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1   **Intraspecific differences in the diet of Kuril harbor seals (*Phoca vitulina stejnegeri*) in Erimo, Hokkaido, using**

2   **DNA barcoding diet analysis**

3

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17

18   **Abstract**

19   Competition between Kuril harbor seals (*Phoca vitulina stejnegeri*) and salmon set-net fishing industries has become a

20   serious problem with the recent increase in the number of seals in Erimo, Hokkaido, Japan. We aimed to understand the

21   detailed dietary structure of Kuril harbor seals focusing on intraspecific differences and verify whether "problem seals"

22   who habitually use salmon set-nets could be characterized by intrinsic factors such as sex and maturity. We estimated the

23   diet of Kuril harbor seals in two fishing seasons using DNA barcoding diet analysis on colon contents and verified

24   intraspecific differences in their diet. In spring, their diets showed different tendencies between maturity stage; each adult

25   seal fed on different prey items, suggesting that they avoid the intra-species competition over food during the breeding

26   season. Additionally, it was implied that some adult females habitually stole from salmon set-nets. Our dietary analysis

27   showed dietary changes of Kuril harbor seals with different tendencies depending on maturity or sex, suggesting that

28   problem individuals who habitually use set-nets can be characterized by intrinsic factors. This detailed dietary information

29   can offer an accurate assessment of seal predation effects on fishing targets and the selective management of Kuril harbor

30   seals, especially in mitigating seal–commercial fishery conflicts.

31

32   **Keywords:** mitochondrial COI, NGS, food habit, seal-fishery conflict

33

2

Introduction

Competition between pinnipeds and coastal fisheries occurs in various places worldwide (e.g. Yodzis 1998; Lance et al. 2012). Fishing grounds in shallow areas could be easy foraging sites for pinnipeds who can learn to steal fish from fishing gear such as set-nets, gillnets, and longlines. In coastal areas where pinnipeds reside throughout the year, such conflicts cause more serious problems, as is the case in Hokkaido, Japan.

Kuril harbor seals (*Phoca vitulina stejnegeri*) are the only year-round resident pinniped in Japan. They are distributed on the eastern coastline of Hokkaido, Japan (Shaughnessy and Fay 2009; Kobayashi et al. 2014), and Cape Erimo is their largest haul-out site (Fig. 1). They show a high level of philopatry and use the same rocky shore throughout the year. The competition between the seals and coastal fisheries has recently become a serious problem due to the increase in seal numbers in Erimo. The most part of damage to the fishery is damage to catches of salmon set net such as biting off the head, abdomen and other parts of the salmon (Fig. 2), which results in economic loss as the damaged catch becomes worthless as a commodity. Salmon set-net fishing operates twice a year in spring (May to early July) and fall (September to early November) in Erimo. Although depredation by seals is observed in both seasons, the scale of the fishery and the damage are greater in the fall. The economic impact of seal depredation on salmon in set-net fishing is especially prominent (Hokkaido Government 2015). Previous study has suggested that most seals do not tamper with fishing gear; generally, individual "problem seals" cause most of the damage to salmon set-net fishing (Masubuchi et al. 2017). However, it is challenging to verify this hypothesis and characterize these individuals because the available information on their diets has been limited.

Diet estimations on Kuril harbor seals in this area have mainly been conducted through stomach content analysis (the

53 Ministry of Environment, Japan 2017). However, this analytical method is likely to yield inaccurate results because prey

54 species identification from undigested hard parts (e.g., fish otoliths or bones and cephalopod beaks) is highly biased due

55 to rapid and differential prey digestion (Bowen 2000). Salmon otoliths are smaller and easier to digest than otoliths of other

56 fish; therefore, few of them remain in the stomach as undigested substances (Jobling and Breiby 1986; Boyle et al. 1990).

57 Additionally, Kuril harbor seals do not swallow salmon whole, but they bite parts of the salmon body (Fig. 2). Therefore,

58 in most cases, salmon otoliths are rarely ingested by seals even if seals eat salmon. Previous studies note that salmonids

59 were rarely detected as undigested substances even though the stomach samples were collected from seals caught by salmon

60 set-nets as bycatch (the Ministry of Environment, Japan 2017).

61   In order to deal with this problem, we used DNA barcoding analysis to accurately identify prey organisms. DNA

62 barcoding (Herbert and Gregory, 2005) is a powerful tool to analyze the community structure of a sample containing

63 various intermingled organisms (e.g. Deagle et al., 2009; Tollit et al., 2009). Organisms are identified on the basis of short

64 base sequences in particular gene regions, called the "DNA barcode," reflecting differences between species. With this

65 technique, researchers can detect and identify prey organisms with high accuracy via amplicon sequencing on prey-derived

66 DNA in predator feces. Prior to this study, Hui et al. (2017) showed that DNA barcoding techniques are more accurate than

67 hard parts techniques in detecting salmonids in fecal samples. Therefore, we reassessed the diet of Kuril harbor seals using

68 DNA barcoding analysis.

69   We also verified intraspecific differences in the diet of the Kuril harbor seals. Some researchers indicated that harbor

70 seal food habits varied depending on age and sex (e.g. Lewis et al. 2006; Beck et al. 2007). Such variation in diet could be

71 due to foraging ability, prey preference, or both (Smith and Metcalfe 1997; Bundy et al. 2000). Differences in energy

72   demands based on sexual body size dimorphism or breeding costs might also influence prey preference (Beck et al. 2003;

73   Breed et al. 2006). If such intraspecific differences in food habits occur in Kuril harbor seals in Erimo, we might be able

74   to characterize the sex and maturity of the specific individuals damaging the salmon set-nets. Therefore, we compared their

75   diet with intrinsic factors (e.g., sex and maturity) in each fishing season.

