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Harmful cyanobacterial aerosolization dynamics in the airshed of a eutrophic estuary

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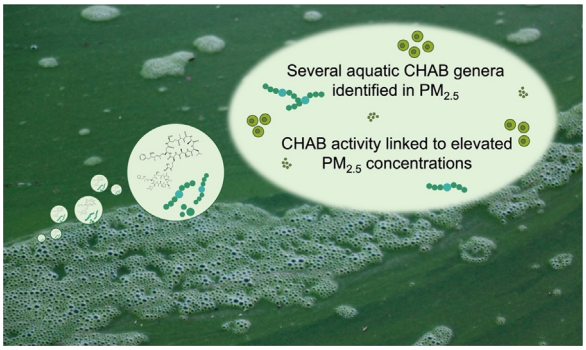
HIGHLIGHTS

- No microcystins were quantified in the airshed of the Chowan River in 2020.
- Several toxigenic, bloom forming cyanobacteria were identified in $PM_{2.5}$.
- Aquatic cyanobacteria influence the abundance of airborne cyanobacteria.
- *Dolichospermum*, *Microcystis*, and *Aphanizomenon* were aerosolized concurrently.
- CHAB activity was linked to elevated $PM_{2.5}$ mass concentrations.

GRAPHICAL ABSTRACT



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ABSTRACT

In addition to obvious negative effects on water quality in eutrophic aquatic ecosystems, recent work suggests that cyanobacterial harmful algal blooms (CHABs) also impact air quality via emissions carrying cyanobacterial cells and cyanotoxins. However, the environmental controls on CHAB-derived aerosol and its potential public health impacts remain largely unknown. Accordingly, the aims of this study were to 1) investigate the occurrence of microcystins (MC) and putatively toxic cyanobacterial communities in particulate matter ≤ 2.5 μm in diameter ($PM_{2.5}$), 2) elucidate environmental conditions promoting their aerosolization, and 3) identify associations between CHABs and $PM_{2.5}$ concentrations in the airshed of the Chowan River-Albemarle Sound, an oligohaline, eutrophic estuary in eastern North Carolina, USA. In summer 2020, during peak CHAB season, continuous $PM_{2.5}$ samples and interval water samples were collected at two distinctive sites for targeted analyses of cyanobacterial community composition and MC concentration. Supporting air and water quality measurements were made in parallel to contextualize findings and permit

Abbreviations: ASV, amplicon sequence variant; AF, aerosolization factor/aerosol enrichment factor; CHAB, cyanobacterial harmful algal bloom; CSS, cumulative sum scaling; LC-MS, liquid chromatography coupled with triple quadrupole mass spectrometry; MC, microcystins; MC-LA, MC-Leucine Alanine; MC-LF, MC-Leucine Phenylalanine; MC-LR, MC-Leucine Arginine; PM_{10}, particulate matter with aerodynamic diameters < 10 μm; $PM_{2.5}$, particulate matter with aerodynamic diameters < 2.5 μm; SOA, secondary organic aerosol; VOC, volatile organic compound.

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statistical analyses of environmental factors driving changes in CHAB-derived aerosol. MC concentrations were low throughout the study, but a CHAB dominated by *Dolichospermum* occurred from late June to early August. Several aquatic CHAB genera recovered from Chowan River surface water were identified in $PM_{2.5}$ during multiple time points, including *Anabaena, Aphanizomenon, Dolichospermum, Microcystis,* and *Pseudanabaena.* Cyanobacterial enrichment in $PM_{2.5}$ was indistinctive between subspecies, but at one site during the early bloom, we observed the simultaneous enrichment of several cyanobacterial genera in $PM_{2.5}$. In association with the CHAB, the median $PM_{2.5}$ mass concentration increased to 8.97 μg m^{-3} (IQR = 5.15), significantly above the non-bloom background of 5.35 μg m^{-3} (IQR = 3.70) ($W = 1835$, $p < 0.001$). Results underscore the need for highly resolved temporal measurements to conclusively investigate the role that CHABs play in regional air quality and respiratory health risk.

1. Introduction

Water security across the globe is threatened by the recent expansion of toxin-producing cyanobacterial harmful algal blooms (CHABs) in freshwater and estuarine ecosystems due to anthropogenic impacts from global warming, eutrophication, and more frequent extreme rainfall and drought cycles (Chapra et al., 2017; Paerl and Huisman, 2008). Cyanobacterial genera such as *Aphanizomenon*, *Dolichospermum*, and *Microcystis* are the frequent focus of CHAB studies due to their production of cyanotoxins and other metabolites (Buratti et al., 2017), which are linked to adverse human health outcomes (Carmichael and Boyer, 2016; Fujiki and Suganuma, 2012; Hernandez et al., 2022; Wood, 2016). CHABs typically bloom in summer when environmental conditions favor cyanobacteria to other aquatic microalgae (Paerl and Huisman, 2008), but cyanotoxin production is spatiotemporally inconsistent. This poses a significant public health challenge from CHABs, as it is difficult to forecast toxin occurrence and potential exposures (Bullerjahn et al., 2016). People, wildlife, and pets can be exposed to cyanotoxins through the ingestion of contaminated drinking water or food, dermal contact during swimming, and inhalation of aerosolized cyanotoxins, which is the most understudied pathway of exposure (Funari and Testai, 2008).

In addition to obvious negative effects on water quality, CHABs impact air quality via emissions carrying cyanobacterial cells and cyanotoxins (Backer et al., 2010; Murby and Haney, 2016; Olson et al., 2020). Cyanobacterial cells and their toxins, like other aquatic microbes and their compounds, are emitted to the atmosphere when air bubbles burst at the water surface via wind stress, breaking wave action, or physical disturbances from human activities such as boating (Backer et al., 2008, 2010; Prather et al., 2013; Quinn et al., 2015; Sharma et al., 2006b). At present, it is unclear to what extent airborne CHAB cells and CHAB-derived biogenic compounds are impacting respiratory health (Plaas and Paerl, 2021). Beyond exposure to cyanotoxins in general, the inhalation of particulate matter with aerodynamic diameters ≤ 2.5 μm ($PM_{2.5}$) also poses significant health risks, regardless of aerosol composition (Laden et al., 2006; Tomczak et al., 2016; Wang et al., 2017).

