

Communication

# Blowing in the Wind: Using a Consumer Drone for the Collection of Humpback Whale (*Megaptera novaeangliae*) Blow Samples during the Arctic Polar Nights

Helena Costa <sup>1,\*</sup>, Andrew Rogan <sup>2</sup>, Christopher Zadra <sup>2</sup>, Oddbjørn Larsen <sup>1</sup>, Audun H. Rikardsen <sup>3,4</sup> and Courtney Waugh <sup>1</sup>

- <sup>1</sup> Faculty of Biosciences and Aquaculture, Nord University, 8026 Bodø, Norway
- <sup>2</sup> Ocean Alliance, Gloucester, MA 01930, USA
- <sup>3</sup> Department of Arctic and Marine Biology, UiT The Arctic University of Norway, 9037 Tromsø, Norway
- <sup>4</sup> Norwegian Institute of Nature Research (NINA), the Fram Centre, 9296 Tromsø, Norway
- \* Correspondence: helena.s.costa@nord.no; Tel.: +47-91360752

**Abstract:** Analysis of cetacean blow offers a unique potential for non-invasive health assessments of their health. In recent years, the use of uncrewed aerial vehicles (UAVs) has revolutionized the way these samples are collected. However, the high cost and expertise associated with purpose-built waterproof UAVs, paired with the challenges of operating during difficult meteorological conditions, can be prohibitive for their standardized use worldwide. A pilot study was conducted in a Northern Norwegian fjord during winter, to assess the feasibility of using a minimally modified and affordable consumer drone to collect blow samples even during the polar nights' challenging weather conditions. For each flight, six petri dishes were attached with velcro to a DJI Mavic 2 Pro. The flights were conducted under temperatures ranging from -1 to -18 degrees Celsius, wind speeds ranging from 9 to 31 km/h, and with the absence of the sun. During the 6-day-long boat survey, 16 blow samples were successfully collected from 11 distinct groups of humpback whales (*Megaptera novaeangliae*). With this study, we further validated the use of a consumer drone as a practical, affordable, and simplified tool for blow collection, functional under harsh meteorological conditions.

Keywords: exhaled breath; humpback whale; UAV; arctic; Norway

### 1. Introduction

After the century-long modern whaling industry led to critical declines in many baleen whale populations across the planet, the International Whaling Commission (IWC) moratorium on commercial whaling came into effect in 1986, abolishing it in most countries and allowing population recoveries to begin [1,2]. Despite that, marine species habitat and health still continue to be under threat, now suffering with the effects of cumulative anthropogenic stressors and with the progressively higher pressures of a changing climate [3–8]. Being able to monitor whale health via non-invasive methods is, therefore, vital for continuing to manage the recovery of their populations. However, physiology studies on large whales are inherently challenging, due to the inability of capturing or restraining a live large whale and, owing to their pelagic nature, limited opportunities to sample them at the surface [9]. Thus, there is a need for the development, improvement, and standardization of non-invasive and cost-effective methods to measure free-ranging whales' health.

In recent years, the collection of cetacean exhaled breath or "blow" samples has been raising interest as a promising tool for non-invasively examining cetacean health [10–19]. Exhaled breath, across species, is composed not only of volatile organic compounds (VOCs) but also of aerosol droplets that carry lung epithelial cells and compounds including proteins, lipids, oxidants and nucleic acids [20]. Some of these are related to processes taking



**Citation:** Costa, H.; Rogan, A.; Zadra, C.; Larsen, O.; Rikardsen, A.H.; Waugh, C. Blowing in the Wind: Using a Consumer Drone for the Collection of Humpback Whale (*Megaptera novaeangliae*) Blow Samples during the Arctic Polar Nights. *Drones* **2023**, *7*, 15. https:// doi.org/10.3390/drones7010015

Academic Editor: Diego González-Aguilera

Received: 23 November 2022 Revised: 17 December 2022 Accepted: 23 December 2022 Published: 26 December 2022



**Copyright:** © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/).



place in the respiratory system and the airways in particular (respiratory biomarkers), while others reflect metabolic activity and the state of the organism, acting as convenient biomarkers for a wide range of systemic conditions [21]. In cetaceans, exhaled breath has been used for detection of hormones related to pregnancy or chronic stress [11–14,16,18,19], metabolomics [15], microbiological studies [10,22–26], genetic markers to determine sex, species, or individual identification [23,27] and have strong potential to be used for transcriptomic and gene expressions analysis [28]. Cetacean exhaled breath samples have been collected placing a surface over the stream of blow, such as petri dish, nylon gauze or a plastic sheet, either held directly over the blowhole, in the case of captive or restrained small cetaceans, or mounted on the end of a long pole when sampling in the wild [10,15,17]. Recently, the use of uncrewed aerial vehicles (UAVs), or drones, has risen as a valuable replacement for the sampling pole and has revolutionized the way these samples are collected from free-ranging cetaceans [17,22–24,26,29–31]. The use of a UAV allows not only for a larger distance to be kept from the boat to the individuals and for a faster and more efficient collection [17], but it also allows for the recording of the behavior of the individual during sampling [32], from a perspective hard or even impossible to obtain from a boat, and the measurement of its body condition using aerial photogrammetry [33–35].

