higher alpha diversity or species richness (Observed and Shannon) during the chick rearing period. The less diverse diet before hatching could be due to a higher specialization, that would require a higher investment in time and energy for the adult birds while they do not have to take care of the chicks. In contrast, one would expect a more generalist diet during chick rearing, at a time when fishing trips must be limited in time to take care of the chicks and energy management is crucial for the adults. Moreover, it has been shown that a higher diversity and, consequently, a wider range of nutrients (Hillebrand et al., 2009) increases chicks growth rate as well as their probability of fledging (van Donk et al., 2017). Another potential explanation is the fact that petrel colonies

comprise more non-breeding adults towards the end of the breeding season i.e. during chick rearing. These non-breeding individuals may cause a variation in the diet at the population level.

Contrary to our expectation, we found significant differences between both sub-colonies. A possible explanation of these differences is the fact that seabirds from nearby sub-colonies forage in different locations, possibly to avoid or decrease inter-colony competition (Cecere et al., 2015; Grémillet et al., 2004; Wakefield et al., 2013). Also, the diet could change every day, depending on the resource availability or the foraging behaviour and habits could be different depending on the sub-colonies. However, in order to clarify the origin of these differences in prey community composition (not in diversity) between sub-colonies, further studies on population dynamics and foraging ecology of the Westland petrel should be conducted.

Sustainable management of worldwide fishery industry needs information regarding the overlap of marine organisms, such as seabirds, with fishing industry (Frederiksen et al., 2004; McInnes et al., 2017b; Okes et al., 2009). Seabirds scavenge food from fishery waste and results in a high number of incidental kills through bycatch, potentially disturbing on population dynamics (Brothers, 1999; McInnes et al., 2017b; Sullivan et al., 2006; Tuck et al., 2011; Watkins et al., 2008; Waugh et al., 2008; Waugh and Wilson, 2017). But, also, the diet of seabirds relies on this commercial activity, as fishery waste is a nutritious prey, naturally unreachable by seabirds. That is why understanding these interactions is essential for seabird conservation and efficient ecosystem-based fishing regulation (Becker and Beissinger, 2006; Freeman, 1998; Furness, 2003; Furness and Tasker, 2000; McInnes et al., 2017b; Phillips et al., 1999; Waugh et al., 2008). In this context, non-invasive dietary studies can provide knowledge to assess risks as well as the needs of these species that may rely heavily on commercial fishing activity (Gaglio et al., 2018; McInnes et al., 2017a, 2017b). This issue is particularly urgent in the case of endangered species, such as the Westland petrel, and, in this study, we show a link between fisheries in New Zealand and the diet of the petrel, that could be taken into account in management strategies.

Our results should draw attention to the complexity that lies in the implementation of fishing and conservation regulations. In the case of Westland petrel, these regulations should take into account, not only the close link between the commercial fishing and the diet preferences of the birds, but also the high number of birds' deaths happening every year through bycatch, as the Westland petrel is the fourth seabird species in terms of bycatch risk in New Zealand (OpenSeas, 2019). Several mitigation solutions have been suggested by practitioners or already included in conservation reports, to limit the number of accidental kills in seabirds and find a sustainable equilibrium between fishery industry and threatened species. Thus, research on how seabirds in general, and Westland petrel in particular, interact with the fishing gear would help to develop bycatch reduction techniques and using or developing gear less dangerous for the seabirds.

Author contribution

Designed the study: SB. Obtained funding: SB. Collected samples: SB. Performed laboratory analyses: MCL, SB. Analysed the data and prepared the figures: MQ. Wrote the first draft of the manuscript MQ, SB. All authors contributed to the writing of the final manuscript.

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Table 1. OTU list after filtering the contaminants, low quality sequences and the sequences that gave no hits. For each OTU, the taxonomical classification is given, together with the standard parameters provided by the BLAST search against the NCBI database. The penultimate column indicates whether the OTU was identified in previous studies or not. The last column gives the depth at which each OTU is naturally found, and coloured rows indicates OTUs whose depth range overlaps with the dive depth of the Westland petrel.

