Research article

Explosive exhalations by common bottlenose dolphins during *Karenia brevis* red tides

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ABSTRACT

Harmful algal blooms (HABs) such as those produced by *Karenia brevis* have acute negative impacts on common bottlenose dolphins (*Tursiops truncatus*) in Florida coastal waters, frequently causing illness and death. However, much less is known about chronic, sub-acute effects on these important sentinel species. This study investigates whether bottlenose dolphin behavior in Sarasota Bay, Florida is influenced by the presence of severe red tide events, focusing on respiratory and other behaviors likely affected by abundant toxin aerosols produced during these blooms. Through focal animal behavioral follows, we observed free-ranging dolphin respiratory behavior, activity budgets, and movement patterns relative to *K. brevis* abundance in the study area. We compared behavior from dolphins observed during a 2005 *K. brevis* bloom to those observed during inter-bloom conditions where *K. brevis* was present at background concentrations. We found that the rate of "chuffing", an explosive type of exhalation, was significantly greater in dolphins observed during the bloom. No apparent effect on respiratory rate, heading change rate or activity budgets was observed. We propose that this chuffing behavior is analogous to symptoms of respiratory irritation observed in humans exposed to such red tide events, and suggest that this may be a type of disturbance response. With an observed increase in both the frequency and severity of HABs, such disturbance responses may have large-scale chronic impacts to the health and fitness of bottlenose dolphins in regions where such HABs are common.

1. Introduction

As one of the major natural stressors to coastal marine ecosystems, harmful algal blooms (HABs) can have severe negative effects on marine wildlife, especially marine mammals (Fire and Van Dolah, 2012; Broadwater et al., 2018) that serve as sentinels of ocean health (Wells et al., 2004). HABs that affect marine mammals are typically dense aggregations of marine phytoplankton capable of producing natural toxins that accumulate in a wide variety of marine organisms, and at multiple trophic levels (Landsberg, 2002; Doucette et al., 2006). When HAB species "bloom" in areas inhabited by marine mammals, the corresponding algal toxins are often transferred via inhalation or the food web to these top-level predators, resulting in adverse impacts such as acute illness and large-scale mortality events (Anderson and White, 1989; Scholin et al., 2000; Brodie et al., 2006). Brevetoxins, the potent neurotoxins naturally produced by the HAB species *Karenia brevis*, have been implicated as the cause for several large-scale marine mammal mortality events occurring in Florida (Geraci, 1989; Mase et al., 2000; Flewelling et al., 2005;

Twiner et al., 2012; Litz et al., 2014; Fire et al., 2015). Blooms of *K. brevis* ("Florida red tides") occur with near-annual frequency in western Florida coastal waters (Steidinger et al., 1998), and its distribution overlaps with that of the common bottlenose dolphin (*Tursiops truncatus*), a key sentinel species abundant throughout the southeast United States. Despite this overlap, little is known about the effects of brevetoxins on dolphins aside from investigations of direct impacts observed during mortality events. A growing body of work suggests that other sublethal impacts of toxin accumulation are possible, as evidenced by elevated brevetoxin loads in dolphin carcasses and prey items during non-bloom conditions (Fire et al., 2007, 2008a, b). In addition, the indirect impacts of *K. brevis* blooms have potentially harmful consequences, including changes to prey fish community structure (Gannon et al., 2009), or changes in juvenile dolphin activity budgets and sociality (McHugh et al., 2011). Investigating the potential indirect impacts of *K. brevis* blooms on bottlenose dolphin behavior sets the framework for the present study.

In response to certain disturbance stimuli, many terrestrial mammals move away from the source of the disturbance or change their behaviors

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and activities, resulting in a variety of negative impacts such as reduced fitness or withdrawal from suitable habitat (Freddy et al., 1986; Tyler, 1991; Cassirer et al., 1992; Cheyne et al., 2016). Negative impacts from disturbance responses are also observed in marine mammals, including mass strandings of beaked whales in response to naval sonar, and increased energetic costs to killer whales from whale watching vessel traffic (Williams et al., 2006; Harris et al., 2018). In Florida bottlenose dolphins, disturbance from nearby watercraft can result in changes to breathing patterns, heading (direction of travel), swimming speed, interanimal distance and acoustic behavior (Nowacek et al., 2001; Buckstaff, 2004). The presence of HAB toxins in marine habitats can also induce disturbance responses in a variety of marine organisms, including marine mammals (Turner and Tester, 1997; Bargu et al., 2003; Kvitek and Bretz, 2005). In southeastern Alaska, the widespread presence of HAB toxins in the butter clam *Saxidomus giganteus* drastically altered the feeding behavior of sea otters (*Enhydra lutris*) that were able to detect the presence of toxins in shellfish tissues, and in turn altered sea otter habitat use and geographic distribution over time (Kvitek et al., 1991). However, little is known about behavioral responses of dolphins to *K. brevis* blooms or the brevetoxins produced during these frequent events.

