

Diurnal patterns of airborne algae in the Hawaiian Islands: a preliminary study

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Abstract Although the literature on the diversity of airborne algal communities in various locations around the world is increasing, little is known about their temporal and spatial patterns. We compared airborne algal communities from Honolulu, Hawai‘i, USA, over three 24-h sampling periods to examine diurnal patterns in diversity and abundance. Using a culture-based approach, 192 algal colonies were characterized and identified as 31 operational taxonomic units. A combination of microscopy and Sanger sequencing (of the UPA marker) was used for characterizations. More airborne algal colonies were identified from nighttime collections (127 of 192 colonies) than daytime collections (65 of 192 colonies) ($p < 0.0001$). Similarly, 95% of the daytime collections were Cyanobacteria, and 87% of the nighttime collections were Chlorophyta, and the trends of more Cyanobacteria being collected during the day and more Chlorophyta at night were significant ($p < 0.0001$). Meteorological analyses for the sampling periods indicated that air masses sampled during the three trials consistently arrived in the Hawaiian

Islands on a northeast trade wind pattern, but with different origins in the Pacific Ocean, and that low-to-trace levels of rain fell during the sampling periods. Land breeze and sea breeze effects, which are common temperature-driven phenomena on tropical islands, may have played a role in the diurnal pattern observed in the current study.

Keywords Chlorophyta · Cyanobacteria · Green algae · Trade winds · Universal Plastid Amplicon

1 Introduction

Airborne algae, while increasingly studied in recent years, have long been underrepresented in the aerobiota literature, possibly because airborne algae make up a very small proportion of all aerobiological particles (Després et al. 2012). However, airborne algae have wide-ranging impacts on human health, local economies, and the ecology of the communities that they colonize, making study of these organisms important (Sharma et al. 2007). Human health can be adversely impacted by airborne algae through contamination of drinking water (Sharma et al. 2007). This process is facilitated by lysis of toxin-producing algal cells and the deposition of these toxins into bodies of water, thereby contaminating drinking water, agricultural, or aquacultural water supplies

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(Sharma et al. 2007). Airborne algae can also carry a significant threat of allergenicity, and their inhalation has been widely reported as a causal agent in respiratory disease (Sharma et al. 2007; Genitsaris et al. 2011; Tesson et al. 2016); it has been estimated that humans inhale approximately 1500 algal cells per day (Schlichting et al. 1971), which makes them crucial but understudied components of the aerial microbiome. Of all identified airborne algal taxa considered in a 2011 review of the subject, 15% were reported to be allergy-inducing (38 of 353) or toxin-inducing (14 of 353), with the most common harmful airborne algal genera being *Chlorella* and *Scenedesmus* (Genitsaris et al. 2011). Frequently observed symptoms from airborne algal inhalation include respiratory issues and skin irritation. It is also unclear to what extent airborne algae can act as carriers of other harmful airborne agents, such as radionuclides, heavy metals, pesticides, and carcinogens. Additionally, airborne algae have been reported to allow propagation of certain strains of bacteria, such as *Legionella pneumophila*, that can be a strong risk to human health (Genitsaris et al. 2011).

Airborne algae can have severe economic impacts through degradation of art sculptures or architectural pieces (Sharma et al. 2007). Airborne algae can serve as initial colonizers of terrestrial substrata or marine or freshwater bodies; however, they can also adversely affect aquatic habitats by serving as the depositional source of algal blooms (Sharma et al. 2007). In addition, airborne algae can be used as bioindicators to assess ozone and atmospheric health (Roy-Ocotla and Carrera 1993).

The presence of algae in the atmosphere was first reported in the mid-1800s, when diatoms that were presumed to originate from Saharan Africa were identified from samples of airborne dust collected on board ships (one being the *Beagle*) on the Atlantic Ocean (Darwin 1845). The first known attempts to cultivate airborne algae were published approximately 90 years later from samples collected by airplane at several altitudes over the Netherlands (van Overeem 1936). Airborne algal research in the Hawaiian Islands began in the 1960s with culture studies of airborne algal diversity along a highway transect over the Koʻolau Mountain Range on the island of Oʻahu (Brown et al. 1964; Brown 1971). As of 2011, 353 distinct airborne algal taxa representing 175 genera had been identified worldwide, with the majority

belonging to the Cyanobacteria (37.4%) and Chlorophyta (35.4%) (Genitsaris et al. 2011).