76

77   **Materials and methods**

78   *Field sampling*

79   We collected dietary samples from Kuril harbor seals that were caught by the salmon set-nets as bycatch during the

80   spring (late May to early July) and fall (late August to early November) fishing seasons in 2014–2017 in Erimo, Hokkaido,

81   Japan. We also collected samples from seals caught by seal trap nets in the spring of 2017 under the Erimo Area Kuril

82   Harbor Seal Specified Rare Wildlife Management Plan (Ministry of Environment, Japan 2018). In order to trap seals, we

83   used salmon set-net with grid nets at the entrance of a bag net. These grids are 20 cm on a side, allowing salmon to pass

84   through, but not seals. However, in one place, one square is 60 cm or 80 cm long, and a funnel-shaped net is attached so

85   that if a seal enters it, it cannot get out (Ministry of Environment, Japan 2017). Bodyweight was measured, and sex was

86   recorded. Age was determined based on a count of cementum growth layers (Mansfield and Fisher, 1960) of the seals'

87   upper right canine teeth after sectioning 10–16 μm and staining by Delafield's hematoxylin (Kobayashi et al. unpublished).

88   Kuril harbor seals undergo secondary growth at age 4–5 years, and almost all individuals start breeding at age 5 (Suzuki

89   and Yamashita 1986). Thus, we classified their maturity into three levels: pups (age 0), juveniles (age 1–4), and adults (age

90   ≥ 5).

91   Samples for dietary analysis were collected as the contents of approximately 15 cm of the colon, which was removed

92   from the anal side of each harbor seal (hereafter called "feces"). The colon samples were collected from dead seals within

93   12 hours of death and kept in a freezer (−20°C) until they were used for analysis. Feces were taken from the colon sample

94   and mixed well; then 0.15–0.43 g soft feces component (i.e., without the undigested hard parts) was used for DNA

95   barcoding diet analysis.

96

97   *DNA barcoding diet analysis*

98   DNA extraction from feces was conducted using the QIAamp DNA Stool Mini Kit (QIAGEN Inc.). All steps followed

99   the "Isolation of DNA from Stool for Human DNA Analysis" protocol recommended by the manufacturer (QIAamp DNA

100   Stool Handbook, June 2012) with slight modification (i.e., the amount of Buffer AE for DNA elution at the last step was

101   set to 100 µL). The concentration of each extracted DNA sample was measured and adjusted to 10 ng/µL (hereafter, these

102   samples are called "template DNA").

103   Mitochondrial COI was chosen as the barcoding region for prey species identification (Hebert et al. 2003), because Kuril

104   harbor seals are thought to prey on various animals such as fish, cephalopods, and crustaceans. We prepared an amplicon

105   library for next-generation sequencing using the two-step tailed PCR method. In step 1, amplification of the target region

106   was performed with a combination of the forward primer *ml intF* (5′-ACA CTC TTT CCC TAC ACG ACG CTC TTC

107   CGA TCT GGW ACW GGW TGA ACW GTW TAY CCY CC-3′) (Leray et al. 2013) and the reverse primer *HCOm* (5′-

108   GTG ACT GGA GTT CAG ACG TGT GCT CTT CCG ATC TTA HAC TTC NGG GTG KCC RAA RAA TCA-3′) which

109   was modified from the universal primer HCO 2198 for the COI region (Folmer et al. 1994). Using these primer sets, 360 bp

6

110   in latter half of the region amplified by the universal primers for COI region was amplified. Blocking primer (5′-TAT CCT

111   CCC CTA GCT GGG AAC CTG GCT CAT GCA GGA-3′) was also used to suppress the amplification of host-derived

112   DNA. The 20-µL first PCR amplification reaction contained 1 ng of template DNA, 0.05 U of ExTaq polymerase (TaKaRa

113   Bio Inc.), 1 × PCR buffer, 0.2 mM dNTP, 0.5 µM of each COI primer, and 4.0 µM of blocking primer. The thermal cycling

114   conditions were as follows: an initial denaturation of 94°C for 2 min followed by 35 cycles of 96°C for 30 s, 67°C for 15 s,

115   52°C for 30 s, and 72°C for 30 s, and a final cycle of 72°C for 5 min. The first PCR products were examined by 2% agarose

116   gel electrophoresis. After the amplification was confirmed, we measured the amount of the first PCR products and purified

117   it by adding the same amount of AMpure XP (Beckman Coulter, Inc.). The first PCR products were purified using the same

118   amount of AMPure XP (Beckman Coulter, Inc.). In step 2, the 20-µL second PCR amplification reaction contained 1 ng of

119   the purified first PCR products, 0.05 U of ExTaq polymerase (TaKaRa Bio Inc.), 1 × PCR buffer, 0.2 mM dNTP, and

120   1.0 µM of each tailed primer (second forward primer: 5′-AAT GAT ACG GCG ACC ACC GAG ATC TAC AC- Index2 -

121   ACA CTC TTT CCC TAC ACG ACG-3′ and second reverse primer: 5′-CAA GCA GAA GAC GGC ATA CGA GAT-

122   Index1 –GTG ACT GGA GTT CAG ACG TGT G-3′). The thermal cycling conditions were as follows: an initial

123   denaturation of 94°C for 2 min followed by 12 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s, and a final cycle