However, while drones are becoming powerful tools for biodiversity studies and wildlife monitoring [36–41], there may be serious challenges for sampling in marine environments, namely humidity, sun flare, swell, and constant movement of the boat. Further, blow collection using drones is still in its infancy, and as a relatively novel procedure, questions persist about the most adequate equipment, collection protocols, and method limitations.

Thus far, different UAVs have been used for this purpose. Acevedo-Whitehouse et al. (2010) [17] used a radio-controlled 3-kg model helicopter; Pirotta et al. (2017) [26] used a custom-built quadopter; Apprill et al. (2017) [24] used an hexacopter drone; Raudino et al. (2019) [30] used a waterproof custom-built quadcopter drone. However, the high cost and expertise associated with purpose-built waterproof UASs, paired with the behavior impacts associated with the use of a larger drone when flown close to the surface of the water [42], can be prohibitive for the standardized use of this method. Atkinson et al. [23], under the research program SnotBot (https://whale.org/snotbot, accessed 29 August 2022), used two relatively small and minimally modified consumer drones (DJI Inspire 2 and DJI Mavic Pro) for the successful collection of blows from humpbacks whales (Megaptera novaeangliae) in the USA (Alaska and Massachusetts), Gabon and Dominican Republic, during the summer. Considering this is a cost-effective, easily reproductible, user-friendly and relatively lownoise method, it has a huge potential to be used as a standard for blow collection worldwide. However, there are specifically remote and logistically difficult habitats that can challenge it, especially for marine mammals that are permanent or temporarily found in cold and harsh arctic regions [43].

One such habitat is the Arctic region of northern Norway. Since 2010, a humpback whale feeding aggregation has been observed in some specific northern Norwegian fjords, driven by the presence of overwintering Norwegian spring spawning-herring (*Clupea harengus*) during the two-month polar night period [44]. North-Eastern Atlantic humpback whales breed during late winter and spring in tropical waters and feed at high arctic latitudes during summer and autumn in relatively remote regions in offshore waters [45]. Thus, when the whales started to aggregate close to the coast of northern Norway, this was presented as a unique occasion for the study and sampling of this population. Yet, the conditions in the Arctic during winter can be extreme, with temperatures dropping far below zero degrees Celsius, the absence of sun (polar nights) and often strong winds and swells.

Thus, the aim of the present study was to assess the feasibility of using an affordable, minimally modified and small-sized consumer drone during the polar nights' challenging weather conditions to collect blow samples from humpback whales during this Norwegian winter-feeding aggregation.

## 2. Materials and Methods

## 2.1. Sampling Equipment

A minimally modified consumer drone, DJI Mavic 2 Pro (DJI, Shenzhen, Guangdong, China), equipped with a pair of foam floaters (STARTRC, Shenzhen, Guangdong, China was used throughout the study. Strips of Velcro (Velcro USA, Inc., Manchester, NH, USA) were glued to the top of the drone, front and frame of the floaters (Figure 1a). Before each flight, six closed sterilized petri dishes (Sterilin 50mm, Thermofisher, Waltham, MA, USA), with Velcro strips glued to the back (prepared beforehand) were attached to the drone. Care was taken to not place the petri dishes in any place that would interfere with flight, camera visual or path of propellers.



**Figure 1.** (a) The drone model used in this study, a DJI Mavic Pro 2, with a pair of foam floaters and six petri dishes attached; (b) the drone (red circle) in position for the collection of a blow sample from a humpback whale.

The drone was flown in manual mode, with "Forward and backward obstacle sensing" and "Downward vision positioning" options disabled. These sensors were disabled to allow the drone to fly close to the water and to allow a crew member to catch it, without automatically avoiding the obstacles. Two spare batteries, charging cables and a charging port were available in the research vessel during sampling. The drone was piloted by an experienced drone operator, but with no previous experience with cetacean blow sampling.

