Faecal sampling using detection dogs to study reproduction and health in North Atlantic right whales (*Eubalaena glacialis*)

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ABSTRACT

Conservation and management of many cetaceans is hindered by the difficulty of acquiring samples from free-swimming individuals to obtain essential data on health, diet, reproduction and physiological impacts of environmental and anthropogenic stressors. This is particularly true for large whales, which are logistically difficult to live-capture for sampling. In North Atlantic right whales (*Eubalaena glacialis*), a significant decline in reproduction and health in the 1990s led to the application of faecal-based analyses to study stress and reproductive endocrinology, marine biotoxin exposure and prevalence of disease-causing protozoa. However, this approach was limited by low sample acquisition rates with opportunistic faecal (scat) collection methods. The work presented here evaluates the relative sampling efficiency of scent detection dogs trained to locate North Atlantic right whale scat versus opportunistic scat collection during photo-identification surveys. Three years of sample collection using both detection dogs and opportunistic methods are summarised. Faecal sample collection rates using detection dogs were over four times higher than opportunistic methods. The use of detection dogs for scat collection from free-swimming right whales has for the first time provided adequate numbers of samples for statistical analyses. The endocrine, disease, genetic and biotoxin studies currently being performed on these samples markedly improve the ability to address fundamental questions vital to effective conservation and management of highly endangered right whales.

KEY WORDS: SAMPLING STRATEGY; NORTH ATLANTIC RIGHT WHALE; HORMONES; REPRODUCTION; GENETICS; DISEASE

INTRODUCTION

Effective conservation and management of many cetaceans has been hindered by insufficient non-lethal methods to acquire data on feeding ecology, reproductive parameters, individual and population health and the physiological impacts of environmental and anthropogenic stressors (e.g. marine biotoxins, contaminants, global climate change). This has been particularly problematic for large whales, which are elusive and extremely difficult to live-capture for sampling of blood or tissues. While remote biopsy darting provides samples for genetic, contaminant and fatty acid analyses, the data that can be obtained from skin and blubber cores are limited.

A significant decline in reproduction and health in the western North Atlantic right whale population (*Eubalaena glacialis*) in the late 1990s raised concern among managers and researchers (Kraus *et al.*, 2001; Pettis *et al.*, 2004; Hamilton and Marx, 2005). In response, the International Whaling Commission (IWC) Workshops on the Comprehensive Assessment of Right Whales, and Status and Trends of Western North Atlantic Right Whales (IWC, 2001a; b) gave priority recommendations to develop methods for assessing health, stress and reproductive failure. Subsequently, a suite of faecal-based studies were validated and applied to northern right whales to assess the reproductive status of individual whales, and to study factors potentially affecting health and fecundity.

Measurement of faecal metabolites of steroid hormones has now been used to determine reproductive status of free-swimming right whales (Rolland *et al.*, 2005). That study showed that concentrations of reproductive hormone metabolites were reliable predictors of gender, pregnancy and lactation in females and sexual maturity in males. Current extensions to this work involve identifying individuals by creating genetic profiles using right whale DNA isolated from their faeces (R. Gillett, unpublished data) and measuring metabolites of adrenal hormones to assess relative stress levels (Hunt *et al.*, 2006). Faecal parasitology studies have shown that right whales have the highest prevalence of infection with potentially pathogenic protozoa (*Giardia* spp. and *Cryptosporidium* spp.) of any marine mammal yet examined (Hughes-Hanks *et al.*, 2005). In that study, over 70% of the faecal samples collected from right whales were *Giardia* spp. positive and 24% were positive for *Cryptosporidium* spp. Finally, faecal measurements of the paralytic shellfish poisoning (PSP) toxins produced by the 'red tide' organism *Alexandrium* showed that sampled right whales were being exposed to this potent neurotoxin by feeding. In some cases, toxin levels reached 0.5μg saxitoxin equivalents g^{-1} faeces, near the levels at which human advisories for shellfish are issued, although the biological effects on right whales remain unknown (Doucette *et al.*, 2006). All of these studies were derived from multiple assays of the same faecal (scat) samples where the individual whale can frequently be identified either photographically (by comparison with the North Atlantic Right Whale Catalogue; Hamilton and Martin, 1999) or genetically (by comparing scat DNA profiles to biopsy DNA profiles of known whales). Preliminary results show that at least 14 whales have been sampled more than once within a field season and/or in multiple years (R. Rolland, unpublished data). These studies represent the foundation of an individual-based profile of health and reproductive status, that when integrated with the Right Whale Catalogue, provide insights into population-based models of reproduction, health, mortality and trends.

