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ARTICLE

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Respiration cycle duration and seawater flux through open blowholes of humpback (*Megaptera novaeangliae*) and North Atlantic right (*Eubalaena glacialis*) whales

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Abstract

Little is known about the dynamics of baleen whale respiratory cycles, especially the mechanics and activity of the blowholes and their interaction with seawater. In this study, the duration of complete respiration cycles (expiration/inhalation events) were quantified for the first time in two species: North Atlantic right whale (NARW) and humpback whale (HW) using high resolution, detailed imagery from an unoccupied aerial system (UAS). The mean duration of complete respiration cycles (expiration/inhalation event) in the NARW and HW were 3.07 s (SD = 0.503, n = 15) and 2.85 s (SD = 0.581, n = 21), respectively. Furthermore, we saw no significant differences in respiration cycle duration between age and sex classes in the NARW, but significant differences were observed between age classes in the HW. The observation of seawater covering an open blowhole was also quantified, with NARW having 20% of all breaths with seawater presence versus 90% in HW. Seawater incursion has not been described previously and challenges the general consensus that water does not enter the respiratory tract in baleen whales. Prevalent seawater has implications for the analysis and interpretation of exhaled respiratory vapor/

mucosa samples, as well as for the potential inhalation of oil in spills.

KEYWORDS
humpback whale, North Atlantic right whale, respiratory cycle, respiratory health, unoccupied aerial systems

1 | INTRODUCTION

Breath-holding mammals have a very different respiratory physiology compared to nonbreath-holding mammals given their diving behavior (Fahlman et al., 2015; Goldbogen et al., 2013, 2015). There is limited knowledge on the respiratory physiology of breath-holding mammals, especially baleen whales (Fahlman et al., 2016, 2018; Kooyman, Kerem, Campbell, & Wright, 1971; Reed et al., 2000; Ridgway, Scronce, & Kanwisher, 1969; Sumich, 1983, 2001). This lack of information is due to practical limitations of studying free-living baleen whales, which include the lack of routine method for capturing and restraining large baleen whales, restricting the collection of physiological samples. (Fahlman et al., 2018; Hunt et al., 2013). Furthermore, large baleen whales cannot be accommodated in captive environments like pinnipeds and odontocetes, which are often studied in captivity, so we rely on their brief periods of time at the surface to collect physiological information (Fahlman et al., 2018, 2019; Hunt et al., 2013). Despite these challenges, previous studies have demonstrated associations between respiratory physiology, energy requirements, and metabolic rates of cetaceans. Specifically, respiratory rates are linked to energy requirements, assuming tidal volume and oxygen exchange is constant (Fahlman et al., 2016, 2018; Sumich, 1983, 2001). Respiratory rates (breaths per minute) have been quantified in some baleen whale species (Blix & Folkow, 1995; Sumich, 1983, 2001; van der Hoop et al., 2014) and studies have shown the effects of anthropogenic (e.g., whale watching boats in Christiansen, Rasmussen, & Lusseau, 2014) and nonanthropogenic (e.g., gull attacks in Fazio, Argüelles, & Bertellotti, 2015) disturbances on respiratory rates.

Most studies on respiratory physiology have only accurately represented respiration rates (breaths per minute) (Blix & Folkow, 1995; Goldbogen et al., 2008; Kvadsheim et al., 2017; Roos, Wu, & Miller, 2016; Williams & Noren, 2009). One noninvasive and low disturbance method of investigating respiratory behavior is using unoccupied aerial systems (UAS) (A. Hodgson, Peel, & Kelly, 2017; J. C. Hodgson, Baylis, Mott, Herrod, & Clarke, 2016). Not only can UAS provide detailed imagery on blowhole activity and mechanics, but they also can be used to collect samples of respiratory vapor and mucosa, known as blow samples (Apprill et al., 2017; Hunt et al., 2013). Blow samples can be analyzed giving fundamental information on the physiology of large baleen whales, such as hormone levels and pregnancy status (Apprill et al., 2017; Hunt et al., 2013; Hunt, Rolland, & Kraus, 2014). Using UAS to obtain respiratory physiological measurements is novel and expands the use of the UAS tool (Christiansen et al., 2014; Christiansen et al., 2019; Durban et al., 2016; A. Hodgson et al., 2017).

Unlike respiration rates (breaths per minute), the respiratory cycle duration (RCD), an expiratory/inspiratory event (total duration of a single breath, does not include the breath hold phase), has not been defined formally in the literature. Additionally, the relationship between RCD and respiration rate, energy expenditure, and metabolic rate is unknown for baleen whales (Fahlman et al., 2015, 2016; Roos et al., 2016).

Furthermore, little is known about the specific activity of cetacean blowholes in relation to surrounding surface seawater. This is significant in regards to blow sampling as one common obstacle in analyzing blow samples is contamination and dilution from water vapor and seawater (Apprill et al., 2017; E. A. Burgess, Hunt, Kraus, & Rolland, 2018; Hogg et al., 2009; Hunt et al., 2014). Despite previous studies attempting to correct for dilution from seawater, the source of the seawater that leads to high salinity in drone-captured blow samples has not been determined (E. A. Burgess et al., 2018). The presence of seawater in blow samples has been assumed to be entrained from skin surfaces adjacent to the blowhole during exhalation (E. A. Burgess et al., 2018) but also could be from seawater coming from inside the blowhole cavity. Either or both scenarios could affect blow sample dilution and contamination (Apprill et al., 2017; E. A. Burgess et al., 2018; Hogg et al., 2009; Hunt et al., 2014). If seawater resides in the upper respiratory tract for the duration of a dive cycle, it would likely be warmed by body heat, and acquire cellular and molecular constituents of the respiratory tract mucosa. The presence of seawater in the blowhole cavity could be a normal occurrence, a scenario that has not been discussed to date.

The aim of the study was to describe quantitatively the duration of complete respiratory cycles (exhalation/inhalation event) for two species of baleen whales: NARW and HW using aerial video collected by UAS. The study also investigated and quantified the presence of seawater entering the blowholes of these species, as evident in the same video.

2 | METHODS

2.1 | UAS Surveys

An Inspire 1 RAW UAS (http://www.dji.com) with a LIDAR altimeter (Dawson, Bowman, Leunissen, & Sirguey, 2017) was flown over groupings of NARW and HW during approaches for collecting samples of blow. Sampling was conducted off the coast of Massachusetts in the southwest Gulf of Maine (Figure 1). Whales were first located by vessel, which also served as the platform for UAS deployment. NARW sampling was conducted on 5 days in 3 years (2017–2019, Table S1) and HW sampling took place on 4 days in 1 year (2017, Table S2).

A 25 mm micro 4/3 F1.8 Olympus lens was used on the UAS to record video imagery. Flights were made with the camera angle vertical to the whales and altitudes ranged from 10 to 50 m. However, consistent accurate altimetry was not available for all flights. NARW were documented at 24 or 60 frames per second versus 30 frames per second for HW (Tables S1 and S2).