124   of 72°C for 5 min. The second PCR products were examined by 2% agarose gel electrophoresis. After the amplification

125   was confirmed, we measured the amount of the second PCR products and purified it by adding the same amount of AMpure

126   XP (Beckman Coulter, Inc.). We measured the amplicon library concentration and checked the quality using the Fragment

127   Analyzer system (Advanced Analytical Technologies Inc.).

128   The DNA samples were paired-end sequenced (2 × 250 bp) on a MiSeq platform (Illumina, Inc.) at the the

129   Bioengineering Lab. Co., Ltd., Kanagawa, Japan. Quality filtering of the reads was performed using fastq_barcode_splitter

130   in the FASTX-Toolkit version 0.0.14 (http://hannonlab.cshl.edu/fastx_toolkit/). The reads in which the beginning of the

131   sequence exactly matched the used primer sequences were selected, and the used primer sequences were trimmed from the

132   reads. After that, the sequences with quality values less than 20 or with lengths of less than 40 bases were discarded using

133   sickle tools version 1.33 (Joshi and Fass, 2011; http://gensoft.pasteur.fr/docs/sickle/1.33). The paired-end merge script

134   FLASH version 1.2.11 (Magoč and Salzberg, 2011; http://gensoft.pasteur.fr/docs/FLASH/1.2.11) was used to merge the

135   sequences that passed the quality filtering. The merging conditions were: merged fragment length 320 bases, read fragment

136   length 230 bases, and minimum overlap length 10 bases. The sequences were assigned to operational taxonomic units

137   (OTUs) using the commands fastx_uniques, sortbysize, cluster_otus, and usearch_global in the USEARCH version

138   8.1.1861 (Edgar, 2010; http://www.drive5.com/usearch/manual8.1/) under the condition of 97 sequence homology, and a

139   BLAST search for each OTU sequence was performed against the full NCBI nucleotide (nt) database for prey species

140   identification.

141   The obtained OTU with BLASTN E-value > 1e-20 or Identity <90% were treated as errors following Deagle et al. (2013),

142   OTUs with a read count < 10 were customarily treated as contamination, and these OTUs were excluded from subset

143   analysis. Fishes (Actinopterygii), cephalopods (Cephalopoda) and crustaceans (Decapoda) were considered to be seal prey

144   organisms based on previous knowledge (the Ministry of Environment, Japan 2017; Hui et al. 2017). Data about the prey

145   species were treated as occurrence data, and it was noted whether the prey organisms were detected (1) or not detected (0)

146   in the fecal sample.

147

148   *Statistical analysis*

149   Samples were grouped by season (spring and fall), sex, maturity (pup, juvenile, and adult), seal catch type (bycatch and

150   capture), and the combination of those categories (season × sex, season × maturity, sex × maturity, sex × catch type, and

151   maturity × catch type). The percent frequency of occurrence (hereafter referred to as "*FO*") of certain prey items was

152   calculated in the respective diet groups:

153

154
$$FO_{Ai} = \frac{n_{Ai}}{n_A}$$

155

156   where n_A is the number of samples in which at least one prey species is detected and n_{Ai} is the number of samples in

157   which the prey item i is detected in group A.

158   The relative *FO* (hereafter referred to as "*RFO*") and the Shannon–Wiener diversity index (hereafter referred to as "*H'*")

159   (Newton-Fisher, 1999) were calculated to assess prey composition in the respective diet groups:

160

161
$$RFO_{Ai} = \frac{n_{Ai}}{\sum_i^s n_{Ai}}$$

162
$$H'_A = \left(-\sum_i^s RFO_{Ai} \cdot \log RFO_{Ai} \right)$$

163

164   where s is the total number of prey items in group A. Higher values of H' indicate the increased number of prey species

165   and the prey's homogeneity coefficient.

166   To verify which factors ("season," "sex," "maturity," and "catch type") affect the seals' diet structure, the prey

167 composition of each group were compared using permutational multivariate ANOVA (PERMANOVA) based on Horn's

168 dissimilarity index (1-C_H) (Anderson 2001; Doi and Okamura 2011). The Horn index (hereafter referred to as "C_H"), which

169 indicates the similarities of prey composition between groups, was calculated following Krebs (1999):

170

171
$$C_H = \frac{2 \sum_i^s RFO_{Ai} \cdot RFO_{Bi}}{\left(\sum_i^s RFO_{Ai}{}^2\right) + \left(\sum_i^s RFO_{Bi}{}^2\right)}$$

172

173 This statistical analysis was conducted using the "adonis" function in the *vegan* package (Okasen et al. 2017) in R

174 ver.3.3.3 (R Core Team, 2017).

175 Additionally, the Jaccard similarity coefficient (hereafter referred to as "J") (Jaccard 1908) was calculated as an indicator

176 of inter-individual overlap of preys:

177

178
$$J'(x, y) = \frac{S_{xy}}{S_x + S_y - S_{xy}}$$

179

180 where S_x and S_y are the total numbers of prey species detected from seal x and seal y, respectively. S_{xy} is the number of

181 prey commonly detected in both seals.

182

183 **Results**

184 *Prey items*

10

185   Sixty-eight fecal samples were collected in our study (Table 1). Seven samples of the total 68 samples were not carried

186   out for sequencing analysis because amplification of the target product was not confirmed. The sequencing of total 61 fecal

187   samples remained after quality filtering yielded 3,243,915 reads in total and 14,787-124,826 reads per sample. Prey

188   organisms were detected in 53 fecal samples. The average number of detected prey species per sample was 2.5 ± 0.2

189   (average $\pm$ standard error, range: 0–8). We identified 19 fish species, 5 cephalopod species, and 1 crustaceans (Table 2).

