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NOTE

Marine Mammal Science

Behavioral responses of Bigg's and Southern Resident killer whales (*Orcinus orca*) to uncrewed aerial vehicle-based breath sample collection

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Uncrewed aerial vehicles (UAV), or drones, are one of many technological advances that are enabling researchers to minimize anthropogenic disturbance when studying vulnerable species (Gonzalez et al., 2016). For cetaceans, UAVs have been used for aerial photogrammetry (Christiansen et al., 2020; Fearnbach et al., 2019; Kotik et al., 2023; Leslie et al., 2022; Palacios & Cantor, 2023; Stewart et al., 2022), photo-identification (Leslie et al., 2022; Palacios & Cantor, 2023; Ryan et al., 2022), and biological sampling (Centelleghe et al., 2020; Horton et al., 2019). Not only do UAVs provide an enhanced dorsal view on animals that are often elusive (Barreto et al., 2021; Torres et al., 2018), they also enable marine mammal researchers to collect data while keeping their boat at a greater distance than a more traditional specimen collection technique would require (e.g., breath sample collected by telescoping pole from a boat; Raverty et al., 2017).

Technology has created smaller and quieter UAVs over time (Costa et al., 2023; Pirotta et al., 2017; Raudino et al., 2019) making current day animal response to UAVs difficult to compare to historical responses. While not accounting for changes in UAVs over time, studies show some marine mammals have frequent and pronounced responses to UAVs, while others do not. For example, no apparent behavioral responses to UAVs were found in studies on bowhead whales (*Balaena mysticetus*; Koski et al., 2015), humpback whales (*Megaptera novaeangliae*; Christiansen et al., 2016), gray whales (*Eschrichtius robustus*; Torres et al., 2018), southern right whales (*Eubalaena australis*; Christiansen et al., 2020), and Risso's dolphins (*Grampus griseus*; Hartman et al., 2020). An important variable associated with UAV-induced behavioral response, where detected, is UAV altitude, which may reflect increased noise level or visual detection at lower altitudes. For example, free-ranging common bottlenose dolphins (*Tursiops truncatus*) in Belize showed behavioral responses to UAV flights at altitudes between 11 and 30 m (Ramos et al., 2018). Aubin et al. (2023) observed that St. Lawrence beluga whales (*Delphinapterus leucas*) showed more evasive reactions to UAVs flying at lower (<23 m) altitudes than at higher ones and, based on a literature review, recommended an altitude of ≥30 m to minimize UAV disturbance of cetaceans. Bottlenose dolphins and common dolphins (*Delphinus delphis*) off the southern coast of Portugal also exhibited a response and changed direction when the UAV was flying at 5 m, but not when it was higher (Castro et al., 2021). Antillean manatees (*Trichechus manatus manatus*) displayed strong evasive responses when repeatedly followed by a UAV (Ramos et al., 2018). Behavioral responses (e.g., faster swimming, rolling, bucking, pectoral fin slaps) specific to UAV breath sampling at an altitude of 3 m were observed in blue whales (*Balaenoptera musculus*) in the Gulf of California and humpback whales in the Gulf of Guinea, Frederick Sound, and the Caribbean Sea, but not in humpback whales in Stellwagen Bank or in a single killer whale (*Orcinus orca*) in Frederick Sound (Atkinson et al., 2021). Specific studies on UAV breath sampling also have shown no observable behavioral responses in some cetaceans, including fin whales (*Balaenoptera physalus*, 13 m; Acevedo-Whitehouse et al., 2010), gray whales (13 m, Acevedo-Whitehouse et al., 2010), humpback whales (0.5–3 m and at 13 m; Acevedo-Whitehouse et al., 2010; Costa et al., 2023; Pirotta et al., 2017), blue whales (5 m, Domínguez-Sánchez et al., 2018), bottlenose dolphins (3 m, Centelleghe et al., 2020), and a sperm whale (*Physeter macrocephalus*, 3 m and 13 m; Acevedo-Whitehouse et al., 2010; Centelleghe et al., 2020). This wide variation in behavioral response suggests that in addition to UAV altitude and flight pattern, species specific response differences may exist (Raudino et al., 2019). For example, eye position and extent of upward field of view may enable some species to better detect UAVs. Other environmental and demographic factors like time of day, angle of sun and shadows, season, temperament (e.g., predator vs. prey species), and the animal's behavioral or reproductive state could also influence its response to a UAV (Domínguez-Sánchez et al., 2018; Ramos et al., 2018).

