Incidence of prey DNA types in Bryde's whale scats

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Abstract

The diet of whales is an important aspect of their ecology and also a very difficult one to study. We have previously demonstrated that DNA can be used to study the diet of blue whales (*Balaenoptera musculus*) and fin whales (*Balaenoptera physalus*) through identification of prey item DNA found in whale faeces. We apply similar DNA based methods here to samples of faeces from ten Bryde's whales (*Balaenoptera edeni*) to determine the presence or absence of important prey groups. DNA from ray-finned fish was present in all samples tested (n=10). Krill and amphipod DNA were present in 7/10 samples and copepod DNA was present in 3/10 samples. This study is an example of using DNA based prey detection as a rapid, cheap and non-invasive way of studying whale diet.

Introduction

Determining the diet of whales has always been a challenging task. It is difficult to get unbiased estimates of whale diet from direct observation. We have previously suggested that DNA-based species identification of prey items in whale faeces may be a useful method for studying whale diet (Gales & Jarman, 2001; Gales & Jarman, 2002). This has been demonstrated in studies of the diet of blue whales (Jarman et al., 2002) and fin whales (Jarman et al., 2004). We have also isolated prey DNA from minke whale *(Balaenoptera acutorostrata),* longfinned pilot whale *(Globicephala melaena)* and bottlenose dolphin (*tursiops truncatus*) faeces, which indicates that cetacean digestion does not generally destroy prey DNA to the point that it is unrecoverable.

This paper describes the detection of DNA from prey groups likely to be important in the diet of Bryde's whales (*Balaenoptera edeni*). Group-specific PCR primer sets were used to detect DNA from ray-finned fish (Actinopterygii), Amphipoda, Cephalopoda, Copepoda, krill (Euphausiacea), Gastropoda, Isopoda and Ostracoda as these groups are potential prey of *B. edeni.* Small, swarming fish or crustacean species such as clupeidae, arraginidae, or krill are thought to be the main feeding targets of *B. edeni* (Tershy, 1992; Ridgeway & Harrison, 1985; Kato 2002). The other groups such as copepods, cephalopods, isopods and amphipods were chosen because they are commonly found in the epipelagic zone. Gastropods were included as a group because pteropods belong to this order and these are often found in plankton trawls of epipelagic water. Tests for DNA from these groups gave a simple set of presence / absence results for prey items in these samples.

Materials and Methods

Sample collection and DNA purification

Samples of whale faeces were collected from near feeding whales found in the Hauraki Gulf, approximately 30 km NE of Auckland, New Zealand. Ten samples were collected by trawling a 500 µM mesh net through the plume of material made by defaecating whales. The faeces was then preserved in ~ 75% ethanol until required for DNA purification. After checking the samples for the presence of undigested metazoans that may have been inadvertently collected along with the faeces, DNA was purified from 30 µL of each sample of whale faeces using a Faecal DNA extraction kit (BIO101).

DNA amplification

DNA from ray-finned fish (Actinopterygii) was amplified with novel primers designed for this study. The primers were designed on an alignment of mitochondrial small subunit rDNA genes from diverse fishes and other vertebrates. Primers for amplifying a short rDNA region only from Actinopterygii and not from other vertebrates were then designed using Amplicon software (Jarman, 2004). The primers were tested on diverse fish, mammal and bird DNAs to establish their specificity for fish DNA. Optimal annealing temperature was determined by gradient PCR on several fish DNA templates.

Prey DNA from other groups was amplified using primers published in previous studies, the copepoda set being published in Bissett et al. (2005); the Euphausiacea set in Jarman et al. (2002); and all others being published in Jarman et al. (2006). The primer sets used in this study are shown in Table 1.

