Aerosol toxins emitted by harmful algae blooms susceptible to complex air-sea interactions

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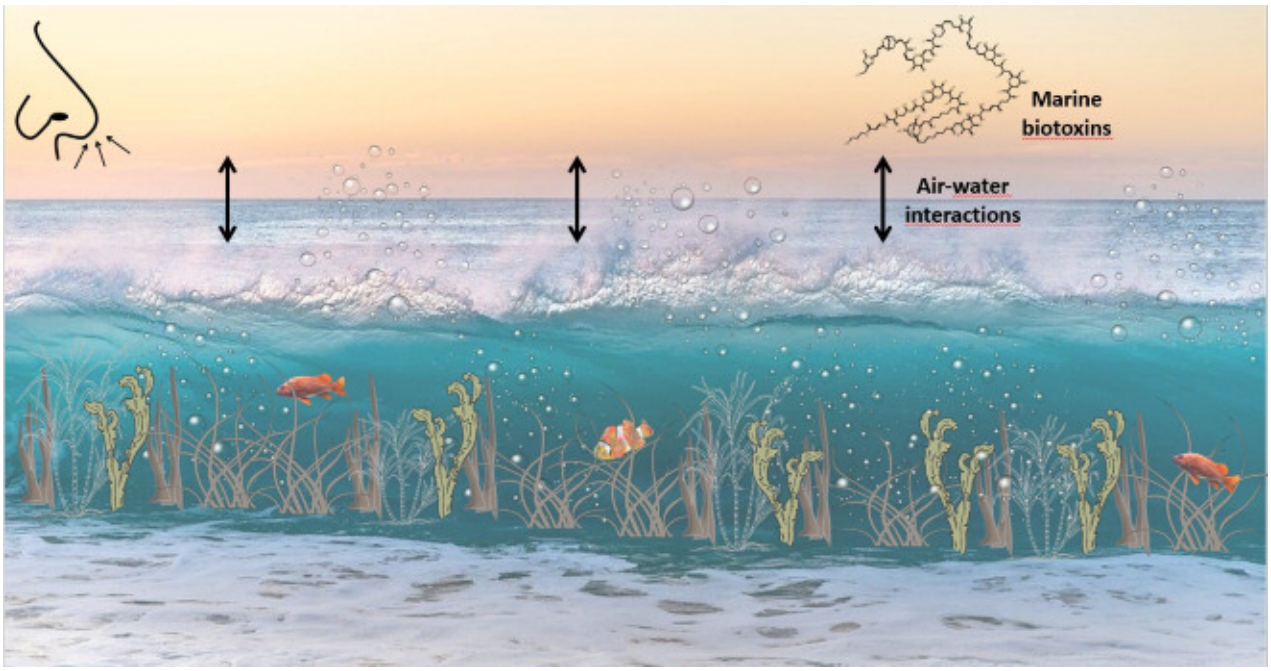

Abstract

Marine harmful algal blooms (HABs) produce toxins that impact marine ecosystems and human health. Little is known for both mechanisms of toxin production and transfer to the atmosphere. Here, we run five experiments to investigate simultaneous water and atmospheric concentrations of toxins synthesized by *Ostreopsis* cf. *ovata*, a tropical microalgae whose recurrent blooms have been related to mild but acute respiratory symptoms in some Mediterranean beaches. We detected *Ostreopsis* produced toxins in both water and atmospheric aerosol generated in all experiments; however two clear groups could be separated. Exp. 1 and Exp. 2 showed the highest toxin concentration in the atmospheric generated aerosols (49-69 $pg \cdot L_{air}^{-1}$) with moderate water toxin concentration ca. 4 x 10^6 $pg \cdot L_{water}^{-1}$, generated by *Ostreopsis* cell numbers in typical summer Mediterranean coastal environments (about 10^5 $cells \cdot L^{-1}$). By striking contrast, the remaining three experiments showed about 15 folder lower atmospheric concentrations (3-4 $pg \cdot L_{air}^{-1}$), regardless the fact much higher cell abundances and toxin concentration (respectively, about 1 10^6 $cells \cdot L^{-1}$ and 2 10^8 $pg \cdot L_{water}^{-1}$) were used. Offline spectroscopic analysis performed by nuclear magnetic resonance (NMR) spectroscopy showed that the particulate organic carbon (POC) matter is drastically different from that of bubble bursting aerosols from the tank experiments, suggesting selectively transfers organic compounds from seawater into the atmosphere. It is concluded that - beside the eco-physiological state of the microalgae and the responses of the cells to the incubation setup - foam accumulation and complex air-sea dynamics in the surface microlayer could difficult aerosol production and diffusion of the marine toxins in the atmosphere.