Researchers have flown UAVs at relatively high altitudes (35–40 m) to collect photogrammetry data from Southern Resident killer whales (SRKWs) and mammal-eating transient/Bigg's killer whales (Durban et al., 2015; Fearnbach et al., 2019; Kotik et al., 2023) without noting behavioral responses (Durban et al., 2015). To collect breath from killer whales, however, UAVs must be flown as low as 3 m. Breath is an important biological sample for evaluating the health of free-ranging cetaceans where hands-on examination is not possible (Acevedo-Whitehouse et al., 2010; Hunt et al., 2013) and is a growing discipline in human and in veterinary medicine (Zamuruyev et al., 2016). The presence and quantity of some inflammatory markers and microorganisms in breath likely reflect similar concentrations in the blood that perfuses the lung alveoli and are known to correlate with various systemic or local metabolic, endocrine, and inflammatory processes (Nollens et al., 2019; Robeck & Nollens, 2013). Breath collection by UAV has been attempted on a number of cetaceans including fin whales (Acevedo-Whitehouse et al., 2010), sperm whales (Acevedo-Whitehouse et al., 2010; Centelleghe et al., 2020), humpback whales (Acevedo-Whitehouse et al., 2010; Atkinson et al., 2021; Costa et al., 2023; Pirotta et al., 2017), gray whales (Acevedo-Whitehouse et al., 2010), blue whales (Atkinson et al., 2021; Domínguez-Sánchez et al., 2018), killer whales (Atkinson et al., 2021), bottlenose dolphins (*Tursiops aduncus* and *Tursiops truncatus*; Centelleghe et al., 2020; Raudino et al., 2019), and humpback dolphins (*Sousa sahulensis*; Raudino et al., 2019).

We evaluated the behavioral response to close-proximity approaches of UAVs while capturing breath in two sympatric but distinct proposed ecotypes/species of killer whales: an endangered population of fish-eating whales (SRKWs), and a growing population of mammal-eating Bigg's whales (de Bruyn et al., 2013; Morin et al., 2024). Video recorded from the UAV and from a boat-based camera were analyzed to determine if these killer whales would have a behavioral response to UAVs modified for breath sampling. We hypothesized that, if present, behavioral responses to UAVs would be highest during the collection phases of aerial focal follows.

A UAV (DJI MavicPro; Dà-Jiāng Innovations [DJI] Co. Ltd.) was modified by adding a three-dimensional printed plastic platform attached by a reusable zip tie on each of the UAVs front arms for mounting of a 100 mm × 15 mm sterile petri dish (Figure 1). The UAV designed to collect breath samples was deployed in Washington State waters in the Salish Sea, during daylight hours in September 2021 and 2022. All flights were conducted during calm weather conditions at a Beaufort sea state <3 and with no rain. Operations, including launching and retrieval of the UAV, as well as high resolution boat-based videography, were conducted and led by a professionally trained, NOAA-certified Pilot in Charge from an 8-m Zodiac Hurricane 733 rigid hull inflatable boat following the NOAA guidelines for operating a UAV from a NOAA vessel around killer whales. Specifically, this included requirements to operate at a minimum altitude of 15.2 m, descend to no lower than 1.8 m for blow sampling, and maintain a minimum distance of 8,047 m from other UAVs.

