Role of Particle-Associated Bacteria in Aggregate Formation in the Ocean

by

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ABSTRACT

The biological carbon pump in the ocean is initiated by the photosynthetic fixation of atmospheric carbon dioxide into particulate or dissolved organic carbon by phytoplankton. A fraction of this organic matter sinks to depth mainly in the form of microaggregates (5-60 μm) and visible macroaggregates. These aggregates are composed of cells, minerals, and other sources of organic carbon. Exopolymeric substances (EPS) are exudated by heterotrophic bacteria and phytoplankton and may form transparent exopolymeric particles (TEP) that act as a glue-like matrix for marine aggregates. Heterotrophic bacteria have been found to influence the aggregation of phytoplankton and in some cases result in an increase in TEP production, but it is unclear if marine heterotrophic bacteria can produce TEP and how they contribute to aggregation. *Pseudoalteromonas carrageenovora, Vibrio thalassae,* and *Marinobacter adhaerens* HP15 are heterotrophic marine bacteria that were found associated with sinking particles in an oligotrophic gyre station in the subtropical North Atlantic. These bacteria were grown in axenic cultures to determine growth, TEP production, and aggregation. They were also inoculated into roller tanks used to simulate open ocean conditions to determine their ability to form macroaggregates. Treatments with added kaolinite clay simulated aeolic dust input from the Sahara. *M. adhaerens* HP15 had the highest TEP concentration but the lowest cell-normalized TEP production at all growth stages compared to the other bacteria. Additionally, *M. adhaerens* HP15 also had the lowest microaggregate formation. The cell-normalized TEP production and microaggregate formation was not significantly different between *P. carrageenovora* and *V. thalassae*. All bacteria formed visible macroaggregates in roller tanks with clay addition and

exhibited high sinking velocities (150-1200 m d^{-1}) that are comparable to those of aggregates formed by large mineral ballasted phytoplankton. Microaggregates in the clay treatments declined during incubation, indicating that they aggregated to form the macroaggregates. The findings from this study show for the first time that heterotrophic bacteria can contribute to aggregation and the export of organic carbon to depth in the ocean.

DEDICATION

To my family. All the time and help you have offered me over the years can never be repaid.

ACKNOWLEDGMENTS

I express my thanks and gratitude first and foremost to all those around me that have helped me reach this amazing goal.

I thank my committee, Susanne Neuer, Hilairy Hartnett, and Hinsby Cadillo-Quiroz, for their time and guidance that has buoyed me to this point. Specifically, I would like to further thank Dr. Neuer for taking a chance on me during my undergrad that led to almost three years in her lab.

Thank you to Dr. Bianca Cruz and Catrina Shurtleff for all their time spent training me and answering an uncountable number of questions. A special acknowledgement for Catrina, thank you for doing everything first. Additionally, thank you to everyone else in the Neuer Lab for their help over the years.

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Introduction

The biological carbon pump, part of the global carbon cycle, is driven by the photosynthetic fixation of inorganic carbon into particulate and dissolved organic carbon (POC and DOC respectively) by phytoplankton (Neuer et al. 2014). The resulting organic carbon is exported through the water column to depth by marine snow, fecal pellets, and other oceanic debris (Alldredge and Silver 1988). Previously, the contribution of smaller particles to the export flux was overshadowed by larger particles due to the difference in size and density (De La Rocha and Passow 2007). Picoplankton (0.2-2 μm) were thus relegated in importance until more recent evidence indicated that picoplankton are capable of contributing to export production at rates proportional to their primary production (Richardson and Jackson 2007). As subtropical gyres expand beyond the previously recorded range and experience a shift in phytoplankton taxa towards smaller sizes, the ability for picoplankton to form aggregates increases in importance for biological carbon export (Polovina et al. 2008, Morán et al. 2010, Signorini et al. 2015).