While rainfall, air temperature, humidity, wind speed and direction, source vegetation and site characteristics all impact airborne algal distributions, no single factor is entirely responsible for the dispersal patterns of airborne algae, but rather a combination of these various factors (Sharma et al. 2007). A number of studies have demonstrated that the Cyanobacteria are more common in tropical regions, while the Chlorophyta dominate in temperate regions (Schlichting 1961; Brown et al. 1964; Sharma et al. 2006; Sharma and Singh 2010; Genitsaris et al. 2011; Ng et al. 2011; Sherwood et al. 2017). Short sampling periods of approximately 2–4 h have proven effective for airborne algal sampling, as this time interval minimizes weather and site variation and reduces the chances of damage to either the collecting medium or the algae (Sharma et al. 2007).

Hawaiʻi has been proposed as a model system for the study of airborne algae for a number of reasons. The extreme isolation of the islands (3200 km from the nearest continent) allows airborne patterns to be examined in a context that is relatively free from interference from continental regions (Schlichting 2000). The relatively consistent northeast trade winds (Carson and Brown 1976) also allow for a degree of predictability in studies of Hawaiian airborne algal dispersal. Additionally, tropical areas, such as the Hawaiian Islands, have greater airborne algal diversity and abundance than temperate or polar areas (Schlichting 1974). More recently, however, studies have demonstrated climatic changes in wind and temperature patterns that emphasize the need to study how wind dispersal can construct and affect microbial communities in these isolated islands (Mahowald 2011; Garza et al. 2012).

Many groups of algae are known to exhibit cryptic diversity. This issue is compounded for the microbial forms that can become airborne, rendering their identification based on morphological features unreliable (Tesson et al. 2016). DNA sequencing has been enthusiastically embraced by the algal community, resulting in an explosion of DNA sequence data for algae; yet, a declining proportion of these sequences available in public databases have been assigned to a Latin binomial (De Clerck et al. 2013). Few species of algae can be confidently identified using a single molecular marker for several reasons: (1) the lack of a

well-populated and well-curated reference dataset and (2) the lack of a single DNA barcode that effectively delimits algal species across their breadth of phylogenetic diversity. Despite a long history of study of airborne algae, only a few investigations thus far have employed molecular methods of identification (but see Woo et al. 2013; Genitsaris et al. 2014; Sherwood et al. 2014a, 2017 for exceptions). The first study to apply molecular identification techniques to Hawaiian airborne algae (Sherwood et al. 2014a) investigated diversity along a transect across the Ko'olau Mountain Range of O'ahu, modeling the study design of Brown (1971). Sherwood et al. (2014a) employed morphological observations and Sanger sequencing (of the UPA marker, 18S rRNA gene, and 16S rRNA gene) from cultures and noted that 97% of their airborne algal operational taxonomic units (OTUs) were previously unreported in Hawai'i; this despite a recent and large effort to characterize the non-marine algal diversity of the Hawaiian Islands (e.g., Sherwood et al. 2012, 2013, 2014b). The UPA marker (Sherwood and Presting 2007) has been used a number of times in surveys of marine, freshwater, and airborne algal communities (Conklin et al. 2009; Steven et al. 2012; Sherwood et al. 2014a, b; Letendu et al. 2014; Yoon et al. 2016; Sherwood et al. 2017). Benefits of the UPA marker for algal DNA barcoding or metabarcoding purposes include its length (slightly less than 400 base pairs, in most taxa) and its ability to be simultaneously amplified and sequenced from Cyanobacteria and eukaryotic algae (Presting 2006; Sherwood and Presting 2007), allowing the characterization of the majority of the algal community.

Few studies have explicitly examined diurnal patterns in the dispersal of airborne algae. However, some researchers have noted diurnal patterns as a subset of larger studies examining a wide range of meteorological factors, although these have yielded inconclusive or conflicting results (Tormo et al. 2001; Schlichting 1961, 1964; Smith 1973). Here, we examine diurnal patterns in diversity and abundance of airborne algae on the island of O'ahu, Hawai'i, USA, over three 24-h sampling intervals.