2.2 | Respiration cycle duration analysis

Respiration cycle duration (RCD) was defined as: "the initial frame at which respiratory vapor (blow) was visible or the first frame at which bubbles were seen trailing from the submerged blowhole as the start of a respiration cycle up to the frame where both blowholes were fully closed at the end of the respiration cycle." There were cases where the blowhole was still open before submergence at the end of a respiratory cycle. In these cases, it was clearly visible that both blowholes were fully closed underwater after submergence so the end of the respiration cycle (when both blowholes were fully closed) could be recorded. This definition was used in this study due to the high resolution of video which made it possible to observe the blowhole opening and closing during respiration cycles. Other descriptions of respiration cycles in baleen whales were not considered due to the disparity in methodology and the lack of previous studies using UAS to study respiratory cycles in baleen whales (Lafortuna, Jahoda, Azzellino, Saibene, & Colombini, 2003). RCD in decimal seconds was calculated by multiplying the number of frames observed for each separate respiration cycle by the frames per second setting on the camera, divided by sixty.

2.3 | Respiratory cycle behavior

To investigate whether the left and right blowhole opened and closed together, the relative area of both left and right blowholes for each frame of a full respiration cycle was measured according to the respiration cycle duration definition for this study. Absolute area was not calculated due to the lack of consistent accurate altimetry available

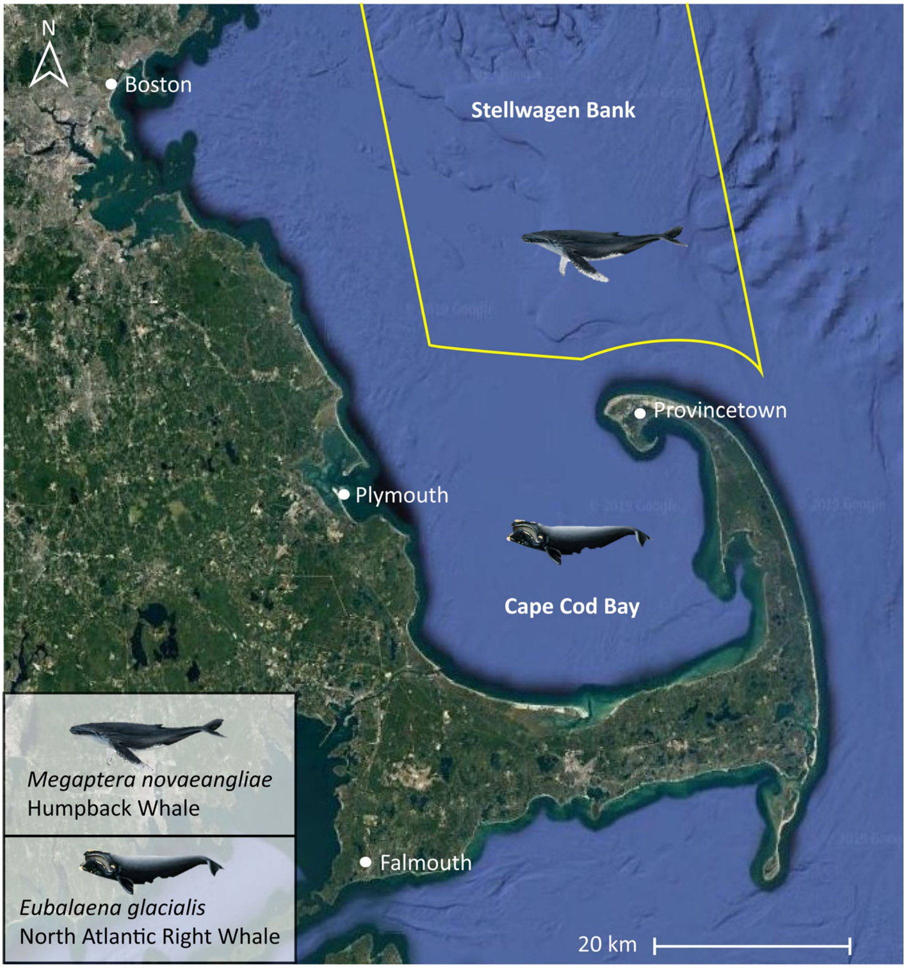

FIGURE 1 Study areas for UAS surveys of NARW and HW. NARW were present in Cape Cod Bay. The yellow line shows the borders of Stellwagen Bank Marine Sanctuary.

for all flights. Each blowhole was measured by outlining the blowhole perimeter using the polygon tool in ImageJ Analyze and Measure (https://imagej.nih.gov/ij/index.html; Figure 2). Respiratory vapor did not obscure the perimeter of the blowholes in most cases.

Relative area was corrected if altitude changed during the respiration cycle using the ratio: altitude for the frame/initial altitude. Blowhole areas were normalized to range from 0 (fully closed blowhole) and 1 (fully open blowhole, maximum measured area) by dividing the actual blowhole area measurement by the maximum measured blowhole area.

Differences in blowhole activity between species were explored by examining each respiration cycle for whether the blowhole was submerged at the beginning of the respiration cycle (presence of bubbles underwater before the whale breaks the surface; Figure 3) and at the end of the respiration cycle (whale submerged with blowhole partially open enabling seawater entrance; Figure 4). Then, each frame was checked for seawater covering an open blowhole (Figure 5; videos available at https://darchive.mblwhoilibrary.org/handle/1912/25606) and it was noted at which stage of the respiration cycle this occurred (exhalation or inhalation).

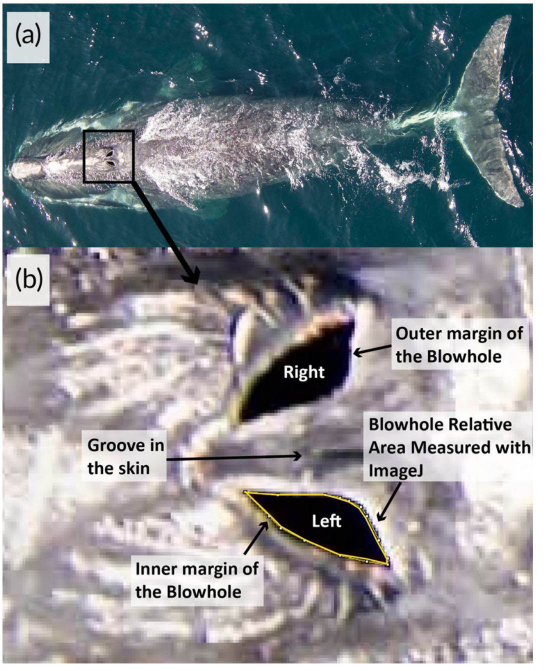

FIGURE 2 Relative blowhole area measurement using ImageJ of left blowhole. (A) The UAS full-frame view of entire NARW individual (#3823 from NEAq Catalog). (B) zoomed in view of blowholes showing the outline of left blowhole where area inside outline was measured.

