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Original Article

Exposure-response relationship between K. brevis blooms and reporting of upper respiratory and neurotoxin-associated symptoms

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ABSTRACT

In southwest Florida, *Karenia brevis* (*K. brevis*) blooms occur frequently, can be very intense and persist over several years. Individuals living in coastal communities around the Gulf of Mexico are particularly vulnerable to brevetoxins released by *K. brevis* in seawater and carried inland within marine aerosol. Exposure to *K. brevis* occurs during residential, recreational, and occupational activities and has been associated with upper respiratory tract (URT) symptoms in healthy and medically vulnerable individuals. Additionally, ingestion of brevetoxin-contaminated seafood causes neurotoxic shellfish poisoning (NSP), and severe headaches prompting emergency department visits which occur in excess during *K. brevis* blooms. The current study examined a dose-response relationship between *K. brevis* in coastal waters and URT and NSP-like symptoms and headaches among southwest Florida residents. Data on past medical history (PMH) and medical symptoms were collected from the participants ($n = 258$) in five southwest Florida counties between June 2019 to August 2021. A dose-response relationship was observed between *K. brevis* blooms and reporting of URT and NSP-like symptoms and headaches. Reporting of NSP-like symptoms was higher among participants with a PMH of migraines, chronic fatigue syndrome (CFS) and mild memory loss, while the association of headaches with *K. brevis* blooms was accentuated among individuals with a PMH of migraines. These results suggest further investigations into the threshold of aerosolized brevetoxin dose required to elicit URT, headaches and/or NSP-like symptoms. These symptoms ultimately cause significant public health safety concerns, primarily among vulnerable populations with preexisting neurological conditions.

1. Introduction

The red tide in southwest Florida is caused by the dinoflagellate *Karenia brevis* (*K. brevis*) and occurs along the coasts of the Gulf of Mexico, typically beginning in fall and persisting until winter (Steidinger, 2009). Exposure to these blooms is associated with an increased reporting of respiratory symptoms among those with preexisting respiratory disorders or healthy adults in occupational settings (Fleming et al., 2007; Kirkpatrick et al., 2006). The Florida Department of Health (FDOH), along with other state agencies, have developed an integrated public health response plan that relies on known health impacts from brevetoxin exposures, through both marine aerosols and ingestion of molluscan shellfish, to reduce red tide impacts (Reich et al., 2015). However, FDOH surveillance systems are not designed to identify and capture non-traditional symptoms and other health impacts. Previous research has demonstrated the association between *K. brevis* blooms and an increased reporting of headaches prompting hospital emergency department visits (Diaz et al., 2019). While the adverse respiratory impacts from exposure to *K. brevis* blooms among individuals with respiratory illnesses and in healthy lifeguards are well documented (Backer et al., 2005), there remains a need for studies to evaluate the association of systemic and neurological symptoms in relation to *K. brevis* blooms in coastal communities of southwest Florida.

Compared to *K. brevis* blooms prior to the 1990s, those occurring

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after 1994 frequently span multiple years and present with an atypical occurrence in the summer and fall months, extending through the winter and into the spring with cell counts in medium (>100,000–1,000,000 cells/L) to high (>1,000,000 cells/L) ranges (Brand et. al., 2007). During the years 2017–2019 and 2020–2021, *K. brevis* blooms were responsible for killing tons of thousands of marine life and affecting large swaths of southwest Florida coastal regions including Sarasota, Manatee, Charlotte, Lee, and Collier counties. Estimates from the clean-up efforts during the 2021 *K. brevis* blooms suggest that over 800 tons of dead fish, dolphins, and manatees had washed ashore in southwest Florida. This indicates that *K. brevis* blooms are not only toxic to fish but also deadly to marine mammals. Ultimately, this raises significant concerns among the scientific and public health communities about the overall effects of *K. brevis* blooms on the exposed population.

Brevetoxins are lipid-soluble polycyclic polyether neurotoxins stored within *K. brevis* and released upon cell lysis (Patel et al., 2020; Pierce et al., 2000). In people with preexisting asthma, emphysema, and bronchitis, inhalation of brevetoxins transmitted in marine aerosols causes upper respiratory tract (URT) symptoms, such as coughing and throat irritation (Cheng et al., 2005). Changes in pulmonary function have also been documented in people with these respiratory illnesses following short exposures during *K. brevis* blooms (Fleming et al., 2011). However, it remains unknown whether a dose-response relationship exists between the level of brevetoxin exposure and the number of URT symptoms experienced during *K. brevis* blooms. A recent geographic correlational study, using hospital emergency department records, showed an increase in the primary diagnosis of headaches among coastal southwest Florida residents when elevated *K. brevis* cell counts were present (Diaz et al., 2019). In addition, consumption of brevetoxin-contaminated molluscan seafood is the major cause of neurotoxic shellfish poisoning (NSP), resulting in gastrointestinal and neurological symptoms, which include headaches, paresthesias (numbness, tingling etc.), ataxia (gait disturbances) and dizziness (Watkins et al., 2008). Nonetheless, it remains unclear whether aerosolized brevetoxin reaches sufficient levels to cause NSP-like symptoms in humans. This study sought to determine whether aerosolized brevetoxin triggers NSP-like symptoms, and if so, whether a dose-response relationship exists between brevetoxin exposure and reporting of URT or NSP-like symptoms in a general residential population of southwest Florida. As this study population included residents from approximately 6,000 square miles of southwest Florida, air monitoring for brevetoxin was not feasible. Therefore, *K. brevis* cell counts in coastal waters were used to estimate exposure, since past studies have shown a high correlation between *K. brevis* cell counts and the levels of brevetoxin in marine aerosols (Cheng et al., 2005; Pierce et al., 2005).