2 Materials and methods

2.1 Sample collection

Samples of airborne algae were collected during three 24-h periods (June 14–15, 2017, June 18–19, 2017, and June 21–22, 2017) with collection periods extending from 18:00 to 18:00. To assess diurnal patterns of airborne algae, 12 collections were made during each 24-h collection period, with each sample collected for 2 h. One set of samples was collected on the north side of the balcony of the sixth floor of the St. John Plant Sciences Laboratory on the campus of the University of Hawai'i at Mānoa in Honolulu, Hawai'i, USA (21.302013°, – 157.815171°), and one set on the south side. At each site, two petri dishes were fastened to a ledge of ca. 1.5 m height above the balcony surface to collect airborne algae ($n = 48$ petri dishes per 24-h collection period). One petri dish contained 1.5% agarized Alga-Gro freshwater growth media (Carolina Biological Supply, Burlington, NC, USA), while the other contained 1.5% agarized Alga-Gro growth media made with artificial seawater (35 ppt). After collection, petri dishes were sealed with parafilm and allowed to grow in the laboratory for 3 weeks under ambient light and temperature conditions (ca. 21 °C) by a north-facing window. Subsequently, and using sterile techniques, individual algal colonies were isolated. Half of each colony was placed onto a microscope slide for morphological examination with light microscopy, and the other half was placed into a sterile Eppendorf tube and stored at – 20 °C for molecular analysis.

2.2 Meteorological analyses

NOAA's Air Resources Laboratory HY-SPLIT Trajectory Model (<http://ready.arl.noaa.gov/HYSPLIT.php>) was used to compute back-trajectories of air masses for the sampling location for a 14-day time frame, for each 2-h interval of the three sampling periods. These data were used to estimate the potential source of air masses sampled during the study. The model was run using the GDAS1 dataset at 50, 500, and 1000 m above ground level (AGL) with the vertical motion selected as model vertical velocity. Back-trajectories were displayed as .kml files on Google Earth. In addition, temperature, wind speed, wind direction and rainfall data were downloaded ([!\[\]\(e474458956c9a37fbf9586ddb60a7fa1_img.jpg\) Springer](http://</p>
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weather.hawaii.edu), and the amount of sunlight was measured with an Apogee Model MQ-200 quantum meter.

2.3 Molecular analysis of airborne algal colonies

DNA was extracted using the Qiagen DNeasy Plant Mini Kit (Qiagen, Germantown, Maryland USA) according to the manufacturer's instructions, with the following modifications: 25 µL of pelleted material was used as the starting material and the final elution was performed with 30 µL of nanopure water due to the microbial nature of the samples. The UPA marker was PCR-amplified for each extract following Sherwood and Presting (2007). Successful PCR products were purified and sequenced as described in Conklin et al. (2009). Newly generated sequences were assembled and edited using Geneious (Drummond et al. 2010). Sequences were compared using the NCBI's BLASTn function against the GenBank nucleotide database (Zhang et al. 2000). Top BLAST results for each sequence were manually inspected to assign a lowest possible taxonomic identification, with identification assigned if above 94% identity. New sequences from this study have been deposited in GenBank under accession numbers MG595796–915.

Weighted betadiversity analyses using Bray–Curtis dissimilarity were performed in Qiime version 1.9.1 (Caporaso et al. 2010) using the `beta_diversity.py`, `principal_coordinates.py`, and `make_emperor.py` with integrated biplot commands (Online Resource 1). Secondary summary betadiversity analyses were run with data merged into 6-h morning (6:00–12:00), afternoon (12:00–18:00), evening (18:00–24:00), and night (24:00–6:00) periods.

Differences in the overall abundance of algae identified from daytime versus nighttime collections, as well as for Chlorophyta and Cyanobacteria, were tested using binomial (sign) tests with GraphPad (<https://www.graphpad.com/quickcalcs/>).

2.4 Morphological analysis of algae

Semipermanent microscope slides were made by adding 2.5% CaCO₃-buffered glutaraldehyde to the algal colony on the slide as a fixative, followed by a graded series of corn syrup at higher concentrations until the coverslip hardened to the slide with no air bubbles introduced. Airborne algae were examined

and photographed using a Zeiss Axio Imager.A1 compound light microscope (Carl Zeiss, Göttingen, Germany). Morphological characters were compared to those in the phycological literature, and an identification to the lowest possible taxonomic level based on morphology was assigned, using standard algal taxonomic references (e.g., Prescott 1951; Ettl and Gärtner 1988; Komárek and Anagnostidas 1999; Wehr and Sheath 2003; Komárek and Anagnostidas 2005; John et al. 2011). Slide vouchers are currently stored in the Sherwood laboratory at the University of Hawai'i and will ultimately be deposited at the Bernice P. Bishop Museum (BISH). A conservative approach was taken in naming OTUs, recognizing that many of the taxa require characterization beyond the scope of this study for species-level identification and determination of whether they represent named species; individuals were assigned to an OTU based on molecular identity (UPA BLAST results) in combination with their morphological identification. Some morphologically distinct colonies were identified based only on their morphology, but only in the case of abundant OTUs for which many sequences were available (e.g., Chlorophyta uncultured alga clone L016 23S ribosomal RNA gene).

