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Feeding ecology of two deep-sea skates bycaught on demersal longlines off Kerguelen Islands, Southern Indian Ocean

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Abstract :

Elasmobranchs, and particularly skates (Rajiforms), are commonly caught in fisheries worldwide as targeted catch or bycatch. Their life history traits make them particularly sensitive to elevated fishing mortality, especially in slow-growing deep-sea species. Knowledge of their ecology is key to ensure effective conservation and mitigation measures, especially dietary references to understand trophic relationships within the ecosystem. This study presents the first comprehensive description of diet composition and trophic position of two skate species (Bathyraja eatonii and B. irrasa) caught as bycatch in the French Patagonian toothfish (Dissostichus eleginoides) fishery in the Indian sector of the Southern Ocean (Kerguelen waters). Stomach content and stable isotopic analyses were performed on both species according to sex and maturity stage. DNA barcoding analysis was performed on prey tissue samples to identify or confirm identification of prey items found in stomachs. Overall, both skate species were generalist feeders with high δ15N values. The higher δ15N values of B. irrasa result in a higher estimated trophic position than B. eatonii. The diet of B. eatonii mainly consisted of fish, pelagic crustaceans and squids, while B. irrasa preyed more on benthic crustaceans, fish and annelids. While sex had no effect on diet and trophic position for both species, maturity stage influenced the prey composition of B. irrasa. Annelids and isopods were prevalent in the stomachs of immature individuals of B. irrasa, while mature individuals fed more on decapods and fish. Our results revealed a wide range of prey items for these two generalist predators (>57 species). Understanding the diet, trophic position and trophic relationships of B. eatonii and B. irrasa is critical to a better comprehension of the impact of the exploitation of top predators on the subantarctic demersal ecosystem.

Highlights

► First study of feeding ecology of two deep-water skates in Kerguelen waters. ► 73% of prey items identified at the species level using traditional methods and DNA barcoding. ► *Bathyraja eatonii* and *B. irrasa* are generalist predators (>57 species) with high trophic positions. ► Both species feed mostly on benthic preys and the proportion of pelagic preys such as myctophids is higher for *B. eatonii.* ► *B. irrasa* shows ontogenetic shift in feeding ecology related to maturity stage.

Keywords : Trophic niche, Top predators, Bathyraja, Stable isotopes, DNA barcoding, stomach contents

1. Introduction

For the last decades, elasmobranch populations have experienced strong declines in large part due to overfishing (Barbini et al., 2020; Oliver et al., 2015; Worm et al., 2013). Elasmobranchs and especially skates (Rajiforms) are commonly caught in fisheries as targeted but also as non-targeted catch (termed "bycatch") and discarded (Oliver et al., 2015; Stevens et al., 2000). Furthermore, their specific life-history traits – long life span, slow growth and late age at maturity – make them intrinsically sensitive to elevated fishing mortality (Brander, 1981; Davidson et al., 2016; Dulvy et al., 2014; Dulvy and Reynolds, 2002; Field et al., 2009). Bycatch has become an issue for global fisheries management and conservation due to increasing awareness of anthropogenic impacts on the environment (Soykan et al., 2008). Following the holistic approach of ecosystem-based fisheries management, there is increasing pressure on the fishing industry to address the unintended consequences of fishing (Pikitch et al., 2004; European Union, 2013). Managing environmental impacts of fishing is therefore a priority, including improving baseline knowledge on species affected by fisheries (Stobutzki et al., 2001; Tschopp et al., 2020).

An important step in the conservation and management of skate populations is to understand their feeding ecology, which can be done by studying their feeding habits and trophic interactions. Although skates are commonly considered as top predators alongside pinnipeds, seabirds and large teleost fish, important differences in their feeding strategies exist between species, with some skates being considered as generalists and other species as specialist consumers (Cortés, 1999; Barría et al., 2018). Generalist species are likely to be less vulnerable to anthropogenic pressures, such as fishing, because of their wide trophic niche (Barbini et al., 2020; Gallagher et al., 2015; Matich et al., 2011). On the contrary, diet specialization is associated with narrow trophic niche and thus specialist species are likely to be more vulnerable to environmental changes in marine ecosystems, especially if their main prey becomes less abundant in the environment (Barbini et al., 2020). Stomach content analysis is widely used in feeding ecology studies and provides information about the prey species consumed;

76 however, different rates of prey digestion and rare prey items may bias diet determination (Brown et

77 al., 2012). For this reason, stable isotope analysis (SIA) have been used as a complementary method

78 for a more integrative diet study (Matich et al., 2011). In most cases, SIA does not allow prey

79 determination at the species level, but it provides information about trophic pathways through food

80 webs and patterns in resource use (Blanco-Parra et al., 2012; Crook et al., 2019). In marine ecological

81 studies, carbon (δ^{13}C) and nitrogen (δ^{15}N) isotopic values of predator tissues are frequently used. δ^{13}C

82 varies little along the food chain and therefore provides an indication of the primary production source

83 responsible for the energy flow in a food web. In contrast, consumers are enriched in δ^{15}N relative to

84 their food and thus δ^{15}N indicates their relative trophic position within the food web (Cherel and

85 Hobson, 2007; Hussey et al., 2012).

86   Three species of skates are caught as bycatch in the demersal longline fisheries targeting

87 Patagonian toothfish (*Dissostichus eleginoides*) within the area of the Southern Indian Ocean under

88 regulation of the Convention for the Conservation of Antarctic Marine Living Resources (CCAMLR;

89 CCAMLR Secretariat, 2014). Two of them, the Eaton's skate (*Bathyraja eatonii*) and the Kerguelen

90 sandpaper skate (*B. irrasa*), are frequently caught on the slopes of the Kerguelen Plateau (49°S, 69°E),

91 where a French fishery operates between 500 and 2,000 m deep (Nowara et al., 2017; Ziegler et al.,

92 2021). They are large deep-sea skates (up to 115 cm total length (TL) for *B. eatonii* and 130 cm (TL)

93 for *B. irrasa*) with partially overlapping bathymetric ranges (100 – 1,200 m for *B. eatonii* and 800 –

94 1,800 m for *B. irrasa*) and constitute a major component of benthic fish biomass on the Kerguelen

95 Plateau (Duhamel et al., 2019, 2005; Nowara et al., 2017). *B. eatonii* is found on continental shelves

96 and slopes of all subantarctic islands and the Antarctic continent while *B. irrasa* is endemic to the

97 Kerguelen Plateau (Stehmann and Bürkel, 1990). In the highly regulated Kerguelen fishery, measures

98 have been implemented to reduce skate bycatch such as move-on rules, allocation of individual fishing

99 quotas based on the amount of skate bycatch, as well as the provision of support tools for spatial

100 avoidance of high-density areas (TAAF, 2019). Despite these mitigation measures, skate bycatch still

101 represents about 250 tons (5% of total catch) in the Kerguelen longline fishery, of which 90% are

102 released at sea with potentially high survival chance (CCAMLR Secretariat, 2022). Given that their

103 post-released survival is unknown, our ability to assess the impact of the fishery on skate populations

104 and demersal ecosystems remains limited (Valls et al., 2011; Wetherbee and Cortés, 2004).

105   Studying food habits of these two species is one of the prerequisites to understand their

106 ecological role in the ecosystem and subsequently the impact of their exploitation on demersal

107 communities (Blanco-Parra et al., 2012; Brown-Vuillemin et al., 2020; Cailliet et al., 2005; Matich et

108 al., 2011; Navia et al., 2007). Deep-sea skates are often generalists, with diets composed of annelids,

109 crustaceans, cephalopods and fish; but prey group contribution varies with skate size. Many skate

110 species show ontogenetic changes in diet, with small individuals often feeding on crustaceans and

111 large individuals on fish (Barbini et al., 2018; Belleggia et al., 2008; Brown et al., 2012; Koen Alonso,

112 2001; Main and Collins, 2011). Furthermore, ecological niche partitioning can arise among species

113 sharing similar morphology and habitat when they are competing for limited resources, as described

114 in *Bathyraja* species found over the Patagonian shelf, which specialize either on fish, annelids or

115 crustaceans (Tschopp et al., 2020). Understanding the trophic relationship of coexisting species can

116 indicate whether they share similar ecological niches, and whether they should be considered as a

117 functional unit, or should be managed separately (Barría et al., 2018).

118   Unraveling feeding ecology of *B. eatonii* and *B. irrasa* on the Kerguelen Plateau is therefore

119 crucial to assess their vulnerability and the potential ecosystem impact of skate biomass reduction

120 (Bizzarro et al., 2007; Ebert and Bizzarro, 2007). As a leader in ecosystem-based fishery management,

121 CCAMLR emphasizes the need to ensure efficient ecosystem fishery management and biodiversity

122 conservation of skate bycatch species (CCAMLR Secretariat, 2014). Thus, in this study we undertook

123 the first comprehensive study of feeding ecology of *B. eatonii* and *B. irrasa* off the Kerguelen Plateau

124 through stomach content and SIA analyses. The identification of prey species was confirmed using

125 DNA barcoding when muscle tissues were available. Our main objectives were to i) describe diet of

126 *B. eatonii* and *B. irrasa*; ii) test for potential differences in their feeding ecology related to sex and

127 maturity stage, and iii) provide information on their role in the marine food web. Finally, we discuss

128 our results in the context of ecosystem-based management and bycatch mitigation.