Neurological effects of brevetoxicosis in mammals include numbness, vomiting, seizures, and respiratory distress (Poli et al., 2000; Kirkpatrick et al., 2004). Brevetoxins are also strong respiratory irritants that become incorporated into marine aerosols during *K. brevis* blooms (Pierce et al., 2003, 2005; Cheng et al., 2005; Abraham et al., 2005). Symptoms of brevetoxin inhalation in humans include coughing, a burning sensation in the nose, throat and lungs, nasal discharge, eye irritation and bronchoconstriction (Backer et al., 2003, 2005; Fleming et al., 2005). It is unknown whether dolphins display similar neurological or respiratory symptoms when exposed to *K. brevis* or brevetoxins, although anecdotal evidence suggests they modify their behavior in the presence of dense, visible patches of *K. brevis* in offshore waters (R. Griffin, Mote Marine Laboratory, pers. comm.). In general, inshore resident dolphins remain within their long-term community home range during *K. brevis* blooms (Bassos-Hull et al., 2013; Wells, 2014), however juveniles were observed to expand their ranging patterns somewhat during the bloom season, while remaining within their community home range (McHugh et al., 2011). However, it is unknown whether dolphins can detect and avoid patches of high cell density or if they preferentially utilize habitats with lower *K. brevis* cell concentrations.

A major challenge in studying the effects of *K. brevis* blooms on living, free-ranging dolphins is the difficulty in estimating the degree of exposure based solely upon the presence or absence of detectable *K. brevis* cells in their habitat. Biological processes such as positive phototaxis (Heil, 1986) and diel changes in swimming behavior (Schofield et al., 2006) can concentrate *K. brevis* vertically in the water column. In addition, physical processes such as tides, winds, and currents concentrate *K. brevis* cells horizontally into dense aggregations in some locations, while nearby surface water may have much lower cell densities (Landsberg, 2002). This results in highly variable cell concentrations that can differ by 1–2 orders of magnitude at sampling sites located only meters apart (personal observation), and thus dolphins are likely exposed to a 'patchy' bloom distribution as they move about within their range. Variable cell density, and the condition of cells within patches, in turn result in a wide range of dissolved and aerosolized brevetoxin concentrations, ultimately causing highly variable exposure levels for dolphins. Unfortunately, sampling efforts for *K. brevis* during Florida blooms generally occurs over large sections of the coastal ocean with little fine-scale sampling performed in regions heavily utilized by dolphins.

The purpose of the present study was to address the above data gaps through 1) behavioral observation of a resident dolphin community frequently exposed to toxic *K. brevis* blooms, as well as 2) conducting fine-scale sampling of *K. brevis* cell densities to which dolphins are exposed during such events. Here we present data on dolphin surface behavior in the field, collected using focal animal behavioral follows (Altmann, 1974) in conjunction with *K. brevis* cell densities obtained

simultaneously along tracks taken by the dolphins being observed. We compared dolphin behaviors and *K. brevis* cell densities encountered by dolphins during *K. brevis* blooms with those from dolphins followed during non-bloom periods. We investigated whether certain aspects of bottlenose dolphin behavior are affected by the presence of elevated *K. brevis* cell densities, and if these animals demonstrate behaviors or behavioral states indicative of neurotoxic symptoms.

2. Methods

The location selected for this study was Sarasota Bay, Florida (27° N, 82° W, Figure 1), a coastal barrier island estuary of approximately 125 km² that experiences frequent, severe *K. brevis* blooms and is a region of continuous HAB monitoring by multiple phytoplankton ecology research programs (FWC, 2019; MML, 2019). Sarasota Bay is also inhabited by a community of approximately 150 year-round resident bottlenose dolphins with high site fidelity to this region (Scott et al., 1990; Wells, 2003; Tyson and Wells, 2016). Through long-term (>49 yrs) study of this population, the majority of these animals are individually identifiable in the field, are of known sex and age, and have known spatial use and ranging patterns (Scott et al., 1990; Wells, 2003, 2014).

2.1. Behavioral data collection

A series of behavioral observations was conducted on individual resident Sarasota Bay dolphins between June 2004 and March 2005, using well-established focal animal sampling methods (Altmann, 1974; Mann, 1999). This study period, as well as a portion of the focal animal sampling sessions (referred to as "focal follows" hereafter), coincided with an intense, long-duration *K. brevis* bloom occurring in Sarasota Bay and throughout the Gulf of Mexico coast of Florida. The bloom was present from January 2005 to November 2005 and was associated with mass mortalities of finfish and multiple species of marine mammals (Litz et al., 2014). In total, 69 h of continuous behavioral data were collected during 66 focal follows, involving 45 known individuals.

A first set of focal follows (n = 48) was conducted between 25 June 2004 and 21 October 2004, a period with no *K. brevis* bloom present, and over 4 months following the termination of the most recent bloom in the study area. These focal follows will be referred to as the "inter-bloom group" (IB), and will represent the baseline, or 'unexposed' dataset. A second set of focal follows (n = 18) was conducted from 26 January 2005 to 24 March 2005, under severe *K. brevis* bloom conditions in the study area. These focal follows will be referred to as the "during-bloom group" (DB), and will represent the HAB-exposed group of dolphins.

The focal follow observation platform was a 7 m center-console Grady-White research vessel equipped with a 225-hp Yamaha outboard engine and a 2.5 m observation tower. Systematic surveys of Sarasota Bay were performed utilizing routes established by the Sarasota Dolphin Research Program (SDRP) which maximized coverage of the study site, and evenly distributed spatial and temporal effort across daylight hours (Figure 2). The vessel was crewed by 4 observers, rotating duties among the various data collection, sample collection, and observation activities described below.