2. Methods

2.1. Study design and procedures

Approval for the study was obtained from the Western Institutional Review Board (WIRB) and Copernicus Group IRB (WCG-IRB). Recruitment efforts consisted of IRB-approved flyers and public service announcements, and newspaper coverage of the study. Recruitment began in July 2019 following the intense 2017–2019 *K. brevis* bloom, ending in February 2019 (https://www.flickr.com/photos/myfwc/sets/72,157,635,398,013,168/). Self-referring residents of the Sarasota, Manatee, Lee, Charlotte, and Collier counties were recruited in the study. A clinical research coordinator (CRC) conducted the informed consent process using the International Committee on Harmonization of Good Clinical Practice (ICH GCP) guidelines. Participants were contacted by the study staff to arrange a study visit over a 26-month period from June 2019 to August 2021. Participants in the current analyses were enrolled during a funding period from June 2019 to August 2021. Seventy of the 258 total participants were seen twice during this period. This ecological study design also included a longitudinal subcomponent consisting of participants who returned for follow-up visits over 2 years of the study. Inclusion criteria included an age of 18 or older, while exclusion criteria included an inability to speak and/or write in English.

A health survey was used to collect self-reported information on age, education, sex, ethnicity, diet, use of dietary supplements, and past medical history (PMH), as well as a systems review and an inventory of symptoms related to organ/function. Self-reported information regarding exposure to marine waters or consumption of local seafood was also collected. The study coordinator contacted the study participants on a quarterly basis between in-person visits via scheduled telephone calls, texts, or emails to check on their health status and to update them of any new information about the study.

K. brevis cell counts for counties of interest were made available to the Roskamp Institute by the Florida Fish and Wildlife Commission (FWC) through the Florida Fish and Wildlife Research Institute (FWRI) Harmful Algal Bloom Monitoring Database. These databases contain cell counts since 1954 and were composed of over 125,000 records from 190 state and county agencies, private research institutions, universities and FWC staff. Location-level data include cell counts with latitude/longitude coordinates (see FWRI website for additional information, https://myfwc.com/research/redtide/monitoring/database/).

Given the correlation between *K. brevis* cell counts and brevetoxin concentrations in seawater (Pierce et al., 2005) and of brevetoxin in seawater with brevetoxin in marine aerosols (Cheng et al., 2005), we generated a surrogate variable (Surrogate Brevetoxin Exposure or SBE) based on gathered *K. brevis* cell counts within a circle that has a radius comprised of the distance from each participant's home address to the shoreline, plus 10 km. On average, our study participants were within 3 km of the shoreline for the SBE categories. The single highest cell count value in these generated circles during the two weeks preceding the study visit was used as the SBE (Fig. 1). Data for SBE values were further stratified into background, low, medium, and high categories similar to that defined by the FWC. A cell count < 1,000 cells per liter (cells/L) was considered "background"; low was 1,000 to 100,000 cells/L, medium was 100,000 to 1,000,000 cells/L, and high was > 1,000,000 cells/L. Seventy participants out of 258 were also returned for follow-up visits and symptoms were evaluated in relation to the changes in SBE between visits.

The outcome variables of interest were illnesses, such as emphysema, pneumonia, and asthma and symptoms including upper respiratory tract (URT) symptoms that were previously associated with *K. brevis* exposure (Backer et al., 2005; Kirkpatrick et al., 2006; Kirkpatrick et al., 2008). A composite variable for URT symptoms (Composite URT) consisted of either the absence or the presence of one or more of the following four symptoms: sore throat, coughing, nasal congestion, and sneezing. Among the central nervous system (CNS) symptoms, reporting of headaches was examined for the same association. Since there is a considerable overlap between symptoms of shellfish poisoning (SP) associated with ciguatera (CTX) and neurotoxin shellfish poisoning (NSP) (Watkins et al., 2008) and as a CTX survey was previously described by Chateau-Degat and colleagues, we used the questions described in the survey (Chateau-Degat et al., 2007) to ensure that a variety of SP/NSP-like (hereon referred to as NSP-like) symptoms were captured as part of the current survey. These included weakness, fever, chills, muscle pain, stiff neck, headaches, and nervous system symptoms. Additional data were collected on a PMH of migraine, chronic fatigue syndrome (CFS), contact allergies, drug allergies, food allergies, irritable bowel syndrome (IBS) and mild memory loss to examine their possible independent influences on the NSP-like symptom presentation.

2.2. Statistical analysis

Demographic characteristics were compared using either ANOVA or the Chi-square test, as applicable. The exposure variable was SBE and the outcomes of interest were those described above and presented in Fig. 2. The outcome variables included URT, neurological and NSP-like

Fig. 1. SBE and air PbTx estimates using K. brevis cell counts. This image depicts the use of K. brevis cell counts for calculating SBE. Image was generated using Biorender.com.

