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Rising bubbles enhance the gelatinous nature of the air–sea interface

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Abstract

Bubbles rising through the water column are known to scavenge organic material and microorganisms, and transport them through the air–sea interface after bursting. This mechanism has important implications for air–sea exchange processes. However, little is known about how bubbles influence the chemical and biological properties of the sea-surface microlayer (SML), a gelatinous film at the air–sea interface. We used floating mesocosms in the coastal Baltic Sea and a laboratory tank filled with seawater from the North Sea to study the effect of bubbling on the gelatinous nature of the SML. Bubbling was found to always increase concentrations of transparent exopolymer particles (TEP) in the SML. In the field, TEP in the SML already increased after 2 min (53% ± 63%) and 10 min (19% ± 12%) bubbling, respectively. During the tank experiment, TEP enriched in the SML by 312% (± 244%) after > 3 h of bubbling. Therefore, bubbling is a highly efficient mechanism for TEP enrichment in the SML. Bubbling caused enrichment and depletion of microbial abundances (prokaryotes, flagellates, eukaryotes) in the SML. However, the incorporation of ^{3}H-thymidine (i.e., bacterial carbon production) was consistently stimulated after 10 min of bubbling in the field experiment, indicating a bubble-induced import of unstressed bacteria and fresh organic substrates into the SML. Overall, our results suggest that the gelatinous matrix of the SML is re-formed within min after disruption by bursting bubbles, and, thus, highlights the importance of biogeochemical interactions within the air–sea interface.

In 1983, John McN. Sieburth first proposed the idea and importance of a biofilm-like sea-surface microlayer (SML) at the air–sea interface (Sieburth 1983). The composition and role of this SML has since been extensively studied and has been shown to have unique chemical, biological, and physical properties, which separate it from underlying waters (ULWs; Sieburth 1983; Cunliffe et al. 2013; Wurl et al. 2016). Many parameters are highly enriched and distinct in the SML compared to the ULW such as phytoplankton, bacteria, and surfactants (Hardy 1982; Liss and Duce 1997; Stolle et al. 2011; Wurl et al. 2011*b*). Further studies have led to the understanding of this layer as a gelatinous film (Wurl and Holmes 2008; Cunliffe and Murrell 2009), composed of both biological and chemical material held together by extracellular polymeric substances (EPS). One of the most predominant forms of EPS is a group of acidic polysaccharides known by their staining with Alcian Blue (Alldredge et al. 1993). These transparent exopolymer particles (TEP) are formed either abiotically or biotically. When formed abiotically, physical movement of the water forces the precursor colloidal material to stick together and form the aggregates and size fraction defined as TEP (Wurl et al. 2011*a*). The biotic pathway is formed by the breakdown and secretion of precursor material from organisms including bacteria and phytoplankton and it is this second pathway, which forms the majority of TEP and other EPS (Alldredge et al. 1993).

One of the most important roles of TEP comes in its relationship with phytoplankton. Phytoplankton release precursor TEP material during both their growth and senescent phase, resulting in TEP peaks which correspond with phytoplankton blooms (Alldredge et al. 1993; Passow 2002; Bartual et al. 2017). Precursor material can also be released as phytoplankton become stressed, e.g., due to nutrient depletion (Passow 2002; Chen and Thornton 2015). Studies have shown a phytoplankton group dependence on TEP concentrations, with diatoms believed to release the largest faction (Kiørboe and Hansen 1993; Passow and Alldredge 1994). However, Passow (2002) found that some nondiatom species can also produce comparable amounts of TEP. All of this dissolved precursor material, via physical forcing, can then spontaneously adhere to itself and form colloids and eventually TEP aggregates. In turn phytoplankton, bacteria and other organic matter will also adhere to these gel like matrices and based on the buoyancy, will either sink down to form what is termed "marine snow" (Alldredge et al. 1993; Logan et al. 1995) or ascend to the

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sea surface (Azetsu-Scott and Passow 2004; Mari et al. 2017) to support the formation of a biofilm-like SML (Wurl and Cunliffe 2016). An increase in biofilm like properties can greatly affect air–sea interactions, for example, air–sea gas exchange (Wurl and Cunliffe 2016). While phytoplankton has the largest role in TEP production, bacteria have been found to be important TEP colonizers (Simon et al. 2002; Taylor and Cunliffe 2017). Whereas it is most likely that all TEP aggregates are colonized to some extent by bacteria, some studies reported that up to 68% of total bacterial cells found in water were attached to EPS (Mari and Kiorboe 1996). A recent study suggests that this might have implications for the transport of bacterial communities between different waterbodies (Busch et al. 2017). During aggregation, heterotrophic bacteria may change TEP quantity and composition by extracellular enzyme activities (Smith et al. 1995) and/or uptake of TEP microgels (Taylor and Cunliffe 2017). Bacteria have also been found to release TEP precursor material and assist in TEP formation from precursor material due to their mobility and sticky coating material on the membrane (Van Loosdrecht et al. 1989). Passow (2002) found TEP production from shear forces or bubbling to increase in water where only bacteria were present.

The SML is disturbed and mixed by physical forces. High wind speeds can create mixing effects; while under moderate wind speeds (< 5 m s^{-1}), surface active material can dampen capillary waves (Espedal et al. 1998). In addition, as wind-driven waves break, they cascade jets of bubbles several meters into the water (Deane and Stokes 2002). These bubbles, which can range in size from micrometers to millimeters (Blanchard and Woodcock 1957; Deane and Stokes 2002), carry with them organic material from the lower depths and transport it to the surface, known as bubble scavenging, and partly into the atmosphere via bubble bursting (Garrett 1967; Bigg and Leck 2008; Wang et al. 2017). It is well known that particles can be easily formed by bubbling seawater (Baylor and Sutcliffe 1963; Riley et al. 1963; Johnson and Cooke 1980), and are affected by bubbling time (Tseng et al. 1992). Zhou et al. (1998) have investigated the formation of TEP through the aggregation of dissolved precursor material on rising bubbles within a 200 cm high bubble adsorption column. They found that bubbling seawater is the dominant process in TEP aggregation during high phytoplankton activities. Others have used bubbling to produce fresh TEP required for their experiments (i.e., Mopper et al. 1995; Mari 1999; Mari et al. 2004). Wotton (1996) suggests that aggregation of colloidal exopolymer particles (EPS < 1 μm) to larger aggregates is promoted by bubbling. The SML may also become disproportionably enriched in high-molecular organic compounds by selective enrichment on rising bubbles, which are presumably more surface-active and therefore prone to aggregation (Johnson and Cooke 1980; Gershey 1983). However, there have been very few studies that have looked at the effect of bubbling and bubbling time on TEP directly (Zhou et al. 1998). Additionally, Zhou et al. (1998) focused on total TEP concentrations formed in foam instead of the abundance within the SML.

Bubble scavenging is also one major factor for bacterial transport toward the SML (Blanchard 1978). Previous studies have shown that cell properties such as hydrophobicity or pigmentation are critical for bacterial attachment to bubbles (Blanchard 1978; Blanchard and Syzdek 1978). Schmale et al. (2015) collected air bubbles in seawater that were emitted from methane seeps and reported that bubbles effectively transport methanotrophic bacteria from the sediment into the water column. Bubble size and rising distance affect the numbers of bacteria transported into air (Blanchard 1978). These mechanisms most likely affect the abundance of bacteria (or other microorganisms) in the SML as well. Aller et al. (2005) performed simultaneous measurements of microorganisms from bulk water, the SML, and aerosols, which were produced by in situ bubbling. They concluded that the SML is an important source for aerosolized bacteria. However, this and many other studies reporting the bubble-driven water–air transport of microorganisms have not investigated the effects of bubbles on the abundance and activity of organisms in the SML.