During surveys, dolphin groups were approached at low speed and digital photography of dorsal fins for identification of known individuals was performed. The identity of each individual was determined in the field by matching unique dorsal fin features to existing photos of fins in a photo-ID catalog. Following methods used in Nowacek et al. (2001), initial sighting data collection typically lasted 10–15 min, and focal follows did not begin until at least 15 min after the focal animal was approached, in order to standardize the data and acclimate dolphins to the presence of the observation vessel. Focal animals were selected primarily based on the observer's judgment of how likely the animal could be quickly identified and distinguished from other animals in a group, and followed at a close distance without interruption. Since the highly fluid nature of dolphin aggregations (Connor et al., 2000) results in
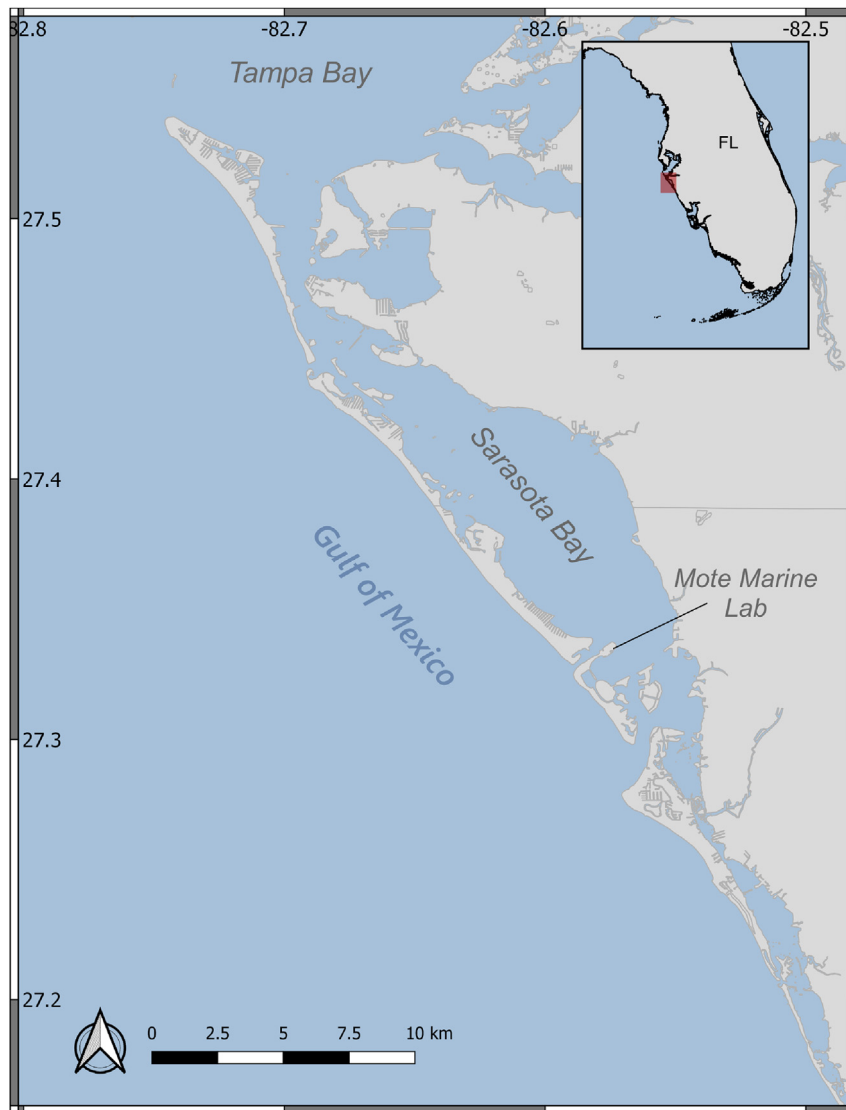
Figure 1. Study area - Sarasota Bay, Florida.

frequent changes to group size, group composition and position of dolphins relative to the observer, we used the photo-ID catalog to identify potential focal animals by selecting the individual in the group with the most distinctive set of dorsal fin features. Dolphin avoidance of the observation vessel was also taken into account when selecting focal animals. Typically Sarasota Bay dolphins can be approached and followed successfully for periods of up to 2 h (Buckstaff, 2004), however some groups of dolphins actively avoid an approaching vessel regardless of its distance, impeding photo identification and selection of focal animals. Focal follows were not attempted on such groups or on groups with insufficiently distinctive dorsal fin features.

Once a distinctive focal animal was selected and did not obviously try to avoid the observation vessel for the initial 15-minute period, the dolphin was followed, and collection of behavioral data was initiated. Typical water quality conditions in Sarasota Bay preclude boat-based observation of submerged dolphins, therefore all behavioral data in this study were collected while focal animals were visible at the surface. During focal follows, the observation vessel matched the speed and heading (direction of travel) of the group while maintaining a position of approximately 45° behind and to one side of the focal dolphin. The distance between the observation vessel and the focal animal was typically maintained between 5 and 15 m. The focal animal's movements were recorded throughout the follow using a handheld GPS receiver,

utilizing the trackline of the observation vessel as a proxy for the path of the dolphin.