The composition of $PM_{2.5}$ varies regionally, but diverse lines of evidence suggest that CHABs can influence aerosol composition and concentration. Several toxigenic cyanobacterial genera sourced from waterbodies have been identified in aerosol during variable environmental conditions (Lewandowska et al., 2017; Sharma et al., 2006b, 2006a; Wiśniewska et al., 2022). Three cyanotoxins have been reported in aerosol, including microcystins (MC) on numerous occasions (Backer et al., 2008, 2010; Gambaro et al., 2012; Murby and Haney, 2016; Wood and Dietrich, 2011; Yung et al., 2007), nodularin (Wood and Dietrich, 2011) and most recently, anatoxin-a (Sutherland et al., 2021). Other studies determined that cyanobacteria increase biochemical tracers in spray aerosol (May et al., 2018) and aerosol number concentration in respirable size fractions (Olson et al., 2020).

Much research remains to better characterize the conditions which promote the emission of CHAB-derived aerosol and subsequent human health effects. Accordingly, the aims of this study were to: 1) investigate the occurrence of MC and putatively toxic cyanobacterial communities in $PM_{2.5}$, 2) elucidate the environmental conditions promoting their aerosolization, and 3) identify associations between CHAB events and $PM_{2.5}$ concentrations in the airshed of the Chowan River-Albemarle Sound, North Carolina, USA.

The Chowan River-Albemarle Sound is a eutrophic estuary in coastal North Carolina. This region is ideal to study CHAB-derived aerosol because its fetch spans up to 14 miles in some locations, promoting wind-driven turbulence and breaking wave action. It has faced recurrent CHABs since the 1980's (Stanley and Hobbie, 1981) with recent high occurrences of MC in association with *Microcystis* blooms. In summer 2019, the North Carolina Department of Environmental Quality reported MC concentrations up to 620 μg L^{-1} in some recreational locations (North Carolina Department of Human Health Services, 2019), exceeding USA Environmental Protection Agency recreational water guidelines by >60 times (U.S. Environmental Protection Agency et al., 2019). Further, a recent study found that counties surrounding the estuary experience amongst the highest asthma related emergency departments visits per year in North Carolina (Dieu et al., 2018), suggesting poor air quality in the region.

2. Material and methods

In summer 2020, from June 11th to October 1st, a field campaign was conducted on the Chowan River-Albemarle Sound. Continuous $PM_{2.5}$ samples and interval water samples were collected for targeted analyses of bacterial community composition and MC concentration. Supporting air and water quality measurements were made in parallel for statistical analyses of environmental factors driving changes in CHAB-derived aerosol. Detailed information on data cleaning, quality assurance, and statistical methods using RStudio can be found in the Supplementary material and found at https://github.com/haleyplaas/CR-AS_2020/blob/v0.1/CR-AS%20data%20cleaning%2C%20stats%2C%20figures.R.

2.1. Study sites

Two sites were selected along the Chowan River-Albemarle Sound; Site A was located on the open, upper Chowan River (36.212593 N, 76.715342 W) and Site B near the western most part of the Albemarle Sound in an embayment near the town of Edenton (36.057583 N, 76.620208 W) (Fig. 1B). Sites were chosen because they were known CHAB hotspots adjacent to recreational and residential areas where people are more likely to be exposed CHAB aerosol, and because they were secure areas with electrical power required for autonomous operation of the aerosol sampling equipment (Fig. 1C).

2.2. Field collection methods

2.2.1. Aerosol sampling

$PM_{2.5}$ was sampled at each site for 12 h daily via two, high-volume $PM_{2.5}$ samplers (Tisch Environmental, Polyurethane Foam Sampler, Model #1000D-BL) (Fig. 1C), which were pre-calibrated and ran autonomously from 07:00–19:00 universal time daily, except for August 3rd to August 12th, when samplers were stored indoors to protect against Hurricane Isaias. $PM_{2.5}$ was size fractionated via a cyclonic inlet and impacted onto 102 mm pre-combusted quartz fiber filters (2 μm pore-size).

