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Title	Intraspecific differences in the diet of Kuril harbor seals (Phoca vitulina stejnegeri) in Erimo, Hokkaido, using DNA barcoding diet analysis
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Citation	Mammal Research, 66(4), 553-563 https://doi.org/10.1007/s13364-021-00586-3
Issue Date	2021-07-14
Doc URL	http://hdl.handle.net/2115/86359
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Туре	article (author version)
File Information	Jimbo_et_al2021_MammRes.pdf



1	Intraspecific differences in the diet of Kuril harbor seals (Phoca vitulina stejnegeri) in Erimo, Hokkaido, using
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3	
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17	

18 Abstract

19	Competition between Kuril harbor seals (<i>Phoca vitulina stejnegeri</i>) 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

34 Introduction

35Competition between pinnipeds and coastal fisheries occurs in various places worldwide (e.g. Yodzis 1998; Lance et al. 36 2012). Fishing grounds in shallow areas could be easy foraging sites for pinnipeds who can learn to steal fish from fishing 37gear such as set-nets, gillnets, and longlines. In coastal areas where pinnipeds reside throughout the year, such conflicts 38cause more serious problems, as is the case in Hokkaido, Japan. 39Kuril harbor seals (Phoca vitulina stejnegeri) are the only year-round resident pinniped in Japan. They are distributed 40 on the eastern coastline of Hokkaido, Japan (Shaughnessy and Fay 2009; Kobayashi et al. 2014), and Cape Erimo is their 41 largest haul-out site (Fig. 1). They show a high level of philopatry and use the same rocky shore throughout the year. The 42competition between the seals and coastal fisheries has recently become a serious problem due to the increase in seal 43numbers in Erimo. The most part of damage to the fishery is damage to catches of salmon set net such as biting off the 44 head, abdomen and other parts of the salmon (Fig. 2), which results in economic loss as the damaged catch becomes 45worthless as a commodity. Salmon set-net fishing operates twice a year in spring (May to early July) and fall (September 46 to early November) in Erimo. Although depredation by seals is observed in both seasons, the scale of the fishery and the 47damage are greater in the fall. The economic impact of seal depredation on salmon in set-net fishing is especially prominent 48(Hokkaido Government 2015). Previous study has suggested that most seals do not tamper with fishing gear; generally, 49individual "problem seals" cause most of the damage to salmon set-net fishing (Masubuchi et al. 2017). However, it is 50challenging to verify this hypothesis and characterize these individuals because the available information on their diets has 51been limited.

52 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 μ L). The concentration of each extracted DNA sample was measured and adjusted to 10 ng/ μ L (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

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.), $1 \times$ PCR buffer, 0.2 mM dNTP, 0.5 μ M of each COI primer, and 4.0 μ M 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 μ M 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.

148 Statistical analysis

Samples were grouped by season (spring and fall), sex, maturity (pup, juvenile, and adult), seal catch type (bycatch and capture), and the combination of those categories (season × sex, season × maturity, sex × maturity, sex × catch type, and maturity × catch type). The percent frequency of occurrence (hereafter referred to as "*FO*") of certain prey items was calculated in the respective diet groups:

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

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.

- 183 **Results**
- 184 Prey items

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	(<i>Enteroctopus dofleini</i> , $FO = 90.6\%$), the Japanese common squid (<i>Todarodes pacificus</i> , $FO = 47.2\%$), the Okhotsk atka
192	mackerel (<i>Pleurogrammus azonus</i> , $FO = 37.7\%$), the chestnut octopus (<i>Octopus conispadiceus</i> , $FO = 13.2\%$), the black
193	plaice (<i>Pseudopleuronectes obscurus</i> , $FO = 13.2\%$), a small type of shrimp (<i>Crangon spp.</i> , $FO = 11.3\%$), the masu salmon
194	(<i>Oncorhynchus masou</i> , $FO = 9.4\%$), the rock greenling (<i>Hexagrammos lagocephalus</i> , $FO = 7.5\%$), and the Japanese dace
195	(<i>Tribolodon hakonensis</i> , $FO = 5.7\%$). 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.

