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RESOURCE ARTICLE

Collecting baleen whale blow samples by drone: A minimally intrusive tool for conservation genetics

Éadin N. O'Mahony[1,2] | Angela L. Sremba[3,4] | Eric M. Keen[2,5] | Nicole Robinson[6] | Archie Dundas[6] | Debbie Steel[3] | Janie Wray[2] | C. Scott Baker[3] | Oscar E. Gaggiotti[1]

[1]Centre for Biological Diversity and Scottish Oceans Institute, School of Biology, University of St Andrews, St Andrews, Fife, UK

[2]North Coast Cetacean Society, Alert Bay, British Columbia, Canada

[3]Marine Mammal Institute, Hatfield Marine Science Centre, Oregon State University, Newport, Oregon, USA

[4]Cooperative Institute for Marine Ecosystem Resources, Oregon State University, Newport, Oregon, USA

[5]Sewanee: The University of the South, Sewanee, Tennessee, USA

[6]Gitga'at Oceans and Lands Department, Hartley Bay, British Columbia, Canada

Correspondence
Éadin N. O'Mahony, Centre for Biological Diversity and Scottish Oceans Institute, School of Biology, University of St Andrews, St Andrews, Fife KY16 9TH, UK.
Email: enao1@st-andrews.ac.uk

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Abstract

In coastal British Columbia, Canada, marine megafauna such as humpback whales (*Megaptera novaeangliae*) and fin whales (*Balaenoptera physalus velifera*) have been subject to a history of exploitation and near extirpation. While their populations have been in recovery, significant threats are posed to these vulnerable species by proposed natural resource ventures in this region, in addition to the compounding effects of anthropogenic climate change. Genetic tools play a vital role in informing conservation efforts, but the associated collection of tissue biopsy samples can be challenging for the investigators and disruptive to the ongoing behaviour of the targeted whales. Here, we evaluate a minimally intrusive approach based on collecting exhaled breath condensate, or respiratory 'blow' samples, from baleen whales using an unoccupied aerial system (UAS), within Gitga'at First Nation territory for conservation genetics. Minimal behavioural responses to the sampling technique were observed, with no response detected 87% of the time (of 112 UAS deployments). DNA from whale blow ($n = 88$ samples) was extracted, and DNA profiles consisting of 10 nuclear microsatellite loci, sex identification and mitochondrial (mt) DNA haplotypes were constructed. An average of 7.5 microsatellite loci per individual were successfully genotyped. The success rates for mtDNA and sex assignment were 80% and 89% respectively. Thus, this minimally intrusive sampling method can be used to describe genetic diversity and generate genetic profiles for individual identification. The results of this research demonstrate the potential of UAS-collected whale blow for conservation genetics from a remote location.

KEYWORDS
baleen whales, conservation genetics, drones, respiratory blow, unoccupied aerial systems

1 | INTRODUCTION

As recognized by the International Union for Conservation of Nature (IUCN), Earth's biodiversity needs to be conserved across three levels: genetic diversity, species diversity and ecosystem diversity (Frankham, 1995). It is a given that these are not mutually exclusive, and major threats to biodiversity, such as overexploitation, habitat loss and anthropogenic climate change, can have profound and

lasting effects across all three tiers (Frankham, 1995; Rockström et al., 2009). There are many genetic issues associated with reduction of population size, including but not limited to inbreeding depressions, the accumulation of deleterious mutations, and the fragmentation of populations, resulting in uncertain taxonomic identification (Frankham, 1995). In light of the global surge in anthropogenic perturbations and habitat fragmentation and/or loss, understanding biodiversity loss at the genetic level and its management implications – a discipline known as 'conservation genetics' – has never been more important (Chase et al., 2020; Di Marco et al., 2018; Díaz et al., 2019; Vigdor, 2021).

Conservation genetics is particularly valuable for elusive or inaccessible species, such as marine fauna (Eizirik et al., 2008; Holderegger et al., 2019). For cetaceans, conservation genetics has proven critical for the identification of genetic divergence, population structure and diversity, as well as reproductively isolated subpopulations, which inform the delineation of management stocks and resulting strategies for recovery (Hoelzel, 1992; Waples et al., 2018). Examples of this include the delineation of the North Pacific fin whale subspecies (*Balaenoptera physalus velifera*) based on mitogenome sequences and a small number of single nucleotide polymorphisms (SNPs; Archer & Brownell, 2019), and the demonstration of impact that maternal fidelity and natal philopatry have on the population structure of North Pacific humpback whales (*Megaptera novaeangliae*) using maternally inherited mitochondrial (mt) DNA haplotypes and microsatellite genotyping (Baker et al., 1998, 2013).

Genetic material can be obtained using a variety of methods, some more invasive than others. Each collection method carries its own set of strengths and weaknesses, depending on research objectives. Conservation and population genetics of marine megafauna have been typically carried out through direct tissue sampling, for example, by the collection of fin clips and tissue biopsy hole punches from sharks and rays (Kashiwagi et al., 2012, 2015; Larson et al., 2015), or with the use of a dart from an air gun or crossbow to collect skin and blubber tissue samples from cetaceans (Marsili et al., 2000; Noren & Mocklin, 2011). These samples generally yield high-quality and large-quantity genetic material and the metadata available have shown that biopsy wounds heal quickly in some species (Krutzen et al., 2002; Noren & Mocklin, 2011). However, these sampling methods require direct contact with target individuals, eliciting behavioural responses of varying strengths and requiring both training of investigators and application for collection permits prior to sampling (Garrigue & Derville, 2021; Noren & Mocklin, 2011). The strength of behavioural responses has also been shown to vary by species, for example, in one study, humpback whales and minke whales (*Balaenoptera acutorostrata*) were shown to be more sensitive to biopsy sampling than fin whales and blue whales (*Balaenoptera musculus*; Gauthier & Sears, 2006).

Over the last few decades, there has been a surge of interest in less intrusive sources of DNA (Adams et al., 2019) ranging from sloughed skin from sperm whales (*Physeter macrocephalus*; Rendell et al., 2012; Whitehead et al., 1990) and humpback whales (Valsecchi et al., 1998), faecal plumes in dolphins (Parsons et al., 1999) to mucus from manta rays (*Mobula birostris*; Kashiwagi et al., 2015). The collection and filtering of seawater as a source of environmental (e)DNA is also a rapidly expanding field with applications in cetology (e.g. Alter et al., 2022; Baker et al., 2018, 2023; Foote et al., 2012; Parsons et al., 2018; Pinfield et al., 2019; Székely et al., 2021; Valsecchi et al., 2020). For cetaceans specifically, exhaled breath condensate, commonly referred to as 'spout' or 'blow', has been used as a non-invasive source of biological material over the last decade (Hunt et al., 2013). The blow is a mucous mixture of aerosol droplets, seawater and gases expelled from the respiratory system of cetaceans (Yeates et al., 2020). It has relatively recently been examined for a range of scientific fields, including its use as a general marker for health and disease surveillance (Acevedo-Whitehouse et al., 2010; Apprill et al., 2017; Atkinson et al., 2021; Cumeras et al., 2014; Groch et al., 2020; Pasamontes et al., 2017). It presents an attractive biological sampling option, not only to minimize disturbance to the target animal but also to assess pollutants such as micro- or nanoplastics, petroleum products or environmental contaminants (Yeates et al., 2020).

Studies targeting genetic material within blow samples taken from small cetaceans, such as wild dolphins, have struggled to extract and sequence DNA (Robinson & Nuuttila, 2020). Researchers highlighted the small plume size and quick erratic movements of the species as the primary causes of challenge (Raudino et al., 2019; Robinson & Nuuttila, 2020). On the other hand, larger cetaceans, such as rorquals (Balaenopteridae), have a much larger blow size and move in a slower and more predictable pattern than small cetaceans. This lends itself to potentially better sampling opportunities (Atkinson et al., 2021; Groch et al., 2020; Würsig et al., 2009).

To date, blow has most commonly been collected using a close vessel approach to the target individual and with the use of various custom-designed devices fastened on the end of a long pole (Acevedo-Whitehouse et al., 2010; Burgess et al., 2018; Cumeras et al., 2014; Groch et al., 2020; Hogg et al., 2009; Hudson et al., 2021; Hunt et al., 2014; Mingramm et al., 2019; Robinson & Nuuttila, 2020; Thompson et al., 2014; Vendl et al., 2019, 2020). Sampling devices have included petri dishes (Robinson & Nuuttila, 2020; Schroeder et al., 2009), nylon fabric (Hogg et al., 2009) and even tulle bridal veil fabric (Hunt et al., 2014). Unoccupied Aerial Systems (UASs), commonly called drones, are a relatively novel tool for conservation biology (Koh & Wich, 2012; Linchant et al., 2015) and in particular, blow sampling of cetaceans (Apprill et al., 2017; Atkinson et al., 2021; Costa et al., 2022; Pirotta et al., 2017).

Commercially available UASs are becoming increasingly affordable, durable to adverse weather conditions, with longer battery life (i.e. increased total flight time), and are rapidly increasing in popularity (Christie et al., 2016). Remote-controlled aerial systems provide the opportunity to collect genetic material from whales without having to approach the target individual in a vessel or make physical contact (Acevedo-Whitehouse et al., 2010), thereby minimizing disturbance to the target animal (Atkinson et al., 2021; Domínguez-Sánchez et al., 2018). In addition, they allow for the simultaneous

collection of photogrammetry data and informative behavioural footage (Fettermann et al., 2022; Keen et al., 2021, 2023).

The study of marine mammals is frequently carried out in remote locations with no direct access to laboratory facilities. Thus, it is important to evaluate if UAS-based blow sampling represents a cost-effective approach for the collection of genetic samples, which is less invasive than biopsy sampling (or other close-approach sampling techniques) but can still be used in such locations to eventually generate genetic datasets. Given the limitations of 'off-grid' power supplies and large distances to laboratory-grade freezers, we set out to test blow sampling of humpback and fin whales with a UAS from an off-grid and remote field station. We aimed to determine the efficacy of baleen whale blow to provide adequate DNA for downstream population genetics questions. We extracted DNA from the blow samples, sequenced the variable region of the mtDNA control region to define haplotype, amplified regions of the ZFX and SRY genes for sex identification, targeted nuclear DNA polymorphic regions well suited for individual identification and population genetics analyses and explored behavioural responses of whales to the UAS collection technique.

We explored the feasibility of this approach within coastal British Columbia (BC), Canada, whereby these culturally and ecologically important rorqual species are faced with increasing stressors of predicted unsustainable impact (Keen et al., 2023). Within the marine territory of the Gitga'at First Nation, humpback and fin whales utilize a fjord system, known in the literature as the Kitimat Fjord System (KFS; Figure 1), as important foraging grounds (Keen et al., 2018), and this area has therefore been proposed as critical habitat (Ashe et al., 2013; Nichol et al., 2010). As with other humpback whale populations, high levels of site fidelity result in the return of known individuals after migration from tropical breeding grounds, year-by-year (Wray et al., 2021). The KFS is thought to be the only known fjord system used regularly by fin whales, which also show strong site fidelity since their return to the area in 2005–2006 (Keen et al., 2021).

The return and growth of both the humpback and fin whale populations within this region have been documented by the North Coast Cetacean Society (NCCS) photo-identification catalogues since 2004 (Ashe et al., 2013; Keen et al., 2021) and both species are listed as 'Special Concern' by the Committee on the Status of Endangered Wildlife in Canada. However, ongoing research and monitoring of these species are critical, given (1) the recent decline in the humpback whale calving rate locally (Wray & Keen, 2020), in south-eastern Alaska (Neilson & Gabriele, 2019) and at the Hawaiian breeding ground for this aggregation (Cartwright et al., 2019), which play a role in the recent population decline of an estimated 20% across the North Pacific between 2012 and 2021 (Cheeseman et al., 2024); (2) Unusual Mortality Events (UME) of fin and humpback whales in Alaska and BC between 2015 and 2016 (Savage, 2017); as well as (3) the impending liquefied natural gas tanker route through the KFS which is estimated to increase large vessel traffic in the study area 4.2 times above the 2019 baseline rate (Keen et al., 2023). Within Gitga'at territory, it is predicted that whale–ship co-occurrences will increase 30-fold by 2030 for such large ships (length > 180 m), resulting in a projected ship-strike mortality rate increase of 2.3 times for fin whales and 3.9 times for humpback whales, likely exceeding the potential biological removal for the North Coast sector of the BC exclusive economic zone (Keen et al., 2023).