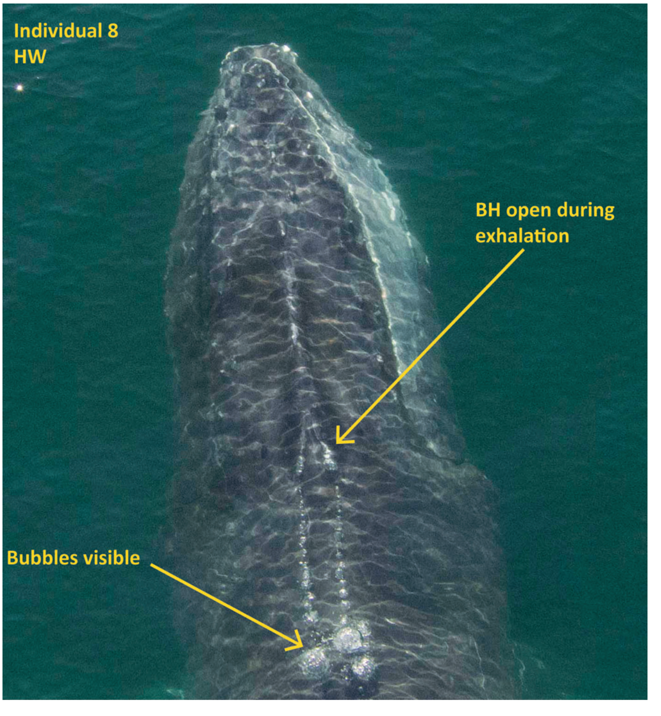

FIGURE 3 Example of humpback whale blowhole open and exhaling before surfacing where bubbles are visible.

2.4 | Identification of individuals

All individual whales were identified and respiration cycles matched to each individual (Tables S1 and S2). The NARWs were identified based on callosity patterns, lip ridges, and prominent scars (Hamilton & Mayo, 1990; Kraus

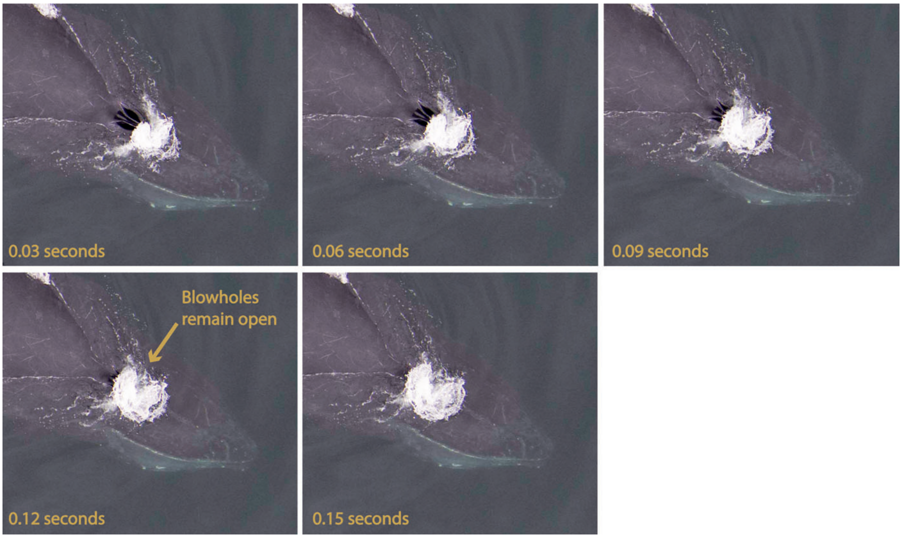

FIGURE 4 Example of blowhole open as the humpback whale submerged. One frame is 0.03 s.

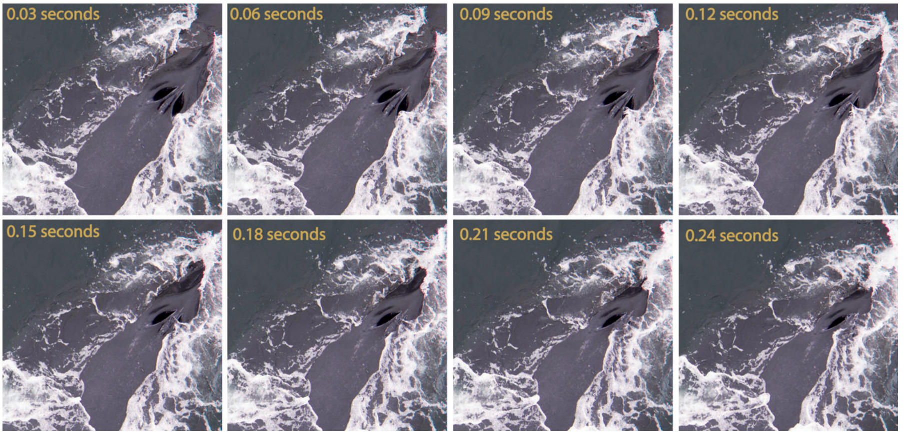

FIGURE 5 Example of seawater covering open blowhole of a humpback whale. One frame is 0.03 s. Videos available at https://darchive.mblwhoilibrary.org/handle/1912/25606.

et al., 1986), then matched to the New England Aquarium Right Whale Catalog (NEAq Catalog, http://rwcatalog.neaq.org; Hamilton & Martin, 1999) to obtain age and sex in the year of sampling (Figure S1). When the individual was not sighted as a calf and where exact age could not be calculated, minimum age was calculated using sighting history data (Hamilton, Knowlton, Marx, & Kraus, 1998). Sex was determined by the examination of the urogenital region, genetic techniques, or whether a calf is present with a female (Brown, Kraus, Gaskin, & White, 1994).

HWs were matched to the Gulf of Maine Humpback Whale Catalog (Center for Coastal Studies, Provincetown, MA) to obtain data on age and sex. However, HWs are usually cataloged based on the ventral fluke pigmentation, trailing edge shape, and dorsal fin features, and these were generally not visible in the available documentation. Instead, matching was based on a combination of permanent and temporary marks on the dorsal body, such as scars, scuffing, lesions, and other marks. Each discrete feature was confirmed to have been present in independent photo-identification data collected in the same area on the same or adjacent days (Figure S2). Sexes of cataloged whales were determined as described above for NARW. Age at the time of sampling was known for individuals first cataloged as a dependent calf. Otherwise, a minimum age was calculated assuming that the individual was alive no less than the year before the first sighting (Robbins, 2007).

2.5 | Statistical analysis

2.5.1 | Respiration cycle duration

For the respiration cycle duration analysis, subsequent respiration cycles from the same individual present in the same UAS video were not considered to be independent samples. Respiration cycles from the same individual were only considered independent samples if they were from different UAS videos recorded at different times. Therefore, for those individuals where more than one respiration cycle was present in the same UAS video, only the first respiration cycle was picked to avoid pseudo replication (n = 15 for NARW and n = 21 for HW). There were instances where the same individual was captured respiring in more than one UAS video and in other cases individuals were only captured in one UAS video (Tables S1 and S2). The first respiration cycle was selected as it was the respiration cycle number present for all individuals, while second or third respiration cycles were not present for all individuals. Mean RCD was then calculated for each species (NARW and HW).

After confirming normality of residuals and heteroscedasticity, a one-way ANOVA and a Bonferroni correction was conducted to test for differences in mean RCD between species.

For the NARW, individuals were grouped by sex and age. The age classes were (1) sexually mature adults (≥7 years old) and (2) sexually immature calves, juveniles up to 5–6 years old, defined by Hamilton and Mayo (1990). A two-way ANOVA was conducted to test for significant differences in mean RCD due to age and sex.