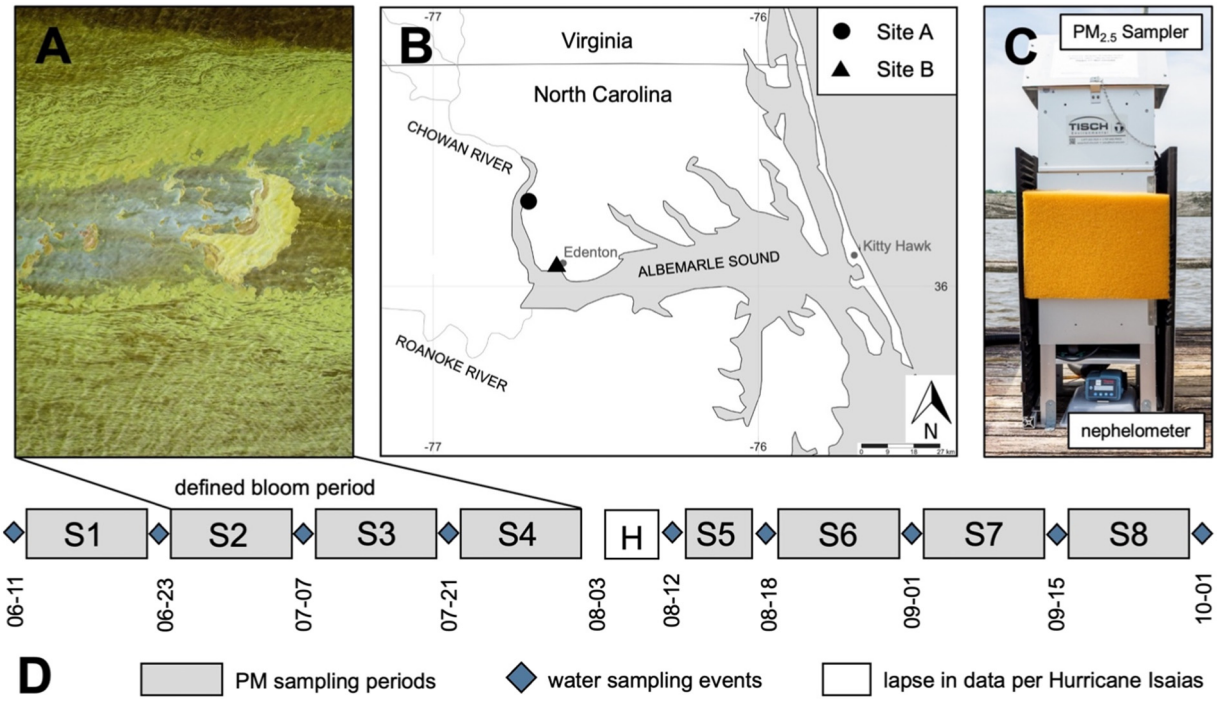

Fig. 1. A) A drone-based image of the CHAB surface scum on July 7th (during S2–4). B) A map of the Chowan River-Albemarle Sound sites where samples were collected. This map was created with https://www.simplemappr.net. C) The $PM_{2.5}$ equipment set-up. The blue nephelometer was housed underneath the high-volume $PM_{2.5}$ sampler. D) A schematic demonstrating the data collection timeline, with $PM_{2.5}$ sampling periods spanning 8–14 days and water sampling occurring in between. The bloom period was defined as S2–4. Data was not collected from August 3rd to 12th, 2020, per the storage of all equipment during Hurricane Isaias, which made landfall on August 4th, 2020 in North Carolina.

Single $PM_{2.5}$ samples were integrated over two-week periods on filters; finer scale variation was not resolved in attempt to meet analytical detection limits for MC and DNA. These bi-weekly periods were denoted as aerosol sampling periods (S1–8) and ranged from 8 to 14 days (Fig. 1D). Between sampling periods, filters were replaced, data were downloaded, filter cassettes were cleaned with alcohol wipes, and a dynamic field blank was collected in a field cassette protected by an antistatic bag. $PM_{2.5}$ samples were removed from filter cassettes in a portable glove box, stored in pre-combusted aluminum foil packets, transported to the lab, and stored at −80 °C until extraction.

Alongside $PM_{2.5}$ sampling, real-time $PM_{2.5}$ mass concentrations were measured with a nephelometer with a $PM_{2.5}$ size-selective inlet cyclone (Thermo Scientific MIE pDR-1500, Model #105983-00). Nephelometers operated continuously beneath each high-volume sampler (Fig. 1C) and 60-second readings were quality assured and reduced to 5-minute averages. Meteorological data were recorded via a single Davis Weather Station (Precision Water Station Vantage Pro2, Model #6162) at Site B and readings were acquired as 5-minute averages. Meteorological parameters recorded included atmospheric pressure, air temperature, cumulative precipitation, relative humidity, solar irradiance, and wind direction and speed.

2.2.2. Water sampling

At the start and end of each $PM_{2.5}$ sampling period (Fig. 1D), water was collected from the top 0.3 m of the water column at each site. Water was collected prior to 09:00 AM and dispensed into pre-cleaned carboys for later processing. Water samples were vacuum filtered onto Whatman glass fiber filters (0.7 μm pore-size) in triplicate and stored according to specific methods for each variable, including particulate and soluble nutrients and MC. Single plankton biomass samples were collected via the same filtration method on SUPOR polyethersulfone filters (0.2 μm pore-size) for DNA extraction. Water samples were transported to the lab and stored at −20 °C until extraction. At the time of water sampling, real-time water conditions were recorded at depths between 0.3 and 1.0 m (maximum depth at either site) with a multi-probe sonde (YSI 6600, Yellow Springs Inc., Ohio). Parameters recorded included dissolved oxygen concentration, pH, salinity, turbidity, and water temperature.

2.3. Analytical methods

2.3.1. Nutrient determination

Soluble and particulate nutrients in water samples were analyzed via flow injection (Lachat Instruments QuickChem 8000) for dissolved nutrient concentration, including nitrate plus nitrite, ammonium, soluble reactive phosphate, silicate, and total dissolved nitrogen. Filters were analyzed for particulate and intracellular nutrients including particulate organic carbon and particulate nitrogen using high-temperature combustion (Costech ECS 4010 analyzer). Detailed method numbers and standard protocols for nutrient analyte quantitation can be found in (Paerl et al., 2020).

2.3.2. Bacterial community composition determination

DNA was extracted from $PM_{2.5}$ filters and SUPOR filters with biomass from near-surface waters using a phenol chloroform extraction method (Djurhuus et al., 2017). Amplified segments of DNA (amplicons), were generated from extracts via polymerase chain reaction using primers targeting the v4-v5 region of the 16S rRNA gene (515F and 926R) (Naqib et al., 2018; Parada et al., 2016; Quince et al., 2011). Amplicons were sequenced using the Illumina platform (MiSeq 300 PE; UIC Genome Research Core) and analyzed in RStudio. Amplicon sequence variants (ASVs), i.e., individual amplicon sequences representing individual populations, were determined and taxonomically assigned using the 'dada2' algorithm. Four ASVs of high interest (0108, 0196, 0389, and 1999) could not be assigned beyond the genus level using 'assignTaxonomy' in 'dada2', and were instead taxonomically assigned using an online BLASTn search (Altschul et al., 1990) against the NCBI NR/NT database. Contaminant ASVs identified on quartz fiber filter field blanks were completely removed from analysis, but contaminant ASVs on extraction blanks were removed via 'decontam' (Davis et al., 2018). ASV abundance (counts) were normalized using cumulative sum scaling (CSS) and visualized via 'phyloseq' (McMurdie and Holmes, 2013)

at several taxonomic levels. A detailed workflow of DNA sequencing and ASV table cleaning can be found in the Supplementary material.

2.3.3. Microcystins quantification

$PM_{2.5}$ and water samples were analyzed for concentrations of eight congeners of MC. MC was extracted via sonication in methanol and quantified using reverse phase high pressure liquid chromatography coupled with triple quadrupole mass spectrometry (LC-MS) via methods adapted from Gambaro et al. (2012). Detailed extraction specifications and instrument method settings can be found in Gaston et al. (2021). Leucine enkephalin acetate (Sigma Aldrich L9133) was used as an internal standard to measure extraction/ionization efficiency and signal. MC congeners were quantified from a calibration curve generated from a dilution series of commercial MC standards (Enzo Life sciences). Final MC concentrations were reported as individual congeners and as bulk MC, calculated by adding the values of each congener. The eight MC congeners analyzed and limits of detection specific to each run and congener and can be found in the Supplementary material but ranged between (0.1–0.9 ng L^{-1} or 0.1–0.9 μg m^{-3}).

