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Preliminary analysis of New Zealand scampi (*Metanephrops challengeri*) diet using metabarcoding

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Deep sea lobsters are highly valued for seafood and provide the basis of important commercial fisheries in many parts of the world. Despite their economic significance, relatively little is known about their natural diets. Microscopic analyses of foregut content in some species have suffered from low taxonomic resolution, with many of the dietary items difficult to reliably identify as their tissue is easily digested. DNA metabarcoding has the potential to provide greater taxonomic resolution of foregut and hindgut contents of the New Zealand (NZ) scampi (*Metanephrops challengeri*), but a number of methodological concerns need to first be overcome to ensure optimum DNA metabarcoding results.

In this study, a range of methodological parameters were trialled to determine the optimum protocols for DNA metabarcoding, and provide a first view of the NZ scampi diet. Several PCR protocols were trialled, using two universal primer pairs targeting the 18S rRNA and COI genes, on DNA extracted from frozen and ethanol preserved samples of both foregut and hindgut digesta.

The selection of appropriate DNA polymerases, buffers and methods for reducing PCR inhibitors (including use of BSA) were found to be critical. Amplification from frozen or ethanol preserved gut contents appeared similarly dependable, but metabarcoding results showed that ethanol samples resulted in better results from the COI gene. The COI gene was found to be more effective than 18S rRNA gene for identifying large eukaryotic taxa from the digesta, however, it was less successfully amplified. The 18S rRNA gene was more easily amplified, but identified mostly smaller marine organisms such as plankton and parasites. This preliminary analysis of the diet of the NZ scampi identified a range of species, which included the ghost shark (*Hydrolagus novaezealandiae*), silver warehou (*Seriolella punctate*), tall sea pen (*Funiculina quadrangularis*) and salp (*Ihlea racovitza*), suggesting that they have a varied diet, with a high reliance on scavenging a diverse range of pelagic and benthic species from the seafloor.

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Abstract

Deep sea lobsters are highly valued for seafood and provide the basis of important commercial fisheries in many parts of the world. Despite their economic significance, relatively little is known about their natural diets. Microscopic analyses of foregut content in some species have suffered from low taxonomic resolution, with many of the dietary items difficult to reliably identify as their tissue is easily digested. DNA metabarcoding has the potential to provide greater taxonomic resolution of foregut and hindgut contents of the New Zealand (NZ) scampi (*Metanephrops challengeri*), but a number of methodological concerns need to first be overcome to ensure optimum DNA metabarcoding results.

In this study, a range of methodological parameters were trialled to determine the optimum protocols for DNA metabarcoding, and provide a first view of the NZ scampi diet. Several PCR protocols were trialled, using two universal primer pairs targeting the 18S rRNA and COI genes, on DNA extracted from frozen and ethanol preserved samples of both foregut and hindgut digesta.

The selection of appropriate DNA polymerases, buffers and methods for reducing PCR inhibitors (including use of BSA) were found to be critical. Amplification from frozen or ethanol preserved gut contents appeared similarly dependable, but metabarcoding results showed that ethanol samples resulted in better results from the COI gene. The COI gene was found to be more effective than 18S rRNA gene for identifying large eukaryotic taxa from the digesta, however, it was less successfully amplified. The 18S rRNA gene was more easily amplified, but identified mostly smaller marine organisms such as plankton and parasites. This preliminary analysis of the diet of the NZ scampi identified a range of species, which included the ghost shark (*Hydrolagus novaezealandiae*), silver warehou (*Seriolella punctate*), tall sea pen (*Funiculina quadrangularis*)

and salp (*Ihlea racovitza*), suggesting that they have a varied diet, with a high reliance on scavenging a diverse range of pelagic and benthic species from the seafloor.

Introduction

Commercial fisheries for deep sea lobster species, those typically captured below 50 m depth, are occurring in many parts of the world, currently producing total annual landings of around 50,000 t (Jeffs 2010; FAO 2016). Many of the targeted species are of high value, with wholesale export prices of over \$40 kg^{-1} common for some species, such as the New Zealand (NZ) scampi *Metanephrops challengeri* (Balss, 1914) (Seafood New Zealand 2017). Despite the economic importance of deep sea lobsters, the knowledge of their feeding ecology and diet is limited. Improved information on diet in deep sea lobsters has potential uses in identifying effective baits for trap fishing, development of suitable feeds for aquaculture, as well as understanding differences in growth rates in natural populations to help improve the management of the fisheries.

Species in two genera of deep-sea lobster, *Metanephrops* and *Nephrops*, are widely targeted by commercial fisheries, share similar ecology and are genetically closely related (Tshudy, Chan & Sorhannus 2007). The Norway lobster, *Nephrops norvegicus,* has a varied diet, which is necessary to achieve optimal growth (Cristo & Cartes 1998; Mente 2010). Similar varied diets have been suggested for some *Metanephrops* species (excluding *M. challengeri*), and include fish, crustaceans, polychaetes and amphipods, although these have only been identified at a crude taxonomic level using microscopic analysis of foregut contents (Choi et al. 2008; Sahlmann, Chan & Chan 2011; Wahle et al. 2012; Bell, Tuck & Dobby 2013). A significant problem with microscopic analysis of gut contents is poor taxonomic resolution, due to difficulties in identification of partly-digested specimens, which typically require an expert for reliable taxonomic identification (Dunn et al. 2010; Pompanon et al. 2012; Zhan et al. 2013; Berry et al. 2015; Young et al. 2015; Crisol-Martínez et al. 2016; Harms-Tuohy, Schizas & Appeldoorn

59 2016; Sousa et al. 2016). Also, soft-bodied animals are frequently suspected of being under-
60 represented in such analyses as they are highly digestible (Bell, Tuck & Dobby 2013).

61 A more promising tool for diet analysis, DNA metabarcoding, combines universal DNA primers
62 with high-throughput (next-generation) sequencing to identify a variety of species from a
63 mixture of gut content DNA (Kress et al. 2015). A single universal primer pair has the ability to
64 amplify a diverse range of species by targeting a single gene region that has been conserved
65 through time (e.g., the cytochrome oxidase I [COI] region in the mitochondrial DNA or the 18S
66 ribosomal RNA [rRNA] region in the nuclear DNA) (Aylagas et al. 2016). This powerful
67 molecular method has been successfully applied in diet studies of a variety of lobster larvae and
68 marine fish species (O'Rorke et al. 2012; O'Rorke et al. 2014; Berry et al. 2015; Harms-Tuohy,
69 Schizas & Appeldoorn 2016). One of the major advantages of this method of gut analysis is that
70 a high taxonomic resolution can be achieved. Also, the digested or liquid gut content, which
71 would normally be of no value for morphological identification, can provide additional dietary
72 information that would otherwise have been missed. The potential difficulties of this approach
73 include the sequencing costs, the sensitivity of detection, and the taxonomic coverage of the
74 reference sequence databases at the time of study. These difficulties will likely fade as research
75 progresses in this field (Cowart et al. 2015; Srivathsan et al. 2016).

76 With sequencing cost and sensitivity being the major restrictions, it is in the best interest of
77 researchers to first address methodological issues before a full analysis is conducted, in order to
78 ensure the best possible results are obtained. One of the potential difficulties in metabarcoding is
79 polymerase chain reaction (PCR) inhibition, which has been found often when using template
80 genomic DNA extracted from biological material with a high proportion of organic (i.e., bile
81 salts) or inorganic (i.e., calcium ions) compounds, or from body fluids or some difficult organs

82 (Rossen et al. 1992; Kreader 1996; Rådström et al. 2004; Farell & Alexandre 2012; Schrader et
83 al. 2012). Another matter to address is determining which DNA polymerases are more
84 susceptible to the effects of specific PCR inhibitors present that impede DNA amplification
85 (Rådström et al. 2004). A third potential problem is the taxonomic coverage and resolution
86 possible from the different reference sequences in the databases available, with some databases
87 targeting different genes or different organisms. Other sampling factors may also be important in
88 determining the success of metabarcoding for diet analysis. These include the preservation
89 method (e.g., frozen or in ethanol) and the location of the gut contents to be analysed (from the
90 foregut or hindgut), with the potential for these two locations to provide different results due
91 either to the state of DNA degradation or the differences in timing of intake and digestive
92 processing of dietary items.

93 The natural diet of the NZ scampi is largely unknown, despite forming the basis of an important
94 commercial fishery in New Zealand, and currently being of some interest for aquaculture
95 development. The aim of this study is to provide a preliminary examination of their natural diet
96 using metabarcoding methods, by first addressing the optimisation of methodological factors,
97 including issues of tissue choice and preservation, target gene, PCR inhibition, PCR reagents and
98 reference database coverage.

Materials & Methods

Sample Collection and Locations

NZ scampi samples were collected in September of 2016 from the seafloor of the Chatham Rise, New Zealand, at depths ranging from 200 – 500 m from the R.V. Kaharoa by towing scampi benthic trawl nets with a 45 mm cod-end (Fig. 1). Upon landing the trawl on the deck, the NZ scampi that were intact were euthanized by immersing them in chilled 95% ethanol as soon as possible to avoid any degradation. The samples were transferred to the University of Auckland's laboratories (Auckland, New Zealand) for further examination. A single trawl location (start - 42.9952°, 177.2367° at a depth of 322 m; end -43.0210°, 177.1800° at a depth of 252 m) and the individuals collected there were selected for the purpose of this study (Fig. 1). For comparison, frozen individuals of commercially harvested NZ scampi were supplied by Sanford Ltd (Auckland, New Zealand), their location of harvesting unknown.

A special permit (#549) for NZ scampi collection was given by New Zealand's Ministry of Primary Industries. The specimens for this study were collected in accordance with approvals under New Zealand's Animal Welfare Act 1991. The transport and holding of the scampi, as well as the experimental procedures, were approved by the Animal Ethics Committee of the Nelson, Marlborough Institute of Technology (AEC2014-CAW-02).

Specimen Dissection, Gut Content Removal and DNA Extraction

The ethanol preserved individuals were placed on paper towel to remove the excess ethanol, and the frozen individuals were thawed at room temperature until each individual's gut contents could be removed separately. Sterile dissection kits were used for each collection of NZ scampi (frozen and ethanol) and the dissection tools were thoroughly cleaned between every individual in each collection; as well as between every individual's hindgut and foregut digesta removal.

122 The gut digesta remained separated throughout the extraction process for each individual, and
123 were placed into separate 1.5 mL microcentrifuge tubes, prior to DNA extraction.

124 The samples of gut contents were each mixed by vortexing briefly before a subsample of each
125 was taken to be extracted using the Gentra Puregene Extraction Kit (Qiagen®, Hilden, Germany)
126 following the manufacturer's instructions. The DNA concentration was measured using the
127 NanoPhotometer® N60 (Implen, Munich, Germany).

128 **Primer Selection and PCR Protocol**

129 Two universal primer pairs were identified and selected from the literature: mlCOIintF (Leray et
130 al. 2013) and jgHCO2198 (Geller et al. 2013) targeting a 313 bp region of the mitochondrial COI
131 gene, and Uni18SF and Uni18SR (Zhan et al. 2013) targeting a 425 bp region of the nuclear 18S
132 rRNA V4 variable region. All primers included Illumina Nextera™ library transposase adapters
133 (Table 1).