For the HW, individuals were also grouped by sex and age class. The age classes were (1) all individuals known to be younger than the minimum age at first calving (5 years old), known as sexually immature juveniles and (2) individuals known to exceed that age (≥5 years old), known as sexually mature adults (Clapham, 1992; Robbins, 2007). A two-way ANOVA was conducted to test for significant differences in mean RCD due to age and sex.

2.5.2 | Respiratory cycle behavior

Differences in blowhole activity between species were explored by examining each respiration cycle for whether the blowhole was open and exhaling before surfacing at the beginning of the respiration cycle (presence of bubbles underwater before the whale breaks the surface; Figure 3) and at the end of the respiration cycle (whale submerged with the blowhole partially open enabling seawater entrance; Figure 4).

A prevalence for the presence of open and exhaling blowhole before surfacing for each species (when bubbles were seen underwater before the whale breaks the surface) was calculated and expressed as a percentage as follows:

number of respiration cycles with open and exhaling blowhole prior to surfacing/total number of respiration cycles multiplied by 100. A Pearson chi-squared test for independence was used to test for any significant association between species (NARW, HW) and prevalence of blowhole being open and exhaling before surfacing. A prevalence for the occurrence of the blowhole being open during submergence at the end of the respiratory cycle was calculated and expressed as a percentage as follows:

number of respiration cycles with blowhole open during submergence/total number of respiration cycles multiplied by 100. A Pearson chi-squared test for independence was used to test for a significant association between species (NARW, HW) and prevalence of blowhole being open during submergence.

Respiratory cycle curves (relative left blowhole, right blowhole, and mean blowhole area versus time) were plotted for each individual whale to detect if both right and left blowhole opened, reached a point of maximum area, and closed together. This is significant in the context of seawater presence analysis because often only one blowhole was covered by seawater. The time point at maximum area was defined as the point of inflection (POI) and was identified for both blowholes in all respiratory cycle curves. A mean POI between left and right blowhole was also taken.

In each respiration cycle, the percentage of time before and after the POI was calculated. The percentage before POI was calculated as: time in seconds up to POI/total respiration cycle duration, multiplied by 100. The percentage after POI was calculated as: time in seconds after POI to the end of the respiratory cycle/total respiration cycle duration, multiplied by 100. The percentage before and after POI added to 100%. The time points of the POI and percentage of time before and after POI were used as reference points on the respiratory cycle curves. The percentage before POI relates to the opening of the blowholes and exhalation. The percentage after POI relates to the closing of the blowholes and inhalation. In this study, the POI was assumed to be the switch from exhalation to inhalation. This assumption is based on the knowledge that blowholes increase in area during exhalation and decrease in area during inhalation (Buono, Fernández, Fordyce, & Reidenberg, 2015).

A mean for POI, percentage before and after POI was calculated for each individual whale in both species. Given that percentage before POI and after POI both add to 100%, they were shown to be complementary so only percentage before POI was used for further analysis. A one-way MANOVA was conducted to test for differences in POI and percentage before POI between species as a method to determine if the two species have similar timings (in terms of exhalation, inhalation, and the switch between the two) in their respiration curves.

2.5.3 | Seawater presence analysis

We assumed that observation of seawater over an open blowhole during the inhalation phase of the respiratory cycle, however briefly, must imply that water will enter the upper respiratory tract (blowhole, vestibule, external bony nares, and external bony fossa; Buono et al., 2015; Gil, Lillie, Vogl, & Shadwick, 2020), given the force of gravity and also the force from inhalation. This assumption comes from the knowledge that water particles move at 9–10 m/s (Corbet, 2002), which means that during the time that we record seawater covering an open blowhole (approximately between 1 and 1.5 s), this is sufficient time for the water particles to move from outside the blowhole to inside. To quantify this observation, a prevalence of seawater presence over an open blowhole, and whether it was during the expiratory or inspiratory stage, was calculated and expressed as a percentage as follows:

number of respiration cycles with seawater presence over open blowhole/total number of respiration cycles, multiplied by 100. This prevalence included the cases where the whale kept the blowhole open during submergence, which allowed seawater entrance, and in all cases, the source of seawater was from the splash from waves. A Pearson chi-squared test for independence was used to evaluate differences in seawater presence between species (NARW, HW).

To investigate further whether seawater could be entering the blowhole, salinities of selected blow samples were measured as further indication of the presence of seawater. Blow samples from five NARWs were collected using a UAS, and salinity was determined by measuring specific gravity measurements with a refractometer (Milwaukee MR100ATC, Rocky Mount, NC). Control measurements were taken for tap water (n = 1), pure seawater (n = 7), and seawater flown on a drone for 403 s (n = 5). These control measurements were taken in order to compare the NARW blow samples in terms of salinity. The NARW blow samples were also compared to standard mammalian plasma, saliva, and respiratory fluid, which are typically saline (human plasma = 1.022–1.026; Mathew & Varacallo, 2019; saliva = 1.002–1.012; McDonald, 1950; serous fluid = 1.016; Keohane, Otto, & Walenga, 2019); however, if higher levels of salinities were recorded in the NARW blow samples, it could be due to seawater entry in the blowhole.

3 | RESULTS

3.1 | Respiratory cycle duration

We measured respiratory cycle duration, RCD for 12 NARW (*n* = 15 samples) and 11 HW individuals (*n* = 21 samples). Across individuals, the mean respiratory cycle duration (RCD) was similar between the two species, with NARW having a slightly higher RCD than HW (Figures 6, S1, S2). The NARW showed a mean RCD of 3.07 s (*SD* = 0.503, CV(%) = 16.38, range = 2.38–4.04 s) and the HW showed a mean RCD of 2.85 s (*SD* = 0.581, CV(%) = 20.39, range = 1.97–4.43 s). Similar standard deviations and coefficients of variation also show low variability in RCD measurements between individuals.

No significant difference was found in mean RCD between species (*F* = 1.416, *p* = .24 after Bonferroni correction, *df* = 1)

3.2 | NARW metadata

In the NARW, mean RCD between the two age classes were disparate, with the sexually mature adults having a higher mean of 3.13 s (*SD* = 0.500) compared to the sexually immature adults (mean = 2.69, *SD* = 0.442). Males had a higher respiration cycle duration compared to females (males: mean = 3.11, *SD* = 0.540 and females: mean = 2.94, *SD* = 0.481), however, we found no significant differences in mean RCD between the age (*F* = 5.272, *p* = .0508, *df* = 1) or sex (*F* = 1.645, *p* = .2356, *df* = 1) classes (Figure 7).