190   The number of prey species with more than 5% FO was 9; these species included the North Pacific giant octopus

191   (*Enteroctopus dofleini*, $FO = 90.6\%$), the Japanese common squid (*Todarodes pacificus*, $FO = 47.2\%$), the Okhotsk atka

192   mackerel (*Pleurogrammus azonus*, $FO = 37.7\%$), the chestnut octopus (*Octopus conispadiceus*, $FO = 13.2\%$), the black

193   plaice (*Pseudopleuronectes obscurus*, $FO = 13.2\%$), a small type of shrimp (*Crangon spp.*, $FO = 11.3\%$), the masu salmon

194   (*Oncorhynchus masou*, $FO = 9.4\%$), the rock greenling (*Hexagrammos lagocephalus*, $FO = 7.5\%$), and the Japanese dace

195   (*Tribolodon hakonensis*, $FO = 5.7\%$). Salmonids were only detected in six adult seals caught by seal trap nets in spring

196   2017: five females and one male.

197

198   *Seasonal and intraspecific differences in the diet structure*

199   Fourteen species from 10 families and 7 orders were detected in fall (the prey diversity; $H' = 2.16$), and 20 species from

200   10 families and 9 orders were detected in spring ($H' = 2.35$). The inter-individual prey overlap (J') was significantly higher

201   in spring ($J' = 0.72 \pm 0.23$ SD) than in fall ($J' = 0.62 \pm 0.18$ SD) (*t*-test: $t_{784} = -4.34$, $P < 0.01$) (Table 3).

202   The results of testing each factor's effects ("season," "maturity," "sex," and "catch type") on prey composition using

203   PERMANOVA were shown in Table 4. Prey compositions showed the differences between "season" in the adult group.

11

204   Additionally, it was indicated that the factors caused intraspecific differences in the diet to vary with each season.

205   In spring, prey composition differed depending on "maturity" and "catch type" but not "sex" (Fig. 3 and Table 4). Prey

206   diversity was highest in the adult group ($H' = 2.24$) and lowest in the pup group ($H' = 1.80$). The inter-individual prey

207   overlap of the adult group ($J' = 0.83 \pm 0.20$ SD) was significantly higher than that of the juvenile group

208   ($J' = 0.64 \pm 0.21$ SD) and the pup group ($J' = 0.57 \pm 0.23$ SD) (Tukey test: $P < 0.01$). The *RFO* of each prey item showed

209   similar composition between the pup group and the juvenile group; octopuses, squids, crustaceans, and greenlings

210   accounted for 80% of their diet. The adult group's diet showed different tendencies; salmon occurred at a high frequency

211   (RFO = 21.4%) in addition to the aforementioned prey items (Fig. 5).

212   In fall, the prey compositions did not differ significantly depending on "sex" and "maturity" (Fig. 4 and Table 4).

213   Octopuses, squids, greenlings, and flounders accounted for 80% of the fall diet.

214

215   **Discussion**

216   Despite the fact that all seals used in our dietary analysis were caught in salmon set-nets, the prey organism with the

217   highest *FO* was the North Pacific giant octopus, not Salmonids (Table 2). This result might correspond to the fact that

218   colon contents reflected the food record from 2.5 to 6 h ago (Markussen 1993). Considering that the average staying time

219   of the seals in the set-net is 24.1 ± 43.0 min (Masubuchi et al. 2017), the prey organisms detected in the colon contents

220   were eaten outside the salmon set-net, inside the salmon set-net during the last visit, or inside another salmon set-net. Since

221   bycatch of octopus in the salmon set-net is very rare, it is likely that most of seals preyed on the North Pacific giant octopus

222   outside the set-net and then enterd the set-net.

12

223   Seasonal variation in the diet was not shown clearly in this study although it has been suggested in the previous studies

224   (e.g. Hui et al. 2017; Kobayashi unpublished). Our sample size was unbalanced between seasons, and may not be sufficient

225   to figure out the diets of harbor seals in the fall. As many researchers indicated, harbor seals are generally opportunistic

226   predators, and their food habits are influenced by the biomass and availability of prey, which changes spatially and

227   temporally in the ocean (Brown and Pierce 1998; Hall et al. 1998; Andersen et al. 2004). Since such an environmental

228   situation would be a major factor influencing the diet of Kuril harbor seals, conducting additional studies with larger sample

229   sizes to get a more accurate perspective of the fall diet is needed. On the other hand, no large population dynamics of

230   marine organisms affecting seal diets have been reported in the four years between 2014 and 2017, when the samples were

231   collected, therefore sampling bias between years is not expected to have a significant effect on the results.

232   Seasonal variation in the diet was significantly shown only in the adult group. The prey compositions of spring differed

233   depending on "maturity," and the adult group's diet was clearly different from that of the pup group and the juvenile group.

234   Spring, late May to early July, is the breeding season of Kuril harbor seals (Niizuma 1986). During the breeding season, it

235   is known that harbor seals, especially adults, stay near haul-out sites because they have a lek-type mating system (Boness

236   et al. 2006; Dietz et al. 2013). Despite their narrow home range, the prey diversity of the adult group was high, and inter-

237   individual prey overlap was low. This result might suggest that adults avoid competition over prey organisms during the

238   breeding season by consuming different prey items.

