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INTERNATIONAL WHALING COMMISSION

Field and analytical protocols for the comparison of using lethal and non-lethal techniques under the JARPNII with preliminary application to biopsy and faecal sampling

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ABSTRACT

In response to the report of the Expert Panel for the final review of the western North Pacific Japanese Special Permit programme (JARPNII), we provide the field and analytical protocols for the comparison of using lethal and non-lethal techniques. The study is conducted for three years in the coastal water off Sanriku and Kushiro, and offshore. Primary questions are (1) whether a tissue and other samples can be obtained by a non-lethal method; (2) whether enough samples for statistical analysis can be obtained by the non-lethal method; (3) whether the sample obtained by the non-lethal method can produce scientific information compared to that produced by a lethal sampling method; and (4) whether the cost for obtaining the sample/producing scientific information is reasonable. As a result of preliminarily applying the field and analytical protocols to the data obtained in 2014 and 2015, we provisionally concluded that sampling efficiency of faeces was very low, and also that the estimation based on DNA analysis are unreliable at this stage because prey species different from stomach contents were identified from DNA analyses of large intestine. As for biopsy sampling, the samples could be obtained from swimming animals, though sampling efficiency will be different by species. We will evaluate the feasibility and practicability of non-lethal means by applying the same approach, using data in the comparative study including those obtained this year.

KEYWORDS: SCIENTIFIC PERMITS; COMMON MINKE WHALE; BRYDE'S WHALE; SEI WHALE

INTRODUCTION

The decision to finalize the JARPNII program was made taking account of the International Court of Justice (ICJ) Judgment in the case concerning Whaling in the Antarctic (Australia v. Japan: New Zealand intervening). The ICJ Judgment on 31 March 2014 states that "It is to be expected that Japan will take account of the reasoning and conclusions contained in this Judgment as it evaluates the possibility of granting any future permits under Article VIII, paragraph 1, of the Convention" (paragraph 246). Therefore, while JARPNII was not included within the subject matter of the case, the Government of Japan reviewed the design of the ongoing JARPN II under its decision described in the Statement by Ministry for Agriculture, Forestry and Fisheries on 18 April 2014.

The purpose of the changes in sample sizes includes conducting comparative research between lethal and non-lethal methods. With regard to implications of the adjustment for meeting the objectives of the programme, the comparative research is designed to verify a hypothesis that lethal methods could be replaced by non-lethal methods. In other words, if non-lethal methods adopted since 2014 produce equivalently useful data as the lethal method, there will be no hindrances to meeting the original objectives of the program. While the proponents will submit the provisional results of the comparative research to the 2016 IWC SC, the results of the verification will be reported after the three years (2014-2016) research period.

The review panel for JARPNII recommended that the proponents provide the field and analytical protocols for the comparison of using lethal and non-lethal techniques for each key parameter taking into account the advice provided in 2009 (IWC, 2016). Following the preliminary response by the proponents that they would provide the protocols at the SC 66b meeting (Tamura *et al*, 2016a), we provide the field and analytical protocols to evaluate the feasibility and practicability of non-lethal means, especially biopsy and faecal sampling under the prioritized objective. We also provide results obtained to date, which is an update of the preliminary results reported to the SC 66a meeting, and apply the protocol to the data available.