2 | METHODS

2.1 | Study area

Blow samples were collected by Transport Canada licenced drone pilots using an UAS within the marine territory of the Gitga'at First Nation, where foraging rorquals utilize a fjord system that stretches 140 km inland from the open North Pacific Ocean (Keen et al., 2017). The KFS comprises a number of large islands, one of which serves as the research base for NCCS (Fin Island Research Station (FIRS), 53°13′18.94″ N, 129°22′34.77″ W). Blow sampling flights were conducted under Department of Fisheries and Oceans research permit XMMS 22018 between July and October 2019, both from land (at research base, FIRS) or from the research vessel, a 7 m outboard skiff, during daylight hours (earliest sample: 6:30 AM; latest sample: 6:00 PM). Vessel-based sampling occurred predominantly within Squally Channel and Verney Passage, two known hotspots for foraging humpback whales (Figure 1). Blow sampling and associated metadata collection were approved by the University of St Andrews' School of Biology Ethics Committee (Ref: SEC20030).

2.2 | Unoccupied aerial system flight protocols and sample collection

A range of commercially available UAS models have been utilized for cetacean blow sampling. Here, we chose to work with the DJI Mavic 2 Pro (www.dji.com) due to its relatively small size (354 mm unfolded diagonal length) and durability, given harsh sampling conditions. The Mavic 2 Pro has an acoustic footprint of 45.5 dBA at 30 m height (Brunton et al., 2019) and benefits from DJI software reliability, off-the-shelf affordability and probable likelihood of withstanding moisture from the blow itself. This model is a multirotor quadcopter, capable of hovering in place over a target individual and has an approximate flight time of 30 min in wind-still conditions. This provides ample opportunity to launch, locate and sample an individual whale and then retrieve the UAS. Sampling flights were generally kept below 5 min, to minimize disturbance. We built attachments for the Mavic 2 Pro to raise the body further off the ground, creating space to both attach sterile petri dishes and create a handle to catch the UAS in the air (Figure S1).

Blow samples were collected non-systematically, taking every sampling opportunity available, to maximize the study's sample size. Flights were only conducted in good weather conditions (no rain and a Beaufort Sea State < 3). The exhalant can be quickly dispersed or flattened by the wind, such that sampling becomes more difficult in high wind conditions. For sampling, the vessel was turned upwind

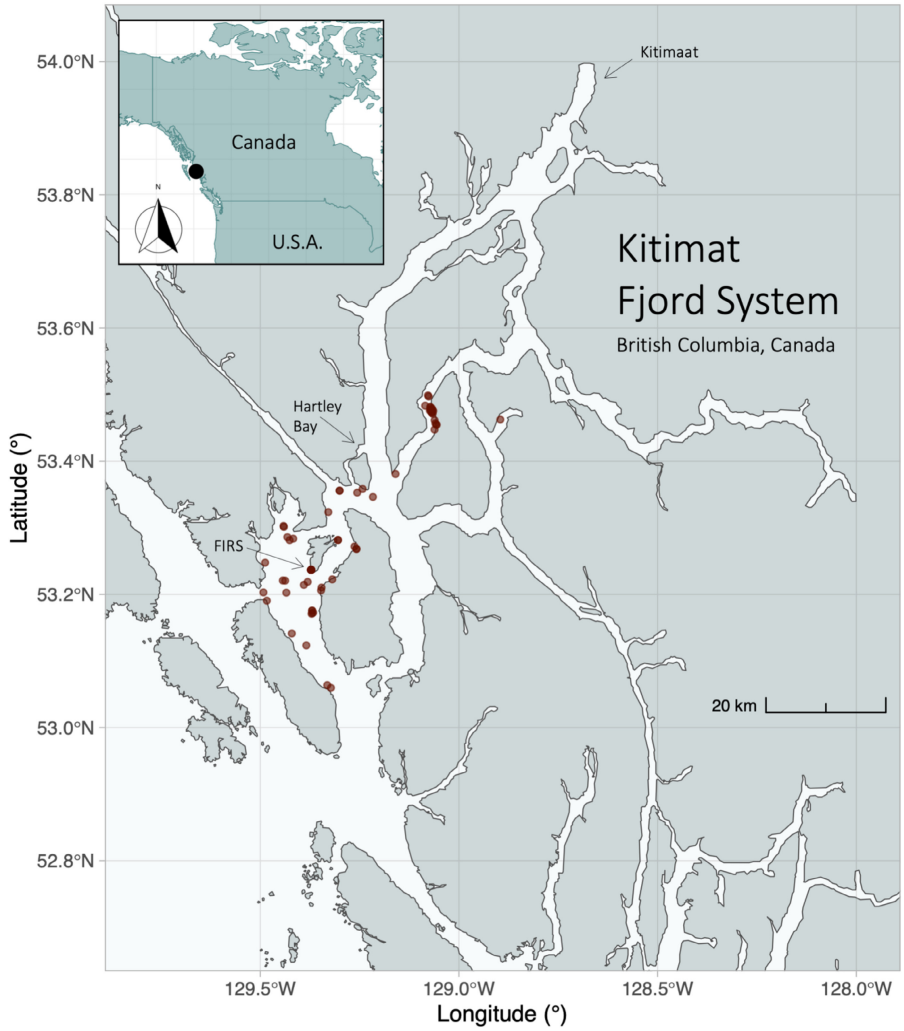

FIGURE 1 Blow sampling locations in the Kitimat Fjord System, British Columbia, Canada. Map includes marine territories of the Gitga'at, Kitasoo/Xai'xais and Haisla First Nations. The field station (Fin Island Research Station = FIRS), the Gitga'at population centre (Hartley Bay) and the principal community centre for the Haisla (Kitimaat) are labelled. Figure produced with R packages *bangarang v. 1.3.3* (Keen, 2016) and *ggplot2* (Wickham, 2016).

and left idling, while the UAS was launched from the lid of a plastic storage container (preventing magnetic interference of flight controls from the metal hull of the skiff).

If sampling was to occur from a group of whales, the farthest upwind individual was targeted first to prevent sample contamination. A group is here defined as two or more individuals within two body lengths of each other and coordinating their behaviour and/or breathing pattern for at least one surfacing (Wray et al., 2021). Contamination prevention is critical for later individual identification using DNA genotyping from whale blow samples. The exhalant travels downwind, likely contaminating the airspace above the downwind individuals (Figure S2). If it was wind still (Beaufort 0, wind speed <1 km/h), other group members were consequently sampled, otherwise only group members with sufficient distance of a minimum of approximately one body length between each other were sampled.

The target individual was approached at a height of ~20 m, to minimize disturbance and chance of petri dish contamination from other whales. Once above the target whale, the UAS was lowered to ~10 m and positioned aft of the blowhole. Flying in First-Person-View allowed for the breath to be pre-empted, so that on each breath, the UAS was lowered down to 2–3 m and flown through the visible cloud of droplets (Costa et al., 2022). The aim was to fly through as many breaths per flight as possible, but ultimately the total number of breaths sampled per flight was determined by behavioural response, diving behaviour, breath force, weather or remaining flight time.

Identification of rostrum markings and dorsal fins using the UAS's First-Person-View was used by the drone pilot in real time to ensure that all members of a group were sampled across different flights. These unique rostra proved a critical element of the sampling workflow, as typically the sample is collected *prior* to the field crew seeing the tail fluke for identification (ID). This allowed for the targeted resampling of a whale when a previous sample was deemed inadequate (based on droplet quantity visible in the petri dish), or, as aforementioned, to ensure that every member of a group was sampled. An image of the ventral side of the sampled whale's tail fluke

was captured for photo-ID as often as possible, using a standard DSLR camera and telephoto lens, adopting established protocols for these species (Ashe et al., 2013; Calambokidis et al., 2008; Wray et al., 2021). Whenever possible, the ventral fluke was also captured in 4K video by the UAS, to cross-reference the ID of the sampled whale between the UAS footage and the DSLR photographs. Fluke photographs were manually matched by two observers to the NCCS catalogues, and unmatched whales were given a new identifier.

Behavioural responses of the whale to a combination of the UAS and outboard noise were carefully monitored in real time and ranked by the UAS pilot using a scale adapted from Weinrich (1991; Table 1). A response was classed as any abrupt change in behaviour as the UAS neared the target individual and therefore was visible through the live video feed of the UAS. The grade of response was then determined based on the specific behavioural change (e.g. a response grade of 2 was assigned if the target whale clearly changed its swimming direction once the UAS approached the individual; Figure 3). When a response was noticed by the pilot (grade ≥ 1), the flight was terminated and that individual was not flown over again that day.

Blow samples were considered 'retainable' if droplets were visible to the naked eye in at least one-half of the petri dish. These were then labelled, photographed and sealed in their petri dishes using Parafilm®, and stored in a cooler on ice until later processing at the field station. The UAS was sterilized with disinfectant between all sampling events and a new sterile petri dish was attached in preparation for the next flight. All sample handling was carried out wearing a fresh pair of silicone gloves to prevent cross-contamination. Metadata were recorded (date, time, flight number, weather conditions, whale behaviour, behavioural response, group size and sample description). Sample descriptions were based on the droplet size and concentration within the petri dish (larger and more abundant droplets = 'wet'; smaller and less abundant droplets = 'dry'; Figure 2). This qualitative descriptor was also informed by the breath force visible by the pilot in First-Person-View and the amount of blow visible on the UAS body after the flight. This qualitative descriptor was chosen because it seemed to be the most visually evident difference across samples, as we aimed to find and test an in-field predictor of sample quality.

As soon as possible, and always within the same calendar day, samples were processed for more permanent storage. Wearing silicone gloves and using a new sterile scraper per sample, blow droplets were diluted with 1.5 mL dH_2O and 0.5 mL Longmire's solution (100 mM Tris, 100 mM EDTA, 10 mM NaCl, 0.5% SDS and 0.2% sodium azide), pipetted into a 2 mL cryovial and sealed with Parafilm®. Samples were frozen in a solar-powered UGP-65L portable fridge/freezer. This was powered via a 12V flooded battery and ProStar solar charge controller by a 120W solar panel. Approximately once per week, samples were transferred from the off-grid site into Hartley Bay, where they were stored in a household freezer at ~ −20°C until the end of the field season (mid-October 2019). Samples were then transferred on dry ice to the Cetacean Conservation and Genomics Laboratory (CCGL) at Oregon State University, OR, USA, where they were stored in a laboratory grade −20°C freezer for ~5 months until DNA extraction and sequencing.

2.3 | DNA extraction and quantification

Total genomic DNA was extracted using the QIAGEN DNeasy® Blood and Tissue Kit spin-column extraction method (QIAGEN, 2006). At the lysis step, 20 μL of proteinase K was added directly into each sample containing the Longmire's Buffer, dH_2O and blow and incubated for 1 h with rocking at 56°C. Standard protocols were then followed, except volumes were modified for an increased volume of ~2 mL per sample. Extractions were run in six subsets of between 15 and 22 samples, with a negative control. Each subset contained a representative number of samples from throughout the field season. DNA was eluted in 200 μL water and 200 μL AE buffer and then concentrated by evaporation to 50 μL prior to further laboratory analysis.

Droplet digital (dd) PCR was used to quantify the concentration of mtDNA in the blow samples, using mtDNA-specific primers and probes designed by Baker et al. (2018). Results are given as the average number of amplified target copies per microlitre of ddPCR reaction, that is, copies/μL, for each sample.

2.4 | Mitochondrial DNA amplification and sequencing

Previously published primers (forward primer M13Dlp1.5; 5′-TGTAAAACGACAGCCAGTTCACCCAAAGCTGRARTTCTA-3′; Dalebout et al., 1998) and reverse primer Dlp5R (5′-CCATCGAGATGTCTTATTTAAGGGGAAC-3′; Dalebout et al., 1998) were used to amplify a 500-base-pair (bp) portion of the mitochondrial control region (D-loop) for mtDNA haplotype assignment. The PCR master mix

TABLE 1 Behavioural response grading system used for all whale approach UAS flights, adapted from Weinrich (1991).

Grade	Description
0	No detectable behavioural response.
1	Minor behavioural response detectable (e.g. stronger breath force or change in surface breath pattern).
2	Medium behavioural response detectable (e.g. change in swimming direction, early fluking).
3	Strong behavioural response detectable (e.g. tail slaps, breaching, pectoral fin slapping).