239   In fall, their diet did not differ depending on neither "maturity" nor "sex." However, this result is likely due to the small

240   sample size used in this study, so it is premature to conclude that there is no variation in the fall diet. Higher specialization

241   in the fall diet of females has been reported in Salish Sea harbor seals (Voelker et al. 2020). Differences in diet were likely

242   because this species has different foraging strategies between males and females (Thompson et al. 1998; Wilson et al.

243   2014). Sexual and seasonal differences in harbor seal home range sizes are reported in Kattegat, Denmark (Dietz et al.,

244   2013), and Salish Sea (Peterson et al., 2012). In addition, females are more likely to perform deeper foraging dives because

245   of their high consumption of demersal fish (Wilson et al. 2014; Schwarz et al. 2018). Although the fall foraging behavior

246   of adult seals was not investigated in our study field, we propose further dietary analysis and to reveal whether their

247   foraging strategy differ depending on sex and maturity.

248   A clear difference in diet between seals caught as bycatch and seals captured intentionally in the seal trap nets was shown.

249   A noteworthy result is that salmonids were not detected in seals caught as bycatch; they were only detected in individuals

250   intentionally caught by seal trap nets in spring 2017. It might be difficult for seals to catch free-ranging salmon outside the

251   set-net because salmon are large and have high swimming capabilities. Therefore, the seals whose colon contents included

252   salmonids might eat salmon inside the salmon set-net and be captured by seal trap nets afterward. These suggest the

253   effectiveness of seal trap nets to selectively eliminate "problem seals" who repeatedly enter and leave salmon set-nets and

254   cause serious damage to the nets. On the other hand, individuals who enter the set-net for the first time or are unfamiliar

255   with stealing from the nets are caught as bycatch.

256   Selective wildlife management has been proposed as an effective way to mitigate human-wildlife conflicts (Swan et al.

257   2017). To reconcile conservation and the mitigation of fishery damage, it is important to aim for the selective removal of

258   "problem seals" rather than random culling for population adjustment. Some previous studies in other regions have shown

259   that male seals have higher rates of access to fishing nets or use of salmonids than female seals (e.g. Kauhala et al. 2015,

260   Schwards et al. 2018), however, our dietary analysis showed a different tendency. Although the number of samples of

14

261   salmon feeders is still insufficient to confirm the characteristics of problem seals, we indicated that some adult females

262   habitually eat salmon during spring. This result indicates that some adult females may be dependent on the catch of fishing

263   nets for the energy they need to give birth and breastfeeding, and they are more likely to be involved in the problem than

264   adult males, at least in spring. This may also suggest that the harbor seals in Cape Erimo are very accustomed to fishing

265   nets, considering that females are generally more cautions than males.

266   The identification of problem seals in fall, when fisheries damage is more serious than in spring, remains a future

267   issue. The samples used for the fall diet analysis might be not sufficiently comprehensive because no fall seal trap

268   nets were conducted during our study periods. Therefore, it is necessary to increase the sample size including seals

269   caught by seal trap nets in the fall and re-examine how intrinsic factors affect their feeding habits in the fall. Although

270   the DNA barcoding dietary analysis on colon contents showed high advantage in detecting the problem seals, this

271   dietary analytical method is not suitable for quantitative diet analysis. In order to assess the potential impact on prey

272   and fisheries, it is necessary to verify the extent to which the problem seals were actually dependent on salmonids.

273   For this purpose, a more detailed understanding of foraging ecology at the individual level is required through

274   behavioral analysis, fatty acid analysis, and stable isotope analysis.

275   Recommendations for future management are to minimize random culling based on bycatch as much as possible and to

276   base it on the characterization and selective removal of problem individuals. Researches should be conducted to determine

277   how effective it is to eliminate the problem individuals from the population. The high dietary diversity and low inter-

278   individual overlap of adult seals in the spring indicate that adults may avoid food competition in the spring. Therefore, it

279   should also be examined whether this is related to dependence on fishing nets, and studied whether the eliminate of problem

280   individuals results in their niches being quickly occupied by other individuals. Taking measures to mitigate the feeding

281   damage based on such scientific grounds will be important not only in Erimo, but also in all conflicts between pinnipeds

282   and coastal fisheries worldwide.

290

291   **Author's contributions:** All authors contributed to the study conception and design. Material preparation, data collection

292   and analysis were performed by Mina Jimbo, Yuki F. Kita, Mari Kobayashi, and Yoko Mitani. The first draft of the

293   manuscript was written by Mina Jimbo and all authors commented on previous versions of the manuscript. All authors read

294   and approved the final manuscript.

295

299

300   **Data availability** The datasets generated during and/or analyzed during the current study are available from the

301   corresponding author on reasonable request.

302

303

Compliance with ethical standards

Conflicts of interest: The authors declare that the research was conducted in the absence of any commercial or financial

relationships that could be construed as a potential conflict of interest in the preparation of this article.

Ethics approval All harbor seal samples were collected as part of a project conducted by the Ministry of the Environment,

Japan, under the Erimo Area Kuril Harbor Seal Specified Rare Wildlife Management Plan.

Consent for participate All authors agreed to participate in this study and co-authorship.

Consent for publication All authors agreed with the content and that all gave explicit consent to submit.

Code availability: Not applicable

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435

436 **Tables**

Table 1 Sampling result. P = pup, J = juvenile, A = adult. The figures in parentheses indicate the number of samples in which the target region was not amplified or no prey was detected. *Total 25 samples from spring 2017 were collected from seals caught by seal trap nets, and all other samples from 2014 to 2016 were collected from seals caught as bycatch.