Killer whales typically surface 3–4 times sequentially at 20–30 s intervals followed by a longer breath hold and dive of approximately 2–3 min (SRKW) and 5–6 min (Bigg's). Accordingly, during longer dives when the whales were out of visual observation range from the UAV, we attempted to match their course and speed with the boat while remaining within 100–150 m at approximately a 45° position behind the whale. When the whale of interest resurfaced, we repositioned the boat to within 20–50 m of the whale. UAV sampling during aerial focal follows consisted of initially trailing whales at an altitude of 15–20 m, followed by a decrease in altitude to as low as 2.0–2.5 m to fly through the breath plume when the whale surfaced to exhale/inhale. When conditions allowed, whales were followed by the UAV at an altitude of 5–10 m in between surface intervals for repeated samplings. Individual killer whales were identified by their unique combination of dorsal fin, eye patch, and saddle patch morphology to assign focal follows to one of two proposed killer whale ecotypes/proposed species (Bigg, 1987; Ford et al., 2000; Morin et al., 2024). All activities were documented in high resolution 4 K video imagery using a Sony FDR AX53 (NTSC 60i) handheld camcorder from the boat and a 12.35-megapixel camera and 4 K video recorder integrated into the UAV payload. The camera on the UAV used a 1/2.3" CMOS sensor with a 28-mm lens and a 78.8° FOV.

High resolution videos of focal follows recorded by the UAV and from the boat were processed and separated into aerial focal follows ranging from <1 s to 47 s. Aerial focal follows were defined as four sequential phases: (1) presample, (2) collection, (3) interval, or (4) postsample. "Collection" was defined as the period when the UAV was near (2.0–2.5 m) the focal whale attempting to obtain a breath sample. "Presample" was defined as the period before the first collection attempt. "Intervals" were the periods between each collection and "Postsample" was the period after the last collection attempt.

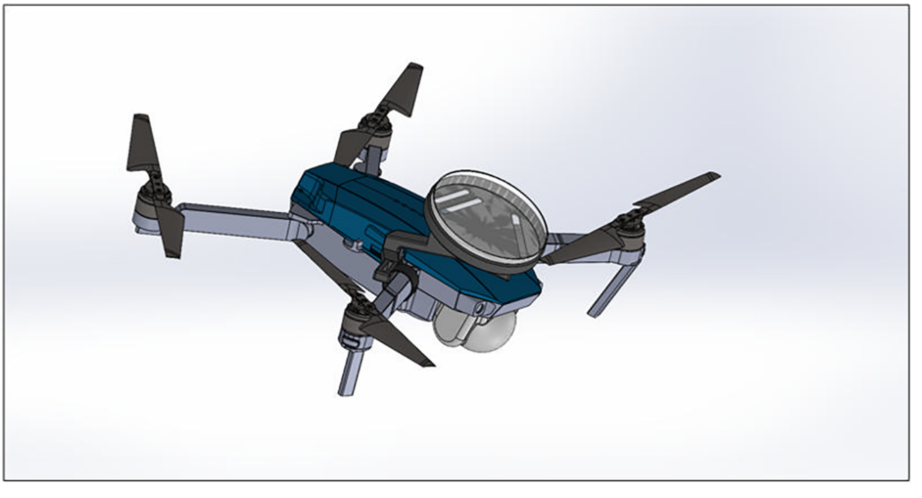

FIGURE 1 DJI Mavic Pro with a modified top mounted three-dimensional printed platform to fit a sterile petri dish for breath collection.

Full-length video recordings of all aerial focal follows recorded by the UAV and from the boat were divided into presample, collection, interval, and postsample aerial focal follows, as defined above. Often, more than one animal was in view of the recordings. When two or more animals were in view at the same time, we used a focal animal sampling approach and assigned each animal their own focal follow phase and a binary response score (1 = response, 0 = no response). Responses were recorded for all killer whales observed, not just the individual targeted for breath sampling.

Response scores were assigned independently to aerial focal follows by three biologists proficient in interpreting killer whale behavior (B.N., M.S., and M.W.). Based on known killer whale behaviors and prior published reports investigating cetacean behavioral responses to UAVs (e.g., Aubin et al., 2023; Castro et al., 2021; Ramos et al., 2018), the biologists monitored for a suite of predefined behaviors (Table 1).

In the first tier of review, high-resolution UAV and boat-based video imagery of each aerial focal follow were evaluated independently by two biologists. Each full-length video was first viewed in full before scoring to understand the sequence of events and the context from the sampling day. The biologists then reviewed each aerial focal follow and identified behavioral responses. A visible change in behavior during an aerial focal follow was scored as "response" whereas the absence of a visible change in behavior was scored as "no response."