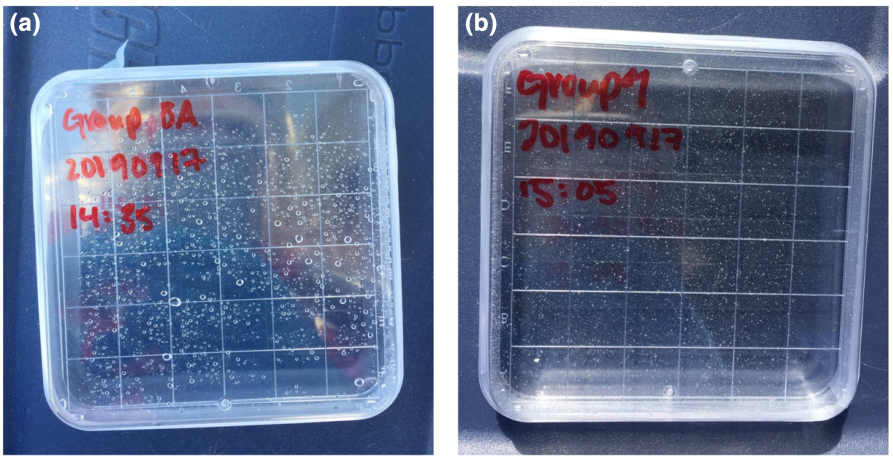

FIGURE 2 Post-sampling petri dishes photographed in the field, depicting the difference between the qualitative wet/dry variable used in correlation analyses. (a) This sample is classed as 'wet', while (b) is classed as 'dry', based on the blow droplet sizes visible in real time, the drone footage and the petri dishes.

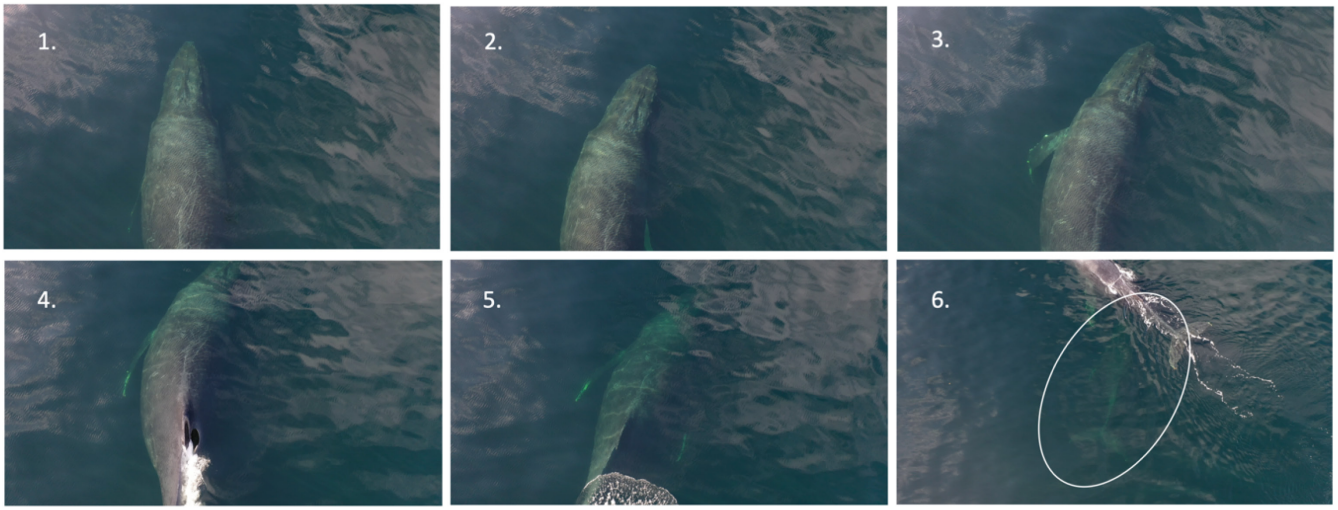

FIGURE 3 An observable behavioural response (grade 2), likely due to the presence of the unoccupied aerial system (UAS) for blow sampling. See text for detailed description.

consisted of 1× buffer (Invitrogen), 2.5 mM Mg^{2+}, 0.4 μM of each primer, 0.2 μM dNTPs, 1/8 Unit of Platinum *Taq* DNA Polymerase (Invitrogen) and 2 μL of template DNA. Reactions were made up to a total volume of 25 μL with nuclease-free and DNase-free water.

All sample batches were run with a positive odontocete control (Hector's dolphin, *Cephalorhynchus hectori*, a species endemic to New Zealand) and a PCR blank. PCR amplification consisted of a denaturation period of 3 min at 93°C, followed by 30 cycles of 94°C for 30 s, annealing temperature of 55°C for 45 s and extension of 72°C for 60 s. There was a final extension step at 72°C for a further 10 min.

Sequencing was carried out with BigDye v3.1 chemistry and run on an ABI 3730xl sequencer (Applied Biosystems). mtDNA D-loop sequences were visually inspected and aligned, and haplotypes were assigned using SEQUENCHER v4.6 (Gene Codes). Haplotypes were defined from a 500 bp segment and assigned through a comparison to previously published sequences (e.g. Baker et al., 2013). SEQUENCHER was used to assign ABI base quality scores, at an error rate of lower than 1 in 1000, that is, a quality cut-off equivalent to a PHRED score of greater than 30 (Baker et al., 2013; Ewing & Green, 1998). Missing data were attributed to either (1) failure to amplify for the D-loop primers (and so there was no band on the gel) or (2) the quality of the sequence fell below the 90% ABI score, and thus were excluded.

2.5 | Sex determination

A multiplex PCR was used to determine the sex of the individual, using primers P1-5EZ and P2-3EZ, amplifying a 443–445 bp region of the ZFX gene (Aasen & Medrano, 1990), in addition to primers Y53-3C and Y53-3D, amplifying a 224 bp region of the SRY gene (Gilson et al., 1998). PCR conditions were similar to the mtDNA conditions listed above, but 5 μL of DNA was used as a template. PCR amplification consisted of a denaturation period of 2 min at 93°C, followed by 30 cycles of 94°C for 45 s, annealing temperature of 60°C for 45 s and extension of 72°C for 60 s. There was a final extension step at 72°C for a further 10 min. Sex was

determined based on banding visible after agarose gel electrophoresis (two bands = male; single band = female). The sex ratio across humpback whale samples (with duplicates removed) was compared to an expected 1:1 ratio (for feeding grounds of this species) using an exact binomial test. Females identified using genetics were also cross-referenced with historical social data of known mothers based on fluke ID and documented in the KFS by NCCS anytime since 2004.

2.6 | Microsatellite genotyping

An effort was made to genotype all blow samples at 10 published microsatellite loci: EV14, EV37, EV96 (Valsecchi & Amos, 1996), GATA28, GATA417 (Palsbøll, Allen, et al., 1997), GT211, GT23, GT575 (Bérubé et al., 2000), rw4-10 and rw48 (Waldick et al., 1999; see Table S1). These loci were chosen due to their extensive use in similar studies, particularly within the Pacific Ocean basin-wide study Structure of Populations, Levels of Abundance and Status of Humpback Whales in the North Pacific, or 'SPLASH' (Calambokidis et al., 2008), and genetic extension led by Baker et al. (2013).

DNA amplifications were performed in a total volume of 10 μL, containing 1× reaction buffer, 1.5 to 3.5 mM $MgCl^2$, 0.4 μM of each primer, 0.2 mM dNTP's, ¼ U of Platinum *Taq* (Invitrogen) and ~2 μL of DNA. Primers were fluorescently labelled and co-loaded in sets of five loci on an ABI 3730xl sequencer (Applied Biosystems) with formamide and 500 LIZ™ size standard (Applied Biosystems). Following this, GENEMAPPER v3.7 software (Applied Biosystems) was used to size and bin alleles. Peaks and automated bins were manually assessed, as is standard protocol (Ewen et al., 2000). Based on this, a genotype was called for each sample.

The total number of successfully amplified loci became the primary quality control (QC) threshold for retaining samples for the final dataset. Samples which failed at three or more microsatellite loci were excluded from further analyses. The final QC dataset used for downstream analysis excluded duplicate samples identified using photo-ID of the ventral fluke and replicates identified in genetic analyses (as discussed below), where the sample retained was chosen based on higher amplification success, that is, between duplicates, the sample with more loci amplified was retained.

2.7 | Microsatellite allelic dropout, false and null alleles

Multiple extractions were not possible as the entire blow sample was consumed in the extraction due to the anticipated low concentration of DNA preserved in the samples. Therefore, there was a limited volume of DNA used to target microsatellite loci. PCRs were repeated for samples that failed at certain loci until all DNA was exhausted. MicroChecker v. 2.2.3 was used to assess the presence of null alleles and error due to stutter (Dewoody et al., 2006; Van Oosterhout et al., 2004).

2.8 | Statistical analyses: Population genetics

Given that our goal was to determine the efficacy of blow to provide adequate DNA for downstream population genetics questions, we calculated a range of standard population genetics metrics. Allele and null allele frequencies, allelic diversity, linkage disequilibrium among pairs of loci and deviations from Hardy–Weinberg equilibrium (HWE) were calculated for each locus using the QC dataset in Genepop v. 4.7.5 (2016), through the R package *genepop* v. 1.1.7 (Rousset, 2008). Exact tests for HWE (Guo & Thompson, 1992; Weir, 1996) were computed using the complete enumeration algorithm (Louis & Dempster, 1987), as the QC dataset has less than 1000 samples ($n = 41$; Rousset, 2020). Estimation of exact *p*-values was by the Markov chain method. Expected heterozygosity (H_E), observed heterozygosity (H_O) and minor allele frequencies (MAF) were calculated using R package *adegenet* v. 2.1.3 in R (Jombart, 2008).

To identify individual whales that had been sampled more than once, the program Cervus was used to find matching genotypes (Kalinowski et al., 2007). For each match, a probability of identity ($p_{(ID)}$) was calculated using GenAlEx 6.5 (Peakall & Smouse, 2006, 2012), where $p_{(ID)}$ is the probability that two randomly drawn individuals from a population will have the same genotype at multiple loci (Waits et al., 2001). All genotype matches were then cross-referenced with photo-IDs from the field, as well as mtDNA haplotypes and sex, as a measure of laboratory and field data accuracy. A low $p_{(ID)}$ would allow for the genetic re-identification of humpbacks within and between projects, and therefore across their migratory routes (Waits et al., 2001).

2.9 | Statistical analyses: Predictors of sample quality

Next, we set out to establish if certain field and laboratory variables can act as predictors of sample 'success'. To do this, we examined the relationships between the number of breaths flown through per sample per flight and a visual quantification of DNA yield ('wet' or 'dry') as predictors of either DNA concentration as measured by the ddPCR, or the number of microsatellite loci amplified per sample. These predictors might then allow a field team to make more informed decisions, such as whether an individual should be resampled, as well as cut down on downstream laboratory time and costs associated with processing poor-quality samples. First, we explored if a higher breath count resulted in a more complete genetic profile. To do this, we used a Poisson generalized linear model (GLM; McCullagh & Nelder, 2019) to model the relationship between the total number of microsatellite loci amplified per sample and the total number of breaths sampled per sampling event. A Poisson regression was chosen because the dependent variable was count data.

Second, a standard linear regression was used to establish if the number of breaths collected in a sampling event could predict mtDNA concentration as determined by the ddPCR. In this analysis, the dependent variable log(ddPCR) was modelled on the (standardized) total number of breaths per sampling event.

Third, a point biserial correlation analysis was used to quantify the strength of relationship between the DNA concentration measured by the ddPCR results and the binary wet/dry variable describing blow sample quality. This method allows for the calculation of Pearson's product–moment correlation (rho) between a continuous and a dichotomous variable (Kornbrot, 2005) and provides an associated *p*-value, indicating the statistical significance of rho.

Finally, we asked if ddPCR mtDNA concentration estimations correlated with high microsatellite loci amplification, allowing ddPCR to be a useful tool for determining sample suitability for later nuclear DNA studies. Using a GLM, the number of loci amplified per sample was modelled on log(ddPCR) of each corresponding sample.

All explanatory variables were standardized to a log scale to compare their effect sizes. All scripts were run in R v. 4.0.2 (R Core Team, 2020).

3 | RESULTS

3.1 | Sample collection and behavioural responses

The UAS was deployed 112 times for blow sampling flights. A total of 88 blow samples were collected between July and November 2019, 87 samples from humpback whales and 1 sample was opportunistically collected from a fin whale. The majority (79.5%) of blow sampling flights were conducted from the bow of the research vessel, and of these 89 boat-based sampling attempts, 74 (83.15%) yielded visible blow droplets to justify retaining the sample. Of the 23 sampling flights conducted from land, 14 (60.9%) yielded retainable samples. The fin whale sample volume was less than expected, given it resulted from a high number of breaths ($n = 6$). For humpback whales, the average number of breaths collected was 3.68 per individual (min = 1; median = 3; max = 9). The time interval between sample collection and processing with Longmire's ranged from 13 min to 10.5 h (mean = 5.2 h). Samples collected on land were processed more quickly than those collected while on a marine survey (land-based mean = 0.4 h; boat-based mean = 6.1 h).