Behavioral observations during focal follows utilized a combination of continuous sampling and instantaneous point sampling (Altmann, 1974) of preselected behavioral events and activity states, following standardized protocols. Behavioral events, activity states and metrics of interest (Table 1) included traveling, milling, socializing, foraging, chuffing (i.e. explosive exhalation), inter-breath interval (IBI, seconds), and change of direction (>90° heading change) during travel. For continuous sampling, each surfacing of the dolphin that resulted in a breath was recorded as a data point that included the time, activity state, number of vessels (including vessel and propulsion type) within 100 m, and any associated behavioral events from those described above. If the focal animal surfaced in a manner that prevented positive identification via dorsal fin features (e.g., due to glare, distance, angle, obstruction by other dolphins), a break in the continuous data was recorded. Instantaneous point sampling at 3-minute intervals was used to record behavioral activity state, time and water depth, and to collect surface water samples for subsequent determination of *K. brevis* cell densities (Altmann, 1974). If the focal animal was submerged at the 3-minute mark, the data recorded corresponded to the conditions observed when the animal next surfaced. If the focal animal did not surface within 120 s of the 3-minute mark, no data were collected, and
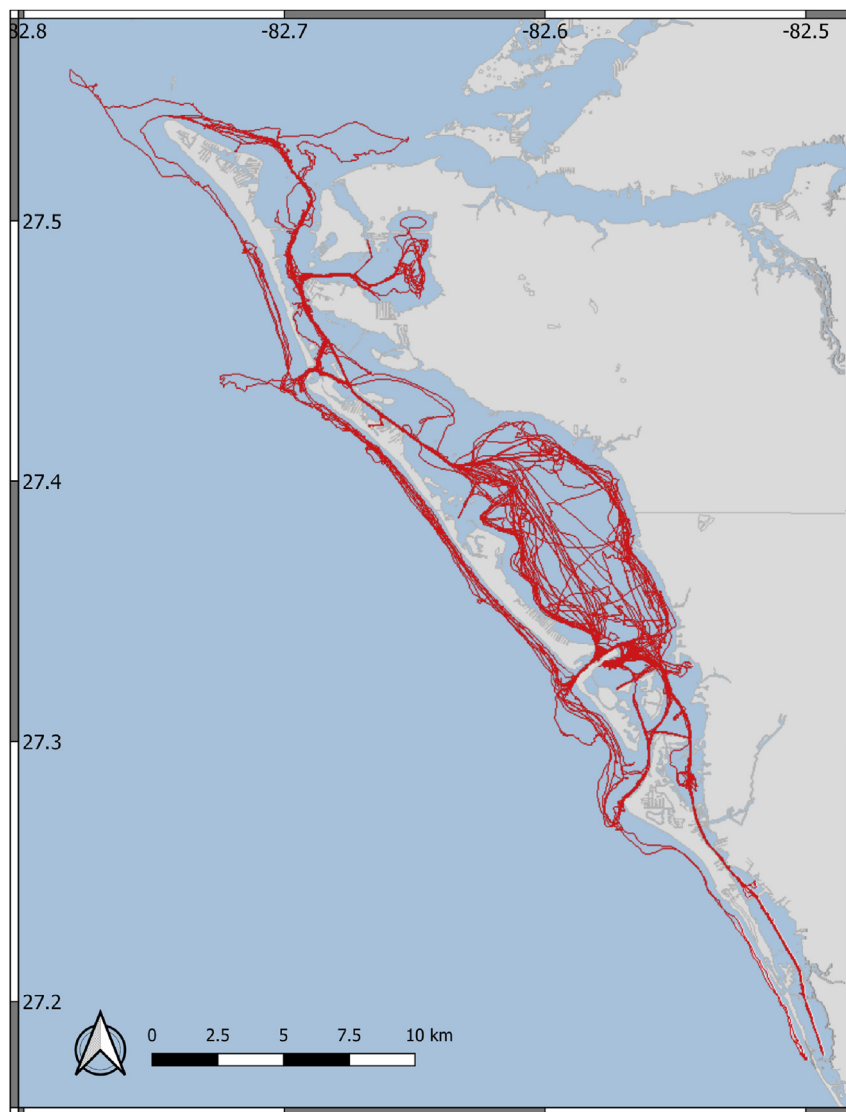
Figure 2. Survey routes and focal dolphin follow tracklines.

after two consecutive missed points the focal animal was considered 'lost' and the follow was terminated. Follows were limited to 2 h to eliminate bias due to observer fatigue, but were also terminated early for inclement weather or if the focal animal entered inaccessible waters or demonstrated avoidance of the observer vessel.

2.2. K. brevis data collection

Seawater samples (20 mL) were collected at each 3-min instantaneous behavioral data collection point, and were used to determine *K. brevis* cell

concentrations to which focal animals were exposed. Samples were collected by hand from the sea surface alongside the observation vessel (<0.5 m from boat, <10 cm depth) using 20-mL screw-cap glass vials. *K. brevis* cells were preserved and stained with 2 drops of Utermöhl's solution (Hallegraeff et al., 2003). Time, latitude, longitude, salinity (psu), water temperature (°C) and depth (m) were recorded for each sample. In the lab, a 1 mL sample aliquot was allowed to settle for 10 min in a settling chamber and viable *K. brevis* cells were enumerated using inverted light microscopy and a handheld counter following methods outlined by Pierce et al. (2005).