3.3 | HW metadata

In HWs, the mean RCD for sexually mature adults was significantly higher than the mean for the sexually immature adults (2.99 s, *SD* = 0.584 versus 2.37 s, *SD* = 0.313; *F* = 7.935, *p* = .0130, df = 1; Figure 7). We failed to find a

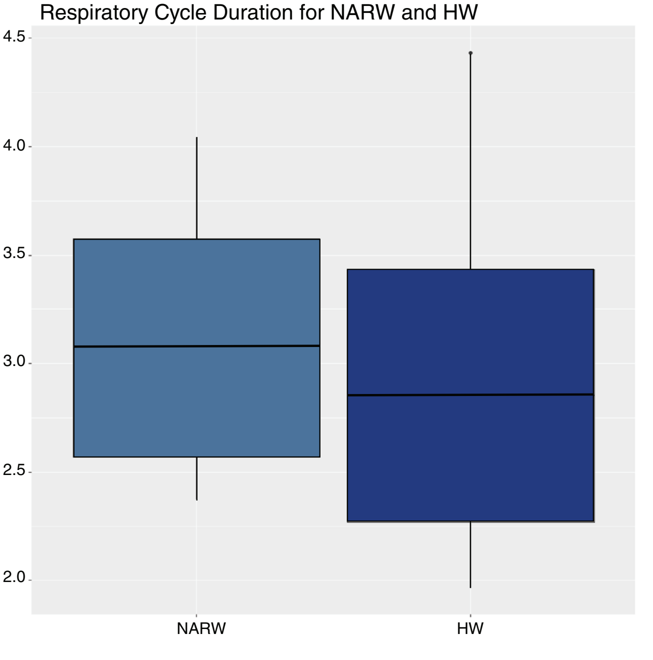

FIGURE 6 Boxplot showing mean and 95% confidence interval for respiratory cycle duration (RCD) for North Atlantic right whale (NARW) and humpback whale (HW). Middle line shows the mean, bottom and top of boxplot show 95% confidence interval, and whiskers show maximum and minimum values.

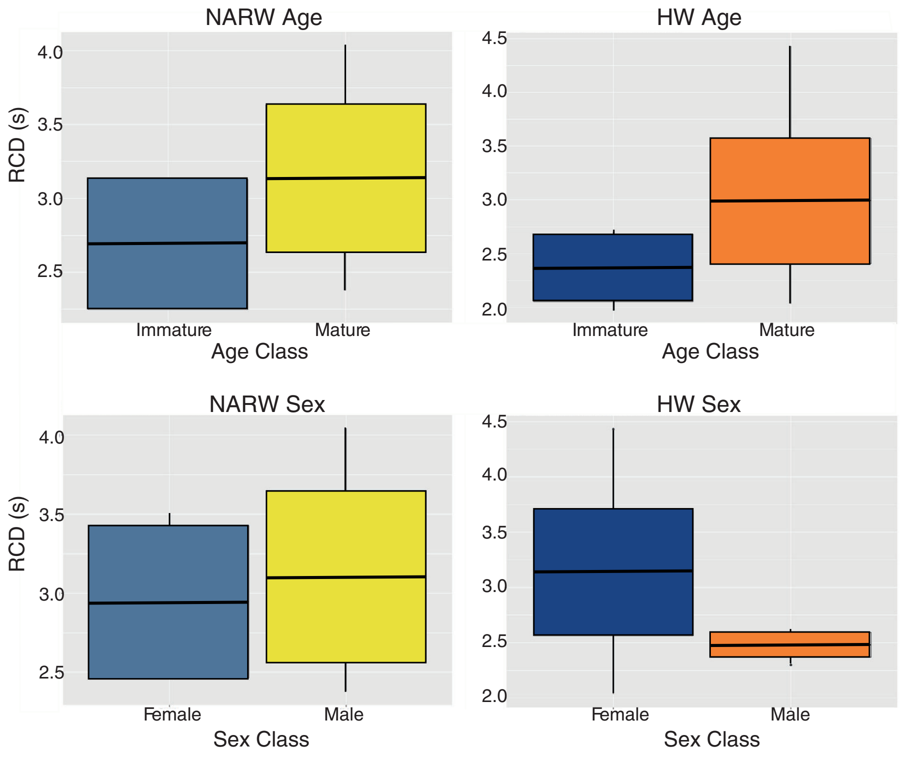

FIGURE 7 Boxplots showing mean and 95% confidence interval for respiratory cycle duration between age and sex classes for both North Atlantic right whale (NARW) and humpback whale (HW). Middle line shows the mean, bottom, and top of boxplot show 95% confidence interval and whiskers show maximum and minimum values.

difference in RCD between sex classes ($F = 4.235$, $p = .0574$, $df = 1$; females: mean = 3.13, $SD = 0.565$ compared to males: mean = 2.48, $SD = 0.112$).

3.4 | Respiratory cycle behavior

3.4.1 | Blowhole open and exhaling before surfacing

Blowholes were open and exhaling before surfacing in one of 15 respiratory cycles across all NARW individuals (6.67%), suggesting it is not a common behavior in that species. Conversely, blowholes were open and exhaling before surfacing in three of 21 respiratory cycles (14.29%), in three HWs (Figure 8). This difference between species was not statistically significant ($\chi^2(1, N = 36) = 0.032$, $p = .858$).

3.4.2 | Blowhole open during submergence

There were no cases where the blowholes were open during submergence at the end of the respiratory cycle in the NARW. Conversely, this blowhole activity was observed in 15 of 21 HW respiratory cycles (71.43%; Figure 8). There was a significant difference in the prevalence of this activity between species ($\chi^2(1, N = 36) = 15.546$, $p < .001$).

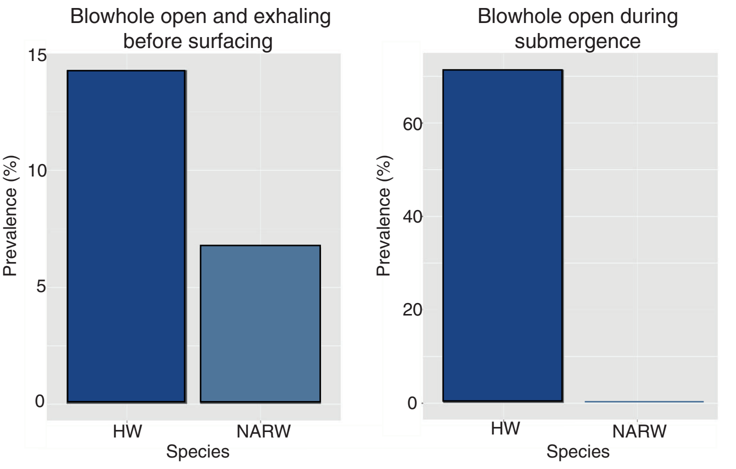

FIGURE 8 Summary of prevalence of blowhole submerged during exhalation at the beginning of the respiratory cycle and BH open during submergence towards the end of the cycle for North Atlantic right whale (NARW) and humpback whale (HW).

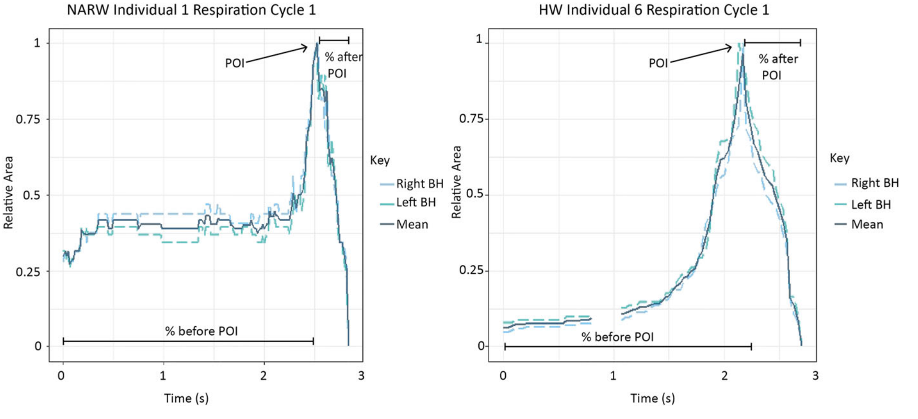

FIGURE 9 Example of the respiration cycle curve: relative area plotted against time for NARW Individual 1 and HW Individual 3, which both only had one respiration cycle recorded. BH-blowhole, HW-humpback whale, NARW-North Atlantic right whale, POI-point of inflection. Gaps in trend indicate water covering the blowhole and area could not be measured.