FIELD AND ANALYTICAL PROTOCOLS

1. Procedure for sampling

Biopsy sampling

The equipment for biopsy skin and/or blubber tissue were a crossbow or Larsen gun system (Larsen, 1998). The open sight of Larsen gun system was replaced with an electronic aiming device (red-dot-sight), which allows faster aiming and thus faster shooting. The biopsy darts (4 inches) consists of a carbon fibre shaft, which is high-pressure moulded to a polyethylene float that also functions as a stop to limit penetration into the tissue. In the float end of the dart, a threaded insert is used for attaching the screw-on biopsy-sampling tip. The biopsy tip is a stainless cylinder with a 9mm outer diameter, an internal diameter of 7mm and three internal barbs for sample retention. To avoid sampling failure or to improve the sensitivity of analysis, re-sampling from the identical individuals are conducted. These samples should be independently collected and not divided after sampling to increase the sample size. Information on time taken, sea state, and swell was recorded to enable a plausible measure of effort to be developed. The researcher should remove the specimen from the biopsy-sampling tip with a sterile needle or single-use tweezers. And place it in a 99% ethanol filled tube or in a small zip plastic bag (−20 ºC). Each of the specimens is separately labelled with sample names (e.g. J16YS1M001).

Faecal sampling

Observation of excretion from the identified whales and sampling of excreted faeces were conducted for common minke, sei and Bryde's whales. The observed time is defined as the duration of approaching to the whale within 0.2 n.mile (confirmation of whale species) to end of chasing or observation. If an observer found faeces near the surface of the sea water, the faeces were sampled by circle net with 100μm mesh size. The sampled faeces were stored using polyethylene bottles at −20°C.

Blubber samples

In order to answer Q3 below, it is useful to compare data from the same animal which can be obtained only lethally (*e.g*. stomach contents) to that which could be obtained non-lethally (*e.g*. blubber tissue sample). For that purpose, skin and blubber tissue samples from lethal techniques are stored at −20°C.

Contents of large intestines

In the same vein, for an experiment to determine the practicability of contents of large intestines contents of large intestines are collected from the lethally sampled whales and stored at −20°C.

2. Preparation of data from lethal and non-lethal samples

Tissue samples from skin and blubber

Stable isotope and fatty acid analysis on the skin and blubber samples are performed to evaluate their comparability to stomach content data. The stable isotope analysis is conducted in collaboration with the Japan Chemical Analysis Centre, Chiba Prefecture, Japan.

Faecal samples and contents of large intestines

DNA analyses on faecal samples and contents of large intestines are performed to evaluate their comparability to stomach content data. Total genomic DNA of each individual is extracted using the standard phenol/chloroform

extractions protocol of the GENTRA PUREGENE DNA extraction kit (QIAGEN) following the company's manual. Extracted DNAs are stored in the TE buffer. After PCR amplification, the products are analysed using an Illumina MiSeq (Next-generation DNA sequencers) to identify the prey species. These results are compared to those observed from stomach content on an individual base.

Stomach content data

The data is obtained from the analysis of stomach contents. Details of the analysis are given in Tamura *et al*, (2016b).

3. Comparison of lethal and non-lethal means/samples (common for biopsy and faecal sampling)

Proponents specified the following four questions to be answered in order to evaluate the feasibility and practicability of non-lethal means, especially biopsy and faecal sampling under the prioritized objective (Mogoe *et al*, 2015);

(1) whether a tissue and other samples can be obtained by a non-lethal method (e.g. biopsy sampling, faeces collection);

(2) whether enough number of samples for statistical analysis can be obtained by the non-lethal method;

(3) whether the sample obtained by the non-lethal method can produce scientific information comparable to that produced by a lethal sampling method; and

(4) whether the cost for obtaining the sample/producing scientific information is reasonable.

Questions 1 and 2 above are technical, Question 3 is analytical, and Question 4 is a logistical evaluation.

Systematic application of the questions

The fundamental question as to whether research objectives are achievable non-lethally can be answered through these questions. A flow chart (see Figure 1) articulates how these four questions are applied to the biopsy and faecal sampling/samples, and specifies the condition where we can conclude biopsy and/or faecal sampling can replace lethal take. The systematic application of the questions forms a basis to objectively discuss the feasibility and practicability of non-lethal means, particularly from a perspective of whether research objectives are achievable through non-lethal means. The objective basis of discussion precludes repetitive discussion as to the feasibility and practicability of non-lethal means, which has taken place to date.

Criteria to answer each of the questions

For Question 1, the criteria is simple. If at least one sample could be taken during the research period, the answer is yes and otherwise the answer is no.