The Sargasso Sea, characterized by dynamic borders due to a lack of land boundaries, is regarded as a representative of oligotrophic oceans due to its low primary production and autotrophic biomass (DuRand et al. 2001). Picoplankton and nanoplankton, mostly cells <5 μm, are the most abundant cells in this ocean region, and the taxonomic groups reflect the variability in seasons and depths (DuRand et al. 2001, Lomas et al. 2013). Aeolian clays derived from continental weathering impact the Sargasso Sea (Jickells et al. 1998). These clays can act as ballasting minerals and clays such as kaolinite have been found to influence and increase aggregation (Deng et al. 2015, Cruz and Neuer 2019, Passow and De La Rocha 2006, Hamm 2002, De La Rocha et al. 2008).

Exopolymeric substances (EPS) can be produced by both bacteria and phytoplankton. Bacterial EPS contain higher portions of uronic acid and nitrous compounds compared to EPS produced by phytoplankton (Santschi et al. 2020). Environmental and physiological factors, such as nutrient availability and growth phase, can impact the production of EPS by cells (Bhaskar and Bhosle 2005). Transparent exopolymer particles (TEP), a subcategory of EPS, are composed of acidic polysaccharides and form gel-like matrices that are able to hold together cells, minerals, and other particles, including zooplankton molts, to form marine aggregates. Therefore, it is thought that TEP can increase the size and density of particles composed of small phytoplankton and bacteria by aggregating with other cells and ballasting minerals (Richardson 2019). Furthermore, studies using cultured phytoplankton and bacteria cultured together observed an increase in TEP precursor material (Gärdes et al. 2012).

Heterotrophic bacteria have been found to influence aggregate formation when introduced into phytoplankton cultures (Cruz and Neuer 2022, Gärdes et al. 2012, and Tran et al. 2020). Specifically, *Marinobacter adhaerens* was found to influence the aggregation of *Thalassiosira weissflogii* and the tiny diatom *Minutocellus polymorphus,* respectively, along with enhancing TEP production in co-cultures of *Thalassiosira weissflogii* and *M. adhaerens* (Gärdes et al. 2012, Cruz and Neuer 2022). The findings by Cruz and Neuer (2022) on the interaction between phytoplankton and heterotrophic bacteria has supported the hypothesis that bacteria play a role in the formation of phytoplankton aggregates (Simon et al. 2002). Furthermore, previous studies have demonstrated that co-cultures of heterotrophic bacteria with pico-cyanobacteria such as axenic *Synechococcus* and axenic *Prochlorococcus*, resulted in an increase in

microaggregate formation without and with an increase in TEP production respectively (Cruz and Neuer 2019).

Cruz and Neuer (2022) showed the impact of bacteria on the aggregation of phytoplankton can vary to the species level. The bacteria *Pseudoalteromonas carrageenovora* and *Vibrio thalassae* that were found associated with sinking particles in the Sargasso Sea (Cruz et al. 2021), in addition to *Marinobacter adhaerens* HP15, had differing effects on the aggregation and TEP production of the tiny diatom *M. polymorphus*. Solely *M. adhaerens* HP15 enhanced aggregate formation in their study.

An open question from these studies is whether these bacteria produce TEP themselves and are able to form aggregates. Hence, in my study, I investigated the growth, aggregation potential, and TEP production of the heterotrophic bacteria *P. carrageenovora*, *V. thalassae*, and *M. adhaerens* HP15. I cultured the bacteria in media that simulated open ocean growth conditions. L1 media is commonly used to culture phytoplankton isolated from the Sargasso Sea (Guillard and Hargraves 1993) and phytoplankton exudates can be simulated by adding a mixture of simple sugars to the L1 media as carbon source (Cho et al. 2004).