For the present study, we investigated the effect of bubbling and bubbling time on the concentration of TEP and the abundance and activity of microorganisms to substantiate the common claim that wave breaking can enrich the SML with such material and organisms, and therefore, contributes to the SML's rapid reformation. We present experiments from an open mesocosm floating on the sea surface and a lab tank to mimic natural effects from bubbling of seawater. First, the effect of short bubbling time (minutes) in an open water mesocosm "in situ bubbling experiment" was examined to see what minimal timeframe is needed for wave breaking affects. Second, a closed tank experiment "in vitro bubbling experiment" was implemented to observe the effect of long-time periods (hours) of bubbling on the movement of TEP and bacteria between and within the SML and ULW. The effect of both short and long bubbling periods on the composition of the SML and its relation to biofilms enriched from breaking waves are discussed.

Materials and methods

In situ bubbling experiment

To study the effects of bubbling in the natural environment, we applied a floating mesocosm in the coastal Southern Baltic Sea offshore Warnemünde, Germany on the following dates: 23–25 July 2013, 12 September 2013, and 8 October 2013. The mesocosm was homemade from an inflatable swimming pool with a diameter of 4 m. The bottom of the pool was cut out leaving a floating ring with a skirt of approximately 30 cm length; this enclosed the SML during the experiments. The mesocosm was attached to the side of a small boat so that it could be positioned away from shore. Bubbles were generated with sintered glass frits (pore size: 15–25 μm) placed at a depth of 1 m with a metal structure immersed. Glass frits producing bubble size spectrums resembling those produced by a breaking wave were used (Haines and Johnson 1995; Zhou et al. 1998). An air pump

(Schego M2K3) was connected to the glass frits using polypropylene tubing. The air pump had a flow rate of 350 L h^{-1} (without air stone or glass frits) as specified by the manufacturer.

We applied two different bubbling times, 2 and 10 min, except on 23 and 25 July, when only 2 or 10 min bubbling was conducted. We collected samples from the SML in the mesocosm before bubbling, and after each bubbling event. Prior to each new experiment (i.e., bubbling event), the mesocosm was lifted for several minutes to renew the sea surface with fresh ambient SML. Generally, on each day, we performed each experiment in triplicates, except on 23 July, when only the bubbled SML was sampled in triplicates, and on 25 July, when all samples were only taken in one replicate. The SML was collected with a glass plate (Harvey and Burzell 1972) attached to an approximately 1.5 m long rod to reach the central part of the mesocosm. According to standard procedure (Cunliffe and Wurl 2014), the glass plate was immersed vertically and withdrawn at a speed of 5–6 cm s^{-1}. The SML was removed from the plate with a squeegee and dripped into a high-density polyethylene bottle. The procedure was repeated until the required volume of 250–350 mL was collected, accounting for a maximum of 45% of the total surface area of the mesocosm when assuming SML layer thickness to be 60–80 μm (Falkowska 1999). For each SML sample, a corresponding subsurface sample at 1 m depth was collected using a large volume syringe with attached and weighted polypropylene tubing. Prior to sampling, we washed all sampling equipment with 10% HCl and rinsed with ultra-pure water. Samples were placed on ice immediately after sampling and processed in the laboratory within 6–8 h.

In vitro bubbling experiment

We performed a laboratory study with natural seawater from the North Sea to study the effect of bubbling during the onset of a phytoplankton bloom under controlled conditions. The experiment was conducted in Bremen, Germany from 23 November 2016 to 1 December 2016. The tank was designed to study the bubble-driven transfer of organic material from the bulk water via the SML into the aerosol phase. It consisted of a 1400-liter basin with a 500-liter aerosol chamber on top. Eight fluorescent lamps providing a photon density of 1000–1200 μmol m^2 s^{-1} were placed above the tank to simulate day night cycles. The basin (120 cm length × 110 cm width × 100 cm height) was made of 10-mm polyvinyl chloride (PVC) plates, held together by an aluminum frame. One side was transparent allowing for visual inspection. As far as possible, all surfaces in contact with seawater were made from Teflon fluorinated ethylene propylene (FEP) or Teflon perfluoroalkoxy (PFA). To minimize contamination from the tank walls, the entire basin was lined with a Teflon FEP bag. Inlet and sampling ports were made from Swagelok stainless steel fittings. PVC in contact with seawater was rinsed with artificial seawater for at least 2 weeks prior to use inside the basin. The head air space was made of Teflon FEP and had a total volume of 500 L (60 cm length, 110 cm width, 60 cm height).

Sampling ports for bulk water (ULW) and the SML were located on top of the basin. ULW samples were taken from 50 cm above the bottom and sampled into 1-liter Duran glass bottles through a Teflon PFA tube using the hydrostatic pressure. Prior to each sampling, the tubing was first flushed with 100 mL of bulk water. SML samples were taken as described above with a boron silicate glass plate (4 mm × 15 cm × 60 cm) via a slit in the enclosure. The sampling volume was limited to about 55 mL, accounting for a maximum loss of the SML by about 50% of the total surface area of the tank when assuming SML layer thickness to be 60–80 μm (Falkowska 1999). The glass plate was cleaned with ultrapure water and ethanol prior to each sampling.

Bubble-driven transport of organic material was simulated using a skimmer pump (aquamedic) placed at the bottom of the tank. The pump entrains air from the enclosure at a flow rate between 300 and 720 L h^{-1} that is regulated via a needle valve. The air is dispersed via a needle wheel resulting in a homogeneous bubble size distribution. While the resulting bubble size distribution could not be measured, particle size distribution of aerosols released into the head space via bubble bursting centered around 200 nm, which resembled sizes found in the larger mode of natural marine aerosols (Quinn et al. 2015). The skimmer pump was run for an average bubbling time of 251 min (± 28, $n = 7$) each day. On each experimental day, the SML and ULW were sampled in the morning. After water sampling, bubbles were produced for around 3–4.5 h. After bubbling was stopped, we immediately sampled the SML and ULW again.

The seawater for the experiment was collected in proximity to the island of Helgoland, North Sea. To supply enough inorganic nutrients for a developing phytoplankton bloom, the concentrations of nitrate/nitrite, silicate, and phosphate were adjusted on 22 November 2016 to approximately 41 μmol L^{-1}, 12 μmol L^{-1}, and 4 μmol L^{-1}, respectively. As the bloom development was not as fast as anticipated during the first days, organic matter was added to the tank to counteract general limitation of dissolved organic matter in the seawater due to bubbling activities. To do so, 500 mL of foam from adjacent aquariums was added in the evening of 25 November 2016 (experimental day 3). This foam had TEP amounts of > 10,000 μmol C L^{-1}, and prokaryotic cell counts of 6.5×10^8 cells mL^{-1} (i.e., a total of 3.3×10^{11} cells). To add additional precursor organic material, 20 L of seawater from the North Sea with unknown phytoplankton community composition was added on 28 November 2016 (experimental day 6) during bubbling.

Sampling

Preparation of TEP samples

TEP were filtered from water samples by low vacuum (< 200 mmHg) filtration onto 25 mm diameter 0.2 μm pore size polycarbonate filters (Nuclepore, Whatman), which had been soaked in 1 mol HCl solution for 24 h for cleaning and reducing background staining of the filter. Low vacuum (< 200 mmHg)

was used to prevent the breaking of delicate TEP structures and a pull through effect with subsequent loss of TEP (Passow and Alldredge 1994). TEP were stained with 1 mL of Alcian blue 8GX (Sigma-Aldrich) solution at pH 2.5 (0.02% in 0.06% acetic acid) (Passow and Alldredge 1994). The dye was allowed to stain the TEP retained on the filter for 5 s and then it was drawn through the filter at low vacuum. Excess dye was rinsed through the filter with two 1 mL rinses of Milli-Q water. Working solutions of Alcian Blue were prepared fresh each day by making a 1/50 dilution from the stock solution. The working solution was prefiltered to counter spontaneous aggregation of the stain. All filters were prepared in triplicate and stored in Eppendorf tubes at −20°C until analysis.

