Infection of phytoplankton by aerosolized marine viruses

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Marine viruses constitute a major ecological and evolutionary driving force in the marine ecosystems. However, their dispersal mechanisms remain underexplored. Here we follow the dynamics of Emiliania huxleyi viruses (EhV) that infect the ubiquitous, bloom-forming phytoplankton E. huxleyi and show that EhV are emitted to the atmosphere as primary marine aerosols. Using a laboratory-based setup, we showed that the dynamic of EhV aerial emission is strongly coupled to the host–virus dynamic in the culture media. In addition, we recovered EhV DNA from atmospheric samples collected over an E. huxleyi bloom in the North Atlantic, providing evidence for aerosolization of marine viruses in their natural environment. Decay rate analysis in the laboratory revealed that aerosolized viruses can remain infective under meteorological conditions prevailing during E. huxleyi blooms in the ocean, allowing potential dispersal and infectivity over hundreds of kilometers. Based on the combined laboratory and in situ findings, we propose that atmospheric transport of EhV is an effective transmission mechanism for spreading viral infection over large areas in the ocean. This transmission mechanism may also have an important ecological impact on the large-scale host–virus “arms race” during bloom succession and consequently the turnover of carbon in the ocean.

Significance

Marine viruses constitute a major ecological and evolutionary driving force in marine ecosystems and are responsible for cycling of major nutrients; however, their dispersal mechanisms remain underexplored. By using one of the most established host–pathogen planktonic model systems we provide strong evidence that specific viruses of marine coccolithophores can be transmitted and stay infective as marine aerosols. Being transported by the wind, phytoplankton viruses can be conveyed long distances and transmit the infection to remote locations to which coccolithophore blooms can be extended. We show that this effective transmission mechanism that has been studied in human, animal, and plant diseases could play an important role in host–virus dynamics during phytoplankton blooms in the ocean.

Author contributions: I.K. and A.V. conceived the basic ideas and supervised the project; S.S., M.T., Y.L., Y.K., I.K., and A.V. developed the concept and designed experiments; S.S. and M.T. performed experiments; D.S., Y.L., M.J.F., and S.B.-D. performed additional lab, in situ and satellite analyses; K.D.B. was the chief scientist on the NA-VICE cruise; and S.S., M.T., I.K., and A.V. wrote the paper.

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Oceanic phytoplankton blooms are the major primary producers that constitute the base of marine food webs, and are key components of large biogeochemical cycles in the ocean (1). Emiliania huxleyi (Prymnesiophyceae, Haptophyta) is a dominant, bloom-forming phytoplankton that plays a pivotal role in cycling of major nutrients; however, their dispersal mechanisms remain underexplored. Here we follow the dynamics of Emiliania huxleyi viruses (EhV) that infect the ubiquitous, bloom-forming phytoplankton E. huxleyi and show that EhV are emitted to the atmosphere as primary marine aerosols. Using a laboratory-based setup, we showed that the dynamic of EhV aerial emission is strongly coupled to the host–virus dynamic in the culture media. In addition, we recovered EhV DNA from atmospheric samples collected over an E. huxleyi bloom in the North Atlantic, providing evidence for aerosolization of marine viruses in their natural environment. Decay rate analysis in the laboratory revealed that aerosolized viruses can remain infective under meteorological conditions prevailing during E. huxleyi blooms in the ocean, allowing potential dispersal and infectivity over hundreds of kilometers. Based on the combined laboratory and in situ findings, we propose that atmospheric transport of EhV is an effective transmission mechanism for spreading viral infection over large areas in the ocean. This transmission mechanism may also have an important ecological impact on the large-scale host–virus “arms race” during bloom succession and consequently the turnover of carbon in the ocean.

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Marine viruses constitute a major ecological and evolutionary driving force in marine ecosystems and are responsible for cycling of major nutrients; however, their dispersal mechanisms remain underexplored. By using one of the most established host–pathogen planktonic model systems we provide strong evidence that specific viruses of marine coccolithophores can be transmitted and stay infective as marine aerosols. Being transported by the wind, phytoplankton viruses can be conveyed long distances and transmit the infection to remote locations to which coccolithophore blooms can be extended. We show that this effective transmission mechanism that has been studied in human, animal, and plant diseases could play an important role in host–virus dynamics during phytoplankton blooms in the ocean.

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Viral Infectivity of Aerosolized Viruses. To test whether the aerosolized viruses are infective, we linked the outflow of the bubbling system containing infected *E. huxleyi* culture using the previous setup to the headspace of two noninfected *E. huxleyi* cultures (Fig. 2A). These targeted cultures consisted of *E. huxleyi* strain RCC 1216, which is susceptible to *EhV* infection, and a resistant strain, RCC 373. The latter served as a control host to validate that if cell lysis occurs it is triggered only by infective aerosolized *EhV,* and not by other contaminants (20) (SI Materials and Methods). In the susceptible targeted host, *E. huxleyi* cells grew to densities of ∼1 × 10^9^ cells per mL before cell lysis occurred 3 days after the initial exposure to the aerosols emitted from the infected culture source (Fig. 2B and C). Concomitantly, virus concentration in the culture media of the target susceptible host increased to a maximal value of ∼6 × 10^9^ viruses per mL. In contrast, the resistant target *E. huxleyi* cells continued to grow rapidly, reaching a concentration of ∼4 × 10^7^ cells per mL, and no viral production was detected (Fig. 2B and C). These findings indicate that aerosolized *EhV* remain infective when transmitted through air and can lead to efficient lysis of noninfected adjacent *E. huxleyi* populations. Furthermore, we tested the infectivity of aerosolized viruses at lower aerial concentrations that are more ecologically relevant (∼10–100 viruses per L of air) and found similar results (SI Materials and Methods, Figs. S2 and S3, and Table S1).