129

130 **2. Materials and Methods**

131 *2.1. Data collection*

132 Sampling was carried out onboard the French commercial longliners *Ile de la Réunion II* (March to

133 June 2020, and March to May 2021) and *Cap Kersaint* (March to June 2021) within the Kerguelen

134 Exclusive Economic Zone (EEZ) in the Indian sector of the Southern Ocean (Figures 1.A and 1.B) at

135 depths ranging from 500 to 1600 m (Figures 1.C and 1.D). Stomachs and tissues were collected from

136 dead individuals. All individuals were identified, sexed, weighed and measured (total and pelvic

137 lengths) following guidelines provided by the French Southern Ocean Fishery Observer Program

138 (Gasco, 2011). Following these guidelines, maturity stage was also determined using the internal

139 development of the reproductive system for females: immatures have small and undeveloped ovaries

140 with no visible ovarian follicles, while maturing females present developing follicles, typically small

141 and white, and mature females show big, fully developed ovaries with the presence of large and yellow

142 yolk-filled follicles. Immature males, have undeveloped testes, and claspers smaller than pelvic fins,

143 while maturing males present developing testes associated with noncalcified claspers longer than

144 pelvic fins, and matures show big and fully developed testes with the presence of large lobules with

145 long and fully calcified claspers. Whole stomachs were collected after palpation to minimize the risk

146 of collecting empty stomachs. When a stomach contained food items, strings were tied around the

147 esophagus and the gut before removing it. A total of 101 and 98 stomachs were collected from *B.*

148 *eatonii* and *B. irrasa*, respectively. Tissue samples from the dorsal-anterior muscle of the body were

149 collected for stable isotope analysis from individuals from which stomachs had also been collected. A

150 total of 69 and 73 muscle samples were collected from *B. eatonii* and *B. irrasa*, respectively. Stomachs

151 were stored deep-frozen (-20°C) and tissue samples were kept in ethanol (90%). Location and depth

152 were reported for each capture event.
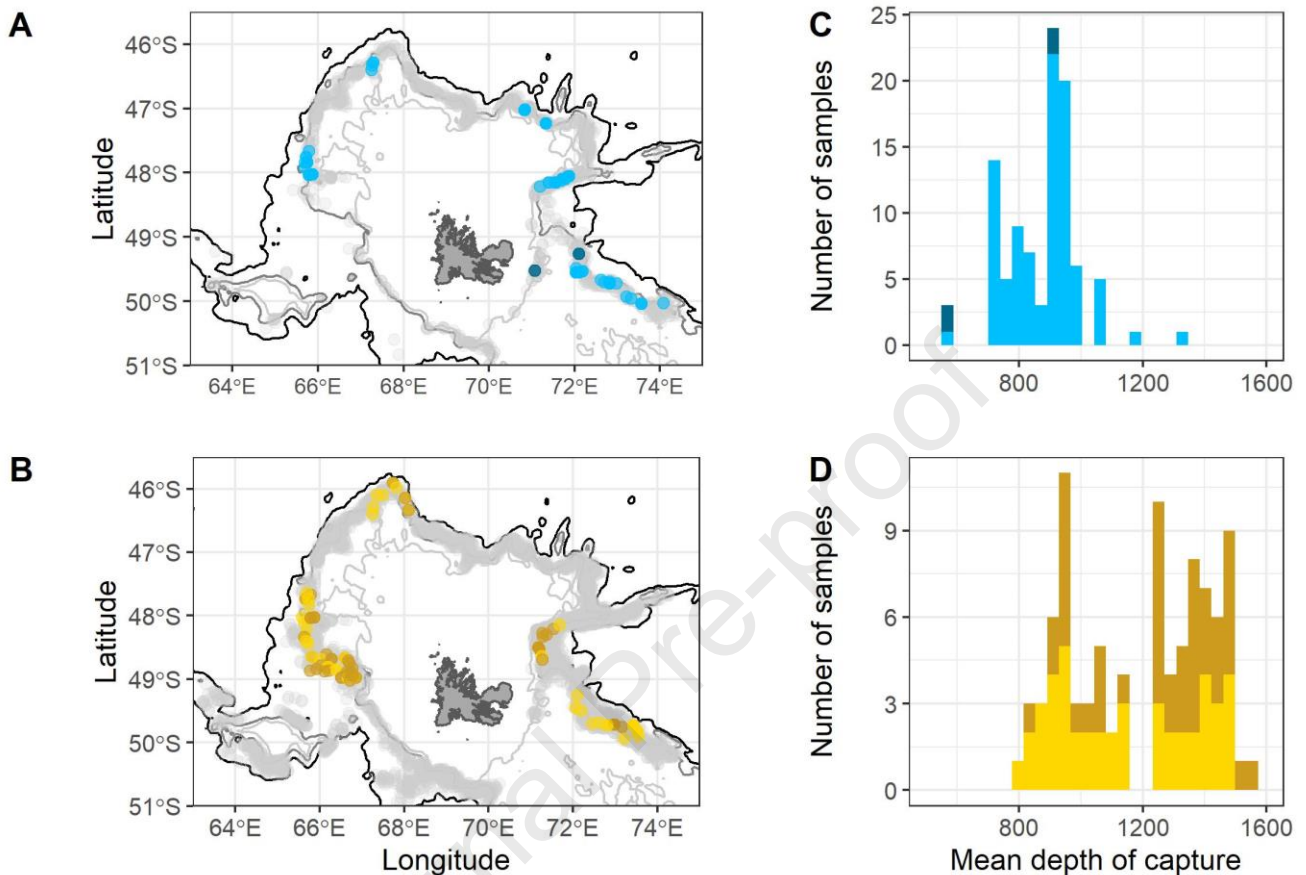
153
154 Figure 1. **(Two-column fitting image)** Location of skate bycatch (grey) and samples collected
155 for A) *Bathyraja eatonii* (in blue) and C) *B. irrasa* (in yellow). Isobaths 500, 1,000 and 2,000 m
156 are indicated on the map. Distribution of mean depth of hauls where samples of B) *B. eatonii* and
157 D) *B. irrasa* were collected. Dark and light shading indicate immature and mature individuals,
158 respectively.

159
160 *2.2. Stomach content analysis*

161 A few empty stomachs were discarded from the analysis (n = 5 for *B. irrasa*). Whole stomachs were

162 thawed and their content was weighed to the nearest 0.01 g using an electronic scale. Accumulated

163 prey items (mainly cephalopod beaks without flesh attached, fish eye lenses and worn otoliths) were

164 subsequently sorted and analyzed separately from fresh prey items (Cherel et al., 2000). Fresh remains

165 were sorted into four prey classes (fish, cephalopods, crustaceans and others) that were weighed to the

166 nearest 0.01 g. If present, the bait (the Atlantic mackerel *Scomber scombrus* that does not occur in the

167 fishing area) was also weighed and counted, but it was not included in further analyses. Identification

168 of prey was based on the examination of the remaining hard structures, including sclerotized beaks for

169 cephalopods, otoliths, jaws and caudal bones for fishes, and exoskeletal remains for crustaceans. Prey

170 items were counted and identified to the lowest taxonomic level using reference materials held in the

171 Centre d'Etudes Biologiques de Chizé (CEBC-CNRS, France) and by reference to the available

172 literature including Williams and McEldowney (1990) and Duhamel et al. (2005) for fish, Boltovskoy

173 (1999) for crustaceans, and Xavier and Cherel (2021) for cephalopods. Species names of cephalopods

174 followed a recent review on Southern Ocean squids that includes taxonomic revisions conducted over

175 the last decades (Cherel, 2020).

176

177   *2.3. DNA barcoding and prey identification*

178 Tissue samples (dorsal muscle in fish and mantle muscle in cephalopods) were collected either from

179 nearly intact and well identified prey items to build up a reference collection, or from digested and

180 undetermined prey items to help identification. Muscle samples (~10 mg) were stored in ethanol (90%)

181 and DNA barcoding was conducted on 47 prey items. DNA was extracted using Qiagen DNA

182 extraction kit (QIAamp DNA Micro Kit). The COI region was amplified using the following primer

183 sets: (a) FishF1 and FishR1; (b) TelF1 and TelR1, (c) LCO1490, HCO2198, mlCOIintF, dgHCO2198

184 (Table A.1). PCR amplifications were performed in a final volume of 20 µL with 0.625 U of Qiagen

185 Taq DNA polymerase containing 3 ng DNA, $1 \times$ reaction buffer, 2.5 mM MgCl2, 0.26 mM dNTP,

186 0.3 mM of each primer, 5% DMSO. The PCR thermal regime consisted of an initial denaturation step

187 of 5 min at 94°C followed by 40 cycles of denaturing (40 s, 94°C), annealing (30 s, 52°C), and

188 elongation (30 s at 72°C) with a final extension step of 5 min at 72°C. PCR products were purified and

189 Sanger sequenced in both directions by Eurofins sequencing (Germany). The obtained sequences were

190 visualized, assembled and edited using Geneious 11.1.5. (Genes Code Corporation - Kearse et al.,

191 2012). A BLASTn search was performed to identify sequences with the best hits from the genomic

192 sequence databases, NCBI and BOLD systems (Altschul et al., 1990; Benson et al., 2005;

193   Ratnasingham and Hebert, 2007). Species identification was confirmed when the degree of similarity

194   between the obtained sequences and reference sequences was higher than 99%.

195

196   *2.4. Diet comparisons*

197   Diet comparisons were conducted using fresh items, meaning that the few stomachs containing

198   accumulated prey items only (n = 3 for *B. eatonii*) were considered empty. In the same way, stomachs

199   containing only the bait were discarded (n = 3 for *B. eatonii,* and n = 1 for *B. irrasa*) and not included

200   in further analyses. Euphausiacea, Mysida and small amphipods such as Gammaridea spp. and

201   *Themisto gaudichaudii* were considered as secondary prey that were incidentally ingested with primary

202   prey, because they are known to be major food items of bathypelagic fish and cephalopods in the

203   Southern Ocean (Cherel and Hobson, 2005; Gaskett et al., 2001). These species were often associated

204   with damaged visceral cavity of their likely predators but this information was not reported. They were

205   consequently not considered as skate prey and excluded from statistical analysis; this was also the case

206   for parasites (Table A.2). Some stomachs containing only incidentally ingested prey or parasites were

207   therefore excluded (n = 4 for *B. eatonii,* and n = 1 for *B. irrasa*). Thus, a total of 91 stomach contents

208   were statistically analyzed for both *B. eatonii* and *B. irrasa*.

209   Prey items were grouped into one of the following seven ecological dietary categories: pelagic

210   fish, benthic and demersal fish, pelagic crustaceans, benthic crustaceans, squids, octopus and worms

211   (including Polychaeta and Priapulida). Cumulative prey curves were constructed using the prey species

212   and prey categories for each skate species against the randomly pooled number of analyzed stomachs

213   to check if a sufficient number of stomach contents had been collected to accurately describe the diet

214   of each predator (Cortés, 1997). Curves were generated after 100 randomizations of the original data

215   using the Vegan Community Ecology package (Oksanen et al., 2020) in R-Studio, version 4.1 (R Core

216   Team 2021). When curves approached an asymptote, it was considered that sufficient stomach samples

217   had been processed to describe the diet. To statistically assess the adequacy of sample size, a linear

218 regression was performed on the final five points of the curve. The levelling off of the prey curve was

219 considered acceptable when the slope was $b < 0.05$ (Brown et al., 2012). To test dietary differences,

220 males and females of each species were separated into maturity classes, with immature individuals

221 separated from maturing and mature individuals (hereafter 'mature individuals').