134 Two DNA polymerases and buffer mixes were tested, Platinum *Taq* (Invitrogen™, Thermo
135 Fisher Scientific Inc.) and the MyTaq™ Red Mix (Bioline, London, UK). The PCR reaction for
136 the former contained 2.5 µl buffer (10x; Invitrogen™, Thermo Fisher Scientific Inc.), 1.25 µl
137 $MgCl^2$ (50 mM; Invitrogen™, Thermo Fisher Scientific Inc.), 0.2 µl deoxyribonucleotide
138 triphosphate mix (dNTPs; 20 mM; Bioline), 2 µl 1% bovine serum album (BSA; MP
139 Biomedicals, California, USA), 0.05 µl Platinum *Taq,* 0.4 µl of reverse primer (10 µM), 0.4 µl of
140 the forward primer (10 µM) and 1 µl genomic DNA, made up to 25 µl using PCR grade water.
141 The standard Bioline reaction contained 12.5 µl of MyTaq™ Red Mix, 2 µl 1% BSA, 1 µl of
142 each reverse and forward primer (10 µM), 1 µl genomic DNA, made up to 25 µl with PCR grade
143 water.

144 The PCR cycle that resulted in optimum amplification for both the COI and 18S primers was as
145 follows: initial denaturation of 95 °C for 60 seconds, 20 cycles at 95 °C for 30 seconds, 45 °C
146 for 30 seconds ("touchup" - increasing 1 °C every cycle) and 72 °C for 30 seconds, 20 cycles at
147 95 °C for 30 seconds, 65 °C for 30 seconds ("touchdown" - decreasing 1 °C every cycle) and 72
148 °C for 30 seconds, 10 cycles at 95 °C for 15 seconds, 45 °C for 20 seconds and 72 °C for 30
149 seconds, with a final extensions at 72 °C for 60 seconds. Each PCR cycle included a negative
150 control (no DNA added) to check PCR reagents were not contaminated. The PCR products were
151 run on 1.6% agarose gel and viewed in a Gel Doc™ XR+ (Bio-Rad Laboratories Inc., California,
152 United States). The PCR products were made visible by using 3 µl of loading dye with stain (50
153 µl BlueJuice™ [10x; Invitrogen™, Thermo Fisher Scientific Inc.], 550 µl Tris/Borate/EDTA
154 [TBE; 0.5x; DUCHEFA Biochemie B.V., Haarlem, Netherlands]) and 2 µl Gel Red® (Biotium,
155 California, USA) and mixing it with 5 µl of PCR product.

156 The effect of both genomic DNA template concentration and BSA concentration were tested to
157 try to reduce PCR inhibition. 1% BSA was trialled with volumes of 1 µl, 2 µl and 5 µl per 25 µl
158 reaction. A range of different DNA dilutions (1:10, 1:50 and 1:100) were also tested.

159 Inconsistent PCR amplification of both genes was observed in the gut content samples, but not
160 from muscle DNA extracts from the same individuals, likely due to the presence of PCR
161 inhibitors. To test the effects of the presumed PCR inhibitors, gut content template DNA (1µl)
162 was added with tail muscle template DNA (1 µl of 10 ng μl^{-1}) in the standard PCR reactions.
163 Each individual was tested in three replicate PCR reactions.

164 **DNA Metabarcoding and Analyses**

165 ***Selection of individuals for metabarcoding***

166 The PCR products for sequencing were selected from six individuals (70.2, 70.3, 70.9, Fro1,
167 Fro2 and Fro3), as they provided consistent and strong amplifications for both COI and 18S
168 genes, as well as encompassing the range of methodological factors that were to be addressed
169 (Table 2). Individual 70.9 was used for comparison of the PCR reactions to determine which
170 DNA polymerase and buffer mix was better (i.e., samples 1 and 2 versus 3 and 4). Preliminary
171 data suggested the Platinum *Taq* reactions were inferior, so the remainder of the comparisons
172 focussed on the Bioline reactions. The taxa that were identified from the digesta of the foregut
173 and the hindgut were compared for the individuals 70.2 and 70.9 (i.e., samples 1 and 5 versus 2
174 and 6) to identify if the hindgut digesta could provide useful additional information about the
175 diet, i.e., successive meals may be represented in the hindgut contents. The ethanol and frozen
176 preservation methods were compared using three individuals each (i.e., samples 1, 2, 5, 6 and 7
177 versus 8 and 9), to assess the effects (if any) of differential DNA degradation of the digesta. All
178 individuals were used for the comparison of the database identifications for the COI and 18S
179 genes and thus a preliminary analysis of the diet was evaluated. Potential diet false positives
180 were identified by evaluating the DNA negative.

181 ***DNA purification and pooling of selected samples***

182 NucleoSpin® Gel and PCR Clean-up (Macherey-Nagel, Düren, Germany) was used according to
183 manufacturer's instructions to purify the PCR products and remove fragments of less than 164 bp
184 in length for all selected individuals. The amplified DNA concentration of those products was
185 determined using Qubit™ dsDNA HS Assay Kit (Invitrogen™, Thermo Fisher Scientific Inc.)
186 following the manufacturer's protocol.

187 The purified COI and 18S PCR products from each sample were pooled and brought to equal
188 molarity where possible. Sequencing was done through New Zealand Genomics Ltd (Auckland,
189 New Zealand) at Massey University (Palmerston North, New Zealand) where indexing occurred
190 using the Nextera™ DNA library Prep Kit (Illumina, San Diego, CA, USA) before sequencing
191 on an Illumina MiSeq™ System (2 × 250 pair pair-end protocol).

192 ***Metabarcoding protocol***

193 The raw Illumina sequences were analysed through the New Zealand eScience Infrastructure
194 (NeSI; Auckland, New Zealand) high performance computing (HPC) facility at the University of
195 Auckland. The resulting Illumina metabarcoding sequenced reads were processed by firstly
196 removing the primers (no mismatch tolerated) using fastq-multx (version 1.3.1) and by pairing
197 the reads with SolexaQA++. Low quality 3' end sequences (Phred scores below 3) were
198 truncated and reads merged using the sequence analysis tool VSEARCH version 2.3.0 (Rognes
199 et al. 2016), allowing a maximum of five non-matching nucleotides in the overlap region.
200 Merged reads were quality filtered on the expected error value (<1) and dereplicated. Chimera
201 checking and removal was performed on the dereplicated sequences with the QIIME
202 package (Caporaso et al. 2010) based on the *de novo* and reference based methods of
203 Usearch61 (Edgar 2010). Reference based chimera detection of 18S was performed with the
204 largest curated database available (SILVA; (Quast et al. 2013)) and for COI, using the Midori
205 database (Machida et al. 2017). Sequences were clustered into OTUs with the
206 Swarm methodology (clustering threshold of d2; (Mahé et al. 2014)).

207 ***Sequence assignment and analysis***

208 Representative OTU sequences (seed sequence of each OTU) were taxonomically assigned with
209 the Ribosomal Database Project (RDP) classifier (minimum confidence level of 80%; (Wang et

210 al. 2007)) using the curated databases Protist Ribosomal Reference (PR2; (Guillou et al. 2013))
211 and SILVA for 18S, and Midori database for COI. Representative sequences were also assigned
212 to reference sequences from the National Center for Biotechnology Information
213 (NCBI) Genbank database (Benson et al. 2013), using the megablast option of blastn (Morgulis
214 et al. 2008) with an e-value threshold of 0.001. Only the best hit for each sequence was kept.
215 NCBI Genbank is an all-inclusive database and thus was used for both the 18S gene sequences
216 and COI gene sequences, however, sequences were only assigned to a taxon if the sequence
217 could be assigned to a genus or species. NCBI Genbank is also not a curated database. The
218 Midori database is specifically for metazoan mitochondrial DNA sequences. The PR2 database
219 consists mainly of nuclear-encoded protistan sequences, and SILVA is an aligned database of
220 small and large subunit rRNA genes. Taxonomically assigned OTUs were filtered for potential
221 PCR or sequencing artefacts by selecting OTUs with five or more sequence reads ("hits"). The
222 resulting filtered OTUs for COI were then further filtered to keep only the OTUs that could be
223 identified to either a genus or species level by Midori and the NCBI databases. The same was
224 done for 18S with PR2, SILVA and NCBI. The resulting data for genus or species were used for
225 assessing the methodological factors and preliminary diet which was analysed using Rstudio® (R
226 Core Team 2017) and the R packages tidyverse (Wickham 2017).

227 To determine a list of identified taxa that were most likely to form part of the true diet of NZ
228 scampi, taxa identified as contamination were removed from the final data. Any sequence reads
229 identified as lobster (Astacidea) were presumed to be host contamination and removed. Sequence
230 reads were also removed from further analysis if they matched to any taxa identified from the
231 sequenced DNA negative sample, or to any taxa that could not be part of the NZ scampi diet

232 (e.g., terrestrial species). The final "hit counts" pertaining to digesta are referred to as "diet hit
233 counts".

234 Taxa were defined as "exclusive" if they were identified in only a single source, such as taxa
235 identified in the foregut but not in the hindgut, or solely identified in one PCR reaction but not
236 another.

237 **Results**

238 **Factors Affecting PCR Amplification Success**

239 Several important factors were identified that affected PCR amplification success prior to
240 metabarcoding. These factors included PCR inhibition (and its reduction with BSA), optimal
241 template DNA dilution, PCR reagents (particularly the DNA polymerase and buffer), and the
242 success of amplification of different target genes (18S or COI). The success of the PCR reactions
243 was determined by the presence and intensity of an appropriately-sized fragment on an agarose
244 gel.

245 PCR inhibition was minimised (visible increase in DNA amplification on agarose gel) when an
246 optimal volume of 2 µl of 1% BSA was used in each 25 µl PCR reaction, with some beneficial
247 effect when using 1 µl or 5 µl of 1% BSA. PCR inhibition was found to occur inconsistently at a
248 range of DNA concentrations, even with the addition of BSA. The inhibitory effect of the DNA
249 extracted from gut material was demonstrated when amplifying both genes with DNA extracted
250 from tail muscle tissue: PCR amplification from muscle DNA alone was usually strong, whereas
251 PCR amplification was often dramatically reduced when DNA extracted from the gut (digesta
252 DNA) was added. Amplification from digesta DNA was often very inconsistent between PCR
253 replicates. Different dilutions of template DNA (1:10, 1:50 and 1:100) were tested in an attempt
254 to reduce PCR inhibition, with the 1:10 dilution proving to result in the strongest DNA
255 amplification overall.

256 Two different sets of PCR reagents (which differed primarily in their DNA polymerase and
257 buffer) were compared. The Bioline reaction was generally more reliable in DNA amplification,
258 as not only was the intensity of the products generally greater, but the digesta DNA was more
259 likely to produce PCR products for both the COI gene and 18S gene.

260 When comparing PCR amplification of the two target genes from digesta DNA, in general the
261 COI gene did not amplify as well as the 18S. For several individuals, no PCR products were
262 seen for the COI gene, while for the same DNA, the 18S gene was amplified well. In addition,
263 DNA from hindgut contents amplified more readily than DNA extracted from the foregut
264 contents.