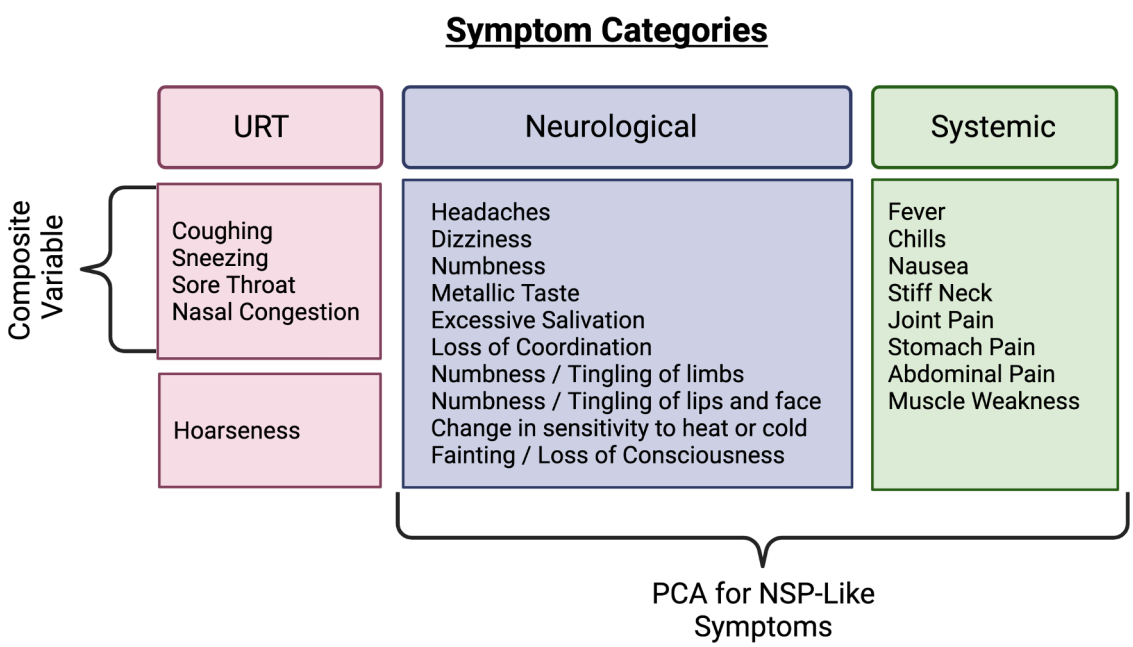

Fig. 2. Relationship between individual symptoms and composite/component variables. The URT symptoms in composite URT include sore throat, cough, hoarseness, and sneezing. Principal component analysis of 25 NSP-like symptoms listed in the survey to reduce multicollinearity effects. Image was generated using Biorender.com.

symptoms (Fig. 2). Given that NSP-like symptoms consisted of a large number of variables, Principal Component Analysis (PCA) was applied to minimize multicollinearity and achieve dimension-reduction for NSP-like symptoms as previously described (Abdullah et al., 2020). A total of 10 components were identified based on the internal correlation between 25 NSP-like symptoms and a linear variable was generated for each component identified by PCA (Fig. 3).

Descriptive analyses: These analyses were performed using the Chi-square test to examine the associations between SBE, general demographic characteristics, PMH of emphysema, asthma, pneumonia, allergies and migraine and the reporting of URT and neurological symptoms as well as control variables (frequency of urination and hearing loss). The dichotomous Component 1 variable was based on the absence of any of the symptoms in Component 1 or the presence of any one or more of the symptoms. The same conversion process was used for Component 6.

Regression analyses: For logistic regression analysis, the Composite URT variable was further dichotomized as an absence of any of the four symptoms or the presence of one or more of the four symptoms. Logistic regression analyses were then performed to examine the influence of SBE on the outcome of individual URT symptoms, Composite URT and PMH of migraines and respiratory conditions. Linear regression analyses were performed to examine the associations between SBE (as linear variable), PMH, and the linear interactions between them for the outcome of NSP-like symptom Components. Since we did not have *a priori* hypotheses for whether PMH of allergies, CFS, contact allergies, drug allergies, food allergies, IBS and mild memory loss are independent predictors or whether they interact with SBE on the outcome of NSP-like symptoms, the linear regression models included SBE and these PMH as independent predictors and also included an interactive term for SBE and these PMH for determining their influence on the outcomes Components 1 and 6. Another linear regression model was constructed where seafood consumption and SBE were included as independent predictors and an interactive term between SBE and seafood consumption for examining their influence on the outcomes Components 1 and 6. Similarly, a separate linear regression was also performed where the time spent in water near the home zones and SBE were included as independent predictors as well as an interaction between them to examine their influence on the outcomes Components 1 or 6. The Benjamini-Hochberg procedure was used for multiple testing correction of linear