222   Percent frequencies of occurrence and number of each prey group were calculated for each

223 skate species by sex and maturity stage. Comparisons of diet composition (frequency of occurrence of

224 each prey category) between species, maturity stages and sexes were assessed using nonmetric

225 multidimensional scaling (nMDS) ordination based on Bray-Curtis dissimilarities using species

226 presence/absence data (Brown-Vuillemin et al., 2020). Ordination mean plots were constructed though

227 bootstrap averages (n = 100). We also tested the correlation of environmental variables (longitude,

228 latitude and depth of sampling) with the ordination configuration to investigate environmental drivers

229 for the composition of dietary samples and test for sensibility to sampling. The significance of fitted

230 vectors was assessed using a permutation of environmental variables (n = 999). Analyses of similarities

231 (ANOSIM) also based on Bray-Curtis dissimilarities were used to test the significance of the observed

232 patterns in the NMDS (Somerfield et al., 2021). When a difference was established by ANOSIM, a

233 similarity percentage analysis (SIMPER) was performed to investigate which prey category was

234 mainly responsible for diet differentiation within groups. nMDS, ANOSIM and SIMPER were

235 performed using R-Studio and the Vegan Community Ecology package (Oksanen et al., 2020).

236

237   *2.5. Stable isotope analysis*

238 Muscle samples were dried and powdered before extracting lipids using cyclohexane (C_6H_{12}). Since

239 lipids are depleted in ^{13}C compared to proteins, lipid content can significantly affect the relative

240 abundance of ^{13}C in consumer tissues (Bodin et al., 2007; Hussey et al., 2012; Post et al., 2007).

241 Relative abundance of ^{13}C and ^{15}N were obtained from ~0.5 mg of delipidated samples packed in tin

242 capsules, that were then measured by continuous flow on a Flash EA 2000 elemental analyzer (Thermo

243 Scientific, Milan, Italy) coupled to a Delta V Plus, isotope ratio mass spectrometer (Thermo Scientific,

244 Bremen, Germany) at the Pôle Spectrométrie Océan (Plouzané, France). For reference standards, an

245 in house standard Thermo-Acetanilide, USGS-61 (certified values : $\delta^{15}N$ = -2.87 ± 0.04 ‰ / $\delta^{13}C$ = -

246 35.05 ± 0.04 ‰ and measured values : $\delta^{15}N$ = -2.94 ± 0.07 ‰ / $\delta^{13}C$ = -35.07 ± 0.06 ‰), USGS-62

247 (certified values : $\delta^{15}N$ = +20.17 ± 0.06 ‰ / $\delta^{13}C$ = -14.79 ± 0.04 ‰ and measured values : $\delta^{15}N$ = -

248 20.13 ± 0.12 ‰ / $\delta^{13}C$ = -14.77 ± 0.09 ‰) and USGS-63 (certified values : $\delta^{15}N$ = +37.83 ± 0.06 ‰

249 / $\delta^{13}C$ = -1.17 ± 0.04 ‰ and measured values : $\delta^{15}N$ = +37.84 ± 0.15 ‰ / $\delta^{13}C$ = -1.15 ± 0.0 9 ‰) were

250 used for isotopic corrections and to assign the data to the appropriate isotopic scale. Results were

251 reported in δ unit notation and expressed as parts per thousand relative to international standards

252 (atmospheric N_2 for nitrogen and Vienna-Pee Dee Belemnite for carbon). Analytical precision based

253 on replicate measurements after every six samples using the in house standard Thermo-Acetanilide

254 was < 0.17 ‰ for both $\delta^{13}C$ and $\delta^{15}N$ values. C:N mass ratios were determined from percentage

255 element weights.

256 Values are mean ± SD. Statistical tests were conducted using R-Studio and the Pipe-Friendly

257 Framework for Basic Statistical Tests package (Kassambara, 2021). Normality was assessed using a

258 Shapiro-Wilk test. We evaluated possible differences in $\delta^{13}C$ and $\delta^{15}N$ values between sexes and

259 maturity classes within species and between species using Student's t-test when data were normally

260 distributed; otherwise Mann-Whitney test was used. Assuming a level of significance of 0.05, the null

261 hypothesis was rejected if the *p* value was < 0.05.

262

3. Results

263

264 *3.1. Prey identification*

265 One thousand and fifteen fresh prey items were found in the pooled stomach contents. Most were

266 identified to species level based on morphological identification: 61% for fish (n = 74 prey items),

267 63% for cephalopods (n = 25 prey items) and 71% for crustaceans (n = 473 prey items). Among these

268 specimens, 47 identifications were checked by DNA barcoding. The method failed in nine samples,

269 but it worked on the 38 remaining samples, thus confirming the species identification of 25 fish and

270 five cephalopods. Among morphologically unidentifiable prey items, four fish (*Gymnoscopelus*

271 *piabilis*, *Luciosudis normani*, *Macrourus whitsoni* and *Paraliparis thalassobathyalis)* and four

272 cephalopods (three *Muusoctopus thielei* and one *Filipovia knipovitchi*) were identified through

273 barcoding analysis. At the family level 8.4% (n = 85) of prey items remained undetermined and only

274 one prey item remained unidentified at all taxonomic levels. This remaining unidentified prey item

275 was the only occurrence in the stomach in which it was found; the sample was therefore not included

276 in further analysis (n = 1 for *B. eatonii*).

277

278   *3.2. Diet composition and intraspecific comparison*

279 While stomach contents were rarely empty when analyzed in the laboratory, more than half of the

280 skates sampled onboard were considered to have empty stomachs (based on palpation), and thus were

281 not sampled. Cumulative prey curves indicated sample sizes did not reach asymptote (*b* < 0.05) at the

282 species level but did using prey categories (Fig. A.1 and Fig. A.2). Sample sizes were thus sufficient to

283 describe the overall diet composition of *B. eatonii* and to investigate differences between sex groups,

284 but not sufficient for maturity groups with only four samples within the group of immature individuals

285 (Fig. A.3 and Fig. A.4). For *B. irrasa*, sample sizes were sufficient to describe the overall diet

286 composition at the prey category level, as well as to investigate differences between both sex and

287 maturity groups. Thus, only mature individual diet composition was analyzed for intraspecific

288 differences between sexes for *B. eatonii*, while interspecific differences were explored for mature

289 individuals only.

290

291   *3.2.1. Bathyraja eatonii*

292 A total of 90 individuals (64 females and 26 males) of *B. eatonii* were used for diet composition and
293 intraspecific comparison. *B. eatonii* ranged from 54 to 108 cm TL with only four stomachs collected
294 from immature individuals (Figure 2.A).
295
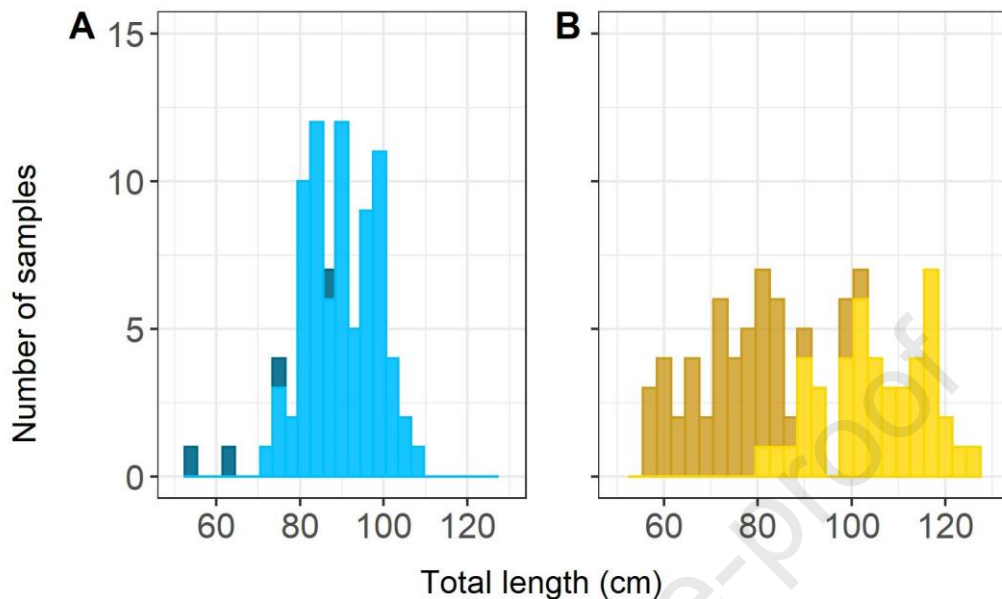
296
297 Figure 2. **(1.5-column fitting image)** Length distribution of individuals of A) *Bathyraja eatonii*
298 (in blue) and B) *B. irrasa* (in yellow). Dark and light shading indicate immature and mature
299 individuals in stacked bars, respectively.
300

301   Stomach content analysis indicated that the diet of *B. eatonii* was dominated by fish, which

302 accounted for 68% of the diet by fresh mass (Table 1). Other significant food sources were crustaceans

303 (18%) and cephalopods (13%), while worms, mostly polychaetas, were negligible (<1% by mass). On

304 average, stomach samples contained 2.4 prey items. For mature individuals only (n = 86), a total of

305 210 fresh prey items from at least 44 species were found. Crustaceans were predominant, occurring in

306 55 food samples (64%) and the most prevalent by number (54% - Table 2). Fish and cephalopods

307 occurred in 54 and 14 dietary samples (63% and 16% respectively) and ranked second and third by

308 number (35% and 9%, respectively).

309   *Bathyraja eatonii* mature individuals fed on a large diversity of crustaceans (11 species), with

310 the isopod *Ceratoserolis trilobitoides* being the most important crustacean prey (30% by number).

311 Other crustacean prey items were the decapod *Campylonotus capensis* (7%) and specimens from the

312 genus *Pasiphaea* (5%). The second most important group by number was fish, with 25 species. Two

13

313   families dominated the fish diet. Myctophids ranked first (7% by number, at least 6 different species),

314   with *Gymnoscopelus piabilis* being the most abundant species (2%). Paralepidids were also common

315   prey (7%), but their high level of digestion precluded identifying them to the species level, except for

316   two individuals of *Magnisudis prionosa*. Nonmetric multidimensional scaling (nMDS) ordinations and

317   statistical analyses were unable to identify differences in dietary composition between females and

318   males for *B. eatonii* (ANOSIM, global *R* statistic 0.03, p = 0.151 – Figure 3.A).