### 2.2. Sampling Method and Processing

During the last week of November 2021, a six-day pilot-study was conducted in the fjord of Kvænangen, that runs next to the village of Skjervøy, Northern Norway  $(70^{\circ}02'1.64'' \text{ N}, 20^{\circ}58'25.14'' \text{ E}).$ 

The work was conducted on board a seven meters research vessel (Merry Fischer Marlin 795) from where members of the team visually scanned the area for humpback whales. Once an individual, or it's blow, was detected, the vessel approached moving at moderate speed to minimize any behavioral impact of an approaching vessel [46]. The boat remained at a minimum of 300 m from the group at all times, unless the animals approached the boat, in which case the engines were switched off. The whale(s)' behaviour, surface interval and respiratory rate were then observed from a distance, before deciding whether it was suitable to launch the drone for a sampling attempt. The decision depended on the whales having a regular surfacing interval and having more than two exhalations per each surfacing occasion. When sampling was decided, the petri dishes were opened and the drone was launched from the hands of a team member equipped with headwear and protective gloves, from the back of the research vessel. From the moment the petri dishes were opened, all the team members were using protective face masks. The drone pilot would then attempt to follow the whales at high altitude (20 m), directed by spotters on the

vessel and with the aid of the live video from the UAVs camera. The drone would approach the whale from behind and descend to 0.5–3 meters during or immediately following an exhalation (Figure 1b), going back to high altitude immediately after collection. If more than one humpback was present, multiple blows from different individuals were collected in the same sample (group sampling). Multiple attempts were made while the whales kept at the surface or until drops of sample would be visible in the drones' camera, marking a positive sample.

For each drone flight, a range of metadata was collected, including date, time, coordinates, environmental conditions (ambient temperature and mean wind speed), duration of the flight, group size, life stage of individuals (adult or calf), fluke photoidentification and sampling success or unsuccess. The video feed of the drone was recorded for each sampling.

Once a sample was collected or once the whales dove deeper, the drone was returned to the vessel and retrieved by a crew member. The sample from the petri dishes was then dry swabbed with one or more swabs (COPAN Diagnostics, Murrieta, California, USA), depending on the volume of the sample, until the petri dish appeared dry. Each swab was put in an individual tube with 2 mL of RNA later (Sigma-Aldrich, St. Louis, Missouri, USA), labeled and stored at room temperature until the return to the laboratory facilities where they were kept at -80 degrees Celsius. The drone was wiped with a cloth and hydrogen peroxide between sampling events.

Five negative controls were collected to account for environmental contamination, using the same methodology, but in the absence of any whales.

## 3. Results

During the six-day survey, we undertook a total of 34 flights. Of these, 16 flights were successful for the sampling of a total of 11 different groups of humpback whales, and five flights were correspondent to the collection of control samples (Figure 2; Table 1).



**Figure 2.** Map of the study area and tracks of successful (green), unsuccessful (red), and control flights (yellow).

Day	Flight	Start	Total	Successful/ Unsuccessful	Group ID	Group Size (Indiv. Sampled)	Hours of Light	Τ°C	Wind (km/h)
23 November 2021	1	10 h 06	4 min	Unsuccessful	-	-	1 h 32 of daylength (6 h 05 of CV)	-5/-6°C	21–26 km/h
	2	10 h 17	5 min	Unsuccessful	-	-			
	3	10 h 28	1 min	Unsuccessful	-	-			
24 November 2021	5	12 h 29	12 min	Unsuccessful	-	-	1 h 02 of day length (5 h 58 of CV)	-1/-8°C	9–19 km/h
25 November 2021	6	08 h 02	4 min	Unsuccessful	-	-	- - - - Sundown all day	−8/−11 °C	24–27 km/h
	7	08 h 09	5 min	Successful	Group 1	4 (2)			
	8	08 h 38	5 min	Control	-	-			
	9	09 h 12	5 min	<u>Successful</u>	Group 2	5 (3)			
	10	9 h 38	3 min	Unsuccessful	-	-			
	11	09 h 49	3 min	Unsuccessful	-	-			
	12	09 h 53	6 min	Successful	Group 3	12-14 (2)	(5 h 52 of CV)		
	13	10 h 09	4 min	<u>Successful</u>	1	1			
	14	10 h 51	2 min	Unsuccessful	-	-			
	15	11 h 26	4 min	<u>Successful</u>	Group 4	6-8 (3)			
	16	11 h 47	8 min	Control	-	-			
	17	12 h 06	13 min	Unsuccessful	-	-			
26 November 2021	18	08 h 07	2 min	Unsuccessful	-	-	Sundown all day (5 h 45 of CV)	-8/-9 °C	21 km/h
	19	08 h 22	3 min	<u>Successful</u>	Group 5	4 (1)			
	20	08 h 52	4 min	<u>Successful</u>	Group 6	2 (1)			
	21	09 h 54	4 min	Successful		3(1)			
	22	10 h 43	2 min	Unsuccessful	-	-			
	23	11 h 52	4 min	Successful	Group 7	5 (3)			
	24	12 h 03	5 min	Successful					
	25	12 h 17	3 min	Successful					
	26	12 h 30	5 min	Control	-	-			
27 November 2021	27	10 h 06	9 min	<u>Successful</u>	Group 8	2 (2)	Sundown all day (5 h 38 of CV)	−7/−9 °C	17–30 km/h
	28	10 h 43	14 min	<u>Successful</u>		_ (_)			
	29	11 h 51	2 min	Successful	Group 9	6 (3)			
	30	11 h 53	4 min	Unsuccessful	-	-			
	31	12 h 21	2 min	<u>Successful</u>	Group 10	2 (1)			
	32	12 h 41	6 min	Control	-	-			
28 November 2021	33	11 h 20	3 min	Control	-	-	Sundown all day (5 h 32 of CV)	1/ 18 °C	31 km/h
	34	11 h 30	4 min	Successful	Group 11	6-7 (2)		-1/-10 C	

**Table 1.** Drone flights undertaken at Kvænangen fjord between 23rd and 28th November 2021 for the collection of humpback whale blow samples. CV= civil twilight; T °C = minimum and maximum ambient temperature.