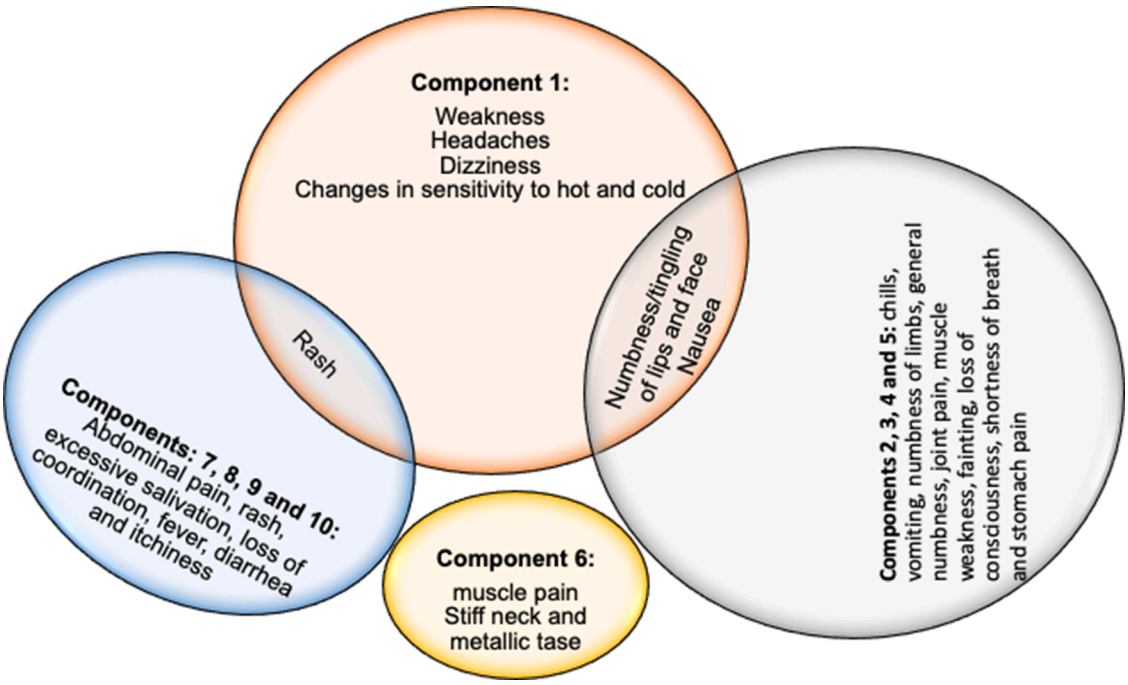

Fig. 3. PCA based identification of components of NSP-like symptoms. There were 25 NSP-like symptoms in the survey which were grouped using PCA into 10 components. Component 1 explained 10% of the variance in the dataset and was associated with SBE. Components 2, 3, 4, and 5 each explained 7% of the variance but were not associated with SBE. Since these components 2 – 5 had equal variance each, these were combined into one group. Component 6 explained 6% of the variance and was associated with SBE. Components 7 through 10 explained 5% of variance each and were grouped together for the figure.

regression analyses. For all main analyses and the false discovery rate (FDR), p values ≤ 0.05 were used as a cut-off for statistical significance. All analyses were conducted using SPSS version 24 (IBM, NY).

3. Results

3.1. Reporting of upper respiratory tract symptoms and headaches is associated with K. brevis cell counts

General demographic characteristics of the 258 study participants presented in Table 1 are stratified by SBE. The average two week maximum cell count (cells/L) for each of the SBE category was: background = 275 ± 28 SE; low = 28,285 ± 4,246 SE; medium = 343,537 ± 48,557 SE; and high = 4,668,052 ± 1,286,314 SE. The maximum difference in radius was 1.24 km across these categories, using which, a difference of 8% surface area of the water being sampled was noted. There was no correlation between the maximum *K. brevis* cell counts and the distance to the shoreline nor was there a significant difference in the distance across the SBE categories. There were no significant differences between SBE with age, sex, and race of the study participants. The study participants were, on average, 63 years old (± 13 SD), 57% female, and almost exclusively white (Table 1).

Table 2 shows that SBE was associated with sore throat, coughing, nasal congestion and sneezing. The Composite URT category, consisting of these four symptoms, was also associated with SBE (see Table 2, $p < 0.05$). No association existed between SBE and the control symptoms of frequency of urination and hearing loss ($p > 0.05$, Table 2). Logistic regression analyses showed an association of SBE with the Composite URT symptoms (low OR = 2.5, 95% CI [1.3–5.0], $p < 0.01$; medium OR = 8.0, 95% CI [2.4–25.9], $p < 0.01$; and high OR = 34.1, 95% CI [7.7–150.3], $p < 0.001$, see Table 3). Among reported PMH, emphysema was significantly associated with SBE ($p < 0.05$, Table 2). However, there was no association between SBE and reporting of prior diagnoses of asthma or pneumonia ($p > 0.05$).

The reporting of one or more symptoms from the Composite URT was associated with the reporting of headaches ($\chi2 = 31.4$, $p < 0.001$). The logistic regression analyses showed an increased prevalence of headache reporting in the medium SBE category (OR = 4.7, 95% CI [1.5–14.9], $p < 0.01$). The same was not true for the low or high categories ($p > 0.05$). About 58% of individuals with a PMH of migraines reported headaches compared to the 12.7% of individuals who reported headaches, but not migraines ($\chi2 = 42.3$, $p < 0.001$). Reporting of Composite URT was also associated with migraines where 68.4% of those with migraines also experienced one or more of the symptoms in Composite URT compared to the 45% of those who did not report migraines on their PMH ($\chi2 = 7.1$, $p < 0.01$). In addition, there was an increased prevalence of migraine reporting on the PMH survey by individuals in the medium SBE (OR = 4.7, 95% CI [1.5–15.1], $p < 0.01$).

Table 1
General demographic characteristics of the study participants by SBE.

	Background (n = 155)	Low (n = 48)	Medium (n = 18)	High (n = 37)
Sex				
Females	91 (59%)	26 (54%)	10 (56%)	21 (57%)
Race				
White	152 (98%)	48 (100%)	18 (100%)	37 (100%)
African American	2 (1%)	0 (0%)	0 (0%)	0 (0%)
American Indian	1 (1%)	0 (0%)	0 (0%)	0 (0%)
Ethnicity				
Hispanic	4 (3%)	2 (4%)	0 (0%)	1 (3%)
		Mean ± SE		
Age	63.7 (1.1)	65.9 (1.8)	63.7 (2.7)	58.3 (2.9)

Note: Surrogate brevetoxin exposure (SBE) is stratified by maximum K. brevis cell counts in proximity to participants' home address within 2 weeks of the study visits. Not present (<1000), low (>1000 to 100,000), medium (>100,000 to 1000,000) and high (>1000,000) were evaluated to generate SBE variable.

Table 2
Symptom presentation and past medical history by SBE.