Table 1. Focal animal activity states and descriptions.

Activity Type	Code	Description
Travel	TR	Consistent forward movement, including occasional directional changes
Mill	ML	Relatively stationary, or lack of obvious directional movement from a given location
Forage	PF	Observed behaviors consistent with pursuit of prey or foraging
Social	SO	Observable physical interactions with conspecifics
Interact with Boat	WB	Approaching, actively avoiding or otherwise directing behaviors towards a vessel
Other	OT	Combined miscellaneous, infrequent behaviors

Datasets maintained by the Mote Marine Laboratory (MML) Phytoplankton Ecology Program and the Florida Fish and Wildlife Conservation Commission (FWC) HAB Monitoring Database were also utilized to provide additional data on *K. brevis* bloom conditions in the Sarasota Bay area. These ongoing monitoring programs provided *K. brevis* abundance data from water samples collected on a near-daily basis (~5 days/wk) from MML sampling stations throughout the Sarasota Bay area and from FWC event-response sampling stations throughout Florida since 1990 (Figure 3). Water samples collected opportunistically during other SDRP field operations taking place during focal follow efforts were also used to supplement *K. brevis* abundance data at various sites throughout the study area.

2.3. Data analysis

Analyses of focal follow behavioral data and *K. brevis* abundance data were performed using Prism 4.0 (GraphPad Software, San Diego, CA) or in R 3.6.1 using the packages stats (R Core Team, 2019), vegan 2.5–5 (Oksanen et al., 2018), and BiodiversityR 2.11-2 (Kindt and Coe, 2005). Statistical significance for all tests was set at $\alpha = 0.05$. Focal follows that ended early due to loss of visual contact or boat avoidance by the focal animal introduced a high degree of variance when calculating mean values per follow for IBI and activity states. Follows of less than 30 min

were therefore excluded from these analyses. Only blocks of continuous breath data were used in calculations for chuffing rates and IBI. Data points obtained during times when powered boats were present within 100 m were also removed from respiratory-based analyses to eliminate bias due to previously reported effects of boat traffic on respiratory behaviors (Nowacek et al., 2001).

2.4. Chuffing rate

Chuffing was analyzed as a proxy for the behavioral response to respiratory irritation in focal animals, analogous to coughing in humans exposed to brevetoxin aerosols. A chuff was defined as a brief, forceful exhalation above the water that produces a sound distinct from that of a typical respiration (Lusseau, 2006). The focal animal was used as the sampling unit for this analysis, due to unequal sample sizes for each animal. The chuffing rate for each focal follow was calculated as the number of discrete chuffs observed divided by the follow duration (chuffs/min). Chuff rates were compared between IB dolphins and a subset of DB dolphins using a Mann-Whitney U test for unequal sample sizes and variances. Only DB follows with a mean *K. brevis* cell density of 5×10^4 cells/L or more were included in this analysis, based on previously described human respiratory irritation thresholds for varying *K. brevis* cell densities (FWC, 2019).
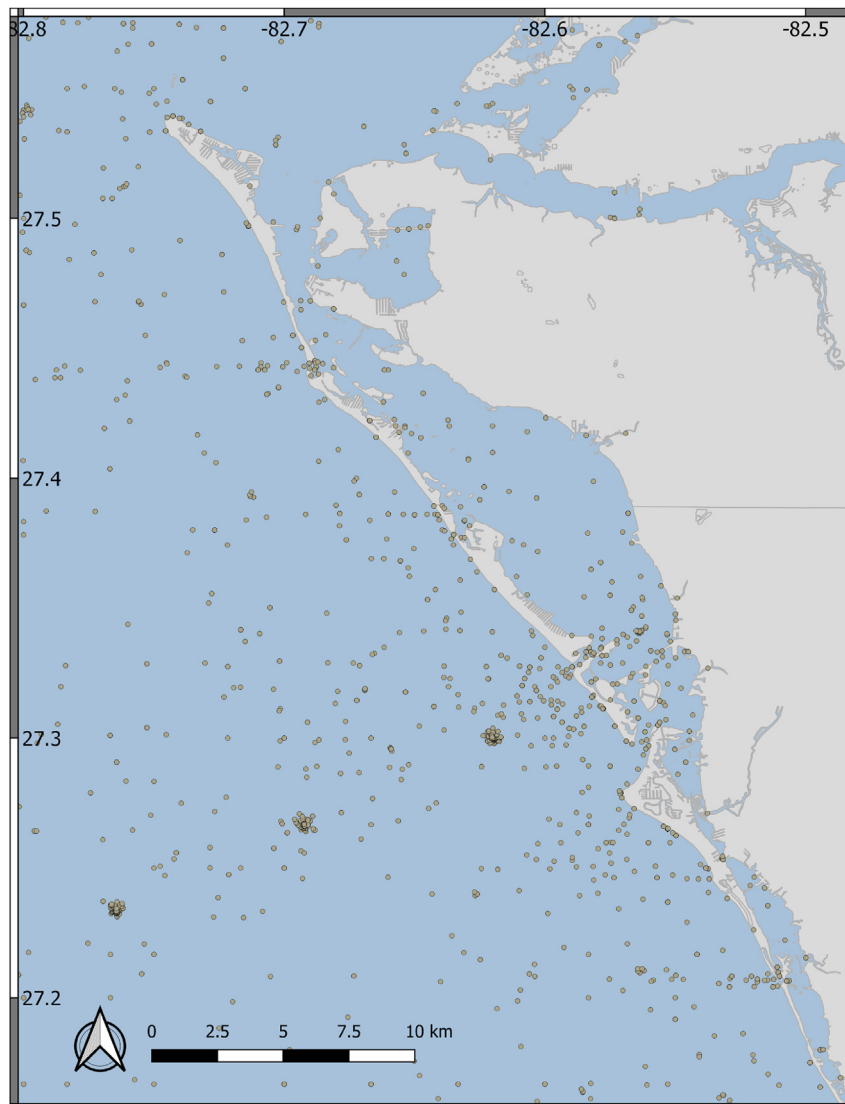