For Question 2, sampling efficiency needs to be compared between lethal and non-lethal means. According to the past studies, biopsy sampling efficiency was examined by the number of samples obtained per shot (Isoda *et al*, 2016). In this study, we regard sampling efficiency as a combination of effort to obtain samples and success rate. Effort and success rate are defined as follows:

Effort: Sum of time from 'confirmation of whale species' to 'obtain sample' for whales successfully sampled

+ Sum of time from 'confirmation of whale species' to 'lose whale' for those targeted but not sampled

Success Rate:

Success Rate 1 (# of sampled whales/ #of targeted whales)

Success Rate 2 (# of samples/ # of shots for each whale)

Question 2 can be answered by taking account of all these results.

For Question 3, whether prioritized research objectives, i.e. prey consumption, prey preference, and ecosystem modelling, can be achieved with data from non-lethal means needs to be examined. Under these objectives, it is required that species composition in diet is quantified accurately and that information is available as model inputs. This aspect will be examined with data obtained from non-lethal means.

For Question 4, reasonableness can be evaluated by dividing the overall cost for research by the number of samples obtained.

APPLICATION OF THE PROTOCOL TO THE DATA OBTAINED

Effort for non-lethal research activity

Non-lethal survey activities were carried out in three different areas within the 2014 and 2015 JARPNII. Table 1 shows a searching 'on effort' (time) with the data obtained by the each research activity. The Sighting and Sampling Vessels (SSV) of offshore components were *Yushin Maru* type (Bando *et al*, 2016). Different survey platforms (research vessels) were used for the Offshore and Coastal components. Smaller vessels were used in the coastal survey (Mogoe *et al*, 2016; Kishiro *et al*, 2016). The Sighting Vessels (SVs) used were *Yushin Maru* type in the sighting survey of offshore components. Total searching time of non-lethal and lethal were 1,238.4 and 2,134.1 hours, respectively. Percentages of non-lethal effort were from 8.4 to 61.8%, except for SV data.

Sampling efficiency of biopsy and faecal sampling (Q1 and Q2)

Data obtained from biopsy and faecal sampling in 2014 and 2015 were used for preliminary evaluation of sampling efficiency.

Of a total of 157 biopsy sampling attempts, we collected 114 samples (38 samples from sei, 68 from Bryde's, and 8 from common minke whales). Table 2 summarises the success rates of biopsy sampling. Success rate 1 for the sei, Bryde's and common minke whales were 47.1%, 71.6% and 38.1%, respectively. These results indicate that biopsy samples are obtainable from the species, that is, the answer to the Question 1 is 'YES', for all species. The results, however, suggests that sampling efficiency will be different among the species. Biopsy sampling effort (time) was compared with the effort required for obtaining lethal samples in each species. The average sampling time of sei whales was 55.7 minutes for biopsy sampling, while it was 31.4 minutes for lethal sampling; for Bryde's whales, the average sampling time was 26.8 minutes for biopsy sampling, while it was 25.6 minutes for lethal sampling; for common minke whales off Kushiro, the average sampling time was 149.6 minutes, while it was 72.1 minutes for the lethal sampling. Sampling efficiency of biopsy sampling is thought to be higher for Bryde's whales than that for the other two species. We will evaluate efficiency of biopsy sampling by species, by adding data obtained from the 2016 surveys, to answer the Question 2.

Regarding the faecal sampling, proponents conducted a total of 1808 experiments (1,179 for sei, 393 for Bryde's and 236 for common minke whales), for 377.2 hours (Table 3). Throughout the experiments, excretion was observed for 38 individuals (30 for sei, 6 for Bryde's, and 2 for common minke whales). Of these, faeces was obtained successfully only from 5 sei whales. For Bryde's and common minke whales, faeces sampling was failed, due to sink or spread of faeces before sampling. Our results indicate that, at the present, the answer to the Question 1 is 'YES', only for sei whales. Table 3 shows that encounter rate with faeces at the sea is extremely low (2.1% of all experiments). Sampling efficiency of faecal sampling from swimming animals will not be so high, especially for Bryde's and common minke whales. The efficiency will be evaluated further, by adding the 2016 survey data.