1. Introduction

Harmful algal blooms (HABs) are proliferations of certain microalgae, cyanobacteria or macroalgae that cause direct or indirect negative impacts to aquatic ecosystems, coastal resources, and human health (GEOHAB 2001). Blooms of the benthic dinoflagellate genus *Ostreopsis* constitute nowadays a particular challenge and paradigm of HABs due to its negative impacts on marine fauna and to the production of toxins involved in exceptional but dramatic seafood intoxications and more general mild respiratory and cutaneous irritation in humans. This unicellular microalga grows in relatively shallow and well illuminated waters attached to benthic surfaces (rocks, sands, corals, macroalgae) by a self-produced mucopolysaccharide substance (Honsell, G. et al. 2013). Under suitable light, temperature, nutrients and turbulence conditions, *Ostreopsis* cells proliferate and benthos appears covered by a rusty mucilaginous microfilm containing high *Ostreopsis* cell numbers. As part of the life cycle and with the help of certain water motion, aggregates are released from the benthos and can be found floating at surface as dense brown foams. Blooms of *Ostreopsis* have been linked to massive mortalities of benthic organisms (starfish, sea urchins, crabs, mussels; Sansoni, G. et al. 2003; Shears and Ross, 2009; Totti, C. et al. 2010) in temperate ecosystems of the Mediterranean, Brazil and New Zealand coasts. The involved mechanisms are still poorly understood, but they could involve a combination of low oxygen availability associated to the microalgal bloom and exposure to *Ostreopsis* produced toxic compounds (Carella et al. 2015; Migliaccio et al. 2016; Neves and Nascimento 2018). *Ostreopsis* synthesizes palytoxin (PLTX) analogues, namely, putative PLTX (pPLTX), ostreocins (OSTR) and ovatoxins (OVTX). PLTX is the most potent nonbacterial toxin that has been attributed to dramatic seafood intoxications in tropical areas (Alcala, A. C. et al. 1988; Randall, J. E. 2005). OVTXs and pPLTXs have been detected in some marine fauna in the Mediterranean Sea (Amzil, Z. et al. 2012; Biré, R. et al. 2013) raising concern due to the recurrence of *Ostreopsis* blooms in this area reported in the last 20 years. Seafood intoxications have not been detected yet in the Mediterranean while the mid to late

summer massive blooms of *Ostreopsis* have been associated to mild respiratory illness and skin irritation on beach users (Gallitelli, M. et al. 2005; Durando, P. et al. 2007; Tichadou, L. et al. 2010; Pfannkuchen, M. et al. 2012; Vila, M. et al. 2016). In order to prevent health impacts, regular monitoring is conducted in Italy, France and Spain, and has driven beach closures with subsequent lost revenue to tourist-dependent coastal communities (Lemée, R. et al. 2012; ICOD, 2012). Concern has increased also among the scientific community due to the apparent increase in the biogeographic distribution of this tropical genus to more temperate latitudes (Rhodes, L. 2011; Tester, P. et al. 2020) likely linked to global warming and anthropogenic forcing in the coastal areas.

It has been hypothesized that pPLTX and OVTXs would be the causative agents of the respiratory disorders and the toxic effects were observed specially in lungs of mice exposed to these aerosolized compounds (Poli et al. 2018). However, despite the recurrence of the *Ostreopsis* blooms during the summer vacation period in the Mediterranean, and the reported respiratory irritation cases, PLTX analogues have been rarely detected (Ciminiello, P. et al. 2014). In the small-scale monitoring along the Tuscan coast (Italy) on 2010, 2.4 pg OVTX per liter of air were measured on August 11 and trace levels on August 12, concurrently to low *Ostreopsis* cell numbers in the water (ca. 12000 cells·L^{-1} and 30000 cells·g FW of macroalgae^{-1}) and *Ostreopsis* DNA detected in the filters used to sample aerosols. Although no human health symptoms were reported, this study constituted the first finding of *Ostreopsis* cf. *ovata* in marine aerosols. Vila, M. et al. (2016) aimed to establish a link between Ostreopsis cf. ovata blooms and human health impacts by using an ecology and epidemiology interdisciplinary approach. It was suggested that human respiratory impacts were not directly related to high *Ostreopsis* cell abundances in the algae blooms. Hence, other factors including the mechanism for the aerosolization of the toxic material from the water phase to the atmospheric one may play an important rule. To test this, marine aerosols were produced in the laboratory using an aerosol generation tank. The objective of this study is to show – for the first time – the simultaneous detection of toxin compounds in the water and the atmospheric phases. Furthermore, we analyze the chemical composition of submicron (PM_1) aerosol samples of both Particulate Organic Carbon

seawater samples and bubble-bursting aerosols. The results provide new hints on the distributions and transfer of organic toxic material from the water into the atmosphere.