Analysis of TEP

TEP were analyzed using the spectrophotometric method (Passow and Alldredge 1995). Stained TEP filters were placed in 25 mL glass vials and 5 mL of extraction solution (80% H_2SO_4) was added. Vials were incubated for 2 h with gentle shaking to reduce bubble formation. Changes in the pH of the Alcian Blue solution can shift the wavelength of the absorbance spectrum. However, due to logistical constraints, the pH of the working solution could not be tested each time before use, so absorbance of Alcian Blue in solution was measured using a full spectrum scan to identify the peak absorbance wavelength, the peak ranged between 780 and 790 nm. Concentrations of TEP are then shown in relation to a xanthan gum standard and reported as micrograms of xanthan gum equivalents per liter. The equation from (Engel et al. 2009) was used to convert TEP concentrations from μg Xeq L^{-1} to micromoles of carbon equivalent.

Chlorophyll a

Photoautotrophic pigments from the tank experiment were determined by filtering 480–490 mL of a water sample onto GF/F filters (Whatman) and chlorophyll *a* (Chl *a*) was analyzed according to the method described by Wasmund et al. (2006).

Determination of microbial cell counts

The total abundance of prokaryotic and small autotrophic cells was determined by flow cytometry following a modified protocol from Marie et al. (2000). Sample water (900 μL) was fixed using paraformaldehyde (1% final concentration) and glutaraldehyde (0.05% final concentration) (Sigma-Aldrich) in the dark for 15 min at 4–6°C. In both experiments, samples were directly fixed after sampling. After fixation, the samples were frozen in liquid nitrogen and stored at −80°C until analysis in the home lab using a Becton & Dickinson FACSCalibur flow cytometer equipped with a laser emitting at 488 nm (Becton, Dickinson and Company, Heidelberg, Germany) with a constant flow rate of 35 μL min^{-1}.

For analysis, prokaryotes were stained with SYBR Green I (2.5 mmol L^{-1} final concentration, Molecular Probes, Schwerte, Germany) for 30 min in the dark. Yellow-green latex beads (Polysciences, Eppelheim, Germany) were used as an internal standard. Prokaryotes were detected by their signature in a plot of side scatter (SSC) vs. green fluorescence (FL1). Their cell counts are presented as total prokaryotic cell numbers (TCN) and include heterotrophic and photoautotrophic prokaryotes. Total prokaryotic cells of the tank experiment were additionally differentiated between cells with high and low green fluorescence to define high nucleic acid-containing cells (HNA) and low nucleic acid-containing cells, respectively (Lebaron et al. 2001).

Small autotrophic cells were counted after addition of red-fluorescent latex beads (Polysciences, Eppelheim, Germany) and were detected by their signature in a plot of red (FL3) vs. orange (FL2) fluorescence, and red fluorescence vs. side scatter (SSC). While this approach generally allows discrimination between different groups of prokaryotic and eukaryotic autotrophs (Marie et al. 2010), any further differentiation of the eukaryotes or detection of photoautotrophic prokaryotes was hampered as they were partly or fully below the detection limit. This was especially profound in the samples from the tank experiment, which water was taken in the North Sea during November, a period known to harbor very low abundances of picoplankton (Lucas et al. 2015).

Flagellate numbers

The number of flagellates was counted only during the in situ bubbling experiments offshore Warnemünde. Thirty milliliter of each water sample was fixed with Glutardialdehyde (1% final concentration) for 1–5 h at 4–6°C. After fixation, 10 mL was filtered onto black polycarbonate filters (0.8 μm pore size, Whatman, Germany), stained with 4′,6-diamidino-2-phenylindole (DAPI) solution and counted using an Epifluorescence microscope (Zeiss, Germany).

Bacterial carbon production

During the in situ bubbling experiments, the incorporation of ^{3}H-methyl-thymidine (^{3}H-Thy, 20.5 Ci $mmol^{-1}$, 50 nmol L^{-1} final concentration, Hartmann Analytics, Göttingen, Germany) was determined for assessing heterotrophic bacterial carbon production (BCP) according to the method of Chin-Leo and Kirchman (1988). Triplicate 5 mL water samples were incubated for 40–120 min at the in situ temperature in the dark. The incorporation was finalized by adding formaldehyde (10% v/w) (Sigma-Aldrich) and fixation in the dark for 1–12 h at 4–6°C. A fourth sample served as a blank and was fixed for at least 10 min before the addition of ^{3}H-Thy. All samples were filtered on 0.2 μm polycarbonate filters (Whatman, Germany) and 4 mL of scintillation cocktail (PerkinElmer, Groningen, Netherlands) were added to each filter. Afterward, the incorporated substrates were counted in a scintillation counter (Packard, PerkinElmer). Results are presented by estimating BCP using a conversion factor of 10^{18} cells mol^{-1} (Riemann and Bjornsen 1987) and a mean C-content of 20 fg C $cell^{-1}$ (Lee and Fuhrman 1987).

Statistical analysis

Enrichment within the SML was operationally defined as the ratio of abundance or concentration in the SML compared to the abundance or concentration in the ULW. Enrichment factor (EF) with $EF > 1$ shows enrichment in the SML and $EF < 1$ shows depletion in the SML. The effect of bubbling was

operationally defined as the ratio of a given concentration or abundance after bubbling compared to before bubbling and is given as an after/before factor (A/B) where A/B > 1 shows positive effects from bubbling and A/B < 1 shows negative effects from bubbling. Two-tailed *t*-tests were run pairwise between groups (SML vs. ULW or before bubbling in SML vs. after bubbling in SML) to determine if mean differences were significant and were run using Prism Graphpad software with $p < 0.05$ as the significance cutoff.

Results

In situ bubbling experiment

General condition

The effect of bubbling on SML properties was investigated in in situ experiments offshore Warnemünde by comparing the undisturbed SML with SML samples taken after 2 min or 10 min of bubbling, respectively. Absolute concentrations of TEP in ULW showed strong variability between all sampling days (12.2–52.8 μmol C L^{-1}) and even from one day to the other (23 July compared to 24 July, Table 1). Nevertheless, TEP were always enriched by a factor of 1.07–2.00 (mean EF = 1.71 ± 0.4; $n = 5$) in the undisturbed SML compared to the ULW irrespective of sampling date but were not statistically significant (Fig. 2, Table 1; *t*-test, $p = 0.05$, df = 4).

Absolute abundance of TCN in the ULW showed some difference between the sampling dates (Table 1). TCN in the ULW ranged from 6.3×10^6 to 8.2×10^6 cells mL^{-1} in July; whereas numbers in September and October were lower (3.4×10^6 cells mL^{-1} and 4.6×10^6 cells mL^{-1}, respectively). In the undisturbed SML, TCN were always significantly higher (i.e., EF > 1, *t*-test, $p = 0.01$, df = 4) (Fig. 2, Table 1). The EF of TCN in the undisturbed SML was higher in September (EF = 1.76) and October (EF = 2.20) compared to July (EF from 1.17 to 1.48; mean EF = 1.31 ± 0.1, $n = 3$). Along with TCN also the rate of thymidine incorporation as a measure of BCP was higher in July in samples of the ULW (3.0–5.3 μg C L^{-1} h^{-1}) and SML (0.8–2.2 μg C L^{-1} h^{-1}) compared to October (ULW: 1.0 μg C L^{-1} h^{-1}; SML: 0.6 μg C L^{-1} h^{-1}). Overall, thymidine incorporation in the undisturbed SML was always significantly lower compared to the ULW irrespective of the sampling date (Fig. 2, Table 1; *t*-test $p = 0.03$ df = 3).