Aggregates formed by cells and other materials are commonly separated into two categories, micro- and macro-aggregates. Microaggregates, previously referred to as suspended aggregates, are aggregates in the 5-60 μm size range and they require special techniques and equipment to characterize them due to their small size. Aggregates of this size range have been found to contribute to carbon flux and their presence increased with depth (Durkin et al. 2015). Small particles, including microaggregates, can further form

macroaggregates through aggregation (Durkin et al. 2015). Macroaggregates are visible aggregates larger than 0.1 mm in size. These macroaggregates are of particular interest as their size and density allows them to sink to depth faster than microaggregates. The potential formation of these aggregates in an environment that simulates the particle collision that occurs in the ocean can be simulated by roller tanks (Shanks and Edmondson 1989). These cylindrical tanks are designed to reach solid body rotation, the point at which the wall of the tank and the water within rotates at an equivalent speed. The addition of specific species and ballasting minerals, such as clays, in a relatively sterile environment allow for the study of aggregation in controlled settings (Deng et al. 2015, Deng et al. 2016, Cruz and Neuer 2019, De La Rocha 2008, Passow and De La Rocha 2006, Iversen and Ploug 2010).

Based on the results from earlier studies, I hypothesize that *Marinobacter adhaerens* HP15 will produce the highest TEP concentrations and therefore higher microaggregate volume concentrations among the three bacteria species investigated. I tested this hypothesis by conducting growth experiments with triplicate cultures of axenic *P. carrageenovora*, *V. thalassae*, and *M. adhaerens* HP15 to compare growth rate, TEP production, and microaggregate formation. I also hypothesize that all bacteria will form macroaggregates in the presence of kaolinite clay acting as a ballasting material. I tested the second hypothesis by performing roller tank experiments using bacterial cultures and kaolinite clay to compare macroaggregate formation between the different species of bacteria studied.

Methods

Origin and maintenance of bacterial cultures

Cultures of *Pseudoalteromonas carrageenovora* and *Vibrio thalassae* were obtained from the German Collection of Microorganisms and Cell Cultures GmbH (DSMZ) and a culture of *Marinobacter adhaerens* HP15 was obtained from Dr. Matthias S. Ullrich of Jacobs University, Bremen, Germany. Axenic *P. carrageenovora* (DSM6820), *V. thalassae* (DSM102810), and *M. adhaerens* HP15 cultures were grown and maintained on Marine Both Agar (BD Difco 2216, Becton Dickinson, NJ; ZoBell, 1941) plates at 24 ± 1°C.

Growth experiments

In preparation for experimentation, bacterial pre-cultures were grown in Marine Broth (BD Difco 2216, Becton Dickinson, NJ; ZoBell, 1941) on a shaker at approximately 150 RPM for 48 hours at 24 ± 1°C before being washed and resuspended in sterile L1+1x mixed carbon to reduce carry-over of nutrients. L1 media is commonly utilized in experiments as it simulates the nutrient availability of oligotrophic waters and is regarded as a typical phytoplankton media (Guillard and Hargraves 1993). Mixed carbon was added to the media to simulate phytoplankton exudates (Cho et al. 2004). The mixed carbon mixture consisted of D-glucose, D-ribose, succinic acid, pyruvic acid, glycerol, N-acetyl D-glucosamine, and ethanol. Throughout the experiments, the cultures were kept axenic and axenicity was monitored by microscopy, by colony morphology, and by periodic 16S rRNA gene sequencing. In sterile conditions, cultures of axenic *Pseudoalteromonas carrageenovora, Vibrio thalassae,* and *Marinobacter adhaerens* HP15 were used to inoculate 1 L flasks containing 750 mL of L1+1x mixed carbon

media. Batch cultures were set up in triplicate. Cultures were prepared at a concentration of $1x10^5$ cells mL^{-1} to be consistent with prior experiments (Cruz and Neuer 2022, Cho et al. 2004, Gärdes et al. 2010, and Gärdes et al. 2012). Replicates were grown at 24 ± 1°C on a shaker at 150 RPM. Samples from each culture were taken every two days, beginning from inoculation until the end of the growth cycle. These samples were then used to determine cell abundance, microaggregate formation and volume, and TEP concentration.