**Decay Rate of Airborne *EhV.*** Unlike the laboratory system, viruses in the natural atmosphere may become inactive owing to structural damage upon exposure to UV radiation and changes in temperature and relative humidity (21, 22). To estimate the time scale over which *EhV* can remain infective in the atmosphere, we measured viral decay rate after exposure to atmospheric conditions typical for daytime clear-sky North Atlantic spring blooms. We used the most probable number (MPN) method (23) for calculations of viral infectivity and found that *EhV* infectivity decays exponentially with a calculated decay rate of k = ∼0.033 min^-1^, corresponding to a half-life time of 20 min (Fig. 3). This decay rate was detected under simulated atmospheric conditions of temperature 15.9 ± 0.2 °C, relative humidity 65–75%, and light intensity 700 μmol photons m^-2^ s^-1^ provided by a halogen lamp with a spectrum simulating sunlight (400–700 nm) (SI Materials and Methods). This result is comparable to previous studies that reported nonmarine viruses can remain infective in the atmosphere for several hours (23–26). In addition, the half-life of infectious *EhV* in the ocean under similar prevailing condition is, as expected, much longer, ∼35 h (13). Unlike bacteria and algae, viruses have no active DNA repair systems; consequently, their inactivation rates are usually higher than those of other microorganisms (25). Nevertheless, they may remain infective for a longer time during nighttime or overcast conditions, when they are not exposed to radiation. Therefore, we hypothesize that our calculated half-life for clear-sky daytime conditions represents a lower limit of the time that *EhV* can remain infective in the marine atmosphere.

**Detection of Airborne *EhV* over a Natural Bloom in the North Atlantic.** To assess the ecological significance of our laboratory findings under natural algal bloom conditions, we examined aerosol samples collected during an *E. huxleyi* spring bloom in the North Atlantic (SI Materials and Methods). The aerosols were collected on July 3 and 4, 2012, at a sampling site where high abundance of *E. huxleyi* cells (∼1.4 × 10^7^ cells per mL, Fig. 4C) and *EhV* (∼5 × 10^9^ EhV per mL, Fig. 4C) were observed in the top 40 m of the water column (location: 61.90°N, 33.70°W). These values were associated with similar patterns of chlorophyll fluorescence (Chl) and particulate inorganic carbon (PIC) retrieved from the moderate resolution imaging spectroradiometer (MODIS) satellite (Fig. 4A and B). The satellite images, together with high *E. huxleyi* and *EhV* abundances in the water column, were indicative of an active viral infection during *E. huxleyi* bloom (10). TEM analysis of collected aerosol samples revealed large virus-like particles (LVLPs) with morphology and size comparable to...
those of EhV (Fig. 4D). Furthermore, a clear EhV DNA signature was obtained from these collected aerosol samples, when using specific primers to the conserved viral phosphoglycerate mutase (PGM) gene in PCR analysis. These primers were used extensively to examine viral diversity in the ocean (27). Phylogenetic analysis of the PGM sequences clearly identified these amplicons as EhVs that cluster together with other known EhV-PGM sequences but are significantly different from them (Fig. 4E and Fig. S4). An EhV with an identical PGM sequence was isolated from seawater samples at 50 m depth on the same cruise (13).

Meteorological data revealed that on the same dates and location about half of the time the wind speed exceeded the minimum threshold of 4 m s\(^{-1}\) for marine aerosol production by bubble bursting (28). Therefore, it is reasonable to assume that the EhV found in the aerosol samples is likely to be emitted from nearby EhV-rich seawater (28).

**Discussion**

Although virus-like particles were previously found in marine aerosols (15, 18), our study presents conclusive genetic and morphological evidence for primary emission of viruses infecting a specific bloom-forming algal host. Furthermore, we suggest that such emissions to the atmosphere may play a critical role in the dispersal of viral infection over large-scale *E. huxleyi* blooms in the ocean (10).

To estimate the potential extent of infection dispersal following EhV emission, we performed a calculation combining our laboratory and in situ findings. We found that for an average wind velocity (\(\sim 8\) m s\(^{-1}\), SI Materials and Methods and Table S2) EhV concentration in the air is expected to be six orders of magnitude less than its abundance in the water (Fig. 1B and C and Fig. S5). Our in situ measurements revealed that during an open ocean *E. huxleyi* bloom, EhV can reach seawater concentrations of \(~10^4\) EhV per mL (Fig. 4C). Therefore, under close to steady-state conditions, we can estimate the EhV concentration in the lower atmospheric boundary layer to be \(~10\) viruses per L of air. Although a typical *E. huxleyi* bloom occupies thousands of square kilometers (29), we consider only a limited area of 1 km\(^2\) of sea surface and 10 m of a well-mixed atmosphere above it, yielding a parcel of \(10^{10}\) L of air with an estimated EhV population of over \(10^{11}\) aerosolized EhV. Using 20 min as the lower limit for the

\[ \text{EhV decay rate} = \frac{\text{C}_t}{\text{C}_0} \]

\[ \ln(C_t/\text{C}_0) = -0.0335x + 0.1676, R^2 = 0.9818. \]
**EhV** half-life (under daytime atmospheric conditions, Fig. 3), we calculate that the infective virus concentration will be reduced by an order of magnitude within 1 h. Moreover, owing to the high dilution factor in the atmosphere, and the long lifetime of submicron aerosols, only a small fraction of the airborne EhV is expected to be deposited back to the ocean within 1 d (30, 31). Mayol et al. (32) have recently reported that ∼10% of microbes in the atmospheric boundary layer were airborne for 4 d post-emission, traveling up to 11,000 km before deposition. Using $7\, m\cdot s^{-1}$ as the typical surface wind speed over the North Atlantic (28) we estimate that an air parcel with $10^{11}$ viruses can disperse through the air and convey $10^{7}$ infective viruses after traveling for hundreds of kilometers. A case study over the measurement area for July 3, 2012, revealed a very consistent wind trajectory for 12 h, suggesting that that the emitted virus particles are likely to be advected over narrow sectors for several hours and therefore most of the deposition would be concentrated over it (Fig. S6 and SI Materials and Methods).

Dispersal through wind-driven processes may contribute to fast and efficient infection at higher rates than other dispersal mechanisms such as diffusion, mixing, advection, and currents (33). Although estimation of dispersal of passive tracers in the ocean is challenging, most estimations suggest that the characteristic distances by which the released viruses will spread per unit of time will be orders of magnitude smaller in the water than through the air. For example, tracing the propagation of an iron-fertilized patch in the Southern Ocean (34) showed a dispersal scale of 150 km after 6 weeks, which is comparable to 1-d dispersal in the atmosphere. In cases where algal blooms are confined to patches in the open ocean (10), the best measure for dispersion accounting for turbulence is defined as eddy diffusivity (35). Considering viral release from infected cells as a passive tracer, the characteristic propagation velocity of the front of the volume containing the tracers can be estimated as the ratio between the characteristic eddy diffusivity coefficient to the characteristic length scale. It was shown that the high-end values of the eddy diffusivity coefficient are on the order of $1,000\, m^{2}\cdot s^{-1}$ for scales of $10–100\, km$, yielding a propagation velocity in the order of $\sim1\, cm\cdot s^{-1}$ for a 100-km scale, as opposed to $\sim1–10\, m\cdot s^{-1}$ in the atmosphere (35, 36).