OTU_ID	Phylum	Class	Order	Family	Genus	Species	Size	E-value	% of identity	Alignment length	Query cover	Previously identified	Depth (m)
OTU_40	Arthropoda	Branchiopoda	Anostraca	Artemiidae	*Artemia*	*Artemia franciscana*	1871	1.14E-88	0.994595	185	0.978723	NO	0.1-0.6
OTU_391	Arthropoda	Branchiopoda	Diplostraca	Daphniidae	Not_identified	Not_identified	2	2.08E-26	0.860656	122	0.636842	NO	-
OTU_1	Arthropoda	Malacostraca	Amphipoda	Talitridae	Not_identified	Not_identified	346004	9.69E-25	0.802260	177	0.982857	NO	0-0.1
OTU_4	Arthropoda	Malacostraca	Amphipoda	Talitridae	Not_identified	Not_identified	150978	3.49E-24	0.793296	179	0.988889	NO	0-0.1
OTU_7	Arthropoda	Malacostraca	Amphipoda	Talitridae	Not_identified	Not_identified	74647	3.44E-34	0.831522	184	0.988950	NO	0-0.1
OTU_10	Arthropoda	Malacostraca	Amphipoda	Talitridae	Not_identified	Not_identified	54540	3.51E-19	0.777174	184	0.983607	NO	0-0.1
OTU_16	Arthropoda	Malacostraca	Amphipoda	Talitridae	Not_identified	Not_identified	22487	3.44E-34	0.828729	181	0.988950	NO	0-0.1
OTU_22	Arthropoda	Malacostraca	Amphipoda	Talitridae	Not_identified	Not_identified	10161	5.71E-37	0.834254	181	0.983425	NO	0-0.1
OTU_23	Arthropoda	Malacostraca	Amphipoda	Talitridae	Not_identified	Not_identified	9847	5.88E-17	0.773481	181	0.983333	NO	0-0.1
OTU_28	Arthropoda	Malacostraca	Amphipoda	Talitridae	Not_identified	Not_identified	5507	9.69E-25	0.797753	178	0.983333	NO	0-0.1
OTU_35	Arthropoda	Malacostraca	Amphipoda	Talitridae	Not_identified	Not_identified	3213	4.51E-23	0.796610	177	0.982857	NO	0-0.1
OTU_36	Arthropoda	Malacostraca	Amphipoda	Talitridae	Not_identified	Not_identified	2757	1.61E-27	0.809783	184	0.988889	NO	0-0.1
OTU_37	Arthropoda	Malacostraca	Amphipoda	Talitridae	Not_identified	Not_identified	2757	3.51E-19	0.777174	184	0.983607	NO	0-0.1
OTU_44	Arthropoda	Malacostraca	Amphipoda	Talitridae	Not_identified	Not_identified	1624	4.48E-28	0.811111	180	0.983146	NO	0-0.1
OTU_48	Arthropoda	Malacostraca	Amphipoda	Talitridae	Not_identified	Not_identified	1404	1.61E-27	0.807692	182	0.983051	NO	0-0.1
OTU_52	Arthropoda	Malacostraca	Amphipoda	Talitridae	Not_identified	Not_identified	1303	5.88E-17	0.775956	183	0.982857	NO	0-0.1
OTU_53	Arthropoda	Malacostraca	Amphipoda	Talitridae	Not_identified	Not_identified	1260	7.49E-26	0.803279	183	0.988889	NO	0-0.1
OTU_54	Arthropoda	Malacostraca	Decapoda	Pilumnidae	*Pilumnus*	*Pilumnus hirtellus*	986	1.89E-96	0.994975	199	0.990000	NO	10-80
OTU_56	Arthropoda	Malacostraca	Decapoda	Penaeidae	*Penaeus*	*Penaeus merguiensis*	913	5.21E-102	1.000000	206	0.990338	NO	10-45
OTU_58	Arthropoda	Malacostraca	Amphipoda	Talitridae	Not_identified	Not_identified	902	7.55E-21	0.786885	183	0.983425	NO	0-0.1
OTU_76	Arthropoda	Malacostraca	Amphipoda	Talitridae	Not_identified	Not_identified	407	1.63E-17	0.771739	184	0.983607	NO	0-0.1
OTU_87	Arthropoda	Malacostraca	Amphipoda	Talitridae	Not_identified	Not_identified	264	4.51E-23	0.794444	180	0.983333	NO	0-0.1