		Male			Female			
		P	**J**	**A**	**P**	**J**	**A**	**Total**
2014	Spring						1	1
2015	Spring	7	6		7	5	1	26
	Fall	1	3	5		2		11
2016	Fall		2	2	1			5
2017	Spring*		3 (3)	5 (4)	1 (1)	2 (2)	14 (5)	25 (15)
	Spring total	7	9 (3)	5 (4)	8 (1)	7 (2)	16 (5)	52 (15)
	Fall total	1	5	7	1	2		16
	Total	**8**	**11 (3)**	**12 (4)**	**9 (1)**	**9 (2)**	**16 (5)**	**68 (15)**

437

26

Table 2. List of fishes, cephalopods, and crustaceans detected from feces using DNA metabarcoding analysis and classification of each taxonomic group. Species in bold-faced type had FO > 5% and were considered the dominant prey of the harbor seal population in Erimo.

Class	Order	Family	Group name	Species	Spring	Fall	FO
Cephalopoda	Octopoda	Octopodidae	Octopuses	*Enteroctopus dofleini*	+	+	**90.6**
				Octopus conispadiceus	+	+	**13.2**
	Teuthida	Enoploteuthidae	Squids	*Watasenia scintillans*	-	+	3.8
		Loliginidae		*Heterololigo bleekeri*	-	+	1.9
		Ommastrephidae		*Todarodes pacificus*	+	+	**47.2**
Malacostraca	Decapoda	-	Crustaceans	-	+	+	**9.4**
		Crangonidae		*Crangon sp.*	+	+	**11.3**
Actinopteri	Perciformes	Hexagrammidae	Greenlings	*Hexagrammos lagocephalus*	-	+	**7.5**
				Pleurogrammus azonus	+	+	**37.7**
		Cottidae	Sculpins	*Enophrys lucasi*	+	-	1.9
				Gymnocanthus galeatus	+	-	1.9
				Hemilepidotus papilio	+	-	1.9
				Myoxocephalus polyacanthocephalus	+	-	1.9
				Triglops nybelini	+	-	3.8
		Liparididae	Snailfishes	*Liparis bathyarcticus*	-	+	3.8
		Trichodontidae	Sandfishes	*Arctoscopus japonicus*	+	-	1.9
	Pleuronectiformes	Pleuronectidae	Flounders	*Cleisthenes pinetorum*	+	-	3.8
				Pseudopleuronectes herzensteini	-	+	1.9
				Pseudopleuronectes obscurus	+	+	**13.2**
	Salmoniformes	Salmonidae	Salmons	*Oncorhynchus keta*	+	-	1.9
				Oncorhynchus masou	+	-	**9.4**
				Oncorhynchus tshawytscha	+	-	1.9
	Cypriniformes	Cyprinidae	Corps	*Tribolodon hakonensis*	+	-	**5.7**
	Gadiformes	Gadidae	Cods	*Eleginus gracilis*	-	+	1.9
	Uranoscopiformes	Ammodytidae	Sand lances	*Ammodytes hexapterus*	+	-	3.8
	Clupeiformes	Clupeidae	Herrings	*Sardinops melanostictus*	-	+	1.9

438

Table 3 Number of samples (*N*), the number of samples in which at least one prey species was detected (*n*), the total number of detected prey species (*s*), the Shannon–Wiener index (*H'*), and the average Jaccard similarity index (*J'*) ± *SD* in each group.

| Group	Spring							Fall							Total						
	N	*n*	*s*	*H'*	*J'*	±	*SD*	*N*	*n*	*s*	*H'*	*J'*	±	*SD*	*N*	*n*	*s*	*H'*	*J'*	±	*SD*
Pup	15	14	10	1.80	0.57	±	0.23	2	2	11	2.35	n/a			17	16	16	2.18	0.61	±	0.23
Juvenile	16	11	11	1.99	0..64	±	0.21	7	7	8	1.83	0.63	±	0.17	23	18	14	2.11	0.63	±	0.20
Adult	21	12	12	2.24	0.83	±	0.20	7	7	7	1.68	0.55	±	0.21	28	19	15	2.26	0.76	±	0.23
Male	21	14	10	1.79	0.57	±	0.22	13	13	12	2.05	0.62	±	0.17	34	27	15	2.04	0.60	±	0.20
Female	31	23	18	2.41	0.77	±	0.22	3	3	9	2.09	0.77	±	0.11	34	26	23	2.55	0.77	±	0.21
Bycatch	27	27	13	1.93	0.58	±	0.24	16	16	14	2.16	0.62	±	0.18	43	43	20	2.18	0.60	±	0.21
Capture	25	10	12	2.56	0.84	±	0.22	n/a	n/a	n/a	n/a	n/a			25	10	12	2.56	0.84	±	0.22
Total	52	37	19	2.28	0.70	±	0.24	16	16	14	2.16	0.62	±	0.18	68	53	26	2.39	0.68	±	0.23

439

Table 4 Results of PERMANOVA. The prey compositions were compared between "season", "sex", "maturity", and "catch type," based on Horn's dissimilarity index. Bold numbers indicate a significant difference ($P < 0.05$). df = degree of freedom, SS = sum of squares, MS = mean of squares, F = F statistics, P = P-value.