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Despite the wealth of data available from these analyses, this approach has been restricted by the difficulty of opportunistically locating scat at sea, limiting the number of available samples. This led to evaluating the use of domestic dogs (*Canis familiaris*) professionally trained to detect wildlife scat (Wasser *et al.*, 2004) to increase sample collections from right whales. In terrestrial studies, detection dogs significantly increased scat collection rates from kit foxes (*Vulpes macrotis mutica*; Smith, D.A. *et al.*, 2003), grizzly (*Ursus arctos*) and black bears (*U. americanus*; Wasser *et al.*, 2004). In those studies, dogs located scat from targeted wildlife with 100% accuracy (based on genetic species confirmation), and increased sampling rates four-fold, compared to experienced human observers. This paper describes the use of detection dogs to locate faecal samples from right whales over three years. Faecal sampling efficiency of surveys with dogs is compared to opportunistic methods and species identity is confirmed genetically for a subset of samples.

METHODS

Study area and survey methods

This work was conducted during August and September, 2003-05 in the waters around Lubec, Maine (training) and in the Bay of Fundy, Canada (surveys), where right whales congregate seasonally to feed (Murison and Gaskin, 1989). Faecal sample collection surveys using detection dogs were conducted aboard a 6.4m boat with a global positioning system (GPS) chart plotter. The chart plotter was used to mark the location of tracklines and positions where dogs detected scent from right whale scat, and helped orient the boat relative to wind and tide direction to locate samples. The crew included one dog and three to four people (dog handler, driver, photographer/data recorder). In addition, opportunistic faecal sample collections occurred aboard a 9.0m vessel with a crew of six to eight people conducting standardised right whale photo-identification surveys.

Surveys used two detection dogs alternately in 2003 and 2004, and a single dog in 2005. Given the demands of working on a boat, dogs that had good physical stability, persistence in locating samples and a calm disposition were selected. Scat detection dog training follows techniques used for narcotic, search and rescue and bomb detection dogs (Wasser *et al.*, 2004). When the dog detects the targeted scent there is a characteristic change in behaviour (recognised by tense body posture and ear position), motivated by the expectation of a reward. Scent from right whale scat was added to these dogs' repertoires through initial exposure using a scent box (Wasser *et al.*, 2004), followed first by searches on land, then from the bow of a boat. Previously collected scat samples from male and female right whales of varied ages were used for training. Initial training occurred over a period of nine days, and 'refresher' work for both handlers and dogs occurred annually for one or two days prior to the start of each field season.

All surveys using dogs were conducted with a Beaufort sea state $\leq$3 and wind speeds $\leq$10 knots. Boat transects were conducted perpendicular to the wind direction at a speed of five to seven knots, downwind from aggregations of right whales or areas where right whales had been previously sighted. The dogs were positioned on the bow for the duration of the trial. On land, the dog leads the handler directly to the sample by following the scent cone along an increasing odour gradient. On the water, since the dog could not lead the handler, the helmsman steered according to the direction indicated by the dog (as interpreted by the handler) until the sample was located (Fig. 1). If the dog lost the scent during the approach, perpendicular transects were resumed until the dog's behaviour indicated that the vessel was back in the scent cone from the sample (Fig. 2). When faecal samples were successfully collected, the dog was rewarded immediately by playing with a tennis ball on a string.