The length of the successful flights was on average 4.8 min, ranging between a minimum flight time of 2 min and a maximum of 14 min. One, two, or three attempts were made per humpback group.

The flights were conducted under temperatures ranging from -5 to -19 degrees Celsius, wind speeds ranging from 9 to 31 km/h and the absence of sun that led to low light conditions (civil twilight) that lasted for 5–6 hours.

## 4. Discussion

The remote nature and unstable weather conditions of polar research sites can present researchers with logistical challenges when using aerial techniques, especially during the winter. Nonetheless, with our results, we validate the use of a relatively low cost (less than 2000 USD) small consumer drone as an adaptable and simplified tool for large whale blow collection that is functional and practical even under the harsh meteorological conditions in northern Norway during late November. To our knowledge, this study is the first to successfully sample baleen whales' exhaled breath at these northern latitudes during winter and polar night.

It is important that some characteristics be taken into account when choosing a UAV for the purpose of collecting cetacean blow samples. Cetaceans can react to drones in different ways depending on various factors, including the species being sampled, the environment, the drone's proximity to the water surface, the model of the drone, and the pilot's flying skills [31]. While reports of baleen whales (such as humpback whales) reacting to drones are more unusual than for other species, such as toothed whales, behavioral reactions have been previously linked to situations where the drone was flying in front of the individual's head [47,48]. Further, during this study, due to strong winds that would carry the blow sideways instead of upwards, we found, at times, the necessity to fly very close to the surface of the water (approximately 1 meter altitude or less). This proximity can potentially lead to a higher disturbance of the animal, if we are to compare it to photogrammetry studies, where the UAV is maintained at a higher altitude (more than 10 meters). Considering this, we decided to use a drone model of a small size (the DJI Mavic Pro 2), as smaller drones are reported to cause less noise disturbance [42,47]. Nonetheless, regardless of the size of the drone chosen, we believe this method causes considerably less disturbance than the alternative blow collection method using a long pole, as it allows the boat, and the associated motor noise, to keep at a greater distance from the animal. Choosing a small-sized UAV can also bring the researcher additional benefits, as it is portable and light enough to be easily transported to the field and in a boat by only one person, besides being easily and safely launchable and retrievable by someone standing on the vessel's deck. A smaller-sized drone also tends to be less expensive, which makes this protocol more widely reproducible globally, as it can be afforded by more groups, and also allows for a less problematic replacement in the event of a crash or malfunction. Under the research program Snotbot, this drone model and similar ones (e.g., Phantom and Inspire) have been used for different field work locations throughout the years [23]. Thus, even if the wear associated with flying this drone under challenging weather conditions can diminish its maximum lifespan, we still believe it is a cost-effective choice for blow sampling throughout different seasons.

Other characteristic of this model that proved to be of great advantage during sampling was the fact that it is user-friendly and operable with minimal training. Our drone operator had no previous experience with blow sampling or with flying at such a low altitude over water, but within a few days the sampling success improved notably, even while the weather conditions worsened throughout the week. This model proved able to withstand a wide range of environmental conditions, such as wind, humidity, low light, and low temperatures. Using the present method, we found it acceptable to fly under all the different weather conditions we encountered in the field, including temperatures ranging from -1 to -18 degrees Celsius, wind speeds ranging from 9 to 31 km/h, and absence of direct sunlight. However, it is important to keep in mind that the presence of rain would be a limiting factor for flying a drone to collect blow samples.

A smaller sized drone, such as the model we used can, on the other hand, also present some disadvantages during challenging weather, since the lighter weight can make it less stable at higher wind speeds and more difficult to maneuver. Together with increased swell and high speed winds can make the hand launch and recovery of a smaller and less stable drone somewhat more difficult. In our experience, the most difficult sections of all our flights were presented during the launching and retrieving of the drone, as during this time there is a higher likelihood of a crash against the boat. During the sampling itself, we found the likelihood of crashing quite low, as long as there is a spotter on the boat maintaining permanent eye contact with the drone and as long as the lowering of the drone is performed relatively slowly under 3 meters of altitude. However, since the propellers are much less powerful than those of a larger UAV, we still believe this is a safe drone to handle during landing, even under difficult wind conditions. As mentioned before, higher wind speeds will also lead to the blow being swept to the side instead of upwards. In this situation, it is important that the drone pilot is able to position the drone sideways and behind the whale's blowhole, in the direction of the wind, to maximize the chance of a successful sampling.