2.4. Statistical analyses

To directly compare interval water samples and continuous meteorological readings with integrated $PM_{2.5}$ samples, the environmental data were transformed. To estimate water conditions during each $PM_{2.5}$ sampling period, water samples collected at the start and end were combined and averaged, e.g., MC concentration measured on June 11th and June 23rd was averaged to determine a representative MC value for S1. Robustness tests were conducted using start-only and end-only (interval) water sample values to ensure statistical findings were consistent with the averaged values (results in the Supplementary material). For continuous $PM_{2.5}$ concentration and meteorological condition measurements other than wind, average daily values were calculated from diurnal measurements (07:00 to 19:00 universal time), and final values were determined by averaging daily averages across each $PM_{2.5}$ sampling period. Wind speed and direction measurements were vectorized into east-west and north-south components from which overall air mass movement was calculated, and these values were also averaged across each sampling period.

To evaluate the variable aerosolization of cyanobacterial genera from water to $PM_{2.5}$, aerosolization factors (AF) were determined by calculating the ratios of CSS normalized ASV abundance (counts) in air to water (Harb et al., 2021; Michaud et al., 2018).

$$\text{Aerosolization Factor (AF)} = \frac{ASV\ abundance\ in\ PM_{2.5}\ sample}{ASV\ abundance\ in\ water\ sample}$$

Only cyanobacterial ASVs that occurred in both water and air samples were used to calculate AFs, because it could be inferred that an ASV's presence in both media indicated its water to air transfer. While it is also possible that these ASVs were transferred from air to water, we find this unlikely because the subspecies identified are known aquatic cyanobacteria (Whitton and Potts, 2002). To compare differential aerosolization potentials, a pairwise-Wilcoxon signed rank test was used to evaluate statistical differences between AF values for each cyanobacterial subspecies, site, and sampling period.

To evaluate associations between CHAB-derived aerosol and environmental conditions, a series of univariate linear regressions was conducted. Four CHAB-derived aerosol outcomes including 1) all cyanobacterial abundance in $PM_{2.5}$, 2) *Dolichospermum* abundance in $PM_{2.5}$, 3) *Microcystis* abundance in $PM_{2.5}$, and 4) $PM_{2.5}$ mass concentrations, were evaluated as a function of individual environmental predictor variables during each sampling period (300 models total, n = 9–14). A comprehensive list of the environmental predictor variables examined can be found in the Supplementary material.

To investigate the relationship between ambient $PM_{2.5}$ and bloom activity, another Wilcoxon test was used to evaluate changes to median $PM_{2.5}$ mass concentrations between defined bloom and non-bloom periods. We define the CHAB as occurring during S2–S4. It is challenging to track precise start and end dates for CHABs due to the complex ecophysiology and spatiotemporal heterogeneity of blooms (Erdner et al., 2008; Wilkinson et al., 2019), but this timeframe was chosen due to the heightened abundance of *Dolichospermum* in water samples during S2–4 when compared to other sampling periods (Fig. 4). Visible cyanobacterial colonies became apparent in the surface waters during S2 (Fig. 1A) but were presumably dissipated by the hurricane following S4. During S2 *Dolichospermum* was identified via microscopy as the primary CHAB genera on site and later confirmed via 16S sequencing. Further justification of the defined bloom period can be found in the Supplementary material.

3. Results and discussion

3.1. Microcystin congeners in $PM_{2.5}$ and water samples

Throughout this study, all quantifiable MC concentrations in Chowan River-Albemarle Sound water samples remained low (<1 μg L^{-1}), which is likely explained by the relatively low abundance of *Microcystis* in water samples, as explored in Section 3.2. Accordingly, no MC was detected in any $PM_{2.5}$ sample, but in water samples, three distinct congeners of MC were quantified during five sampling days, including MC-Leucine Alanine (LA), -Leucine Arginine (LR), and -Leucine Phenylalanine (LF) (Table 1). Bulk MC peaked during the suspected exponential growth phase of the CHAB (S2). MC-LA was most frequently reported at both sites throughout the campaign, MC-LR was detected at Site A during S2–S3, and MC-LF was detected at Site B between S3–S4 (Table 1). Although no MC was quantified in $PM_{2.5}$, it is notable that MC-LA was the most abundant congener detected in water samples, as previous research found that MC-LA has the highest AF for all MC congeners (Olson et al., 2020). MC-LA is nearly 2.5 times as likely to be enriched in aerosol compared to MC-LR, and 200 times as likely compared to less hydrophobic congeners like MC-RR (Olson et al., 2020).

3.2. Comparison of $PM_{2.5}$ and aquatic bacterial communities

3.2.1. Amplicon sequencing results

In $PM_{2.5}$ and water samples, 3423 and 4854 distinct ASVs were respectively detected. Of these, 3222 were unique to $PM_{2.5}$ and 4654 were unique to water, but 201 ASVs occurred in both $PM_{2.5}$ and water samples, suggesting transfer of select bacterial communities between media. Of these 201 ASVs, 15 were assigned to the taxonomic class of cyanobacteria. The relative abundance of cyanobacteria in $PM_{2.5}$ samples ranged from 0.34 to 11.9 % of the 16S bacterial community, with the maximum occurring during S2 at Site B. Several CHAB genera were identified in $PM_{2.5}$ samples, including *Anabaena, Aphanizomenon, Dolichospermum, Microcystis,* and

Table 1
Calculated MC concentration (ng L^{-1}) by congener and site during each sampling period.

Sampling period	Site	Bulk MC PM2.5 (ng m^{-3})	Bulk MC water (ng L^{-1})	MC-LA (ng L^{-1})	MC-LF (ng L^{-1})	MC-LR (ng L^{-1})
S1	A	0	2.07	2.07	0	0
	B	–	6.36	6.36	0	0
S2	A	0	10.66	2.07	0	8.6
	B	0	6.36	6.36	0	0
S3	A	0	8.6	0	0	8.6
	B	0	1.36	0	1.36	0
S4	A	0	0	0	0	0
	B	0	1.36	0	1.36	0
S5	A	0	0	0	0	0
	B	0	3.57	3.57	0	0
S6	A	–	0	0	0	0
	B	0	3.57	3.57	0	0
S7	A	0	0	0	0	0
	B	0	0	0	0	0
S8	A	0	1.93	1.93	0	0
	B	0	7.31	7.31	0	0

Pseudanabaena, but most cyanobacteria in $PM_{2.5}$ were unable to be assigned a genus (Fig. 2). $PM_{2.5}$ cyanobacterial community composition was highly variable between sites and sampling periods, but in water samples, *Cyanobium* dominated the cyanobacterial community. Although, during the CHAB, the abundance of *Dolichospermum* increased considerably, along with *Microcystis, Aphanizomenon,* and *Caenarcaniphilales,* a non-phototrophic genus related to cyanobacteria (Monchamp et al., 2019) (Fig. 2).