| Source	df	SS	MS	F	R^2	P
Season	1	0.453	0.453	1.778	0.034	0.063
Sex	1	0.395	0.395	1.544	0.029	0.129
Maturity	2	0.794	0.397	1.571	0.059	0.059
Spring						
Sex	1	0.337	0.337	1.240	0.034	0.290
Maturity	2	1.143	0.572	2.232	0.116	**0.008**
Catch type	1	1.440	1.440	5.994	0.146	**0.001**
Fall						
Sex	1	0.079	0.079	0.362	0.025	0.931
Maturity	2	0.505	0.252	1.249	0.161	0.256
Male						
Season	1	0.242	0.242	1.235	0.047	0.287
Maturity	2	0.281	0.140	0.692	0.054	0.760
Catch type	1	0.315	0.315	1.631	0.061	0.061
Female						
Season	1	0.231	0.231	0.725	0.029	0.714
Maturity	2	1.011	0.506	1.691	0.128	**0.026**
Catch type	1	1.170	1.170	4.180	0.148	**0.001**
Pup						
Season	1	0.402	0.402	2.000	0.125	**0.015**
Sex	1	0.114	0.114	0.514	0.035	0.815
Juvenile						
Season	1	0.220	0.220	1.010	0.059	0.446
Sex	1	0.216	0.216	0.987	0.058	0..457
Adult						
Season	1	0.684	0.684	2.313	0.120	**0.020**
Sex	1	0.563	0.563	1.860	0.099	0.062
Catch type	1	1.178	1.178	4.417	0.206	**0.001**

440

Figure
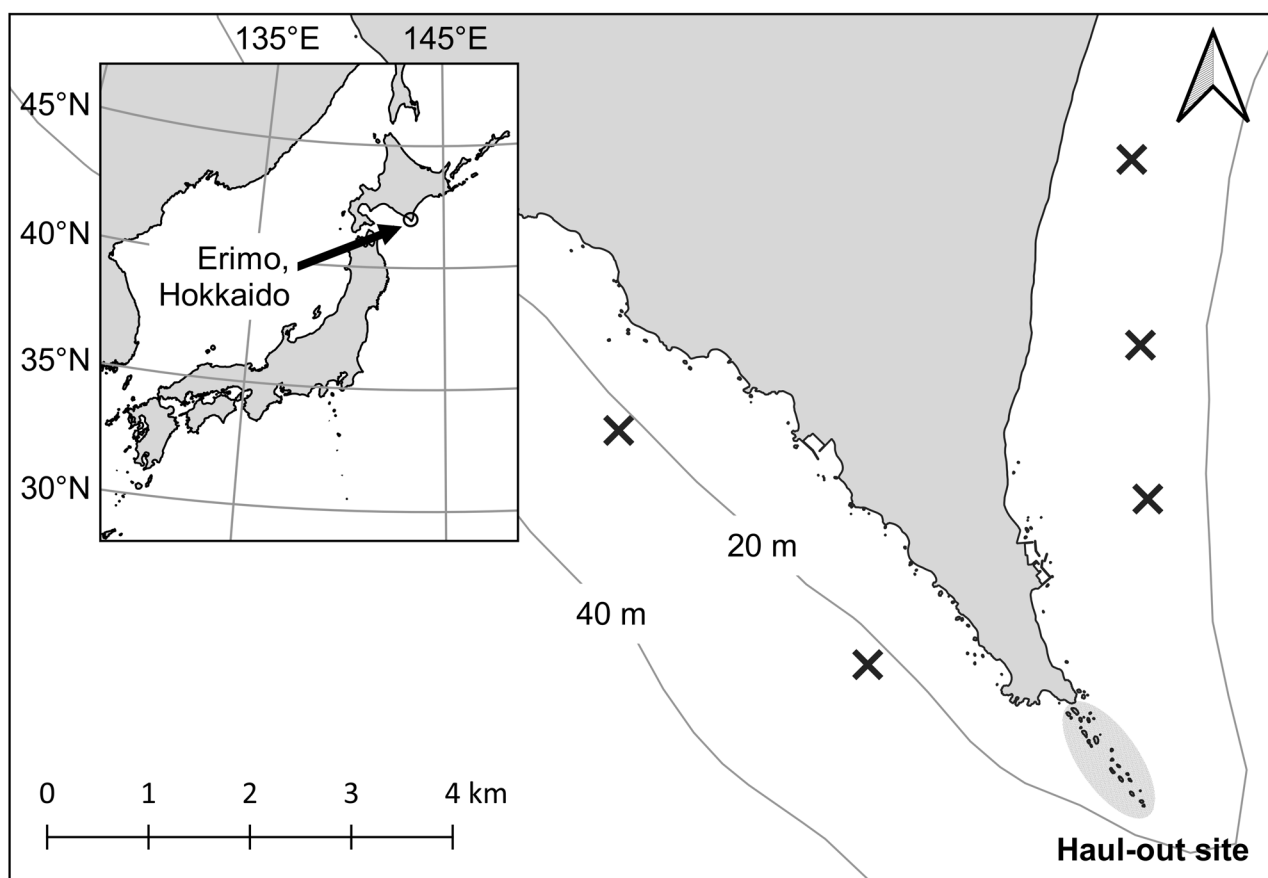
444 **Fig. 1** Map of Cape Erimo, Hokkaido, Japan. The rocky shore area (shaded in grey) is a Kuril harbor seal haul-out site;