Fig. 1. The dog handler signalling to the helmsman the direction to steer as indicated by the detection dog during a search for a right whale scat sample.

Sample collection

Floating pieces of clumped right whale scat were collected using a 300μm nylon dipnet (Sea-Gear Corp., Melbourne, Florida, USA; Rolland *et al.*, 2005). Scat samples were identified in the field by size, shape, brown-orange colour, characteristic odour and presence of fine baleen hairs. Salt water was drained off the faeces, samples were stored in polypropylene jars and placed on ice until frozen at −20°C for subsequent analyses. The date, time and position of collection were recorded for each sample. When defecation was witnessed, the whale was photographed for subsequent photo-identification analysis (Kraus *et al.*, 1986).

Comparison of sample collection methods

The sampling efficiency of the detection dog surveys was calculated by dividing the number of faecal samples collected per day by the total time that the dog was working. Hours of dog survey effort were defined as the total time the dog was working 'on watch' during transects. These results were compared with opportunistic faecal sample collections made during right whale photo-identification surveys. Opportunistic collections occurred when whales were observed defecating at the surface or observers detected scat by odour. Hours of opportunistic effort were defined as the time observers were 'on watch' between the first and last whale photographed that day. Samples collected per hour of survey effort were calculated over three years (2003-05). Comparisons between opportunistic surveys and detection dog surveys were only made on days when both vessels were working to control for variability in weather conditions and whale density.

Data analysis was performed using *SPSS* 13.0 (SPSS Inc., Chicago, Illinois, USA). The data were not normally distributed, thus non-parametric tests were used. Differences