Comparison of data between faecal samples and stomach contents (Q3)

The studies on faecal steroid metabolites published for free-living whales are limited by the difficulty in obtaining samples except for North Atlantic right whales (Gillett *et al*, 2008). Zooplankton in faecal samples should be identified using by a dissecting microscope to determine species composition, quantity and developmental stages (Swaim *et al*, 2009). However, faecal contents of sei and Bryde's and common minke whales showed that the contents derived from zooplankton (such as copepods) tend to float while the faeces from whales feeding fish tend to sink (Mogoe *et al*., 2015).

Preliminary findings from the DNA analyses of contents of intestine and faeces using next-generation sequencing (NGS) technologies (Table 4) are as follows:

1. The prey species compositions identified in the contents of large intestine were quite different from those in the stomach contents.

2. Although this method provides some prey information even from a whale without stomach contents, in some cases, no prey species were identified in the contents of large intestine of the individuals notwithstanding the full stomach contents. In addition, the prey identification rate differed among the whale species.
3. Prey of the prey species were also detected (e.g., Copepoda: *Acartia clausii* in the individual 14NPCS-M019 is known as a major prey of sand lance and not as a prey of common minke whales). Such contamination of prey of prey species causes a critical problem in feeding ecology study of whales.
4. The results of prey ID from the contents of upper part and middle part in the small intestine were similar to contents of the stomach rather than that of the large intestine.
5. The prey ID could not be obtained from faecal samples of sei whales by using NGS.

The next step to be conducted especially for points 1 and 2 above is to examine whether these results were due to either biological, technical or both reasons. One of the technical reasons which we encountered in this study was that almost all of the PCR products predominantly contained the fragments of the whale sequences, causing low prey species identification rate in the samples. Development of a method which avoids amplifying the whale sequences (*e.g.*, Shehzad *et al*, 2012) is now under way, so a better resolution will be obtained in near future. Likewise, a solution should be found to separate amplification of prey of the prey species. This preliminary study clearly indicates that the genetic prey ID only in the contents of large intestine (and faeces) is insufficient to understand feeding habits of the whales at this stage.

With regard to the comparison between stable isotopic data and stomach contents data, according to Icelandic research whaling in the North Atlantic, results from the stable isotope analysis showed a considerably lower trophic level in food consumption compared to the trophic levels indicated by the stomach content analysis (Víkingsson *et al*, 2013). They concluded that depending solely on stable isotope results, in general, may not be able to give an accurate profile of diet in the case of a highly generalist predator such as common minke whales (Ólafsdóttir *et al*, 2013).

Currently, stable isotope analyses on skin/blubber samples are being conducted and proponents will provide results of the comparison between stable isotopic data and stomach contents data in the North Pacific.

CONCLUSION

Proponents developed the field and analytical protocols specifically for the comparative study, but they are also universally applicable to evaluate the feasibility and practicability of non-lethal means. Systematic application of the protocols are an efficient and constructive way because, even though the feasibility and practicability of non-lethal means have been repeatedly discussed, the conclusion was often obscure due to a lack of an objective evaluation scheme.

A few faeces of sei whales were obtained, whereas no faeces of Bryde's and common minke whales were obtained. Furthermore, encounter rates of faeces at sea in the three species were extremely low. Compositions of prey species based on our DNA analysis of large intestine contents of whales were quite different from those based on observation of stomach contents. And also, we could not identify prey species from faeces of three sei whales. Therefore, sampling efficiency of faeces was very low, and also the estimation based on DNA analysis are unreliable at this stage.