The difference between the two transport processes is of two to three orders of magnitude, indicating that aerial dispersal can be an efficient transmission mechanism, which can contribute to the observed large-scale, rapid, and synchronized *E. huxleyi* bloom demise attributed to viral infection (5, 6, 9, 10).

Therefore, we suggest that aerosolization and consequent aerial dispersal can be a common mechanism for epidemics of marine pathogens. These findings may have important implications for the factors determining microbial composition, flow of nutrients in marine food webs, and large biogeochemical cycles in the ocean (37, 38).

**Materials and Methods**

The experimental setup and the methods are described fully in SI Materials and Methods.
E. huxleyi and EHV Dynamics in the Culture and Airborne EHV Quantification. Four liters of E. huxleyi susceptible strain RCC 1216 cultures were grown in a 10-L carboy in f/2 media and infected with EHV201 during the exponential growth phase. Simultaneously, a control culture that was not infected was grown under the same conditions. Cultures were continuously bubbled at a rate of 3 L min⁻¹. The emitted aerosols from the infected and the control cultures were collected on nitrocellulose filters every 24 h, extracted, and analyzed for viral DNA abundance. Quantification of EHV in the culture and in the aerosols was determined by quantitative PCR (qPCR) for the EHV major capsid protein gene (MCP). E. huxleyi cell analysis was performed with an Eclipse (Cyt) flow cytometer.

Viral Infectivity of Aerosolized Viruses. Similar to the previous experiment, 4 L of E. huxleyi susceptible strain RCC 1216 cultures were grown and continuously bubbled in a 10-L carboy in f/2 media and infected with EHV201 during the exponential growth phase. The outflow from the carboys containing the infected cultures was split with a stainless steel flow splitter into two 2-L Erlenmeyer flasks, each containing 1 L of E. huxleyi culture. One Erlenmeyer flask contained the susceptible E. huxleyi RCC 1216 strain (n = 4), and the other contained the resistant E. huxleyi RCC 373 strain (n = 2). The cultures were further incubated for 5–6 d postexposure to airflow from the infected carboy. The resistant strain was used to demonstrate that the demise is due to virus infection following due to other stresses or toxic contaminants rising from the infected population in the carboy. Cultures were harvested for cell and viral enumeration in the carboy and in the two Erlenmeyer flasks every 24 h. Host and virus quantification was performed as described above.

Decay Rate Experiment. EHV201 was introduced onto polyester filters using a vacuum pump. The filters were incubated under regulated atmospheric conditions as follows: temperature 15.9 ± 0.2 °C, 65–75% relative humidity, light intensity of 700 μmol m⁻² s⁻¹ (400–700 nm). Filters were collected at different time points: 0, 40, 80, and 120 min (n = 7). Viruses were extracted from the filters, and a series of 10-fold dilutions were used to infect host cultures (n = 12). After incubation we used the MPN method (23) to determine the number of infective viruses at each point.

Oceanographic Cruise Water and Air Sampling. Water was collected from 61.90°N, 33.70°W on July 3 and 4, 2012, during the North Atlantic Virus Infection of Coccolithophore Expedition (NA-VICE; KN207-03, www.bco-dmo.org/project/2136), aboard the RV Knorr. Water samples were obtained from the water column using a Sea-Bird SBE 911plus CTD carrying 10-L Niskin bottles, and genomic DNA was isolated from filtered biomass using an adapted phenol–chloroform method. Air was continuously collected during the cruise by pulling through PM10 inlet heads placed on a 15-m-high ship mast. Aerosols were collected for 24 h on 47-mm nitrocellulose and PVDF filters and kept at 4 °C until analysis. DNA from the collected filters was extracted and tested for the presence of EHV using primers designed for the PGm gene (Fig. 3).

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Supporting Information

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SI Materials and Methods

**Bubbling System.** All of the bubbling experiments were performed using 10-L polycarbonate carboys (Nalgene; Thermo scientific). The carboys were filled with 4 L of sample, which resulted in a headspace of 6 L. A glass tube was fixed from the top of the carboy into the center of the solution with a PBS adapter to help keep the bubbling conditions constant. Filtered air was pumped into the carboy with a continuous airflow of 3 L-min⁻¹. The carboy was sealed and the pressure differences due to the pumping resulted in air being pulled through the glass tube, leading to bubbling of the solution at a constant rate of 3 L-min⁻¹. The solution was constantly mixed with a magnetic stirrer at a rate of 4 rpm (Fig. S1).

**Collection of Airborne Particles.** The outward airflow from the carboy was directed into a filter holder (ferrule nut 3/8 inch; PFA), and the aerosols were collected on 0.45-µm nitrocellulose filters (Millipore). The humidity in the system was reduced to 50–70% relative humidity using a silica gel column before reaching the filter to avoid blockage of the system (Fig. S1).

**Culture Growth.** The *Emiliania huxleyi* strains used in this study are the virus-susceptible strains RCC 1216 and RCC 374 and the resistant strain RCC 373. All strains were purchased from the National Center of Marine Algae and Microbiota (formerly known as RCC). Cultures were grown in filtered, sterilized seawater that was collected from Michmoret, Israel (32.24°N, 34.52°E), and enriched with f/2 medium (1). The cultures were grown in polycarbonate carboys at 18 °C with a 16:8 h light:dark cycle. Light was provided by cool-white light-emitting diode lights at an intensity of 80 µmol photons·m⁻²·s⁻¹.

**Virus Stock.** The viruses used for this study are *E. huxleyi* virus (*EhV*) 201, isolated by Schroeder et al. (2). Viral stocks were prepared by infecting *E. huxleyi* cultures until lysis and filtering out the cell debris from the infected cultures using 0.45-µm PVDF filters (Millipore). Viral stocks were kept in the dark at 4 °C.