2. Materials and methods

2.1. Study site and samplings

Experiments were run on natural epiphytic microbenthic communities obtained along the *Ostreopsis* cf. *ovata* bloom in the rocky beach of Sant Andreu de Llavaneres, in the Catalan coast of Spain (41°33.13′N; 2°29.54′E; Fig. 1. Photo or Map). The bloom, that recurrently occurs along the summer - fall season was monitored as described in previous studies (Vila, M. et al. 2016). Briefly, samples for the estimations of epiphytic *Ostreopsis*, were collected from the dominant macroalgae (*Ellisolandia elongata* Ellis & Solander and *Jania rubens* (Linnaeus) J.V.Lamouroux). Between 10 and 20 g of fresh macroalgae were collected and transferred into a 250 mL capacity plastic bottle and filled with a known volume of the surrounding seawater. The sample was then vigorously shaken for 1 minute, and subsequently sieved through a 200 µm mesh. A percolated water subsample, ca. 125 mL, containing the microalgae community was fixed with iodine Lugol's solution and cell numbers were estimated as described in section 2.3. Cell abundances were expressed as cells·g^{-1} of fresh weight (FW) of macroalga. When cell numbers were above 200,000 cells·g^{-1} considered the alert value (Lemée et al., 2012), samples for the experiments were taken. In particular, natural samples for Experiments #1 and #2 were obtained on 24 and 25 of July, 2019 while those for Experiments #3, #4 and #5, on 5, 6 and 7 of August, 2019.

For the experiments, macroalgae (ca. 200-500 grams) were collected on several 8 L plastic beakers containing 5 L natural seawater and transported to the laboratory within 1 hour after collection. Microbenthic organisms were released from the macroalgae by intensively shaking each beaker and subsequent filtration through a 200 µm sieve. All samples were merged into a single container and *Ostreopsis* cell numbers were estimated to calculate the inoculation volume in order to conduct the experiments with different initial *Ostreopsis* abundance.

2.2. Aerosol Generation Tank

An airtight high grade stainless steel tank (150 l) was filled with about 50 L of seawater (0.2 µm filtered) obtained from Llavaneres beach on the same day. Water was circulated with a peristaltic pump at 50 rpm, dropped from the top of the tank as a plunging jet at a flow rate of 12 L min^{-1}, entrained air formed bubbles that - by bursting processes - produce sea spray aerosols. Particle-free compressed air was blown into the tank headspace (60 L min^{-1}), which had outlet ports leading to an PM_1 aerosol sampler (37 L min^{-1}), the rest as excess flow. Running time was 45 minutes for Experiment #1, 4 hours for Experiment #2, and 21h for Experiments #3, #4 and #5. The PM_1 aerosol collecting filter was frozen at -80ºC. The tank was open, water was carefully mixed and samples for *Ostreopsis* cell numbers and toxin concentration (duplicate GF/F filters) were obtained.

2.3. *Ostreopsis* cell counts

Water samples (10 mL) were fixed with iodine Lugol's solution and *Ostreopsis* cells numbers were estimated using a Sedgewick-Rafter cell chamber under a Leica inverted light microscope.

2.4. Toxin collection, extraction and analysis in seawater and aerosol samples

Seawater samples were filtered through 25 mm GF/F filters (Whatman, Clifton, NJ, US). PM1 aerosol was collected on 47 mm Whatman QM-A quartz filters, pretreated by washing twice on 1% nitric acid, followed by rinsing twice with pure water, and subsequent drying at 30°C (Ackerman et al. 2018). Both water and aerosol samples were immediately immediately frozen at -80C and kept in these conditions until analysis. Toxin extraction was conducted on 2 mL of MeOH:H_2O (1:1, *v/v*) (previously cooled down to -80°C) with three 10 min ultrasonic pulses in an ice-cooled bath, with vortex shaking between ultrasound pulses. The extract was centrifuged (4500 rpm, 4°C, 5 min), the supernatant was transferred to a new tube and a second extraction was conducted on the pellet. The two extracts were merged, filtered through 0.22 µm Nylon membranes

(Whatman, Clifton, NJ, US) and stored on ambar glass vials at -80°C until their analysis by UHPLC–HRMS.

Ultra-High Performance Liquid Chromatography coupled to High Resolution Mass Spectrometry