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 $(J' = 0.83 \pm 0.20 \text{ SD})$ was significantly higher than that of the juvenile group
208	$(J' = 0.64 \pm 0.21 \text{ SD})$ and the pup group $(J' = 0.57 \pm 0.23 \text{ SD})$ (Tukey test: $P < 0.01$). The <i>RFO</i> 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 prev organism with the

ıg s, the prey թ ' y гy org 217highest FO was the North Pacific giant octopus, not Salmonids (Table 2). This result might correspond to the fact that 218colon contents reflected the food record from 2.5 to 6 h ago (Markussen 1993). Considering that the average staying time 219of the seals in the set-net is 24.1 ± 43.0 min (Masubuchi et al. 2017), the prey organisms detected in the colon contents 220were eaten outside the salmon set-net, inside the salmon set-net during the last visit, or inside another salmon set-net. Since 221bycatch of octopus in the salmon set-net is very rare, it is likely that most of seals preyed on the North Pacific giant octopus 222outside the set-net and then enterd the set-net.

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

261salmon feeders is still insufficient to confirm the characteristics of problem seals, we indicated that some adult females 262habitually eat salmon during spring. This result indicates that some adult females may be dependent on the catch of fishing 263nets for the energy they need to give birth and breastfeeding, and they are more likely to be involved in the problem than 264adult males, at least in spring. This may also suggest that the harbor seals in Cape Erimo are very accustomed to fishing 265nets, 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 267issue. The samples used for the fall diet analysis might be not sufficiently comprehensive because no fall seal trap 268nets were conducted during our study periods. Therefore, it is necessary to increase the sample size including seals 269caught by seal trap nets in the fall and re-examine how intrinsic factors affect their feeding habits in the fall. Although 270the DNA barcoding dietary analysis on colon contents showed high advantage in detecting the problem seals, this 271dietary analytical method is not suitable for quantitative diet analysis. In order to assess the potential impact on prey 272and fisheries, it is necessary to verify the extent to which the problem seals were actually dependent on salmonids. 273For this purpose, a more detailed understanding of foraging ecology at the individual level is required through 274behavioral analysis, fatty acid analysis, and stable isotope analysis. 275Recommendations for future management are to minimize random culling based on bycatch as much as possible and to 276base it on the characterization and selective removal of problem individuals. Researches should be conducted to determine how effective it is to eliminate the problem individuals from the population. The high dietary diversity and low inter-277278individual overlap of adult seals in the spring indicate that adults may avoid food competition in the spring. Therefore, it

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
- damage based on such scientific grounds will be important not only in Erimo, but also in all conflicts between pinnipeds
- and coastal fisheries worldwide.

283	Acknowledgments We are deeply grateful to the Erimo Seal Club, Erimo Fisherman's Cooperative, Erimo Town Office,
284	the Ministry of the Environment, Japan, and the members of Laboratory of Aquatic Wild Life Management, Tokyo
285	University of Agriculture, for their support during our fieldwork and their efforts to collect samples. We are also grateful
286	to the members of Kita Laboratory, Tokai University, Mr. K. Nakano and the Bioengineering Lab. Co., Ltd., for assisting
287	in DNA analysis. The authors would like to thank MARUZEN-YUSHODO Co., Ltd. (https://kw.maruzen.co.jp/kousei-
288	honyaku/) for the English language editing. We are also grateful for two anonymous reviewers; their comments greatly
289	contributed to improving this study.
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	
296	Funding This study is partly supported by the Environment Research and Technology Development Fund of the Ministry
297	of the Environment, Japan, and Japan Society for the Promotion of Science KAKENHI Grant Number 15H05709 and
298	16KT0140.
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.

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304 Compliance with ethical standards

- **Conflicts of interest:** The authors declare that the research was conducted in the absence of any commercial or financial
- 306 relationships that could be construed as a potential conflict of interest in the preparation of this article.
- 307 Ethics approval All harbor seal samples were collected as part of a project conducted by the Ministry of the Environment,
- 308 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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436 Tables

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

		Male			Female			
		Р	J	Α	Р	J	Α	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
1	Total	8	11 (3)	12 (4)	9 (1)	9 (2)	16 (5)	68 (15)

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	darodes pacificus + +				
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		

	Spring							Fall						Total							
Group	Ν	n	s	H'	J'	±	SD	Ν	n	s	H'	J'	±	SD	Ν	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	064	±	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

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'*) \pm *SD* in each group.

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).

	Source	df	SS	MS	F	R^2	Р
	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
Sprin	g						
	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
Fema	le						
	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
Juver	nile						
	Season	1	0.220	0.220	1.010	0.059	0.446
	Sex	1	0.216	0.216	0.987	0.058	0457
Adult	t						
	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

df = degree of freedom, SS = sum of squares, MS = mean of squares, F = F statistics, P = P-value.



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



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



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

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

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