**Quantification of *E. huxleyi* and *EhV*.** Exponential phase *E. huxleyi* cultures were infected with *EhV* at a multiplicity of infection (MOI) of five viral particles per cell. The cultures were further incubated for 5–6 d. Culture harvesting for cell and viral enumeration and collection of aerosol on filters were performed every 24 h. *E. huxleyi* cell analysis was performed with Multisizer 4 Coulter Counter (Beckman Coulter). We used the qPCR method to count viruses in the culture media. For this, 0.5-mL samples were filtered through a 0.45-µm Milllex-HV filter (Millipore) and boiled for 20 min. One microliter of the viral lysate was taken for each reaction. *EhV* DNA was quantified using primers against the Major Capsid Protein (mcp) gene, mcpFw: 5′-agcagctactcaggagtggag-3′ and mcpRv: 5′-agcagctactcaggagggag-3′. All reactions were carried out in triplicates. For all reactions Platinum SYBR Green qPCR SuperMix-UDG with ROX (Invitrogen) was used as described by the manufacturer. Reactions were performed on StepOnePlus real-time PCR Systems (Applied Biosystems) as follows: 50 °C for 2 min, 95 °C for 2 min, 40 cycles of 95 °C for 15 s, 60 °C for 30 s. Results were calibrated against serial dilutions of *EhV*201 DNA at known concentrations, enabling exact enumeration of viral abundance.

Aerosols collected on the filters were preserved at 4 °C until DNA extraction. Filters were cut into halves and DNA was extracted according to the protocol in Boström et al. (3) with minor changes: filters were inserted into the 1-mL lysis buffer containing 40 mM EDTA (pH 8), 50 mM Tris-HCL (pH 8), and 0.75 mM sucrose and incubated at –20 °C until extraction. Before extraction, filters were dissolved and lightly vortexed for 40 min. After shaking the filters were incubated at 37 °C for 30 min. After incubation 50 µL of proteinase K (final concentration of 0.5 mg·mL⁻¹), and 100 µL 10% (vol/vol) SDS (final concentration of 1%) were added and incubated at 55 °C for 1 h followed by 70 °C for 5 min. Following this procedure, a fixed volume of 850 µL was used for DNA precipitation. Precipitation was made using 1 µL of glycen (5 mg·mL⁻¹), 0.1 volumes of 3 M Na-acetate, and 0.6 volumes of isopropanol (Sigma-Aldrich). The DNA was dried and eluted with 50 µL nuclease-free water. In this method, DNA recovery efficiencies are reported to be 92–96% (3). *EhV* enumeration was conducted as above with the exception that the qPCR was performed using 5 µL of the extract for each reaction.

**EhV Aerosol Concentration Calculations.** The following equations were used to calculate the total number of viruses on the filter, and consequently the aerosolized virus concentrations from qPCR retrievals (viruses per microliter):

\[
\text{Viruses extracted from the filter} = \text{virus concentration} \times \frac{\text{viruses} \cdot \mu L}{1,000}/0.85 \tag{S1}
\]

\[
\text{Aerial virus concentration} = \frac{\text{viruses extracted from the filter}}{\frac{\text{flow rate} \cdot \text{L of air}}{\text{min}}} \cdot \frac{1}{\text{min}} \cdot \text{sampling time(min)} \tag{S2}
\]

Each half of the filter was multiplied by 2 to obtain total mass on the filters. The viral concentration used in the equations was obtained by qPCR. One thousand microliters were used as the volume of elution solution, and 0.85 is the volume used for DNA extraction (milliliters).

**Lysate Bubbling Experiment: Validation of the System.** Viral lysate was used for system validation in the following manner: Four liters of virus lysate at three different concentrations of 2 × 10⁸, 3 × 10⁸, and 2 × 10⁹ viruses per mL were continuously bubbled at a rate of 3 L·min⁻¹ in the 10-L carboy, as in previous experiments. The emitted aerosols were collected on nitrocellulose filters at different time lapses (5–25 h), extracted, and analyzed for viral DNA abundance. Quantification of *EhV* in the culture and in the aerosols was determined by qPCR for the *EhV* major capsid protein gene (MCP).

**SI Results**

**Virus Emission to the Air As a Function of Lysate Concentration.** To optimize the experimental setup that allows continuous production and sampling of aerosols, we measured the number of viruses collected on a filter from the air as a function of collection time and viral concentration in the lysate solution. We found that for every lysate concentration, the viral concentration in the aerosol is proportional to the concentration in the lysate solution, with a consistent difference of seven orders of magnitude (Fig. S2 and Table S1).
concentrations that are similar to environmental conditions in the seawater and air, we conducted an additional experiment: We used the same setup described above (also see Fig. 2A), but instead of bubbling infected host we bubbled 4 L lysate solution containing \(10^9\) virus per mL. The outflow that we calculated to contain \(~10^{-10}\) viruses per L of air based on extrapolation of the data from Fig. S2 and Table S1 was continuously directed into the headspace of new, healthy \(E. huxleyi\) cultures. The host–virus dynamic was followed in a susceptible \(E. huxleyi\) culture RCC 1216 strain (n = 2) and in a resistant \(E. huxleyi\) culture RCC 373 strain (n = 2).

Viral Infectivity of the Emitted Viruses. The outflow from carboys containing infected cultures was split with a stainless steel flow splitter into two 2-L Erlenmeyer flasks, each containing 1 L of \(E. huxleyi\) culture. One Erlenmeyer flask contained the susceptible \(E. huxleyi\) RCC 1216 strain (n = 4), and the other contained the resistant \(E. huxleyi\) RCC 373 strain (n = 2). The cultures were further incubated for 5–6 d postexposure to the infected carboy. The resistant strain was used to demonstrate that the demise is due to viral infection and not due to other stresses or toxic contaminants rising from the infected population in the carboy. The total outflow from the carboy was 3 L·min\(^{-1}\), and the split resulted in a flow of 1.5 L·min\(^{-1}\) into each Erlenmeyer flask (see Fig. 2A). Cultures were harvested for cell and viral enumeration in the carboy and in the two Erlenmeyer flasks every 24 h. Host and virus quantification was performed as described below.