In the second tier of the review process, a third biologist experienced in killer whale behavior scored only UAV aerial focal follows. Responses were so rarely observed from the boat-based footage that there was no need to initiate a second-tier review process. The third biologist reviewed a subset ($n = 100$) of 1,133 total UAV aerial focal follows. This included all aerial focal follows where one or both biologists noted a behavioral response to the UAV, as well as a set of randomly selected aerial focal follows where no behavioral response was noted. The sequences were randomized such that the third reviewer was blind to the first two reviewers' scoring. An aerial focal follow where a whale response was identified by at least two of the three biologists was considered a "response" in the final count.

TABLE 1 A list of behavioral responses and their definitions reported in other cetaceans and monitored for by biologists.

Behaviors	Definition
Tail slap	Whale is ventral or dorsal side up and raises fluke out of the water and hits it on the surface.
Spy hop	Vertical rise of the whale's rostrum and head.
Herding	Whales observed traveling apart and then moving.
Scattering	Whales observed traveling in close proximity and then separating.
Ventral (underside up) tight swimming	Group of whales synchronized swimming upside down.
Rolling over another whale in tight formation	Within one body length of each other.
Bubble blowing	Whale emits a trail of bubbles from the blowhole underwater.
Lunging	Whale rises out of the water directed towards the UAV.
Traveling toward or following the UAV	Swimming towards, approaching or trailing the UAV.
Rapid dive	Sudden submersion with subsequent strong undulation of the peduncle.
Positioning the head or body to look at the UAV	Based on change in position of the eye patch (larger proportion of the eye patch coming into view that indicates the head turning upward) and angle of the dorsal fin (indicates body at a sideways angle).
Startle response	Sudden body movement that is not directed at the UAV.
Sudden change in direction	Animal abruptly changes swimming direction.

We fit a Bayesian generalized linear mixed model (GLMM) to assess the difference in probability of a behavioral response between SRKWs and Bigg's killer whales and between the four aerial focal follow phases (presample, collection, interval, postsample) with date, flight number, and individual (or family of) whale as random effects using the R package "brms." Priors were specified using a standard normal prior on all fixed effects (ecotype and aerial focal follow phase). Model selection, diagnostics, and convergence was assessed by balancing the effective sample sizes, R-hat statistics, ESS, and stability of the estimated posterior distribution for each parameter. The final model ran a total of four chains with 2,000 iterations and adapt delta was set to 0.98. Adapt delta defines the target step acceptance probability for the Markov chain Monte Carlo (MCMC) sampling procedure. Increasing adapt delta from the default allowed the model to make more careful steps during the sampler and thus eliminate the number of divergent transitions against the posterior draws. All statistical analyses were performed using R (R Core Team, 2022).

A total of 35 UAV flights were conducted during seven of 20 days of field effort in September 2021 and 2022. Of these flights, 12 were flown over SRKWs and 23 were flown over Bigg's killer whales. The total flight time over SRKWs and Bigg's killer whales was 90:26 (mm:ss) and 265:31 (mm:ss), respectively. In total, there were 131 aerial focal follows over SRKWs and 1,002 aerial focal follows over Bigg's (Table 2), and 19 breath samples were collected.

During the first-tier review, reviewer one and two identified responses in 1.6% (18 of 1,133) and 1.1% (13 of 1,133) of aerial focal follows, respectively. Of the total 31 identified responses, they agreed on seeing a response in five individual aerial focal follows. In tier two, the third reviewer, who was blinded to the tier 1 reviews, identified a response in four of the five aerial focal follows where both biologists also had previously seen a response showing a high degree of agreement on confirmed responses. In total, two or more reviewers agreed on seeing a response in six SRKW (4.6%) and three Bigg's aerial focal follows (0.3%).

The GLMM showed weak statistical evidence that Bigg's killer whales had a lower rate of response than SRKWs (difference ± $SE = -0.75 \pm 0.93$, posterior P[Bigg's < SRKW] $= 0.79$; Figure 2). This could be due to their different foraging ecology and social structure (Baird & Whitehead, 2000). SRKWs routinely engage in subsurface and surface-active behaviors that include rolling (Noren et al., 2009; Tennessen, Holt, Hansen, et al., 2019; Tennessen, Holt, Ward, et al., 2019) possibly to enhance their chances of detecting stimuli at the surface. Consequently, it is not certain that the behavioral responses observed in SRKW in the presence of the UAV were, in fact, caused by the UAV.