265 **Factors Affecting Metabarcode Sequencing Results**

266 ***PCR reagents (DNA polymerase and buffer)***

267 A comparison between Platinum *Taq* and the Bioline PCR reagents was made for individual
268 70.9, for which both sets of reagents were used (Table 2). Metabarcode sequences from both
269 foregut and hindgut were pooled for each set of reagents (thus comparing samples 1 and 2 with 3
270 and 4). The total number of taxa identified and the total number of exclusive taxa identified (i.e.,
271 those found only in one category) was greater for both the COI and 18S genes when using the
272 Bioline reaction (11 different taxa in total and six exclusive taxa), compared to the Platinum *Taq*
273 reaction (eight different taxa identified and three exclusive taxa) (Table 3). The total diet hit
274 count (number of sequence reads matching a potential dietary reference sequence in that
275 database – i.e., excluding host matches) was also greater for the Bioline reaction.

276 Five of the taxa identified by Platinum *Taq* were a subset of those found in Bioline, which had a
277 low summed hit count of 54 compared to Bioline's 468 hit count for the same taxa. Moreover,
278 Bioline identified a higher number of exclusive taxa which averaged a hit count of ~32 hits per
279 taxa whereas Platinum *Taq* only averaged 8 hits per taxa.

280 ***Hindgut versus foregut digesta***

281 The taxa identified from the hindgut and foregut of two individuals were compared (i.e., samples
282 1 and 5 versus 2 and 6). The hindgut digesta contained a higher number of both total taxa and
283 exclusive taxa compared to the foregut, when the sequences were matched to the COI gene
284 databases (Table 4). Conversely, the foregut contained a higher number of total taxa and
285 exclusive taxa with the 18S gene databases.

286 ***Sample Preservation Method***

287 The effects of potential differential DNA degradation due to the method of preservation were
288 more difficult to assess directly, as the same individuals could not be compared across the two
289 conditions, and the comparison was somewhat confounded by being collected in different
290 locations. Instead, broad comparisons were made between the pooled results from the three
291 individuals preserved for each of the ethanol and frozen methods. Firstly, the potential
292 differential effects on preservation of diet DNA was assessed by calculating the diet hit count as
293 a percentage of the overall hit count (for samples 1, 2, 5, 6 and 7 versus 8 and 9) (Table 5). A
294 noticeable difference in percentage between frozen (0%) and ethanol (6%) individuals is seen in
295 the diet hit count for the COI databases. No difference was seen for the ethanol and frozen
296 individuals when using the 18S databases, as both had a diet hit count of 1%.

297 **Database Comparison and Preliminary Assessment of NZ Scampi Diet**

298 All individuals and their digesta amplified using the Bioline PCR reactions were used to provide
299 a preliminary assessment of the diet. A total of 13,553 sequences were used to analyse the diet
300 (24 taxa from 10,623 COI sequences, and 23 taxa from 2,930 18S sequences). Among the 24
301 taxa identified from the COI databases were seven fish taxa (*Epinephelus epistictus*, *Helicolenus*
302 *barathri, Hydrolagus novaezealandiae*, *Macruronus magellanicus*, *Notophycis marginata*,

303 *Seriolella punctate* and *Thyrsites atun*), crab taxa (*Neosarmatium fourmanoiri*, *Microphrys*
304 *branchialis* and *Homola* sp.), a marine worm (*Laonice cirrata*) and an anemone (*Diadumene*
305 *leucolena*) (Fig. 2). The 18S databases identified 23 taxa, which included a sea pen (*Umbellula*
306 sp.), salps (*Pyrosoma* sp. and *Ihlea racovitzai*) and many parasites (i.e., apicomplexan *Eimeria*
307 *variabilis* and dinoflagellate *Duboscquella*) (Fig. 3). The databases (18S verse COI) did not
308 confidently identify any of the same organisms and the taxonomic resolution and level of
309 confidence varied for the same OTU between databases when assessing the same gene (Table S1
310 and Table S2).

311 **Discussion**

312 **Methodological Issues in PCR Amplification Success**

313 ***PCR inhibition***

314 PCR inhibition is a substantial factor in this study. BSA has been used as a successful additive to
315 reduce PCR inhibition by increasing the amplification efficiency. An optimal volume of 1% BSA
316 (2 µl) facilitated gut content DNA amplification in the 25 µl PCR reactions, thus reducing
317 inhibition. Even with the enhancing effect of BSA in the PCR amplification, it was still found
318 that DNA amplification was inconsistent. An additional step in the reactions was taken to try and
319 alleviate the PCR inhibition, by diluting the template genomic DNA, and thus diluting the PCR
320 inhibitors it presumably contained. The best overall DNA dilution was a 1:10 of the original
321 DNA.

322 An inconsistency of PCR amplification between reactions was observed, as the DNA of the gut
323 content of single individuals did not amplify consistently among replicate reactions. This
324 suggests that the reaction conditions were bordering on adequate, and that stochastic PCR
325 dynamics resulted in only a fraction of amplifications being successful. This needs to be taken
326 account of, as it may indicate that individual template components in the genomic DNA
327 extractions (i.e., different species in the diet) may also vary in their amplification from one
328 reaction to another. Consequently, PCR inhibition appears to be notably different among
329 replicate PCR reactions.

330 It has been implied in previous studies that it can be difficult to eliminate PCR inhibition from
331 DNA extracts of digesta (Juen & Traugott 2006; Liu 2009; O'Rorke et al. 2013; Harms-Tuohy,
332 Schizas & Appeldoorn 2016). The presence of organic compounds in the foregut (i.e., bile salts
333 in the gastric juices) is thought to be one of the main contributors to PCR inhibition which

334 cannot be alleviated by BSA (Lorenz 2012; Schrader et al. 2012; Harms-Tuohy, Schizas &
335 Appeldoorn 2016). Bile salts often found in vertebrate gastric juices, is similar to an organic
336 compound in crustaceans such as crabs, lobsters and crayfish and suggests why BSA is not an
337 alleviator in all DNA amplifications trialled here (Borgström 1974). Different diets would also
338 affect the secretion of the gastric juices, which may play a role in determining which PCR
339 inhibitors are present and in what concentration in different parts of the gut (Rotllant et al. 2014).
340 Thus it may ideally require PCR optimisation for each DNA extract to ensure adequate
341 amplification of target DNA fragments. This demonstrates the problematic, high levels of PCR
342 inhibition present in many genomic DNA extracts, and its influence on successfully amplifying
343 gut content DNA. Increased quantitative monitoring of the PCR success (such as by quantitative
344 PCR) may be required in future.

345 One solution to PCR inhibition may be to pool replicate PCR products together, if final DNA
346 concentration after clean-up is low (less than 1 ng μl^{-1}). Also, if no amplification appears to be
347 occurring, additional PCR conditions can be explored, such as addition of the T4 gene 32
348 protein (gp32), which can be effective against inhibitors for which BSA is not, such as sodium
349 dodecyl sulfate (SDS), sodium chloride and bile salts (Schrader et al. 2012).

350 ***PCR reagents (DNA polymerase and buffer)***

351 Choosing the correct DNA polymerase and buffer can be greatly beneficial to the success of
352 amplifying poorer quality DNA from the digesta. Different DNA polymerases and buffers are
353 available and are two of the key reagents for DNA amplification, each being susceptible to
354 various components in the biological environment in which they amplify, thus affecting their
355 performance (Rådström et al. 2004; Wolffs et al. 2004). A substantial difference could be seen
356 visually in the DNA amplification when using the Bioline reaction compared to the Platinum *Taq*

357 reaction. The Platinum *Taq* reaction appeared to be more susceptible to the effects of PCR
358 inhibitors, and the DNA amplification was not as effective as in the Bioline reaction.

359 ***Amplification of the loci***

360 The 18S gene region (~425 bp), in general, amplified more successfully compared to the COI
361 gene region (~300 bp) targeted here. It is not common for shorter gene fragments to be more
362 difficult to amplify, but it is relatively common to see some difficulties in amplification of the
363 COI gene in some species, likely due to the greater sequence variation in the primer annealing
364 sites (Chen, Jiang & Qiao 2012; Lv et al. 2014). However, when both genes amplified, their
365 PCR product intensity appeared consistent between sample conditions. For example, where the
366 PCR product concentration was higher from the DNA extracted from the foregut compared to the
367 hindgut from a single individual using the COI gene, this was consistent when looking at the
368 PCR product concentration from the 18S gene.

369 **Methodological Issues in Metabarcoding Success**

370 ***PCR reagents (DNA polymerase and buffer)***

371 Quantification of the PCR amplification can only suggest if amplification is occurring and not
372 necessarily what the quality of amplification is, or whether all templates are being amplified. So
373 although it was visually determined that the Bioline reaction generally resulted in stronger PCR
374 amplifications, it was possible that it was amplifying only a subset of the possible templates
375 compared to that of the Platinum *Taq* reaction. Thus, DNA metabarcoding was undertaken on
376 both of the PCR reaction products from the same samples to ascertain comparative DNA
377 amplification. Individual 70.9 amplified equally well for both the Platinum *Taq* and Bioline
378 reactions, and also for both the hindgut and the foregut. The DNA sequencing revealed that the
379 Bioline reaction amplified a higher number of taxa from the same individual's gut content DNA.

380 This was likely due to a higher tolerance of PCR inhibitors from the DNA polymerase and buffer
381 in Bioline and thus having a greater amplification of DNA. This is suggested by the taxa that the
382 two sets of PCR reagents have in common, as Bioline (hit count 468) amplified approximately
383 nine times more diet fragments than Platinum *Taq* (hit count 54). The exclusive taxa, presumed
384 to have the lower DNA concentrations, identified by Platinum *Taq* has ~8 hit counts per taxon,
385 whereas Bioline had ~32 hit counts per exclusive taxon, thus indicating that the Bioline reaction
386 appears to be more efficient at amplifying DNA from the digesta of NZ scampi.

387 ***Foregut versus Hindgut***

388 The examination of both the foregut and hindgut digesta provided a broader overview of the NZ
389 scampi diet. There was an increase in the number of taxa when the entire gut digesta was used,
390 compared to using digesta from only the hindgut or the foregut. As ingested food takes a few
391 hours to pass through the foregut and into the hindgut, the examination of the digesta from both
392 may permit the assessment of successive meals over a broader time period before capture (Simon
393 & Jeffs 2008; Lee, Hartstein & Jeffs 2015; Kamio et al. 2016). Also, as the chemical
394 environment (i.e. pH) and degree of degradation of digesta differ between the gut sections, an
395 assessment of both foregut and hindgut digesta appears to maximise the opportunity to detect the
396 widest possible breadth of diet per individual.

397 It was initially expected that the hindgut contents may provide lower quality sequencing results,
398 because it was more difficult to eliminate host tissue contamination during dissection, and the
399 gut content clearly contained a higher concentration of sediment compared to dietary items. In
400 addition, most previous studies that used gut digesta from marine fish and lobsters in diet
401 analysis have generally used only the foregut, because of concerns about the extent of
402 degradation of food items by the time they reach the hindgut (Choi et al. 2008; Sahlmann, Chan

403 & Chan 2011; Wahle et al. 2012; Berry et al. 2015; Harms-Tuohy, Schizas & Appeldoorn 2016).
404 The results here reveal that, within individual NZ scampi, there are taxa that are detected
405 exclusively in the hindgut and in the foregut, and that combining gut contents will provide more
406 complete results than the foregut by itself. A higher number of taxa of smaller marine organisms
407 (i.e. dinoflagellates) were identified in the foregut than in the hindgut and a higher number of
408 taxa of larger marine organisms (fish) were identified in the hindgut than in the foregut. Thus, to
409 dismiss either the foregut or the hindgut would have narrowed the number of taxa that were able
410 to be identified. This difference may be due to detecting taxa from successive meals or
411 potentially from different gut conditions favouring the DNA amplification of different taxa in
412 each.