319   Analysis of accumulated beaks increased the number of cephalopod prey, with the

320   onychoteuthid *Filipovia knipovitchi* being predominant (n = 5). Accumulated beaks added two taxa to

321   the skates' diets, namely the ommastrephid *Todarodes* sp. (n = 2) and the histioteuthid *Histioteuthis*

322   *eltaninae* (n = 3). Fish accumulated items included five individuals from counting eye lenses, and

323   seven individuals from analyzing worn otoliths, four of which were morid fish.

324
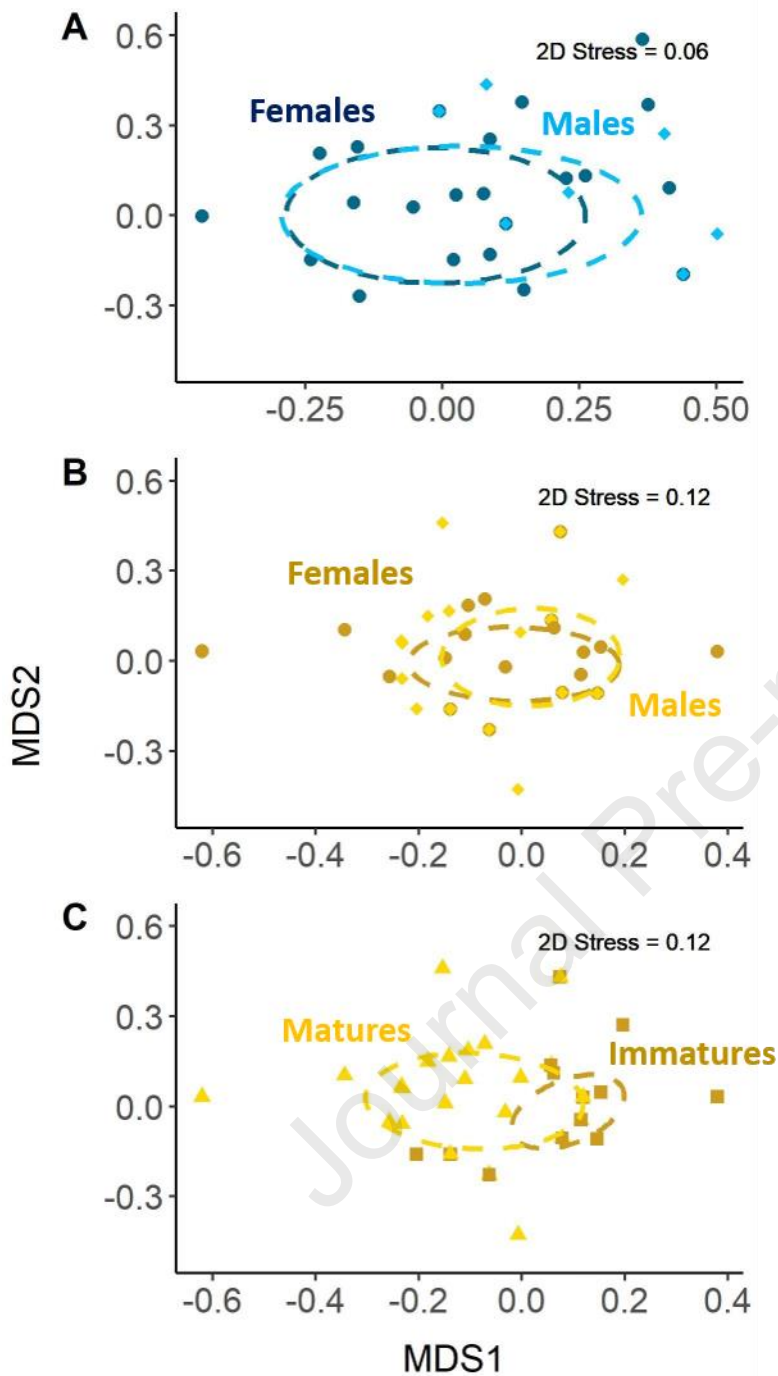
325
326 Figure 3. **(Two-column fitting image)** Nonmetric multidimensional scaling (nMDS) ordinations
327 of the occurrence of different prey categories in dietary samples of: A) *Bathyraja eatonii* with
328 S.E. ellipses based on sex and *B. irrasa* with B) S.E. ellipses based on sex and C) S.E. ellipses based
329 on maturity. Females are in dark shades (plain circles) and males in light shades (diamonds).
330 Immature individuals are in dark shades (square) and matures in light shades (triangles).
331

332

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15

334   3.2.2. *Bathyraja irrasa*

335 A total of 91 individuals (49 females and 42 males) of *B. irrasa* were used for diet composition and

336 intraspecific comparison. *B. irrasa* ranged from 56 to 125 cm TL (Figure 2.B).

337   According to stomach content analysis, the diet of *B. irrasa* was dominated by crustaceans and

338 cephalopods, which accounted for 45% and 33% by fresh mass of the diet, respectively (Table 1).

339 Other significant food sources were fish (19%), while worms were only minor prey items (3%). In

340 average, stomach samples contained 8.8 prey items. A total of 797 fresh prey items from at least 39

341 prey species were found. Prey items were dominated by crustaceans (548 prey items) occurring in 81

342 food samples (89%) and ranked first by number (69%). Worms ranked second by occurrence and

343 number (63% by occurrence and 23% by number) and fish ranked third (37% by occurrence and 6%

344 by number).

345   *Bathyraja irrasa* fed on a large diversity of crustaceans (13 species), of which isopods were the

346 main prey taxon. Statistical analyses were unable to identify differences in the dietary composition

347 between females and males (ANOSIM, global *R* statistic -0.005, *p* < 0.571 – Figure 3.B). However,

348 nMDS ordinations indicated dietary segregation between immature and mature individuals (n = 46 and

349 n = 45) (ANOSIM, global *R* statistic 0.134, *p* < 0.001 – Figure 3.C). The most important prey species

350 was the isopod *Brucerolis bromleyana*, although they were found in greater occurrence and abundance

351 in immature (70% and 35%, respectively) than mature skates (33% and 26%). Other isopods were

352 recorded almost exclusively in the diet of immature individuals, namely *Sursumura* sp. (57% and 21%)

353 and *Ceratoserolis trilobitoides* (15% and 8%). In contrast, the decapod *Thymopides grobovi* was less

354 frequently found in the diet of immature (17%) than mature individuals (58%). This is also highlighted

355 by the SIMPER test showing that benthic crustaceans (including Isopoda) had a substantial

356 contribution to the dietary difference between maturity groups (SIMPER, 16% among

357 immature/mature – Figure 4). The second highest group in abundance was Polychaeta, of which two

358 families were particularly abundant in the overall diet, Polynoidae and Nephtyidae. Polychaeta were

16

359   more frequently encountered in the diet of immature individuals (72% and 48% respectively) than

360   mature individuals (29% and 20%). This difference in stomach contents composition between

361   immature and mature individuals is also supported by the SIMPER test showing that worms were

362   responsible for the major difference in diet composition between maturity groups (SIMPER, 19%

363   among immature/mature). *B. irrasa* mature individuals also fed on a large diversity of fish (n = 19

364   species) along with the octopuses *Muusoctopus thielei* and *Graneledone gonzalezi* (7% by number),

365   in contrast with immature individuals. *Depth* was a significant vector (r^2=0.15, $p < 0.005$) explaining

366   the ordination of samples as a function of the axis MDS2.
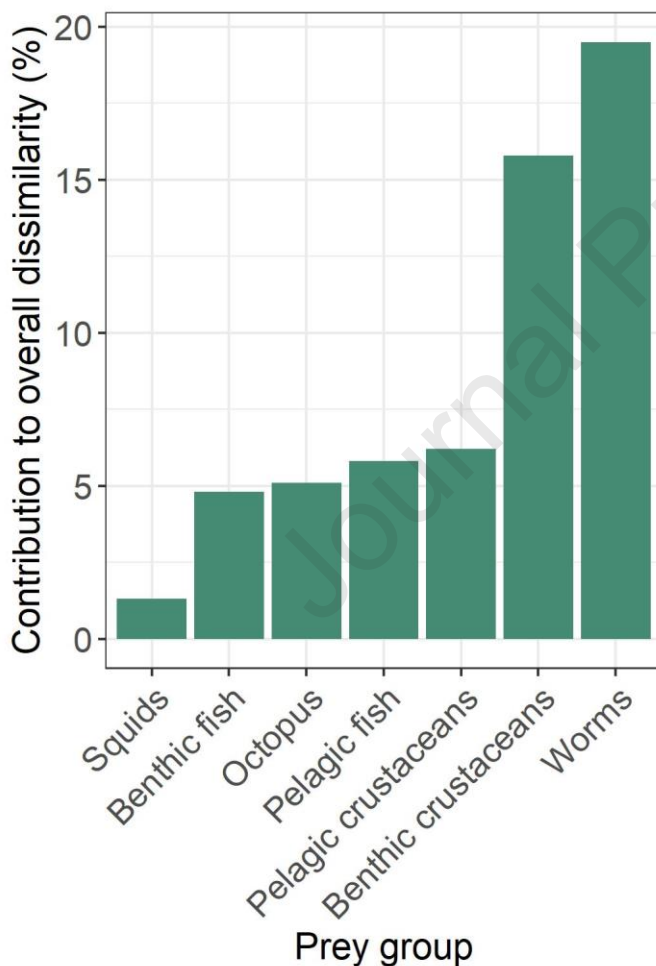
367
368   Figure 4. **(Single-column fitting image)** Average contribution of prey groups to overall
369   dissimilarity (%) between mature and immature individuals of *Bathyraja irrasa.*
370

371   The analysis of accumulated prey items increased the number of cephalopod prey, with three

372   additional squid species (*Todarodes* sp., *Gonatus antarcticus* and *Galiteuthis glacialis*), which

17

373   totalized 10 of the 13 accumulated beaks. Analysis of accumulated fish items included nine prey found

374   by counting pairs of eye lenses and vertebral remains, and 15 prey identified by heavily worn otoliths

375   that include those of an additional myctophid species, *Gymnoscopelus microlampas* (n = 1). Samples

376   also contained a small gastropod and a foraminifera, as well as pebbles and mud that were considered

377   incidentally ingested.