Other contributing factors that can impact a successful sampling event are the battery life-time and a correct assessment of the best time to launch the drone. In fact, all the unsuccessful flights of the present study were due to the drone not reaching the individuals in time before their dive. This happened either because the individuals surfaced at a larger distance from the boat than expected or because their surfacing times were not correctly assessed. Thus, having spotters in the boat, assessing whale behavior, and surfacing and diving intervals, led to the ability to choose the best time to launch, improved sampling efficiency, and minimized flight times. In our experience, the best time to launch proved to be one to two minutes before the whales' expected re-surfacing. The drone model used is described to have a maximum battery life of 31 minutes, which is expected to be lowered substantially in cold environments. Nonetheless, considering that our flights ranged from 1 minute to a maximum of 12 minutes, with an average of 4.8 minutes, we found that the battery life was enough for one sampling attempt followed by a safe and non-rushed retrieving of the drone back to the boat (battery higher that 30%). However, we would not recommend attempting more than one flight per battery, being essential to have spare charged batteries, kept warm at all times, and the capability of charging them between flights. During sampling, it was also very important to have a team member call out the height of the drone to the pilot, since the altimeter of the drone is unreliable over the water. For the same fact, it is important to keep in mind that the method described here should only be used for photogrammetry studies with the addition of a laser or radar altimeter to the drone. The placement of the petri dishes is also a factor to consider, as the propeller movement of the drone model concentrated most of the sample on the top of it. Therefore, the placement of two petri dishes on the top of the drone is recommended, with the addition of two frontal and two bottom petri dishes to maximize the amount of sample collected.

Finally, this model is also capable of collecting live footage, not only allowing for a first-person view in real time, which allows the boat to keep greater distance from the whales, but also allows for the collection of very useful footage for later determine how many exhales are represented in a sample, if there is a chance of a mixed sample (i.e., if a second animal seems close), account for behavioral reactions that were missed from the boat and give additional data on body condition. This footage can also be used to identify repeated samples from a single individual by observing specific markings on an animal's back and complementing photo ID data.

Blow samples have previously been collected for the study of a range of different health metrics and used for the successful extraction of hormones [11–14,16,18,19], DNA [23,24,27], and RNA [22,28]. While, our blow collection methodology has the potential to be used for all these different purposes, it is important to keep in mind that the sample storage method might need to be adapted depending on the aim of the research. Additionally, the collection of control samples from both air and saltwater is recommended, especially for microbiological and hormonal studies. Furthermore, for our study, we did not target individual animals and we were interested in group sampling. If samples from individual whales are required, we would recommend the sampling of isolated individuals, surfacing alone, as it is very challenging to avoid contamination of other individual's blows when the drone is sampling in the middle of a group.

Our study shows that it is feasible to use a small minimally modified consumer drone to collect blow samples from free-ranging large whales in conditions that in the past might have been seen as too challenging due to the cost, the remote location, or the weather, such as the ones found during polar nights in northern latitudes. The creation of cost effective, non-invasive, and uncomplicated sampling techniques that any researcher, anywhere in the world, can have access to, are essential to allow for the establishment of long-term, standardized cetacean health surveillance programs. These programs are vital for collecting baseline data which can then enable monitoring the health of populations and to provide an early warning system for potential future changes. Ultimately, this data collected will be critical for understanding how large whale populations will respond to progressively higher anthropogenic changes in the marine and Arctic ecosystems in the future.

**Author Contributions:** Conceptualization, H.C. and C.W.; methodology, A.R., C.Z., O.L. and H.C.; validation, O.L. and H.C.; data curation, O.L. and H.C.; writing—original draft preparation, H.C.; writing—review and editing, H.C., A.R., C.Z., A.H.R. and C.W.; supervision, C.W. and A.H.R.; project administration, H.C. and C.W.; funding acquisition, H.C. and C.W. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by the Nord University under the PhD funding no. 224000-169 and by the Wildlife Conservation Research Grant 2021 of the European Wildlife Disease Association (EWDA).

Acknowledgments: We are very grateful to Charlie Lavin, Cesc Gordó-Vilaseca, Anne-Fleur Brand, Sam Steyaert and Audun Rikardsen's team for their assistance on the field, and to Charlie Lavin and Alex Détain for contributing with photos.

Conflicts of Interest: The authors declare no conflict of interest.