413 ***Preservation Methods***

414 In this study, host DNA amplification did not completely outcompete the amplification of prey
415 DNA. However, the overall impact of host contamination can be reduced by introducing several
416 molecular techniques that will help decrease any biased amplification of the host's DNA, and
417 broaden the taxonomic analysis (Polz & Cavanaugh 1998; Green & Minz 2005; Vestheim &
418 Jarman 2008; Pompanon et al. 2012; Harms-Tuohy, Schizas & Appeldoorn 2016). PCR
419 "clamping", using additional DNA or peptide nucleic acid (PNA) oligonucleotide primers to
420 bind to and inhibit PCR amplification of host DNA, is a favoured method for suppressing host
421 DNA amplification in PCRs, and has been successful in diet studies (Egholm et al. 1993; Orum
422 et al. 1993; Vestheim & Jarman 2008; Chow et al. 2011; O'Rorke et al. 2012). Laboratory
423 contamination can be further reduced by taking additional precautions, such as working in a
424 laminar flow hood. Consideration for diet analysis work should include PCR clamping to reduce

host DNA amplification, as well as very strict lab sterilization protocols and procedures during gut material extraction and PCR preparations.

The quality of DNA extracted from the digesta is affected by the state of degradation due to the digestion rate in the gut (Rådström et al. 2004; Deagle & Tollit 2007; Troedsson et al. 2009). As the two different preservation methods used would have different rates in which the digesta was preserved, it was thought that one method may provide better preservation of digesta than the other. Determining which preservation method, frozen or ethanol, is better was relatively problematic to assess, due to the relatively high level of inter-individual variation and the confounded nature of the sample collection. By necessity, different preservation methods could only be undertaken on different individuals taken from different locations.

If DNA degradation was more prevalent in one preservation method over the other, it should be noticeable in the diet hit count percentage, as this proportion would be expected to be higher in the better preserved sample.

The major difference between preservation methods was seen within the COI database results as the proportion of sequences matching diet taxa was 0% in the frozen individuals and 6% in the ethanol individuals. However, the diet hit count percentage from the 18S databases were both ~1% for ethanol and frozen individuals. Although not a particularly robust test, it may suggest that the frozen preservation method may increase the DNA degradation rate in the digesta.

Amplification of the loci

Although there were difficulties in gaining equal success in the DNA amplification from both genes, together they provided a much broader overview of the NZ scampi diet at the highest resolution possible. The 18S gene and the COI gene identified a variety of taxa (Fig. 2 and Fig.

447 3). The COI gene is more commonly used than the 18S gene in studies of molecular taxonomy of
448 large eukaryotes and is one of the pillars of DNA barcoding, making it a sought-after gene region
449 for the discrimination of closely related Metazoan species (Hebert, Ratnasingham & deWaard
450 2003; Derycke et al. 2010; Tang et al. 2012). Consequently, the COI databases have, in general,
451 a higher diversity of sequences of large metazoan species. The 18S gene is more frequently used
452 in molecular taxonomic studies for microbial organisms (i.e., micro-plankton and parasites) (Wu,
453 Xiong & Yu 2015), and provides additional insight into taxa not present in the COI databases.
454 For further analysis of the NZ scampi diet, both genes should be used, as this lobster, being
455 relatively small in size, is likely to take advantage of the availability of both large and small
456 prey, both dead and alive.

457 ***Database review***

458 NCBI's Genbank is a user friendly, non-curated, multipurpose database. It does have curated
459 sequences within its database – RefSeq and Barcode of Life Data Systems (BOLD) – but these
460 were not singled out in this study (Pruitt, Tatusova & Maglott 2007). NCBI's Genbank does
461 provide a very useful platform for gene identification and characterisation, as new sequences of
462 species are continually being added, and the database also provides functional information
463 readily available for researchers (Pruitt 2002). This leads to the database growing rapidly, but
464 also leaves room for error if sequences have been mislabelled or misidentified. For this reason
465 this study also used the curated databases Midori (COI), PR2 and SILVA (both 18S) for
466 comparison against NCBI's Genbank. However, it must still be noted that many original species
467 misidentifications may exist in these curated databases, as many species have not been identified
468 by taxonomic experts.

469 Using a combination of all databases to identify the OTUs to genus or species level proved
470 invaluable; where the curated databases could not provide a high resolution identification (i.e.
471 only to order or family), NCBI was usually able to.

472 Caution should still be taken when proposing the taxa that match to each OTU, as this match
473 may be just a closely related species, if the true species is not yet in the databases. The taxa
474 identified in the 18S databases included the majority of the planktonic and parasitic taxa. The
475 majority of the fish species were identified in the COI databases. For future diet analysis, a
476 combination of databases should be considered to provide the most informative results.

477 **Preliminary Assessment of NZ Scampi Diet**

478 This study undertook the first assessment of the NZ scampi diet using DNA metabarcoding
479 methods. Although this study investigated only a small number of individuals in this preliminary
480 analysis, it has already provided a fascinating snapshot of their diet, confirming some previous
481 expectations, and providing a broader understanding of their feeding ecology.

482 The components of the diet of the six selected individuals were identified at a much finer
483 taxonomic level than any other previous studies undertaken microscopically on any
484 *Metanephrops* species. The diet of NZ scampi was found to include a range of pelagic fish
485 species, crabs, sea pens and dinoflagellates. This range of species have been identified at the best
486 taxonomic resolution possible. This compares to the diets determined microscopically at a low
487 taxonomic resolution from other *Metanephrops* species, which consisted of crustaceans, fish,
488 annelids and bivalves (Choi et al. 2008; Sahlmann, Chan & Chan 2011; Wahle et al. 2012). NZ
489 scampi are thought to be benthic foragers and scavengers, relying heavily on chemosensory
490 detection of potential food items (Major & Jeffs 2017). They are likely to be scavengers of fish

remains whether it is from trawl debris, sunken carcases or faeces and forage for smaller dietary such as sea pens. Using metabarcoding methods on digesta also allowed identification of parasites that are either from the diet source or from the NZ scampi individuals sampled. Below, we examine some of the most interesting and prominent taxa identified in the NZ scampi diet, and suggest likely species identifications for those taxa not classified to species or those whose counterparts are more likely to be the closest match.

NZ scampi have been reported to reside at a depth ranging from 140 – 640 m (Holthuis 1991). Diet taxa were closely matched to several common fish species (ethanol individuals only) that reside within this depth range on the Chatham Rise, including *H. novaezealandiae* (ghost shark), *N. marginata* (dwarf codling), *S. punctata* (silver warehou) and *T. atun* (snoek) (Francis 1998; Ministry for Primary Industries 2006b; Ministry for Primary Industries 2006a; Luna 2008; Ministry for Primary Industries 2008; Priede 2017). The OTU identified as *M. magellanicus* is likely to be *Macruronus novaezelandiae* (New Zealand hoki), as *M. magellanicus* is located off the southern coast of Chile and Argentina and *M. novazealandiae* is located in the Chatham Rise at depths from 209 – 904 m (D'Amato 2006; Connell, Dunn & Forman 2010; Kobayashi, Mizuguchi & Matsuoka 2014). The OTU identified as *H. barathri* (sea perch) is likely to be *Helicolenus percoides,* a sea perch (Scorpaenidae) found at depths between 250 – 700 m in the Chatham Rise (Anderson 1998; Horn, Forman & Dunn 2012). *Epinephelus epistictus* (grouper) is another OTU that is probably instead its counterpart found in New Zealand waters at the appropriate depth, *Epinephelus octofasciatus*.

The only sea pen to be identified in the COI databases, *Funiculina quadrangularis*, is a common tall deep-sea sea pen that grows on muddy substrates and has been found between 20 – 2000 m in New Zealand waters (Hughes 1998). *Umbellula* sp. is a sea pen taxa identified by the 18S

514 databases and has a world-wide distribution in depths 200 – 6260 m (Williams 1995; Williams
515 2014).

516 There are several OTUs identified as belonging to the Brachyuran crabs, found only in the gut of
517 ethanol preserved NZ scampi. Many families of this infraorder occur in New Zealand waters in
518 the area of the Chatham Rise (Wilkens 2015), but it is not possible at this stage to identify the
519 OTUs to their likely species from this region. OTUs identified to species in this infraorder
520 include *Microphrys branchialis* (Majidae, spider crabs – 12 species found on the Chatham Rise,
521 according to the Ocean Biogeographic Information System [OBIS; (OBIS 2017)]), and
522 *Neosarmatium fourmanoiri* (Grapsidae), although none are known to be found in New Zealand.
523 It is likely that these OTUs belong to genetically similar species that exist at the depth in which
524 NZ scampi are found on the Chatham Rise. The *Homola* sp. may be *Homola orientalis*, as this
525 crab is known to be distributed in New Zealand waters, living at depths of 500 m (Eldredge
526 1980).

527 The marine polychaete OTU was identified as *Laonice cirrata*, but this species has not been
528 found in New Zealand waters, and it is likely to be DNA signal from a closely related benthic
529 worm. Numerous plankton were identified, such as the deep-water salp, *I. racovitza*
530 (tunicate), which can grow to over 20 mm in diameter and are an appropriate size for NZ scampi
531 to handle with their feeding appendages (Pakhomov et al. 2011). This species is known to more
532 commonly occur in the high Antarctic cold-water zone, but has been found to occur in waters
533 south of Australia (Casareto & Nemoto 1986; Pakhomov et al. 2011; Ono & Moteki 2013).
534 *Ebria tripartita* (Rhizaria) has been reported world-wide, has oval cells 21-35 pm in length and
535 13-25 pm in width, and is likely to be consumed unintentionally due to their size (Tong et al.

536 1998). It is known to be a grazer of phytoplankton (nannoplanktonic diatoms and dinoflagellates)
537 and has been reported in the Hauraki Gulf, New Zealand (Gordon et al. 2012).

538 It is noticeable that no molluscs were detected, which may be due to more rapid degradation of
539 their soft tissues (Bell, Tuck & Dobby 2013). It will be interesting to examine the ratios of OTU
540 hit counts across a larger number of individuals from several locations.

541 It is difficult to infer importance in the diet from the number of metabarcode sequence reads for
542 each OTU. Many factors may impact the hit counts, and without further experimental
543 comparisons, these cannot be directly interpreted as providing any reliable measure of the
544 importance of each OTU in the diet of NZ scampi, particularly without a large number of
545 individuals sampled. However, the hit counts may provide at least some indication of the relative
546 concentration of taxa in the DNA extracted from the digesta of these sampled individuals.

547 At present it is impossible to entirely differentiate between species that were directly consumed
548 by NZ scampi, and those that were consumed by their prey, i.e. secondary predation. However, it
549 is expected that, due to rapid decomposition, secondary predation is likely to account for only a
550 very small proportion of the taxa detected in the NZ scampi digesta.

551 The parasites that have been identified are likely to be residing either within the gut of the NZ
552 scampi or the individuals that were consumed. Parasites known to infect fish were identified,
553 such as *Eimeria percae* which is known to parasitize the European perch (*Perca fluviatilis*)
554 (Molnár et al. 2012). Syndiniales Dino-Group I and II are known to parasitize a variety of marine
555 organisms, including crustaceans (Guillou et al. 2010). Previous studies of gut contents of lobster
556 larvae using DNA methods have also identified a range of microscopic parasites suggesting they
557 may be common component of the gut (O'Rorke et al. 2012; O'Rorke et al. 2015).