Target taxon	Primer sequences	Annealing	Reference
Actinopterygii	for-CGGTAAAACTCGTGCC	55°C	This paper
	rev-CCGCCAAGTCCTTTGGG		
Amphipoda	for-CTGCGGTTAAAAGGCTCGTAGTTGAA	51°C	Jarman et al. (2006)
	rev-ACTGCTTTRAGCACTCTGATTTAC		
Cephalopoda	for-TGCGGTATTWTAACTGTACT	52°C	Jarman et al. (2006)
	rev-TTATTCCTTRATCACCC		
Copepoda	for-TGTGTGGTGGTAAACGGAG	61°C	Bissett et al. (2005)
	rev-CCGCCGACCTACTCG		
Euphausiacea	for-TCTCAGCGCTGGCAAGGTGTCA	61°C	Jarman et al. (2002)
	rev-CTCGGGGACGTTTTATCCGGGACGAG		

Gastropoda	for-GCGGYAACGCAAACGAAGT	52°C	Jarman et al. (2006)
	rev-CGAAAWTMACACCGTCTCCG		
Isopoda	for-TCTATGATTYATGGGATGT	51°C	Jarman et al. (2006)
	rev-AAGACCTCAGCGCTCGGC		
Ostracoda	for-GTGACAAGAAGACCCTARGAG	46°C	Jarman et al. (2006)
	rev-AATCCAACATCGAGGTCA		

Table 1. Oligonucleotides used in this study for group-specific PCR amplification of prey DNA. Each PCR primer set targets a short DNA region from one prey group. Forward primers are designated by 'for' and reverse primers by 'rev.' The annealing temperature used during thermal cycling with each primer set is given as well as the paper in which it was published.

PCRs were run on a Chromo4 real time fluorescence detecting thermal cycler (MJ Research). Reaction components were 10 mM Tris-HCl (pH 8.8 @ 25°C), 50 mM KCl, 0.1% Triton® X-100, 1.5 mM $MgCl_2$, 2 mM each dNTP, 2 units DynaZyme II thermostable DNA polymerase (Finnzymes), 1 x bovine serum albumin (New England Biolabs) and 1 x EvaGreen (Biotium). 4 µL template DNA solution purified from whale faeces was added and water to bring the volume to 20 µL. Thermal cycling conditions were an initial 94°C DNA denaturation step for 2 min. This was followed by 50 cycles of 94°C for 10 s for denaturation; annealing at a temperature given in table 1 for 30 s; and polymerization of new DNA at 72°C for 30 s. Fluorescence in the 520-530 nm band was measured immediately following the polymerization step.

For each batch of PCRs, a positive control containing DNA purified directly from a member of the target group was included to check that the reaction worked. A negative control containing no template DNA was also run to test for sample cross contamination. PCRs were assembled using aerosol free pipette tips in a laminar flow hood after destroying DNA on the surfaces of pipettes and plasticware with UV exposure.

Results

Ten samples were collected, preserved and posted to the Australian Antarctic Division for analysis. DNA was purified from all samples after checking for whole metazoans that might have been derived from the water column rather than from the whale faeces, which were found in samples 8 and 9 shown in Table 2.

Sample	Date	Location	Whales present	Sample description
1	04/01/2003	36.31.78S, 175.03.07E	3	Pink slurry with some white particles
2	18/07/2002	36.38.23S, 175.11.32E	1	Fine pink sediment
3	30/11/2002	36.35.50S, 175.03.30E	4	Fine pink sediment with dark lumps
4	10/04/2003	36.35.63S, 175.12.11E	1	Small white particles
5	15/04/2002	36.30.66S, 174.55.61E	1	Fine pink sediment with dark lumps
6	07/10/2003	36.34.86S, 175.01.74E	3	Greyish sediment with white lumps
7	01/11/2004	36.36.34S, 175.06.06E	2	Fine white sediment

8	11/01/2005	36.32.28S, 174.38.62E	2	Fine white sediment, some whole copepods
9	13/01/2005	36.31.47S, 174.54.32E	1	Pink sediment and some whole krill
10	15/01/2005	36.33.46S, 174.57.64E	2	Mixed small pink and white particles

Table 2. Sampling details for ten Bryde's whale faecal samples. The time and place of collection are given as well as how many whales were present where the faeces was collected and what the sample looked like.

Results of PCRs to detect prey are given in table 3 below. Samples were considered to be positive if they had amplification monitored by real time fluorescence that was significantly above the baseline provided by the template free negative control. All samples tested positive for DNA from ray-finned fish, which was consistent with expectations as Bryde's whales are frequently seen feeding on them in the area where the samples were collected. Krill and amphipod DNA were both identified in 7/10 samples. The presence of krill DNA in sample 9 cannot definitely be ascribed to predation by the whale as whole krill were present in this sample and were removed before DNA extraction. However, the absence of copepod DNA in sample 8, from which whole copepods were removed prior to DNA purification, suggests that this may be effective in preventing contamination from whole metazoans inadvertently collected in the plankton nets. The low incidence of copepods in these samples (3/10) was slightly surprising given their prevalence in most near-coastal epipelagic waters.