As for biopsy sampling, we could obtain the samples from all the three species by using Larsen gun. The results suggest that the answer to the Question 1 is 'YES' to date. Our results also suggest that sampling efficiency of biopsy sampling will be different among the species. The sampling for Bryde's whales is thought to be more efficient than that for the other two species. We will collect further data, because our data are insufficient. At the same time, we will start to investigate estimation of prey composition and trophic level using skin sample of JARPNII. Proponents will evaluate biopsy sampling by applying the same approach, using data obtained in the comparative study, in addition to the data collected in 2016 surveys.

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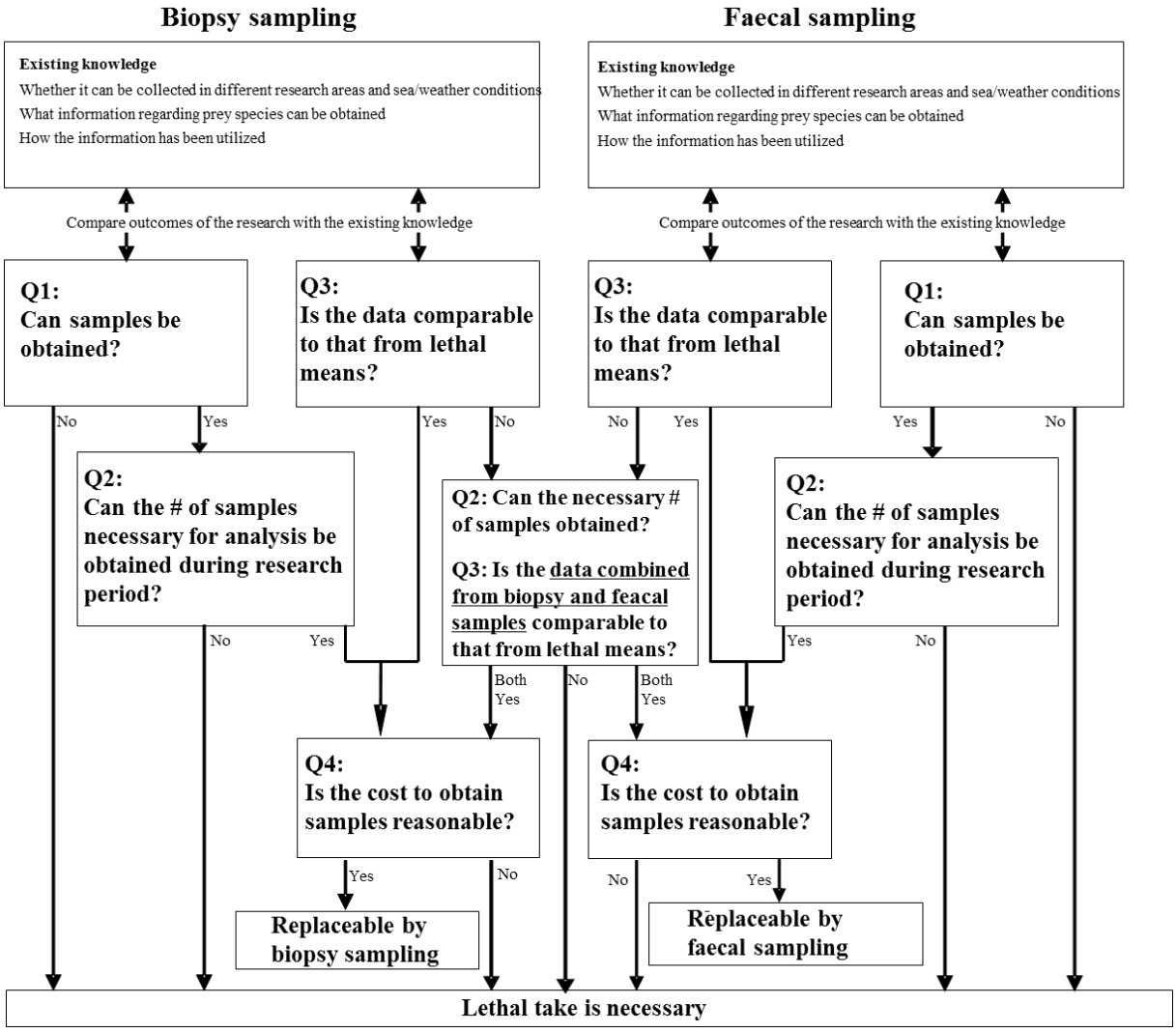