558 There was a small proportion of OTU sequences that were not matched to known sequences in
559 the databases and demonstrates the limitations of the reference databases. This is largely due to
560 the small amount of material from New Zealand deep-sea marine life that has been sequenced
561 and uploaded to the various databases. A possible short term solution for the analysis of the diet
562 would be to collect samples of bycatch species from the trawls, identify them, extract their DNA
563 and sequence the genes of interest. This would create a database against which the diet sequences
564 could be compared, potentially revealing more specific matches or even assigning identities to
565 unassigned sequences. Further insight into the NZ scampi diet will come with a greater number
566 of individuals analysed. Data on the availability of prey will be needed in order to determine diet
567 preference, as relative concentration in the diet may simply be related to what is more prevalent
568 in their foraging area.

570 **Conclusion**

571 Many methodological issues have been addressed in this study, which has provided solutions for
572 alleviating them, such as template DNA dilution and BSA addition for minimising PCR
573 inhibition. The study has also identified considerable variation in PCR inhibition among
574 individual samples, which indicates that it will be necessary to optimise PCR amplification for
575 each sample. The study has also determined the optimum PCR reagents (particularly the DNA
576 polymerase and buffer) and the benefit of using a selection of different databases for assigning
577 OTUs to taxa for different genes.

578 The preliminary insight from this study into the varied diet of the NZ scampi provides a
579 foundation for both the production of a nutritional feed for aquaculture and an attractive bait for
580 fishery pots. However, further examination of the diet is clearly needed. Given the variability
581 among individuals, the minimum sample number that is analysed per collection site should be of
582 around 10 individuals that have a moderate amount of digesta for DNA extraction. This will help
583 in quantifying if NZ scampi have a diet preference and if it is related to sex, size and/or location.
584 A future study should also proceed in collecting by-catch when NZ scampi are trawled, which
585 will assist in determining prey availability, and provide a more complete sequence reference
586 DNA database for comparison. It is clear that there is considerably more insight provided from
587 identification using metabarcoding than from traditional microscopic identification. A further
588 benefit is the store of diet DNA sequences that can be retained for future analysis (against
589 updated reference databases), whereas microscopy results cannot be further analysed. Overall,
590 this study shows there is great promise in analysing NZ scampi diet using metabarcoding
591 methods.

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599 **References**

600 Anderson O, Bagley NW, Hurst RJ, Francis MP, Clark MR, McMillan PJ. 1998. *Atlas of New Zealand fish*
601 *and squid distributions from research bottom trawls.* Wellington, NZ: NIWA.
602 Aylagas E, Borja Á, Irigoien X, and Rodríguez-Ezpeleta N. 2016. Benchmarking DNA metabarcoding for
603 biodiversity-based monitoring and assessment. *Frontiers in Marine Science* 3.
604 https://doi.org/10.3389/fmars.2016.00096
605 Bell M, Tuck I, and Dobby H. 2013. Nephrops Species. In: Phillips B, ed. *Lobsters: Biology, Management,*
606 *Aquaculture & Fisheries*. 2nd ed. Oxford, UK: John Wiley & Sons Ltd., 357-413.
607 Benson DA, Cavanaugh M, Clark K, Karsch-Mizrachi I, Lipman DJ, Ostell J, and Sayers EW. 2013. GenBank.
608 *Nucleic Acids Res* 41:D36-42. https://doi.org/10.1093/nar/gks1195
609 Berry O, Bulman C, Bunce M, Coghlan M, Murray DC, and Ward RD. 2015. Comparison of morphological
610 and DNA metabarcoding analyses of diets in exploited marine fishes. *Marine Ecology Progress*
611 *Series* 540:167-181. https://doi.org/10.3354/meps11524
612 Borgström B. 1974. Bile Salts—Their physiological functions in the gastrointestinal tract. *Acta Medica*
613 *Scandinavica* 196:1-10. https://doi.org/10.1111/j.0954-6820.1974.tb00958.x
614 Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Pena AG,
615 Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA,
616 McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA,
617 Widmann J, Yatsunenko T, Zaneveld J, and Knight R. 2010. QIIME allows analysis of high-
618 throughput community sequencing data. *Nat Methods* 7:335-336.
619 https://doi.org/10.1038/nmeth.f.303
620 Casareto BE, and Nemoto T. 1986. Salps of the Southern Ocean (Australian sector) during the 1983-84
621 summer, with special reference to the species *Salpa thompsoni*, Foxton 1961. *Memoirs of*
622 *National Institute of Polar Research Special issue* 40:221-239.
623 Chen R, Jiang L-Y, and Qiao G-X. 2012. The Effectiveness of Three Regions in Mitochondrial Genome for
624 Aphid DNA Barcoding: A Case in Lachininae. *PLoS ONE* 7:e46190.
625 https://doi.org/10.1371/journal.pone.0046190
626 Choi JH, Kim JN, Kim MH, Chang DS, Yoo JT, and Kim JK. 2008. Population biology and feeding habits of
627 the nephropid lobster *Metanephrops thomsoni* (Bate, 1888) in the East China Sea. *Journal of*
628 *Environmental Biology* 29:453-456.
629 Chow S, Suzuki S, Matsunaga T, Lavery S, Jeffs A, and Takeyama H. 2011. Investigation on natural diets of
630 larval marine animals using peptide nucleic acid-directed polymerase chain reaction clamping.
631 *Marine Biotechnology* 13:305-313. https://doi.org/10.1007/s10126-010-9301-3
632 Connell AM, Dunn MR, and Forman J. 2010. Diet and dietary variation of New Zealand hoki *Macruronus*
633 *novaezelandiae*. *New Zealand Journal of Marine and Freshwater Research* 44:289-308.
634 https://doi.org/10.1080/00288330.2010.515232
635 Cowart DA, Pinheiro M, Mouchel O, Maguer M, Grall J, Miné J, and Arnaud-Haond S. 2015.
636 Metabarcoding Is Powerful yet Still Blind: A Comparative Analysis of Morphological and
637 Molecular Surveys of Seagrass Communities. *PLoS ONE* 10:e0117562.
638 https://doi.org/10.1371/journal.pone.0117562
639 Crisol-Martínez E, Moreno-Moyano LT, Wormington KR, Brown PH, and Stanley D. 2016. Using Next-
640 Generation Sequencing to Contrast the Diet and Explore Pest-Reduction Services of Sympatric
641 Bird Species in Macadamia Orchards in Australia. *PLoS ONE* 11:e0150159.
642 https://doi.org/10.1371/journal.pone.0150159

643 Cristo M, and Cartes JE. 1998. A comparative study of the feeding ecology of *Nephrops norvegicus* (L.),
644 (Decapoda: Nephropidae) in the bathyal Mediterranean and the adjacent Atlantic. *Scientia*
645 *Marina* 62:81-90.
646 D'Amato ME. 2006. Demographic expansion and subtle differentiation in the longtailed hake
647 *Macruronus magellanicus*: evidence from microsatellite data. *Marine Biotechnology* 8.
648 https://doi.org/10.1007/s10126-005-5075-4
649 Deagle BE, and Tollit DJ. 2007. Quantitative analysis of prey DNA in pinniped faeces: potential to
650 estimate diet composition? *Conservation Genetics* 8:743-747. https://doi.org/10.1007/s10592-
651 006-9197-7
652 Derycke S, Vanaverbeke J, Rigaux A, Backeljau T, and Moens T. 2010. Exploring the Use of Cytochrome
653 Oxidase c Subunit 1 (COI) for DNA Barcoding of Free-Living Marine Nematodes. *PLoS ONE*
654 5:e13716. https://doi.org/10.1371/journal.pone.0013716
655 Dunn MR, Szabo A, McVeagh MS, and Smith PJ. 2010. The diet of deepwater sharks and the benefits of
656 using DNA identification of prey. *Deep-Sea Research Part I: Oceanographic Research Papers*
657 57:923-930. https://doi.org/10.1016/j.dsr.2010.02.006
658 Edgar RC. 2010. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 26:2460-
659 2461. https://doi.org/10.1093/bioinformatics/btq461
660 Egholm M, Buchardt O, Christensen L, Behrens C, Freier SM, Driver DA, Berg RH, Kim SK, Norden B, and
661 Nielsen PE. 1993. PNA hybridizes to complementary oligonucleotides obeying the Watson-Crick
662 hydrogen-bonding rules. *Nature* 365. https://doi.org/10.1038/365566a0
663 Eldredge L. 1980. Two Species of Homola (Dromiacea, Homolidae) from Guam. *Micronesica* 16:271-277.
664 FAO. 2016. Fisheries and aquaculture software. FishStatJ - software for fishery statistical time series.
665 *Available at http://www.fao.org/fishery/statistics/software/fishstatj/en* (accessed October
666 2017).
667 Farell EM, and Alexandre G. 2012. Bovine serum albumin further enhances the effects of organic
668 solvents on increased yield of polymerase chain reaction of GC-rich templates. *BMC Research*
669 *Notes* 5:257-257. https://doi.org/10.1186/1756-0500-5-257
670 Francis MP. 1998. New Zealand shark fisheries: Development, size and management. *Marine and*
671 *Freshwater Research* 49:579-591.
672 Geller J, Meyer C, Parker M, and Hawk H. 2013. Redesign of PCR primers for mitochondrial cytochrome c
673 oxidase subunit I for marine invertebrates and application in all-taxa biotic surveys. *Mol Ecol*
674 *Resour* 13:851-861. https://doi.org/10.1111/1755-0998.12138
675 Gordon DP, Diggles BK, Meisterfeld R, Hollis C, and Buchanan PK. 2012. Phylum Cercozoa: cercomonads,
676 filose testate amoebae, Phaeodaria, plasmodiophoras, Gromia, haplosporidians, and kin. In:
677 Gordon D, ed. *New Zealand inventory of biodiversity*. Christchurch, New Zealand: Canterbury
678 University Press, Christchurch, New Zealand, 233-241.
679 Green SJ, and Minz D. 2005. Suicide Polymerase Endonuclease Restriction, a Novel Technique for
680 Enhancing PCR Amplification of Minor DNA Templates. *Applied and Environmental Microbiology*
681 71:4721-4727. https://doi.org/10.1128/AEM.71.8.4721-4727.2005
682 Guillou L, Alves-de-Souza C, Siano R, and González HE. 2010. The ecological significance of small,
683 eukaryotic parasites in marine systems. *Microbiology Today* May 2010:93-95.
684 Guillou L, Bachar D, Audic S, Bass D, Berney C, Bittner L, Boutte C, Burgaud G, de Vargas C, Decelle J, del
685 Campo J, Dolan JR, Dunthorn M, Edvardsen B, Holzmann M, Kooistra WHCF, Lara E, Le Bescot N,
686 Logares R, Mahé F, Massana R, Montresor M, Morard R, Not F, Pawlowski J, Probert I, Sauvadet
687 A-L, Siano R, Stoeck T, Vaulot D, Zimmermann P, and Christen R. 2013. The Protist Ribosomal
688 Reference database (PR(2)): a catalog of unicellular eukaryote Small Sub-Unit rRNA sequences
689 with curated taxonomy. *Nucleic Acids Research* 41:D597-D604.
690 https://doi.org/10.1093/nar/gks1160