OTU_99	Arthropoda	Malacostraca	Amphipoda	Talitridae	Not_identified	Not_identified	197	2.11E-16	0.798611	144	0.842424	NO	0-0.1
OTU_103	Arthropoda	Malacostraca	Amphipoda	Talitridae	Not_identified	Not_identified	188	7.60E-16	0.767956	181	0.988889	NO	0-0.1
OTU_120	Arthropoda	Malacostraca	Amphipoda	Talitridae	Not_identified	Not_identified	125	3.46E-29	0.807692	182	0.983516	NO	0-0.1
OTU_144	Arthropoda	Malacostraca	Amphipoda	Talitridae	Not_identified	Not_identified	60	3.54E-14	0.763441	186	0.983607	NO	0-0.1
OTU_148	Arthropoda	Malacostraca	Amphipoda	Talitridae	Not_identified	Not_identified	56	7.55E-21	0.786096	187	0.983607	NO	0-0.1
OTU_155	Arthropoda	Malacostraca	Amphipoda	Talitridae	Not_identified	Not_identified	51	1.27E-13	0.762162	185	0.983333	NO	0-0.1
OTU_175	Arthropoda	Malacostraca	Amphipoda	Talitridae	Not_identified	Not_identified	34	2.07E-31	0.817680	181	0.983146	NO	0-0.1
OTU_177	Arthropoda	Malacostraca	Amphipoda	Talitridae	Not_identified	Not_identified	33	1.62E-22	0.791209	182	0.988889	NO	0-0.1
OTU_188	Arthropoda	Malacostraca	Amphipoda	Talitridae	Not_identified	Not_identified	28	1.63E-17	0.774194	186	0.983607	NO	0-0.1
OTU_192	Arthropoda	Malacostraca	Amphipoda	Eusiridae	Not_identified	Not_identified	27	5.92E-12	0.809091	110	0.675159	NO	1-3
OTU_195	Arthropoda	Malacostraca	Amphipoda	Talitridae	Not_identified	Not_identified	25	2.08E-26	0.806630	181	0.983051	NO	0-0.1
OTU_202	Arthropoda	Malacostraca	Amphipoda	Talitridae	Not_identified	Not_identified	23	1.61E-27	0.804469	179	0.988889	NO	0-0.1
OTU_204	Arthropoda	Malacostraca	Amphipoda	Talitridae	Not_identified	Not_identified	22	1.27E-13	0.760870	184	0.983516	NO	0-0.1
OTU_207	Arthropoda	Malacostraca	Amphipoda	Talitridae	Not_identified	Not_identified	21	7.49E-26	0.802260	177	0.983146	NO	0-0.1
OTU_215	Arthropoda	Malacostraca	Amphipoda	Talitridae	Not_identified	Not_identified	18	9.83E-15	0.768817	186	0.983240	NO	0-0.1
OTU_218	Arthropoda	Malacostraca	Amphipoda	Talitridae	Not_identified	Not_identified	16	7.55E-21	0.782609	184	0.983607	NO	0-0.1
OTU_219	Arthropoda	Malacostraca	Amphipoda	Talitridae	Not_identified	Not_identified	16	7.55E-21	0.785714	182	0.988889	NO	0-0.1
OTU_222	Arthropoda	Malacostraca	Amphipoda	Talitridae	Not_identified	Not_identified	13	1.27E-13	0.759563	183	0.983516	NO	0-0.1
OTU_226	Arthropoda	Malacostraca	Amphipoda	Talitridae	Not_identified	Not_identified	12	9.69E-25	0.801105	181	0.983051	NO	0-0.1
OTU_236	Arthropoda	Malacostraca	Amphipoda	Talitridae	Not_identified	Not_identified	11	2.13E-11	0.761364	176	0.987805	NO	0-0.1
OTU_237	Arthropoda	Malacostraca	Amphipoda	Talitridae	Not_identified	Not_identified	11	2.64E-40	0.846995	183	0.983425	NO	0-0.1
OTU_238	Arthropoda	Malacostraca	Amphipoda	Talitridae	Not_identified	Not_identified	11	1.26E-18	0.775956	183	0.978142	NO	0-0.1
OTU_246	Arthropoda	Malacostraca	Amphipoda	Talitridae	Not_identified	Not_identified	10	2.68E-30	0.821229	179	0.988439	NO	0-0.1
OTU_274	Arthropoda	Malacostraca	Decapoda	Axiidae	Not_identified	Not_identified	7	2.14E-06	0.737624	202	0.989418	NO	0-200
OTU_275	Arthropoda	Malacostraca	Amphipoda	Talitridae	Not_identified	Not_identified	7	2.10E-21	0.788660	194	0.984293	NO	0-0.1
OTU_276	Arthropoda	Malacostraca	Amphipoda	Talitridae	Not_identified	Not_identified	7	1.60E-32	0.827027	185	0.988889	NO	0-0.1
OTU_300	Arthropoda	Malacostraca	Amphipoda	Talitridae	Not_identified	Not_identified	5	1.23E-38	0.840659	182	0.983425	NO	0-0.1