High-quality photo-IDs were captured for 76 of the samples with the DSLR camera from the boat or land, with six whales not fluking (and therefore no photo-ID possible) and five photos deemed as too poor quality for a confident match. Of 112 sampling flights, 50 flights (44.6%) succeeded in additionally capturing the photo-ID with the UAS. All UAS-captured photo-IDs matched those captured by the DSLR, cross-validating each photo type as a reliable source of identification. Behavioural responses to the UAS were rare but not completely absent. A grade 0 response (i.e. no detectable response) was observed 86.5% of the time, with grades 1, 2 and 3 ranking at 10.6%, 2.9% and 0% respectively. Due to a low number of behavioural responses (i.e. low sample size), we did not explore correlations between behavioural response grade with prior behavioural state. However, we noticed that foraging behaviours generally resulted in higher volume, 'wet' samples, when compared with resting behaviours, and seemed not to elicit behavioural responses. An example of a high behavioural response included a target individual clearly altering its swimming course while completely submerged (Figure 3 [Frames 2–5]), followed by a shallow dive beneath the second individual of this group (Figure 3 [Frame 6]). The other individual had already been sampled (response grade 0) and showed no behavioural change during this second sampling flight. The target whale was observed resurfacing 45 seconds after its initial behavioural change and returned to its prior behavioural state, thus was graded as a behavioural response of 2.

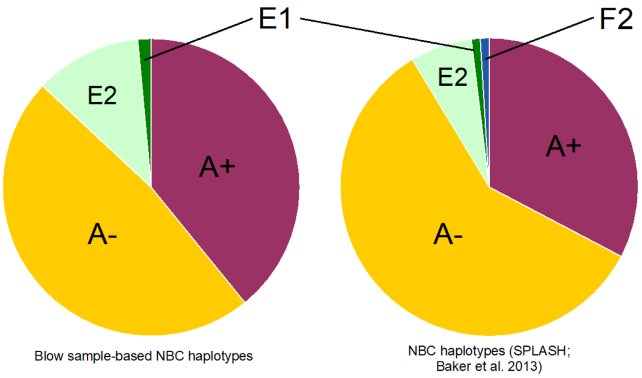

FIGURE 4 Mitochondrial (mt)DNA haplotype frequencies of the blow sample humpback whale dataset from the Kitimat Fjord System of northern British Columbia (left), in comparison with those found through biopsy sampling in northern British Columbia, as presented by Baker et al. (2013; right).

3.2 | Mitochondrial DNA and sex PCR

Of the 88 blow samples, 70 (79.55%) were amplified for the 500 bp fragment of the maternally inherited mtDNA D-loop region. Sixty-nine of the 70 samples that were amplified were identified as humpback whales by comparison of the sequenced D-loop region to internal databases at CCGL. Of these, 33 (48%) were haplotype *A*–, 27 (39%) were *A*+, 8 (12%) were *E2* and 1 (1%) was the *E1* haplotype (all haplotype nomenclature following Baker et al., 2013). The single fin whale sample was identified as haplotype *G* (Hatch et al., 2006). The diversity of mtDNA haplotypes of humpback whales sequenced in this study is low, with *A*– and *A*+ haplotypes representing 87% of the samples. This low diversity is not a product of the sample format (i.e. blow) and instead is likely due to the overall low levels of mtDNA diversity in humpback whales of Northern British Columbia (NBC) and South-East Alaska (SEAK; Baker et al., 2013). In fact, of the 8 breeding grounds and 10 feeding grounds sampled during this SPLASH study, NBC-SEAK haplotype diversity was found to be the lowest. The frequency distribution of haplotypes found here is very similar to that found through biopsy sampling in the NBC region by

SPLASH, which was 56%, 31%, 6% and 1% for *A–*, *A+*, *E2* and *E1*, respectively, with an additional 1% represented by an *F2* haplotype not found in this study (Figure 4; Baker et al., 2013). Sequences representing each of the four haplotypes can be found on GenBank (KF477244, KF477245, KF477249 and KF477256) with haplotype frequency information submitted to the Dryad Digital Repository (DOI: 10.5061/dryad.6djh9w185).

Sex was determined for 78 samples: 34 males and 44 females (88.64% success rate). No sex could be determined for the fin whale. An exact binomial test showed no significant difference to the expected 1:1 sex ratio, after removal of duplicate samples (female:male sex ratio of 1.25:1, $n=63$, $p=.45$). Fourteen of the females are known mothers who have brought calves to the KFS. All duplicate samples (based on photo-ID and genetic matching, $n=7$ pairs of samples) had the same sex and mtDNA haplotype assignment.

3.3 | Microsatellite genotyping

Of the 88 samples, 53 samples were genotyped for ≥8 microsatellite loci, 46 for ≥9 loci and 36 for all 10 loci. The average number of loci genotyped per sample was 7.5 of 10 targeted loci (see Table S3 for genotype information). The dataset before QC has a mean of 7.5 distinct alleles per locus, with a minimum of 5 (rw4-10) and a maximum of 13 (both EV37 and GATA417; Table 2). The percentage missing loci across all individuals drops to 3.41% in the QC dataset, which has photo-ID and genetic duplicates removed and data filtered to retain samples with a minimum of 8 of 10 microsatellite loci genotyped ($n=41$). All analyses discussed hereafter utilize this QC dataset (unless otherwise specified).

The MAF for each locus is presented in Table 2, providing insight into the threshold between common and rare variants (Linck & Battey, 2019). Often a threshold of 0.05 is used to identify rare variants (International HapMap Consortium, 2003) and here all MAF values >0.05 (0.22 ± 0.08, mean ± SD). Table 2 includes comparative columns between the North Pacific humpback whale SPLASH study and this dataset, comparing the total number of alleles genotyped per locus in the NBC region. This study recovered a comparatively similar number of alleles despite a lower sample size and lower input DNA concentration (Table 2).

MicroChecker found no evidence of significant allelic dropout or error due to stutter using a 95% confidence interval for the Monte Carlo simulations. Exact tests for HWE using complete enumeration yielded 2 of 10 loci that differed significantly from HWE (GT211, $p<.05$; rw48, $p<.0001$; Table 3). The inbreeding coefficient, F_{IS}, for each locus, is presented in Table 3, after methods presented by Weir and Cockerham (1984). Locus rw48 shows the highest levels of inbreeding ($F_{IS}=0.4453$) as well as the most significant deviation from HWE. However, this could be due to allelic dropout, given the low concentrations of DNA.

3.4 | Duplicate samples and photo-identification

All genetic matches found within the blow dataset were confirmed using photo-ID of either the fluke or rostrum markings, where genetic sample pairs were accepted as matches if they were mismatching at a maximum of one locus, as long as the photo-ID of these samples was a match, to allow for some error arising from allelic dropout. One exact match based on microsatellite loci was found between one blow sampled whale and an individual sampled by biopsy during the 2006–2008 SPLASH study ($p_{(ID)}=1.11E\text{-}13$). Within this study, three pairs of blow samples were identified as genetic matches matching at all microsatellite loci and seven pairs matched at all but one locus. In a perfect match, one of the whales did not fluke during the sampling occasion and therefore no photo-ID was obtained. UAS footage was then used to corroborate these findings using rostrum markings, demonstrating additional photo-ID potential and benefits to UAS-based sampling due to the bird's eye perspective.

TABLE 2 Allele ranges and number of observed alleles per locus (presented for SPLASH study, population 'northern British Columbia' (NBC; Baker et al., 2013) and this study 'blow data'). Expected heterozygosity (H_E), observed heterozygosity (H_O) and minor allele frequencies (MAF) are calculated using R package *adegenet* v 2.1.3 in R (Jombart, 2008).

Locus	Allele range	# of alleles (NBC SPLASH data, $n=123$)	# of alleles (blow data, $n=88$)	H_O	H_E	MAF
EV14	129–139	6	6	0.49	0.63	0.26
EV37	190–226	14	13	0.74	0.87	0.13
EV96	147–167	10	7	0.74	0.77	0.28
GATA28	143–187	6	7	0.25	0.27	0.06
GATA417	191–274	16	13	0.76	0.86	0.20
GT211	102–118	8	8	0.55	0.74	0.33
GT23	109–119	7	6	0.63	0.77	0.19
GT575	143–165	11	10	0.61	0.69	0.20
rw4-10	194–206	7	5	0.54	0.61	0.26
rw48	112–122	6	6	0.40	0.71	0.29

TABLE 3 Exact tests for deviation from Hardy–Weinberg equilibrium (HWE) for each locus, as well as the per-locus inbreeding coefficient, F_{IS}.

Locus	HWE exact test *p*-value	Std. error	F_{IS} (W&C)
EV14	.1512	0.0025	0.2355
EV37	.0761	0.0041	0.1682
EV96	.1854	0.0034	0.0442
GATA28	.3124	–	0.0856
GATA417	.3221	0.0088	0.1286
GT211	.0064	0.0005	0.2726
GT23	.1393	0.0023	0.1996
GT575	.1348	0.0044	0.1224
rw4-10	.3889	0.0035	0.1373
rw48	.0000	0.0000	0.4453

TABLE 4 Generalized linear models (GLMs; models 1 and 3) and standard linear regression (model 2) exploring the relationships between DNA concentration in samples (ddPCR); the total number of breaths sampled per sampling event; and the number of microsatellite loci amplified.

Model	Slope	Std. error	*p*-value
1. (Loci amplified) ~ (std. breaths)	0.18	0.040	***
2. (Std. ddPCR) ~ (std. breaths)	0.74	0.185	***
3. (Loci amplified) ~ (std. ddPCR)	0.33	0.045	***

Abbreviations: Std., standardized explanatory variables; Std. error, standard error of the slope.

***Highly significant *p*-value <.0001.

3.5 | Droplet digital PCR and sample quality prediction

The range of ddPCR concentrations for targeted mtDNA was 0–375 copies/μL, with a median value of 6.05 copies/μL (21.19 ± 48.32, mean ± SD, $n = 88$). With the QC dataset, the mean increased to 34.23 copies/μL (± 59.18 copies/μL, ± SD, $n = 53$). ddPCR results are presented in Table S2.

The Poisson GLM showed that there was a significant positive relationship between the total number of breaths per sampling event and the total number of microsatellite loci amplified per sample (slope = 0.18, std. error of the slope = 0.040, $p < .0001$; Table 4). Linear regression of log(ddPCR) on the standardized number of breaths demonstrated a significant effect, where an increase in the number of breaths sampled increases the total mtDNA concentration of samples (slope = 0.74, std. error = 0.185, $p = .00015$; Table 4).

The point biserial correlation analysis between the ddPCR results and the binary wet/dry variable yielded a significant positive correlation, whereby 'wet' samples were correlated with higher quantities of mtDNA concentration (Pearson's product–moment correlation coefficient (r) = .32, $p = .002164$). The ratio of wet to dry samples was 34 wet to 54 dry samples. Finally, to corroborate the use of ddPCR to select samples for microsatellite amplification, the GLM showed that there was a significant positive relationship between the mtDNA concentration as measured by ddPCR and the total number of microsatellite loci that amplified per sample (slope = 0.33, std. error of the slope = 0.045, $p < .0001$; Table 4).

4 | DISCUSSION

Through the collection of 88 blow samples from humpback whales and fin whales, we demonstrate the feasibility of blow sampling with a small Unoccupied Aerial System (UAS) in a remote, off-grid location – the temperate Great Bear Rainforest – for the conservation genetics of baleen whales. A primary objective was to collect and preserve blow samples on a low budget, using commercially available equipment (e.g. a solar-powered freezer and the Mavic 2 Pro UAS) to facilitate collaboration between academic and/or federal laboratories and non-profit organizations (NPOs). Costs were kept down by using an off-the-shelf, low-cost UAS; by collecting as many samples as possible from the shore without the need for a boat and associated crew; and samples were stored in regular household and solar-powered freezers before the transfer to the laboratory.

This study builds upon the protocols described in Atkinson et al. (2021), with promising results demonstrating the potential of blow samples for conservation and population genetics research. Compared to this previous work, we were able to score a larger number of microsatellites, on average 7.5 loci of 10 were amplified with 36 samples amplified at all 10 loci. Of the 88 samples collected, 78 (~89%) succeeded in sex determination and 70 samples (~80%) had successful mtDNA haplotype assignment. As expected, a higher number of breaths flown through with the UAS generally resulted in a higher mtDNA concentration and a larger number of amplified microsatellite loci ($p < .0001$). Additionally, 'wet' samples resulted in higher mtDNA concentrations. The UAS pilot blow sampling baleen whales should thus aim for as many breaths as possible in one sampling event (while monitoring behavioural responses in real time) and consider targeting behavioural states which result in 'wet' sample types (e.g. in our case, foraging whales).