3.4.3 | Left versus right blowhole

In both species, the respiration cycle curves were similar for both blowholes; they both opened, reached a point of maximum area (point of inflection, POI) and closed together at the same or similar times. There were no cases of individuals where the left and right blowhole opened or closed independently (Figure 9, Tables S1 and S2).

3.4.4 | Comparison between NARW and HW respiratory cycle curves

Points of inflection (POI) on the respiration cycle curves identified the point of maximum blowhole area (presumably the switch from exhalation to inhalation), the percentage of time before POI showed the blowholes opening (exhalation), and the percentage of time after POI showed the blowholes closing (inhalation). According to the MANOVA

TABLE 1 Specific gravity of blow samples and controls for the North Atlantic right whale (n = 5) in 2016, seawater flown on drone (n = 3), seawater not flown on drone (n = 7), tap water (n = 1).

Type of sample	Mean specific gravity measurement	*SD*	Range
Blow collected by drone	1.033	0.002	1.030–1.037
Seawater flown on drone	1.034	0.001	1.033–1.035
Seawater not flown on drone	1.024	0.003	1.016–1.026
Tap water	1.000	0.000	1.000

results, no difference in POI or percentage before POI was detected between species ($F(2, 33) = 0.901$, $p = .4158$, Pillai = 0.0518).

3.4.5 | Seawater presence over open blowhole

For those cases in which it was observed, seawater covering an open blowhole occurred only during the inhalation phase (blowhole area decreasing). In the NARW, 3 of 15 respiratory cycles (20.00%) had water present over open blowholes. This was observed in individuals 4714, BK01RB13, and 3823. Presence of seawater over open blowholes was much higher in the HW than in NARW, with seawater over open blowhole in 19 out of 21 respiratory cycles (90.48%; Table S5). This was observed in all individuals except individual 6, which was a calf (Table S2, Figure S2).

The Pearson chi-squared test showed a significant difference between the prevalence of seawater over an open blowhole between the two species ($\chi^2(1, N = 36) = 15.442$, $p < .001$).

3.5 | Blow samples

The blow samples from NARW 2016 (Tables 1 and S3) showed mean specific gravity readings of 1.033. The blow samples had higher specific gravity measurements compared to seawater. However, the specific gravity of seawater, after being flown on the drone, was comparable to that of the drone collected blow samples.

The NARW blow samples also had a higher mean specific gravity compared to the reference specific gravity measurements of other body fluids (human saliva = 1.002–1.012; McDonald, 1950; human plasma = 1.022–1.026; Mathew & Varacallo, 2019; serous fluid = 1.016; Keohane et al., 2019), suggesting the blow samples were more saline.

4 | DISCUSSION

4.1 | Respiratory cycle duration

In this study we measured the respiratory cycle duration (RCD), a complete expiration/inhalation event, for two baleen whale species for the first time using UAS. RCD has been quantified for other breath-holding mammals using other technologies, such as short distance video from boat-based surveys (Lafortuna et al., 2003) and acoustic data loggers with external hydrophones to record the sounds of respiration (Le Boeuf et al., 2000). However, until now, RCD data were lacking for baleen whales versus odontocetes and pinnipeds, which expands the knowledge of respiratory dynamics for this group of marine mammals (W. C. Burgess, Tyack, Le Boeuf, & Costa, 1998; Craig & Påsche, 1980; Fahlman et al., 2016, 2018).

We measured RCD for 12 NARW (3% of the total population; Pace, Corkeron, & Kraus, 2017), and 11 HW and found no difference between the two species. Body size, and by extension, lung volume and tidal volume, may influence RCD (Blix & Folkow, 1995; Fortune et al., 2012; Hill, Wyse, Anderson, & Anderson, 2004; Sumich, 2001). However, the smaller lung and tidal volume of calves (Fortune et al., 2012; Hill et al., 2004) did not seem to affect RCD for the two calves measured (NARW: 4,714 and HW: Individual 6). Despite not detecting significant differences in RCD between age and sex classes for the NARW, differences were detected between age classes in the HW. Larger sample sizes may be needed to understand these relationships more clearly, especially between age classes, given the limited number of juveniles and calves in the samples. Differences in respiration rate between lactating females, other adults, neonates, and calves in HW have been shown in previous literature, however, how this relates to RCD has not been explored (Bejder et al., 2019).

The literature on breath-holding mammals (specifically cetaceans) has focused on respiratory rates (breaths per minute) and these have been determined by observer counts (van der Hoop et al., 2014), radio transmitters (Blix & Folkow, 1995), and bioenergetics models (Christiansen et al., 2014). For nonbreath-holding mammals, RCD is expected to change with respiration rates if respiratory flow rates remain constant (Hill et al., 2004; Van Diest et al., 2014). A longer duration of expiratory/inspiratory events would suggest a decreased respiratory rate (breaths per minute) (Hill et al., 2004; Van Diest et al., 2014). Whether this can also be extrapolated to breath-holding mammals (specifically baleen whales in this case) is unknown. However, if found that RCD and respiratory rates had similar relationships in breath holding mammals and further association with physiological processes, such as metabolic rate and energy expenditure, which are calculated from respiratory rates, RCD could be a useful additional metric to monitor baleen whale physiological health, especially in calves, where changes in energy expenditure can impact survival (Kooyman, 2009; Lafortuna et al., 2003; Mortola & Limoges, 2006; Shaffer, Costa, Williams, & Ridgway, 1997; Sumich, 2001). Previous studies have already documented negative effects in respiratory rates in response to stressors (Christiansen et al., 2014; Fazio et al., 2015) but the way in which stressors impact the RCD has not been assessed. Furthermore, understanding metabolic respiratory rates is also of interest in understanding the physiological limitations for survival in a species (Fahlman et al., 2018). Gas exchange in a species will depend on respiratory rate, respiratory NOT blowhole cycle duration, gas flow rate, and the extent of gas exchange (Fahlman et al., 2016, 2018, 2019). With respiratory rate and respiratory NOT blowhole cycle duration now quantified, the next major unknown is how respiratory gas flow rates and compositions change in mysticetes. For a better understanding of baleen whale respiratory physiology, data on respiratory flow rate, expiratory oxygen and carbon dioxide concentrations are required, in addition to measures of respiratory rate and RCD (Fahlman et al., 2015, 2016).

Future studies on RCD using UAS are recommended at both feeding and breeding grounds, and should also concentrate on obtaining imagery of multiple breaths from the same individual whale, as many recordings in this study were limited to one full respiratory cycle per whale. RCD calculated from UAS and from acoustic tagged (DTAG) animals would also be an interesting comparison to investigate, given larger sample sizes are obtained.