The absolute abundance of eukaryotes (as determined by flow cytometry) and flagellates (as determined by epifluorescence microscopy) in the ULW ranged from 2.2×10^4 to 6.5×10^4 cells mL^{-1} and from 7.5×10^3 to 15.6×10^3 cells mL^{-1}, respectively (Table 1). Flagellates were overall enriched in the undisturbed SML with EF values ranging from 1.05 (23 July) to 2.35 (8 October) but were not statistically significantly different from ULW (*t*-test, $p = 0.09$, df = 4). The strongest enrichment of eukaryotes was EF = 2.71 but showed overall more variability with several depletions occurring as well (lowest EF = 0.72, mean EF = 1.38 ± 0.79, $n = 5$) (Fig. 2).

Effect of in situ bubbling

Positive effects of bubbling on particulates could already be observed via microscopic visualization (Fig. 1) Bubbling caused enhanced concentrations of TEP in the SML as can be seen from higher EFs of TEP after bubbling in the field mesocosm (Fig. 2A,B) and were found to be significantly different to EFs of TEP in the undisturbed SML (*t*-test, $p = 0.04$, df = 4). This effect was independent of the bubbling time applied (Fig. 2A,B). Furthermore, the direct comparison of TEP concentrations in undisturbed SML to SML after bubbling additionally shows the positive effect of TEP accumulation in the SML due to bubbling (Fig. 2C). TEP concentration in the SML after 2 min bubbling increased by 53% (± 63%, $n = 4$), whereas 10 min bubbling caused TEP concentration to increase by 19% (± 12%, $n = 4$). So, while both bubbling times had a positive effect on the enrichment of TEP in the SML, there was a higher increase of TEP in the SML after 2 min of bubbling.

TCN in the SML were not significantly different affected after 2 or 10 min of bubbling (2 min: *t*-test, $p = 0.32$, df = 6; 10 min: *t*-test, $p = 0.26$, df = 6). A slight mean reduction was observed for eukaryotes after 2 min (–22%) and 10 min (–8%) of bubbling. Cell counts for flagellates in the SML showed no consistent effect by bubbling (2 min: –2%, 10 min: +4%) and were not significantly different (*t*-test, $p = 0.76$, df = 6). BCP in the SML did not show a consistent behavior after 2 min of bubbling, because both increasing and decreasing BCP were observed. However, BCP in the SML consistently increased by 24% after 10 min bubbling time. Nevertheless, even after bubbling, BCP in the SML was still lower compared to the ULW (Table 1) (*t*-test, $p = 0.0395$, df = 3).

In vitro bubbling experiment

General condition

Chl *a* concentrations had a steady increase over all sampling days and reached a final concentration of 3.93 mg m^{-3} (Fig. 2A). Two simple linear regressions were performed for Chl *a* increase from day to day ($F_{1,4} = 65.15$, $p < 0.001$) and increases from 251 min (± 28, $n = 7$) bubbling ($F_{1,5} = 22.74$, $p < 0.005$). The increase rate of Chl *a* from day to day was twice as high (slope: 10 ± 1.24) as the increase from bubbling (slope: 5 ± 1) ($p = 0.015$), suggesting that any effects of bubbling on phytoplankton growth were masked by an overall bloom occurrence.

Total concentrations of TEP over all sampling days showed a higher increase over time in the SML (14.4–1030 μmol C L^{-1}) than in the ULW (2.43–48.2 μmol C L^{-1}) (Fig. 3B). TEP concentrations in the ULW decreased from day to day with probable transfer to the SML via bubbling, except on days 3 and 6 when addition of organic material (foam and 20 L seawater, respectively) into the ULW occurred. Overall concentrations of TEP in the SML were higher than in the ULW (*t*-test, $p = 0.0143$, df = 13) with a positive EF of TEP ranging from 1.13 to 68.8 magnitude (mean = 16.9 ± 21.8, $n = 14$) showing a high enrichment of TEP within the SML over the whole experiment. The only exception was at day 2 after bubbling, when TEP concentrations were low (14–43 μmol C L^{-1}).

Table 1. Absolute values from in situ bubbling experiment in the coastal Baltic Sea.

Date	Sample	TEP (μmol C L^{-1}) $\pm$ SD	TCN (10^6 cells mL^{-1}) $\pm$ SD	Eukaryotes (10^4 cells mL^{-1}) $\pm$ SD	Flagellates (10^3 cells mL^{-1}) $\pm$ SD	BCP (μg C L^{-1} h^{-1}) $\pm$ SD
23 Jul 2013	ULW	12.2	8.2	2.9	15.6	3.0
	SML without bubbling	18.8	10.5	7.8	16.4	0.8
	SML 2 min bubbling	31.1 $\pm$ 8.6	8.1 $\pm$ 0.3	2.7 $\pm$ 0.4	19.6 $\pm$ 2.9	1.9 $\pm$ 0.1
24 Jul 2013	ULW	47.0 $\pm$ 22.5	6.9 $\pm$ 0.3	4.2 $\pm$ 0.9	10.4 $\pm$ 1.6	5.3 $\pm$ 0.7
	SML without bubbling	94.0 $\pm$ 19.0	8.1 $\pm$ 0.4	3.0 $\pm$ 0.4	13.6 $\pm$ 2.8	2.2 $\pm$ 0.7
	SML 2 min bubbling	100.9 $\pm$ 7.6	7.6 $\pm$ 0.4	2.8 $\pm$ 0.2	13.9 $\pm$ 0.8	1.9 $\pm$ 0.1
	SML 10 min bubbling	128.4 $\pm$ 8.4	8.0 $\pm$ 0.7	3.0 $\pm$ 0.2	11.4 $\pm$ 1.3	3.0 $\pm$ 0.2
25 Jul 2013	ULW	52.8	6.3	6.5	16.1	5.1
	SML without bubbling	101.4	9.3	6.1	17.2	2.2
	SML 10 min bubbling	109.6	9.5	6.0	19.5	2.6
12 Sep 2013	ULW	21.0 $\pm$ 5.0	3.4 $\pm$ 0.2	2.2 $\pm$ 0.7	7.8 $\pm$ 3.5	
	SML without bubbling	22.5 $\pm$ 1.7	6.0 $\pm$ 1.8	2.5 $\pm$ 0.6	8.8 $\pm$ 3.2	
	SML 2 min bubbling	53.3 $\pm$ 13.2	6.9 $\pm$ 1.3	2.1 $\pm$ 0.3	7.0 $\pm$ 0.6	
	SML 10 min bubbling	26.4 $\pm$ 0.7	5.3 $\pm$ 0.4	1.6 $\pm$ 0.2	7.3 $\pm$ 1.4	
08 Oct 2013	ULW	42.9 $\pm$ 5.8	4.6 $\pm$ 0.3	4.9 $\pm$ 0.4	7.5 $\pm$ 0.5	1.0 $\pm$ 0.2
	SML without bubbling	85.9 $\pm$ 3.0	10.1 $\pm$ 2.0	7.0 $\pm$ 2.4	17.7 $\pm$ 5.4	0.6 $\pm$ 0.3
	SML 2 min bubbling	86.5 $\pm$ 22.7	8.7 $\pm$ 0.8	6.9 $\pm$ 1.5	16.5 $\pm$ 3.0	0.5 $\pm$ 0.1
	SML 10 min bubbling	98.5 $\pm$ 14.6	8.5 $\pm$ 0.9	5.5 $\pm$ 0.4	20.5 $\pm$ 5.7	0.7 $\pm$ 0.1

Mean values $\pm$ SD from three replicated experiments are shown. In cases when only one experiment was conducted, no SD is given.