An ultra-high-performance liquid chromatography (UHPLC) system (Accela; Thermo Fisher Scientific, San José, CA, US) equipped with Accela 1250 quaternary pump, an Accela autosampler and column oven (Thermo Fisher Scientific) was used coupled to a Q-Exactive Orbitrap Fourier-transform mass spectrometer (FTMS) system (Thermo Fisher Scientific) equipped with a heated electrospray source (HESI) operated in positive ion mode. The vaporizer and ion transfer tube temperatures were set at 350ºC and 275ºC, respectively. The spray voltage was stablished at +3 kV and a S-lens RF level of 70 a.u. was used. The chromatographic separation was performed in a Hypersil GOLD^{TM} C18 column, (100 mm x 2.1 mm id., 1.9 µm particle size) filled with totally porous silica particles from Thermo Fisher Scientific. The mobile phase consisted on acetonitrile (solvent A) and water (solvent B) both containing 0.1% formic acid as additive. The gradient elution program was: 0–1.5 min, a isocratic step at 30:70 (acetonitrile:water 0.1% formic acid, v/v); 1.0–13-5 min, linear gradient up to 35:65; 13.5–15.0 min, linear gradient up to 90:10 and then returns to initial conditions. The mobile phase flow-rate was 300 µL min^{-1} and the column temperature was held at 23ºC. The UHPLC system was coupled to a Q-Exactive Orbitrap FTMS system operating in full scan MS mode (m/z 800 – 1500) at a mass resolution of 70,000 full width half maximum (FWHM). The Xcalibur software v2.1 (Thermo Fisher Scientific) was used to control the UHPLC–HRMS system and to acquire and process the MS data. Calculation of elemental formulae was performed on the mono-isotopic peak of each ion cluster with a mass tolerance of 5 ppm. The isotopic pattern of each ion cluster was taken into consideration in assigning molecular formula. Extracted ion chromatograms for PLTX and OVTXs were obtained selecting the most intense peaks of the toxin ion cluster. The quantitative results are expressed as the sum of the putative PLTX and all OVTX forms (a-g).

2.5 ^{1}H-NMR spectroscopy

Two POC filter samples and two sea-spray generation experiments from EXP.1 and EXP. 2 were extracted with deionized ultra-pure water (Milli-Q) in a mechanical shaker for 1 h and the water extract was filtered on PTFE membranes (pore size: 0.45 μm) in order to remove suspended particles, details of the procedure can be found elsewhere (Decesari et al., 2019). On the basis of the range of frequency shifts, the signals can be attributed to H-C containing specific functionalities (Decesari et al., 2000, 2007).

3. Results and discussions

3.1 Water and air Toxin concentrations

Fig. 2 shows the cell abundance of *Ostreopsis* (Figure 2, bottom) and toxin concentrations (Figure 2, centre) in the seawater samples at the beginning (T_0) and at the end (T_1) of each experiment, as well as the toxin concentration retained in the PM_1 aerosol collecting filter during the duration of the experiment ($T_{0\text{-}1}$) (Figure 2, top). The black arrow lines help to visualize the sea-air interface. Overall, all the experiments were conducted on *Ostreopsis* cell concentrations in the water commonly found in Mediterranean waters during the peak bloom season (Mangialajo et al., 2011).

In Experiment #1, after 45 min incubation, both *Ostreopsis* cell numbers and total toxin concentrations increased from 0.72 x 10^5 to 1.82 x 10^5 cells·L_{water}^{-1} and from 3.52 x 10^6 to 6.23 x 10^6 pg·L_{water}^{-1}, respectively, while in the air total toxin concentration accounted for 69.01 pg·L_{air}^{-1} (69% OVTX-a, 31% OVTX-b). In Experiment #2, after 4 hours incubation, both cell numbers and total toxin decreased slightly from 1.35 x 10^5 cells·L_{water}^{-1} to 1.05 x 10^5 cells·L_{water}^{-1} and from 4.86 x 10^6 pg·L_{water}^{-1} to 3.64 x 10^6 pg·L_{water}^{-1}, respectively. Total toxin concentration retained in the filters accounted for 49.13 pg·L_{air}^{-1} (53% OVTX-a, 28% OVTX-b, 4% OVTX-c, 8% OVTX-d, 7% OVTX-e).

Opposite trends were observed in Experiments #3, #4 and #5 run with one order of magnitude higher cell concentrations in the tank than the previous experiments #1 and #2. Regarding Experiment #3, after 21 hours incubation, both *Ostreopsis* cells numbers and total toxin concentration in the water slightly increased from 3.26 x 10^6 cells·L_{water}^{-1}

to 3.83 x 10^6 cells·L_{water}^{-1} and from 2.30 x 10^8 pg·L_{water}^{-1} to 2.93 x 10^8 pg·L_{water}^{-1}. In Experiment #4, after 21 hours incubation, *Ostreopsis* cells numbers slightly increased from 4.69 x 10^6 cells·L_{water}^{-1} to 5.21 x 10^6 cells·L_{water}^{-1} and total toxin concentration in the water increased from 1.33 x 10^8 pg·L_{water}^{-1} to 3.15 x 10^8 pg·L_{water}^{-1}. Finally, in Experiment #5, after 21 hours incubation, *Ostreopsis* cells numbers slightly decreased from 3.52 x 10^6 cells·L_{water}^{-1} to 3.15 x 10^6 cells·L_{water}^{-1} and total toxin concentration in the water increased from 1.87 x 10^8 pg·L_{water}^{-1} to 2.34 x 10^8 pg·L_{water}^{-1}. Opposite to what was expected, we found very low total toxin concentration retained in the PM_1 aerosol filters for the three experiments (about 3-4 pg·L_{air}^{-1}; 70% OVTX-a, 30% OVTX-b).