3 Results

3.1 Overview of airborne algal identifications

No algal colonies were identified on the petri dishes containing marine growth medium after the three-week period, and thus only the freshwater dishes were analyzed in this study. Of the 240 total algal colonies harvested from the petri dishes from the three trials, 120 colonies were successfully sequenced for the UPA marker. Using a combination of BLAST to compare the UPA sequences of the colonies to known reference data, and microscopic comparisons to other morphologically identical colonies resulted in the identification of 113 colonies as Chlorophyta (green algae), 79 as Cyanobacteria, 9 as ferns, and 1 as a moss. For the remainder of analyses, non-algal colonies and sequences were disregarded. The 192 algal identifications were classified into 31 OTUs, consisting of 24 Cyanobacterial OTUs, five Chlorophyta OTUs, and two uncultured organisms that were not classified at the phylum level based on sequence data.

Cyanobacterial diversity included 12 Nostocophycidae, seven Oscillatoriophycidae, four Synechococcophycidae, and one classified at the level of Cyanobacterium. Four Trebouxiophyceae and one Chlorophyceae were identified in the Chlorophyta. The most common identification in this study was “uncultured algal clone L016” within the Trebouxiophyceae of the Chlorophyta (107 colonies). The most common Cyanobacterial identification was “uncultured Pleurocapsales Cyanobacterium OTU00085” (18 colonies). The final identifications to the lowest possible taxonomic level are presented in Table 1. No other algal lineages were present in the collections.

3.2 Diurnal patterns of airborne algae

Results were analyzed both separately and in combination for the three trials to examine diurnal patterns in airborne algae. Nighttime collections were defined as those collected from 18:00 to 6:00, and daytime collections as those collected from 6:00 to 18:00. Overall, more airborne algal colonies were identified from nighttime collections (127 of 192 colonies, or 66%) than daytime collections (65 of 192 colonies, or 34%), and this difference was statistically supported ($p < 0.0001$). A principal coordinates plot illustrated the difference between daytime and nighttime collections and identified several OTUs as being most important in accounting for the pattern (Fig. 1). Of the daytime collections, 95% were Cyanobacterium (62/65), and of the nighttime collections, 87% were Chlorophyta (110/127). The total number of algal colonies peaked sharply for collections between the hours of 18:00–20:00 for two of the three trials, with 75 of the total 192 colonies identified during this period (Fig. 2). Of the colonies identified as Chlorophyta, 97% were collected between 18:00 and 6:00, while, in contrast, 78% of the Cyanobacteria were collected between 6:00 and 18:00 (Fig. 3). Given that the average time of sunrise during the study was at 5:50, and the average sunset was at 19:15, the Chlorophyta were almost entirely collected during nighttime hours and Cyanobacteria were mostly collected during the day. This trend was consistent across all three trials (Online Resource 2). Again, these results were statistically significant; we rejected the null hypothesis of equal collections of Chlorophyta during daytime and nighttime ($p < 0.0001$), and the corresponding null hypothesis for Cyanobacteria ($p < 0.0001$). The only

period in which both Chlorophyta and Cyanobacteria colonies were collected in high abundance was between the hours of 18:00–20:00, just before and immediately following sunset (Online Resource 3). Thus, the time period of 18:00–20:00 showed a clear transition from collecting airborne Cyanobacteria colonies to Chlorophyta colonies.

Forty-three of the 72 petri dishes had airborne algal colonies observed and sampled. While overall a greater number of the airborne algal colonies were identified as Chlorophyta, Cyanobacteria colonies appeared on a greater number of the petri dishes. Cyanobacterial colonies were harvested from 36 of the 43 petri dishes with colonies (84%), while Chlorophyta colonies were found on 12 of the 43 petri dishes (28%). Five petri dishes contained both Chlorophyta and Cyanobacterial colonies; these petri dishes were associated with the sampling periods of 18:00–20:00 (trial 1), 22:00–24:00 (trial 2), 18:00–20:00 (trial 3), 14:00–16:00 (trial 3), and 16:00–18:00 (trial 3).

3.3 Meteorological analyses

Fourteen-day back-trajectories using the NOAA HY-SPLIT model demonstrated that air masses entered the Hawaiian Islands as northeast trade winds during all three trials, but the point of origin for the air masses differed depending on time and altitude of the model run (Online Resources 4, 5, 6). Air masses arriving during the first and second sampling trials were less consistent than the third in that they originated from a number of directions around the Pacific Ocean, as opposed to the NW to NE direction of origin for the third trial. Rainfall data collected at the University of Hawai‘i at Mānoa meteorological station demonstrated minimal rainfall during the collection periods (ranging from trace quantities to 0.4 cm over the three trials). Wind speed and wind direction were relatively consistent across all three trials (Online Resource 7).