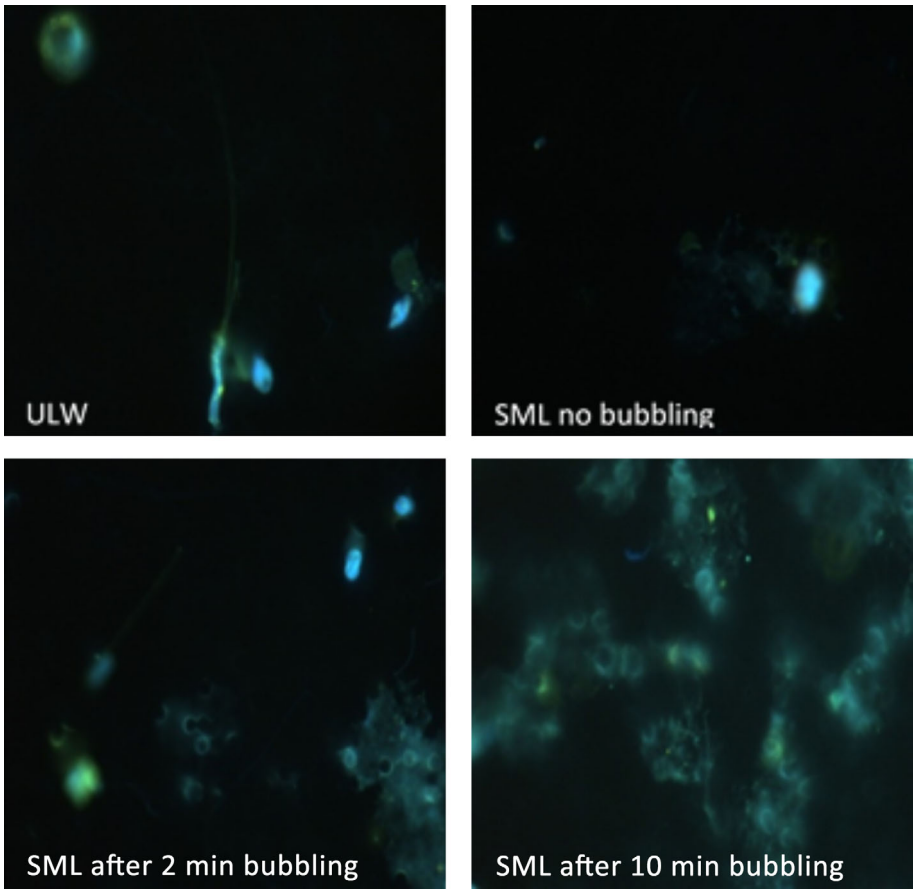

Fig. 1. Microscopic images of DAPI-stained samples taken during the in situ bubbling experiment in the Baltic Sea. Samples from ULW, SML before bubbling, and SML after bubbling for 2 and 10 min are shown.

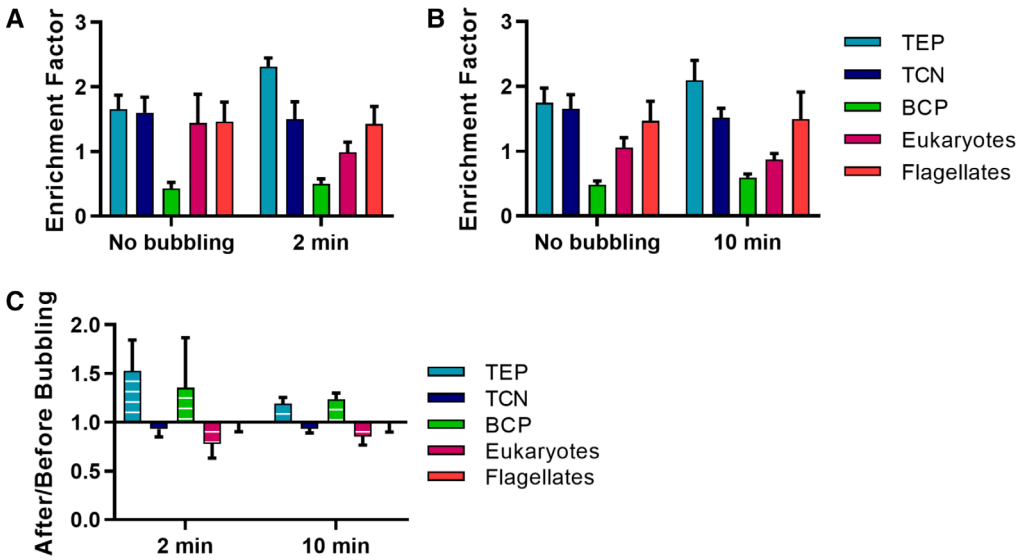

Fig. 2. Results from the in situ bubbling experiments in the Baltic Sea showing relative concentration of TEP, abundance of TCN, eukaryotes, and flagellates as well as uptake of thymidine incorporation. Results are given in relative values between SML and ULW (i.e., enrichment factor) in the undisturbed SML compared to 2 min bubbling (**A**) and 10 min bubbling (**B**). The effect of bubbling on the parameters in the SML is shown in (**C**) for both bubbling times. Absolute values are given in Table 1.