The most important key observation of this study is the fact that the sea-air experiments using the highest cell abundance in the water - also producing the highest water toxins concentrations - are the ones with the lowest toxin concentrations detected in the sea spray bubble bursting aerosols. In other words, the obtained data showed that despite the increase in the toxin concentration in the water in Experiments #3, 4 and 5 - with high cell abundances - toxins in the atmospheric aerosols were low. Whilst this is only a laboratory experiment, some analogous results were found by Ciminiello et al., 2014 – the higher concentrations of toxins in the air were found during days with low toxin abundance in the water, although a detailed explication was not given. Our results are also consistent with the observations that high *Ostreopsis* cell concentration in the water may not be necessarily cause respiratory symptoms on beach users as described by Vila M. et al., 2016. These authors suggested that the complex physiological processes influenced by environmental factors (e.g. nutrients, temperature, water motion) may modulate toxin production by the cells, its release and aerosolization.

3.2 Organic composition of POC water and bubble bursting aerosols.

Further insights from the primary aerosol production come from the chemical analysis of the POC water and bubble bursting aerosols. Figure 3 (top) shows the proton NMR spectra of two POC samples from EXP1 and EXP2, respectively. Our interpretation of these unique POC spectra is reported along a comparison with other datasets from

surface ocean water and from melted sea-ice samples investigated in studies carried out in the North Atlantic (Decesari et al., 2011) and in the Weddell Sea (Decesari et al., 2019). Similarly to the previous studies, the POC composition is dominated by peptides (traced by aminoacids like Ala, Thr, Val, Ile and Leu) and to sugars or polyols (with signals in the H-C-O region between 3.3 and 4.2 ppm of chemical shift). In contrast with the previous studies, we could not detect specific signals of anomeric hydrogen atoms characteristics of most common monosaccharides (in the range 5.2 to 5.5 ppm of chemical shift). This can be due to the occurrence of sugars as large polimers (polysaccharides) and to significant contributions from polyols as well as from alpha-hydrogen atoms of aminoacids to the H-C-O spectral region. Other resonances of organic markers common to all POC samples shown in Figure 3 (top) are isobutyric acid (IsoBu), acetic acid (Ace), dimethylamine (DMA) and N-osmolytes (Bet: betaine; Cho: choline) as well as the glycerol (Glc). The specific NMR resonances of palytoxins cannot be resolved from the complex matrix signal. The spectra of the aerosolized algae-enriched sea-water (algae-BB spectra, Figure 3 bottom) show little overlaps with the POC spectrum, except for a few resonances (such as the triplet at 3 ppm, which however it has never been identified unambiguously). The spectra of the bubble bursting samples (Figure 3) show intense signals from isobutyric acid (IsoBu) and glycerol (Glc). Glycerol occurs both in free form and chemically bound in esters. The glycerol resonances overlap with a broader band in the H-C-O region between 3.4 and 3.9 ppm attributable to oligo-/poly-saccharides. In the spectral region of alkyls (H-C, 0.7 - 1.9 ppm) the resonances of lactic acid and isobutyric acid overlap with the broad bands of linear aliphatic compounds, like short-chain fatty acids. These occur in much smaller proportions with respect to sugars when comparing to the bubble bursting experiments conducted in the Weddell Sea. Another important difference is the scarcity of N-osmolytes (i.e., betaine, choline, etc.) which, in the Weddell Sea samples, were also associated to phospholipids in nascent sea-spray particles.

In summary, our NMR chemical profile results show that the process of aerolization in the tank enriches primary marine aerosols with lipids, smaller oligo-sugars while depleting them of free aminoacids and peptides in general. This implies that a foam layer continuously broken and recreated by a plunging jet may play a role in aerosol particle

307 production, resulting in an aerosol population in the atmosphere with a very different
308 chemical composition than the bulk water.

309

310 **3.3 From the benthic-pelagic compartments to the atmospheric implications**

311

312 This study examined the toxin concentrations of aerosol generated from HAB seawater
313 samples with varying *Ostreopsis Cv Ovata* cell concentrations. For the first time we were
314 able to measure toxin concentrations simultaneously in the water and in the air in an
315 aerosol marine tank. Regardless the fact that HAB cells abundance did not vary with the
316 time of each experiment, we found a higher toxin concentrations at the end of the bubble
317 bursting experiment relative to the concentration measured at the beginning. This
318 increase - found for the 3 experiments with high cell abundance - suggests that these
319 biological experiments vary with time and complex unknown mechanisms are
320 responsible for the observed variation. Toxin production in phytoplankton can be
321 regulated both by intrinsic and extrinsic factors. High algal cellular toxicity levels are
322 normally associated with stress caused under unfavourable conditions for algal growth
323 (Ogata et al. 1989, Johansson & Granéli 1999, Ashton et al. 2003, Gedaria et al. 2007).