4 Discussion

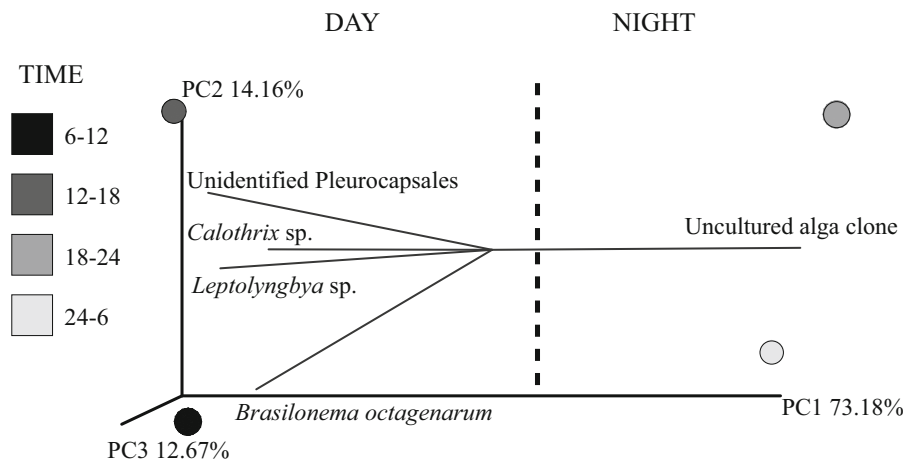
The three trials of the current study examining diurnal patterns of airborne algae deposition suggest a pattern at the phylum level, with mostly Cyanobacteria sampled in daytime collections and Chlorophyta (green algae) during nighttime collections, which should be investigated in greater detail in the future.

Table 1 Taxonomic identification of airborne algal OTUs from the current study

	% Identity (to GenBank accession)	Trial 1 (# colonies)	Trial 2 (# colonies)	Trial 3 (# colonies)
Cyanobacteria				
<i>Brasilonema octagenarum</i> 04186-00001 23S ribosomal RNA gene, partial sequence	99 (KC848590.1)	3		
Uncultured <i>Calothrix</i> sp. isolate OTU 00041 23S ribosomal RNA gene, partial sequence	95 (KM458324.1)	1		
Uncultured <i>Calothrix</i> sp. isolate OTU 00130 23S ribosomal RNA gene, partial sequence	99 (KM458383.1)		1	2
Uncultured <i>Calothrix</i> sp. isolate OTU 00132 23S ribosomal RNA gene, partial sequence	98 (KM458384.1)	6		2
Uncultured <i>Calothrix</i> sp. isolate OTU 00139 23S ribosomal RNA gene, partial sequence	96 (KM458389.1)		1	
<i>Calothrix</i> sp. NIES-4105 DNA, nearly complete genome	100 (AP018290.1)	1	1	3
Uncultured <i>Chroococcidiopsis</i> sp. isolate OTU 00022 23S ribosomal RNA gene, partial sequence	100 (KM458310.1)			1
<i>Chroococcidiopsis</i> sp. SAG 2025 partial ribosomal RNA operon, strain SAG 2025	99 (AM709635.1)		3	1
Cyanobacterium ARS04068_00002 23S ribosomal RNA gene, partial sequence	95 (KM676756.1)			1
Uncultured <i>Leptolyngbya</i> sp. isolate OTU 00142 23S ribosomal RNA gene, partial sequence	99 (KM458392.1)		2	2
<i>Leptolyngbya</i> sp. ATA34QCV6 23S ribosomal RNA gene, partial sequence	99 (KC848638.1)		1	
<i>Leptolyngbya</i> sp. SEV53C28 23S ribosomal RNA gene, partial sequence	96 (KC848645.1)		2	2
<i>Leptolyngbya</i> sp. NIES-3755 DNA, complete genome	97 (AP017308.1)	4	1	1
<i>Nostoc</i> sp. ‘ <i>Peltigera membranacea</i> cyanobiont N6’ 16S ribosomal RNA gene, 16S-23S ribosomal RNA intergenic spacer, tRNA-Ile, tRNA-Ala, 23S ribosomal RNA, and 5S ribosomal RNA genes, complete sequence	97 (JX975209.1)	1		
Uncultured Oscillatoriales Cyanobacterium isolate OTU 00107 23S ribosomal RNA gene, partial sequence	97 (KM504132.1)		2	
Uncultured Pleurocapsales Cyanobacterium isolate OTU 00042 23S ribosomal RNA gene, partial sequence	100 (KM458325.1)		2	
Uncultured Pleurocapsales Cyanobacterium isolate OTU 00085 23S ribosomal RNA gene, partial sequence	99 (KM458351.1)		17	1
Uncultured Pleurocapsales Cyanobacterium isolate OTU 00094 23S ribosomal RNA gene, partial sequence	95 (KM458358.1)			1
Uncultured Pleurocapsales Cyanobacterium isolate OTU 00133 23S ribosomal RNA gene, partial sequence	99 (KM458385.1)	1	1	1
<i>Scytonema hofmanni</i> PCC 7110 partial ribosomal RNA operon, strain PCC 7110	95 (AM709637.1)			1
<i>Scytonema</i> sp. HK-05 DNA, nearly complete genome	99 (AP018194.1)		1	
Uncultured <i>Tolypothrix</i> sp. isolate OTU 00013 23S ribosomal RNA gene, partial sequence	95 (KM458304.1)	1		1
Uncultured <i>Tolypothrix</i> sp. isolate OTU 00124 23S ribosomal RNA gene, partial sequence	98 (KM458379.1)			1
<i>Tolypothrix</i> sp. 04266-00001 23S ribosomal RNA gene, partial sequence	96 (KC848626.1)	1		