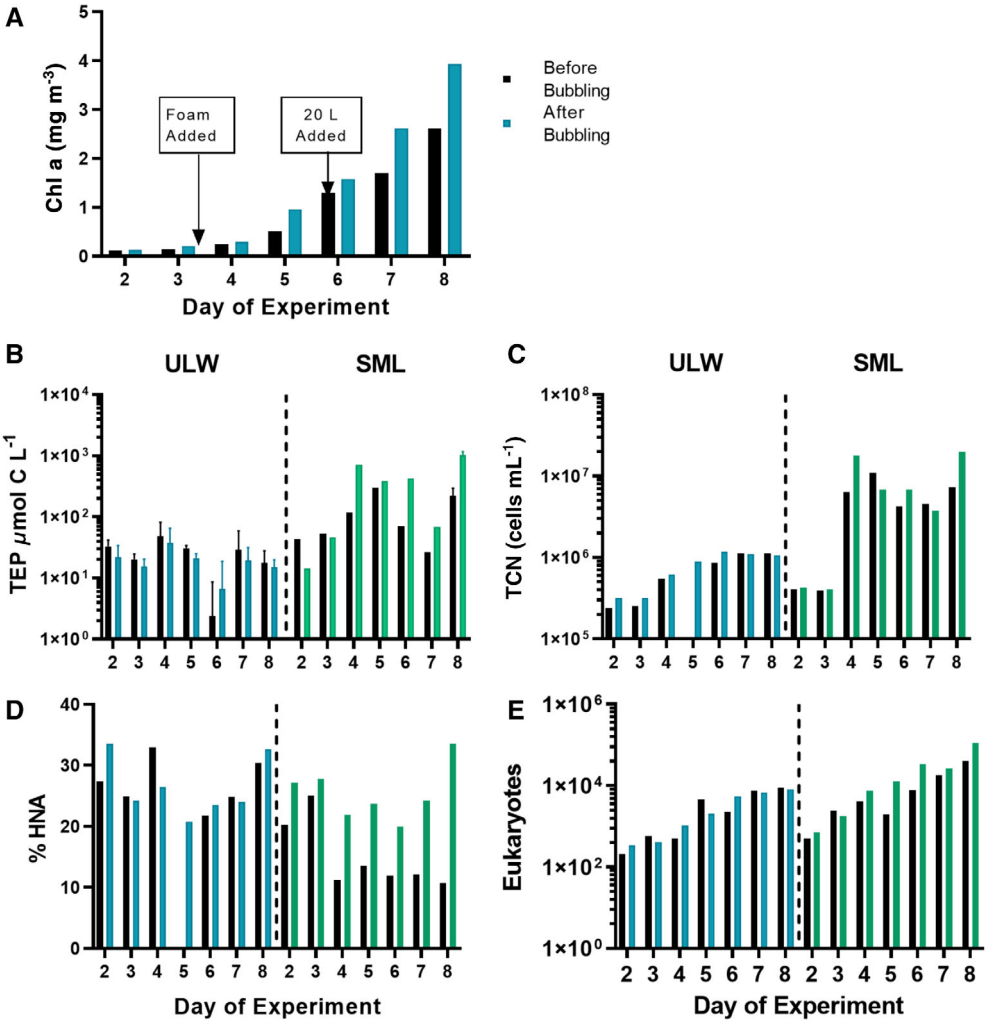

Fig. 3. A controlled tank bubbling experiment using seawater from the North Sea, showing the temporal development of Chl *a* (**A**), TEP (**B**), TCN (**C**), the relative proportion of HNA cells compared to TCN (**D**), and eukaryotes (**E**). Results are shown for samples taken before bubbling (black bars) and after bubbling (colored bars) both from ULW and SML, except for (**A**) where only ULW was sampled. Arrows indicate the time points when additional organic material was added to the tank (**A**, *see* "Materials and methods" section). Absolute values are given in Table 2.

The total abundance of prokaryotic cells (TCN) in the ULW followed the developing phytoplankton bloom in the tank as TCN increased during the experiment from 2.4×10^5 cells mL^{-1} (day 2) to 10.7×10^5 cells mL^{-1} (day 8) (Fig. 3C). TCN increased twofold from day 3 to day 4 which was probably a direct effect of the addition of foam in the evening of day 3, as TCN within the foam suspension was 6.5×10^8 cells mL^{-1}, which theoretically increased TCN in the tank by 2.5×10^5 cells mL^{-1}. The increase in TCN from day 5 to day 8 was, however, most likely related to the increase in photoautotrophic biomass, and, thus, the availability of fresh organic matter. TCN in the SML showed a similar temporal pattern and were generally significantly higher in the SML compared to the ULW (day 2: 4.0×10^5 cells mL^{-1}, day 8: 7.4×10^6 cells mL^{-1}) (*t*-test, $p = 0.01$, df = 12). The addition of foam caused an increase by one order of magnitude of absolute TCN in the SML on day 4 (1.8×10^7 cells mL^{-1}).

Using flow cytometry, we determined the number of HNA cells as a subgroup of TCN with high fluorescent intensities, thus, serving as an indicator of cells with high metabolic activity (Lebaron et al. 2001). The relative proportion of HNA to TCN cells in the ULW (i.e., %HNA cells) varied between 20.8% and 33.5% without any clear trend throughout the experiment (Fig. 3D). In the undisturbed SML, the relative abundance of HNA cells was generally significantly lower compared to the ULW, ranging from 10.7% to 25.1% (Fig. 3D) (*t*-test, $p = 0.02$, df = 5).

The abundance of small autotrophic cells, defined as eukaryotes by flow cytometry, followed the general pattern of the phytoplankton bloom in the tank throughout the experiment (Fig. 3; Table 2). Abundances in the ULW were 2.04×10^2 cells mL^{-1} at day 2 and increased to 80.41×10^2 cells mL^{-1} at day 8. In the undisturbed SML, eukaryotes followed the same temporal development with 5.11×10^2 cells mL^{-1} at day 2 to 406.40×10^2 cells mL^{-1} at day 8 (Fig. 3E). Compared to the ULW, abundances in the SML were

Table 2. Absolute values from controlled tank bubbling experiment.

		ULW						SML				
	Day of experiment	Chl *a* (mg m^{-3})	TEP (μmol C L^{-1})	TCN (10^5 cells mL^{-1})	HNA (10^5 cells mL^{-1})	% HNA	Eukaryotes (10^2 cells mL^{-1})	TEP (μmol C L^{-1})	TCN (10^5 cells mL^{-1})	HNA (10^5 cells mL^{-1})	% HNA	Eukaryotes (10^2 cells mL^{-1})
2	Before bubbling	0.12	32.9 ± 9	2.41	0.66	27.4	2.04	43.0	4.03	0.82	20.3	5.11
	After bubbling	0.13	21.8 ± 12	3.20	1.07	33.5	3.41	14.4	4.28	1.16	27.2	7.16
3	Before bubbling	0.14	20.2 ± 4.6	2.55	0.64	24.9	5.79	54.0	3.95	0.99	25.0	23.85
	After bubbling	0.21	15.3 ± 5	3.20	0.77	24.2	4.09	46.8	4.09	1.14	27.8	17.60
4	Before bubbling	0.24	48.2 ± 33.6	5.48	1.80	32.9	5.11	118.7	63.20	7.08	11.2	41.68
	After bubbling	0.29	37.7 ± 27.6	6.13	1.62	26.4	10.33	715.5	178.00	38.90	21.9	73.64
5	Before bubbling	0.51	30.8 ± 3.1				45.54	296.6	109.00	14.80	13.6	19.46
	After bubbling	0.96	20.7 ± 4.2	8.95	1.86	20.8	20.33	386.6	68.50	16.20	23.7	124.00
6	Before bubbling	1.29	2.43 ± 6.2	8.62	1.88	21.8	22.94	71.2	42.30	5.05	11.9	76.44
	After bubbling	1.58	6.69 ± 12.0	11.80	2.76	23.5	54.97	430.4	68.40	13.60	19.9	332.90
7	Before bubbling	1.70	29.1 ± 29.7	11.20	2.79	24.8	73.71	26.3	46.00	5.58	12.1	179.00
	After bubbling	2.62	19.6 ± 11.9	11.00	2.64	24.0	68.03	68.7	37.40	9.03	24.1	268.40
8	Before bubbling	2.61	17.6 ± 10.3	11.30	3.42	30.4	88.57	221 ± 73	73.50	7.87	10.7	406.40
	After bubbling	3.93	15.1 ± 4.9	10.70	3.51	32.7	80.41	1030 ± 142	200.00	67.10	33.5	1118.00

Values for single measurements are given, except for TEP, where mean values ± SD are shown, when triplicate measurements were done.

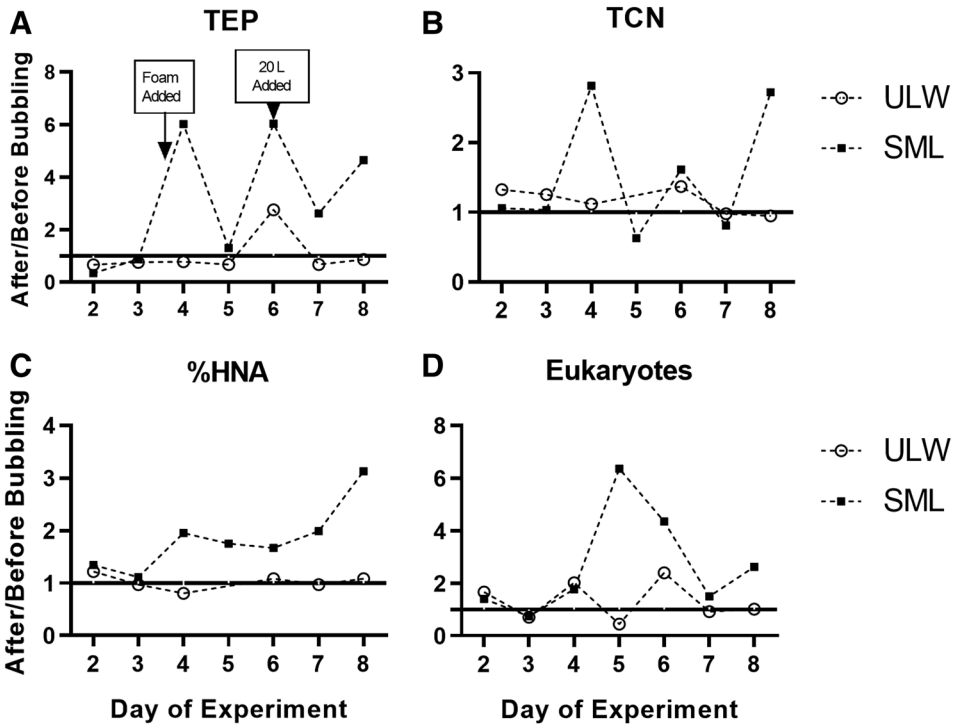

Fig. 4. Temporal development during the controlled tank bubbling experiment conducted with North Sea of TEP (**A**), TCN (**B**), the relative proportion of HNA cells compared to TCN (**C**), and eukaryotes (**D**). Results are shown as the ratio before and after bubbling in either ULW (circles) or SML (squares).