324 This work is particularly important as it suggests that physical-chemical mechanisms
325 could have large effects on toxin aerosol production. In the atmospheric aerosols
326 samples, we expected a simple linear response between the water and the atmospheric
327 phases: the more toxins in the water, the more toxins in the air. By striking contrast, an
328 unexpected consistent trend was found: the higher the cells and toxin concentrations in
329 the water samples, the lower the amount of toxin material sampled in bubble bursting
330 aerosols produced in a sea spray chamber. This implies that there must be a selective
331 transfer of molecules from the water solution phase into the aerosol atmospheric phase
332 during bubble bursting. Further strong support comes from the different chemical
333 composition of the POC water samples and relative bubble bursting aerosols, showing
334 enrichment of some chemical compounds (lipids, small sugars) and depletion of others
335 (aminoacids). Although theories have been developed that describe surface activity of

organic molecules at the air–water interface (Cochran et al., 2016 a, b), we still know too little about the generation of primary marine organic aerosols. This information is essential for improving our currently limited understanding of the potentially wide ranging atmospheric impacts. Honsell et al (2013) reported an integrated approach describing unique features of the mucilage - acid polysaccharides and trischocysts forming large network - abundantly produced by Osctreopsis Cf. Ovata, playing a fundamental role to colonize benthic substrates. In other words, it was suggested that mucilage contributes to the buoyancy of the phytoplankton aggregates and may contribute the formation of toxin aerosols. This may be true as higher amount of particulate organic carbon may be formed via primary aerosol production, however the high amount of cell abundance may contribute to foam limiting the toxin emissions as our studies suggest.

Monitoring the toxic blooms of Ostereopsis is challenging due to the complex variability of the benthic and pelagic compartments (Jauzein et al., 2018). The cells of Ostreopsis are mainly distributed in the benthic environment (sediment surface and some sub-surface layers), but also located on hard substances such as rocks, sand and mollusk shells. These cells are also observed as free cells or agglutinated in mucilaginous aggregates in the water column. Such mucilage seem to play a key role in the vertical distribution and eventually in the sea water surface. During the algae bloom development, higher concentrations of cells are observed in the water column, whilst the main stock of the population is located on the sea bed (Vila et al., 2001; Vila et al., 2016). It seems the proportion of cells loosely attached to biotic substrates is anticorrelated with benthic cell abundances, suggesting dense mucilaginous mats may prevent cells to detach and get suspended in the water column.

Not only the cells and toxins need to move from the benthic to the pelagic environments, but also these need to be aerosolized from the pelagic sea to the atmosphere. Marine aerosol particles are produced at the sea surface primarily due to interaction between wind and waves (bubble bursting). However, the export of organic matter from ocean to atmosphere represents a substantial carbon flux in the Earth system, yet the impact of environmental drivers on this transfer is not fully understood (Van Pinxteren et al., 2017, Engel et al. 2017). Jenkinson et al. (2018) reviewed the biological modification of

mechanical properties of the sea surface microlayer, influencing waves, ripples, foam and air-sea fluxes. Foams – including whitecaps and extensive foams – may have ecological functions but also play a major role in modulating global air-sea aerosol fluxes; they are critical in the production of sea-salt aerosols. In other words, the organic productivity of surface waters, the composition of different ecological communities do influence the amount of polysaccharide-rich and protein-rich particles in the surface microlayer (Matrai et al., 2008; Gao et al., 2012; Aller et al., 2017). Furthermore, additional complexity not only comes from different abundances varying with dominating bloom species, but different phases of blooms. Overall all these difference translate in drastic differences of aerosol mass particle production and different chemical characteristics of the organic component emitted in the atmosphere.

It is worth to remind that aerosol production from wave breaking on freshwater lakes is poorly understood in comparison to sea spray aerosol (May et al., 2016; Axson et al., 2016). Recent studies reported novel results showing that harmful algal blooms (HABs) of Cyanobacteria (blue-green algae) from freshwater lakes can produce toxins that impact human health (May et al., 2018). Human societies concentrated in coastal and freshwater areas benefit from ecosystems. In turn, high population density and activity led to nutrient inputs. Indeed, using a 45 year record of the occurrence and duration of HABs along the chine coast (Xiuo et al 2019), it was shown that warming and eutrophication show further complexity in triggering HAB blooms. The mixing of fresh and salt water in coastal areas add further complexity in the sea spray generation. For example, the Artic riverine organic matter can be directly emitted from surface seawater into the atmosphere via bubble bursting (Park et al., 2019). Salinity may vary drastically in coastal regions due to river inputs, the role of salinity, organic matter, HABs and aerosol production is not fully known (Abdennadher et al., 2017).