OTU_306	Arthropoda	Malacostraca	Amphipoda	Talitridae	Not_identified	Not_identified	4	9.69E-25	0.828571	140	0.857143	NO	0-0.1
OTU_308	Arthropoda	Malacostraca	Amphipoda	Talitridae	Not_identified	Not_identified	4	3.51E-19	0.778378	185	0.983607	NO	0-0.1
OTU_315	Arthropoda	Malacostraca	Amphipoda	Talitridae	Not_identified	Not_identified	4	9.76E-20	0.849057	106	0.656250	NO	0-0.1
OTU_339	Arthropoda	Malacostraca	Amphipoda	Talitridae	Not_identified	Not_identified	3	5.75E-32	0.817680	181	0.983333	NO	0-0.1
OTU_340	Arthropoda	Malacostraca	Amphipoda	Talitridae	Not_identified	Not_identified	3	2.66E-35	0.829670	182	0.983333	NO	0-0.1
OTU_372	Arthropoda	Malacostraca	Amphipoda	Talitridae	Not_identified	Not_identified	2	9.76E-20	0.784530	181	0.982659	NO	0-0.1
OTU_377	Arthropoda	Malacostraca	Amphipoda	Pontogeneiidae	Not_identified	Not_identified	2	9.83E-15	0.763441	186	0.988889	NO	5-90
OTU_381	Arthropoda	Malacostraca	Amphipoda	Talitridae	Not_identified	Not_identified	2	1.25E-28	0.813187	182	0.988889	NO	0-0.1
OTU_384	Arthropoda	Malacostraca	Amphipoda	Talitridae	Not_identified	Not_identified	2	1.62E-22	0.792350	183	0.983051	NO	0-0.1
OTU_69	Arthropoda	Maxillopoda	Calanoida	Candaciidae	*Candacia*	*Candacia armata*	487	4.23E-68	0.993243	148	0.980000	NO	-
OTU_59	Chordata	Actinopterygii	Anguilliformes	Nettastomatidae	Not_identified	Not_identified	877	3.23E-79	0.866906	278	0.992754	NO	deep-sea
OTU_133	Chordata	Actinopterygii	Gadiformes	Euclichthyidae	*Euclichthys*	*Euclichthys polynemus*	84	5.10E-117	0.979839	248	0.991968	NO	250-920
OTU_147	Chordata	Actinopterygii	Gadiformes	Macrouridae	*Coelorinchus*	*Coelorinchus fasciatus*	57	5.10E-117	0.976096	251	0.992063	Freeman, 1998 (genus level); Imber, 1976 (genus level)	400-800
OTU_47	Chordata	Actinopterygii	Gadiformes	Macrouridae	*Coelorinchus*	*Coelorinchus oliverianus*	1434	2.36E-120	0.984064	251	0.992063	Freeman, 1998 (genus level)	400-600
OTU_20	Chordata	Actinopterygii	Gadiformes	Macrouridae	*Lepidorhynchus*	*Lepidorhynchus denticulatus*	15234	1.82E-121	0.984190	253	0.992126	Freeman, 1998: Imber, 1976	270-450
OTU_12	Chordata	Actinopterygii	Gadiformes	Merlucciidae	*Macruronus*	*Macruronus novaezelandiae*	36442	2.36E-120	0.984064	251	0.992063	Freeman, 1998	200-700
OTU_14	Chordata	Actinopterygii	Gadiformes	Merlucciidae	*Merluccius*	*Merluccius australis*	33916	8.47E-120	0.984000	250	0.992032	Freeman, 1998 (family level)	28-1000
OTU_86	Chordata	Actinopterygii	Gadiformes	Moridae	*Mora*	*Mora moro*	277	8.54E-115	0.972112	251	0.992032	Freeman, 1998 (family level)	450-2500
OTU_32	Chordata	Actinopterygii	Ophidiiformes	Ophidiidae	*Genypterus*	*Genypterus blacodes*	3548	1.41E-122	0.988095	252	0.992095	NO	300-550
OTU_15	Chordata	Actinopterygii	Perciformes	Triglidae	*Lepidotrigla*	*Lepidotrigla modesta*	33305	2.36E-120	0.984064	251	0.992063	NO	10-300
OTU_18	Chordata	Actinopterygii	Scombriformes	Trichiuridae	Not_identified	Not_identified	15685	8.60E-110	0.946768	263	0.992424	Imber, 1976 (family level)	0-1600
OTU_84	Chordata	Actinopterygii	Scombriformes	Gempylidae	*Rexea*	Not_identified	285	1.11E-108	0.952941	255	0.992188	NO	100-800
OTU_77	Chordata	Actinopterygii	Zeiformes	Zenionidae	*Capromimus*	*Capromimus abbreviatus*	380	5.06E-122	0.988048	251	0.992063	NO	87-500
OTU_125	Chordata	Actinopterygii	Zeiformes	Cyttidae	*Cyttus*	*Cyttus traversi*	104	5.06E-122	0.988048	251	0.992063	Freeman, 1998 (genus level)	200-978
OTU_17	Mollusca	Cephalopoda	Octopoda	Octopodidae	Not_identified	Not_identified	16331	1.14E-93	0.985000	200	0.990050	Freeman, 1998; Imber, 1976 (genus level)	0-450
OTU_71	Mollusca	Cephalopoda	Oegopsida	Histioteuthidae	*Histioteuthis*	Not_identified	479	5.55E-57	0.896739	184	0.989189	Freeman, 1998; Imber, 1976	300-1400
OTU_6	Mollusca	Cephalopoda	Teuthida	Loliginidae	Not_identified	Not_identified	86867	5.21E-102	0.986047	215	0.990741	NO	0-400