No apparent associations were observed between the abundance of individual cyanobacterial genera in water and $PM_{2.5}$ samples, suggesting that CHAB abundances in water are poor predictors of those in the air. This finding agrees with previous research which found complex relationships between dynamic aquatic microbial activity, surface microlayer composition, and sea spray aerosol formation (Michaud et al., 2018; Tumminello et al., 2021; Urbano et al., 2011; Wang et al., 2015). Several combined environmental conditions more likely to drive CHAB aerosolization rates when compared to CHAB genera abundances in surface waters.

3.2.2. Enrichment of cyanobacteria in $PM_{2.5}$

Of the fifteen cyanobacterial ASVs that occurred in both $PM_{2.5}$ and water samples, no single cyanobacterial subspecies (ASV) was determined to be more likely enriched in aerosol when compared to others (Fig. 3), based on a pairwise Wilcoxon test comparing AF between genera ($p > 0.1$). However, the pairwise test did confirm significant differences between AFs pooled across sites ($p < 0.001$) and select sampling periods (S2 was distinct from S3 and S5; S3 was distinct from S2, S5 and S7, $p < 0.05$). The difference in AF values between sites might be explained by the geographic location of Site B compared to Site A. Site B sits near the mouth of the Albemarle Sound, and it is possible that the aerosol sampler at Site B more frequently collected cells aerosolized from the turbulent Albemarle Sound, where spray aerosolization is more likely compared to upriver.

The AF of nearly every ASV examined was elevated at Site B during S2 at the beginning of the CHAB. However, when the bloom was still ongoing, AF values were largely neutral during S3, and only elevated for some ASVs at Site B during S4 (Fig. 3). We speculate that the significant difference between S2 and S3 might be explained by S2 coinciding with the exponential growth phase of the CHAB (Fig. 3). During this phase, cells exhibit increased production of cyanotoxins and other metabolites such as extracellular polymeric substances or lipopolysaccharides (Gemma et al., 2016; Harland et al., 2015; Merel et al., 2013). MC occurred at both sites during S2 and many of the CHAB genera recorded during the bloom (e.g., *Dolichospermum*, *Microcystis, Aphanizomenon,* etc.) are known to produce a suite of metabolites, which could have influenced AF. Cyanobacterial ASVs other than CHAB genera (e.g., *Cyanobium*) were also more enriched in aerosol at Site B during S2 when compared to other sampling periods (Fig. 3), suggesting some environmental condition not captured by our study design, possibly water chemistry, promoted aerosolization at this site during this period. Cyanobacterial production of metabolites other than MC could have occurred during this period, influencing the exterior properties of cells and subsequent spray aerosolization potential. More research must be conducted to examine the possible

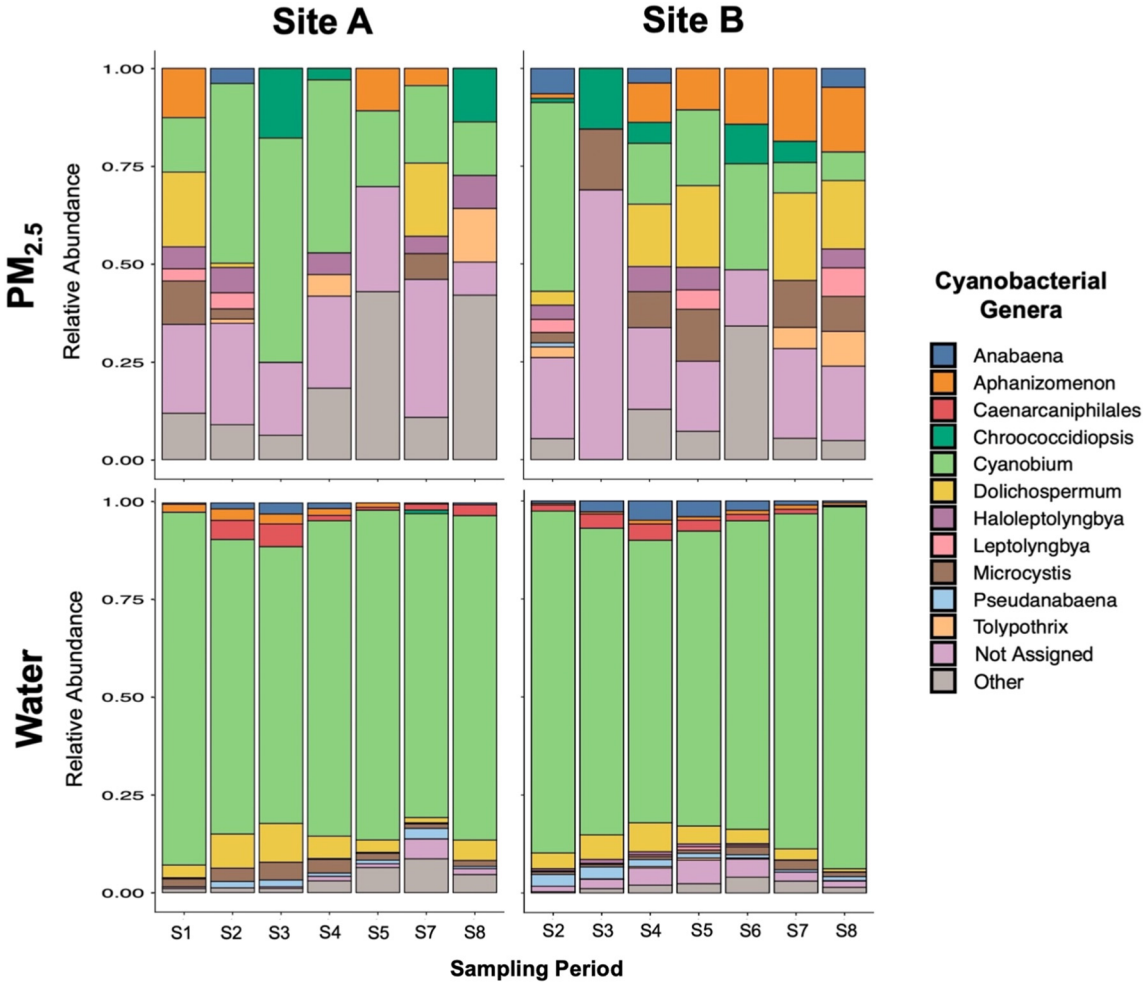

Fig. 2. The relative abundance of cyanobacterial genera detected in 16S rRNA gene libraries from $PM_{2.5}$ and water samples, relative to cyanobacterial genera. Samples are grouped by sampling period, media, and site as denoted. Class level relative abundance data and relative abundance data for each water sampling interval are provided in the Supplementary material.