378

379   *3.3. Interspecific diet comparison*

380   Although analysis of mass composition showed *B. eatonii* fed primarily on fish (68%), while *B. irrasa*

381   preyed primarily on crustaceans and cephalopods (45% and 33% respectively – Table 1), both nMDS

382   ordinations and statistical analyses were unable to identify any differences in dietary composition

383   between the two skate species (ANOSIM, global *R* statistic 0.062, *p* < 0.009 – Figure 5).

384   Environmental variables were fitted to the nMDS ordination and *Depth* appeared to be a significant

385   vector (r^2=0.13, *p* < 0.01) explaining the ordination of samples as a function of the axis MDS2 while

386   *Longitude* explained the ordination of samples as a function of the axis MDS1 (r^2=0.09, *p* < 0.01).
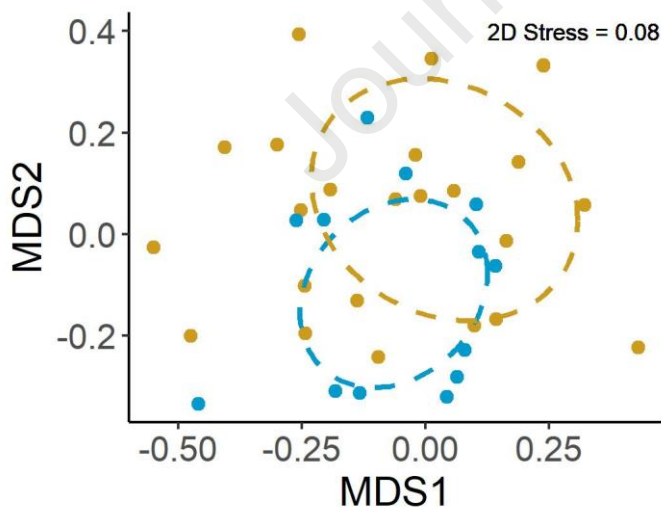
387
388   Figure 5. **(Single-column fitting image)** Nonmetric multidimensional scaling (nMDS)
389   ordinations of the occurrence of different prey categories with S.E. ellipse for *Bathyraja eatonii*
390   (blue) and *B. irrasa* (yellow*).*
391

18

392   *3.4. Stable isotopes*

393   *Bathyraja eatonii* isotopic values ranged from -20.4 to -17.5 ‰ (mean = -19.1 ± 0.6 ‰) for

394     $\delta^{13}C$, and from 9.4 to 12.9 ‰ (mean = 11.1 ± 0.8 ‰) for $\delta^{15}N$ (Table 3). Muscle isotopic values showed

395   no differences between sex classes for $\delta^{13}C$ and $\delta^{15}N$ values (Figure 6.A), but $\delta^{15}N$ values differed

396   between maturity classes (t-test$_{67}$ = -3.1, $p < 0.004$; Table 4). Immature individuals had lower mean

397     $\delta^{15}N$ values than mature individuals (Figure 6.B).

398   *Bathyraja irrasa* isotopic values ranged from -20.4 to -17.5 ‰ (mean = -19.2 ± 0.8 ‰ for $\delta^{13}C$)

399   and from 9.6 to 13.8 ‰ (mean = 12.3 ± 0.8 ‰) for $\delta^{15}N$ (Table 3). One muscle sample was an outlier

400   ($\delta^{13}C$ = -23.13 ‰ and $\delta^{15}N$ = 7.01 ‰), which was therefore removed from the analysis. Muscle isotopic

401   composition between males and females showed no differences for $\delta^{13}C$ and $\delta^{15}N$ values (Figure 6.C).

402   However, $\delta^{13}C$ values differed between maturity classes (U-test = 365, $p < 0.002$; Table 4). Immature

403   individuals had lower mean $\delta^{13}C$ values than mature individuals (Figure 6.D).

404

405   Muscle $\delta^{15}N$ values, but not $\delta^{13}C$ values, differed between species (U-test = 764, $p < 0.005$; Table 4)

406   with *B. eatonii* having lower $\delta^{15}N$ values than *B. irrasa* (Figure 7).

407
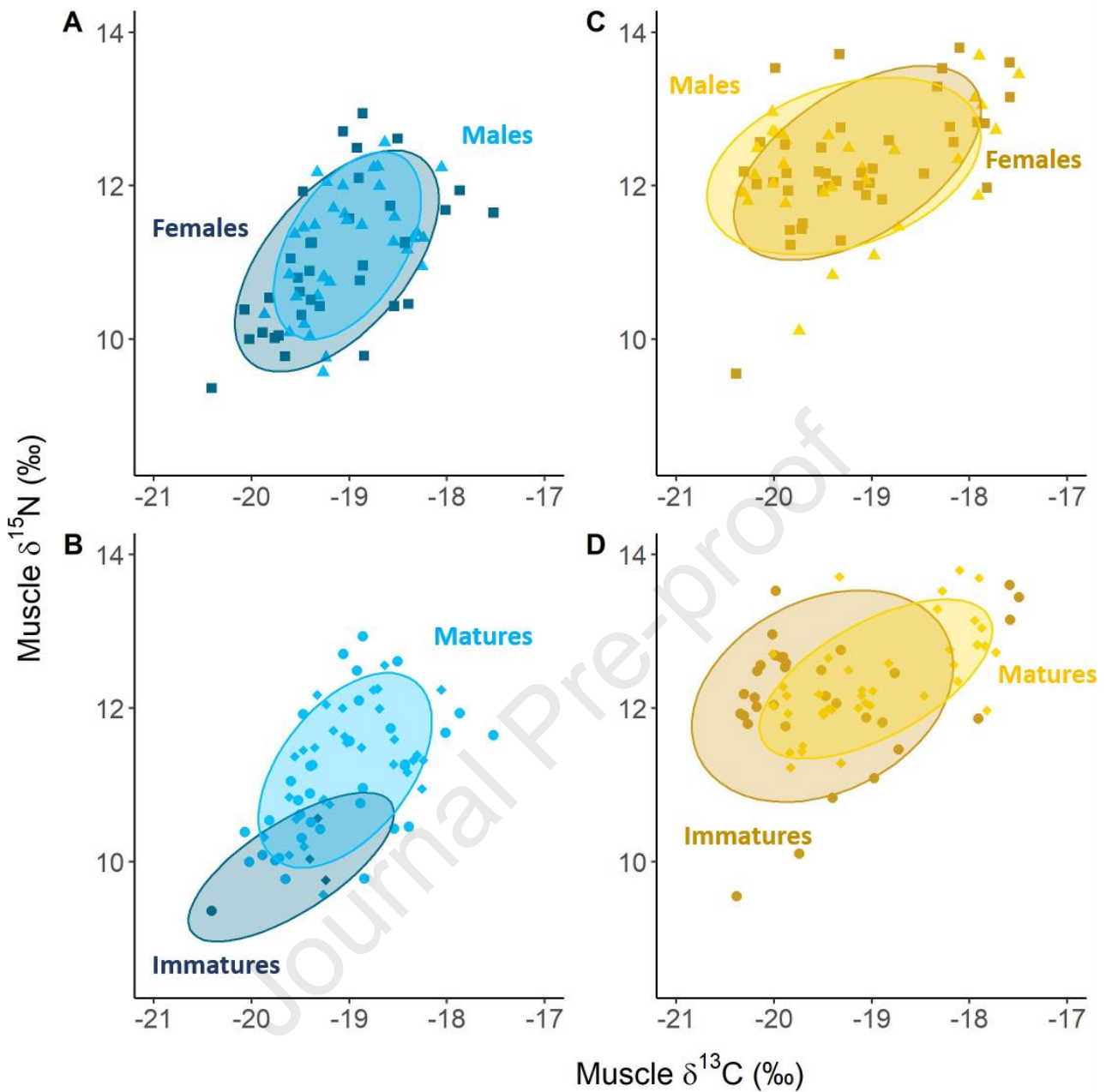
19

Figure 6. **(Two-column fitting image)** Muscle $\delta^{15}N$ and $\delta^{13}C$ values and ellipse areas (70% of the data) of *Bathyraja eatonii* based on A) sex and B) maturity and *B. irrasa* based on C) sex and D) maturity. Females are in dark shades (square) and males in light shades (triangles). Immature individuals are in dark shades (plain circles) and matures in light shades (diamonds).

20
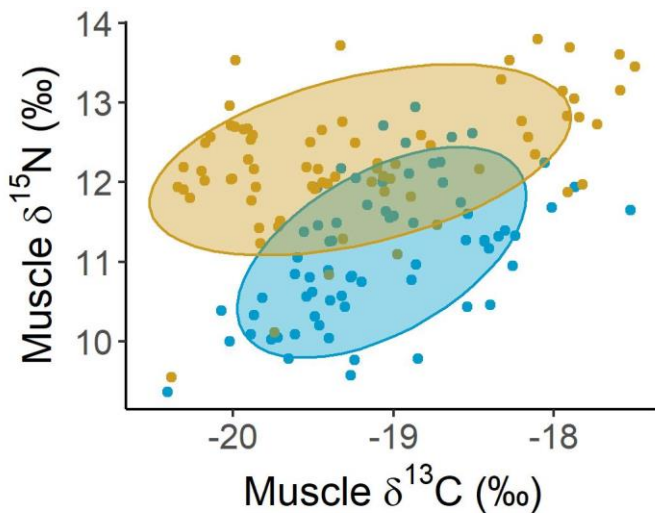
416
417 Figure 7. **(Single-column fitting image)** Muscle δ^{15}N and δ^{13}C values and ellipse areas (70% of
418 the data) of *Bathyraja eatonii* (blue) and *B. irrasa* (yellow).
419

4. Discussion

421 The present study is the first to investigate the feeding ecology of two skate species commonly caught

422 as bycatch in the French toothfish fishery occurring at the Kerguelen Islands. The majority of prey

423 (73%) were identified to the species level, thus allowing a precise description of the diet of *Bathyraja*

424 *eatonii* and *B. irrasa.* The use of barcoding analysis on prey tissues confirmed the morphological

425 identification and allowed identifying well-digested prey items. We acknowledge that demersal

426 longlines engender the risk of stomach washout during hauling, and thus may result in a potential

427 underestimation of prey biomass, as well as introducing bias into the prey composition results (Barnett

428 et al., 2013; Boyle et al., 2012). Nevertheless, the combination of stomach content and stable isotope

429 analyses revealed interspecific differences in the trophic niches of the two skate species, and

430 ontogenetic differences in the main feeding strategies used by different life stages of *B. irrasa*.