OTU_46	Mollusca	Cephalopoda	Teuthida	Loliginidae	Not_identified	Not_identified	1559	1.87E-101	0.985981	214	0.990698	NO	0-400
OTU_134	Mollusca	Cephalopoda	Teuthida	Loliginidae	Not_identified	Not_identified	84	6.88E-86	0.989071	183	0.879227	NO	0-4

Table 2. Taxonomical classification of prey items in the diet of *P. westlandica* along with their Relative Read Abundance (RRA) and Frequency of Occurrence (FOO). Values are presented for the whole sampling and also detailled according to the two different seasons (Before Hatching: BH and Chick Rearing: CR) and according to the two different sites (Paparoa Natural Park: NP and the Private Land: PL).

Phylum	Class	Order	Family	Species	Common name	Total FOO (%)	Total RRA (%)	FOO BH (%)	FOO CR (%)	RRA BH (%)	RRA CR (%)	FOO NP (%)	FOO PL (%)	RRA NP (%)	RRA PL (%)
Arthropoda						86.2069	73.7939	80	91.4894	35.7822	94.1305	95.7447	75	89.7389	60.9839
	Branchiopoda					4.5977	0.1988	2.5	6.3830	0.0755	0.2621	2.1277	7.5	0.0380	0.4346
		Anostraca				3.4483	0.1986	2.5	4.2553	0.0755	0.2621	2.1277	5	0.0380	0.4341
			Artemiidae			3.4483	0.1986	2.5	4.2553	0.0755	0.2621	2.1277	5	0.0380	0.4341
				Artemia franciscana	Brine shrimp	3.4483	0.1986	2.5	4.2553	0.0755	0.2621	2.1277	5	0.0380	0.4341
		Cladocera				1.1494	0.0002	0	2.1277	0	0.0003	0	2.5	0	0.2409
			Daphniidae			1.1494	0.0002	0	2.1277	0	0.0003	0	2.5	0	0.2409
	Malacostraca					86.2069	73.5434	80	91.4894	35.7068	93.7934	95.7447	75	89.7009	60.4248
		Amphipoda				85.0575	73.3412	77.5	91.4894	35.3488	93.6387	95.7447	72.5	89.5089	60.1839
			Eurisidae			3.4483	0.0002	0	6.3830	0	0.0003	4.2553	2.5	0	0.0005
			Pontogeneiidae			1.1494	0.0002	0	2.1277	0	0.0003	0	2.5	0	0.0005
			Talitridae		Landhoppers/sandhoppers	85.0575	73.3381	77.5	91.4894	35.3488	93.6352	95.7447	72.5	89.5065	60.1812
		Decapoda				3.4500	0.2023	5	2.1277	0.3579	0.1547	2.1277	5	0.1920	0.2409
			Axiidae		Thalassinidean crustaceans	1.15	0.0007	0	2.1277	0	0.0011	2.1277	0	0	0.0018
			Penaeidae			2.3	0.0969	5	0	0.3579	0	0	5	0	0.2391
				Penaeus merguiensis	Banana shrimp	2.3	0.0969	5	0	0.3579	0	0	5	0	0.2391
			Pilumnidae			1.15	0.1046	0	2.1277	0	0.1536	2.1277	0	0.1920	0

				Pilumnus hirtellus	Bristly crab	1.15	0.1046	0	2.1277	0	0.1536	2.1277	0	0.1920	0