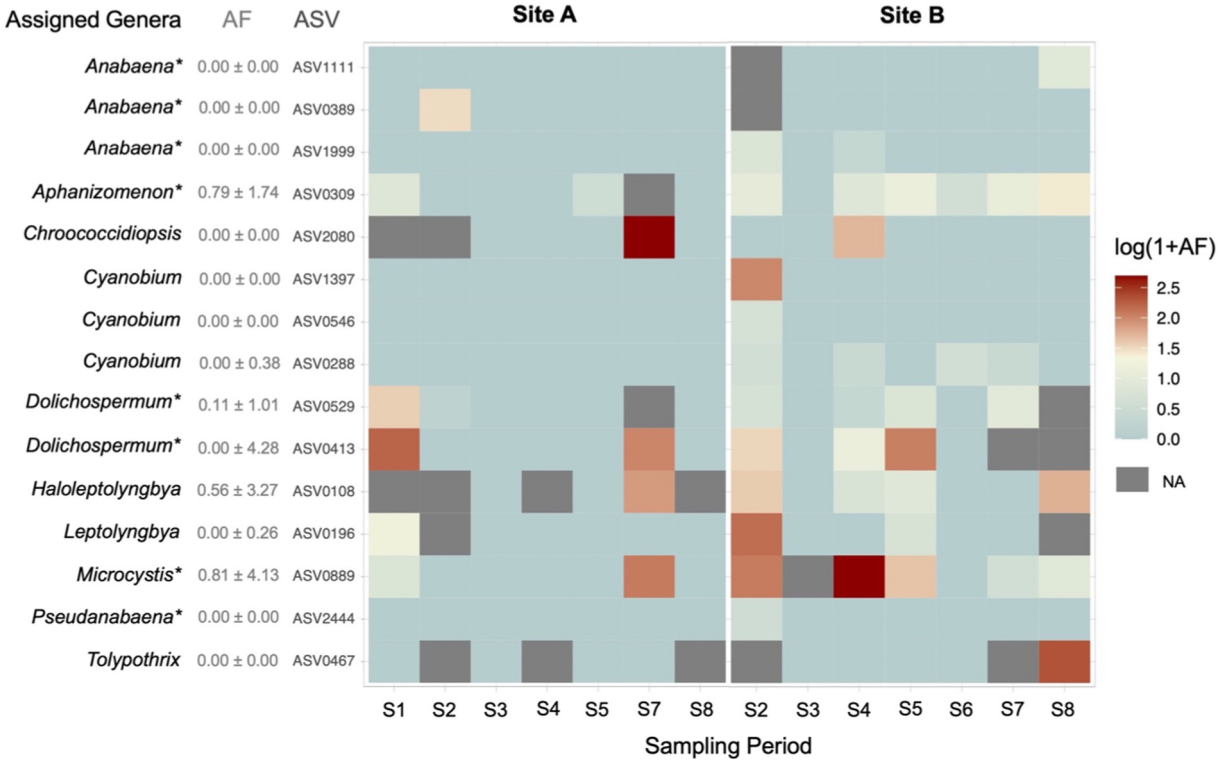

Fig. 3. A heatmap, showing transformed aerosolization factors (AF) for each cyanobacterial ASV identified in both water and $PM_{2.5}$ samples. The specific ASV, median AF with interquartile range, and assigned genus are denoted on the Y-axis. The sampling period (ranging from 1 to 8) and specific site (A or B) for each value are denoted on the X-axs. There were several instances where ASVs were not recorded in either water or $PM_{2.5}$ samples, thus for improved visualization, AF values were modified by +1 and log transformed. AF with no denominator (no identification of the given ASV in water for that event), appear as gray NA values. Putatively toxic genera are denoted with an *.

role of bloom-state and cyanobacterial metabolite production in CHAB aerosolization.

Cyanobacterial ASV enrichment in $PM_{2.5}$ was ultimately indistinct between subspecies, but how genera-specific properties influence the aerosolization and atmospheric fate of aquatic cyanobacteria should be explored further. Although statistically non-significant, we found that *Microcystis* was most often enriched in aerosol when compared to other cyanobacterial genera. Its median AF was highest across the entire campaign (Fig. 3) and during any single sampling period, which also coincided with the bloom (S4; AF = 13.72). This observation might be explained by the persistent presence of *Microcystis* in the surface microlayer at the air-water interface. The presence of gas vesicles and large colony size enable *Microcystis* colonies to achieve buoyancy velocities higher than most other cyanobacteria (Reynolds et al., 1987). This ecological advantage which affords *Microcystis* cells access to more sunlight might also promote more interaction with entrained air bubbles for subsequent aerosolization. We suspect a combination of environmental variables altering cell physicochemical properties such as cell morphology, hydrophobicity, and water column distribution might explain the differential AFs observed between subspecies. More work must be conducted to determine the abiotic and biotic factors that most strongly influence aerosolization differential between CHAB genera if any.

Table 2
Select adjusted R^2 values from the univariate linear regression models, evaluating various CHAB-derived $PM_{2.5}$ as a function of individual environmental predictor variables (CHAB aerosol ~ environmental predictor). The environmental predictor variables shown in this table were determined to have a statistically significant ($p < 0.05$) association with at least one of the outcomes variables. All associations are positive unless otherwise noted. A comprehensive list of predictor variables tested, regression visualizations, and summary statistics for each significant model, along with robustness test results, can be found in the Supplementary material.