Figure 3. Phytoplankton sampling sites within and adjacent to the study area, 1990–2005.

2.5. Respiratory rate

As an additional test for response to brevetoxin aerosols, respiratory rates and IBI variability were compared among focal animals. The respiratory rate (mean IBI for a focal follow) for each individual was calculated, with data for duplicate follows of IB individuals being pooled. Chuffing events were not considered normal breaths and were excluded from this analysis. Data for follows of any given DB animal on different days were treated as independent since their potential *K. brevis* exposure changed throughout the study. Respiratory rates were compared between IB and DB dolphins with a Mann-Whitney U test for unequal sample sizes and variances. Additionally, the variance for the set of IBI values for each follow was calculated and IBI variance values were similarly compared between IB and DB groups.

2.6. Heading change

Should dolphins be able to sense and respond to the presence of brevetoxin aerosols or other noxious stimuli from *K. brevis* cells, heading changes were used as a proxy for dolphins actively avoiding dense (or relatively denser) patches of *K. brevis* during focal follows. A heading change was defined as a change in the animal's heading of greater than 90° from one surfacing to the next, while the animal was continuously exhibiting behavior assigned to the Traveling activity state. Rates of heading change were compared between IB dolphins and a subset of DB dolphins using a Mann-Whitney U test for unequal sample sizes and variances. Only DB animals observed in the "Travel" activity category for at least some portion of the follow were included in this analysis.

2.7. Activity budgets

In addition to discrete behavioral events, multivariate analyses of focal animal activity budgets (the sum of time spent in various ecologically important behavior states) were used to determine a disturbance response to *K. brevis* blooms. The proportion of time spent (minutes in category ÷ total minutes in follow) in each of the following categories was calculated for each focal dolphin: Travel, Mill, Forage, Social, Interact with Boat and Other (Table 1). Using individual dolphins as the sampling unit, the proportion of time dolphins spent in each category during *K. brevis* blooms was compared to the proportion of time spent in the same category during inter-bloom conditions. Activity budgets were compared using an analysis of similarity (ANOSIM) on the Euclidean distances of the untransformed proportions and visualized using a nonmetric multidimensional scaling (MDS).

2.8. Avoidance of elevated K. brevis concentrations

To test whether dolphins preferentially avoid areas with greater levels of red tide, *K. brevis* cell counts corresponding to the location of each focal follow were compared to cell count data for the entire study area, on the same day as each focal follow occurred. Data from seawater samples collected along the path of each focal animal were used to determine both the mean and maximum cell counts for each follow. These values were compared to the daily mean and daily maximum cell counts (respectively) for our study area, taken from the phytoplankton monitoring datasets mentioned previously. A paired t-test was used to compare these values for animals belonging to the DB group of dolphins.

3. Results

Focal follow data used for analyses comparing IB to DB behavioral data comprised 47 follows with durations between 30 min and 120 min. Of these, 32 follows occurring in the 2004 inter-bloom season were used for IB dolphins and 15 focal follows from the 2005 red tide event were used for DB dolphins. IB focal follows consisted of observations of 24 unique individuals with 8 repeat observations, and DB follows consisted of 15 unique individuals with 5 repeat observations. Overall, *K. brevis* cell densities encountered during DB focal follows ranged in concentration from 1×10^3 to 4.7×10^6 cells/L (Figure 4). Cell counts for waters encountered by IB dolphins along their follow tracks ranged from not detected to 1×10^3 cells/L (i.e., baseline levels).

3.1. Chuffing rate

In total, 17 chuffing events were observed across 32 IB focal follows (2,753 total minutes), with a mean IB chuffing rate of 0.0048 ± 0.01 chuffs/min. In total, 54 chuffing events were observed across 15 DB focal follows (1,347 total minutes), with a mean DB chuffing rate of 0.047 ± 0.05 chuffs/min. A Mann-Whitney U test comparing the two groups showed significantly higher rates of chuffing occurred during red tides (U = 152; p = 0.0228).

3.2. Respiratory rate

Comparisons of respiratory rates (mean IBI values for a given follow) for focal follows lasting 30 min or more showed no significant difference between IB and DB groups (p > 0.05; mean for IB group = 36.2 ± 10.7 s, n = 26; mean for DB group = 34.9 ± 7.5 s, n = 15). Comparisons of IBI variances between sample groups also showed no significant difference (p > 0.05; mean for IB group = $1,216 \pm 941$, n = 26; mean for DB group = $1,226 \pm 779$, n = 15).