431

4.1. Interspecific diet comparison

433 The wide variety of prey found in stomach contents (> 58 species) indicated that both *B. eatonii* and

434 *B. irrasa* can be considered as generalist feeders (Tschopp et al., 2020). Diets were mainly composed

435 of crustaceans, fish and annelids, which is consistent with previous studies on the feeding ecology of

21

436   other skate species in subantarctic areas (Brickle et al., 2003; Main and Collins, 2011). Although diets

437   of both species were composed of the same prey groups, the proportions of mass, number and

438   occurrence of each prey group varied among skate species, as well as their specific composition. By

439   mass, fish dominated *B. eatonii* diet whereas crustaceans and cephalopods were most prevalent in the

440   diet of *B. irrasa*. Crustaceans were the main prey group by number. Interestingly, crustacean species

441   composition varied amongst skate species: *B. eatonii* diet was dominated in number by one benthic

442   isopod (*Ceratoserolis trilobitoides*) and two pelagic shrimps (*Campylonotus capensis* and *Pasiphaea*

443   sp.), while *B. irrasa* diet was dominated by different species of benthic isopods (*Brucerolis*

444   *bromleyana* and *Sursumura* sp.) and one species of benthic decapod (*Thymopides grobovi*). These

445   results showed that *B. eatonii* fed on both benthic and pelagic prey, whereas *B. irrasa* fed almost

446   exclusively on benthic prey. This difference was also supported by the importance of other pelagic

447   prey such as myctophid and paralepidid fishes as well as squids in *B. eatonii* diet, whereas other major

448   components of *B. irrasa* diet were benthic and demersal prey such as polychaetas and octopuses. In

449   addition, the occurrence of mud and small pebbles was observed in some *B. irrasa* stomach contents,

450   while none were observed in *B. eatonii* samples, which could be explained by differences in feeding

451   habitat (mud vs rocky bottom substratum). Inter-specific variations in prey composition and prey

452   habitat (pelagic vs benthic) have indeed been well documented in previous studies on feeding habits

453   of co-occurring skate species (Brickle et al., 2003; Tschopp et al., 2020). Unexpectedly statistical

454   analysis of diet composition did not provide evidence for different feeding strategies between *B. irrasa*

455   and *B. eatonii*. However, given the broad prey categories used and that this analysis is based on prey

456   species presence/absence data, variability observed in the abundance of prey species consumed may

457   be masked. Furthermore, the benthic feeding strategy of *B. irrasa* was not supported by muscle δ^{13}C

458   values integrating diet over several months, which showed similar values to *B. eatonii*. In contrast,

459   δ^{15}N value of *B. irrasa* were higher in comparison to *B. eatonii,* which typically reflects a higher

460   trophic position and/or stronger affinity to benthic food webs found to be more enriched in ^{15}N than

22

461   pelagic food web, especially in the deep-sea (Boyle et al., 2012). Differences in ^{15}N values between

462   the two species therefore confirm different feeding strategies.

463   Depth was found to explain a small proportion of the dissimilarities observed between the two

464   skate species. While their distribution overlaps, *B. eatonii* favors the continental shelf and upper slope

465   areas, whereas *B. irrasa* mainly inhabits slopes at greater depths (Duhamel et al., 2005). Hence,

466   variation in diet compositions may be related to the abundance of prey species among the depth ranges.

467   For example, myctophids are pelagic fish known to make important diel vertical migrations; they could

468   be trapped at the bottom during the day, thus vertically connecting the pelagic and benthic food webs

469   in slope areas (Cotté et al., 2022; Drazen and Sutton, 2016; Hunt and Swadling, 2021). Videos made

470   using cameras attached to longlines set on slopes deeper than 1000 m showed myctophids sitting on

471   the sea floor (Pers. Com.) revealing their accessibility to benthic feeders such as skates. Hence

472   partitioning food or habitat resources may be a strategy to avoid direct competition between these two

473   congeneric skate species (Barbini and Lucifora, 2012; Barría et al., 2018).

474   Finally, we observed inter-specific variations in the number of ingested prey, the extent of prey

475   digestion, and the texture of gastric fluids during stomach content analysis, which suggest differences

476   in digestive systems and thus foraging habits (Cyrino et al., 2008). The high number of both

477   accumulated fish and prey items in each stomach of *B. irrasa* (8.8 in average) compared to *B. eatonii*

478   (2.4 in average) supported the hypothesis of greater digestion capability in *B. irrasa* due to its benthic

479   feeding strategy. Crustaceans are indeed harder to digest compared to cephalopods, which are in turn

480   harder to digest than fish (Cyrino et al., 2008). This variation in digestion rates among species would

481   also lead to a potential underestimation of easily digested prey species such as fishes in *B. irrasa,* and

482   an overestimation of prey more resistant to digestion such as crustaceans in *B. eatonii* (Barnett et al.,

483   2013; Boyle et al., 2012).

484

485   *4.2. Intraspecific diet comparisons*

486   Several studies conducted in different species of skates found no significant differences between male

487   and female diets (Blanco-Parra et al., 2012; Brown-Vuillemin et al., 2020). Similarly, *B. eatonii* and

488   *B. irrasa* did not display diet differentiation related to sex, and both sexes occupy similar trophic niches

489   on the Kerguelen Plateau according to SIA analyses, although the arrangement of teeth in the jaw is

490   different between males and females (Pers. Com.).

491   Nevertheless, many authors have documented variability in skate diet composition according to

492   maturity stages, indicating ontogenetic shifts in their feeding habits (Barbini and Lucifora, 2012;

493   Belleggia et al., 2008; Brown-Vuillemin et al., 2020). In this study, we found an effect of maturity

494   stage on *B. irrasa* diet: immature individuals fed mostly on small benthic crustaceans and annelid

495   worms, while mature individuals preyed more upon large crustaceans, teleost fish and cephalopods.

496   This result was consistent with other studies carried out on subantarctic skate diets such as *B.*

497   *albomaculata*, *B. brachyurops* and *B. griseocauda* (Brickle et al., 2003). This ontogenetic variation

498   could be explained by an improved ability to capture larger-sized prey with increased size of the jaw

499   and swimming speed, or changes in habitat and prey availability (Belleggia et al., 2008; Blanco-Parra

500   et al., 2012; Munroe et al., 2022; Orlov, 2003). The latter explanation is unlikely, since immature and

501   mature *B. irrasa* were found in similar habitats. Apart from the predator size, another explanation

502   could be changes in energetic and physiological needs associated to the cost of sexual maturity, with

503   fishes having a higher energy density than crustaceans and annelids (Munroe et al., 2022; Schaafsma

504   et al., 2018). Such variation in diet composition is typically associated with variation in trophic

505   position, with small individuals preying on crustaceans considered as secondary consumers, whereas

506   larger individuals preying on fish and cephalopods being tertiary consumers (Belleggia et al., 2008).

507   Immature and mature *B. irrasa* showed different δ^{13}C values, thus indicating varying sources of

508   primary production such as benthic/pelagic trophic chains. They would therefore be part of two

509   different trophic chains. In contrast, SIA showed no difference in δ^{13}C values for *B. eatonii*, thus

510 allowing a direct comparison of the $\delta^{15}N$ values of immature and mature skates that suggested an

511 ontogenetic shift with a higher position of mature individuals. However, the small number of immature

512 *B. eatonii* highlights the need to collect additional immature specimens, especially outside the fishing

513 area (particularly on the Plateau < 500 m) where they live.

514

515   *4.3. Skates in the Kerguelen marine food web*

516 Our results supported the hypothesis that skates should be considered as top predators (Barbini et al.,

517 2018; Brown-Vuillemin et al., 2020; Ebert and Bizzarro, 2007). Adult individuals of *Bathyraja* species

518 found on the slopes of the Kerguelen Plateau have similar diets to other benthic predators such as the

519 Patagonian toothfish. They also share some prey items with seabirds around Kerguelen Islands, such

520 as fish (Nototheniidae, Myctophidae), cephalopods (*Moroteuthopsis longimana*, *G. antarcticus*,

521 *Slosarczykovia circumantarctica*), shrimps, isopods and annelids (Delord et al., 2010; Lescroël et al.,

522 2004). Despite the lack of detailed studies on the diet of Patagonian toothfish over the Kerguelen

523 Plateau, studies from elsewhere in the Southern Ocean indicated the species fed on high proportions

524 of meso- and bathy-pelagic fish such as myctophids (including *Gymnoscopelus* spp.), and morids and

525 macrourids, cephalopods (including *G. antarcticus*), crustaceans and polychaetes (Duhamel, 1987;

526 Goldsworthy et al., 2001; Troccoli et al., 2020). When comparing their stable isotopics values, skates

527 showed lower mean trophic position ($\delta^{15}N_{B. eatonii} = 11.1‰$ and $\delta^{15}N_{B. irrasa} = 12.3‰$) than toothfish

528 ($\delta^{15}N_{D. eleginoides} > 14.0‰$ (Cherel et al., 2017). However, comparing isotopic niches requires caution,

529 because retention of urea in the body tissue of elasmobranchs may complicate interpretations of stable

530 isotope values. Since urea is enriched in ^{14}N compared to proteins, $\delta^{15}N$ values of skates and sharks

531 tend to be lower than those of bony fishes, which can result in an underestimation of their trophic

532 position (Carlisle et al., 2017; Kim and Koch, 2012). Some skates were found in Patagonian toothfish

533 diet, suggesting that skates and toothfish could have predator-prey interactions within an exploited

534 ecosystem (Duhamel, 1987). Thus, a decline in the abundance of one of these predator species through

25

535 overfishing could trigger an increase in the abundance of the other species through the related release

536 of competition (Barbini and Lucifora, 2012; Brown et al., 2012). Because little is known about trophic

537 interactions resulting from the effects of fishing on skates, dietary composition and trophic information

538 of these bycatch species are essential to understand their role in the ecosystem in order to forecast the

539 possible ecosystem-level effects of fisheries on species composition and diversity (Bizzarro et al.,

540 2007; Brown et al., 2012; Stevens et al., 2000; Subramaniam et al., 2022, 2020). The results of this

541 study support the growing body of knowledge that indicates that crustaceans, cephalopods and

542 myctophids are important prey items for marine predators in the Southern Ocean, particularly over the

543 Kerguelen Plateau that sustains a high diversity of predators in a wide range of habitats (Cherel, 2020;

544 Doubleday et al., 2016; Hulley and Duhamel, 2011; Jackson et al., 1998; Yang et al., 2022).

545

546 *4.4. Catchability and implications for skate bycatch reduction*

547 Relative vulnerability to fishing can be assessed through various parameters related to diet (e.g. feeding

548 strategy), as well as the extent to which skates may be attracted to fishing gear, all of which impact

549 catchability. Because no evidence of bites on prey were found in the diet composition analysis, we

550 assumed that both skate species used suction to capture their prey whole. In addition, both species

551 were found to display partially or fully benthic feeding strategies making them susceptible to longline

552 gear. Our results are thus useful in informing technical improvements to fishing gear, such as on

553 longlines, which would reduce skate catchability (Afonso et al., 2011). Floating snoods enable the

554 hook to float above the sea floor instead of being laid on it, and consequently can allow skates to avoid

555 longlines while foraging on the substrate, as the fully-benthic *B. irrasa*.