constantly enriched with mean EF = 3.65 (± 2.41, $n = 7$) but were statistically not significant (*t*-test, $p = 0.05$, df = 13).

Effect of in vitro bubbling

In the ULW, the effect of 251 min (± 28, $n = 7$) bubbling on TEP, TCN, and %HNA cells was constant over time, whereas for the eukaryotes, it varied from day to day. However, TEP showed a generally significant depletion in the ULW (*t*-test, $p = 0.03$, df = 6), with an average A/B (i.e., the relation of TEP after compared to before bubbling) of 0.7 ± 0.1 ($n = 6$) (with putative outlier excluded due to the addition of plankton-rich seawater at day 6; Fig. 4A). While TCN showed a general increase, with an average A/B of 1.2 ± 0.2 ($n = 6$), both %HNA cells and eukaryotes showed neither with an average A/B of 1.0 ± 0.1 ($n = 6$) and 1.3 ± 0.7($n = 7$), respectively. Thus, on a temporal scale, bubbling had overall minimal effects on material abundance in the ULW, which is important to note as a comparison to the effect of bubbling on these same parameters within the SML.

Increased TEP concentration in the SML due to 251 min (± 28, $n = 7$) bubbling is shown in Fig. 3B. At the beginning of the experiment when TEP and Chl *a* concentrations were low, bubbling had no or a negative effect on TEP concentrations, as seen by an A/B lower than 1 (Fig. 4a). After the addition of organic rich foam however, the A/B stayed above one for the rest of the experiment, showing an overall increase in TEP concentrations in the SML due to bubbling, but was not statistically significant (*t*-test, $p = 0.06$, df = 6). The highest A/B ratios were found on day 4 (A/B = 6.03) and day 6 (A/B = 6.04) directly after organic material was added to the tank, and additionally on day 8 (A/B = 4.65) when Chl *a* concentrations were at the maximum. These high A/B ratio days were then immediately followed by a decrease the next day in the effectiveness of bubbling with A/B ratios on day 5 (A/B = 1.3) and day 7 (A/B = 2.6).

Bubbling for 251 min (± 28, $n = 7$) per se had no clear effect on TCN in the SML with both enrichment and depletion being observed after bubbling and was not significantly different (*t*-test, $p = 0.25$, df = 6). However, the effect of bubbling on TCN in the SML (Fig. 4B) closely matches trends seen for TEP (Fig. 4A), with increases of A/B on days 4 (A/B = 2.8), 6 (A/B = 1.6), and 8 (A/B = 2.7) while subsequently decreasing on days 5 (A/B = 0.6) and 7 (A/B = 0.8). The strong enrichment and depletion on day 4 and day 5, respectively, were most likely affected by foam addition, considering the high cell numbers being added with the foam (see above). Similarly, the addition of foam at day 3 influenced the relative proportion of HNA cells (i.e., %HNA cells), as %HNA cells in the SML were always enhanced by bubbling with A/B = 2.1 ± 0.6 ($n = 5$) (Fig. 4C). However, the enrichment of TCN and %HNA cells in the SML after bubbling at day 8 probably reflected the overall increase of growing cells in the ULW as a response to the onset of the phytoplankton bloom. Bubbling increased the numbers of eukaryotes in the SML constantly throughout the experiment but was not statistically significant (Fig. 4D) (*t*-test, $p = 0.12$, df = 6). This enhancement was

independent of the temporal development of eukaryotes in the tank, with a mean A/B = 2.68 (± 2.00, n = 7).

Discussion

Increase of TEP in the SML from bubbling

Bubbling seawater or liquid media containing phytoplankton exudates has been proven to be an effective mechanism to produce TEP from precursor organic material (Mopper et al. 1995; Mari 1999). Furthermore, bubbles may not only produce TEP in bulk water, but can also cause an enrichment of TEP in surface-floating foam (Zhou et al. 1998) and surface water (Bigg and Leck 2008). The enrichment of TEP in the SML of natural marine habitats has therefore been suggested to be influenced by bubbles rising through the water column and subsequently bursting at the air–sea interface, e.g., after wave breaking (Wurl et al. 2011*a*). However, as previous studies have only looked at total TEP concentrations or abundance in aerosols or bulk water, to our knowledge, our study is the first one to show that bubbling natural seawater increases TEP concentrations in the SML both in the natural environment and during a tank experiment.

We observed that TEP were generally enriched in the SML before bubbling, which confirms previous findings from the field (Wurl and Holmes 2008) and mesocosm experiments (Cunliffe et al. 2009), but bubbling enhanced this enrichment even more. After in situ bubbling in the Baltic Sea, TEP concentration in the SML already increased after a few min by 53% ± 63% (2 min bubbling) and 19% ± 12% (10 min bubbling). in vitro bubbling of North Sea water during the tank experiment enriched TEP in the SML by 312% (± 244%) after > 3 h of bubbling. Thus, our results show that bubbling is generally a highly efficient mechanism to enrich TEP at the air–sea interface.

In Zhou et al. (1998), TEP extraction efficiency from bubbling was found to decrease as bubbling time increased, with the maximum efficiency occurring at the first time point after a few min. It would not be possible to see this decrease in bubbling effectiveness over time in our in situ mesocosm experiment, due to quick replenishment of ULW. However, a similar decrease between 2 and 10 min of bubbling was observed, the cause of which is unknown: with only two time points to compare, any temporal trends cannot be deduced. Yet, in both the Zhou et al. (1998) experiment and our in situ mesocosm experiment, the highest effect of bubbling was observed to occur in as short a time as 2 min, further supporting the short timescale on which breaking waves could increase TEP concentrations. Additionally, Zhou et al. (1998) had positive TEP extraction rates over the entire time of the experiment, up to 5 h. This likewise compliments the tank experiment, which had average bubbling times of 251 min (± 28, n = 7) and had positive effects of bubbling on TEP (after/before bubbling) for all days after the foam, as an additional source of organic material, was added.

There are a few mechanisms in which we propose that TEP might be increased in the SML by bubbling: (1) All TEP formed in the SML is pre-existing and is transported from the ULW either on bubbles or between bubbles. (2) New TEP is formed on top of bubbles or between bubbles from precursor material and transported to the SML (Johnson and Cooke 1980; Gao et al. 2012). (3) Some precursor material is more susceptible or more desirable for bubble scavenging and so this material is scavenged first and forms TEP first (Gershey 1983; Zhou et al. 1998), while less optimal material requires longer to form TEP aggregates or their physicochemical properties do not allow for aggregate formation at all. (4) Bubbles bursting at the air–sea interface may produce TEP from precursor material either originating from the bulk or being already present in the SML by physical forcing (e.g., compression, shear forces).