Figure 1. Systematic application of the four questions to evaluate non-lethal means. It should be noted that when at least one of the arrows reaches the box "lethal take is necessary to achieve research objectives," the Proponents conclude that lethal take is necessary for the research program.

Table 1. Searching effort time (hours) of each survey.

	Offshore SSVs	Offshore SVs	Coastal Sanriku	Coastal Kushiro
2014 JARPNII				
Lethal effort	193.3	—	510.3	250.9
Non-lethal effort	89.6	262.4	60.8	58.6
Total effort	282.9	262.4	571.1	309.5
Rate (%) of non-lethal effort	31.7	100	10.6	18.9
2015 JARPNII				
Lethal effort	61.5	—	596.4	521.7
Non-lethal effort	99.5	547.5	54.6	65.4
Total effort	161	547.5	650.9	587.1
Rate (%) of non-lethal effort	61.8	100	8.4	11.1

Table 2. Success rates and effort required for one sample under biopsy sampling in the 2014 and 2015 surveys.

Species	Ship type	Number of Exp.	Targeted whales (A)	Number of shoots (B)	Number of hits	Number of samples* (C)	Number of sampled whales (D)	Effort (min) (E)	Success rate 1 (D)/(A)	Success rate 2 (C)/(B)	Effort required for one sample (min) (E)/(D)
2014 JARPN II											
Sei	SSVs	33	42	63	21	16	16	1,275	0.381	0.254	80
	SVs	0	—	—	—	—	—	—	—	—	—
Bryde's	SSVs	37	39	67	31	25	25	789	0.641	0.373	32
	SVs	0	—	—	—	—	—	—	—	—	—
C. minke (Offshore)	SSVs	0	—	—	—	—	—	—	—	—	—
	SVs	0	—	—	—	—	—	—	—	—	—
C. minke (Sanriku)	SSVs	0	—	—	—	—	—	—	—	—	—
C. minke (Kushiro)	SSVs	9	9	14	5	5	5	458	0.556	0.357	92
2015 JARPN II											
Sei	SSVs	25	26	44	22	22	16	507	0.615	0.364	32
	SVs	0	—	—	—	—	—	—	—	—	—
Bryde's	SSVs	41	42	90	46	43	33	763	0.786	0.367	23
	SVs	0	—	—	—	—	—	—	—	—	—
C. minke (Offshore)	SSVs	2	2	4	2	2	2	52	1.000	0.500	26
	SVs	2	2	2	2	1	1	20	0.500	0.500	20
C. minke (Sanriku)	SSVs	1	1	1	0	0	0	54	0.000	0.000	—
C. minke (Kushiro)	SSVs	7	7	4	4	0	0	236	0.000	0.000	—
Total											
Sei		58	68	107	43	38	32	1,782	0.471	0.299	56
Bryde's		78	81	157	77	68	58	1,552	0.716	0.369	27
C. minke (Offshore)		4	4	6	4	3	3	72	0.750	0.500	24
C. minke (Coastal)		17	17	19	9	5	5	748	0.294	0.263	150

Table 3. The results of faecal sampling in the 2014 and 2015 surveys.