**Ethics statement:** All flights were performed according to the Civil Aviation Authority Norway (CAAN) regulations.

#### References

- 1. Castellini, M. History of polar whaling: Insights into the physiology of the great whales. *Comp. Biochem. Physiol. -A Mol. Integr. Physiol.* **2000**, *126*, 153–159. [CrossRef] [PubMed]
- 2. Waugh, C.A.; Monamy, V. Opposing lethal wildlife research when nonlethal methods exist: Scientific whaling as a case study. *J. Fish. Wildl. Manag.* **2016**, *7*, 231–236. [CrossRef]
- 3. Sanderson, C.E.; Alexander, K.A. Unchartered waters: Climate change likely to intensify infectious disease outbreaks causing mass mortality events in marine mammals. *Glob. Chang. Biol.* **2020**, *26*, 4284–4301. [CrossRef] [PubMed]
- 4. van Weelden, C.; Towers, J.R.; Bosker, T. Impacts of climate change on cetacean distribution, habitat and migration. *Clim. Chang. Ecol.* **2021**, *1*, 100009. [CrossRef]
- Redfern, J.V.; McKenna, M.F.; Moore, T.J.; Calambokidis, J.; Deangelis, M.L.; Becker, E.A.; Barlow, J.; Forney, K.A.; Fiedler, P.C.; Chivers, S.J. Assessing the Risk of Ships Striking Large Whales in Marine Spatial Planning. *Conserv Biol.* 2013, 27, 292–302. [CrossRef]
- 6. Wise, J.P., Jr.; Wise, J.T.; Wise, C.F.; Wise, S.S.; Zhu, C.; Browning, C.L.; Zheng, T.; Perkins, C.; Gianios, C., Jr.; Xie, H.; et al. Metal levels in Whales from the Gulf of Maine: A One Environmental Health Approach. *Chemosphere* **2019**, *216*, 653–660. [CrossRef]
- Fossi, M.C.; Panti, C.; Guerranti, C.; Coppola, D.; Giannetti, M.; Marsili, L.; Minutoli, R. Are baleen whales exposed to the threat of microplastics? A case study of the Mediterranean fin whale (Balaenoptera physalus). *Mar Pollut Bull.* 2012, 64, 2374–2379. [CrossRef]
- 8. Blair, H.B.; Merchant, N.D.; Friedlaender, A.S.; Wiley, D.N.; Parks, S.E. Evidence for ship noise impacts on humpback whale foraging behaviour. *Biol. Lett.* **2016**, *12*, 20160005. [CrossRef]
- 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.; et al. Overcoming the challenges of studying conservation physiology in large whales: A review of available methods. *Conserv Physiol.* 2013, 1, cot006. [CrossRef]
- Groch, K.; Blazquez, D.; Marcondes, M.; Santos, J.; Colosio, A.; Delgado, J.D.; Catão-Dias, J. Cetacean morbillivirus in Humpback whales' exhaled breath. *Trans. Emerg. Dis.* 2021, 68, 1736–1743. [CrossRef]
- 11. Hogg, C.J.; Vickers, E.R.; Rogers, T.L. Determination of testosterone in saliva and blow of bottlenose dolphins (Tursiops truncatus) using liquid chromatography-mass spectrometry. J. Chromatogr. B. 2005, 814, 339–346. [CrossRef] [PubMed]
- 12. Hogg, C.J.; Rogers, T.L.; Shorter, A.; Barton, K.; Miller, P.J.O.; Nowacek, D. Determination of steroid hormones in whale blow: It is possible. *Mar. Mammal. Sci.* 2009, 25, 605–618. [CrossRef]
- 13. Thompson, L.A.; Spoon, T.R.; Goertz, C.E.C.; Hobbs, R.C.; Romano, T.A. Blow collection as a non-invasive method for measuring cortisol in the beluga (Delphinapterus leucas). *PLoS ONE* **2014**, *9*, e114062. [CrossRef] [PubMed]
- 14. Burgess, E.A.; Hunt, K.E.; Kraus, S.D.; Rolland, R.M. Get the most out of blow hormones: Validation of sampling materials, field storage and extraction techniques for whale respiratory vapour samples. *Conserv. Physiol.* **2016**, *4*, 1–11. [CrossRef]
- Aksenov, A.; Yeates, L.; Pasamontes, A.; Siebe, C.; Zrodnikov, Y.; Simmons, J.; McCartney, M.; Deplanque, J.-P.; Wells, R.; Davis, C. Metabolite content profiling of bottlenose dolphin exhaled breath. *Anal. Chem.* 2014, *86*, 10616–10624. [CrossRef]
- 16. Mingramm, F.M.J.; Keeley, T.; Whitworth, D.J.; Dunlop, R.A. Relationships between blubber and respiratory vapour steroid hormone concentrations in humpback whales (Megaptera novaeangliae). *Aquat. Mamm.* **2019**, *45*, 465–477. [CrossRef]