Symptoms yes (%)	Background (n = 155)	Low (n = 48)	Medium (n = 18)	High (n = 37)
Sore throat*	8 (5%)	10 (21%)	8 (44%)	6 (16%)
Coughing*	19 (12%)	13 (27%)	14 (78%)	25 (68%)
Nasal congestion*	33 (21%)	18 (38%)	13 (72%)	24 (65%)
Sneezing*	15 (10%)	10 (21%)	8 (44%)	17 (46%)
Hoarseness	9 (6%)	3 (6%)	1 (6%)	5 (14%)
Headaches†	20 (13%)	9 (19%)	7 (39%)	7 (19%)
Frequent urination	13 (8%)	4 (8%)	4 (22%)	2 (5%)
Hearing loss	15 (10%)	2 (4%)	1 (6%)	0 (0%)
§Composite URT (1)	27 (17%)	10 (21%)	1 (6%)	11 (30%)
Composite URT (2)	15 (10%)	6 (13%)	1 (6%)	9 (24%)
Composite URT (3)	9 (6%)	3 (6%)	6 (33%)	9 (24%)
Composite URT (4)	2 (1%)	5 (10%)	5 (28%)	5 (14%)
ǯComposite URT*	53 (34%)	24 (50%)	14 (78%)	34 (92%)
Past Medical Histories Yes (%)	Background (n = 155)	Low (n = 48)	Medium (n = 18)	High (n = 37)
Migraine*	15 (10%)	8 (17%)	6 (33%)	7 (19%)
$Emphysema*	2 (1%)	2 (4%)	3 (17%)	2 (5%)
Pneumonia	23 (15%)	9 (19%)	2 (11%)	6 (16%)
Asthma	18 (12%)	9 (19%)	5 (28%)	3 (8%)
Allergies	67 (43%)	23 (48%)	10 (56%)	21 (57%)

Note: *$p < 0.05$, † $p = 0.069$. Hearing loss and frequency of urination are used as control variables. §The Composite URT variable contains either no symptoms (0) 1, 2, 3 or 4 symptoms consisting of either sore throat, coughing, nasal congestion and sneezing. ǯComposite URT variable with individual symptoms was converted to presence of one or more of the 4 URT symptoms listed herein. $Since observed and expected cell counts were below 5, a Fisher exact test was used by generating 2 × 2 contingency table based on presence or absence of SBE.

Table 3
Dose-response relationship between URT symptoms, headaches and past medical history reporting and SBE.

Symptoms	Low	Medium	High
ǯComposite URT	2.5[1.3–5.0]**	8.0 [2.4–25.9]**	34.1 [7.7–150.3]***
Sore Throat	5.0 [1.8–14.0]**	14.4 [4.4–47.4]***	3.6 [1.1–11.3]**
Coughing	2.7 [1.2–6.1]*	23.8 [7.0–51.3]***	20.0 [7.8–51.3]***
Nasal congestion	2.2 [1.1–4.5]*	8.8 [2.9–26.8]***	8.9 [3.7–21.2]***
Sneezing	2.4 [1.0–5.8]	7.0 [2.4–20.7]***	9.7 [4.0–23.5]***
Headaches	1.9 [0.7–4.7]	4.7[1.5–14.9]**	1.2[0.4–3.44]
Past Medical Histories	Low	Medium	High
Migraine	2.1 [0.8–5.5]	4.7 [1.5–15.1]**	1.9 [0.7–5.3]
Respiratory other Ť	1.4 [0.7–2.9]	1.9 [0.7–5.3]	1.0 [1.0–1.0]

Note: *$p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. All odds ratios (OR) are adjusted for age and sex. Ť(emphysema, asthma and pneumonia combined into one variable). ǯComposite URT variable with individual symptoms was converted to presence of one or more of the 4 URT symptoms listed in the table. Logistic regression analyses showed a dose related increase in the OR for Composite URT, as well as nasal congestion and sneezing. The OR for the association between headaches and SBE and migraines and SBE reached significance for the medium level.

3.2. Reporting of NSP-like symptoms are associated with SBE

As mentioned above, the URT symptoms are generally associated with exposure to aerosolized brevetoxins, whereas NSP symptoms have been associated with the ingestion of brevetoxins in contaminated molluscan shellfish. Component 1 included weakness, headaches, dizziness, numbness or tingling in lips and faces, changes in sensitivity to hot or cold, nausea and rash. A linear association between SBE and Component 1 was observed even after adjusting for age and sex (Table 4, $p < 0.05$). Component 6 included muscle pain, stiff neck and metallic taste, and this component also showed a linear association with SBE even after adjusting for age and sex (Table 4, $p < 0.01$). Component 1 explained 10% of the variance in the dataset and was associated with SBE. Components 2 through 5 each explained 7% of the variance and were not associated with SBE (Fig. 3). Component 6 explained 6% of the variance and was associated with SBE. Components 7 through 10 explained 5% of variance each and were not associated with SBE.

There was a significant association between the Composite URT symptom category and the NSP-like symptom Component 1 ($\chi 2 = 36.4$, $p < 0.001$) and Component 6 ($\chi 2 = 6.8$, $p < 0.01$). There was no association between SBE and reporting by participants for spending time in marine waters near their homes ($p > 0.05$). There was no linear association between marine water at the home location with Components 1 or 6 ($p > 0.05$) and there was also no interaction between marine water at home location with SBE for Components 1 and 6 as the outcomes. There was an association between reporting of lower seafood consumption and SBE ($\chi 2 = 5.9$, $p = 0.015$). However, there was no influence of seafood consumption on the linear association between SBE and Components 1 or 6 ($p > 0.05$).