	1	2	3	4	5	6	7	8	9	10
Actinopterygii	+	+	+	+	+	+	+	+	+	+
Amphipoda	+	-	-	+	+	+	+	-	+	+
Cephalopoda	-	-	-	-	-	-	-	-	-	-
Copepoda	+	-	-	-	-	-	+	-	-	+
Euphausiacea	+	+	-	-	+	+	+	-	+	+
Gastropoda	-	-	-	-	-	-	-	-	-	-
Isopoda	-	-	-	-	-	-	-	-	-	-
Ostracoda	-	-	-	-	-	-	-	-	-	-

Table 3. Presence or absence (+/-) of prey DNA from eight groups of metazoans in ten Bryde's whale scat samples as determined by group-specific real time PCR.

Discussion

DNA molecules have a high information content, are present in most animal cells, do not vary between cell types and are easily copied many times from small initial numbers of molecules. These features make DNA an excellent biomarker for species identification purposes and it is an increasingly popular molecule for identifying anonymous biological material. This has been driven in part by the instigation of large projects aimed at increasing the diversity of species that have diagnostic DNA sequences represented in public databases (Hebert at al., 2003; Schander & Willassen, 2005). DNA based markers allow the identification of any life stage of prey

species with equal ease and allow identification of parts of animals, even single cells. The ability to identify species from DNA persists for some time after death. This is the basis for DNA based dietary analyses, which target prey DNA after it has been digested by a predator.

The major initial technical obstacles for DNA based dietary analysis of whale prey were the lack of sequence data for prey and predator species and consequent narrow focus of early studies to well-studied prey groups (Jarman et al., 2002). Since this first DNA based study of whale diet, the number of sequences representing prey species in public databases has expanded enormously. This has allowed the development of novel PCR primer sets that target some of these groups, which is a very convenient methods for analysing dietary samples as it avoids amplifying predator and parasite DNA (Jarman et al., 2004; Jarman et al., 2006). The greater availability of sequences has also, of course, made identification of prey species much simpler as the researcher does not have to sequence DNA of every prey species themselves.

A common and very reasonable criticism of using DNA to identify prey in whale faeces is that whale faecal samples inevitably become contaminated with biological material already in the water column. It is probably more accurate to describe the samples as plankton samples very heavily contaminated with whale faeces. Although this seems problematic, the water column near feeding whales is clearly very rich in whale prey (and its DNA), so biases introduced by this problem are likely to be less significant than expected. Nevertheless, the degree of representation of non-whale ingested prey DNA can be at least partially controlled by removing obviously undigested metazoans from a sample. In future studies we hope to also collect samples from water where the whales had not been defaecating to further investigate this bias.

In this simple analysis of the presence or absence of prey DNA we have shown that Bryde's whales feeding in the coastal waters of the North Island of New Zealand feed predominantly on ray-finned fish, krill and amphipods. About half of the samples tested were collected while whales were feeding on prey aggregations. This was usually in association with other predators, common dolphins (*Delphinus delphis*) and Australasian gannets (*Sula serrator*) in particular. The fish aggregations were identified as pilchard (*Sardinops sagax*) on the occasions when identification was possible. Bryde's whales feed opportunisticically off South Africa, feeding primary on krill in pelagic waters and fish in coastal waters (Kato, 2002). Bryde's whales in the Gulf of California have been observed feeding primarily on fish (Tershy, 1992). There are diverse fish available in near-shore waters around New Zealand (Hendrick and Francis, 2002), with snapper (*Pagrus auratus*) being the dominant species (McKenzie, 1960; Hendrick and Francis, 2002). Krill such as *Nyctiphanes australis* are present in these waters as well.

The analysis presented here is preliminary. The presence or absence data is only for very large taxonomic categories at present, but we can extend the study to identify the prey to species level in most cases. We intend to make clone libraries from the PCR products generated here in order to sequence them and compare the sequences to sequences already deposited in databases such as GenBank. This should provide more detailed information on Bryde's whale diet, which should be interesting as the species consumed by this whale are not thoroughly documented and the fish prey available in the Hauraki gulf is very diverse (Hendrick and Francis, 2002).

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