There was very strong evidence from the GLMM that the probability of a response was higher in the interval phase than the collection phase (difference ± $SE = 2.21 \pm 0.83$, posterior P[interval > collection] > 0.99; Figure 3). We hypothesized that behavioral responses to UAVs would be highest during collection phases yet found that SRKWs primarily responded to the UAV between breath samplings. It is possible that actual collection aerial focal follows did not elicit reactions because the UAV approached animals from behind and usually dropped in altitude

TABLE 2 Summary of total aerial focal follows and phase (presample, collection, interval, and postsample) and number of responses where two or more reviewers agreed that there was a response noted from UAV video for both ecotypes (SRKW and Bigg's).

	Presample	Collection	Intervals	Postsample	Total
Southern Resident killer whales (SRKW)					
Total aerial focal follows (*n*)	16	74	34	7	131
Response (*n*)	0	0	5	1	6
No response (*n*)	16	74	29	6	125
Bigg's killer whales					
Total aerial focal follows (*n*)	662	114	153	74	1,002
Response (*n*)	2	0	1	0	3
No response (*n*)	659	114	152	74	999

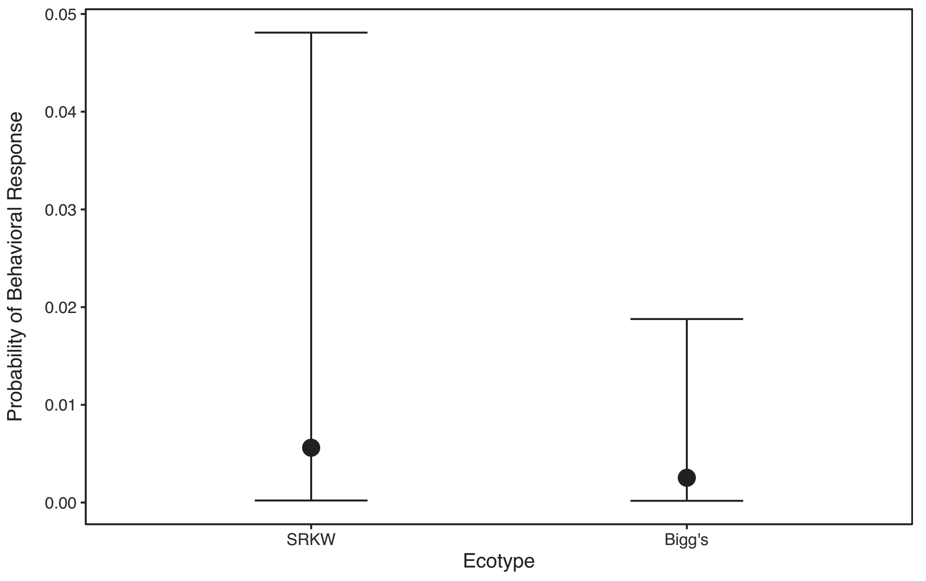

FIGURE 2 Conditional probability of response by ecotype. Estimated probabilities are based on the posterior of a Bayesian Bernoulli mixed-effects model. Points represent posterior means, and error bars indicate 95% credible intervals.