These improvements likely stem from the field setting, given that the samples were collected in the narrow channels of a fjord system (Figure 1). The channels are protected from the open ocean by a network of islands, which prevents oceanic swell from reaching sampling areas. We only attempted blow sampling flights in calm weather conditions (a Beaufort Sea State < 3). Additionally, the behavioural states of the KFS humpback whales might contribute towards better DNA profiling, with foraging at depth as the most commonly sampled behavioural state during this study. In this behavioural state, we noticed that whales resurfaced after a prolonged period submerged (~5–7 min), taking several forceful, 'wet' breaths at the surface, before fluking once more. This diving pattern resulted

in sampling flights of less than 5 min in total length; with high-quality samples and a UAS-based fluke ID.

The median mtDNA concentration, as measured by the ddPCR, was 6.05 copies/μL (mean = 21.19 copies/μL; $n = 88$ samples) but with a large range, indicating that DNA concentration varies among blow samples, some of which are not worth amplifying for the chosen molecular marker. Given that higher ddPCR concentrations were positively correlated with higher numbers of amplified microsatellites ($p < .0001$), ddPCR can be used as a first laboratory step to identify which samples in a dataset should be retained. Going forward, these ddPCR results can be used as a benchmark for comparison to blow samples collected under further optimized field methodologies, or against other non-invasive sources of cetacean DNA such as sloughed skin and eDNA samples from the 'wake of whales', meaning sampling surface water in the fluke print of a whale (Baker et al., 2018). Blow sampling has some clear advantages over these other sample types, most notably that cross-contamination can be minimized and known individuals can be targeted and genetically identified. This is especially true when sampling from groups of whales, where sloughed skin or eDNA detections cannot be confidently attributed to individuals. Furthermore, the genetic profiles assembled from blow, targeting both nuclear and mitochondrial DNA, are more extensive than what is typically possible with eDNA, where species detection using 'mini-barcodes' is often the primary goal (Alter et al., 2022; Baker et al., 2018, 2023; Foote et al., 2012), or, at most, mtDNA haplotypes have been generated (e.g. Baker et al., 2018; Parsons et al., 2018; Székely et al., 2021).

As respiratory blow is a fresh biological sample, we showed sample DNA to be relatively intact but in low concentrations, as it stems from epithelial cells suspended in exhalant. Limitations to using blow therefore do exist – particularly the low amount of DNA obtained. This often led to the entire blow sample needing to be exhausted in the analysis to maximize amplification success. Unlike with direct tissue sampling, no archive of genetic material was retained for later re-extraction. However, considering previous blow sequencing results (Atkinson et al., 2021; Sremba et al., 2019), we can confidently say that we see clear improvements with each iteration of sample collection and laboratory processing.

It is often recommended that when working with non-invasive sources of DNA, such as faecal matter or sloughed skin, one should amplify and genotype samples more than once to obtain reliable results for downstream analyses (Boston et al., 2012). Generally, a consensus genotype is then recorded at each locus based on the rule of thumb that an allele must appear at least twice across repetitions (Boston et al., 2012). This poses a significant challenge for blow due to its relatively low DNA quantity. The only perceived solution to this is to collect replicate samples from each individual (Acevedo-Whitehouse et al., 2010), which seems reasonable given the low behavioural responses found here, and thus allowing separate samples to be amplified, genotyped and then pooled where molecular markers have failed. Nonetheless, the alleles captured within the blow dataset from this localized study area are representative of the spread of alleles found across northern British Columbia as identified by SPLASH (Table 2; Baker et al., 2013), which contributes to our confidence in the data generated with the blow samples. This supports our method to generate complete genetic profiles that can be applied to questions relating to genetic diversity (Richard et al., 2018), kinship (Konrad et al., 2018; Patel et al., 2017), population structure (Schmitt et al., 2014; Valsecchi et al., 1997) and genetic bottlenecks (Waldick et al., 2002).

Although Waits et al. (2001) argue that a minimum of 9–10 microsatellite loci might be necessary to prevent incorrect genetic matches from being made between two related individuals based on $p_{(ID)}$, Palsbøll, Bérubé, et al. (1997) have already demonstrated the feasibility of genetic identification of humpback whales using just 6 microsatellite loci in the North Atlantic. Of the 3060 analysed skin samples, they had 692 genetic 'recaptures' and sufficiently low probabilities of identity. Here also, genetic matches were found from the blow samples and corroborated using photo-ID of the fluke and, more unusually, the scars and tubercles visible in the UAS footage. Interestingly, when a match search was run through the SPLASH genetic profiles, one blow sample matched with an individual from the NBC subpopulation likely sampled using a biopsy dart. This further supports the efficacy of blow as a sample source for individual identification, demonstrating its applicability across studies.

In recent years, genome-wide single nucleotide polymorphisms (SNPs) have gained popularity within the field of population genetics, and have been the marker of choice for research focused on population structure (Lah et al., 2016), individual identification (Morin et al., 2004), sex determination (DeWoody et al., 2017) and relatedness and parentage analysis (Flanagan & Jones, 2019; Hauser et al., 2011; Huisman, 2017). Given the rapidly developing SNP-based techniques, this molecular marker would be of interest to future blow sampling genetics studies, particularly that with the use of next-generation sequencing (NGS) technologies, SNP panels can be quite easily generated, even for non-model organisms such as large marine mammals (Garvin et al., 2010; Helyar et al., 2011). As discussed, the low amounts of DNA in blow pose a limitation for genotyping of high numbers of microsatellite loci. However, SNP genotyping may yield better results for assaying nuclear variation given the nature of the mutations occurring at a single nucleotide (Morin & McCarthy, 2007), although a much larger number of SNPs compared to microsatellites will be required to achieve a $p_{(ID)}$ sufficient for individual identification.

The KFS presents a rare opportunity to sample whales from shore and given a 61% success rate of land-based sample collection (land-based sampling flights, $n = 23$), we consider this a viable option because it further minimizes impact on the target species by removing potential disturbance from a research vessel. Likely due to the fjord morphology of the KFS, humpback whales are regularly observed utilizing the near-shore habitat, making them an easy target for shore-based blow sampling. Owing to the fjords' steep walls and almost immediate depths (Macdonald et al., 1983), this nearshore microhabitat results in upwelling of nutrients and perhaps dense concentrations of humpback prey: small schooling fish such as Pacific herring (*Clupea pallasii*), which is a known species

exploited by bubble netting humpback whales (Sharpe & Dill, 1997). Alternatively, the steep walls may serve as another form of entrapment, allowing foraging humpbacks to capture prey more efficiently.

There was a general lack of behavioural responses to the UAS, with 86.5% of sampling events showing *no* detectable behavioural response. For direct comparison, behavioural responses to tissue biopsy sampling of humpback whales were detected more often, ranging in frequency from 41.4% to 93%, in the studies reviewed by Noren and Mocklin (2011). In one case, a 'moderate' response or above occurred 66.2% of the time (Weinrich et al., 1992), which is equivalent to our highest grade and includes 'hard tail slaps' and breaching. This is a notable difference and is possibly because UAS-based blow sampling does not require a close vessel approach to the target individual, as is the case with pole-based blow sampling and tissue biopsy sampling, and does not need to make direct physical contact with the body of the whale to obtain a sample. Nonetheless, UAS-based blow sampling still carries potential risk of disturbance to target individuals, and thus behavioural response results here should not be extrapolated to other species per se. In fact, it is critical that any interactions, by UAS or otherwise, with animals as research subjects, undergo strict ethics assessments and close monitoring of behavioural disturbance (Perry, 2007). The monitoring of behavioural response is thus highly recommended (Smith et al., 2016).

Behavioural responses of whales to UASs during blow sampling attempts have been measured in some instances. Atkinson et al. (2021) documented 21 behavioural responses of humpback whales of a total of 516 UAS flights (4.07% response incidence) across multiple locations and responses themselves varied from turning to see the UAS, to startle responses including bucking and pectoral fin slapping. Domínguez-Sánchez et al. (2018) examined the behavioural response by blue whales to a DJI Phantom 2 and found no significant deviation in observed diving behaviours. UAS model choice and flight pattern have been determined as important predictors of whale response level (Domínguez-Sánchez et al., 2018; Mulero-Pázmány et al., 2017), with a report of a blue whale turning to look up at the UAS before prematurely diving, when the pilot approached the individual from the head (Domínguez-Sánchez et al., 2018). A similar response was detected here, whereby a humpback turned on its side so that its eye was plainly visible from the UAS's First-Person-View (response grade = 2). Influence of approach angle on behavioural response was outside the scope of our study, but recommendations are made by others to approach the whale from the caudal fin moving towards the head whenever possible (Domínguez-Sánchez et al., 2018; Pomeroy et al., 2015).

Ultimately, UAS sampling of respiratory blow presents a promising approach that can be adapted to other species and systems, perhaps particularly to those species that show heightened behavioural responses to biopsy sampling (Barrett-Lennard et al., 1996; Best et al., 2005; Gauthier & Sears, 2006; Hooker et al., 2001). Highly depleted cetacean species of IUCN Endangered ranking, such as the blue whale (Cooke, 2018), could benefit from a more streamlined data collection protocol. The use of a UAS allows for the simultaneous collection of individual behavioural data, morphological data (e.g. documentation of scarring and injury), morphometrics, sociality information (e.g. group formations and intra-group interactions), photo-ID data as well as blow collection for conservation genetics, epidemiology, virology or endocrinology. This presents a powerful tool for conservation biologists aiming to minimize time with a study species, especially given the fact that direct contact need never be made.

AUTHOR CONTRIBUTIONS

ÉOM and OEG designed the study, while funding was acquired by ÉOM and JW. ÉOM was the drone pilot, with fieldwork carried out by ÉOM, EMK, JW, NR and AD. ALS, DS and ÉOM carried out the laboratory work, which was supported by CSB. ALS and DS carried out the genotype calling. Data were analysed by ÉOM with reviews from OEG, ALS and DS. ÉOM wrote the first draft of the manuscript and all authors contributed to subsequent drafts.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

OPEN RESEARCH BADGES

This article has earned an Open Data badge for making publicly available the digitally-shareable data necessary to reproduce the reported results.

DATA AVAILABILITY STATEMENT

All relevant data are within the manuscript, its Supplementary Materials files and on the Dryad Digital Repository (DOI: 10.5061/dryad.6djh9w185).

ETHICS STATEMENT

Research by the North Coast Cetacean Society (NCCS) is conducted under a formal research agreement with the Gitga'at First Nation. Blow sampling and associated metadata collection were approved by the University of St Andrews' School of Biology Ethics Committee (Ref: SEC20030). The funders of this study had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript. The authors declare no conflict of interest.

ORCID

Éadin N. O'Mahony https://orcid.org/0000-0002-0755-1329

REFERENCES

Aasen, E., & Medrano, J. F. (1990). Amplification of the ZFY and ZFX genes for sex identification in humans, cattle, sheep and goats. *Biotechnology*, *8*(12), 1279–1281.