	Maxillopoda					1.1494	0.0517	0	2.1277	0	0.0751	0	2.5	0	0.1245
		Calanoida				1.1494	0.0517	0	2.1277	0	0.0751	0	2	0	0.1245
			Candaciidae			1.1494	0.0517	0	2.1277	0	0.0751	0	2.5	0	0.1245
				Candacia armata		1.1494	0.0517	0	2.1277	0	0.0751	0	2.5	0	0.1245
Chordata						37.9310	15.0296	45	31.9149	48.2577	2.7135	25.5319	52.5	3.9071	31.5495
	Actinopterygii					37.9310	15.0296	45	31.9149	48.2577	2.7135	25.5319	52.5	3.9071	31.5495
		Anguilliformes				1.1494	0.0931	2.5	0	0.3405	0	0	2.5	0	0.2275
			Nettastomatidae		Duckbill eels	1.1494	0.0931	2.5	0	0.3405	0	0	2.5	0	0.2275
		Gadiformes				25.2874	9.2796	40	12.7660	33.6161	0.1368	14.8936	37.5	1.2149	21.0678
			Euclichthyidae			1.1494	0.0089	0	2.1277	0	0.0130	0	2.5	0	0.0216
				Euclichthys polynemus	Eucla cod	1.1494	0.0089	0	2.1277	0	0.0130	0	2.5	0	0.0216
			Macrouridae			6.8966	1.7749	12.5	2.1277	6.2599	0.0947	4.2553	10	0.1188	0.1188
				Coelorinchus fasciatus	Banded whiptail	1.1494	0.0060	2.5	0	0.0221	0	0	2.5	0	0.0148
				Coelorinchus oliverianus	Hawknose grenadier	2.2989	0.1522	2.5	2.1277	0.5152	0.0173	2.1277	2.5	0.0217	0.3441
				Lepidorhynchus denticulatus	Thorntooth grenadier	6.8966	1.6166	12.5	2.1277	5.7226	0.0773	4.2553	10	0.0971	3.8216
			Merlucciidae			20.6897	7.4764	35	8.5106	27.2511	0.0291	10.6383	32.5	1.0962	16.7955
				Macruronus novaezelandiae	Hoki	14.9425	3.8672	30	2.1277	14.0981	0.0240	8.5106	22.5	1.0936	8.0057
				Merluccius australis	Southern hake	10.3448	3.5992	15	6.3830	13.1530	0.0051	4.2553	17.5	0.0026	8.7899
			Moridae			1.1494	0.0294	2.5	0	0.1051	0	0	2.5	0	0.0702
				Mora moro	Common mora	1.1494	0.0294	2.5	0	0.1051	0	0	2.5	0	0.0702
		Ophidiiformes				3.4483	0.3765	5	2.1277	1.2306	0.0592	2.1277	5	0.0004	0.9196
			Ophidiidae			3.4483	0.3765	5	2.1277	1.2306	0.0592	2.1277	5	0.0004	0.9196
				Genypterus blacodes	Pink cusk-eel	3.4483	0.3765	5	2.1277	1.2306	0.0592	2.1277	5	0.0004	0.9196
		Perciformes				9.1954	3.5343	15	4.2553	12.9452	0.0008	4.2553	15	0.0010	8.6461
			Triglidae			9.1954	3.5343	15	4.2553	12.9452	0.0008	4.2553	15	0.0010	8.6461
				Lepidotrigla modesta	Cocky gunard	9.1954	3.5343	15	4.2553	12.9452	0.0008	4.2553	15	0.0010	8.6461
		Scombriformes				5.7471	1.6947	0	10.6383	0	2.4916	8.5106	2.5	2.6629	0.6003