While this set of laboratory experiments represent a major step forward in simultaneously determining the amount of HABs toxins both in water and air, the role of organic and inorganic components present in the real ambient conditions is currently poorly understood. Further laboratory and field measurements of the relationship between complex ecosystems and marine aerosol production. Harmful Algal Blooms operating at

different spatial-temporal scales are a peculiar example of a number of processes including complex physical-chemical (temperature, wind, waves, surface microlayer, air-sea interactions) and biological (predation, competition, stress, parasitism) ones (Borja et al., 2020; Tester et al., 2020). Additionally, HABs can also affect water quality and water flow (Berdalet et al., 2016) and potentially - once aerosolized, can be transported by the wind. These HABs can potentially convey long distance and potentially expand in other locations (Sharoni et al., 2015). A necessary step toward a better understanding of the ocean/atmosphere is the strong interdependence of multiple biological (production, stress, ecological state, decomposition), chemical (photodecomposition) and physical (aerosol formation, coagulation). Interdisciplinary studies – widely advocated but yet still poorly developed – are needed in order to reduce gaps between ocean and atmospheric sciences.

Acknowledgement

The authors gratefully acknowledge the financial support received by N.I.M-P. from Spanish Ministry of Science, Innovation and Universities under the project PGC2018-095013-B-I00 and from the Agency for Administration of University and Research Grants (Government of Catalonia, Spain) under the project 2017SGR–31. E.B. is supported by project CoCliME an ERA4CS Network (ERA-NET) initiated by JPI Climate, and funded by EPA (IE), ANR (FR), BMBF (DE), UEFISCDI (RO), RCN (NO) and FORMAS (SE), with co-funding by the European Union (Grant No. 690462). CoCliME is endorsed to the international programme on harmful algae GlobalHAB (www.globalhab.info). The study was supported by the Spanish Ministry of Economy through PI-ICE (CTM2017–89117-R) and the Ramon y Cajal fellowship (RYC-2012-11922).

Compliance with ethical standards

Conflict of interest: The authors declare that they have no conflicts of interest.

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Fig. 1: Left: Ostreopsis cf.ovata hot spot (Sant Andreu de Llavaneres beach) in the NW Mediterranean (red dot); center: detail of the rocky beach were macroalgal communities and Ostreopsis cf. ovata proliferate; right: microscopi image of O. cf. ovata cells embedded in the mucilaginous matrix by which cells can attach to surfaces (Photo credit: Rubén Duro).

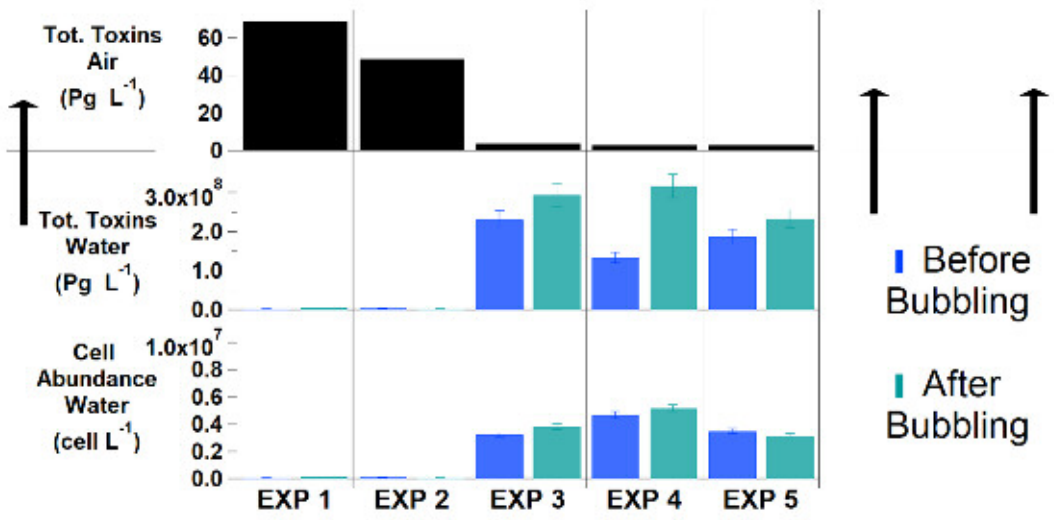

Fig. 2: Ostreopsis cell numbers (a) and toxin concentrations in the water (b) and the beginning and the end of the experiments, and c) toxin concentrations in the air after the aerosol generation time.