Acevedo-Whitehouse, K., Rocha-Gosselin, A., & Gendron, D. (2010). A novel non-invasive tool for disease surveillance of free-ranging whales and its relevance to conservation programs. *Animal Conservation*, *3*(2), 217–225. https://doi.org/10.1111/j.1469-1795.2009.00326.x

Adams, C. I. M., Knapp, M., Gemmell, N. J., Jeunen, G., Bunce, M., Lamare, M. D., & Taylor, H. R. (2019). Beyond biodiversity: Can environmental DNA (eDNA) cut it as a population genetics tool? *Genes*, *10*(3), 192. https://doi.org/10.3390/genes10030192

Alter, S. E., King, C. D., Chou, E., Chin, S. C., Rekdahl, M., & Rosenbaum, H. C. (2022). Using environmental DNA to detect whales and dolphins in the New York Bight. *Frontiers in Conservation Science*, *3*, 820377. https://doi.org/10.3389/fcosc.2022.820377

Apprill, A., Miller, C. A., Moore, M. J., Durban, J. W., Fearnbach, H., & Barrett-Lennard, L. G. (2017). Extensive core microbiome in drone-captured whale blow supports a framework for health monitoring. *mSystems*, *2*(5). https://doi.org/10.1128/mSystems.00119-17

Archer, F. I., & Brownell, R. L. (2019). Revision of fin whale *Balaenoptera physalus* (Linnaeus, 1758) subspecies using genetics. *Journal of Atomic and Molecular Physics*, *100*, 1653–1670. http://doi.org/10.1093/jmammal/gyz121

Ashe, E., Wray, J., Picard, C. R., & Williams, R. (2013). Abundance and survival of Pacific humpback whales in a proposed critical habitat area. *PLoS One*, *8*(9), e75228. https://doi.org/10.1371/journal.pone.0075228

Atkinson, S., Rogan, A., Baker, C. S., Dagdag, R., Redlinger, M., Polinski, J., Urban, J., Sremba, A., Branson, M., Mashburn, K., Pallin, L., Klink, A., Steel, D., Bortz, E., & Kerr, I. (2021). Genetic, endocrine, and microbiological assessments of blue, humpback and killer whale health using unoccupied aerial systems. *Wildlife Society Bulletin*, *45*(4), 654–669. https://doi.org/10.1002/wsb.1240

Baker, C. S., Claridge, D., Dunn, C., Fetherston, T., Baker, D. N., Klinck, H., & Steel, D. (2023). Quantification by droplet digital PCR and species identification by metabarcoding of environmental (e)DNA from Blainville's beaked whales, with assisted localization from an acoustic array. *PLoS One*, *18*(9), e0291187.

Baker, C. S., Steel, D., Calambokidis, J., Falcone, E., Ganzález-Peral, U., Barlow, J., Burdin, A. M., Clapham, P. J., Ford, J. K. B., Gabriele, C. M., Mattila, D., Rojas-Bracho, L., Straley, J. M., Taylor, B. L., Urbán, J., Wade, P. R., Weller, D., Witteveen, B. H., & Yamaguchi, M. (2013). Strong maternal fidelity and natal philopatry shape genetic structure in North Pacific humpback whales. *Marine Ecology Progress Series*, *494*, 291–306. https://doi.org/10.3354/meps10508

Baker, C., Scott, D., Steel, S. N., & Klinck, H. (2018). Environmental DNA (eDNA) from the wake of the whales: Droplet digital PCR for detection and species identification. *Frontiers in Marine Science*, *5*, 133. https://doi.org/10.3389/fmars.2018.00133

Baker, C., Scott, L., Medrano-Gonzalez, J., Calambokidis, A., Perry, F., Pichler, H., Rosenbaum, J. M., Straley, J., Urban-Ramirez, M. Y., & von Ziegesar, O. (1998). Population structure of nuclear and mitochondrial DNA variation among humpback whales in the North Pacific. *Molecular Ecology*, *7*(6), 695–707. https://doi.org/10.1046/j.1365-294x.1998.00384.x

Barrett-Lennard, L., Smith, T. G., & Ellis, G. M. (1996). A cetacean biopsy system using lightweight pneumatic darts, and its effect on the behavior of killer whales. *Marine Mammal Science*, *12*(1), 14–27. https://doi.org/10.1111/j.1748-7692.1996.tb00302.x

Bérubé, M., Martine, H. J. Ø., McEwing, R., & Palsbøll, P. J. (2000). Polymorphic di-nucleotide microsatellite loci isolated from the humpback whale, *Megaptera novaeangliae*. *Molecular Ecology*, *9*(12), 2181–2183. https://doi.org/10.1046/j.1365-294X.2000.105315.x

Best, P. B., Reeb, D., Rew, M. B., Palsbøll, P. J., Schaeff, C., & Brandão, A. (2005). Biopsying southern right whales: Their reactions and effects on reproduction. *The Journal of Wildlife Management*, *69*(3), 1171–1180. https://doi.org/10.2193/0022-541X(2005)069[1171:BSRWTR]2.0.CO;2

Boston, E. S. M., Puechmaille, S. J., Scott, D. D., Buckley, D. J., Lundy, M. G., Montgomery, I. W., Prodöhl, P. A., & Teeling, E. C. (2012). Empirical assessment of non-invasive population genetics in bats: Comparison of DNA quality from faecal and tissue samples. *Acta Chiropterologica/Museum and Institute of Zoology, Polish Academy of Sciences*, *14*(1), 45–52. https://doi.org/10.3161/150811012X654259

Brunton, E., Bolin, J., Leon, J., & Burnett, S. (2019). Fright or flight? Behavioural responses of kangaroos to drone-based monitoring. *Drones*, *3*(2), 41. https://doi.org/10.3390/drones3020041

Burgess, E. A., Hunt, K. E., Kraus, S. D., & Rolland, R. M. (2018). Quantifying hormones in exhaled breath for physiological assessment of large whales at sea. *Scientific Reports*, *8*(1), 10031.

Calambokidis, J., Falcone, E. A., Quinn, T. J., Burdin, A. M., Clapham, P. J., Ford, J. K. B., Gabriele, C. M., LeDuc, R., Mattila, D., Rojas-Bracho, L., Straley, J. M., Taylor, B. L., Urbán, J., Weller, D., Witteveen, B. H., Yamaguchi, M., Bendlin, A., Camacho, D., Flynn, K., … Maloney, N. (2008). *SPLASH: Structure of populations, levels of abundance and status of humpback whales in the North Pacific*. U.S. Department of Commerce.

Cartwright, R., Venema, A., Hernandez, V., Wyels, C., Cesere, J., & Cesere, D. (2019). Fluctuating reproductive rates in Hawaii's humpback whales, *Megaptera novaeangliae*, reflect recent climate anomalies in the North Pacific. *Royal Society Open Science*, *6*(3), 181463. https://doi.org/10.1098/rsos.181463

Chase, J. M., Blowes, S. A., Knight, T. M., Gerstner, K., & May, F. (2020). Ecosystem decay exacerbates biodiversity loss with habitat loss. *Nature*, *584*(7820), 238–243.

Cheeseman, T., Barlow, J., Acebes, J. M., Audley, K., Bejder, L., Birdsall, C., Bracamontes, O. S., Bradford, A. L., Byington, J., Calambokidis, J., Cartwright, R., Cedarleaf, J., García Chavez, A. J., Currie, J., De Castro, R. C., De Weerdt, J., Doe, N., Doniol-Valcroze, T., Dracott, K., Clapham, P. (2024). Bellwethers of change: Population modelling of North Pacific humpback whales from 2002 through 2021 reveals shift from recovery to climate response. *Royal Society Open Science*, *11*(2), 231462. https://doi.org/10.1098/rsos.231462

Christie, K. S., Gilbert, S. L., Brown, C. L., Hatfield, M., & Hanson, L. (2016). Unmanned aircraft systems in wildlife research: Current and future applications of a transformative technology. *Frontiers*

in Ecology and the Environment, *14*(5), 241–251. https://doi.org/10.1002/fee.1281

Cooke, J. G. (2018). "Balaenoptera Musculus (errata Version Published in 2019)." {The IUCN Red List of Threatened Species}. https://doi.org/10.2305/IUCN.UK.2018-2.RLTS.T2477A156923585.en

Costa, H., Rogan, A., Zadra, C., Larsen, O., Rikardsen, A. H., & Waugh, C. (2022). Blowing in the wind: Using a consumer drone for the collection of humpback whale (*Megaptera novaeangliae*) blow samples during the Arctic polar nights. *Drones*, *7*(1), 15.

Cumeras, R., Cheung, W. H. K., Gulland, F., Goley, D., & Davis, C. E. (2014). Chemical analysis of whale breath volatiles: A case study for non-invasive field health diagnostics of marine mammals. *Metabolites*, *4*(3), 790–806.

Dalebout, M. L., Helden, A. V., van Waerebeek, K., & Baker, C. S. (1998). Molecular genetic identification of southern hemisphere beaked whales (Cetacea: Ziphiidae). *Molecular Ecology*, *7*(6), 687–694.

DeWoody, J., Andrew, N. B., Fernandez, A., Brüniche-Olsen, J. D., Antonides, J. M., Doyle, P. S., Miguel, R., Westerman, V. V., Vertyankin, C. A. J., Godard-Codding, C. A. J., & Bickham, J. W. (2017). Characterization of the gray whale *Eschrichtius robustus* genome and a genotyping array based on single-nucleotide polymorphisms in candidate genes. *The Biological Bulletin*, *232*(3), 186–197. https://doi.org/10.1086/693483

Dewoody, J., Nason, J. D., & Hipkins, V. D. (2006). Mitigating scoring errors in microsatellite data from wild populations. *Molecular Ecology Notes*, *6*(4), 951–957.

Di Marco, M., Venter, O., Possingham, H. P., & Watson, J. E. M. (2018). Changes in human footprint drive changes in species extinction risk. *Nature Communications*, *9*(1), 4621.

Díaz, S., Settele, J., Brondízio, E. S., Ngo, H. T., Agard, J., Arneth, A., Balvanera, P., Brauman, K. A., Butchart, S. H. M., Chan, K. M. A., Garibaldi, L. A., Ichii, K., Liu, J., Subramanian, S. M., Midgley, G. F., Miloslavich, P., Molnár, Z., Obura, D., Pfaff, A., … Zayas, C. N. (2019). Pervasive human-driven decline of life on Earth points to the need for transformative change. *Science*, *366*(6471), eaax3100. https://doi.org/10.1126/science.aax3100

Domínguez-Sánchez, C. A., Acevedo-Whitehouse, K. A., & Gendron, D. (2018). Effect of drone-based blow sampling on blue whale (*Balaenoptera musculus*) behavior. *Marine Mammal Science*, *34*(3), 841–850. https://doi.org/10.1111/mms.12482

Eizirik, E., Haag, T., Santos, A. S., Salzano, F. M., Silveira, L., Azevedo, F. C., & Furtado, M. M. (2008). Jaguar conservation genetics. *Cat News*, *4*, 31–34.

Ewen, K. R., Bahlo, M., Treloar, S. A., Levinson, D. F., Mowry, B., Barlow, J. W., & Foote, S. J. (2000). Identification and analysis of error types in high-throughput genotyping. *American Journal of Human Genetics*, *67*(3), 727–736.

Ewing, B., & Green, P. (1998). Base-calling of automated sequencer traces using Phred. II. Error probabilities. *Genome Research*, *8*(3), 186–194.

Fettermann, T., Fiori, L., Gillman, L., Stockin, K. A., & Bollard, B. (2022). Drone surveys are more accurate than boat-based surveys of bottlenose dolphins (*Tursiops truncatus*). *Drones*, *6*(4), 82. https://doi.org/10.3390/drones6040082

Flanagan, S. P., & Jones, A. G. (2019). The future of parentage analysis: From microsatellites to SNPs and beyond. *Molecular Ecology*, *28*(3), 544–567.

Foote, A. D., Thomsen, P. F., Sveegaard, S., Wahlberg, M., Kielgast, J., Kyhn, L. A., Salling, A. B., Galatius, A., Orlando, L., & Gilbert, M. T. P. (2012). Investigating the potential use of environmental DNA (eDNA) for genetic monitoring of marine mammals. *PLoS One*, *7*(8), e41781. https://doi.org/10.1371/journal.pone.0041781

Frankham, R. (1995). Conservation genetics. *Annual Review of Genetics*, *29*, 305–327. https://doi.org/10.1146/annurev.ge.29.120195.001513

Garrigue, C., & Derville, S. (2021). Behavioral responses of humpback whales to biopsy sampling on a breeding ground: The influence of age-class, reproductive status, social context, and repeated sampling. *Marine Mammal Science*, *38*, 102–117. https://doi.org/10.1111/mms.12848

Garvin, M. R., Saitoh, K., & Gharrett, A. J. (2010). Application of single nucleotide polymorphisms to non-model species: A technical review. *Molecular Ecology Resources*, *10*(6), 915–934.

Gauthier, J., & Sears, R. (2006). Behavioral response of four species of balaenopterid whales to biopsy sampling. *Marine Mammal Science*, *15*(1), 85–101.

Gilson, A., Syvanen, M., Levine, K., & Banks, J. (1998). Deer gender determination by polymerase chain reaction: Validation study and application to tissues, bloodstains, and hair forensic samples from California. *California Fish and Game*, *84*(4), 159–169.

Groch, K. R., Blazquez, D. N. H., Marcondes, M. C. C., Santos, J., Colosio, A., Díaz Delgado, J., & Catão-Dias, J. L. (2020). Cetacean morbillivirus in humpback whales' exhaled breath. *Transboundary and Emerging Diseases*, *68*, 1736–1743. https://doi.org/10.1111/tbed.13883

Guo, S. W., & Thompson, E. A. (1992). Performing the exact test of Hardy-Weinberg proportion for multiple alleles. *Biometrics*, *48*(2), 361–372.