We suggest that at least some part of the TEP found in the SML of the tank experiment originated from the ULW, due to decreasing TEP levels in the ULW after bubbling (Fig. 3B). The addition of new organic material to the tank on day 3 (foam suspension) and day 6 (20 L North Seawater) showed an immediate increase of TEP concentration in the ULW, which was decreasing again during the following days, indicating transport of TEP from the ULW into the SML or into aerosols. Because our tank experiment was conducted over multiple days with the same water being sampled from, there was a daily maximum theoretical loss of 50% of the SML, i.e., the amount of SML sample volume compared to the surface coverage of the tank. So, it cannot be known how much of the TEP in the SML was formed from the four previously described pathways. Additionally, there is further loss from sinking of particles and release of particles into the air, the percentage contributions of which cannot be known due to all processes occurring simultaneously. Further controlled tank experiments focusing on a shorter time scale and the composition of precursor material could elucidate where and how the TEP is formed. During the tank experiment, we never observed sinking of TEP into the ULW (as would be noted by EF < 1), but this would be difficult to observe due to the setup of our system. The continuous bubbling decreases the chance for TEP to sink while bubbling is happening. The inclusion of the SML and head space allow for TEP to be transported out of the ULW, increasing the complexity of the system, so that while there might be a total TEP increase within the system, it might not appear in the ULW. Finally, and this would be relative to any similar study, due to the unequal volumes of the ULW and SML (1400 L vs. ~ 100 mL, in the case of our in vitro experiment), it would take a considerably large abundance of TEP sinking from the SML, in order to see an increase in the concentration of TEP in the ULW. However, if sinking is the main source of TEP loss in the SML, then there would be an equal increase of total mass of TEP in the ULW.

Effect of bubbling on microbial abundance and activity in the SML

Bubbles rising through the water column are well-known vectors for bacterial transport (Blanchard 1978) and continuous bubbling of water was shown to effectively concentrate

bacteria in foam at the air–water interface (Carlucci and Williams 1965; Zhou et al. 1998). However, to our knowledge, there is no study reporting how bacterial transport on bubbles affects the abundance of microorganisms in the SML. In the tank experiment, we occasionally observed an enrichment of bacteria in the SML after bubbling, which might be caused by bubble scavenging and transport. However, in the same experiment and in the in situ experiment, we often observed a depletion of cells in the SML due to bubbling as well, showing that the effect of bursting bubbles on abundance of bacteria in the SML (i.e., bacterioneuston) is more complex. The depletion of bacterial cells in the SML after bubbling might be attributed to a transfer of organisms into the air, as aerosols have been repeatedly reported to be enriched in prokaryotic cell numbers compared to the water source (Blanchard and Syzdek 1970; Aller et al. 2005; Rastelli et al. 2017), although the importance of this mechanism remains unclear (Blanchard and Syzdek 1970). Another potential export mechanism of microorganisms could be attachment to sinking particles from the SML into the ULW. Although we cannot fully evaluate the importance of either mechanism, it is interesting to note that although bubbling reduced the abundance of larger microorganisms such as eukaryotes and flagellates to some extent in the SML during the in situ experiment, even after bubbling flagellates were still enriched in the SML compared to the ULW. This might indicate that the gelatinous matrix of the SML is quite stable against bubble bursting and newly formed or imported TEP due to bubbling could be one major contributor for its stability. However, the interaction between bubbles, SML integrity, TEP, and the microbial loop within this biofilm-like habitat (Wurl et al. 2016) clearly warrants further research.

It has been known for decades that different organisms (i.e., different bacterial taxa) show different efficiencies for bubble transport, which may be caused by the hydrophobicity or pigmentation of the cells (Bezdek and Carlucci 1972; Blanchard 1978). Although these factors are of general importance for the bubble transport itself, the relative importance of them to influence bacterioneuston abundance and community composition due to bubble bursting needs further investigations. In this respect, the different effect of bubbling on the abundance of eukaryotic cells in the SML between the two experiments (i.e., in situ vs. tank experiment) might have been as well influenced by differing community composition in the source water. If cell hydrophobicity is of major importance for the transport of microorganisms by bubbles, the amount or composition of dissolved surface-active material in the water column, and thus the coverage of bubble surfaces with surfactants films, may be an additional factor influencing the number of cells attaching to a bubble surface.

During the in situ mesocosm experiment, the bacterioneuston generally showed lower BCP compared to the ULW, which has been frequently observed (Reinthaler et al. 2008; Stolle et al. 2009, 2010). The reason for this might be increased levels of solar radiation in the SML, especially of ultraviolet radiation (UVR), the latter being especially known to exhibit negative effects on bulk BCP in marine systems (Ruiz-Gonzalez et al. 2013). Inhibition of BCP by solar radiation may result from physiological stress, and the bacterioneuston seems to not be specifically adapted to solar radiation (Agogué et al. 2005). UVR is also known to modify the availability of organic substrates for bacterial uptake (Obernosterer et al. 1999), and Reinthaler et al. (2008) found less available amino acids in the SML. In our experiment, the BCP in the SML generally increased after bubbling. Although this effect was not so clear after 2 min bubbling time, BCP was consistently stimulated by 24% after 10 min of bubbling. Although we cannot define which of the above-mentioned factors was dominating in our study, we conclude that bubble bursting causes transport of new, nonstressed bacteria and/or fresh, labile organic material from the ULW and subsequent release into the SML. The tank experiment revealed that the relative proportion of HNA in the SML increased after bubbling. Although HNA cells are not necessarily a general measure for the activity of a complex community (Longnecker et al. 2005), Lebaron et al. (2001) could show that HNA cells are a good indicator for growing cells. Thus, these results strengthen our findings from the in situ experiment that bubbling enhances bacterial productivity in the SML.

Kepkay and Johnson (1989) reported that bubbling bulk seawater enhanced bacterial respiration compared to nonbubbled control treatments, which they discuss to result from dissolved organic matter (DOM) coagulation and scavenging of inorganic nutrients (e.g., phosphorous and nitrogen) on bubble surfaces. This mechanism of organic and inorganic compounds accumulating on bubble surfaces while rising through the bulk water, and their release into the SML upon bubble bursting, could as well explain the effects observed in our study. In the SML, the organic material pool changed after bubbling which is evident from increasing TEP abundances. Thus, new organic material being released into the SML could enhance bacterioneuston activity. Future studies will need to focus on how bubble transport and bursting influences the organic composition as well as the bacterial community composition in the SML, and thus, unravel if unstressed bacteria or new organic material being transported into the SML cause the increase in bacterioneuston activity.

Conclusion

While the accumulation of TEP in the SML is a well-known feature, we can now show that bubble bursting even further enhances TEP abundance in the SML. This enriching effect due to bubbling was obvious for different bubbling times, ranging from 2 and 10 min (in situ bubbling) to > 4 h (in vitro bubbling). Although we cannot differentiate the different production pathways and sinks of TEP in the SML, this study underlines that bubble bursting is a highly efficient mechanism for TEP accumulation in the SML.

The effect of bubbling on microbial abundance in the SML was diverse with depletions and enrichments after bubbling occurring. However, we observed that bubbling stimulates bacterial activity in the SML, even after only a few min, again suggesting that the import of new labile organic substrates and/or cells into this demanding habitat by bursting bubbles is very effective.

We know from literature that surface-active material is immediately retransported into the SML after disruption (due to, e.g., wave breaking). We can now show that TEP, and partly microorganisms, are also transported into the SML within min after disruption. Additionally, bubbling can increase TEP and bacteria in the SML at a faster rate than normal increases caused by diffusion or advection, i.e., calm sea states without bubble transport. Thus, bubbling seems to be a very efficient way to re-establish the gelatinous and biologically active nature of the SML.

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Conflict of Interest

None declared.

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