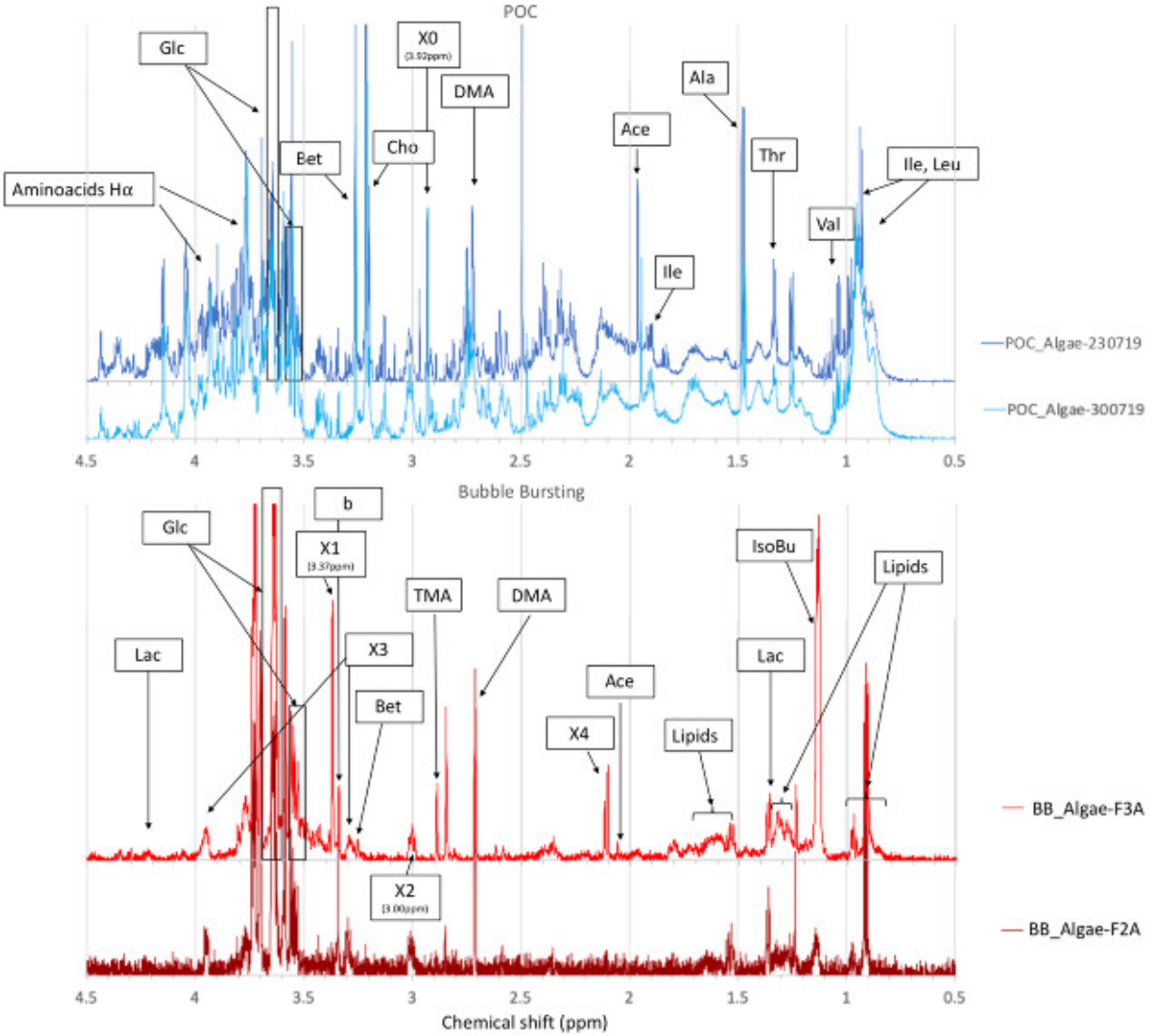

Figure 3: Aliphatic region of the H-NMR spectra of the two POC (upper blueish spectra) and the two Bubble bursting aerosol (lower reddish spectra) samples extracts. In POC samples, specific NMR resonances were assigned to: the residuals of aminoacids (Ala, Thr, Val, Ile, Leu) and their alpha hydrogen atoms, isobutyric acid (IsoBu), acetic acid (Ace), dimethylamine (DMA), N-osmolytes (Bet: betaine; Cho: choline), and glycerol (Glc). A sharp peak at 3.92ppm (labeled as X0) resembles the methyl of the Dimethylsulfoniopropionate (DMSP), not confirmed by the absence of the characteristic triplets at 2.8 and 3.49ppm. In Bubble Bursting samples, specific resonances were assigned to lactic acid (Lac), acetic acid (Ace), isobutyric acid (IsoBu), di- and tri-methylamines (DMA and TMA), glycerol (Glc), N-osmolytes (only Bet: betaine) and to

625 blank contaminations (b). Unresolved mixtures of aliphatic compounds were identified as
626 lipids. Peaks or structures denoted as X# correspond to resonances not attributed yet to
627 specific tracers but already identified in previous studies (Facchini et al., 2008; Decesari
628 et al., 2011 and 2019)
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