Species	Ship type	Number of experiments (school)	Number of experiments (individuals)	Observation effort (hours)	Observation of excretion (number)	Faecal sampling (number)
2014 JARPA II						
Sei	SSVs	192	346	75.1	11	3
	SVs	134	333	5.9	10	0
Bryde's	SSVs	94	116	25.4	1	0
	SVs	30	42	12.7	2	0
C. minke (Offshore)	SSVs	2	2	0.1	0	0
	SVs	2	2	0.2	0	0
C. minke (Sanriku)	SSVs	49	49	44.8	0	0
C. minke (Kushiro)	SSVs	89	89	60.6	1	0
2015 JARPA II						
Sei	SSVs	193	259	51.6	6	2
	SVs	133	241	7.7	3	0
Bryde's	SSVs	113	147	27.4	2	0
	SVs	70	88	2.4	1	0
C. minke (Offshore)	SSVs	2	2	0.9	0	0
	SVs	0	0	0.0	0	0
C. minke (Sanriku)	SSVs	33	33	31.0	0	0
C. minke (Kushiro)	SSVs	59	59	31.4	1	0
Total						
Sei		652	1,179	140.3	30	5
Bryde's		307	393	67.9	6	0
C. minke (Offshore)		6	6	1.2	0	0
C. minke (Coastal)		230	230	167.8	2	0

Table 4. Results of detected prey species in enteral content using next generation DNA sequencers.

Species	ID number	Prey species observed by stomach contents	Prey species estimated by NGS – Upper part of small intestine	Prey species estimated by NGS – Middle part of small intestine	Prey species estimated by NGS – Large intestine
Sei	14NPSE001	Mackerels (90%) and Japanese anchovy (10%)	Mackerels and Japanese anchovy	Mackerels and Japanese anchovy	No identified
	14NPSE006	Copepods (99%) and krill (1%)	Krill	No identified	Krill
	14NPSE018	Mackerels	Mackerels and Pacific saury	Pacific saury	No identified
	14NPSE044	Japanese sardine (50%), Japanese anchovy (40%) and Mackerels (10%)	Japanese sardine and Japanese anchovy	No identified	No identified
	14NPSE048	Copepods (80%) and Pacific saury (20%)	Pacific saury	Pacific saury	Krill
	14NPSE052	Copepods	Pacific saury	Pacific saury	Pacific saury
	14NPSE067	Copepods	No identified	No identified	No identified
	14NPSE070	Mackerels	Mackerels and Pacific saury	Mackerels	No identified
Bryde's	14NPB005	Japanese anchovy	Japanese anchovy	Japanese anchovy	No identified
	14NPB006	Mackerels	Japanese anchovy	No identified	Light fish (*Maurolicus muelleri*)
	14NPB009	Japanese anchovy (90%), Japanese sardine (8%) and Mackerels (2%)	Japanese anchovy	Japanese anchovy	Japanese anchovy, Japanese sardine and krill
	14NPB010	Japanese anchovy	Japanese anchovy	No identified	No identified
	14NPB016	Japanese anchovy	Japanese anchovy	Japanese anchovy	Krill
	14NPB019	Japanese anchovy (99%) and mackerels (1%)	Japanese anchovy	Japanese anchovy	No identified
C.minke	14NPCS-M013	Sand lance	—	—	Sand lance
	14NPCS-M019	Sand lance	—	—	Copepoda (*Acartia clausii*)
	14NPCS-M021	Sand lance	—	—	No identified
	14NPCK-M013	Japanese sardine	—	—	No identified
	14NPCK-M015	Japanese sardine	—	—	No identified
	14NPCK-M016	Japanese sardine	—	—	Japanese sardine
	14NPCK-M017	Walleye pollock and Japanese sardine	—	—	No identified
	14NPCK-M019	Japanese sardine	—	—	Japanese anchovy
	14NPCK-M027	Walleye pollock and Japanese common squid	—	—	Japanese common squid and krill

NGS: next-generation sequencing.

Table 5. Results of detected prey species in faeces using next generation DNA sequencers.

Species	ID Number	Results
Sei	140527SEI	*Oithona similis*
Sei	140528SEI	*Oithona similis*
Sei	150529SEI	Euphausiacea, Calanoida