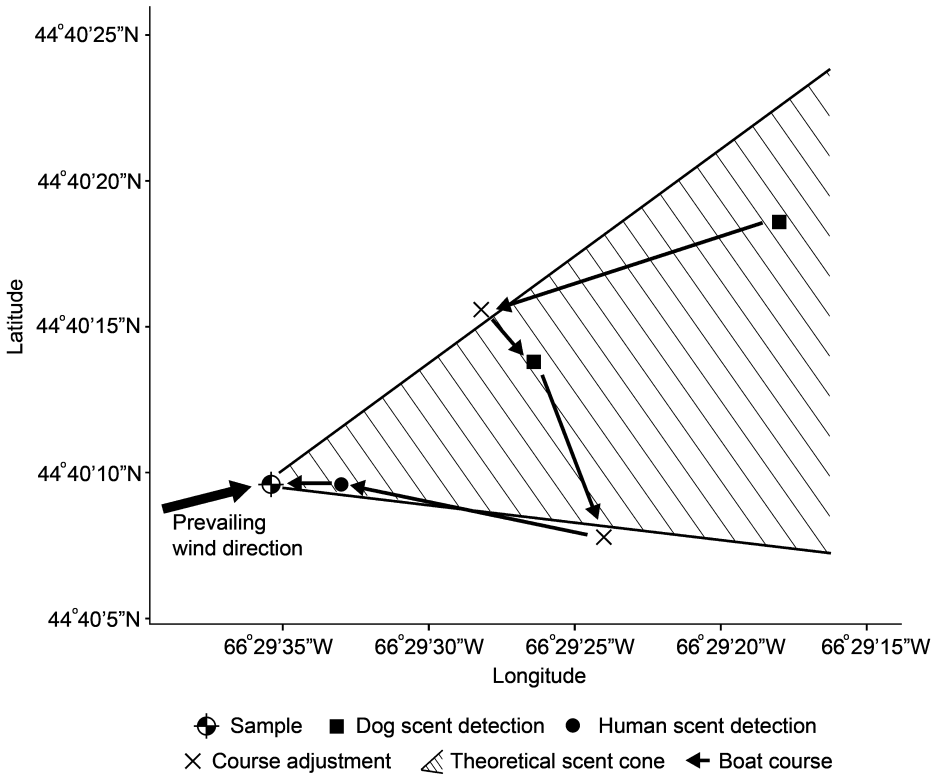

Fig. 2. An example of the search pattern the research vessel followed (→) to locate a right whale scat sample with a detection dog. As the vessel enters the scent cone coming from the sample (striped area), the dog detects the odour (■) as indicated by a change in the dog's ear set and body position, prompting the boat driver to steer into the wind. The dog loses the odour when the vessel leaves the scent cone (X). The vessel then resumes a transect perpendicular to the wind until the dog has another detection, turning into the wind again to find the sample. The human crew smelled the sample just before it was collected (●). The distance from the first scent detection by the dog to the final position of sample collection was ~0.5km.

were considered significant if $p<0.05$. The number of samples collected per day and the sampling efficiency using detection dogs were compared to results from opportunistic collection methods using a Mann-Whitney U test. Differences in sampling efficiency between years were analysed for each method separately using the Kruskal-Wallis test. The detection distance for each sample located by the dogs was estimated by calculating the distance between the GPS positions of the first observed change of behaviour (indicating scent acquisition) and the location of sample collection. These are estimates of distance because tidal motion may have moved the scat (closer or farther depending on the stage) relative to the location of the dog's first detection.

Genetic analyses

Species identity was determined genetically for 54 samples collected in 2003 by extraction and amplification of mitochondrial control region DNA. DNA was extracted in duplicate from frozen, lyophilised faecal samples using a modified Qiagen DNeasy extraction protocol (Qiagen, Valencia, CA). Nucleic Acid Purification Grade Lysis Buffer (1X, 1.6ml; ABI) was added to ~70-90mg of the each sample, then samples were vortexed (1min) and incubated (65°C, 1hr). Following incubation, 25µl Proteinase K (>600mAU ml^{-1}; Qiagen) and 600µL AL buffer (Qiagen) were added. Tubes were inverted and incubated for an additional hour. Ethanol was added (100%, 600µl), the tubes were mixed, and the contents were run through a silica spin column. Samples were washed and eluted following steps four through seven of the Qiagen DNeasy protocol, incubated (65°C, 10min) to evaporate any residual ethanol and frozen at −20°C.

The mitochondrial control region was amplified using the polymerase chain reaction (PCR) with the primers UP098 and LP282 (Malik *et al.*, 2000; Rastogi *et al.*, 2004). Amplification consisted of a 25µl reaction (0.3mg bovine serum albumin, 1X PCR Buffer, 0.2µM of dNTP mix, 2µM magnesium chloride, 0.3µM each primer, 0.1U *Taq* DNA polymerase and ~1.5ng template DNA) with the following cycling conditions: 94°C for 5min; 50 cycles of 94°C for 30s, 52°C for 60s, 72°C for 60s; 60°C for 45min. Extraction and PCR negative controls were included to test for contamination.

RESULTS

Results from the detection dog and photo-identification surveys were compared for 19 days (2003-05) on which both detection dog and opportunistic survey vessels were working. Detection dog surveys located significantly more samples (n=97) compared to the opportunistic method (n=30; Mann-Whitney U test, Z=-3.418, $p<0.001$). Detection dogs located many scat samples in areas where the human crew did not observe whales in close proximity. The mass of faeces collected varied from approximately 20g to 0.5kg or more. Mean sampling efficiency of the detection dog surveys from 2003-05 was 1.1 samples hr^{-1} (range: 0.80 to 1.43 samples hr^{-1}), significantly greater than 0.25 samples hr^{-1} (range: 0.15 to 0.32 samples hr^{-1}) for

opportunistic surveys (Table 1; Mann-Whitney U=5.000, Z=-5.129, p<0.001). Although the sampling efficiency of both methods appeared to be higher in 2005 (Table 1), there were no significant differences between years for either method, indicating consistency in the survey methodologies.

Table 1

Comparison of yearly and overall faecal sample collection rates from right whales (2003-05) using opportunistic methods or detection dogs trained to locate samples. Sampling efficiency using detection dogs was significantly higher than opportunistic sample collection during photo-identification surveys (p<0.001). There is no significant difference in sample collection rates between years for either method.