4.2 | Respiration cycle behavior

HWs in this study often started exhaling before surfacing and kept their blowholes open during submergence at the end of a respiratory cycle. Whether these behaviors are specific to HW is unknown, as this is the first time a UAS was used to detect respiratory activity in baleen whales. When exhalation occurs before surfacing, bubbles are generated underwater as bubble clouds or curtains, which is known to be a visual signal and often an aggressive display in HW (Tyack & Whitehead, 1982), as well as a component of feeding behavior (Hain, Carter, Kraus, Mayo, & Winni, 1982; Wiley et al., 2011). The role of keeping the blowhole open before submergence at the end of the respiratory cycle, which likely allows water to enter the blowhole cavity is unclear but it is possible that this may be common observation that simply has not been reported previously. Previous literature consistently reported that the blowholes close just before submergence to avoid water entering (Berta, Ekdale, & Cranford, 2014; Buono et al., 2015; Maust Mohl, Reiss, & Reidenberg, 2019), with the exception of recent research that also observed blowholes remaining open during submergence (Gil et al., 2020).

The resolution of imagery from higher altitude flights and the lack of accurate altimetry on some flights limited the accuracy of the relative blowhole area calculated. However, when areas were normalized, the same general trend was found between left and right blowhole, which was important to assess because often only one blowhole was covered by seawater. Therefore, given that the right and left blowhole opened, reached a point of maximum area (POI), and closed together, it was evident that when seawater was covering one blowhole, there was no asynchronous blowhole response to prevent water entry.

Regarding the respiratory cycle curves, no differences were detected in POI or percentage before POI between species. However, despite there being no significant differences in POI or percentage before POI between species, there was variation between shapes of individual respiration curves. The basis for this is unclear, it may reflect differences in pulmonary health or dive behavior, or simply be idiosyncratic. The main assumption in the respiratory cycle curves was that the POI represents the switch from exhalation to inhalation, which was based on the knowledge that blowhole area increase/open during exhalation and decrease in area/close during inhalation (Buono et al., 2015; Gil et al., 2020). This could only be confirmed by measuring air flow rate and direction across the blowhole throughout the cycle. The digital acoustic recording tag (DTAG), attached behind the blowhole with suction cups, records all received sound, including those of whale blows from the tagged animal and other close by (Johnson & Tyack, 2003). Therefore, a next step to better understand the POI could be to compare the timing of blowhole area changes and breath sounds recorded on an animal that is also carrying a DTAG. The sounds of neighboring animals could be discarded by their lack of synchrony with the drone video record.

4.3 | Seawater presence

Seawater covering an open inhaling blowhole was observed in 20% of NARW breaths and 90% of HW breaths, implying that both species are potentially inhaling seawater into their blowhole and upper respiratory system. Much of the evolutionary theory about the migration of the nares to a dorsal position, with valve-like structures that seal the blowholes and external ridges and grooves to deflect water entrance have evolved to avoid water entering the blowhole (Buono et al., 2015; Gil et al., 2020; Heyning & Mead, 1990; Reidenberg, 2018). Seawater entering the lower respiratory system of a baleen whale, could lead to asphyxiation and death (Berta et al., 2014; Buono et al., 2015; Heyning & Mead, 1990). Despite previous literature implying that water does not enter the blowhole (Berta et al., 2014; Buono et al., 2015; Maust Mohl et al., 2019; Reidenberg, 2018), our results show that nearly all HW breaths have seawater overlying open blowholes, thereby indicating that seawater may indeed enter the upper respiratory tract. We do not know if this seawater enters the tracheobronchial system. The introduction and partial removal of physiologically normal saline into the lower respiratory tract of mammals is used for diagnostic purposes with bronchoalveolar lavage (including dolphins), and therapy with lung lavage (Hawkins et al., 1997). But in the case of whales inhaling seawater, the salinity is substantially greater than that of mammalian body fluids. Obviously, the seawater volumes these whales may be inhaling either do not reach the lower respiratory tract, or are insufficient to cause problems for them. A further point is that most whale observation data are collected at relatively calm sea states, so therefore, presumably the potential for sea water to enter the blowholes in rougher sea states is even more likely.

The nasal passage of our study species, which are from two baleen whale families (HW from Balaeonopteridae and NARW from Balaenidae) are believed to be similar in anatomy despite the limited knowledge on the nasal anatomy of baleen whales (Berta et al., 2014; Buono et al., 2015; Gil et al., 2020; Maust Mohl et al., 2019). The nasal passage of *Eubalaena australis*, a closely related species to the NARW and HW, is separated into the upper and lower respiratory tracts (Figures 10 and S5; Berta et al., 2014; Buono et al., 2015; Heyning & Mead, 1990). The upper tract consists of: blowhole (valve like, soft connective adipose tissue), vestibule (a cavity just below the blowhole), external

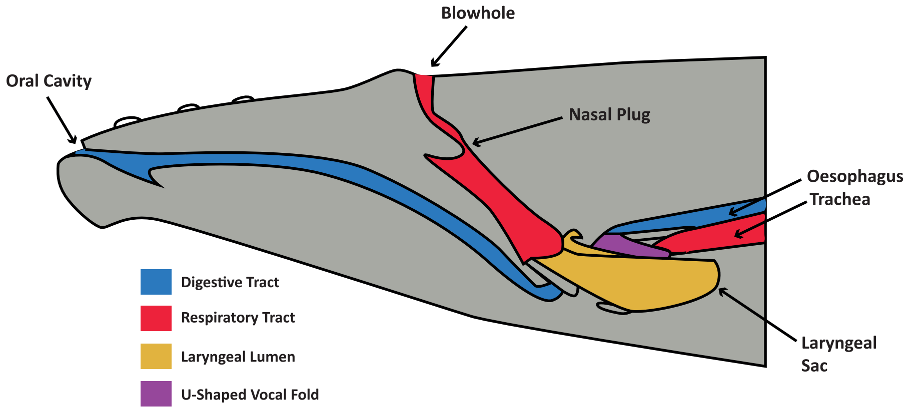

FIGURE 10 Line drawing of upper and lower respiratory and digestive system of a balaenopterid whale (modified from Reidenberg, 2018). Figure S5 shows upper respiratory tract in more detail.

bony nares (distal or external bony opening), and external bony fossa (on the dorsal surface of the skull, which holds the upper nasal passage) (Figures 10 and S5; Buono et al., 2015).

A structure called a nasal plug closes off the upper respiratory tract from the lower respiratory tract and is thought to be key to limiting seawater entry into the lower respiratory tract (Figure 8; Gil et al., 2020; Heyning & Mead, 1990). The nasal plug lines the lumen of the vestibule and is a valve like structure formed by connective and adipose tissue and muscles (Buono et al., 2015; Maust Mohl et al., 2019; Reidenberg, 2018).