556 In addition, we sometimes found more than one bait in stomach contents, suggesting that skates

557 could forage on baits without being captured systematically. Furthermore, *Antimora rostrata* and

558 *Macrourus* sp., which are the most common teleost fish bycatch in the toothfish fishery, also occurred

559 in the diet of skates, suggesting that they could feed on bycaught fish during the soaking time of the

560 line (Barnett et al., 2013; Duhamel et al., 2005). Although *Dissostichus eleginoides* was absent from

561 the diet of both skate species, such interactions with fishing lines would increase skate catchability in

562 the fishery (Georgeson et al., 2020; Stobutzki et al., 2001).

563

564 **5. Conclusion**

565   This study provides a comprehensive analysis of the feeding habits of *Bathyraja eatonii* and *B.*

566 *irrasa* over the Kerguelen Plateau. As top predators and among the 10 most abundant demersal fish

567 species there (Duhamel et al., 2019), significant changes in skate population dynamics due to fishing

568 mortality could influence the structure of the food web. Our results will contribute to ecosystem models

569 being developed in the region by increasing our understanding of deep-sea food webs and predator-

570 prey interactions over the Kerguelen Plateau (Clavareau et al., 2020; Subramaniam et al., 2022).

571 Interspecific differences in diet may be the consequence of competition between these generalist

572 species, but may also reflect differences in the abundance of prey by depth. These disparities in feeding

573 ecology emphasize the importance of considering species separately for conservation programs when

574 designing solutions to reduce catch rates or catchability. Possible competition and niche overlap

575 between skates and toothfish also stress the importance of fully describing toothfish diets over the

576 Kerguelen Plateau, and quantifying skate bycatch mortality to be able to predict its ecosystem impacts.

577

591

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885 Tables

886 Table 1. Mass (mean [min – max]) and composition of the food in stomach contents of *Bathyraja*
887 *eatonii* and *B. irrasa* in Kerguelen waters.

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	Bathyraja eatonii (n = 90)	*Bathyraja irrasa* (n = 91)
Mass (g)	28.0 [0.3 – 330.2]	36.6 [1.1 -213.3]
Fresh prey items (g)	27.9 [0.2 – 330.2]	36.5 [0.1 -213.3]
Fish (g)	14.1 [0 – 330.2]	6.0 [0 – 87.9]
Cephalopods (g)	2.6 [0 – 179.1]	10.2 [0 – 213.3]
Crustaceans (g)	3.7 [0 – 26.4]	14.0 [0 – 89.6]
Worms (g)	0.03 [0 – 1.3]	0.9 [0 – 13.5]
Bait (g)	7.2 [0 – 71.9]	5.4 [0 – 53.5]
Accumulated cephalopods (n)	19	13
Accumulated fishes (n)	12	24
Overall composition by mass (all samples pooled):		
Fish (%)	68.1	19.4
Cephalopods (%)	12.8	32.7
Crustaceans (%)	18.1	44.9
Other organisms (%)	0.1	3.0

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Table 2. Frequency of occurrence and numbers of prey items identified in stomach contents from all 90 samples of *Bathyraja eatonii* pooled and 91 samples of *B. irrasa* pooled, excluding secondary prey.

Species	Bathyraja eatonii								Bathyraja irrasa							
	Immature (M=1, F=3)				Mature (M=25, F=61)				Immature (M=24, F=22)				Mature (M=18, F=27)			
	Occurence		Number		Occurence		Number		Occurence		Number		Occurence		Number	
	(n)	(%)	(n)	(%)	(n)	(%)	(n)	(%)	(n)	(%)	(n)	(%)	(n)	(%)	(n)	(%)
FISH	**3**	**75.0**	**4**	**57.2**	**54**	**62.8**	**73**	**34.8**	**14**	**30.4**	**17**	**2.8**	**20**	**44.4**	**28**	**14.0**
Pelagic fish																
Centrolophidae																
Icichthys australis	-	-	-	-	1	1.2	1	0.5	-	-	-	-	-	-	-	-
Gempylidae																
Paradiplospinus gracilis	-	-	-	-	2	2.3	2	1.0	1	2.2	1	0.2	2	4.4	2	1.0
Melamphaidae																
Poromitra atlantica	-	-	-	-	1	1.2	1	0.5	1	2.2	1	0.2	1	2.2	1	0.5
Microstomatidae																
Nansenia antarctica	-	-	-	-	1	1.2	1	0.5	-	-	-	-	-	-	-	-
Myctophidae																
Electrona carlsbergi	-	-	-	-	2	2.3	2	1.0	3	6.5	3	0.5	2	4.4	3	1.5
Gymnoscopelus bolini	-	-	-	-	2	2.3	2	1.0	1	2.2	1	0.2	-	-	-	-
Gymnoscopelus braueri	-	-	-	-	-	-	-	-	1	2.2	1	0.2	-	-	-	-
Gymnoscopelus hintonoides	-	-	-	-	2	2.3	2	1.0	-	-	-	-	-	-	-	-
Gymnoscopelus microlampas	-	-	-	-	1	1.2	1	0.5	-	-	-	-	-	-	-	-
Gymnoscopelus nicholsi	-	-	-	-	-	-	-	-	-	-	-	-	1	2.2	1	0.5
Gymnoscopelus piabilis	1	25.0	1	14.3	5	5.8	5	2.4	-	-	-	-	2	4.4	2	1.0
Protomyctophum bolini	-	-	-	-	1	1.2	1	0.5	-	-	-	-	1	2.2	1	0.5
Unidentified Myctophidae	-	-	-	-	1	1.2	1	0.5	-	-	-	-	-	-	-	-
Notosudidae																
Luciosudis normani	-	-	-	-	1	1.2	1	0.5	-	-	-	-	-	-	-	-
Paralepididae																
Magnisudis prionosa	1	25.0	1	14.3	1	1.2	2	1.0	1	2.2	1	0.2	3	6.67	3	1.5
Unidentified Paralepididae	-	-	-	-	10	11.6	12	5.7	1	2.2	1	0.2	-	-	-	-
Stomiidae																
Borostomias antarcticus	-	-	-	-	1	1.2	1	0.5	-	-	-	-	-	-	-	-
Unidentified Stomiidae	-	-	-	-	1	1.2	1	0.5	-	-	-	-	1	2.2	1	0.5

Benthic and demersal fish

Achiropsettidae																
Unidentified Achiropsettidae	-	-	-	-	1	1.2	1	0.5	-	-	-	-	-	-	-	-
Bathydraconidae																
Bathydraco antarcticus	-	-	-	-	2	2.3	3	1.4	-	-	-	-	-	-	-	-
Liparidae																
Paraliparis thalassobathyalis	-	-	-	-	1	1.2	1	0.5	-	-	-	-	-	-	-	-
Paraliparis sp.	-	-	-	-	2	2.3	2	1.0	2	4.4	3	0.5	1	2.2	1	0.5
Moridae																
Antimora rostrata	-	-	-	-	2	2.3	2	1.0	1	2.2	1	0.2	1	2.2	1	0.5
Guttigadus kongi	-	-	-	-	1	1.2	2	1.0	-	-	-	-	-	-	-	-
Unidentified Moridae	-	-	-	-	1	1.2	1	0.5	-	-	-	-	-	-	-	-
Macrouridae																
Coryphaenoides armatus	-	-	-	-	-	-	-	-	-	-	-	-	1	2.2	1	0.5
Coryphaenoides ferrieri	-	-	-	-	-	-	-	-	-	-	-	-	1	2.2	1	0.5
Macrourus carinatus	-	-	-	-	1	1.2	1	0.5	-	-	-	-	3	6.7	3	1.5
Macrourus whitsoni	-	-	-	-	1	1.2	1	0.5	-	-	-	-	-	-	-	-
Muraenolepididae																
Muraenolepis marmoratus	2	50.0	2	28.6	3	3.5	3	1.4	-	-	-	-	-	-	-	-
Nototheniidae																
Lepidonotothen squamifrons	-	-	-	-	-	-	-	-	-	-	-	-	1	2.2	1	0.5
Zoarcidae																
Lycenchelys hureaui	-	-	-	-	1	1.2	1	0.5	3	6.5	4	0.7	-	-	-	-
Lycodapus antarcticus	-	-	-	-	3	3.5	3	1.4	-	-	-	-	-	-	-	-
Melanostigma gelatinosum	-	-	-	-	1	1.2	1	0.5	-	-	-	-	1	2.2	1	0.5
Unidentified fish	-	-	-	-	14	16.3	15	7.1	-	-	-	-	4	8.9	5	2.5
CEPHALOPODS	**1**	**25.0**	**1**	**14.3**	**14**	**16.3**	**19**	**9.0**	**3**	**6.5**	**3**	**0.5**	**12**	**26.7**	**17**	**8.5**
Squids																
Brachioteuthidae																
Slosarczykovia circumantarctica	-	-	-	-	3	3.5	3	1.4	-	-	-	-	-	-	-	-
Cranchiidae																
Taonius notalia	-	-	-	-	-	-	-	-	1	2.2	1	0.2	-	-	-	-
Gonatidae																
Gonatus antarcticus	-	-	-	-	1	1.2	1	0.5	-	-	-	-	1	2.2	1	0.5

