PERSPECTIVE

The answer, my friend, is blowin' in the wind: Blow sampling provides a new dimension to whale population monitoring

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Marine mammals play a fundamental role in the functioning of healthy marine ecosystems and are important indicator species. Studying their biology, distributions, behaviour and health are still technically and logistically demanding for researchers. However, the efforts and commitment have not been in vain, since we are witnessing constant and exponential advancement in the study of these animals, thanks to technological progress in numerous fields. These include miniaturization and performance of biologger tags, which are equipped with sensors for measuring physiological parameters, hydrophones, accelerometers, time-depth records and spatial locations; the use of high throughput 'Next Generation' Sequencing to gain genetic information about communities and individual species from nucleic acids in environmental samples at miniscule concentrations; through, to the possibility of monitoring species with autonomous aerial and underwater vehicles. In parallel advances in computing and statistical modelling frameworks support the analysis of increasingly large and complex data sets. In this issue, O'Mahony et al. (2024) draw from at least two of these innovations: (a) the collection of biological material retrieved from large whales' blows using a modified drone and (b) the use of the samples to infer a wide spectrum of genetic information (both nuclear and mitochondrial) about the target animal/population. The methodology is not completely novel, but the study shows an impressive advancement in the amount of data obtained compared to preceding studies using the same approach. In the wake of these promising results, future perspectives are evaluated in relation to alternative sampling methodologies currently in use. It is possible to speculate that, in the next few years, the combination of non-invasive molecular profiling and enhanced drone technology (e.g. assembling increasingly smaller components, thus expanding capacity for autonomous operation) will open up perspectives that were unimaginable at the beginning of this millennium.

The study of marine mammals in their natural environment is based primarily on direct visual or aerial surveys or indirect observation, as through acoustic surveys. Besides observation, which is often challenging for marine species, the best ally in marine mammal studies is the analysis of the biological material and organic compounds found inside the animal, on the animal or in the surrounding environment. The molecules are able to tell us much about populations (e.g. levels of inbreeding and gene flow), individuals (e.g. sex and kinship relationships), their health condition (disease/infection), physiology (e.g. reproductive status, detection of stress transcripts) and ecotoxicology (e.g. pollutants' concentration in body tissues). However, these molecules must be intercepted, collected and analysed. There are various methods with which informative biological samples can be collected: the range of different procedures has been expanding as molecular techniques have become increasingly sensitive, requiring less and less starting material, even using sea water as a template, in the case of environmental DNA.

The possibility of working on increasingly small quantities of biological traces has made it possible to implement the use of non- or minimally-invasive sampling methods, such as the acquisition of sloughed skin, first, and then water or air samples taken near the 'animal. The study presented in this issue by O'Mahony et al. (2024), successfully explores the possibility of acquiring a wide range of

molecular information on a single individual whale from the 'simple' analysis of its blow. The truly innovative component of this study is profiling the 'aerial' DNA sample with both nuclear (microsatellites and sexing) and mitochondrial (D-loop of the control region) markers. The yield was high both qualitatively (a large number of PCR-reactions successfully amplifying the desired DNA fragment) and quantitatively (a minimum of 10–12 enzymatic amplifications were performed per sample). These remarkable outcomes demonstrate that a single sample collected intercepting the whale blow can be sufficient to retrieve the entire range of genetic information that a molecular ecologist might wish to obtain from an individual sample, i.e. specimen characterization, data for kinship analysis and population genetics.

Figure 1 illustrates six main sources of biological material in marine mammal studies and ranks the fields of application in which they perform best. It seems clear that, among all the minimally invasive sampling methodologies (sloughed skin, eDNA, blow and faeces), the collection of samples from the blow is the approach that guarantees greater coverage of analyses (7 out of the 9 applications illustrated), and therefore data acquisition. Not only that, but the data acquired are complementary to those retrieved from other forms of minimally invasive sampling (for example eDNA allows the characterization of the biological community that characterizes the waters in which the whale is found, which cannot be deduced from the blow), suggesting that a comprehensive collection (blow+eDNA+faeces) could provide a complete picture of the biology of the animal investigated which is unprecedented.