Table 1 continued

	% Identity (to GenBank accession)	Trial 1 (# colonies)	Trial 2 (# colonies)	Trial 3 (# colonies)
Chlorophyta				
<i>Coelastrella striolata</i> var. <i>multistriata</i> 23S ribosomal RNA gene, chloroplast	98 (KF355932.1)			1
<i>Nannochloris normandinae</i> strain SAG 9.82 23S large subunit ribosomal RNA gene, chloroplast	100 (JQ921007.1)	1		1
<i>Trebouxia corticola</i> strain UTEX 909 23S large subunit ribosomal RNA gene	99 (JQ921013.1)	1		
<i>Watanabea reniformis</i> culture-collection SAG:211-9b chloroplast, complete genome	94 (KM462863.1)			2
Uncultured alga clone L016 23S ribosomal RNA gene, partial sequence; chloroplast	100 (KF673419.1)	33	25	49
Unknown phylum				
Uncultured organism clone UniqueSequence 1663 23S ribosomal RNA gene, partial sequence	98 (KY916238.1)	1	1	
Uncultured organism clone UniqueSequence 6318 23S ribosomal RNA gene, partial sequence	96 (KY920893.1)		1	1
Total number of colonies		55	62	75

**Fig. 1** Principal coordinates plot illustrating the separation of collections by daytime and nighttime, and the taxa most responsible for the pattern

97% of the Chlorophyta colonies were found between the hours of 18:00–6:00 (nighttime), and 78% of the Cyanobacteria colonies were found between the hours of 6:00–18:00 (daytime). The period between 18:00 and 20:00 was the only window of time in which above average numbers of both Chlorophyta and

Cyanobacterial colonies were sampled and was also when sunset occurred. Although the differences were statistically significant, future trials with increased sample sizes would be useful to explore the pattern in greater depth. Several explanations are possible for the diurnal trends found in the current study.

Fig. 2 Total number of airborne algal colonies collected over the three trials, by 2-h sampling period; dark = trial 1, medium = trial 2, light = trial 3