	Samples collected per hour	
Year(s)	Opportunistic	Detection dogs
2003	0.15	1.07
2004	0.28	0.80
2005	0.32	1.43
Overall 2003-05	0.25	1.10

Estimated detection distances for the dogs ranged 22m to 1.93km (just over one nautical mile). In 2003, the only year that this was measured, humans detected seven samples (by smell) at 56-359m, while the dogs detected the same samples at 150-563m. All faecal samples found by the dogs and humans in 2003 have been confirmed to be from right whales by mitochondrial DNA analyses, and the remainder are currently undergoing analysis.

Statistical comparisons only included a subset of samples collected on days when both research vessels were working in the Bay of Fundy. Another 72 faecal samples were obtained between 2003-05 on other survey days, in other habitats or by other vessels in the Bay of Fundy (total samples from 2003-05 = 199). Prior to using detection dogs (1999-2002), an additional 86 samples were collected opportunistically, bringing the total samples for all faecal-based studies to 285. All samples were collected for reproductive and stress hormone analyses. In many cases sufficient faecal material was collected to allow for sub-dividing of samples for multiple assays, so that 128 of these samples are also being examined for marine biotoxins, and 111 for parasites. Additionally, all samples will eventually be characterised genetically using mitochondrial and nuclear markers to confirm the species of origin and determine individual whale identity.

DISCUSSION

These results demonstrate that scat detection dogs can work from boats to dramatically increase faecal sampling rates from free-swimming right whales. Sampling efficiency of detection dogs was over four times higher than opportunistic collection methods over a three-year period. In addition, dogs detected samples from as far as one nautical mile away, greatly increasing the area that can be sampled. The success of this method depended upon the involvement of a professional dog trainer, an experienced handler and dogs and a boat driver with intimate knowledge of the local tide and wind patterns. It also involved use of a dedicated vessel for detection dog surveys, because of methodological conflicts between visually-based photo-identification surveys and detection dog survey protocols. Nevertheless, using dogs to collect large numbers of scat samples from right whales has significantly increased sample sizes, enhancing the utility of the diversity of faecal analyses in quantitatively assessing this population's status.

These assays and faecal collection methods are potentially useful in multiple species, and can address a wide array of questions. Faeces have been collected opportunistically from bottlenose dolphins (*Tursiops truncatus*) for genetic studies (Parsons *et al.*, 2003), sperm whales (*Physeter macrocephalus*) for feeding ecology research (Smith and Whitehead, 2000), blue whales (*Balaenoptera musculus*) and humpback whales (*Megaptera novaeangliae*) to study marine biotoxin exposure (Lefebvre *et al.*, 2002) and North Atlantic right whales for environmental toxicology (Weisbrod *et al.*, 2000). Faecal analyses provide estimates of exposure to synthetic chemicals and biotoxins, both issues of concern to cetaceans worldwide because of increasing human impacts on the marine environment.

In addition to the assays described here, DNA markers from prey species in scat are being used in cetaceans to identify dietary components and diversity to understand marine food webs with more accuracy than previous work relying on analysis of hard parts of prey in faeces or stomach contents (e.g. Jarman *et al.*, 2002). Recent advances in extraction and amplification of host nuclear and mitochondrial DNA from scat samples permits PCR-based studies using genetic markers to determine species, sex and individual identity (Wasser *et al.*, 2004). Although faecal DNA tends to be more degraded than that obtained by biopsy, in this study 100% of the faecal samples analysed yielded sufficient DNA for species determination.

Many cetaceans are at-risk or poorly studied, and researchers require physiological and biomedical data to assess population health and reproductive status. Such information is not easily obtained using conventional methods. Enhanced sampling of cetacean scat by using detection dogs, coupled with endocrine, toxicological and molecular analyses, opens a new window into the physiology, health and genetic status of free-swimming whales that can contribute greatly to their conservation and management.

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