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Onychoteuthidae																
Filippovia knipovitchi	-	-	-	-	2	2.3	2	1.0	1	2.2	1	0.2	1	2.2	1	0.5
Moroteuthopsis longimana	1	25.0	1	14.3	3	3.5	3	1.4	-	-	-	-	-	-	-	-
Moroteuthopsis sp. B (Imber)	-	-	-	-	1	1.2	1	0.5	-	-	-	-	-	-	-	-
Mastigoteuthidae																
Mastigoteuthis psychrophila	-	-	-	-	1	1.2	1	0.5	-	-	-	-	-	-	-	-
Octopus																
Octopodidae																
Graneledone gonzalezi	-	-	-	-	-	-	-	-	-	-	-	-	2	4.4	3	1.5
Muusoctopus thielei	-	-	-	-	1	1.2	1	0.5	-	-	-	-	5	11.1	5	2.5
G. gonzalezi / M. thielei	-	-	-	-	4	4.7	6	2.9	-	-	-	-	4	8.9	5	2.5
Opisthoteuthidae																
Opisthoteuthis sp.	-	-	-	-	-	-	-	-	-	-	-	-	1	2.2	1	0.5
Unidentified cephalopods	-	-	-	-	1	1.2	1	0.5	1	2.2	1	0.2	1	2.2	1	0.5
CRUSTACEANS	**1**	**25.0**	**2**	**28.6**	**55**	**64.0**	**113**	**53.8**	**43**	**93.5**	**425**	**71.2**	**38**	**84.4**	**123**	**61.5**
Pelagic crustaceans																
Amphipoda																
Pegohyperia princeps	-	-	-	-	1	1.2	1	0.5	-	-	-	-	-	-	-	-
Caridea																
Campylonotus capensis	-	-	-	-	9	10.5	14	6.7	1	2.2	1	0.2	1	2.2	2	1.0
Chorismus antarcticus	-	-	-	-	1	1.2	1	0.5	-	-	-	-	-	-	-	-
Chorismus tuberculatus	-	-	-	-	1	1.2	1	0.5	-	-	-	-	-	-	-	-
Nematocarcinus romenskyi	-	-	-	-	2	2.3	2	1.0	2	4.4	2	0.3	4	8.9	4	2.0
Nematocarcinus sp.	-	-	-	-	-	-	-	-	-	-	-	-	1	2.2	1	0.5
Pasiphaea ledoyeri	-	-	-	-	4	4.7	5	2.4	1	2.2	1	0.2	3	6.7	3	1.5
Pasiphaea rathbunae	-	-	-	-	3	3.5	3	1.4	1	2.2	1	0.2	2	4.4	2	1.0
Pasiphaea scotiae	-	-	-	-	-	-	-	-	-	-	-	-	2	4.4	2	1.0
Pasiphaea sp.	-	-	-	-	3	3.5	3	1.4	-	-	-	-	1	2.2	1	0.5
Unidentified Natantia	-	-	-	-	1	1.2	1	0.5	-	-	-	-	1	2.2	1	0.5
Lophogastrida																
Neognathophausia gigas	-	-	-	-	-	-	-	-	1	2.2	1	0.2	1	2.2	1	0.5
Benthic crustaceans																
Anomura																
Paralomis sp.	-	-	-	-	-	-	-	-	-	-	-	-	1	2.2	1	0.5

Isopoda																
Brucerolis bromleyana	-	-	-	-	2	2.4	2	1.0	32	69.6	210	35.2	15	33.3	51	25.5
Ceratoserolis trilobitoides	1	25.0	2	28.6	25	29.1	63	30.0	7	15.2	46	7.7	2	4.4	2	1.0
Sursumura sp.	-	-	-	-	-	-	-	-	26	56.5	125	20.9	3	6.7	3	1.5
Unidentified Isopoda	-	-	-	-	1	1.2	1	0.5	2	4.4	14	2.4	3	6.7	4	2.0
Reptantia																
Thymopides grobovi	-	-	-	-	2	2.3	2	1.0	8	17.4	12	2.0	26	57.8	38	19.0
Unidentified crustaceans	-	-	-	-	13	15.1	14	6.7	9	19.6	12	2.0	7	15.6	7	3.5
WORMS	-	-	-	-	**3**	**3.5**	**5**	**2.4**	**39**	**84.8**	**152**	**25.5**	**18**	**40.0**	**32**	**16.0**
Polychaeta																
Unidentified Nephtyidae	-	-	-	-	-	-	-	-	22	47.8	52	8.7	9	20.0	11	5.5
Unidentified Nereidae	-	-	-	-	-	-	-	-	2	4.4	2	0.3	-	-	-	-
Unidentified Polynoidae	-	-	-	-	3	3.5	5	2.4	33	71.7	81	13.6	13	28.9	18	9.0
Unidentified Polychaeta	-	-	-	-	-	-	-	-	4	8.7	9	1.5	1	2.2	1	0.5
Priapulida																
Priapulus tuberculaspinosus	-	-	-	-	-	-	-	-	6	13.0	8	1.3	1	2.2	2	1.0
TOTAL	-	-	**7**	**100**	-	-	**210**	**100**	-	-	**597**	**100**	-	-	**200**	**100**

Table 3. Sample size (n) and mean (± standard deviation) of muscle isotopic values of *Bathyraja eatonii* and *B. irrasa* for immature, mature and all individuals sampled.

Species	Groups	n	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	C : N
Bathyraja eatonii	males	34	-19.02 ± 0.48	11.22 ± 0.77	3.18 ± 0.04
	females	35	-19.13 ± 0.66	11.01 ± 0.91	3.17 ± 0.04
	immature	4	-19.60 ± 0.55	9.93 ± 0.50	3.15 ± 0.02
	mature	65	-19.04 ± 0.57	11.19 ± 0.81	3.18 ± 0.05
	all	**69**	**-19.07 ± 0.58**	**11.12 ± 0.85**	**3.18 ± 0.04**
Bathyraja irrasa	males	32	-19.29 ± 0.89	12.26 ± 0.73	3.19 ± 0.06
	females	40	-19.15 ± 0.80	12.30 ± 0.80	3.20 ± 0.07
	immature	33	-19.50 ± 0.85	12.16 ± 0.87	3.19 ± 0.07
	mature	39	-18.96 ± 0.76	12.38 ± 0.66	3.20 ± 0.06
	all	**72**	**-19.21 ± 0.84**	**12.28 ± 0.77**	**3.20 ± 0.06**

Table 4. Statistical results for isotopic differences between species and within species depending on sex and maturity for *Bathyraja eatonii* and *B. irrasa*. Student's t-test (t-test$_{df}$) was used when test assumptions were met; otherwise Mann-Whitney (U-test) was used. Significant results are indicated in bold, $p < 0.05$.

	Groups		Statistic	p-value
	Species	$\delta^{13}C$	U-test = 2899	p > 0.08
		$\delta^{15}N$	**U-test = 764**	**p < 0.005**
Bathyraja eatonii	Sex	$\delta^{13}C$	t-test$_{67}$ = -0.78	p > 0.4
		$\delta^{15}N$	t-test$_{67}$ = -0.99	p > 0.3
	Maturity	$\delta^{13}C$	U-test = 74	p > 0.15
		t-test$_{67}$ = -3.07		**p < 0.004**
Bathyraja irrasa	Sex	$\delta^{13}C$	U-test = 732	p > 0.3
		$\delta^{15}N$	U-test = 651	p > 0.9
	Maturity	$\delta^{13}C$	**U-test = 365**	**p < 0.002**
		$\delta^{15}N$	U-test = 557	p > 0.3

Table A.1. Primers used for the amplification and sequencing of the COI gene.

Primers	Sequences (5'-3)	References
TelF1	TCGACT AATCAYAAAGAYATYGGCAC	Dettai et al., 2011
TelR1	ACTTCTGGGTGNCC AAARAATCARAA	Dettai et al., 2011
FishF1	TCAACCAACCACAAAGACATTGGCAC	Ward et al., 2005
FishR1	TAGACTTCTGGGTGGCCAAAGAATCA	Ward et al., 2005
mlCOIintF1	GGWACWGGWTGAACWGTWTAYCCYCC	Leray et al., 2013
DgHCO2198	TAAACTTCAGGGTGACCAAARAAYCA	Leray et al., 2013

Table A.2. Frequency of occurrence and numbers of incidentally ingested prey items (including secondary prey items, indicated by an asterisk (*)) identified from stomach contents for all 90 samples of *Bathyraja eatonii* pooled and 91 samples of *B. irrasa* pooled.

	Bathyraja eatonii								Bathyraja irrasa							
	Immature (M=1, F=3)				Mature (M=25, F=61)				Immature (M=24, F=22)				Mature (M=18, F=27)			
	Occurence		Number		Occurence		Number		Occurence		Number		Occurence		Number	
	(n)	(%)	(n)	(%)	(n)	(%)	(n)	(%)	(n)	(%)	(n)	(%)	(n)	(%)	(n)	(%)
CRUSTACEANS																
Amphipoda																
Gammaridea spp.*	-	-	-	-	1	1,14	1	0,39	12	26,09	29	4,49	2	4,44	2	0,96
*Themisto gaudichaudii**	-	-	-	-	4	4,55	6	2,36	-	-	-	-	-	-	-	-
Unidentified Amphipoda*	-	-	-	-	1	1,14	1	0,39	2	4,35	5	0,77	-	-	-	-
Copepoda																
Paraeuchaeta sp.*	-	-	-	-	-	-	-	-	2	4,35	2	0,31	-	-	-	-
Unidentified Pennellidae	-	-	-	-	-	-	-	-	-	-	-	-	1	2,22	1	0,48
Euphausiacea																
*Euphausia triacantha**	-	-	-	-	9	10,23	13	5,12	2	4,35	3	0,46	3	6,67	3	1,44
*Euphausia vallentini**	-	-	-	-	-	-	-	-	1	2,17	1	0,15	-	-	-	-
Euphausia sp.*	-	-	-	-	6	2,82	6	2,36	-	-	-	-	-	-	-	-
Isopoda																
Unidentified Flabellifera	-	-	-	-	2	2,27	2	0,79	4	8,70	4	0,62	1	2,22	1	0,48
Unidentified Gnathiidae	-	-	-	-	-	-	-	-	-	-	-	-	1	2,22	1	0,48
Mysida																
Unidentified Mysidacea*	-	-	-	-	10	11,36	16	6,30	5	10,87	5	0,77	1	2,22	1	0,48

Highlights :

- First study of feeding ecology of two deep-water skates in Kerguelen waters.

- 73 % of prey items identified at the species level using traditional methods and DNA barcoding.

- *Bathyraja eatonii* and *B. irrasa* are generalist predators (> 57 species) with high trophic positions.

- Both species feed mostly on benthic species and the proportion of pelagic preys such as myctophids and squids is higher for *B. eatonii.*

- *B. irrasa* shows ontogenetic shift in feeding ecology related to maturity stage.

Declaration of interests

☐ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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