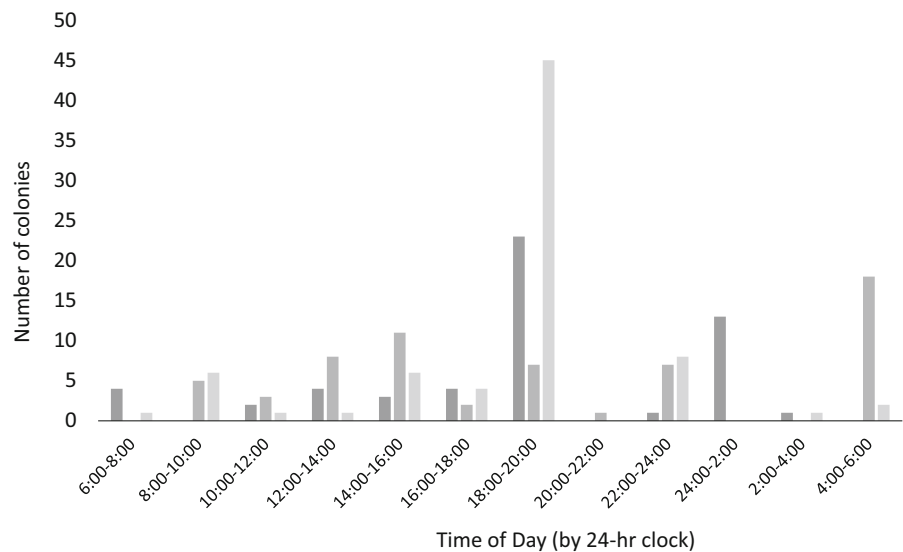
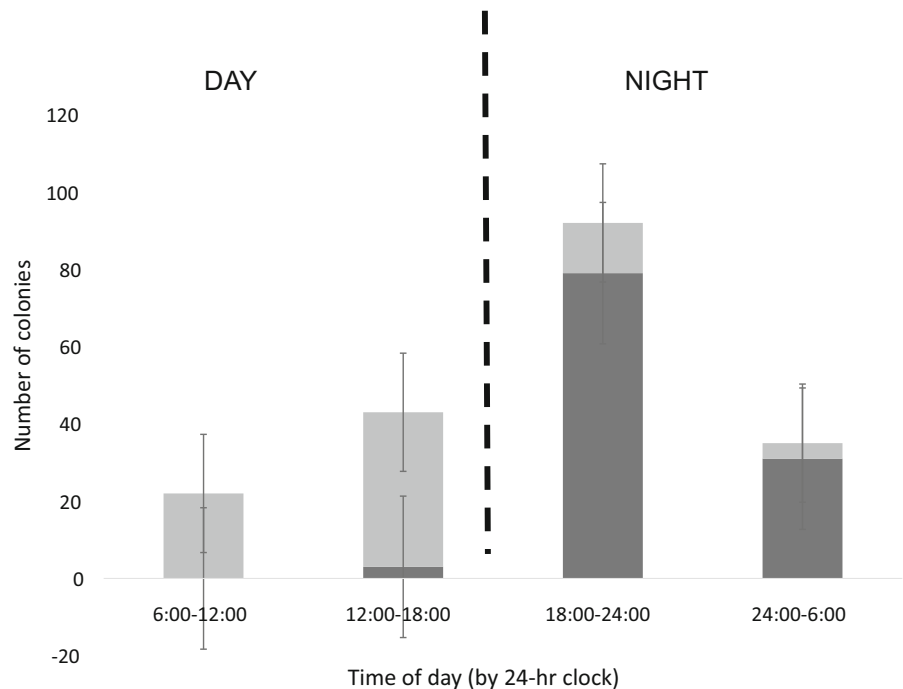


Fig. 3 Number of airborne algal colonies corresponding to Chlorophyta (dark) versus Cyanobacteria (light) for daytime intervals versus nighttime intervals; error bars = standard error



First, temperature-driven land breeze and sea breeze effects commonly interact with prevailing trade winds in tropical insular locations such as the Hawaiian Islands (Leopold 1949). Daytime typically brings sea breezes to the leeward side of the islands in the Hawaiian archipelago, and at night this air flow reverses to bring a land breeze (Leopold 1949). Thus, although the major air masses arriving in the Hawaiian

Islands during the sampling trials may have been transported from other regions of the Pacific (Online Resources 4, 5, 6), local changes in wind direction were likely significant in playing a role in the microclimatic conditions affecting the sampling sites over the course of the study. This may explain the differences in both the overall abundance of airborne algae collected over the three 24-h periods of study

and the apparent differential aerosolization of Chlorophyta versus Cyanobacteria on a diurnal basis.

Alternatively, the differential aerosolization of the two algal groups may be associated with temperature preferences of the algae. It has been well documented that airborne Cyanobacteria tend to be dominant in tropical areas, while Chlorophyta are more abundant in temperate regions (Schlichting 1961; Brown et al. 1964; Sharma et al. 2006; Sharma and Singh 2010; Genitsaris et al. 2011; Ng et al. 2011; Sherwood et al. 2017). Similarly, it has been reported that prokaryotic and eukaryotic algae react differently to atmospheric temperatures, with Cyanobacteria preferring higher temperatures than Chlorophyta, resulting in Cyanobacteria being more abundant during summer months and in warmer climates (Sharma et al. 2007). Our study suggests that these patterns could scale more finely onto a diurnal pattern, where Cyanobacteria aerosolize during the warmer daylight hours with high sun exposure, while Chlorophyta aerosolize more commonly during the colder night hours with no sun exposure. Over the three trials, there was an average difference of 5 °C between the warmest and the coldest time of the day.