- 17. Acevedo-Whitehouse, K.; Rocha-Gosselin, A.; Gendron, D. A novel non-invasive tool for disease surveillance of free-ranging whales and its relevance to conservation programs. *Anim. Conserv.* **2010**, *13*, 217–225. [CrossRef]
- Burgess, E.A.; Hunt, K.E.; Kraus, S.D.; Rolland, R.M. Quantifying hormones in exhaled breath for physiological assessment of large whales at sea. Sci. Rep. 2018, 8, 1–14. [CrossRef]
- 19. Hunt, K.E.; Rolland, R.M.; Kraus, S.D. Detection of steroid and thyroid hormones via immunoassay of North Atlantic right whale (Eubalaena glacialis) respiratory vapor. *Mar. Mammal. Sci.* 2014, *30*, 796–809. [CrossRef]
- Mutlu, G.M.; Garey, K.W.; Robbins, R.A.; Danziger, L.H.; Rubinstein, I. Collection and analysis of exhaled breath condensate in humans. *Am. J. Respir. Crit. Care Med.* 2001, 164, 731–737. [CrossRef]
- 21. Popov, T.A. Human exhaled breath analysis. Ann. Allergy Asthma. Immunol. 2011, 106, 451–456. [CrossRef] [PubMed]
- Geoghegan, J.; Pirotta, V.; Harvey, E.; Smith, A.; Buchmann, J.; Ostrowski, M.; Eden, J.-S.; Harcourt, R.; Holmes, E. Virological sampling of inaccessible wildlife with drones. *Viruses* 2018, 10, 300. [CrossRef] [PubMed]
- Atkinson, S.; Rogan, A.; Baker, C.S.; Dagdag, R.; Redlinger, M.; Polinski, J.; Urban, J.; Sremba, A.; Branson, M.; Mashburn, K.; et al. Genetic, Endocrine, and Microbiological Assessments of Blue, Humpback and Killer Whale Health using Unoccupied Aerial Systems. *Wildl. Soc. Bull.* 2021, 1–16. [CrossRef]
- 24. Apprill, A.; Miller, C.A.; Moore, M.J.; Durban, J.W.; Fearnbach, H.; Barrett-Lennard, L.G. Extensive Core Microbiome in Drone-Captured Whale Blow Supports a Framework for Health Monitoring. *mSystems* **2017**, 2. [CrossRef]
- Raverty, S.; Rhodes, L.; Zabek, E.; Eshghi, A.; Cameron, C.; Hanson, B.; Schroeder, P. Respiratory Microbiome of Endangered Southern Resident Killer Whales and Microbiota of Surrounding Sea Surface Microlayer in the Eastern North Pacific. *Sci. Rep.* 2017, 7, 394. [CrossRef]
- Pirotta, V.; Smith, A.; Ostrowski, M.; Russell, D.; Jonsen, I.D.; Grech, A.; Harcourt, R. An economical Custom-Built drone for assessing whale health. *Front. Mar. Sci.* 2017, 4, 425. [CrossRef]
- 27. Robinson, C.V.; Nuuttila, H.K. Don't hold your breath: Limited DNA capture using non-invasive blow sampling for small cetaceans. *Aquat. Mamm.* **2020**, *46*, 32–41. [CrossRef]
- Richard, J.T.; Schultz, K.; Goertz, C.E.C.; Hobbs, R.C.; Romano, T.A.; Sartini, B.L. Evaluating beluga (Delphinapterus leucas) blow samples as a potential diagnostic for immune function gene expression within the respiratory system. *Conserv. Physiol.* 2022, 10, 1–9. [CrossRef]
- Centelleghe, C.; Carraro, L.; Gonzalvo, J.; Rosso, M.; Esposti, E.; Gili, C.; Bonato, M.; Pedrotti, D.; Cardazzo, B.; Povinelli, M.; et al. The use of Unmanned Aerial Vehicles (UAVs) to sample the blow microbiome of small cetaceans. *PLoS ONE* 2020, *15*, e0246177. [CrossRef]
- 30. Raudino, H.C.; Tyne, J.A.; Smith, A.; Ottewell, K.; McArthur, S.; Kopps, A.M.; Chabanne, D.; Harcourt, R.G.; Pirotta, V.; Waples, K. Challenges of collecting blow from small cetaceans. *Ecosphere* **2019**, *10*, e02901. [CrossRef]
- 31. Domínguez-Sánchez, C.A.; Acevedo-Whitehouse, K.A.; Gendron, D. Effect of drone-based blow sampling on blue whale (Balaenoptera musculus) behavior. *Mar. Mammal. Sci.* **2018**, *34*, 841–850. [CrossRef]
- 32. Torres, L.G.; Nieukirk, S.L.; Lemos, L.; Chandler, T.E. Drone up! Quantifying whale behavior from a new perspective improves observational capacity. *Front. Mar. Sci.* 2018, *5*, 319. [CrossRef]
- Aoki, K.; Isojunno, S.; Bellot, C.; Iwata, T.; Kershaw, J.; Akiyama, Y.; López, L.M.M.; Ramp, C.; Biuw, M.; Swift, R.; et al. Aerial photogrammetry and tag-derived tissue density reveal patterns of lipid-store body condition of humpback whales on their feeding grounds. *Proc. R Soc. B Biol. Sci.* 2021, 288, 20202307. [CrossRef] [PubMed]
- Christiansen, F.; Sironi, M.; Moore, M.J.; Di Martino, M.; Ricciardi, M.; Warick, H.A.; Irschick, D.J.; Gutierrez, R.; Uhart, M.M. Estimating body mass of free-living whales using aerial photogrammetry and 3D volumetrics. *Methods Ecol Evol.* 2019, 10, 2034–2044. [CrossRef]
- 35. Christiansen, F.; Sironi, M.; Moore, M.J.; Di Martino, M.; Ricciardi, M.; Warick, H.A.; Irschick, D.J.; Gutierrez, R.; Uhart, M.M. Estimating body mass of sperm whales from aerial photographs. *Mar. Mammal. Sci.* **2022**, 136–154. [CrossRef]
- 36. Monks, J.M.; Wills, H.P.; Knox, C.D. Testing Drones as a Tool for Surveying Lizards. *Drones* **2022**, *6*, 199. [CrossRef]
- Fettermann, T.; Fiori, L.; Gillman, L.; Stockin, K.A.; Bollard, B. Drone Surveys Are More Accurate Than Boat-Based Surveys of Bottlenose Dolphins (Tursiops truncatus). *Drones* 2022, 6, 82. [CrossRef]
- Jones, I.V.G.P.; Pearsltine, L.G.; Percival, H.F. An Assessment of Small Unmanned Aerial Vehicles for Wildlife Research. Wildl. Soc. Bull. 2006, 34, 750–758. [CrossRef]
- 39. Hodgson, J.C.; Mott, R.; Baylis, S.M.; Pham, T.T.; Wotherspoon, S.; Kilpatrick, A.D.; Segaran, R.R.; Reid, I.; Terauds, A.; Koh, L.P. Drones count wildlife more accurately and precisely than humans. *Methods Ecol. Evol.* **2018**, *9*, 1160–1167. [CrossRef]
- 40. Ramp, C.; Gaspard, D.; Gavrilchuk, K.; Unger, M.; Schleimer, A.; Delarue, J.; Landry, S.; Sears, R. Up in the air: Drone images reveal underestimation of entanglement rates in large rorqual whales. *Endanger. Species Res.* **2021**, *44*, 33–44. [CrossRef]
- 41. Chabot, D.; Bird, D.M. Wildlife research and management methods in the 21st century: Where do unmanned aircraft fit in? *J. Unmanned. Veh. Syst.* **2015**, *3*, 137–155. [CrossRef]
- Christiansen, F.; Rojano-Doñate, L.; Madsen, P.T.; Bejder, L. Noise levels of multi-rotor unmanned aerial vehicles with implications for potential underwater impacts on marine mammals. *Front. Mar. Sci.* 2016, *3*, 277. [CrossRef]
- 43. Hamilton, C.; Lydersen, C.; Aars, J.; Biuw, M.; Boltunov, A.N.; Born, E.W.; Dietz, R.; Folkow, L.P.; Glazov, D.M.; Haug, T.; et al. Marine mammal hotspots in the Greenland and Barents Seas. *Mar. Ecol. Prog. Ser.* **2021**, 659, 3–28. [CrossRef]