There are two intrinsic aspects of the study by O'Mahony et al. (2024) that are undoubtedly interesting: blow and drone. The first is that blow samples have proven to be informative also for aspects other than genetic profiling, such as for assessing whale health conditions based on blow microbiome characterization (Apprill et al., 2017), or for endocrinological studies (Burgess et al., 2016), for instance looking for cortisol (Thompson et al., 2014) or steroid hormones (Hogg et al., 2009) for assessing whale stress status and reproductive function, respectively. Furthermore, it is worthwhile mentioning that we are becoming aware that nucleic acids contain valuable information that goes beyond the genotype itself: genetic material can also be used for aging animals based on the level of DNA demethylation (Barratclough et al., 2024) or to determine the health status based on the expression of genes (RNA) involved in immune functions

sample source	amount of DNA	target	species determination	population	individual	microbiome	transcriptomics (RNA)	bio-community	diet	endocrinology	ecotoxicology
sample from carcasses	lots of DNA	one single individual				C	C	n/a		C	C
biopsy sample	high quality DNA	one single individual				n/a		n/a	n/a		
sloughed skin sample	little, low quality DNA	one single (or a few) individual(s)				n/a	n/a	n/a	n/a	n/a	n/a
marine eDNA sample	traces of (degraded) DNA	thousand of taxa	Ø	C	C	n/a	C		C	n/a	n/a
blow	traces of fresh DNA	one single individual						n/a	n/a		C
feces	traces of fresh DNA	one single individual	Ø			C	n/a	C		n/a	n/a

Legend:
null
faint
mild
medium
good
excellent

C = conditional to sample conditions

n/a = not applicable

FIGURE 1 Different sources of biological material and their expected resolution power in relation to different fields and aspects of cetacean investigation based on detection of specific molecules. From left to right are shown approaches based on: Nucleic acids' analysis (dark grey), molecular or morphological data (grey) and the analysis of organic molecules other than nucleic acids (light grey). Namely, these are: (1) 'species determination'—normally unnecessary when visual determination is possible, although molecular identification can be useful in specific cases (such as species uncertainty due to advanced state of decomposition or in the presence of suspected hybrids or undescribed morphotypes). Note that eDNA may allow species identification also without *de visu* encounters; (2) 'population'—referring to the detection of population specific haplotypes or alleles; (3) 'individual'—genetic profiling (mtDNA haplotypes, nDNA genotyping, sexing, genomics); (4) 'Microbiome'—referring to the community of microorganisms living in association with the study specimen (e.g. lung and gut microbiomes); (5) 'Transcriptomics'—referring to the assessment of physiological conditions based on the examination of expressed genes (RNA analysis); (6) 'Bio-community'—composition of taxa characterizing the environment surrounding the study species; (7) 'Diet'—analysis of stomach content based on molecular or morphological examination of food residues in the intestinal system or faeces; (8) 'Endocrinology'—detection and measurement of hormones in the body tissues or surrounding environment; (9) 'Ecotoxicology'—detection and quantification of pollutants and toxic compounds in the body tissues of the study species. The crossed-out-eye symbol indicates that data can be obtained even without *de visu* encounters.

(Richard et al., 2022). It is therefore conceivable that one unique same sample (or a drone equipped with multiple sampling devices) could be used for all these analyses simultaneously, giving the possibility of relating physiology and health status and genotype (see Atkinson et al., 2021). The second opportunity is given by the use of the drone itself as a sampling platform. Drones, or unoccupied aerial systems (UAVs) are already used in whale population studies for other purposes other than sample collection. For instance, drones can be used to measure the size and physical state of the animal (Dawson et al., 2017), for photo-identification (Degollada et al., 2023) and (O'Mahony et al., 2024) themselves and to place tags on the body of large cetaceans (Wiley et al., 2023). Sampling systems that may incorporate all these aspects in a single device are already a reality and technological improvement are bound to be expected in the next decades.