	Environmental Predictor Variables						Cyanobacterial Genera Abundances									
$PM_{2.5}$ Outcome Variable	**Air Temperature**	**Ammonium**	**C:N molar ratio in water**	**$PM_{2.5}$ Mass Concentration**	**Relative Humidity**	**Solar Irradiance**	***Aphanizomenon* in $PM_{2.5}$**	***Cyanobium* in $PM_{2.5}$**	***Dolichospermum* in $PM_{2.5}$**	***Haloleptolyngbya* in $PM_{2.5}$**	***Leptolyngbya* in $PM_{2.5}$**	***Microcystis* in $PM_{2.5}$**	**Unassigned Cyanos in $PM_{2.5}$**	***Caenarcaphilales* in water**	***Dolichospermum* in water**	***Leptolyngbya* in water**	***Pseudanabaena* in water**
Relative Abundance of Cyanobacteria in $PM_{2.5}$	-	-	-	*0.37	-	-	-	***0.94	-	***0.80	***0.80	***0.68	***0.96	-	-	-	-
Relative Abundance of *Dolichospermum* in $PM_{2.5}$	-	*†0.24	-	-	-	-	***0.71	-		-	-	***0.90	-	-	-	*0.22	-
Relative Abundance of *Microcystis* in $PM_{2.5}$	-	-	-	-	-	-	**0.56	-	***0.90	-	*0.03		-	-	-	*0.32	-
$PM_{2.5}$ Mass Concentration	***0.75	-	**†0.52		**†0.51	**0.68	-	-	-	*0.27	-	-	-	*0.24	*0.36	-	*0.33

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, - indicates a non-significant relationship, † indicates an inverse relationship

3.3. Environmental drivers of CHAB-derived aerosol

3.3.1. Environmental drivers of CHAB ASVs in $PM_{2.5}$ samples

Several environmental parameters were found in association with CHAB-derived $PM_{2.5}$. A complete list of the regression outputs can be found in the Supplementary material, but the environmental conditions determined to be significant are found in Table 2. When cyanobacterial abundance increased in $PM_{2.5}$, so did the abundance of *Cyanobium*, *Haloleptolyngbya*, and *Leptolyngbya*, demonstrating that several aquatic cyanobacteria contribute to an increased abundance of airborne cyanobacteria (Table 2). *Cyanobium* was most consistently identified in $PM_{2.5}$ when compared to other cyanobacterial genera, which might be explained by its frequent presence in the water or its small size, allowing it to more easily aerosolize and/or stay suspended as aerosol. Aerosol morphology is one factor well-known to impact aerosol lifetime and processing (Pham et al., 2017; Valsan et al., 2015; Zhang et al., 2008), and filamentous, colonial, and pico- cyanobacteria likely have different properties in aerosol.

The abundance of *Dolichospermum* increased with both *Aphanizomenon* and *Microcystis* abundances in $PM_{2.5}$ (Table 2). Their co-occurrence in $PM_{2.5}$ further suggests that environmental and ecophysiological conditions influence the aerosolization of these CHAB genera simultaneously. It is well known that wind speed and direction interact to influence the formation and detection of spray aerosol from large waterbodies (Olson et al., 2019; Revell et al., 2021), but interestingly, wind vectors were not significantly correlated with any cyanobacterial abundances in $PM_{2.5}$. Throughout the study, winds prevailed primarily from the south with variability between southwest-southeast, as expected on the USA east coast during summer months. Although not statistically confirmed, southerly winds (169–191° weather wind direction), contributed most prominently to elevated $PM_{2.5}$ mass concentrations, and when winds blew from the southeast (113°–158°), *Dolichospermum* abundances in $PM_{2.5}$ were higher. A similar trend was observed for *Microcystis* in $PM_{2.5}$, suggesting spray aerosol formation most often coincided with southeasterly winds. This observation could be explained by geography as the Albemarle Sound is positioned southeast of the sites (Fig. 1B). Although a southeasterly wind was not directly onshore for either site, such winds spanned a larger fetch across the Albemarle Sound, increasing the distance across which spray aerosol could be formed and possibly increasing the abundance of airborne CHAB cells.

3.3.2. CHAB associations with ambient $PM_{2.5}$ mass concentration

Several meteorological variables well known to influence $PM_{2.5}$ mass concentration including air temperature, solar irradiance, relative humidity, and precipitation were associated with ambient $PM_{2.5}$ mass concentration (with relative humidity and precipitation being inverse) (Table 2). $PM_{2.5}$ was negatively associated with C:N molar ratio in water samples, suggesting that water chemistry and/or nutrient availability impacted aerosol emissions from the surface water.

The abundance of several cyanobacterial genera in water samples were associated with elevated $PM_{2.5}$ mass concentrations, including *Dolichospermum*, *Caenarcaphilales*, and *Pseudanabaena* (Table 2), suggesting that something associated these genera specifically were a source of aerosol. $PM_{2.5}$ mass concentration increased in association with the CHAB occurring during S2–S4; a time-series of the continuous $PM_{2.5}$ concentration readings revealed an increase in the baseline $PM_{2.5}$ during bloom conditions (Fig. 4B). A Wilcoxon signed rank test confirmed this trend statistically, demonstrating a change to median $PM_{2.5}$ mass concentration in response to the CHAB ($W = 1835$, $p < 0.001$). During the non-bloom period, defined as S1 and S5–S8, the median $PM_{2.5}$ concentration was 5.35 μg m^{-3} (IQR = 3.70), but during the bloom, this value increased to 8.97 μg m^{-3} (IQR = 5.15) (Fig. 4A).

Given there was no apparent association between the abundance of individual CHAB genera in $PM_{2.5}$ and water samples, and that no MC was quantified in $PM_{2.5}$, there are likely other unexplored sources of primary aerosol emitted from CHABs that might explain the increase in $PM_{2.5}$ mass concentrations, e.g., cyanotoxins other than MC and secondary metabolites like lipopolysaccharides (Buratti et al., 2017; Gemma et al., 2016; Swanson-Mungerson et al., 2017). Further, another source of $PM_{2.5}$ linked to the CHAB could be secondary organic aerosol (SOA) derived from cyanobacterial volatile organic compound (VOC) emissions. Several studies have observed SOA formation from VOC emissions by marine phytoplankton (Hu et al., 2013; Montoya-Aguilera et al., 2017; Schneider et al., 2019), and over the open ocean, marine phytoplankton activity and aerosol optical density have been linked (Dasarathy et al., 2021; Sanchez et al., 2021; Yu and Li, 2021). CHABs including *Microcystis* are demonstrated to increase VOC concentration in the airshed of eutrophic waterbodies (Liu et al., 2021; Xu et al., 2017; Ye et al., 2018; Zuo et al., 2018), and a recent study found that emissions from marine cyanobacterial blooms in the Baltic Sea are linked to aerosol nucleation events (PM_1) (Thakur et al., 2022).