\(E. huxleyi\) and \(EhV\) Dynamics in the Culture and Airborne \(EhV\) Quantification. Four liters of \(E. huxleyi\) susceptible strain RCC 1216 cultures were grown in a 10-L carboy in f/2 media and infected with \(EhV\) during the exponential growth phase in MOI of approximately five viruses per cell. The culture was continuously bubbled at a rate of 3 L·min\(^{-1}\) using a fixed glass tube in the center of the carboy (Fig. S1). The emitted aerosols were collected on nitrocellulose filters every 24 h, extracted, and analyzed for viral DNA abundance. Quantification of \(EhV\) in the culture and in the aerosols was determined by qPCR for the \(EhV\) MCP. \(E. huxleyi\) cell analysis was performed with an Eclipse (iCyt) flow cytometer.

Decay Rate Experiment. \(EhV\) was introduced onto polyester filters and incubated under regulated atmospheric conditions as follows: temperature 15.9 ± 0.2 °C, 65–75% relative humidity, light intensity of 700 \(\mu\)mol·m\(^{-2}\)·s\(^{-1}\). Filters were collected at different time periods: 0, 40, 80, and 120 min (n = 7). Viruses were extracted from the filters, and a series of 10-fold dilutions was used to infect host cultures (n = 12). After incubation we used the MPN method (4) to determine the number of infective viruses at each point.

Oceanographic Cruise Water and Air Sampling. Water was collected from 61.90°N/33.70°W on July 3 and 4, 2012, during the North Atlantic Virus Infection of Coccolithophore Expedition (NA-VICE; KN07-03), aboard the R/V Knorr (www.bco-dmo.org/project/2136). Samples were obtained from six depths (11, 17, 24, 31, 51, and 150 m) using a Sea-Bird SBE 911plus CTD carrying 10-L Niskin bottles. Biomass from 1 to 2 L of seawater was collected on 0.8-µm polycarbonate filters (Millipore), flash-frozen in liquid nitrogen, and stored at −80 °C until further processing. Genomic DNA was isolated using an adapted phenol-chloroform method previously described by Schroeder et al. (2). Filters were cut into small, easily dissolved pieces and placed in a 2-mL Eppendorf tube. Following addition of 800 µL of GTE buffer (50 mM glucose, 25 mM Tris·Cl (pH 8.0), and 10 mM EDTA), 10 \(\mu\)g·mL\(^{-1}\) protease K, and 100 µL of 0.5 M filter-sterilized EDTA, samples were incubated at 65 °C for 1–2 h. Following incubation, 200 µL of a 10% (vol/vol) stock solution of SDS was added and DNA was then purified by phenol extraction as previously described (5). Finally, air-dried DNA pellets were resuspended in 50 µL DW. Cell abundance was determined by qPCR for the \(E. huxleyi\) cytochrome c oxidase subunit 3 (cox3) gene. Cox3 F1: 5′-agctagaacaccttgagggt-3′, Cox3R1: 5′-tcgcaatgtgagcagtgg-3′.

\(EhV\) abundance (viruses associated to biomass) was determined by qPCR for the \(EhV\) MCP gene. qPCR reactions were performed as described below.

Air was collected continuously during the cruise by pulling through PM10 inlet heads from a 15-m ship mast. The air flowed through stainless steel conductive tubes, minimizing adsorption of particles onto the tube walls. Relative humidity in the inlets was reduced to 20–40%, using silica gel column dryers. The aerosols were collected on 47-mm nitrocellulose and PVDF filters, (0.45 and 0.1 µm; Millipore). Collection duration was 24 h at flow rates of 17 L·min\(^{-1}\) (total capacity of 25 m\(^3\)). Filters were preserved at 4 °C until DNA extraction. DNA was extracted using a PowerWater DNA extraction kit (MoBio). Purified DNA from crushed filters was used as a template for two PCR reactions using primers for the \(EhV\) phosphoglycerate mutase family protein (Pgm) gene EhVPGM_2234F; 5′-attatatcctatgacagc-3′ and EhVPGM_2747R; 5′-aagatgacagttgatg-3′ (2). For each reaction, a reaction mix containing 5 µL of Rapid Ligation buffer (Promega), 4 µL dNTP mixture (2.5 mM each), 0.25 µL of Ex-Taq enzyme (5 U/µL), and nucleases-free water was prepared. Each PCR amplification series included reactions as controls for any contamination occurring during the pipetting and during the extraction of DNA from the aerosols samples. The following steps were performed inside a biological sterile hood to prevent contamination of the samples. All reactions involved initial denaturing (3 min at 95 °C), followed by 40 cycles including denaturing (30 s at 95 °C), primer annealing (45 s at 50 °C), and extension (60 s at 72 °C). Five microliters of each PCR product (including the control reactions) were subjected to a second round of PCR with the same PGM primers. Conditions during the second PCR were identical to those during the first round of PCR. Viral amplicons were sequenced using the ABI 3730 DNA Analyzer.

**Determination of Particle Concentration and Wind Speed Equivalent Mass from Airborne Sea Salt Mass.** Four liters of filtered sterilized seawater (salinity 38–40‰) were bubbled at a rate of 3 L·min\(^{-1}\) in the 10-L carboy, as in previous experiments. The flow was directed into a filter holder (ferrule nut 3/8 inch; PFA), and the sea salt aerosols were collected for 24 h on nitrocellulose filters, pore size 0.45 µm (Millipore) (n = 3). The filters were cut in half and extracted into 4-mL HPLC-grade deionized water for 45 min using a sonicator (7). The extract was inserted, in duplicates, into an ion chromatograph (ICS-3000; Dionex), combined with a conductivity detector (Dionex) for cation analysis. Na\(^+\) was detected using an IonPac column (CS12A, 4 × 250 mm, guard IonPac CG12A 4 × 50 mm) at 30 °C, with 20 mM methanesulfonic acid eluent buffer (Fluka) with an injection volume of 50 µL at a flow rate of 1 mL·min\(^{-1}\); suppressor current was set to 28 mA (CSRS 300 4-mm self-regeneration). Calibration curves for Na\(^+\) were prepared between 10–0.375 ppm. MDL was calculated to be 0.225 µg·m\(^{-3}\). The Na\(^+\) ion was used as a proxy for sea salt, by applying the following equations:

\[
\text{Total sea salt [grams]} = \frac{\text{Na}^+ [\text{ppm} \times 4 \text{ mL}]}{10^6} + \frac{\text{Na}^+ [\text{ppm} \times 4 \text{ mL}]}{10^6} \times 23 \times 35.5 \times 1.16\]  

where ppm = µg/mL, 23 and 35.5 are the atomic weights of Na and Cl, respectively, and 1.16 is the [Cl]/[Na] ratio in seawater (8). Each half of the filters was multiplied by 2 to obtain total mass on the filters.