Nevertheless, some caution should be taken, as equally there are some limitations. For example, the blow-sampling approach is probably not suitable or not so successful on small cetaceans (Robinson & Nuuttila, 2020) and/or in warmer climates, as the blow approach has so far been tested successful mostly in cold waters (Costa et al., 2023). The sampling efficiency depends also on the field setting (with sampling in open sea expanses expected to be less efficient) and on whales' behaviour: when the animals are in feeding grounds milling for food the blows follow long apneas in depth and are therefore more conspicuous than, for example, when the animals are engaged in other activities, e.g. during migration. Finally, the genetic material is probably not sufficient for long-term storage, and it should be tested if biological material is suitable (collection protocol) or enough to perform complementary (ecotox and endocrine) analyses. This does not take away from the approach being extremely promising and opening up new opportunities for monitoring whales in their natural environment.

CONFLICT OF INTEREST STATEMENT

The author declares that the perspective article was written in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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REFERENCES

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), 1–15. https://doi.org/10.1128/msystems.00119-17

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

Barratclough, A., Takeshita, R., Thomas, L., Photopoulou, T., Pirotta, E., Rosel, P. E., Wilcox Talbot, L. A., Vollmer, N. L., Wells, R., Smith, C. R., Rowles, T. K., Horvath, S., & Schwacke, L. (2024). Estimating age and investigating epigenetic changes related to health across multiple bottlenose dolphin populations. *Biological Conservation*, *293*, 110570. https://doi.org/10.1016/j.biocon.2024.110570

Burgess, E. A., Hunt, K. E., Kraus, S. D., & Rolland, R. M. (2016). Get the most out of blow hormones: Validation of sampling materials, field storage and extraction techniques for whale respiratory vapour samples. *Conservation Physiology*, *4*(1), Cow024. https://doi.org/10.1093/conphys/cow024

Costa, H., Rogan, A., Zadra, C., Larsen, O., Rikardsen, A. H., & Waugh, C. (2023). 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*(15), 1–10. https://doi.org/10.3390/drones7010015

Dawson, S. M., Bowman, M. H., Leunissen, E., & Sirguey, P. (2017). Inexpensive aerial photogrammetry for studies of whales and large marine animals. *Frontiers in Marine Science*, *4*, 366. https://doi.org/10.3389/fmars.2017.00366

Degollada, E., Amigó, N., O'Callaghan, S. A., Varola, M., Ruggero, K., & Tort, B. (2023). A novel technique for photo-identification of the fin whale, Balaenoptera physalus, as determined by drone aerial images. *Drones*, *7*(3), 220. https://doi.org/10.3390/drones7030220

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

O'Mahony, É. N., Sremba, A. L., Keen, E. M., Robinson, N., Dundas, A., Steel, D., Wray, J., Baker, C. S., & Gaggiotti, O. E. (2024). Collecting baleen whale blow samples by drone: A minimally intrusive tool for conservation genetics. *Molecular Ecology Resources*, e13957. https://doi.org/10.1111/1755-0998.13957

Richard, J. T., Schultz, K., Goertz, C. E. C., Hobbs, R. C., Romano, T. A., & Sartini, B. L. (2022). Evaluating beluga (Delphinapterus leucas) blow samples as a potential diagnostic for immune function gene expression within the respiratory system. *ConservationPhysiology*, *10*(1), 1–9. https://doi.org/10.1093/conphys/coac045

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. https://doi.org/10.1578/AM.46.1.2020.32

Thompson, L. A., Spoon, T. R., Goertz, C. E. C., Hobbs, R. C., & Romano, T. 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

Wiley, D. N., Zadra, C. J., Friedlaender, A. S., Parks, S. E., Pensarosa, A., Rogan, A., Shorter, K. A., Urbán, J., & Kerr, I. (2023). Deployment of biologging tags on free swimming large whales using uncrewed aerial systems. *Royal Society Open Science*, *10*(4), 221376. https://doi.org/10.1098/rsos.221376