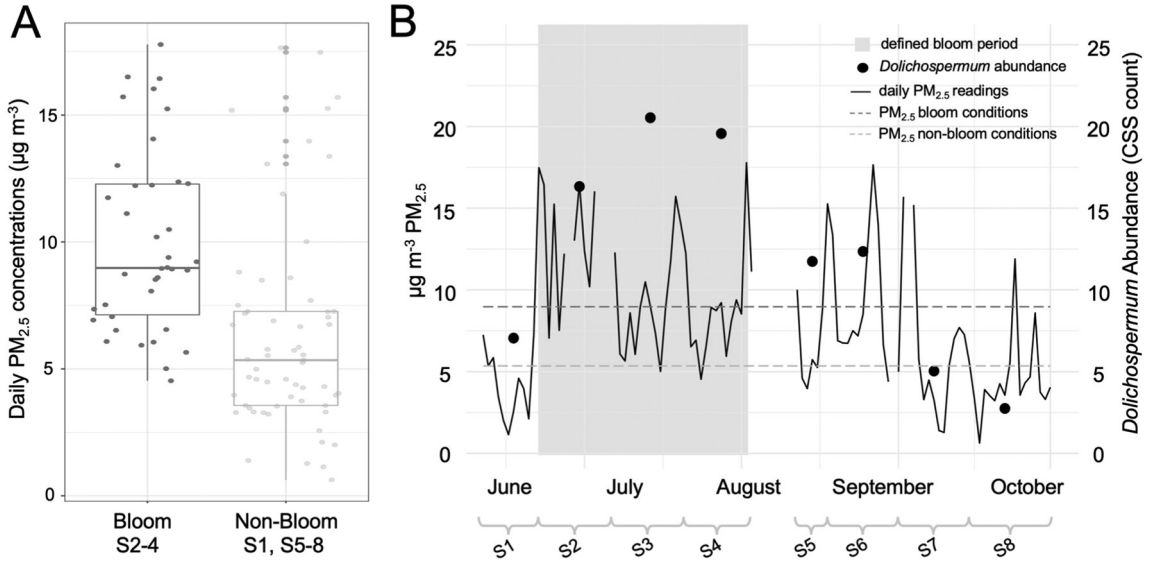

Fig. 4. A) The daily median $PM_{2.5}$ concentrations recorded across the study, grouped by the CHAB event. B) A time-series of daily $PM_{2.5}$ mass concentration readings, plotted with the average abundance of *Dolichospermum* in water samples, averaged across both sites during each sampling period (S1–S8). Median values for $PM_{2.5}$ readings were calculated for both non-bloom (5.35 μg m^{-3}) and bloom (8.97 μg m^{-3}) periods and are plotted as dashed lines. The bloom period (S2–4) is shaded in gray.

Volatile taste and odor compounds emitted by cyanobacteria such as geosmin, 2-methylisoborneol, β-cyclocitral, etc., may also be potential sources of SOA. Due to their chemical structures as terpenes, they are potential candidates for the formation of SOA via atmospheric oxidation (Bernard et al., 2016; Holopainen et al., 2017), but this has not been confirmed in situ. *Dolichospermum*, the dominant CHAB genera in this study, is composed of strains capable of producing diverse volatiles, especially geosmin (Watson et al., 2016). Other cyanobacterial metabolites, including volatile ones, should be investigated as a possible source of aerosol and subsequent respiratory distress or inflammation. Much work remains to characterize the production, ecological functions, and environmental fate of VOCs and potential SOA produced by freshwater cyanobacteria and algae (Achyuthan et al., 2017; Meredith and Tfaily, 2022; Sauer et al., 2021).

4. Conclusions

This study presents new evidence suggesting that freshwater CHABs are associated with elevated $PM_{2.5}$ mass concentrations. Several CHAB genera recovered from Chowan River surface water were identified in $PM_{2.5}$. *Dolichospermum, Microcystis,* and *Aphanizomenon* were aerosolized during the same conditions, and results suggest that aquatic cyanobacteria may be aerosolized at a higher rate during periods of rapid bloom growth. Future work should aim to characterize the composition of airborne emissions from CHABs more comprehensively, considering the potential presence of cyanobacterial metabolites and their derivatives in aerosol.

Although no aerosolized MC was quantified in this study, our findings do not rule out the possible aerosolization of cyanotoxins in the airshed of the Chowan River. LC-MS is a high-resolution tool to quantify cyanotoxins, but our detection limits may have missed any airborne MC present. It is possible that other cyanotoxins we did not investigate may have also occurred in $PM_{2.5}$ and water samples.

In summary, we present novel evidence suggesting that bloom-forming cyanobacteria impact local $PM_{2.5}$ composition and concentration via the direct emission of CHAB cells. Results underscore the need for highly resolved temporal measurements to conclusively investigate the role that freshwater algae, specifically CHABs, play in regional air quality and subsequent respiratory health risk.

CRediT authorship contribution statement

Conceptualization: H.E.P., K.B., N.S.H., **Formal analysis:** H.E.P., R.W·P., K.B., K.J.P., O.L.M., D.J.M., A.N.B., J.S·B, K.L.R., R.S., **Investigation:** H.E.P., R.W·P., K.B., C.K., M.A.B., N.Y.C., N.P.C., H.H., J.S., **Resources:** R.W.P., K.B., C.K., K.J.P., H.W.P., **Writing – original draft:** H.E.P., R.W.P., **Writing – review & editing:** R.W.P., K.B., M.A.B., N.S.H., J.S., H.W.P., **Supervision:** R.W.P., K.B., N.S.H., H.W.P., **Visualization:** H.E.P., **Project administration:** H.E.P., C.K., H.W.P., **Funding Acquisition:** H.E.P., R.W.P., J.S., H.W.P.

Data availability

Code can be found at the Github linked in the article: https://github.com/haleyplaas/CR-AS_2020/blob/v0.1/CR-AS%20data%20cleaning%2C%20stats%2C%20figures.R. Data can be made available upon request.

Declaration of competing interest

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Appendix A. Supplementary data

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