			Gempylidae			1.1494	0.0302	0	2.1277	0	0.0438	2.1277	0	0.0547	0
				Rexea sp.	Snake mackerels	1.1494	0.0302	0	2.1277	0	0.0438	2.1277	0	0.0547	0
			Trichiuridae			5.7471	1.6645	0	10.6383	0	2.4478	8.5106	2.5	2.6082	0.6003
		Zeiformes				4.5977	0.0514	5	4.2553	0.1252	0.0251	2.1277	7.5	0.0279	0.0884
			Cyttidae			1.1494	0.0110	2.5	0	0.0407	0	0	0	0.0000	0.0272
				Cyttus traversi	King dory	1.1494	0.0110	2.5	0	0.0407	0	0	2.5	0	0.0272
			Zenionidae			3.4483	0.0403	2.5	4.2553	0.0845	0.0251	2.1277	5	0.0279	0.0612
				Capromimus abbreviatus	Capro dory	3.4483	0.0403	2.5	4.2553	0.0845	0.0251	2.1277	5	0.0279	0.0612
Mollusca						56.3218	11.1766	57.5	55.3191	15.3105	3.1556	59.5745	52.5	6.3537	7.0328
	Cephalopoda					56.3218	11.1766	57.5	55.3191	15.3105	3.1556	59.5745	52.5	6.3537	7.0328
		Oegopsida				1.1494	0.0508	0	2.1277	0	0.0754	2.1277	0	0.0943	0.0000
			Histioteuthidae		Oegopsida squids	1.1494	0.0508	0	2.1277	0	0.0754	2.1277	0	0.0943	0.0000
		Octopoda				28.7356	1.7330	25	31.9149	2.5363	1.5208	29.7872	27.5	1.7319	1.9191
			Octopodidae		Octopodids	28.7356	1.7330	25	31.9149	2.5363	1.5208	29.7872	27.5	0.0279	0.0612
		Teuthida				31.0345	9.3927	35	27.6596	12.7742	1.6348	34.0426	27.5	4.6218	5.1137
			Loliginidae		Pencil squids	31.0345	9.3927	35	27.6596	12.7742	1.6348	34.0426	27.5	4.6218	5.1137