Hatch, L. T., Dopman, E. B., & Harrison, R. G. (2006). Phylogenetic relationships among the baleen whales based on maternally and paternally inherited characters. *Molecular Phylogenetics and Evolution*, *41*(1), 12–27.

Hauser, L., Baird, M., Hilborn, R., Seeb, L. W., & Seeb, J. E. (2011). An empirical comparison of SNPs and microsatellites for parentage and kinship assignment in a wild sockeye Salmon (*Oncorhynchus nerka*) population. *Molecular Ecology Resources*, *11 Suppl 1*, 150–161. https://doi.org/10.1111/j.1755-0998.2010.02961.x

Helyar, S. J., Hemmer-Hansen, J., Bekkevold, D., Taylor, M. I., Ogden, R., Limborg, M. T., Cariani, A., Maes, G. E., Diopere, E., Carvalho, G. R., & Nielsen, E. E. (2011). Application of SNPs for population genetics of nonmodel organisms: New opportunities and challenges. *Molecular Ecology Resources*, *11 Suppl 1*, 123–136.

Hoelzel, A. R. (1992). Conservation genetics of whales and dolphins. *Molecular Ecology*, *1*(2), 119–125.

Hogg, C. J., Rogers, T. L., Shorter, A., Barton, K., Miller, P. J. O., & Nowacek, D. (2009). Determination of steroid hormones in whale blow: It is possible. *Marine Mammal Science*, *25*(3), 605–618. https://doi.org/10.1111/j.1748-7692.2008.00277.x

Holderegger, R., Balkenhol, N., Bolliger, J., Engler, J. O., Gugerli, F., Hochkirch, A., Nowak, C., Segelbacher, G., Widmer, A., & Zachos, F. E. (2019). Conservation genetics: Linking science with practice. *Molecular Ecology*, *28*(17), 3848–3856.

Hooker, S. K., Baird, R. W., Al-Omari, S., Gowans, S., & Whitehead, H. (2001). Behavioral reactions of northern bottlenose whales (*Hyperoodon ampullatus*) to biopsy darting and tag attachment procedures. Fishery Bulletin. https://dalspace.library.dal.ca/handle/10222/29954

Hudson, J. M., Anderson, W. G., & Marcoux, M. (2021). Measurement of cortisol in blow samples collected from free-swimming beluga whales (*Delphinapterus leucas*). *Marine Mammal Science*, *37*(3), 888–900.

Huisman, J. (2017). Pedigree reconstruction from SNP data: Parentage assignment, sibship clustering and beyond. *Molecular Ecology Resources*, *17*(5), 1009–1024.

Hunt, K. E., Moore, M. J., Rolland, R. M., Kellar, N. M., Hall, A. J., Kershaw, J., Raverty, S. A., Davis, C. E., Yeates, L. C., Fauquier, D. A., Rowles, T. K., & Kraus, S. D. (2013). Overcoming the challenges of studying conservation physiology in large whales: A review of available methods. *Conservation Physiology*, *1*(1), cot006. https://doi.org/10.1093/conphys/cot006.

Hunt, K. E., Rolland, R. M., & Kraus, S. D. (2014). Detection of steroid and thyroid hormones via immunoassay of North Atlantic right whale (*Eubalaena glacialis*) respiratory vapor. *Marine Mammal Science*, *30*(2), 796–809.

International HapMap Consortium. (2003). The international HapMap project. *Nature*, *426*(6968), 789–796.

Jombart, T. (2008). Adegenet: A R package for the multivariate analysis of genetic markers. *Bioinformatics*, *24*(11), 1403–1405.

Kalinowski, S. T., Taper, M. L., & Marshall, T. C. (2007). Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology*, *16*(5), 1099–1106.

Kashiwagi, T., Marshall, A. D., Bennett, M. B., & Ovenden, J. R. (2012). The genetic signature of recent speciation in manta rays (*Manta alfredi* and *M. birostris*). *Molecular Phylogenetics and Evolution*, *64*(1), 212–218.

Kashiwagi, T., Maxwell, E. A., Marshall, A. D., & Christensen, A. B. (2015). Evaluating manta ray mucus as an alternative DNA source for population genetics study: Underwater-sampling, dry-storage and PCR success. *PeerJ*, *3*, e1188.

Keen, E. M., O'Mahony, É. N., Nichol, L. M., Wright, B. M., Shine, C., Hendricks, B., Meuter, H., Alidina, H. M., & Wray, J. (2023). Ship-strike forecast and mitigation for whales in Gitga'at first nation territory. *Endangered Species Research*, *51*, 31–58. https://doi.org/10.3354/esr01244

Keen, E. M. (2016). *Bangarang. R package*. https://github.com/ericmkeen/bangarang.

Keen, E. M., Pilkington, J., O'Mahony, É. N., Thompson, K., Hendricks, B., Robinson, N., Dundas, A., Nichol, L., Alidina, H. M., Meuter, H., Picard, C. R., & Wray, J. (2021). Fin whales of the great bear rainforest: *Balaenoptera physalus velifera* in a Canadian Pacific Fjord system. *PLoS One*, *16*(9), e0256815. https://doi.org/10.1371/journal.pone.0256815

Keen, E. M., Wray, J., Meuter, H., Thompson, K., Barlow, J. P., & Picard, C. R. (2017). 'Whale wave': Shifting strategies structure the complex use of critical fjord habitat by humpbacks. *Marine Ecology Progress Series*, *567*, 211–233. https://doi.org/10.3354/meps12012

Keen, E. M., Wray, J., Pilkington, J. F., Thompson, K. L., & Picard, C. R. (2018). Distinct habitat use strategies of sympatric rorqual whales within a fjord system. *Marine Environmental Research*, *140*, 180–189. https://doi.org/10.1016/j.marenvres.2018.06.014

Koh, L. P., & Wich, S. A. (2012). Dawn of drone ecology: Low-cost autonomous aerial vehicles for conservation. *Tropical Conservation Science*, *5*(2), 121–132.

Konrad, C. M., Gero, S., Frasier, T., & Whitehead, H. (2018). Kinship influences sperm whale social organization within, but generally not among, social units. *Royal Society Open Science*, *5*(8), 180914. https://doi.org/10.1098/rsos.180914

Kornbrot, D. (2005). Point biserial correlation. In *Encyclopedia of statistics in behavioral science*. John Wiley & Sons, Ltd. https://doi.org/10.1002/0470013192.bsa485

Krutzen, M., Barre, L. M., Moller, L. M., Heithaus, M. R., Simms, C., & Sherwin, W. B. (2002). A biopsy system for small cetaceans: Darting success and wound healing in *Tursiops* spp. *Marine Mammal Science*, *18*(4), 863–878. https://doi.org/10.1111/j.1748-7692.2002.tb01078.x

Lah, L., Trense, D., Benke, H., Berggren, P., Gunnlaugsson, Þ., Lockyer, C., Öztürk, A., Öztürk, B., Pawliczka, I., Roos, A., Siebert, U., Skóra, K., Víkingsson, G., & Tiedemann, R. (2016). Spatially explicit analysis of genome-wide SNPs detects subtle population structure in a mobile marine mammal, the harbor porpoise. *PLoS One*, *11*(10), e0162792.

Larson, S., Farrer, D., Lowry, D., & Ebert, D. A. (2015). Preliminary observations of population genetics and relatedness of the *Broadnose sevengill* shark, *Notorynchus cepedianus*, in two Northeast Pacific estuaries. *PLoS One*, *10*(6), e0129278.

Linchant, J., Lisein, J., Semeki, J., Lejeune, P., & Vermeulen, C. (2015). Are unmanned aircraft systems (UASs) the future of wildlife monitoring? A review of accomplishments and challenges. *Mammal Review*, *45*(4), 239–252.

Linck, E., & Battey, C. J. (2019). Minor allele frequency thresholds strongly affect population structure inference with genomic data sets. *Molecular Ecology Resources*, *19*(3), 639–647. https://doi.org/10.1111/1755-0998.12995

Louis, E. J., & Dempster, E. R. (1987). An exact test for Hardy-Weinberg and multiple alleles. *Biometrics*, *43*(4), 805–811.

Macdonald, R. W., Bornhold, B. D., & Webster, I. (1983). The Kitimat Fjord system: An introduction. Proceedings of a workshop on the Kitimat marine environment, Canadian technical report of hydrography and ocean sciences, 18:2–13. Fisheries and Oceans Canada.

Marsili, L., Fossi, M. C., Neri, G., Casini, S., Gardi, C., Palmeri, S., Tarquini, E., & Panigada, S. (2000). Skin biopsies for cell cultures from Mediterranean free-ranging cetaceans. *Marine Environmental Research*, *50*(1–5), 523–526.

McCullagh, P., & Nelder, J. A. (2019). *Generalized linear models* (Vol. *2*). Routledge.

Mingramm, F. M., Keeley, T., Whitworth, D. J., & Dunlop, R. A. (2019). Relationships between blubber and respiratory vapour steroid hormone concentrations in humpback whales (*Megaptera novaeangliae*). *Aquatic Mammals*, *45*(5), 465–477.

Morin, P. A., Luikart, G., Wayne, R. K., & the SNP Workshop Group. (2004). SNPs in ecology, evolution and conservation. *Trends in Ecology & Evolution*, *19*(4), 208–216. https://doi.org/10.1016/j.tree.2004.01.009

Morin, P. A., & McCarthy, M. (2007). Highly accurate SNP genotyping from historical and low-quality samples. *Molecular Ecology Notes*, *7*(6), 937–946. https://doi.org/10.1111/j.1471-8286.2007.01804.x

Mulero-Pázmány, M., Jenni-Eiermann, S., Strebel, N., Sattler, T., José Negro, J., & Tablado, Z. (2017). Unmanned aircraft systems as a new source of disturbance for wildlife: A systematic review. *PLoS One*, *12*(6), e0178448. https://doi.org/10.1371/journal.pone.0178448

Neilson, J. L., & Gabriele, C. M. (2019). *Glacier Bay & Icy Strait humpback whale population monitoring: 2018 update*. National Park Service Resource Brief.

Nichol, L. M., Abernethy, R., Flostrand, L., Lee, T. S., Ford, J., & Department of Fisheries. (2010). *Information relevant to the identification of critical habitats of North Pacific humpback whales* (Megaptera novaeangliae) *in British Columbia*. DFO, Ottawa, ON, Canada.

Noren, D. P., & Mocklin, J. A. (2011). Review of cetacean biopsy techniques: Factors contributing to successful sample collection and physiological and behavioral impacts. *Marine Mammal Science*, *28*(1), 154–199. https://doi.org/10.1111/j.1748-7692.2011.00469.x

Palsbøll, P. J., Allen, J., Bérubé, M., Clapham, P. J., Feddersen, T. P., Hammond, P. S., Hudson, R. R., Jørgensen, H., Katona, S., Larsen, A. H., Larsen, F., Lien, J., Mattila, D. K., Sigurjónsson, J., Sears, R., Smith, T., Sponer, R., Stevick, P., & Oien, N. (1997). Genetic tagging of humpback whales. *Nature*, *388*(6644), 767–769.

Palsbøll, P. J., Bérubé, M., Larsen, A. H., & Jørgensen, H. (1997). Primers for the amplification of tri-and tetramer microsatellite loci in baleen whales. *Molecular Ecology*, *6*(9), 893–895.

Parsons, K. M., Dallas, J. F., Claridge, D. E., Durban, J. W., Balcomb, K. C., III, Thompson, P. M., & Noble, L. R. (1999). Amplifying dolphin mitochondrial DNA from faecal plumes. *Molecular Ecology*, *8*(10), 1766–1768. https://doi.org/10.1046/j.1365-294x.1999.00723-8.x

Parsons, K. M., Everett, M., Dahlheim, M., & Park, L. (2018). Water, water everywhere: Environmental DNA can unlock population structure in elusive marine species. *Royal Society Open Science*, *5*(8), 180537.

Pasamontes, A., Aksenov, A. A., Schivo, M., Rowles, T., Smith, C. R., Schwacke, L. H., Wells, R. S., Yeates, L., Venn-Watson, S., & Davis, C. E. (2017). Noninvasive respiratory metabolite analysis associated with clinical disease in cetaceans: A deepwater horizon oil spill study. *Environmental Science & Technology*, *51*(10), 5737–5746. https://doi.org/10.1021/acs.est.6b06482

Patel, S., Thompson, K. F., Santure, A. W., Constantine, R., & Millar, C. D. (2017). Genetic kinship analyses reveal that Gray's beaked whales

strand in unrelated groups. *The Journal of Heredity*, *108*(4), 456–461. https://doi.org/10.1093/jhered/esx021

Peakall, R., & Smouse, P. E. (2006). GenAlEx 6: Genetic analysis in excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, *6*(1), 288–295.