The following equations were used to obtain sea salt particle mass and number concentrations:
Sea salt mass conc. in the air \( \frac{g}{cm^2} = \frac{\text{Total sea salt}[g]}{4,320,000} \) \[[S4]\]

\[ \# \text{pp} = \frac{\text{Total sea salt}[g]}{2.165[g/L] \times V_{\text{particle}}} \] \[[S5]\]

\[ \text{flux} \left[ \frac{\# \text{pp}}{(m^2 \cdot s)} \right] = \frac{\# \text{pp}}{\text{bubbling system surface area}[m^2] \times 86,400[s]} \] \[[S6]\]

where \( \# \text{pp} \) is number of sea salt aerosols; 4,320,000 is the total volume of air sampled for the 24 h in \( cm^3 \); 2.165 is the density of sea salt (8); and particle volume \( V_{\text{particle}} \) is \( 1.25 \times 10^{-14} \pm 8.64 \times 10^{-15} \ cm^3 \), obtained from scanning mobility particles sizer (SMPS) mode data for online size distribution of sea salt aerosols.

Particle mass concentration was calculated using Eq. S4. Eq. S5 was used to calculate total particle number for submicron particle population. The mean value of the volume distribution \((1.25 \times 10^{-14} \pm 8.64 \times 10^{-15} \ cm^3)\), obtained from online measurements using an SMPS (TSI Inc.), was used to calculate \( V_{\text{particle}} \) (average particle volume) in Eq. S5. Eqs. S4 and S6 were used to obtain the wind speed equivalent mass and fluxes from literature values, respectively (9, 10). Mass concentrations from Table S2 were compared with wind speed equivalent mass concentrations from Woodcock (10); the results show an average wind speed of 8 m s\(^{-1}\). Fluxes were calculated by Eq. S6. The results (Table S2) were compared with fluxes for submicron size distributions in ref. 11 and references therein and were found to be equivalent to a wind speed of 8–10 m s\(^{-1}\).

**Calculation of Viral Decay Rates.** Ten milliliters of E\(h\)\(u\)\(x\)\(l\)\(y\)\(i\) from a fresh virus stock was collected on a 0.45-\(\mu\)m white gridded polyester filter (Millipore) using a vacuum pump. The filters were cut into halves and incubated under atmospheric conditions typical for the North Atlantic spring bloom: temperature 15.9 ± 0.2 °C, relative humidity 65–75%, light intensity 700 \(\mu\)mol photons\(m^{-2}\cdot s^{-1}\), provided by a halogen lamp with a spectrum simulating sunlight (400–700 nm). Radiation was regulated using Fiber Illuminator FL-460 in combination with the Special Fiberoptics 460-F (Heinz Walz GmbH). We used analysis of average atmospheric conditions in the North Atlantic (30–60°N, 15–40°W) during May–June performed using National Oceanic and Atmospheric Administration –National Centers for Environmental Prediction (NOAA–NCEP) Global Data Assimilation System reanalysis data (12, 13) to determine the above experimental conditions. Filters were collected after exposure durations of 0, 40, 80, and 120 min \((n = 7 \text{ for each time point})\). After incubation the filters were extracted by placing them into a tube with 1 mL of filtered seawater followed by vortexing at 8 rpm for 10 min. After extraction, a series of 10-fold dilution steps from 10\(^9\) to 10\(^{-2}\) were made. Aliquots (20 \(\mu\)L) of each dilution were added to 12 wells of a 96-well assay plate, each well containing 180 \(\mu\)L of \(E.\ hu\)\(x\)\(l\)\(y\)\(i\) RCC 374 culture. The assay plates were incubated as described above. Culture density (representing growth or lysis) was measured as optical density at 595 nm. Wells in which lysis occurred were scored, and enumeration of infective viruses was estimated by MPN calculator (4). The decay rate was found to be \(k = 0.0335 \text{ min}^{-1}\) (see Fig. 3).

**Evaluating the Dispersal of Infectious Viruses.** We found that under the specific wind velocity that our laboratory system mimics (8 m s\(^{-1}\), Table S2) E\(h\)\(u\)\(x\)\(l\)\(y\)\(i\) concentration in the air is expected to be six orders of magnitude less than its abundance in the water (see Fig. 1 B and C and Fig. S5). Our in situ measurements revealed that during an open-ocean E. \(hu\)\(x\)\(l\)\(y\)\(i\) bloom, E\(h\)\(u\)\(x\)\(l\)\(y\)\(i\) can reach seawater concentrations of ~10\(^6\) E\(h\)\(u\)\(x\)\(l\)\(y\)\(i\) per mL (Fig. 4C) (14). Therefore, we can estimate the E\(h\)\(u\)\(x\)\(l\)\(y\)\(i\) concentration in the lower atmospheric boundary layer to be ~10 E\(h\)\(u\)\(x\)\(l\)\(y\)\(i\) per L of air. This is in agreement with other studies, where general virus quantification in aerosols collected in a clean marine environment was conducted (15). A typical E. \(hu\)\(x\)\(l\)\(y\)\(i\) bloom occupies thousands of square kilometers (16). To bind our estimations to the lower limit, we consider only an area of 1 km\(^2\), and assuming that only the first 10 m of atmosphere above the water are well mixed. This area yields a parcel of 10\(^10\) L of air with ~10\(^7\) viruses. Next, using 20 min as the lower limit for the E\(h\)\(u\)\(x\)\(l\)\(y\)\(i\) half-life (under daytime atmospheric conditions, see Fig. 3) we calculate, using Eqs. S7 and S8 below, that the infective virus concentration will be reduced by an order of magnitude within a hour. Using 7 m s\(^{-1}\) as the average surface wind speed over the North Atlantic (17), and using Eq. S9 below, allows us to estimate that the given 1 km\(^2\) × 10 m air parcel that contains 10\(^11\) viruses can pass more than 100 km in 4 h, and carry over 10\(^7\) infective viruses.