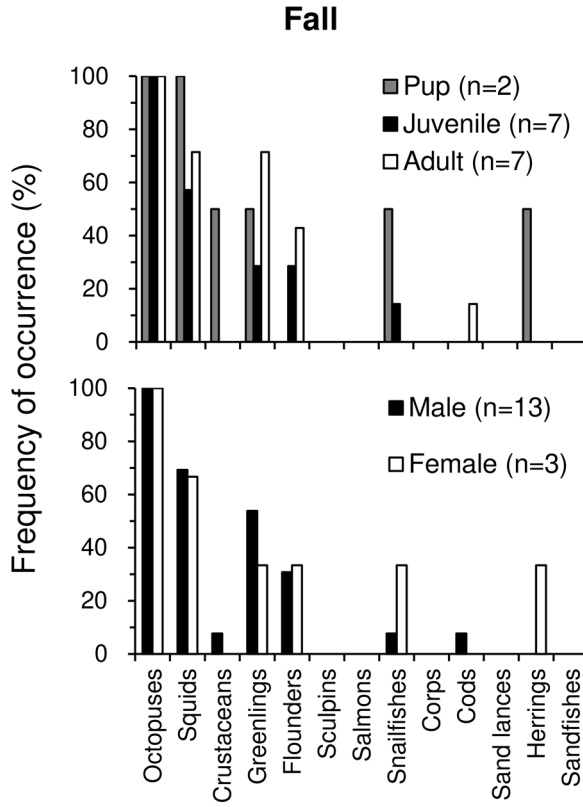445 cross marks indicate salmon set-net locations

446

447   **Fig. 2** Photo of a chum salmon (*Oncorhynchus keta*) that was caught by the set-net and bitten by Kuril harbor seals
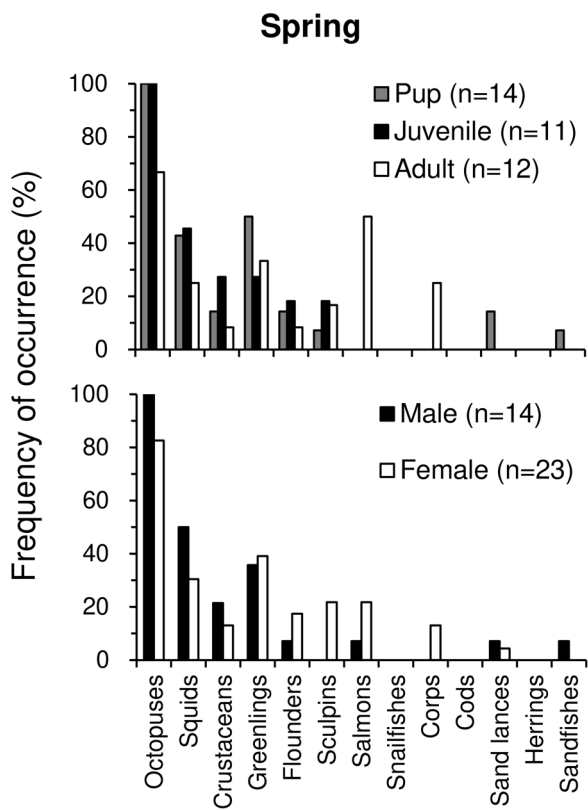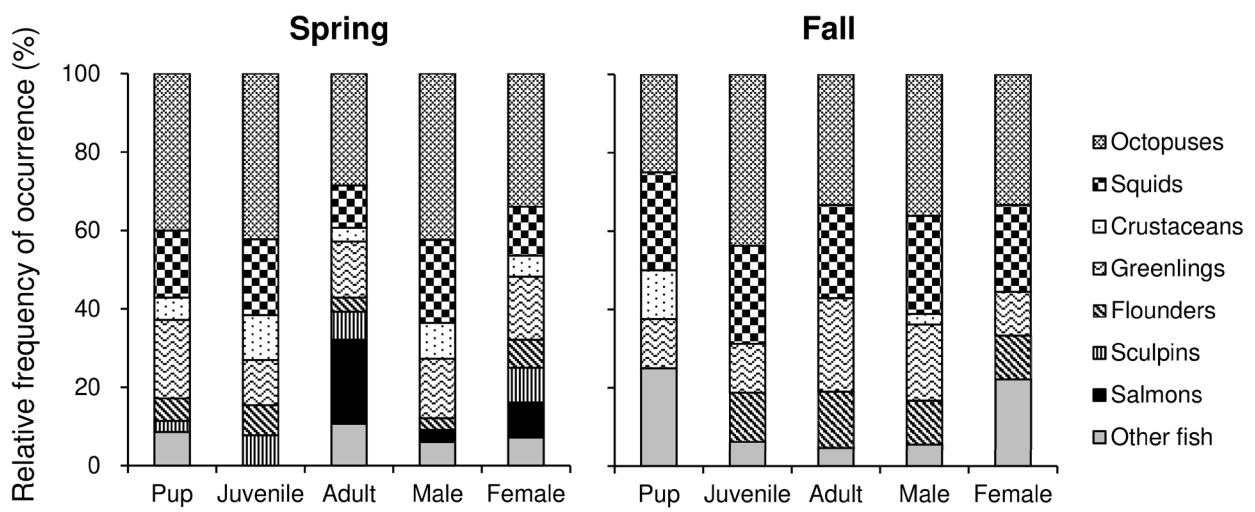
Spring

448

449   **Fig. 3** The percent frequency of occurrence (*FO*) of fish, cephalopods, and crustaceans in spring

Fall

450

451   **Fig. 4** The percent frequency of occurrence (*FO*) of fish, cephalopods, and crustaceans in fall

452

453   **Fig. 5** The relative frequency of occurrence (*RFO*) of each prey item