3.3. Heading change

In total, 219 heading changes were observed across 32 IB focal follows (1,990 min spent in Travel), with a mean rate of 0.12 ± 0.07 heading changes/minute. In total, 95 heading changes were observed across 14 DB focal follows (854 min in Travel), with a mean rate of 0.09 ± 0.07 heading changes/minute. A Welch's t-test for independent samples found no significant difference in mean heading change frequency per follow between the two sample groups (t = 1.3638, df = 29.2410, p = 0.1830).

3.4. Activity budgets

No significant difference was found between IB and DB activity budgets when comparing the proportion of time spent in the various behavioral state categories (ANOSIM R = 0.0552, p-value = 0.1548). MDS visualization of the activity budgets showed no clustering of IB or DB focal follows. Proportions of time spent in each category are as follows: Travel - IB $71 \pm 22\%$ vs. DB $63 \pm 33\%$; Mill - IB $26 \pm 23\%$ vs DB $32 \pm 29\%$; Forage - IB $1.0 \pm 1.3\%$ vs. DB $0.96 \pm 1.2\%$; Social - IB $0.37 \pm 1.0\%$ vs. DB $2.4 \pm 7.8\%$; Other - IB $0.35 \pm 1.2\%$ vs. DB $0.6 \pm 1.1\%$; IB n = 24, DB n = 10.

3.5. Avoidance of elevated K. brevis concentrations

For DB animals, the abundance of *K. brevis* occurring generally in the study area was compared to the abundance of *K. brevis* occurring locally during focal follows. *K. brevis* survey data for the entire study area were grouped into daily subsets for 26 January to 24 March 2005 and the mean and maximum cell densities for each period were calculated. For 12 of the 18 DB follows, the mean focal cell concentration was less than the corresponding daily mean concentration found generally in the study area. However, no significant difference between mean values was detected using the paired t-test (p > 0.05, t = 1.524, df = 17). In 13 of the 18 DB follows, the maximum focal cell concentration was less than the corresponding daily maximum concentration found generally in the study area. Similarly, no significant difference between maximum values was detected (p > 0.05, t = 1.503, df = 17).
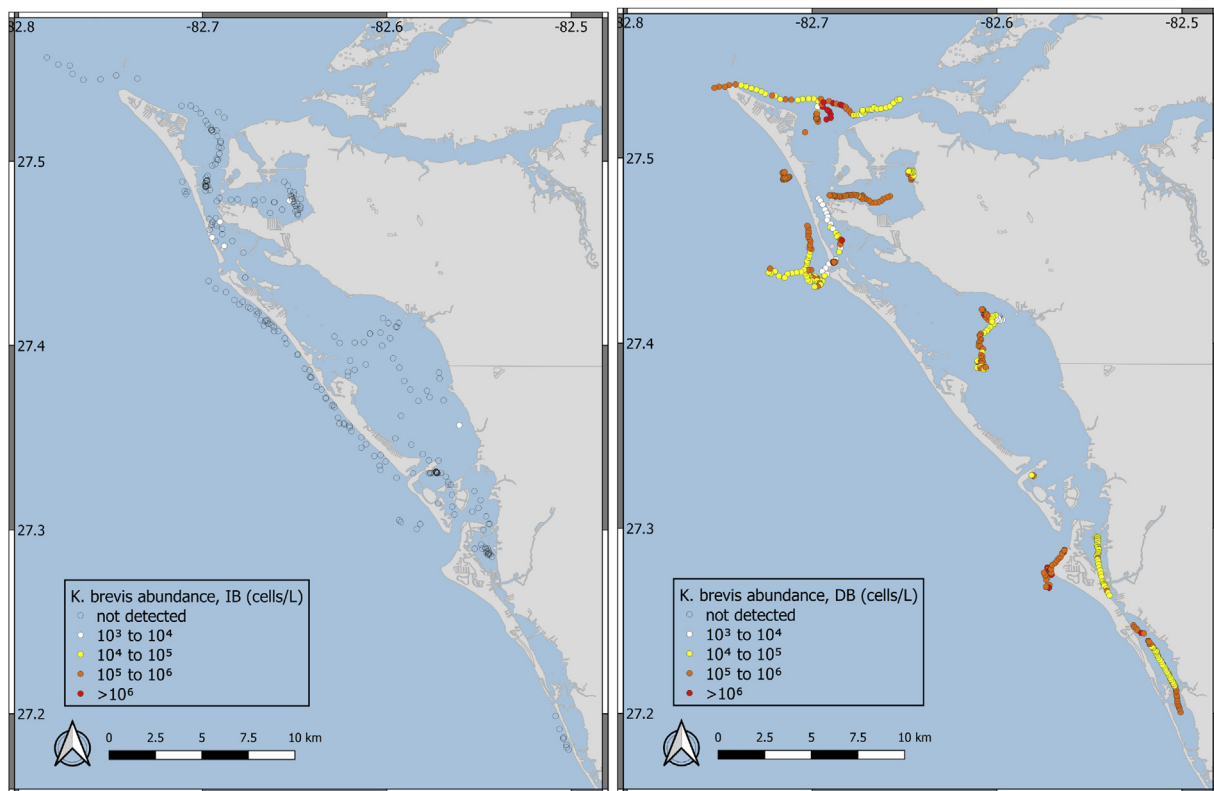
Figure 4. *K. brevis* abundance during IB (left) and DB (right) focal follows.

