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Field and analytical protocol for the evaluation of novel non-lethal techniques in the Japanese whale research programs

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ABSTRACT

This paper describes the ICR field and analytical protocol used to evaluate novel non-lethal techniques, which potentially can be used to respond to scientific questions related to NEWREP-A and NEWREP-NP. The protocol includes four primary questions, which should be responded to in order to evaluate the techniques. This paper also illustrates the use of this protocol to evaluate the use of faeces for studies of feeding ecology of whales (a non-lethal technique).

INTRODUCTION

The International Whaling Commission Scientific Committee (IWC SC) recommended that a field and analytical protocol should be developed to assist the evaluation of the utility of novel non-lethal techniques (IWC, 2016). Such techniques could potentially be used to respond to scientific questions related to the Japanese whale research programs (see Goto and Inoue, this issue). In response to this recommendation, scientists from the Institute of Cetacean Research (ICR) developed a protocol consisting of several questions that should be responded to in order to evaluate novel non-lethal techniques (see Mogoe *et al.*, 2015).

The objective of this paper is to describe this field and analytical protocol. The paper also illustrates the use of this protocol to evaluate the use of faeces for studies of feeding ecology of whales, a non-lethal technique.

DESCRIPTION OF THE FIELD AND ANALYTICAL PROTOCOL

The questions

The protocol consists of four questions, which are listed and explained in this section.

The first question (Q1) is whether a tissue or other kinds of samples can be obtained by a non-lethal technique (for example whether or not faeces can be collected from the sea surface during a research period).

The second question (Q2) is whether a sufficient number of samples for statistical analysis can be obtained by the non-lethal technique.

The third question (Q3) is whether the sample obtained by the non-lethal technique can produce scientific information comparable to that produced by a lethal sampling technique.

The fourth question (Q4) is whether the cost for obtaining the sample and for producing scientific information from the sample is reasonable.

Q1 and Q2 above are technical in nature. Q3 is analytical while Q4 is of a logistic nature.

Formulating the questions to evaluate a non-lethal technique

The flow chart of Figure 1 shows a systematic application of the protocol to evaluate a novel non-lethal technique. The fundamental question to be responded to through this chart is whether a novel non-lethal technique can replace the lethal-sampling.

The formulation of the four questions forms a basis to objectively discuss the feasibility and practicability of non-lethal techniques, particularly from a perspective of whether particular research objectives are achievable through non-lethal techniques.

Criteria for questions

The criteria for Q1 is simple. If at least one tissue or sample can be taken during the research period by the non-lethal technique, the answer is 'yes' otherwise the answer is 'no.'

For Q2 the sampling efficiency of a non-lethal technique needs to be compared with that of a lethal technique. For example in the case of faeces sampling (see below), the number of samples collected by some unit of effort is compared with the number of whales (stomachs)