691 Harms-Tuohy CA, Schizas NV, and Appeldoorn RS. 2016. Use of DNA metabarcoding for stomach content
692 analysis in the invasive lionfish *Pterois volitans* in Puerto Rico. *Marine Ecology Progress Series*
693 558:181-191. https://doi.org/10.3354/meps11738
694 Hebert PDN, Ratnasingham S, and deWaard JR. 2003. Barcoding animal life: cytochrome c oxidase
695 subunit 1 divergences among closely related species. *Proceedings of the Royal Society B:*
696 *Biological Sciences* 270:S96-S99. https://doi.org/10.1098/rsbl.2003.0025
697 Holthuis L. 1991. FAO species catalogue. Marine lobsters of the world: An annotated and illustrated
698 catalogue of species of interest to fisheries known to date. Rome, Italy: FAO.
699 Horn PL, Forman JS, and Dunn MR. 2012. Dietary partitioning by two sympatric fish species, red cod
700 (*Pseudophycis bachus*) and sea perch (*Helicolenus percoides*), on Chatham Rise, New Zealand.
701 *Marine Biology Research* 8:624-634. https://doi.org/10.1080/17451000.2011.653543
702 Hughes DJ. 1998. Sea pens and burrowing megafauna (vol.III): an overview of dynamics and sensitivity
703 characteristics for conservation management of Marine SACs. *Available at*
704 *http://www.ukmarinesac.org.uk/search/sea-pens.htm* (accessed January 2018).
705 Jeffs A. 2010. Status and challenges of advancing lobster aquaculture globally. *The Marine Biological*
706 *Association of India* 52:320-326.
707 Juen A, and Traugott M. 2006. Amplification facilitators and multiplex PCR: Tools to overcome PCR-
708 inhibition in DNA-gut-content analysis of soil-living invertebrates. *Soil Biology and Biochemistry*
709 38:1872-1879. https://doi.org/10.1016/j.soilbio.2005.11.034
710 Kamio M, Wakabayashi K, Nagai H, and Tanaka Y. 2016. Phyllosomas of smooth fan lobsters (*Ibacus*
711 *novemdentatus*) encase jellyfish cnidae in peritrophic membranes in their feces. *Plankton and*
712 *Benthos Research* 11:100-104. https://doi.org/10.3800/pbr.11.100
713 Kobayashi G, Mizuguchi T, and Matsuoka A. 2014. Isolation and autoxidation profile of fish myoglobin
714 from hoki (*Macruronus magellanicus*). *Fukushima J Med Sci* 60:31-34.
715 https://doi.org/10.5387/fms.2014-5
716 Kreader CA. 1996. Relief of amplification inhibition in PCR with bovine serum albumin or T4 gene 32
717 protein. *Applied and Environmental Microbiology* 62:1102-1106.
718 http://www.ncbi.nlm.nih.gov/pmc/articles/PMC167874/
719 Kress WJ, García-Robledo C, Uriarte M, and Erickson DL. 2015. DNA barcodes for ecology, evolution, and
720 conservation. *Trends Ecol Evol* 30:25-35. http://dx.doi.org/10.1016/j.tree.2014.10.008
721 Lee S, Hartstein ND, and Jeffs A. 2015. Characteristics of faecal and dissolved nitrogen production from
722 tropical spiny lobster, *Panulirus ornatus*. *Aquaculture International* 23:1411-1425.
723 https://doi.org/10.1007/s10499-015-9893-8
724 Leray M, Yang JY, Meyer CP, Mills SC, Agudelo N, Ranwez V, Boehm JT, and Machida RJ. 2013. A new
725 versatile primer set targeting a short fragment of the mitochondrial COI region for
726 metabarcoding metazoan diversity: application for characterizing coral reef fish gut contents.
727 *Frontiers in Zoology* 10:34-34. https://doi.org/10.1186/1742-9994-10-34
728 Liu D. 2009. *Handbook of nucleic acid purification*. Florida, USA: Taylor & Francis CRC Press.
729 Lorenz TC. 2012. Polymerase Chain Reaction: Basic Protocol Plus Troubleshooting and Optimization
730 Strategies. *Journal of Visualized Experiments : JoVE*:3998. https://doi.org/10.3791/3998
731 Luna SM. 2008. *Notophycis marginata* (Günther, 1878) Dwarf codling. *Available at*
732 *http://www.fishbase.org/summary/2008* (accessed 27-9-2017 2017).
733 Lv J, Wu S, Zhang Y, Chen Y, Feng C, Yuan X, Jia G, Deng J, Wang C, Wang Q, Mei L, and Lin X. 2014.
734 Assessment of four DNA fragments (COI, 16S rDNA, ITS2, 12S rDNA) for species identification of
735 the Ixodida (Acari: Ixodida). *Parasites & Vectors* 7:93-93. https://doi.org/10.1186/1756-3305-7-
736 93

737 Machida RJ, Leray M, Ho S-L, and Knowlton N. 2017. Metazoan mitochondrial gene sequence reference
738 datasets for taxonomic assignment of environmental samples. 4:170027.
739 https://doi.org/10.1038/sdata.2017.27
740 Mahé F, Rognes T, Quince C, de Vargas C, and Dunthorn M. 2014. Swarm: robust and fast clustering
741 method for amplicon-based studies. *PeerJ* 2:e593. https://doi.org/10.7717/peerj.593
742 Major R, and Jeffs A. 2017. Orientation and food search behaviour of a deep sea lobster in turbulent
743 versus laminar odour plumes. *Helgoland Marine Research* 71:9.
744 https://doi.org/10.1186/s10152-017-0489-8
745 Mente E. 2010. Survival, food consumption and growth of Norway lobster (*Nephrops norvegicus*) kept in
746 laboratory conditions. *Integrative Zoology* 5:256-263. https://doi.org/10.1111/j.1749-
747 4877.2010.00211.x
748 Ministry for Primary Industries. 2006a. Barracouta (BAR). *Available at https://fs.fish.govt.nz* (accessed
749 October 2017).
750 Ministry for Primary Industries. 2006b. Fisheries Assessment: *Silver Warehou* (SWA). *Available at*
751 *https://fs.fish.govt.nz* (accessed October 2017).
752 Ministry for Primary Industries. 2008. Fisheries Assessment: Dark Ghost Shark (GSH). *Available at*
753 *https://fs.fish.govt.nz* (accessed October 2017).
754 Molnár K, Ostoros G, Dunams-Morel D, and Rosenthal BM. 2012. *Eimeria* that infect fish are diverse and
755 are related to, but distinct from, those that infect terrestrial vertebrates. *Infection, Genetics and*
756 *Evolution* 12:1810-1815. https://doi.org/10.1016/j.meegid.2012.06.017
757 Morgulis A, Coulouris G, Raytselis Y, Madden TL, Agarwala R, and Schaffer AA. 2008. Database indexing
758 for production MegaBLAST searches. *Bioinformatics* 24:1757-1764.
759 https://doi.org/10.1093/bioinformatics/btn322
760 O'Rorke R, Jeffs A, Wang M, Waite A, Beckley LE, and D. Lavery S. 2015. Spinning in different directions:
761 Western rock lobster larval condition varies with eddy polarity, but does their diet? *Journal of*
762 *Plankton Research* 37:542–553. https://doi.org/10.1093/plankt/fbv026
763 O'Rorke R, Jeffs AG, Fitzgibbon Q, Chow S, and Lavery S. 2013. Extracting DNA from whole organism
764 homogenates and the risk of false positives in PCR based diet studies: A case study using spiny
765 lobster larvae. *Journal of Experimental Marine Biology and Ecology* 441:1-6.
766 https://doi.org/10.1016/j.jembe.2013.01.003
767 O'Rorke R, Lavery S, Chow S, Takeyama H, Tsai P, Beckley LE, Thompson PA, Waite AM, and Jeffs AG.
768 2012. Determining the diet of larvae of western Rock Lobster (*Panulirus cygnus*) using high-
769 throughput DNA sequencing techniques. *PLoS ONE* 7.
770 https://doi.org/10.1371/journal.pone.0042757
771 O'Rorke R, Lavery S, Wang M, Nodder SD, and Jeffs AG. 2014. Determining the diet of larvae of the red
772 rock lobster (*Jasus edwardsii*) using high-throughput DNA sequencing techniques. *Marine*
773 *Biology* 161:551-563. https://doi.org/10.1007/s00227-013-2357-7
774 OBIS. 2017. Ocean Biogeographic Information System. *Available at www.iobis.org* (accessed October
775 2017).
776 Ono A, and Moteki M. 2013. Spatial distributions and population dynamics of two salp species, *Ihlea*
777 *racovitzai* and *Salpa thompsoni*, in the waters north of Lützow-Holm Bay (East Antarctica) during
778 austral summers of 2005 and 2006. *Polar Biology* 36:807-817. https://doi.org/10.1007/s00300-
779 013-1305-9
780 Orum H, Nielsen PE, Egholm M, Berg RH, Buchardt O, and Stanley C. 1993. Single base pair mutation
781 analysis by PNA directed PCR clamping. *Nucleic Acids Research* 21:5332-5336.
782 http://www.ncbi.nlm.nih.gov/pmc/articles/PMC310567/
783 Pakhomov EA, Dubischar CD, Hunt BPV, Strass V, Cisewski B, Siegel V, von Harbou L, Gurney L, Kitchener
784 J, and Bathmann U. 2011. Biology and life cycles of pelagic tunicates in the Lazarev Sea,

785 Southern Ocean. *Deep Sea Research Part II: Topical Studies in Oceanography* 58:1677-1689.
786 https://doi.org/10.1016/j.dsr2.2010.11.014
787 Pante E, and Simon-Bouhet B. 2013. marmap: A Package for Importing, Plotting and Analyzing
788 Bathymetric and Topographic Data in R. *PLoS ONE* 8.
789 https://doi.org/10.1371/journal.pone.0073051
790 Polz MF, and Cavanaugh CM. 1998. Bias in Template-to-Product Ratios in Multitemplate PCR. *Applied*
791 *and Environmental Microbiology* 64:3724-3730.
792 http://www.ncbi.nlm.nih.gov/pmc/articles/PMC106531/
793 Pompanon F, Deagle BE, Symondson WOC, Brown DS, Jarman SN, and Taberlet P. 2012. Who is eating
794 what: Diet assessment using next generation sequencing. *Molecular Ecology* 21:1931-1950.
795 https://doi.org/10.1111/j.1365-294X.2011.05403.x
796 Priede IG. 2017. *Deep-sea Fishes: Biology, Diversity, Ecology and Fisheries*. Cornwall, UK: Cambridge
797 University Press.
798 Pruitt K, Brown G, Tatusova T, Maglott D. 2002. The Reference Sequence (RefSeq) Database. In:
799 McEntyre J, Ostell J, ed. *The NCBI Handbook*. US: Bethesda (MD): National Center for
800 Biotechnology Information.
801 Pruitt KD, Tatusova T, and Maglott DR. 2007. NCBI reference sequences (RefSeq): a curated non-
802 redundant sequence database of genomes, transcripts and proteins. *Nucleic Acids Research*
803 35:D61-D65. https://doi.org/10.1093/nar/gkl842
804 Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, and Glöckner FO. 2013. The SILVA
805 ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic*
806 *Acids Research* 41:D590-D596. https://doi.org/10.1093/nar/gks1219
807 R Core Team. 2017. R: A language and environment for statistical computing. 1.0.143 ed. Vienna,
808 Austria: R Foundation for Statistical Computing.
809 Rådström P, Knutsson R, Wolffs P, Lövenklev M, and Löfström C. 2004. Pre-PCR processing. *Molecular*
810 *Biotechnology* 26:133-146. https://doi.org/10.1385/mb:26:2:133
811 Rognes T, Flouri T, Nichols B, Quince C, and Mahé F. 2016. VSEARCH: a versatile open source tool for
812 metagenomics. *PeerJ* 4:e2584. https://doi.org/10.7717/peerj.2584
813 Rossen L, Nørskov P, Holmstrøm K, and Rasmussen OF. 1992. Inhibition of PCR by components of food
814 samples, microbial diagnostic assays and DNA-extraction solutions. *International Journal of Food*
815 *Microbiology* 17:37-45. https://doi.org/10.1016/0168-1605(92)90017-W
816 Rotllant G, Mente E, Gisbert E, and Karapanagiotidis IT. 2014. Effects of different diets on the digestive
817 physiology of adult Norway Lobster *Nephrops norvegicus*. *Journal of Shellfish Research* 33:1-9.
818 https://doi.org/10.2983/035.033.0101
819 Sahlmann C, Chan TY, and Chan BKK. 2011. Feeding modes of deep-sea lobsters (Crustacea: Decapoda:
820 Nephropidae and Palinuridae) in Northwest Pacific waters: Functional morphology of
821 mouthparts, feeding behaviour and gut content analysis. *Zoologischer Anzeiger* 250:55-66.
822 https://doi.org/10.1016/j.jcz.2010.11.003
823 Schrader C, Schielke A, Ellerbroek L, and Johne R. 2012. PCR inhibitors – occurrence, properties and
824 removal. *Journal of Applied Microbiology* 113:1014-1026. https://doi.org/10.1111/j.1365-
825 2672.2012.05384.x
826 Seafood New Zealand. 2017. New Zealand Seafood Exports: Seafood exports by species by country.
827 *Available at https://www.seafoodnewzealand.org.nz/publications/export-information/*
828 (accessed August 2017).
829 Simon CJ, and Jeffs A. 2008. Feeding and gut evacuation of cultured juvenile spiny lobsters, *Jasus*
830 *edwardsii*. *Aquaculture* 280:211-219. https://doi.org/10.1016/j.aquaculture.2008.05.019