Few studies have investigated diurnal patterns in the distribution of airborne algae, and those that have were under the scope of larger projects. The findings of previous studies are not consistent and lack consensus on the diurnal patterns of airborne algae (Genitsaris et al. 2011). As a result, it remains unknown to what degree reported diurnal patterns are associated with particular geographical locations and meteorological conditions. For example, Tormo et al. (2001) reported the greatest abundance of airborne algae in southwest Spain between the hours of 17:00 and 19:00, with minimal airborne algae collected between 06:00 and 11:00, while Schlichting (1961) reported no diurnal patterns in airborne algae in Michigan, USA, but found twice as much airborne algae between the hours of 12:00 and 24:00 as compared to 24:00 and 12:00 in Texas, USA (Schlichting 1964). Meanwhile, Smith (1973) reported maxima in airborne algal concentrations in North Carolina, USA, between 08:00 and 12:00, 14:00 and 18:00, and 22:00 and 04:00, and minima between 04:00 and 08:00, and 18:00 and 22:00. Schlichting (1974) later summarized the available data and stated that there is no definite diurnal pattern of variation for airborne algae. The results of the current study, although preliminary, suggest that

there may be distinct diurnal patterns for airborne algae at the phylum level, at least for tropical regions such as the Hawaiian Islands.

Molecular techniques commonly reveal higher levels of algal diversity compared to using only morphological identification (e.g., Suutari et al. 2010; Freshwater et al. 2017). In terms of airborne algae, Genitsaris et al. (2011) reported that only approximately 20% of airborne algae are common in studies independent of location, indicating a great deal of potentially unexplored airborne algal diversity. Sherwood et al. (2014a) reported almost 97% of their airborne algal OTUs as not matching the non-marine algal diversity described in the recently completed Hawaiian Freshwater Algal Biodiversity Survey (Sherwood et al. 2014b), highlighting the large amount of potentially unexplored algal diversity remaining in the Hawaiian Islands. Further, Sherwood et al. (2017) reported that most of their algal *de novo* OTUs did not exactly match available reference sequences and that even some of the reference sequences that matched perfectly were matched to entries that were not classified to the genus or species level, suggesting that additional taxonomic research will reveal new records or undescribed species. Only 14.6% of the OTUs in the present study were identified at 99–100% identity to reference sequences that had been classified to the species level, further highlighting the need for detailed systematic work on airborne algae.

In contrast to previous culture-based studies of Hawaiian airborne algae where Cyanobacteria were the most abundant algal group (Brown 1971; Sherwood et al. 2014a), in this study more colonies were identified as Chlorophyta than as Cyanobacteria. However, prior studies conducted sampling entirely during daytime hours. Our nighttime sampling accounted for 97% of the Chlorophyta colonies analyzed in this study, indicating the importance of round-the-clock sampling for a more accurate representation of the full airborne algal community than is typically revealed by daytime-only collections.

No airborne algal colonies were found on the marine growth medium during the three-week culturing time of the study, and airborne algae studies in general tend to report few marine taxa, if any. Out of the 175 airborne algal genera summarized by Genitsaris et al. (2011), only 13 were marine algae. In contrast, a previous study of airborne algae on O‘ahu

that were sampled by collecting algae on marine and freshwater agarized growth media from the window of a moving vehicle reported substantial numbers of colonies on the marine media plates (ca. 31% of collected airborne algal OTUs grew only on marine media plates) (Sherwood et al. 2014a); however, proportionally very few algal colonies grew on marine medium at sampling sites on the leeward side of the Ko'olau Mountains, which is also the location of the current study. Proximity to marine sources and the presence of onshore winds likely play a large role in the proportion of marine versus freshwater algal colonies. For example, Lee and Eggleston (1989) successfully collected and cultured a number of marine microalgae from the air at sites in New Hampshire and Maine, USA, which are close to the ocean.

The current study provides some of the first temporal data for airborne algae using molecular characterization methods and illustrates an intriguing phylum-level pattern that should be investigated in more detail in the future. Future research should also account for varying meteorological conditions to examine the range of possible diurnal patterns of airborne algae for particular regions. Expanding the study of diurnal patterns of airborne algae to other geographical regions would help to link the influence of topography and meteorology to the patterns of distribution over time of these understudied components of the airborne microbiome.

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