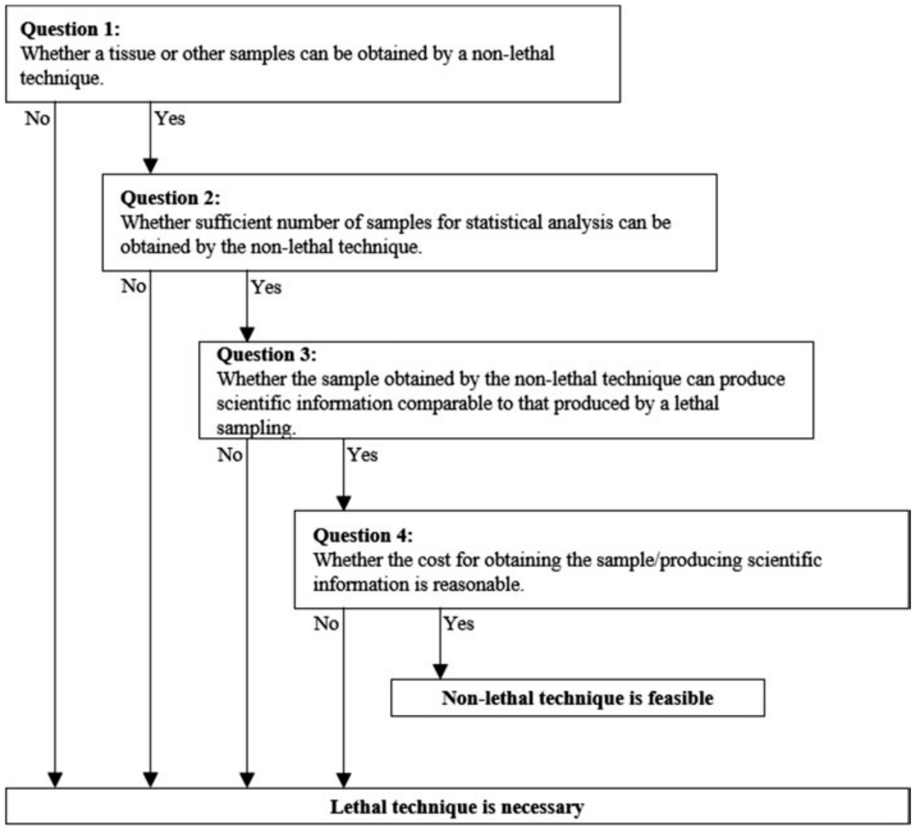

Figure 1. Systematic application of the four questions to evaluate the feasibility of novel non-lethal techniques.

sampled by the same unit.

In Q3 the key issue is whether the analysis of the tissue or samples obtained by the non-lethal technique can respond to specific scientific questions by producing scientific information of similar or better quality than that produced by the lethal-technique. For example in the case of faeces samples (see below), the key question will be whether the analysis of faeces samples can produce similar or better scientific information than the lethal technique (stomach content analysis) to respond questions of prey consumption (qualitative and quantitative) and prey preference, which are important input data for ecosystem models.

Criteria for Q4 require further consideration but the simplest approach for an evaluation could be dividing the overall cost for research by the number of samples obtained.

APPLICATION OF THE PROTOCOL TO EVALUATE THE USE OF FAECES FOR FEEDING ECOLOGY STUDIES

Currently reliable information on prey consumption and prey preferences of whales is obtained through the analyses of stomach contents. The fundamental question is whether the analysis of faeces samples can provide such reliable information.

Field and laboratory data were obtained, which are explained below. Data collected were the basis of responses to the four questions in Figure 1.

Field data

The field experiments on the evaluation of the faeces sampling for studies of feeding ecology of North Pacific common minke, Bryde's and sei whales were carried out during the 2014 and 2015 JARPNII surveys. *Yushin Maru*-type vessels were used for the experiments in the offshore component of JARPNII (Bando *et al.*, 2016) (sighting and sampling vessels: SSVs, and sighting vessels: SVs). Smaller vessels were used in the coastal component (Kishiro *et al.*, 2016).

Table 1 shows a summary of the effort (time) spent on both whale observation and sampling (lethal) and faeces observation and sampling (non-lethal).

Observation and sampling of faeces

Observation of excretion and sampling of faeces in identified individuals of common minke, sei and Bryde's whales were made from the platforms of the survey vessels. The effort was defined as the observation time from confirmation of the whale species (0.2 n.miles) to the end of observation/sampling. If an observer found faeces near the sea surface, the faeces were sampled by circle net

Table 1
Effort (hours) spent in whale observation and sampling (lethal) and faeces observation and sampling (non-lethal) surveys. See text for details.

	Offshore SSVs	Offshore SVs	Coastal Sanriku	Coastal Kushiro
2014 JARPNII				
Whale observation and sampling effort	193.3	—	510.3	250.9
Faeces observation and sampling 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	100.0	10.6	18.9
2015 JARPNII				
Whale observation and sampling effort	61.5	—	596.4	521.7
Faeces observation and sampling effort	99.5	547.5	54.6	65.4
Total effort	161.0	547.5	650.9	587.1
Rate (%) of non-lethal effort	61.8	100.0	8.4	11.1

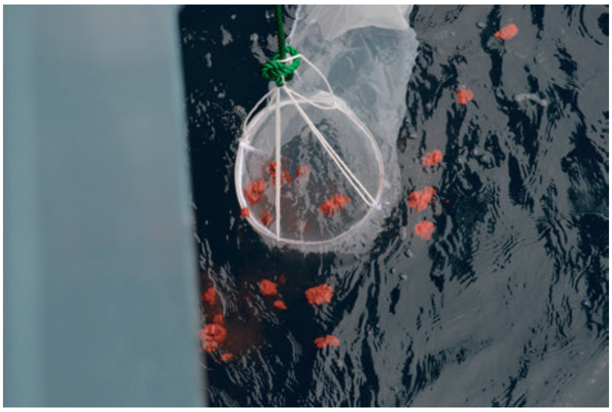

Figure 2. Sampling of sei whale faeces in the 2015 JARPNII offshore survey.

with 100 µm mesh size (Figure 2). The sampled faeces were stored using polyethylene bottles at −20°C.

Observation and sampling of whales
Whales were sighted and sampled along the predetermined track-lines. The effort was defined as the time from confirmation of whale species to the time of the shooting.