831 Sousa LL, Xavier R, Costa V, Humphries NE, Trueman C, Rosa R, Sims DW, and Queiroz N. 2016. DNA
832 barcoding identifies a cosmopolitan diet in the ocean sunfish. *Scientific Reports* 6:28762.
833 http://dx.doi.org/10.1038/srep28762
834 Srivathsan A, Ang A, Vogler AP, and Meier R. 2016. Fecal metagenomics for the simultaneous
835 assessment of diet, parasites, and population genetics of an understudied primate. *Frontiers in*
836 *Zoology* 13. https://doi.org/10.1186/s12983-016-0150-4
837 Tang CQ, Leasi F, Obertegger U, Kieneke A, Barraclough TG, and Fontaneto D. 2012. The widely used
838 small subunit 18S rDNA molecule greatly underestimates true diversity in biodiversity surveys of
839 the meiofauna. *Proceedings of the National Academy of Sciences of the United States of America*
840 109:16208-16212. https://doi.org/10.1073/pnas.1209160109
841 Tong S, Nygaard K, Bernard C, Vørs N, and Patterson D. 1998. Heterotrophic flagellates from the water
842 column in Port Jackson, Sydney, Australia. *European Journal of Protistology* 34:162-194.
843 https://doi.org/10.1016/S0932-4739(98)80027-8
844 Troedsson C, Simonelli P, Nägele V, Nejstgaard JC, and Frischer ME. 2009. Quantification of copepod gut
845 content by differential length amplification quantitative PCR (dla-qPCR). *Marine Biology*
846 156:253-259. https://doi.org/10.1007/s00227-008-1079-8
847 Tshudy D, Chan TY, and Sorhannus U. 2007. Morphology based cladistic analysis of *Metanephrops*: The
848 most diverse extant genus of clawed lobster (Nephropidae). *Journal of Crustacean Biology*
849 27:463-476. https://doi.org/10.1651/S-2777.1
850 Vestheim H, and Jarman SN. 2008. Blocking primers to enhance PCR amplification of rare sequences in
851 mixed samples – a case study on prey DNA in Antarctic krill stomachs. *Frontiers in Zoology* 5:12.
852 https://doi.org/10.1186/1742-9994-5-12
853 Wahle RA, Tshudy D, Cobb JS, Factor J, and Jaini M. 2012. Infraorder Astacidea Latreille, 1802 p.p.: the
854 marine clawed lobsters. In: Schram FR, and von Vaupel Klein JC, eds. *Treatise on Zoology -*
855 *Anatomy, Taxonomy, Biology The Crustacea, Volume 9 Part B*. Boston: Brill, 3-108.
856 Wang Q, Garrity GM, Tiedje JM, and Cole JR. 2007. Naive Bayesian classifier for rapid assignment of
857 rRNA sequences into the new bacterial taxonomy. *Appl Environ Microbiol* 73:5261-5267.
858 https://doi.org/10.1128/aem.00062-07
859 Wickham H. 2017. tidyverse: Easily Install and Load 'Tidyverse' Packages. 1.1.1 ed. p R package.
860 Wilkens S, Ahyong, S. 2015. Coastal Crabs: A guide to the crabs of New Zealand. *Available at*
861 *https://www.niwa.co.nz/coasts-and-oceans/marine-identification-guides-and-fact-*
862 *sheets/Coastal%20Crabs* (accessed October 2017).
863 Williams G, Tracey, D., Mackay, E. 2014. Pennatulacea (sea pens) Descriptions for the New Zeaand
864 Region. A field guide of commonly sampled New Zealand sea pens including illustrations
865 highlighting technical terms and sea pen morphology. New Zealand Aquatic Environment and
866 Biodiversity Report No 132. Wellington. p 22.
867 Williams GC. 1995. Living genera of sea pens (Coelenterata: Octocorallia: Pennatulacea): illustrated key
868 and synopses. *Zoological Journal of the Linnean Society* 113:93-140.
869 https://doi.org/10.1111/j.1096-3642.1995.tb00929.x
870 Wolffs P, Grage H, Hagberg O, and Rådström P. 2004. Impact of DNA Polymerases and Their Buffer
871 Systems on Quantitative Real-Time PCR. *Journal of Clinical Microbiology* 42:408-411.
872 https://doi.org/10.1128/JCM.42.1.408-411.2004
873 Wu S, Xiong J, and Yu Y. 2015. Taxonomic Resolutions Based on 18S rRNA Genes: A Case Study of
874 Subclass Copepoda. *PLoS ONE* 10:e0131498. https://doi.org/10.1371/journal.pone.0131498
875 Young JW, Hunt BPV, Cook TR, Llopiz JK, Hazen EL, Pethybridge HR, Ceccarelli D, Lorrain A, Olson RJ,
876 Allain V, Menkes C, Patterson T, Nicol S, Lehodey P, Kloser RJ, Arrizabalaga H, and Anela Choy C.
877 2015. The trophodynamics of marine top predators: Current knowledge, recent advances and

878 challenges. *Deep Sea Research Part II: Topical Studies in Oceanography* 113:170-187.
879 http://doi.org/10.1016/j.dsr2.2014.05.015
880 Zhan A, Hulák M, Sylvester F, Huang X, Adebayo AA, Abbott CL, Adamowicz SJ, Heath DD, Cristescu ME,
881 and MacIsaac HJ. 2013. High sensitivity of 454 pyrosequencing for detection of rare species in
882 aquatic communities. *Methods in Ecology and Evolution* 4:558-565.
883 https://doi.org/10.1111/2041-210X.12037

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Figure 1(on next page)

Bathymetry map showing the location of NZ scampi sampling on the Chatham Rise, on the east coast of New Zealand.

Map created using marmap (Pante & Simon-Bouhet 2013).

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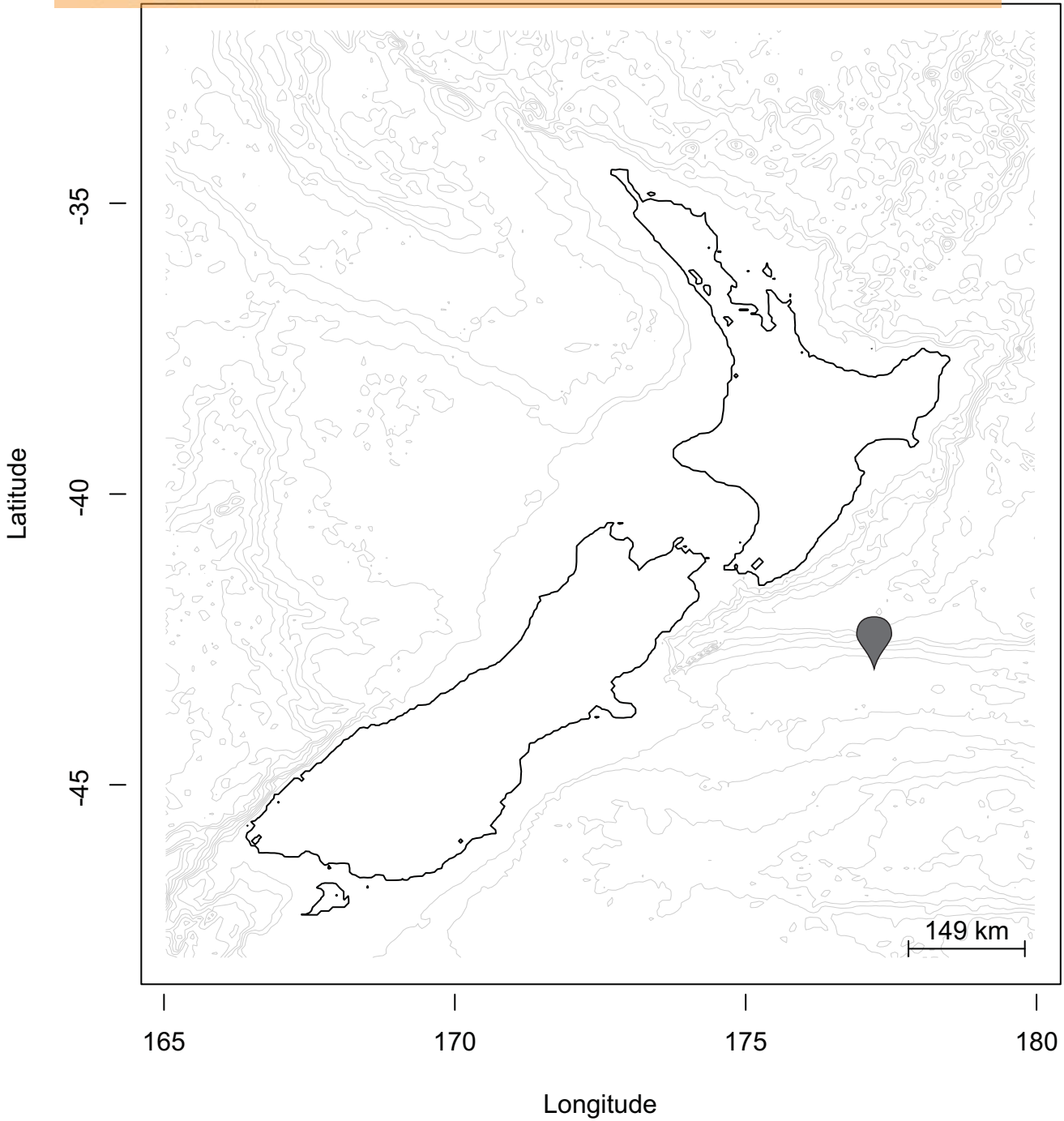

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Figure 2(on next page)

Taxa identified from the NZ scampi diet using Midori and NCBI databases for the COI gene.