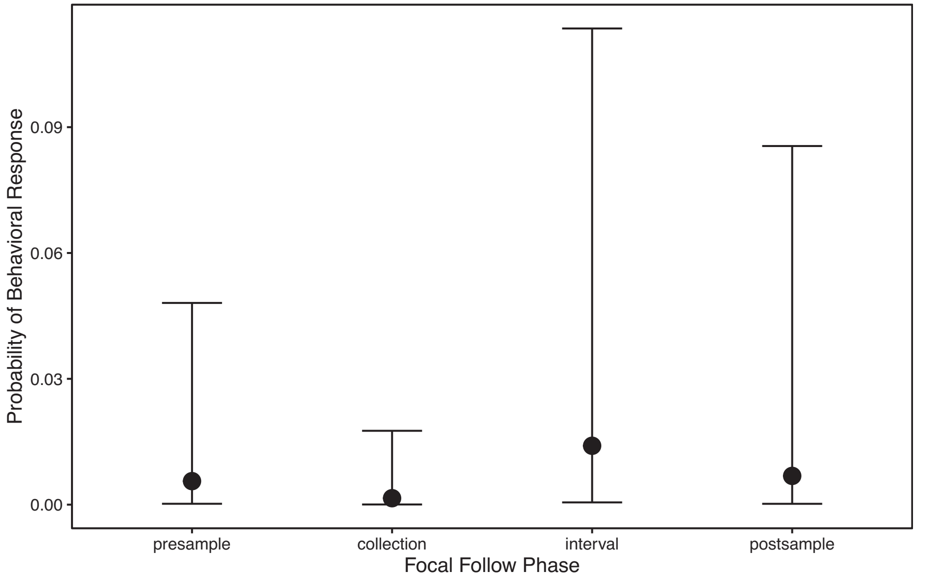

FIGURE 3 Conditional probability of response by sampling phase. Estimated probabilities are based on the posterior of a Bayesian Bernoulli mixed-effects model. Points represent posterior means, and error bars indicate 95% credible intervals.

after the whale was diving, whereas during intervals the UAV was usually trailing the whale overhead. If so, we may be able to further reduce the rare reactions we identified by keeping the UAV higher during interval aerial focal follows. Of the five interval aerial focal follows during which a response was detected, one response was in the first flight interval, two responses were in the second flight interval, one response was in the third flight interval, and one response was in the eighth flight interval, suggesting that number of breath collection attempts in a flight did not influence reactions during the interval aerial focal follow.

We demonstrated that a UAV can be used to collect breath from two eastern North Pacific killer whale ecotypes with minimal disturbance to the animals. Responses were rare and those noted were mild, often reflecting an awareness of the UAV rather than avoidance. No behavioral responses were noted using the boat-based high-resolution video, showing that UAV video is more sensitive than boat-based detection. In future work the concurrent use of a second UAV operated at a higher altitude could record and compare individual and group behavior of whales prior to, during, and following the approaches of a low flying UAV.

Killer whale breath plume is substantially smaller than that of larger cetaceans previously sampled (e.g., humpback whales; Costa et al., 2023, and gray whales Acevedo-Whitehouse et al., 2010), and likely persists more briefly. For example, estimates of blow height for humpback whales ranges from 2.7 and 4.7 m (Horton et al., 2017), whereas for bottlenose dolphins it is <1 m (Raudino et al., 2019). This requires UAVs to fly closer and lower to smaller cetaceans like killer whales to successfully collect sufficient quantities of breath vapor. Here we demonstrate that close-range UAV flights for breath collection rarely caused behavioral responses in mammal-eating and fish-eating killer whales. Eight of the nine behavioral responses observed included rolling the body while maintaining swim speed and direction, in an apparent effort to observe the UAV (Figure 4; SRKW $n = 5$, Bigg's $n = 3$). The ninth behavioral response observed was a SRKW that altered swimming direction, with no other avoidance behaviors or changes in behavioral state detected in 1,133 aerial focal follows.

Using UAVs to collect breath samples permitted us to keep the research vessel substantially further from animals than the close approach (≤7.62 m) required for collecting breath with a petri dish mounted on a pole (Raverty et al., 2017). This is critical considering that vessel presence and proximity can alter the behavioral state of endangered SRKWs during biologically significant activities like foraging and resting (e.g., Giles, 2014; Holt, Tennessen, Hanson, et al., 2021; Holt, Tennessen, Ward, et al. 2021; Lusseau et al., 2009; Tennessen, Holt, Hansen, et al., 2019; Tennessen, Holt, Ward, et al., 2019). The few responses noted in this study were minor compared to evasive reactions to UAVs noted in other species. Aubin et al. (2023) recorded that 4.3% (22/511) of beluga whale reactions to UAVs were sudden dives. Comparably, Atkinson et al. (2021) recorded some deep dives and increased swimming speeds for flights over blue whales. These types of reactions are similar to how SRKWs respond to vessels