Analytical procedures
The idea was to compare the results on prey species in the stomach contents (direct observations) and in faeces samples (DNA analyses). As this comparison was not possible for a same individual, the contents in intestine was used as a proxy for faeces. Therefore prey species in the stomach (direct observation) and intestine (DNA analyses) was compared for a same individual. In addition, prey species in the faeces samples collected were also investigated by DNA analyses.

In the case of genetic analysis, total genomic DNA was extracted using the standard phenol/chloroform extractions protocol of the GENTRA PUREGENE DNA extraction kit (QIAGEN) following the company's manual. Extracted DNAs were stored in TE buffer. After PCR amplification, the products were analyzed using an Illumina MiSeq (next-generation DNA sequencers) to identify the prey species.

Analysis of stomach content followed standard procedure (Tamura *et al.*, 2016).

RESULTS

Response to question 1
A total of 1,808 experiments were made (1,179 for sei, 393 for Bryde's and 236 for common minke whales), for 377.2 h (Table 2). Excretion was observed in 38 individuals (30 sei, 6 Bryde's, and 2 common minke whales). Of these, faeces was obtained successfully from five sei whales. For Bryde's and common minke whales, faeces could not be obtained due to sinking or spreading of faeces before sampling.

Therefore at the present, the answer to Q1 is 'yes' for sei whales and 'no' for common minke and Bryde's whales.

Response to question 2
Table 2 shows that the observation effort and number of excretions observed in the research period. The number of faeces observed is extremely low, 38 cases in 1,808 individual experiments (2.1%) for all surveys and species combined. These percentages were 2.5%, 1.5%, 0.0% and 0.0% for sei, Brydes's, common minke (offshore) and common minke (coastal), respectively.

These percentages contrast with those of the efficiency of whale (stomach) sampling. For all surveys and species combined there were 297 targeted individuals of which 151 were sampled (50.8%). The percentages by species were 93.6%, 89.3% and 50.8% for sei, Bryde's and minke (coastal) whales, respectively.

Sampling efficiency of faecal sampling is substantially lower than the whale (stomach) sampling efficiency. Therefore the response to Q2 is 'no' for sei, Bryde's and common minke whales.

Table 2
The results of faecal sampling in the 2014 and 2015 surveys.

Species	Vessel type	Number of experiments (school)	Number of experiments (individuals)	Observation effort (hours)	Observation of excretion (number)	Faecal sampling (number)
2014 JARPNII						
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 JARPNII						
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

Response to question 3

Comparison between direct observation of stomach contents and DNA analysis of intestine

Table 3 shows a comparison of the prey species found in the stomach (direct observation) and in the intestine (DNA analysis) of sei, Bryde′s and common minke whales. As indicated earlier, intestine was used as a proxy for faeces.

There is no good correspondence between the preys identified in the stomach and the intestine of the same individuals. In several cases the species could not be identified by the DNA analysis notwithstanding the full stomach of the whales.

A prey of prey species was also observed. For example *Acartia clausii* was identified by the DNA analysis in the individual 14NPCS-M019. This species is known as a major prey of sand lance, which is a known prey species of the common minke whale.

The results of prey identification by the DNA analysis was somewhat different between the upper/middle parts of the small intestine and the large intestine.

DNA analysis of faeces

Table 4 shows the results of the DNA analysis for prey identification from faeces in three sei whales. The DNA analysis could not detect the prey species from the faeces of other three sei whales, which suggest low efficiency of this technique to identify prey species from faeces samples.

Therefore the response to Q3 is ′no′ for sei, Bryde′s and common minke whales.

Respond to question 4

This question is not addressed in the present study. However whale field surveys are extremely expensive. Funding for some of the surveys involving sampling of whales are provided in part by the sale of by-products.

CONCLUDING REMARKS

Discussions on the feasibility of novel non-lethal techniques in whale research have so far been controversial and inconclusive. This is due a lack of an objective protocol guiding these discussions. The ICR has developed

Table 3
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 4
Prey species in faeces of sei whales identified by the NGS.

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

a protocol with four questions that should be responded to in order to evaluate the feasibility of novel non-lethal techniques. An objective evaluation of the available data in the context of the four questions will make possible more useful discussions and conclusions on the feasibility of a given novel non-lethal technique. In this study the protocol was used to evaluate the feasibility of the analysis of whale faeces in studies on feeding ecology specifically to respond to questions on prey consumption and prey preferences. Results of the evaluation following the protocol suggest that at this stage of knowledge, such technique is not feasible and therefore cannot replace the analysis of stomach content to respond the same questions.

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