- Ramm, T. Hungry During Migration? Humpback Whale Movement from the Barents Sea to a Feeding Stopover in Northern Norway Revealed by Photo-ID Analysis. 2020. Available online: <u>https://munin.uit.no/handle/10037/19109</u> (accessed on 15 September 2022).
- 45. Kettemer, L.E.; Rikardsen, A.H.; Biuw, M.; Broms, F.; Mul, E.; Blanchet, M.-A. Round-trip migration and energy budget of a breeding female humpback whale in the Northeast Atlantic. *PLoS ONE* **2022**, *17*, e0268355. [CrossRef] [PubMed]
- Sprogis, K.R.; Videsen, S.; Madsen, P.T. Vessel noise levels drive behavioural responses of humpback whales with implications for whale-watching. *Elife* 2020, 9, e56760. [CrossRef]
- 47. Palomino-González, A.; Kovacs, K.M.; Lydersen, C.; Ims, R.A.; Lowther, A.D. Drones and marine mammals in Svalbard, Norway. *Mar. Mammal. Sci.* 2021, 37, 1212–1229. [CrossRef]
- 48. Raoult, V.; Colefax, A.P.; Allan, B.M.; Cagnazzi, D.; Castelblanco-Martínez, N.; Ierodiaconou, D.; Johnston, D.; Yauri, S.L.; Lyons, M.; Pirotta, V.; et al. Operational protocols for the use of drones in marine animal research. *Drones* **2020**, *4*, 64. [CrossRef]

**Disclaimer/Publisher's Note:** The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.