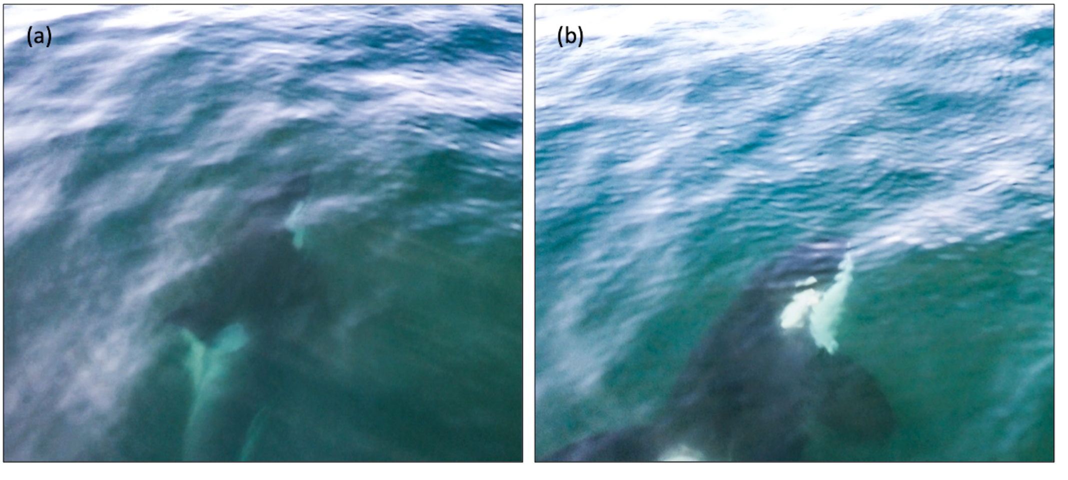

FIGURE 4 Bigg's killer whale T37B1 displaying the most commonly observed behavioral response. (a) T37B1 travels underwater, then (b) rolls to the left to look at the UAV.

(Giles, 2014). Differences in responsiveness to UAVs could also be related to the species (e.g., are they a prey or predator type species). Ford and Reeves (2008) classified different cetaceans as either fight or flight species in response to attacks by killer whales. Therefore, a species' reactiveness to predation or external disturbances could provide insight to different response frequency and response type with UAV-breath sampling. It would be worth comparing results from different methods (boat vs. UAV) for focal follows and breath sampling if given the chance to conduct them on the same individual.

Our findings support the utility of UAVs as a valuable, lower-impact tool than boat-based sampling methods to remotely collect breath samples from SRKWs and Bigg's killer whales. To further minimize disturbance from UAVs during breath collection, we recommend keeping the boat at the greatest distance possible from whales that still permits UAV positioning into the exhaled breath plume. Also, it is important for pilots to balance UAV altitude so that it is high enough to minimize the likelihood of a response and low enough to be able to rapidly drop in to collect breath samples safely and effectively. Future research to improve our understanding of killer whales to close approach of UAVs could include evaluating the direction of the approach (anterior, posterior, or lateral approaches) and study of UAV designs that minimize noise and their visible signature.

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AUTHOR CONTRIBUTIONS

Catherine Feng-Yu Lo: Conceptualization; data curation; formal analysis; investigation; methodology; project administration; resources; supervision; validation; visualization; writing – original draft; writing – review and editing. **Hendrik Nollens:** Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; resources; supervision; validation; visualization; writing – original draft; writing – review and editing. **Brittany Nollens:** Data curation; formal analysis; investigation; methodology; resources; validation; writing – original draft; writing – review and editing. **Maya Sears:** Data curation; formal analysis; investigation; methodology; resources; validation; writing – original draft; writing – review and editing. **Brad Hanson:** Conceptualization; funding acquisition; investigation; methodology; project administration; resources; supervision; validation; writing – original draft; writing – review and editing. **Michael Weiss:** Data curation; formal analysis; funding acquisition; investigation; methodology; validation; writing – original draft; writing – review and editing. **James Sheppard:** Data curation; investigation; methodology; validation; writing – review and editing. **Charlie Welch:** Data curation; investigation; methodology; validation; writing – review and editing. **Robert Friel:** Data curation; investigation; methodology; validation; writing – review and editing. **Joseph Gaydos:** Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; resources; supervision; validation; visualization; writing – original draft; writing – review and editing.

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