The following equations were used to evaluate the dispersal of the infectious viruses:

\[ C_0 = e^{-kt} \] \[[S7]\]

\[ \frac{\ln(\%\text{surviving})}{-k} = T_{\%\text{surviving}}[\text{min}] \] \[[S8]\]

\[ lptD[km] = T_{\%\text{surviving}} \times 7m \cdot s^{-1} \times 0.06 \] \[[S9]\]

where \(C_0\) is the number of infectious viruses at time 0 \((C_0 = 10^{11} \text{ viruses according to the calculation made above})\); \(C_t\) is the number of viruses at time \(t\), and \(k = 0.0335 \text{ min}^{-1}\) is the percent of the viral population which remained infective, \(T_{\%\text{surviving}}\) is the duration, in minutes, for which that percent of the viral population remained infective, and \(D\) is the distance (kilometers) that the remaining infective population traveled at an assumed typical wind speed of 7 m s\(^{-1}\).

**The Deposition of Aerosolized Infectious Viruses Through the Course of Airborne Virus Trajectory: A Case Study.** This case study aims to estimate the potential viral deposition spatial distribution using the atmospheric conditions that were measured during the North Atlantic Virus Infection of Coccolithophore Expedition. To do so we used the NOAA–NCEP reanalysis data of the meteorological conditions over the North Atlantic during the cruise, with the help of the hybrid single-particle Lagrangian integrated trajectory (HYSLPLIT) model to obtain the forward trajectories for an air parcel leaving the source area at 61.90°N, 33.70°W, where E\(h\)\(u\)\(x\)\(l\)\(y\)\(i\) was observed in the atmosphere on July 3, 2012. We followed the air parcel from 10:00 AM to 10:00 PM, by running 12 trajectories, one for each of the 12 h. The results show that during the 12 h modeled the southwestern wind path is consistent with an average speed of 6 ± 0.5 m s\(^{-1}\).

Analyzing the meteorological variables along the trajectories shows that the humidity is between 84–92%, the air temperature is between 11 and 12 °C, and the air pressure along the trajectories slightly increases (from 1,017 to 1,020 hPa).

By combining the data from the previous section with the results from the HYSLPLIT trajectory, we are able to investigate the efficiency of the aerial infection mechanism from an additional perspective. At this section we look for the deposition of aerosolized infectious viruses through the course of the airborne virus trajectory.
Considering exponential time dependence of the aerosol residence time such that
\[ N = N_0 e^{-t/\tau}, \]
where \( N \) is the concentration at time \( t \), \( N_0 \) is the initial concentration, and \( \tau \) is the characteristic residence time. We assume \( \tau \) is on the order of 1 d (the residence time of aerosols in the marine boundary layer (MBL) depends on their size; the lifetime for aerosols in the accumulation mode, as the virus particles, is estimated to be between one and several days (18)). We further assume that, to a first approximation, within the examined 12 h, an air parcel with an initial area of 1 km^2 and height of 10 m traveling along the trajectories will conserve its volume. We base this assumption on the narrow variance between the trajectories in Fig. S6, which suggests that an air parcel will be advected along these trajectories and subjected to a relatively small dissipation.

Our third assumption is that the airborne virus flux over the source is relatively constant (i.e., that at any given time there are \( 10^3 \) viruses over the source area of 10 m \times 1 km^2). Based on these assumptions and the above equation, we calculate that 4% of these viruses would settle within the first hour (\( \Delta T = t_1 - t_0 \)) and 3.6% between the fourth and fifth hour (\( \Delta T = t_5 - t_4 \)), reaching a distance of \( \sim 100 \) km from the source (Fig. S6). If we assume that one parcel of air obtaining \( 10^3 \) viruses originates from the source every hour, then we calculate that within 12 h (equivalent to 12 parcels) \( \sim 50\% \) of the initial number of viruses (i.e., \( 10^3 \times 0.5 \)) originating from the 1-km^2 source will sink at a distance covered in the first hour along the trajectory (12 parcels \( x \) 0.04), and \( 10^4 \times 0.43 \) will sink within the distance covered between the fourth and fifth hour along the trajectory (12 parcels \( x \) 0.036). To summarize, assuming a steady wind speed of 6 m/s, a steady concentration over the source, and negligible dispersion in the first 12 h yields a sinking rate of \( 0.43 \times 10^4 \) per square kilometer, at a distance of \( \sim 100 \) km from the source. We measured a half-lifetime of 20 min for viable viruses in the atmosphere, which suggests that \( 10^3 \) viruses will remain infective after 5 h at 100 km downwind from the source. Now, combining our calculations, \( 10^3 \times 0.43 \) viruses can deposit into the seawater at 100 km from the source while remaining infective within a 1-km^2 parcel.

These crude estimates depend on the three main assumption mentioned above. The assumption that \( 10^3 \) virus particles are suspended over the source area in any given time can be loosened because only 1 km^2 of the source is considered, whereas a realistic North-Atlantic bloom occupies thousands of square kilometers. The key interplay in the above estimates is between the number of infected viruses sinking per unit area versus the dispersion area. In case of small dispersion and when all parcels’ trajectories are similar the deposition sector will narrow, experiencing relatively large deposition rate. However, in the case of large variance between the trajectory directions and large air dispersion the concentration of the sinking viruses per unit area along track will be smaller but the area covered by the trajectories will be larger.

**TEM.** Aerosols were collected on PVD and nitrocellulose filters (0.1 and 0.45 μm; Millipore). The filters were extracted onboard by washing the filters with 1 mL artificial seawater several times using an pipette and placing the extract in an Eppendorf tube. The extract was fixed with glutaraldehyde to a final concentration of 0.5%, incubated at 4 °C for 20 min, then plunged into liquid N\(_2\) and kept at −80 °C until analysis. Formvar-coated nickel grids (EMS) were placed on a 20-μL drop of this extract for 1 h, then air-dried. For TEM imaging of laboratory generated EhV aerosol particles, Formvar-coated nickel grids (EMS) were placed on nitrocellulose filters that collected the aerosols for 72 h from an infected E. huxleyi culture, starting collection from the fourth day of infection.

The grids were stained with 2% (vol/vol) uranyl acetate, washed and air-dried, then examined using FEI Tecnai T12 TEM operating at 120 kV. Images were recorded on an FEI Eagle 2Kx2K CCD camera.

**Surface Chl and Particulate Inorganic Carbon.** Satellite data of surface Chl and particulate organic are derived products from radiance measured by the MODIS aboard the Aqua satellite as described in ref. 19.