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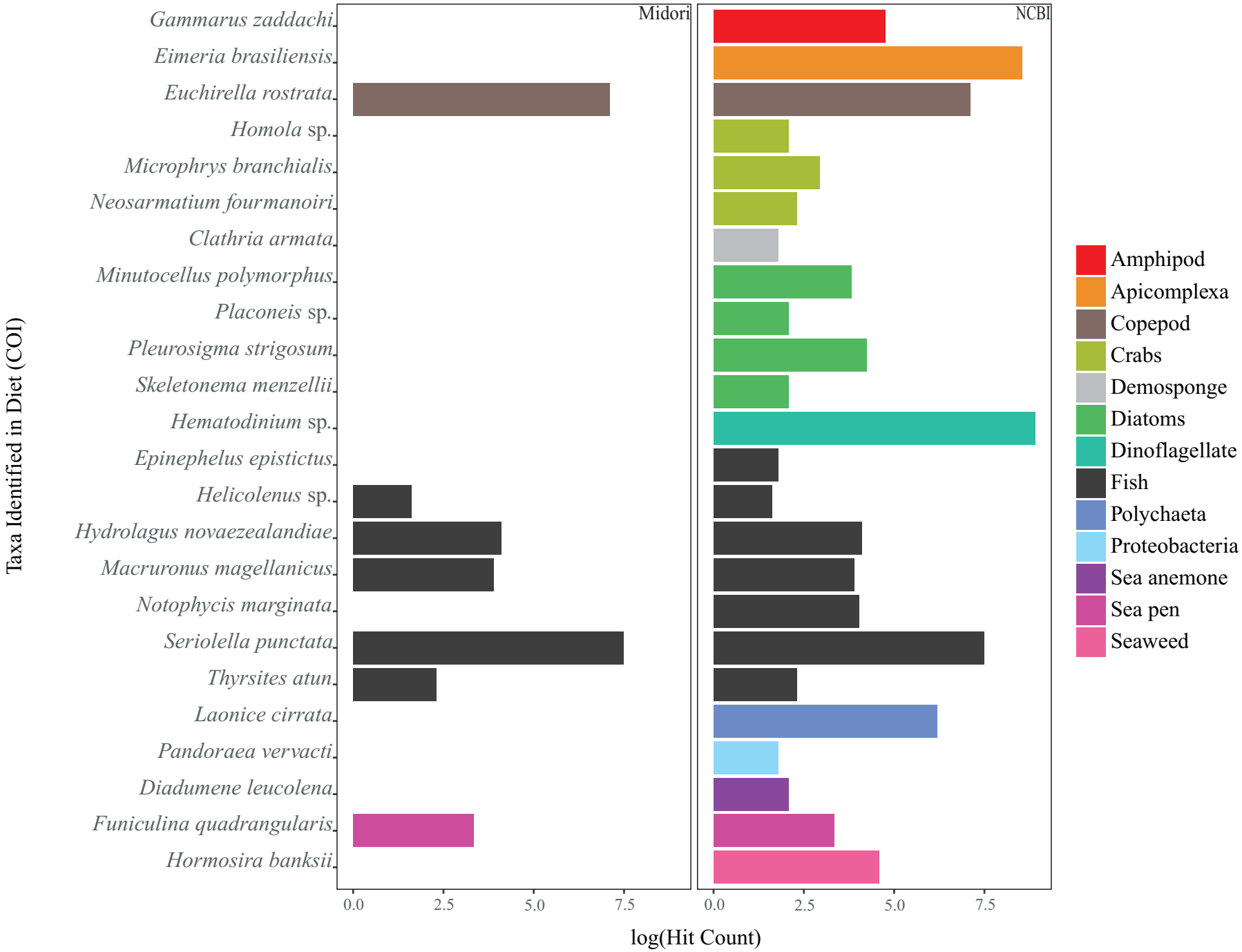

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Figure 3(on next page)

Taxa identified from the NZ scampi diet using PR2, SILVA and NCBI databases for the 18S gene.

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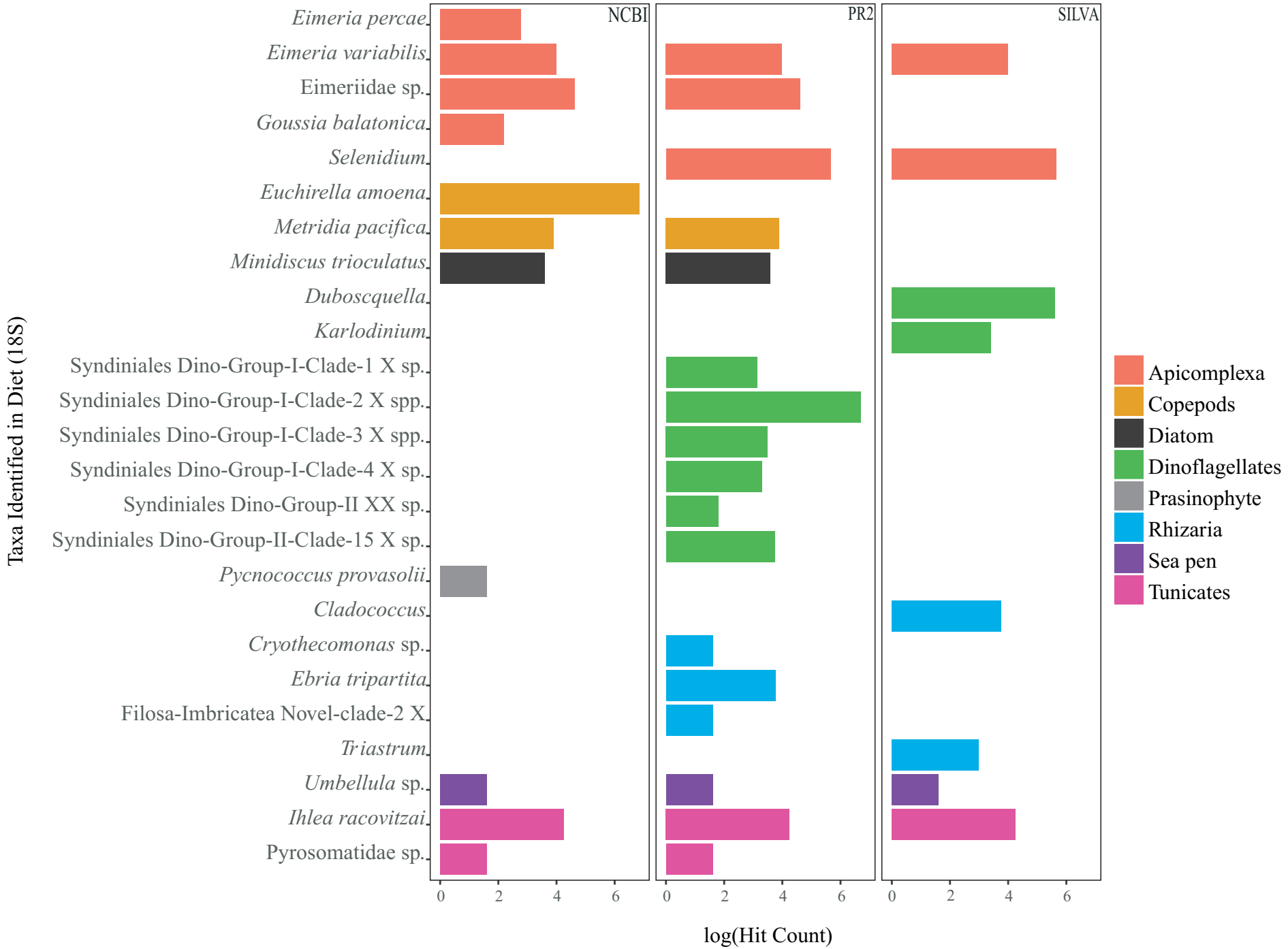

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Table 1 (on next page)

Universal primer pairs adapted with Illumina Nextera™ library transposase adapters, used to target the selected COI and 18S genes.

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Primer Name	Target	Sequence (adapters underlined)
mlCOIintF_NexAd (Forward)	COI 313 bp region	5' TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG GGW ACW GGW TGA ACW GTW TAY CCY CC 3'
jgHCO2198_NexAd (Reverse)	COI 313 bp region	5' GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GTA IAC YTC IGG RTG ICC RAA RAA YCA 3'
Uni18SF_NexAd (Forward)	18S v4 425 bp region	5' TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG AGG GCA AKY CTG GTG CCA GC 3'
Uni18SR_NexAd (Reverse)	18S v4 425 bp region	5' GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GGR CGG TAT CTR ATC GYC TT 3'

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Table 2 (on next page)

Sample selection for DNA metabarcoding.

In total ten samples were sent for DNA metabarcoding, nine selected individuals and one DNA negative control.

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Sample	Individuals	Digesta Source	PCR Reagents	Preservation
1	70.9	Hindgut	Bioline	Ethanol
2	70.9	Foregut	Bioline	Ethanol
3	70.9	Hindgut	Platinum *Taq*	Ethanol
4	70.9	Foregut	Platinum *Taq*	Ethanol
5	70.2	Hindgut	Bioline	Ethanol
6	70.2	Foregut	Bioline	Ethanol
7	70.3	Foregut	Bioline	Ethanol
8	Fro1 and Fro2	Foregut	Bioline	Frozen
9	Fro3	Foregut and Hindgut	Bioline	Frozen
10	DNA Negative	NA	Bioline	NA

1

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Table 3 (on next page)

Comparison of metabarcode results for assigned taxa between Bioline and Platinum *Taq* reactions.

	Taxa Identified in the Bioline Reaction (Diet Hit Count Total[1])	Taxa Identified in the Platinum *Taq* Reaction (Diet Hit Count Total)
Midori/NCBI (COI)	9 (648)	7 (73)
NCBI/PR2/SILVA (18S)	2 (10)	1 (5)
Total Taxa	11 (658)	8 (78)
Total Exclusive[2] Taxa	6 (190)	3 (24)

[1] Diet Hit Count Total: the number of sequence reads matching a potential dietary reference sequence in that database, excluding host matches

[2] Exclusive Taxa: those taxa found only in one category

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Table 4 (on next page)

Comparison of total and exclusive diet taxa identified in the foregut and the hindgut.

Diet hit count is given for the total taxa identified per gut source.

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Database	Total Taxa Identified (Diet Hit Count) in Foregut	Total Taxa Identified (Diet Hit Count) in Hindgut	Exclusive Taxa Identified in Foregut	Exclusive Taxa Identified in Hindgut
Midori/NCBI (COI)	4 (448)	15 (294)	3	14
NCBI/PR2/SILVA (18S)	14 (736)	2 (14)	13	1

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Table 5 (on next page)

Comparison of digesta amplification from ethanol and frozen preserved individuals.

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Database and Preservation	Filtered Hit Count	Diet Hit Count	Diet Hit Count (%)
Midori /NCBI (COI)			
Ethanol	171664	10623	6
Frozen	36691	0	0
NCBI/PR2/SILVA (18S)			
Ethanol	165566	2028	1
Frozen	94218	902	1

1