An evaluation of health conditions reported on the PMH survey showed that for Component 1, there were significant interactions between SBE (as a linear factor) and CFS ($p < 0.001$), migraines ($p = 0.025$), mild memory loss ($p = 0.017$), and dermatitis ($p = 0.019$). Stratified analyses were performed to evaluate whether NSP reporting differed by the level of SBE exposure by individual diagnosed with above mentioned health conditions. These analyses showed that when SBE was at background, the odds of reporting symptoms in Component 1 was not significantly higher in those with a PMH of CFS (OR = 8.8, 95% CI [0.9 – 87.6], $p > 0.05$). By contrast, with any levels of SBE above background, the reporting of symptoms in Component 1 was higher for those with a PMH of CFS (OR = 11.5, 95% CI [1.3 – 99.2], $p = 0.03$). For migraine, when SBE was at background level, the odds of reporting symptoms in Component 1 was not significantly higher in those with a PMH of migraine (OR = 2.7 95% CI [0.9 – 8.0], $p > 0.05$), Again, by contrast, with any levels of SBE above background, the reporting of symptoms in Component 1 was higher for those with a PMH of migraine (OR = 9.1 95% CI [3.0 – 27.7], $p < 0.001$). For mild memory loss and dermatitis stratified analyses did not show statistical significance for SBE at background level or when it was low to high ($p > 0.05$). When Component 6 was used as the outcome, there were significant statistical interactions

Table 4
Association of NSP-like symptom components with SBE.

Cell count (presence)	Background (N = 155)	Low (N = 48)	Medium/high (N = 55)
Component 1 NSP-like symptoms (#)			
0	113 (73%)	35 (73%)	29 (53%)
1	24 (15%)	6 (13%)	12 (22%)
2	8 (5%)	0 (0%)	7 (13%)
3	6 (4%)	3 (6%)	3 (5%)
4	2 (1%)	3 (6%)	2 (4%)
5	1 (1%)	1 (2%)	1 (2%)
6	0 (0%)	0 (0%)	1 (2%)
7	1 (1%)	0 (0%)	0 (0%)
Component 6 NSP-like symptoms (#)	N = 155	N = 48	N = 55
0	146 (94%)	39 (81%)	40 (73%)
1	8 (5%)	3 (6%)	11 (20%)
2	1 (1%)	5 (10%)	4 (7%)
3	0 (0%)	1 (2%)	0 (0%)

Note: Distribution of number of symptoms in each Component by the level of SBE exposure.

between SBE (as a linear factor) and asthma ($p < 0.001$), migraines ($p = 0.013$), IBS ($p = 0.008$), and contact allergies ($p = 0.013$). However, stratified analyses aimed at determining whether the presence or the absence of SBE on the reporting of Component 6 symptoms did not show significant differences with respect to asthma, migraines, IBS and contact allergies ($p > 0.05$).

3.3. Reporting of URT and NSP-like symptoms in relation to red tide blooms between visits in a subset of subjects

Of 258 participants, 70 had follow-up visits and, for these participants, the change in exposure from visit one to visit two was recorded as either "no change" ($n = 23$, of which 20 went from background to background and three went from low to low) or "change" ($n = 47$ either going from no exposure to low, medium, or high or going from a lower exposure to higher exposure level). An examination of symptom reporting over two sequential visits ($n = 70$) with change (as defined above) in SBE were restricted to Composite URT and NSP-like symptoms in Components 1 or 6. Compared to subjects within the group where there was "no change" in SBE between the first and second visits. Those going from no or low-level exposure to a higher SBE level reported significant increases in composite URT symptoms (30% prevalence in "no change" compared to 57% prevalence in "change", $p < 0.05$) and a non-significant increase in reporting of NSP-like symptoms in component 1 or 6 (22% in "no change" to 34% in "change", see Table 5).

4. Discussion

Previous studies have shown that exposure to aerosolized brevetoxins is associated with reporting of upper respiratory symptoms either by individuals with preexisting respiratory illnesses or by healthy individuals in occupational settings. However, this had not been investigated in a general population of southwest Florida residents. Given the unavailability of tools and equipment to accurately quantify personal brevetoxin exposure, the study was performed in relation to ecological level exposure. A proxy for aerosolized brevetoxin was employed which generated an estimate of exposure, SBE, derived from localized *K. brevis* cell counts in relation to the participants' home location. This study shows that in a population of southwest Florida residents, SBE exposure is associated with increased reporting of URT and NSP-like symptoms and headaches, particularly among those with certain preexisting systemic (asthma, dermatitis, contact allergies, and irritable bowel syndrome) and neurological conditions (chronic fatigue syndrome, mild memory loss and migraine). Since the reporting of URT symptoms is well recognized in these communities as a consequence of the *K. brevis* blooms, there is a possibility of reporting bias during the periods of *K. brevis* bloom for these symptoms. However, neither the study staff nor the participants were aware of the baseline or the fluctuations in local *K. brevis* cell counts that were the basis of the SBE calculations, and as such, the possible effects of reporting bias remain minimal. However, given that the study population was small, and composed of self-referring volunteers who may be influenced by self-reporting bias, generalizability to larger population of Florida residents is limited.

Table 5
Association between change in SBE status and reporting of composite URT and component 1 and 6 of NSP-like symptoms.

SBE difference from visit 1 to visit 2	No change ($n = 23$)	Cell count higher ($n = 47$)
Composite URT (any of the 4 symptoms)		
No (%)	16 (70%)	20 (43%)
Yes (%)	7 (30%)	27 (57%)*
Component 1 and 6 (any symptoms)		
No (%)	18 (78%)	31 (66%)
Yes (%)	5 (22%)	16 (34%)

Note: *$p < 0.05$ for dichotomous Composite URT variable and change in SBE.