**Phylogenetic Analysis Based on Phosphoglycerate Mutase Family Protein (PGM) Gene.** Sequences that were generated in this project have been deposited in GenBank (accession nos. KJ620817, KJ620818, KJ620819, KJ620820, KJ620821, and KJ620822).

Additional sequences used for alignment and tree included the following: EhV66, AJ903641.1; PGM-1, HQ412624.1; PGM-2, HQ412625.1; PGM-3, HQ412626.1; PGM-4, HQ412627.1; PGM-5, HQ412629.1; PGM-7, HQ412630.1; PGM-9, HQ412632.1; PGM-10, HQ412633.1; PGM-11, HQ412634.1; PGM-13, HQ412636.1; PGM-14, HQ412637.1; EhV99B1, FN420076.1; and EhV22-F04, HQ412631.1. Other PGM sequences in GenBank were identical to sequences taken for the tree and were not used. EhV163 was too distant to include in the tree. Multiple alignments were performed and phylogenetic trees were built with ClustalW version 2.1. Phylogenetic trees were built with the neighbor-joining algorithm and 1,000 bootstraps, and the trees were visualized using NJPlot. A Phylip distance matrix was constructed on the same alignment using ClustalW, and a PCA was performed using Partek Genomic Suite, version 6.6 (Partek Inc.) (20, 21).


Fig. S1. Experimental setup of *E. huxleyi* and *EhV* dynamics in the culture and airborne *EhV* collection and quantification.

Fig. S2. Concentration of aerosolized viruses as a function of virus concentration in solution. The x axis represents virus concentration of the virus lysate in the solution and the y axis is the corresponding virus concentration in the air. The samples from the air were extracted from the filters and viruses were counted. Each column is an average of different collection time (5, 10, 15, and 20 h, n = 2 for each time point). Error bars are replicates deviation from the average. The linear fit is Y = 1 × 10^{-7} x – 868, R^2 = 0.9892 (Pearson correlation, r = 0.9945), indicating that virus concentration in the air can be predicted by lowering the virus lysate concentration in the seawater by seven orders of magnitude.

Fig. S3. Aerosolized *EhV* in low concentrations infecting healthy *E. huxleyi* populations. Aerosolized viruses at a low concentration of ∼10–100 viruses per L were emitted from a virus lysate (10^5 viruses per mL) (based on Fig. S2 and Table S1). The outflow was continuously directed into the headspace of new, healthy *E. huxleyi* cultures. The host–virus dynamic was followed in (A) a susceptible *E. huxleyi* culture (n = 2), and in (B) a resistant *E. huxleyi* culture (n = 2). Error bars represent the SD of the biological duplicates.
PCA analysis of the PGM sequences found in the aerosol samples collected during the NA-VICE (KN207-03). In both the neighbor-joining tree (see Fig. 4E) and PCA analysis, the most significant split is between the EhV_ice group and all of the others. In the distance matrix, the EhV_ice sequences were clustered because they are very close to each other and cannot be differentiated (blue). The clusters are as follows: (i) EhV_ice01; (ii) EhV_ice 4, EhV_ice 5; and (iii) EhV_ice02, EhV_ice03, EhV_ice06. Shown in red are additional sequences used for alignment and tree (Supporting Information). An animated version of the PCA is available at pca.weizmann.ac.il/pcab/shwpca?ca21c9cc19ae101a2ad47cc34970d11e.
Fig. S5. Correlation between EhV concentration in the culture media and in the air. The x axis represents virus concentration in the seawater and the y axis depicts the corresponding virus concentration in the air. Each point represents a sampling point at a different stage of culture growth (n = 3, each replicate had six time points as shown in Fig. 1 B and C). The linear fit is \( Y = 1 \times 10^{-6} x + 0.4012 \), \( R^2 = 0.4433 \) (Pearson correlation, \( r = 0.6658 \), \( P = 0.0026 \)), indicating that virus concentration in the air can be predicted by lowering the virus concentration in the seawater by six orders of magnitude.

Fig. S6. One-hour-interval forward trajectories presented for 12 h on July 3, 2012, from 10:00 AM to 10:00 PM, at 61.90°N, 33.70°W, at 20 m height. The results were obtained from the HYSPLIT.
Table S1. Virus concentration in the air versus virus concentration in the lysate solution for three different concentrations

<table>
<thead>
<tr>
<th>Virus in the solution (virus per L)</th>
<th>Tank A</th>
<th>Tank B</th>
<th>Tank C</th>
</tr>
</thead>
<tbody>
<tr>
<td>2E+09</td>
<td>3E+10</td>
<td>2E+11</td>
<td></td>
</tr>
<tr>
<td>Virus in the air (virus per L of air)</td>
<td>4E+02</td>
<td>1E+03</td>
<td>2E+07</td>
</tr>
</tbody>
</table>

Table S2. Sea salt mass obtained from filters collected after 24-h bubbling of sterilized sea water (salinity 38–40‰) at a rate of 3 L·min⁻¹

<table>
<thead>
<tr>
<th>Replicate number</th>
<th>NaCl, g/cc air</th>
<th>Bubbling system wind speed equivalent, m/s (^†)</th>
<th>NaCl flux, # pp/(m(^2)·s)(^‡)</th>
<th>Bubbling system wind speed equivalent, m/s (^§)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.75116 \times 10^{-11} ± (9.43227 \times 10^{-13})</td>
<td>8</td>
<td>1.03 \times 10^6</td>
<td>8–10</td>
</tr>
<tr>
<td>2</td>
<td>1.92546 \times 10^{-11} ± (7.13541 \times 10^{-13})</td>
<td>8</td>
<td>1.13 \times 10^6</td>
<td>8–10</td>
</tr>
<tr>
<td>3</td>
<td>2.30185 \times 10^{-11} ± (4.18661 \times 10^{-13})</td>
<td>8</td>
<td>1.36 \times 10^6</td>
<td>8–10</td>
</tr>
</tbody>
</table>

NaCl mass concentration (g/cc air) and NaCl flux (# pp/(m\(^2\)·s)) were used to obtain wind speed equivalent bubbling in our system.

\(^†\)Comparison with Woodcock (1) and Wells et al. (2).

\(^‡\)Particle flux using submicron size distribution calculated from SMPS data according to Eq. S6.

\(^§\)Comparison with Fuentes et al. (3) and references therein.