4. Discussion

Sarasota dolphins displayed changes in their respiratory behavior associated with the presence of a *K. brevis* bloom. The mean chuff rate for focal animals in the DB group was 10 times the rate for animals in the IB group (0.0048 vs. 0.047 chuffs/min). Additionally, 78% of DB animals were observed chuffing during their focal follows, compared to only 21% of IB animals. Chuffing, or explosive exhalation, is a behavior typically expressed during disturbance events (Herman and Tavolga, 1980). However, in the context of the typical respiratory irritation associated with *K. brevis* blooms, it is likely that chuffing is analogous to coughing or sneezing in humans, i.e., a physiological response to irritation by particulate matter in the respiratory tract. Air masses above and adjacent to waters with elevated *K. brevis* concentrations have been shown to have detectable or higher levels of aerosolized brevetoxins (Pierce, 1986; Pierce et al., 2003). Humans exposed to these aerosols experience symptoms such as coughing and wheezing (Kirkpatrick et al., 2004). If our focal animals experienced similar symptoms upon exposure to elevated *K. brevis* concentrations, the observed increase in DB chuff rates may be a physiological respiratory response to elevated levels of brevetoxin aerosols, suggesting a disturbance response to this environmental stimulus. An alternative explanation for chuffing behavior during *K. brevis* blooms may be to clear the air at the sea surface of aerosolized brevetoxins prior to inhalation. This may be analogous to the function of forward-directed exhalations observed for spinner dolphins as a means to avoid inhalation of water droplets at the sea surface (Norris and Johnson, 1994). In addition to potential toxicological concerns, a dolphin producing frequent, explosive exhalations throughout the course of a *K. brevis* bloom may suffer negative health impacts or ecological consequences. As red tides typically last several months (and in some cases over a year), such chronic abnormal respiratory behavior may cause mechanical damage to respiratory organs, or perhaps result in altered predator-prey relationships due to changes in foraging sounds that are detectable by prey fish (Steidinger et al., 1998; Remage-Healey et al., 2006).

High individual variance in respiratory patterns of the focal animals led to inconclusive results in the remaining respiratory-based analyses comparing IB to DB groups. Among focal animals that were observed during baseline *K. brevis* cell densities, the percent standard deviation for individual IBI values ranged from 59% to 165%. Similarly, rates of heading change observed for animals in Travel showed high variability (% std. deviation: IB = 60%; DB = 75%). None of the activity budgets for the focal animals appeared to be influenced by the presence of a *K. brevis* bloom, nor were the cell counts at the focal follow locations significantly different from those observed throughout the study area. With the exception of chuffing, the lack of significant findings among the analyses presented here suggests that there is no major disturbance response in these animals that would affect habitat usage, foraging success or other critical behaviors important in their survival. If this lack of response is typical for all animals in this population, it follows that dolphins exposed to *K. brevis* blooms are unable to modulate the degree of their toxin exposure via changes to movement patterns, behavior, or foraging activities. Therefore, the degree of harmful exposure through inhaled or ingested toxins will likely scale with the severity of the associated *K. brevis* bloom. However, these behaviors are likely influenced by many confounding factors in dolphin behavior, such as social interactions, prey availability, and focal follow location, and merit additional investigation.

Additional studies are necessary to further establish the relationship between the presence of *K. brevis* blooms and the corresponding response in exposed dolphins. Using *K. brevis* cell concentrations as the sole proxy for brevetoxin exposure may not give an appropriate representation of the health risks associated with *K. brevis* blooms. In terms of actual toxin exposure, it is clear that aerosolized brevetoxins can enter the lungs of exposed marine mammals, but it is not known whether a toxicologically significant degree of absorption of these toxins occurs via the respiratory pathway (Bossart et al., 1998; Fire et al., 2015). There are few data available besides detection of toxin in lung tissue from dead-stranded dolphins, and immunohistochemical staining of exposed lung tissue in dead-stranded manatees, that show the presence of the toxin following red tide exposure. Dissolved brevetoxins in seawater rapidly adsorb to

upward-moving air bubbles that subsequently burst at the sea surface, ejecting brevetoxin aerosols and resulting in toxin concentrations in the air up to 50 times greater than those found in the underlying water column (Pierce et al., 1990, 2003). In addition, the proximity of the dolphin blowhole to the surface of the water may result in their exposure to higher concentrations of toxin aerosols relative to humans located up to several meters above the waters' surface. Since the fragile *K. brevis* cells lyse easily, releasing brevetoxins into the water column, the early stages of a bloom produce much lower brevetoxin aerosols relative to later stages (Pierce et al., 2001). Thus the actual respiratory exposure for focal dolphins may depend on the "age" of the bloom in which they were observed. Since it is unknown how chronic exposure to aerosolized brevetoxins may affect dolphin health, further studies investigating respiratory effects and behavioral responses should measure aerosolized toxin concentrations in addition to cell counts associated with focal follows. More generally, we suggest that future efforts to assess overall health and ecological impacts of Florida red tide events on dolphins take into account behavioral impacts on living animals, as these may represent longer-term, indirect effects.

Declarations

Author contribution statement

Spencer E. Fire: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Glenn A. Miller: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Randall S. Wells: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

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