Past studies have shown that exposure to brevetoxins released from *K. brevis* cells is associated with an increased reporting of URT symptoms among people with asthma (Milian et al., 2007). In addition, an occupational exposure study conducted by Backer and colleagues showed increased reporting of URT symptoms during *K. brevis* blooms among healthy lifeguards (Backer et al., 2005). Among these symptoms, there was a significant increase in nasal congestion, throat irritation and coughing (Backer et al., 2005). Using a more generalized population of community participants from southwest Florida, this current study shows that nasal congestion, coughing, sore throat, and sneezing are related to the increasing concentrations of *K. brevis* cell counts in coastal waters. However, to date, it remains unknown whether an increase in URT symptom reporting occurs with increasing intensities of the *K. brevis* blooms. The present study reports a dose-response relationship between increased SBE and increased reporting of URT symptoms (especially sore throat, coughing, nasal congestion and sneezing). Within the range of exposures measured in this study, this dose-dependent relationship appears to be linear. However, this relationship may not hold true outside of the SBE range evaluated herein and may differ in the presence of higher *K. brevis* bloom intensities. Further understanding a dose-response relationship between *K. brevis* blooms and URT symptoms should be a focus of future studies.

Previously, Backer and colleagues revealed a relationship between the reporting of headaches and *K. brevis* blooms, but only among healthy lifeguards (Backer et al., 2005), while another recent study reported increased visits to hospital emergency departments due to headaches during *K. brevis* blooms (Diaz et al., 2019). For the current study, reporting of headaches also increased with the presence of medium SBE. Since URT symptoms are classic indicators of *K. brevis* exposure, concurrent association between URT symptoms and headaches further reinforces the hypothesis that they are both triggered by SBE. Additionally, reporting of headaches with increasing SBE was prevalent among individuals with a PMH of migraines. This suggests that these individuals may be susceptible to headaches during *K. brevis* blooms.

Analyzing a difference aspect of *K. brevis* effects, the consumption of brevetoxin-contaminated molluscan seafood (i.e. clams, oysters and mussels) harvested during red tide blooms has been linked to NSP with gastrointestinal and neurological symptoms. The current study associated SBE with two specific groups of NSP-like symptoms; one group consisted of weakness, headaches, dizziness, nausea, numbness/tingling of lips and face, changes in sensitivity to heat or cold, while the other group consisted of metallic taste, stiff neck and muscle pain. The results highlighted a linear dose-response relationship between SBE and NSP-like symptoms. Furthermore, these NSP-like symptoms could not be attributed to the ingestion of either contaminated water or local seafood. This suggests that exposure to aerosolized brevetoxin may also contribute to NSP-like symptom reporting by otherwise healthy individuals. However, potential confounding effects of other concurrent exposures, such as those emanating from decaying fish, that may occur during the *K. brevis* bloom cannot be ruled out and warrant further investigation. To our knowledge, this is the first study to correlate reporting of NSP-like symptoms with potential exposure to aerosolized brevetoxin. This finding requires additional effort to determine the nature of the relationship between aerosolized brevetoxin dose and NSP-like symptoms. In particular, the dose threshold for emergence of specific symptoms should be estimated as certain symptoms may constitute a greater public health threat than others.

Additionally, individuals experiencing NSP-like symptoms were those who frequently reported migraine and CFS on the PMH survey with increasing SBE. Unfortunately, sample size limitations did not allow the present study to examine this further. However, the possibility that individuals with a prior history of neurological illnesses are susceptible to NSP-like symptoms requires further surveillance of such individuals during *K. brevis* blooms.

In support of the inference from this cross-sectional study that increased reporting of URT and NSP-like symptoms correlates with increased brevetoxin exposure, our longitudinal studies reveal a higher reporting of such symptoms when there is a sequential increase in brevetoxin exposure

5. Conclusions

This study identified a dose-dependent relationship between the magnitude of *K. brevis* blooms in southwest and upper respiratory tract symptoms among residents of southwest Florida. This study also confirmed and extended a previous observation that exposure to *K. brevis* blooms is associated with headaches by showing that those reporting headaches during *K. brevis* blooms were more likely to have a PMH of migraines. Furthermore, a dose-dependent relationship was detected between the magnitude of *K. brevis* blooms and neurotoxic shellfish poisoning-like symptoms. This latter observation suggests that airborne brevetoxin levels may be sufficient to trigger NSP-like symptoms. Furthermore, we found that this relationship is exacerbated in individuals with PMH of specific neurological conditions.

With the effects of climate change potentially increasing the intensity and/or duration of *K. brevis* blooms and with the ever-increasing population in coastal communities, studies, such as this one, are needed to fully evaluate the public health risks which accompany them. Currently, public health messaging only includes generic advisories for residents and visitors to stay away from areas with active blooms; these advisories are based on limited information about acute effects to assumed at-risk populations. Even though this is an understandable initial effort to reduce the most obvious health impacts and ensure an accepted public relations response, a greater effort needs to be applied in order to understand the public health impacts of brevetoxins at various concentrations, exposure periods, exposure pathways and to other at-risk populations. Public health policies can only be set with a clear understanding of risks, especially to the vulnerable populations. This publication provides the first effort to broaden our knowledge on the central nervous system impacts to humans, and thereby identify an approach that leads us in a direction to appreciate the multifaceted adverse impacts of brevetoxins on public health.

Author contributions

LA and MM conceived and supervised the project. LA, SF, DN, GB, DP, MP and CHT collected the data. LA, SF, GB, MP, and DN analyzed the data. LA, SF, BK, AR, AK, AN, FC and MM interpreted data. LA, AR, BK, and MM wrote the manuscript. All authors reviewed and edited the manuscript.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Data Availability

Data will be made available on request.