Peakall, R., & Smouse, P. E. (2012). GenAlEx 6.5: Genetic analysis in excel. Population genetic software for teaching and research – An update. *Bioinformatics*, *28*(19), 2537–2539.

Perry, P. (2007). The ethics of animal research: A UK perspective. *ILAR Journal/National Research Council, Institute of Laboratory Animal Resources*, *48*(1), 42–46. https://doi.org/10.1093/ilar.48.1.42

Pinfield, R., Dillane, E., Runge, A. K. W., Evans, A., Mirimin, L., Niemann, J., Reed, T. E., Reid, D. G., Rogan, E., Samarra, F. I. P., Sigsgaard, E. E., & Foote, A. D. (2019). False-negative detections from environmental DNA collected in the presence of large numbers of killer whales (*Orcinus orca*). *Environmental DNA*, *1*(4), 316–328. https://doi.org/10.1002/edn3.32

Pirotta, V., Smith, A., Ostrowski, M., Russell, D., Jonsen, I. D., Grech, A., & Harcourt, R. (2017). An economical custom-built drone for assessing whale health. *Frontiers in Marine Science*, *4*, 425. https://doi.org/10.3389/fmars.2017.00425

Pomeroy, P., O'Connor, L., & Davies, P. (2015). Assessing use of and reaction to unmanned aerial systems in gray and harbor seals during breeding and molt in the UK. *Journal of Unmanned Vehicle Systems*, *3*(3), 102–113. https://doi.org/10.1139/juvs-2015-0013

QIAGEN. (2006). *DNeasy® blood & tissue handbook – QIAGEN*. July 2006. https://www.qiagen.com/us/resources/resourcedetail?id=6b09dfb8-6319-464d-996c-79e8c7045a50&lang=en

R Core Team. (2020). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.R-project.org/.

Raudino, H. C., Tyne, J. A., Smith, A., Ottewell, K., McArthur, S., Kopps, A. M., Chabanne, D., Harcourt, R. G., Pirotta, V., & Waples, R. (2019). Challenges of collecting blow from small cetaceans. *Ecosphere*, *10*(10), e02901. https://doi.org/10.1002/ecs2.2901

Rendell, L., Mesnick, S. L., Dalebout, M. L., Burtenshaw, J., & Whitehead, H. (2012). Can genetic differences explain vocal dialect variation in sperm whales, *Physeter macrocephalus*? *Behavior Genetics*, *42*(2), 332–343.

Richard, G., Titova, O. V., Fedutin, I. D., Steel, D., Meschersky, I. G., Hautin, M., Burdin, A. M., Hoyt, E., Filatova, O. A., & Jung, J. (2018). Cultural transmission of fine-scale fidelity to feeding sites may shape humpback whale genetic diversity in Russian Pacific waters. *The Journal of Heredity*, *109*(7), 724–734. https://doi.org/10.1093/jhered/esy033

Robinson, C. V., & Nuuttila, H. K. (2020). Don't hold your breath: Limited DNA capture using non-invasive blow sampling for small cetaceans. *Aquatic Mammals*, *46*(1), 32–41.

Rockström, J., Steffen, W., Noone, K., Persson, Å., Chapin, F. S. III, Lambin, E., Lenton, T. M., Scheffer, M., Folke, C., Schellnhuber, H. J., Nykvist, B., de Wit, C. A., Hughes, T., van der Leeuw, S., Rodhe, H., Soerlin, S., Snyder, P. K., Costanza, R., Svedin, U., … Foley, J. (2009). Planetary boundaries: Exploring the safe operating space for humanity. *Ecology and Society*, *14*(2), 32. https://www.jstor.org/stable/26268316.

Rousset, F. (2008). genepop'007: a complete re-implementation of the genepop software for Windows and Linux. *Molecular Ecology Resources*, *8*(1), 103–106. Portico. https://doi.org/10.1111/j.1471-8286.2007.01931.x

Rousset, F. (2020). *Genepop 4.7.5 manual*. https://kimura.univ-montp2.fr/~rousset/Genepop4.7.pdf.

Savage, K. (2017). *Alaska and British Columbia Large Whale Unusual Mortality Event Summary Report*. https://repository.library.noaa.gov/view/noaa/17715.

Schmitt, N. T., Double, M. C., Jarman, S. N., Gales, N., Marthick, J. R., Polanowski, A. M., Baker, C. S., Steel, D., Jenner, K. C. S., Jenner, M.-N. M., Gales, R., Paton, D., & Peakall, R. (2014). Low levels of genetic differentiation characterize Australian humpback whale (*Megaptera novaeangliae*) populations. *Marine Mammal Science*, *30*(1), 221–241. https://doi.org/10.1111/mms.12045

Schroeder, P., Raverty, S., Cameron, C., Zabek, E., Eshghi, A., Bain, D., Wood, B., Rhodes, L., & Hanson, B. (2009). Investigation into the microbial culture and molecular screening of exhaled breaths of endangered southern resident killer whales (SRKW) and pathogen screening of the Seasurface microlayer (SML) in Puget Sound. Report to the Northwest Fisheries Science Center, National Oceanic and Atmospheric Administration, Seattle, WA, USA.

Sharpe, F. A., & Dill, L. M. (1997). The behavior of Pacific herring schools in response to artificial humpback whale bubbles. *Canadian Journal of Zoology*, *75*(5), 725–730. https://doi.org/10.1139/z97-093

Smith, C. E., Sykora-Bodie, S. T., Bloodworth, B., Pack, S. M., Spradlin, T. R., & LeBoeuf, N. R. (2016). Assessment of known impacts of unmanned aerial systems (UAS) on marine mammals: Data gaps and recommendations for researchers in the United States. *Journal of Unmanned Vehicle Systems*, *4*(1), 31–44. https://doi.org/10.1139/juvs-2015-0017

Sremba, A., Kerr, I., Rogan, A., Steele, D., Pallin, L., & Baker, S. (2019). DNA profiling by SnotBot® sampling from the spout of a whale. Presented at the World Marine Mammal Conference, Barcelona, December.

Székely, D., Corfixen, N. L., Mørch, L. L., Knudsen, S. W., McCarthy, M. L., Teilmann, J., Heide-Jørgensen, M. P., & Tange Olsen, M. (2021). Environmental DNA captures the genetic diversity of bowhead whales (*Balaena mysticetus*) in West Greenland. *Environmental DNA*, *3*(1), 248–260. https://doi.org/10.1002/edn3.176

Thompson, L. A., Spoon, T. R., Goertz, C. E., Hobbs, R. C., & Romano, R. A. (2014). Blow collection as a non-invasive method for measuring cortisol in the beluga (*Delphinapterus leucas*). *PLoS One*, *9*(12), e114062. https://doi.org/10.1371/journal.pone.0114062

Valsecchi, E., & Amos, W. (1996). Microsatellite markers for the study of cetacean populations. *Molecular Ecology*, *5*(1), 151–156. https://doi.org/10.1111/j.1365-294X.1996.tb00301.x

Valsecchi, E., Bylemans, J., Goodman, S. J., Lombardi, R., Carr, I., Castellano, L., Galimberti, A., & Galli, P. (2020). Novel universal primers for metabarcoding environmental DNA surveys of marine mammals and other marine vertebrates. *Environmental DNA*, *2*(4), 460–476. https://doi.org/10.1002/edn3.72

Valsecchi, E., Glockner-Ferrari, D., Ferrari, M., & Amos, W. (1998). Molecular analysis of the efficiency of sloughed skin sampling in whale population genetics. *Molecular Ecology*, *7*(10), 1419–1422. https://doi.org/10.1046/j.1365-294x.1998.00446.x

Valsecchi, E., Palsbøll, P., Hale, P., Glockner-Ferrari, D., Ferrari, M., Clapham, P., Larsen, F., Mattila, D., Sears, R., Sigurjonsson, J., Brown, M., Corkeron, P., & Amos, B. (1997). Microsatellite genetic distances between oceanic populations of the humpback whale (*Megaptera novaeangliae*). *Molecular Biology and Evolution*, *14*(4), 355–362. https://doi.org/10.1093/oxfordjournals.molbev.a025771

Van Oosterhout, C., Hutchinson, W. F., Wills, D. P. M., & Shipley, P. (2004). Micro-checker: Software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, *4*(3), 535–538. https://doi.org/10.1111/j.1471-8286.2004.00684.x

Vendl, C., Ferrari, B. C., Thomas, T., Slavich, E., Zhang, E., Nelson, T., & Rogers, T. (2019). Interannual comparison of core taxa and community composition of the blow microbiota from east Australian humpback whales. *FEMS Microbiology Ecology*, *95*(8), fiz102. https://doi.org/10.1093/femsec/fiz102

Vendl, C., Slavich, E., Wemheuer, B., Nelson, T., Ferrari, B., Thomas, T., & Rogers, T. (2020). Respiratory microbiota of humpback whales may be reduced in diversity and richness the longer they fast. *Scientific Reports*, *10*(1), 12645.

Vigdor, N. (2021). Pacific northwest heat wave shatters temperature records. *The New York Times*, June 27, 2021. https://www.nytimes.com/2021/06/27/us/heat-wave-seattle-portland.html.

Waits, L. P., Luikart, G., & Taberlet, P. (2001). Estimating the probability of identity among genotypes in natural populations: Cautions and guidelines. *Molecular Ecology*, *10*(1), 249–256. https://doi.org/10.1046/j.1365-294X.2001.01185.x

Waldick, R. C., Brown, M. W., & White, B. N. (1999). Characterization and isolation of microsatellite loci from the endangered North Atlantic right whale. *Molecular Ecology*, *8*(10), 1763–1765. https://doi.org/10.1046/j.1365-294X.2002.01605.x

Waldick, R. C., Kraus, S., Brown, M., & White, B. N. (2002). Evaluating the effects of historic bottleneck events: An assessment of microsatellite variability in the endangered, North Atlantic right whale. *Molecular Ecology*, *11*(11), 2241–2249.

Waples, R., Rus Hoelzel, A., Gaggiotti, O. E., Tiedemann, R., Palsbøll, P. J., Cipriano, F., Jackson, J., Bickham, J. W., & Lang, A. R. (2018). Guidelines for genetic data analysis. *The Journal of Cetacean Research and Management*, *18*, 33–80.

Weinrich, M. T. (1991). Behavioural response of humpback whales (*Megaptera novaeangliae*) in the southern Gulf of Maine to biopsy sampling. *Report of the International Whaling Commission*, *13*, 3012–3019.

Weinrich, M. T., Lambertson, R. H., Belt, C. R., & Schilling, M. R. (1992). Behavioral reactions of humpback whales *Megaptera novaeangliae* to biopsy procedures. *Fishery Bulletin*, *90*(3), 588–598.

Weir, B. S. (1996). *Genetic data analysis II*. Sinauer.

Weir, B. S., & Cockerham, C. C. (1984). Estimating F-statistics for the analysis of population structure. *Evolution; International Journal of Organic Evolution*, *38*(6), 1358–1370.

Whitehead, H., Gordon, J., Mathews, E. A., & Richard, K. R. (1990). Obtaining skin samples from living sperm whales. *Marine Mammal Science*, *6*(4), 316–326. https://doi.org/10.1111/j.1748-7692.1990.tb00361.x

Wickham, H. (2016). *ggplot2: Elegant graphics for data analysis*. https://ggplot2.tidyverse.org/.

Wray, J., & Keen, E. M. (2020). Calving rate decline in humpback whales (*Megaptera novaeangliae*) of northern British Columbia, Canada. *Marine Mammal Science*, *36*(2), 709–720. https://doi.org/10.1111/mms.12665

Wray, J., Keen, E. M., & O'Mahony, É. N. (2021). Social survival: Humpback whales (*Megaptera novaeangliae*) use social structure to partition ecological niches within proposed critical habitat. *PLoS One*, *16*(6), e0245409. https://doi.org/10.1371/journal.pone.0245409

Würsig, B., Perrin, W. F., & Thewissen, J. G. M. (2009). *Encyclopedia of marine mammals*. Academic Press.

Yeates, L. C., Borras, E., Cumeras, R., & Davis, C. E. (2020). Chapter 28 – Breath analysis in marine mammals. In J. Beauchamp, C. Davis, & J. Pleil (Eds.), *Breathborne biomarkers and the human Volatilome* (2nd ed., pp. 461–472). Elsevier.

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