Five nasal plug muscles, the *musculus dilator naris superficialis*, *m. dilator naris profundus*, *m. constrictor naris*, *m. retractor alae nari*, and the *m. depressor alae nasi*, control the opening and closing of the blowhole in *Eubalaena australis* (Buono et al., 2015) and it is highly likely that similar structures are present in NARW and HW. During the opening of the blowhole (exhalation), three nasal plug muscles (*m. dilator naris superficialis*, *m. depressor alae nasi*, and *m. dilator naris profundus*) contract causing the lumen of the vestibule to widen (Buono et al., 2015). This is the only point in the respiration cycle (exhalation/inhalation event) where the lumen is fully open, and when seawater could potentially enter the lower respiratory tract. However, given that the fatty, spring like structure of the nasal plugs help the muscles return back to their original position quickly, this quick transition from open to closed nasal plug may limit the seawater from entering the lower respiratory tract (Buono et al., 2015; Heyning & Mead, 1990).

Closing of the blowhole after inhalation is accomplished by the contraction of one nasal plug muscle (*m. depressor alae nasi*) and the relaxation of two other nasal plug muscles (*m. constrictor naris*, *m. retractor alae nasi*). The relaxation of the two muscles causes the lumen of the vestibule to return to its original, relaxed closed position (Buono et al., 2015). Water may remain within the vestibule when the nasal plug is relaxed and closed between breaths. Further anatomical research is needed to understand this phenomenon for both species, particularly for HW where 90% of respiratory cycles had water over open blowholes.

Seawater covering an open inhaling blowhole may also imply that many blow samples may be contaminated with seawater. Blow sampling using collection devices on UAS and hand held poles has become a prominent, noninvasive tool for obtaining samples of exhaled breath from free-living cetaceans and can contain sufficient material to assess level of steroid and thyroid hormones which can indicate changes in physiology such as stress levels, pregnancy status and metabolic rate (Apprill et al., 2017; E. A. Burgess, Hunt, Kraus, & Rolland, 2016; E. A. Burgess et al., 2018; Hunt et al., 2014). However, a factor that hinders hormone analysis is dilution from variable amounts of water vapor and seawater, making it difficult to standardize hormone concentrations (E. A. Burgess et al., 2016, 2018; Hogg et al., 2009; Hunt et al., 2014). Previous studies have used an independent biomarker, such as urea, as a dilution indicator, to correct for amount of respiratory vapor present (E. A. Burgess et al., 2016, 2018; Hogg et al., 2009). Seawater both from the upper respiratory tract and from when the whale breaks the surface, could further dilute samples

(Apprill et al., 2017; E. A. Burgess et al., 2018). This is also a point to consider in microbiome analysis of blow where seawater associated bacteria have been found in samples (Apprill et al., 2017). For both hormone and microbiome studies, the potential for seawater, residing in the upper respiratory tract for the duration of a dive, to absorb cellular and molecular constituents of the surrounding mucosa is an important consideration.

The mean specific gravity measurements taken from blow samples of five NARWs in 2016 showed higher specific gravity measurements compared to other body fluids (specific gravity of human saliva = 1.002–1.012, McDonald (1950); human plasma = 1.022–1.026, Mathew & Varacallo, (2019); human serous fluid = 1.016, Keohane et al., 2019), suggesting salinity is increased, and that seawater is likely present. Whether this seawater comes from when the whale breaks the surface and/or from inside the blowhole is unknown. However, with the observation of seawater over open blowhole in the UAS imagery, there is a very high probability that water does indeed enter the blowhole during inhalation and is present in exhaled respiratory blow. An alternative approach to attempt to quantify whether salinities are increased due to seawater entry in exhaled respiratory breath is using a ratio-based approach of analytes such as sodium or urea levels, similar to the method when standardizing hormone concentrations in respiratory blow (E. A. Burgess et al., 2016, 2018; Hogg et al., 2009).

Thermal infrared (IR) imagery could also be of use in future studies on the respiratory system of baleen whales (Churnside, Ostrovsky, & Veenstra, 2009; Horton et al., 2019; Hunt et al., 2013; Zitterbart, Kindermann, Burkhardt, & Boebel, 2013) and may be able to help discern the presence of seawater in the blow. Baleen whales are detected on ship based thermography with the clear signals coming from respiratory vapor, or blow (Boebel & Zitterbart, 2013; Burkhardt, Boebel, & Zitterbart, 2014; Horton et al., 2019; Zitterbart et al., 2013). The blow is easily detectable on thermal IR imagery from cameras looking out from the surface at a shallow angle (i.e., boat or ship based cameras) due to the lower background emissivity (Churnside et al., 2009; Horton et al., 2019). An IR camera from a vessel perspective at close range could detect whether the seawater present in a respiratory event was from inside the respiratory tract or from the surface because presumably that water from inside the respiratory tract would be warmed and show a higher temperature than water that originated from the surface (Boebel & Zitterbart, 2013; Horton et al., 2017, 2019; Zitterbart et al., 2013). However, for the blow to have higher apparent temperature, this could also be from organic fluid or from particles from within the blow. Observing the exhalation with a high-resolution, high-framerate thermal imaging camera, which could determine if an internal apparent temperature distribution exists, might be an appropriate method to discern between these particles and seawater. Thermography could be the next step to further test for the source of high salinity in blow samples.

The implications of this finding of seawater over an open blowhole are varied, from the previously mentioned blow sampling to oil spill dispersants. During oil spills, cetaceans breathing just above the surface are frequently exposed to high concentrations of oil droplets (Takeshita et al., 2017). The high prevalences of seawater entering the blowhole in HW and the behavior of the HW submerging with their blowhole open, would suggest that baleen whales could be inhaling oil droplets on the surface waters and they could be entering the upper respiratory tract. Whether they could also make their way down to the lower respiratory tract is unlikely but still unknown. Therefore, oil dispersants that reduce the oil concentration on the surface waters could minimize surface oil inhalation (Geraci & Aubin, 1988), thereby benefitting cetaceans surfacing within oil slicks. If sea water also enters the blowhole of inhaling small odontocetes, such as the bottlenose dolphin (*Tursiops truncatus*), this would be compatible with the observations of pulmonary disease in that species affected by the Deep Water Horizon oil spill in the Gulf of Mexico (Smith et al., 2017). Surface oil could presumably also be inhaled if oil droplets were nebulized into air at sea level by wind and wave surface stress (Takeshita et al., 2017).

4.4 | Conclusion

Respiration cycle duration (RCD) of the NARW and HW was quantified using a novel UAS approach, which aids in further understanding of the respiratory dynamics of baleen whales, and could be used as a way of monitoring baleen whale health. Seawater presence over open blowholes was also quantified for the first time in this study, with HW having a very high prevalence of breaths with seawater over open blowholes. The phenomenon has not been described previously and can have many implications in future blow sampling methodology and in oil dispersant usage.

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AUTHOR CONTRIBUTIONS

Maria Clara Iruzun Martins: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; software; validation; visualization; writing-original draft; writing-review and editing. **Carolyn Miller:** Conceptualization; data curation; investigation; methodology; project administration; resources; supervision; validation; writing-review and editing. **Philip Hamilton:** Data curation; formal analysis; investigation; methodology; project administration; resources; supervision; validation; visualization; writing-review and editing. **Jooke Robbins:** Conceptualization; data curation; formal analysis; investigation; methodology; project administration; resources; supervision; validation; visualization; writing-original draft; writing-review and editing. **Daniel Zitterbart:** Conceptualization; methodology; resources; supervision; writing-original draft; writing-review and editing. **Michael Moore:** 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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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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