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Bibliography

Abdullah, L., Crawford, F., Tsolaki, M., Borjesson-Hanson, A., Olde Rikkert, M., Pasquier, F., Wallin, A., Kennelly, S., Ait-Ghezala, G., Paris, D., Hendrix, S., Blennow, K., Lawlor, B., Mullan, M., 2020. The influence of baseline Alzheimer's disease severity on cognitive decline and CSF biomarkers in the NILVAD trial. Front. Neurol 11, 149.

Backer, L.C., Kirkpatrick, B., Fleming, L.E., Cheng, Y.S., Pierce, R., Bean, J.A., Clark, R., Johnson, D., Wanner, A., Tamer, R., Zhou, Y., Baden, D.G., 2005. Occupational exposure to aerosolized brevetoxins during Florida red tide events: effects on a healthy worker population. Environ. Health Perspect. 113 (5), 644–649.

Brand, L.E., Compton, A., 2007. Long-term increase in Karenia brevis abundance along the southwest Florida coast. Harmful Algae 6 (2), 232–252.

Chateau-Degat, M.L., Huin-Blondey, M.O., Chinain, M., Darius, T., Legrand, A.M., Nguyen, N.L., Laudon, F., Chansin, R., Dewailly, E., 2007. Prevalence of chronic symptoms of ciguatera disease in French Polynesian adults. Am. J. Trop. Med. Hyg. 77 (5), 842–846.

Cheng, Y.S., Zhou, Y., Irvin, C.M., Pierce, R.H., Naar, J., Backer, L.C., Fleming, L.E., Kirkpatrick, B., Baden, D.G., 2005. Characterization of marine aerosol for assessment of human exposure to brevetoxins. Environ. Health Perspect. 113 (5), 638–643.

Diaz, R.E., Friedman, M.A., Jin, D., Beet, A., Kirkpatrick, B., Reich, A., Kirkpatrick, G., Ullmann, S.G., Fleming, L.E., Hoagland, P., 2019. Neurological illnesses associated with Florida red tide (Karenia brevis) blooms. Harmful Algae 82, 73–81.

Fleming, L.E., Kirkpatrick, B., Backer, L.C., Bean, J.A., Wanner, A., Reich, A., Zaias, J., Cheng, Y.S., Pierce, R., Naar, J., Abraham, W.M., Baden, D.G., 2007. Aerosolized red-tide toxins (brevetoxins) and asthma. Chest 131 (1), 187–194.

Fleming, L.E., Kirkpatrick, B., Backer, L.C., Walsh, C.J., Nierenberg, K., Clark, J., Reich, A., Hollenbeck, J., Benson, J., Cheng, Y.S., Naar, J., Pierce, R., Bourdelais, A. J., Abraham, W.M., Kirkpatrick, G., Zaias, J., Wanner, A., Mendes, E., Shalat, S., Hoagland, P., Stephan, W., Bean, J., Watkins, S., Clarke, T., Byrne, M., Baden, D.G., 2011. Review of Florida red tide and human health effects. Harmful Algae 10 (2), 224–233.

Kirkpatrick, B., Currier, R., Nierenberg, K., Reich, A., Backer, L.C., Stumpf, R., Fleming, L., Kirkpatrick, G., 2008. Florida red tide and human health: a pilot beach conditions reporting system to minimize human exposure. Sci. Total Environ. 402 (1), 1–8.

Kirkpatrick, B., Fleming, L.E., Backer, L.C., Bean, J.A., Tamer, R., Kirkpatrick, G., Kane, T., Wanner, A., Dalpra, D., Reich, A., Baden, D.G., 2006. Environmental exposures to florida red tides: effects on emergency room respiratory diagnoses admissions. Harmful Algae 5 (5), 526–533.

Milian, A., Nierenberg, K., Fleming, L.E., Bean, J.A., Wanner, A., Reich, A., Backer, L.C., Jayroe, D., Kirkpatrick, B., 2007. Reported respiratory symptom intensity in asthmatics during exposure to aerosolized Florida red tide toxins. J. Asthma 44 (7), 583–587.

Patel, S.S., Lovko, V.J., Lockey, R.F., 2020. Red tide: overview and clinical manifestations. J. Allergy Clin. Immunol. Pract. 8 (4), 1219–1223.

Pierce R.H., H. M. S. B., P.C., Hamel S.L., Kirkpatrick B., Cheng Y.S., Zhou Y., Irvin C.M., Naar J., Weidner A., Fleming L.E., Backer L.C., Baden D.G. (2005). "Brevetoxin composition in water and marine aerosol along a Florida beach: assessing potential human exposure to marine biotoxins" 4(6): 965–972.

Pierce, R.H., Henry, M., Blum, P., Payne, S., 2000. Gymnodinium breve toxins without cells: intra-cellular and extra-cellular toxins. Harmful Algal Blooms 421–424.

L. R. Reich, A., Faris, R., Fleming, L.E., Kirkpatrick, B., Watkins, S., Ullmann, S., Kohler, K., Hoagland, P., 2015. Assessing the impact of shellfish harvesting area closures on neurotoxic shellfish poisoning (nsp) incidence during red tide (Karenia brevis) blooms Harmful Algae 43, 13–19.

Steidinger, K., 2009. Historical perspective on Karenia brevis red tide research in the Gulf of Mexico. Harmful Algae 8 (4), 549–561.

Watkins, S.M., Reich, A., Fleming, L.E., Hammond, R., 2008. Neurotoxic shellfish poisoning. Mar. Drugs 6 (3), 431–455.