Staining EPS

The bacteria were stained with Alcian Blue to qualitatively determine the presence of transparent exopolymeric substances. Single colonies were transferred from Difco Marine Broth 2216 agar plates and suspended in filtered ultrapure water. In the case of liquid cultures, samples of the cultures were aliquoted for staining. The Alcian Blue dye was passed through 0.02 μm Acrodisc syringe filters before 30μL of stain was added to the samples. Cells were then fixed with 0.05 mL of 25% glutaraldehyde. Samples were transferred onto microscope slides and glass coverslips were placed on top. Each slide was visualized at 100x magnification with brightfield to observe for the presence or absence of dyed TEP.

Cell abundance

Preparation of cells for epifluorescence microscopy was carried out as in Amacher et al (2009). Cells were fixed with glutaraldehyde (1%; 0.25 mL 100% (v/v) glutaraldehyde / 25 mL sample) and stained with DAPI (4',6-diamidino-2-phenylindole, 0.03M) before being filtered through 0.2 μm polycarbonate filters (GVS Life Technologies). The filters

were then sandwiched in immersion oil on a glass slide and covered with a cover slip. Bacterial cells were visualized by UV excitation under an epifluorescent microscope (Carl Zeiss AxioScope.A1) with 100x magnification. Approximately 150-200 cells were counted across the filter. The growth rate of the experimental cultures was determined based on the natural log of the change in cell concentration over time.

Microaggregation

Microaggregate formation was determined using a Beckman-Coulter Multisizer3 particle counter before the samples were fixed with glutaraldehyde. Culture samples were diluted with Isoton II saline solution (Beckman-Coulter) to a 1-10% final particle concentration. The abundance and volume of microaggregates were determined with a 100 μm aperture tube within a range of 5-60 μm. The volume of the microaggregates was used to ascertain the equivalent spherical diameter (ESD, μm) of the particles. Microaggregate formation was calculated as the change in microaggregate abundance over time.

TEP

The concentration of TEP was determined colorimetrically as described in Passow and Alldredge (1995). Samples were fixed with glutaraldehyde (1%; 0.25 mL 100% (v/v) glutaraldehyde/25 mL sample) before being filtered on 0.2 μm polycarbonate filters and stained with Alcian blue (AB). Filters were placed into 15 mL centrifuge tubes and stored at -40°C until further processing. The dye was calibrated according to the updated Bittar et al (2018) procedure and used for no more than one month following calibration. The TEP on the filters was extracted with 80% sulfuric acid over 3 hours. The absorbance values of the resulting solutions were measured at 787 nm using a spectrophotometer

(Shimadzu UV-1900i). The TEP concentration was determined as described in Iuculano et al. (2017) as follows:

$$\text{TEP } (\mu\text{g XG Eq. mL}^{-1}) = (a_{sample} - a_{blank}) * V^{-1} * F$$

where a_{sample} and a_{blank} are the absorbance of the sample and the blank filter. V is the volume filtered and F is the f-factor, or calibration factor.

Cell-normalized TEP production was calculated for the stationary growth phase with a measurable growth rate as described by Fukao et al. (2012), as follows:

$$\text{Tpr-C } (\mu\text{g XG Eq. cell}^{-1}\text{ day}^{-1}) = \mu * (TEP_t - TEP_0)/(N_t - N_0)$$

where μ is the growth rate, TEP_t is the final TEP concentration, and TEP_0 is the initial TEP concentration. N_t and N_0 are the cell abundances of the sample at the final and initial timepoints.

Macroaggregation

I determined the formation of macroaggregated using roller tank experiments (Fig. 1). Bacterial cultures were incubated in the roller tanks with and without kaolinite clay particles. Bacterial cultures were grown in Marine Broth (BD Difco 2216, Becton Dickinson, NJ; ZoBell, 1941) on a shaker at approximately 150 RPM for 48 hours prior to being washed and resuspended in sterile artificial seawater before inoculation to reduce nutrient carry-over. The pre-cultures were diluted to $1x10^5$ cells mL^{-1} with artificial seawater and were incubated in cylindrical 1.25 L plexiglass tanks. Kaolinite clay was added to the tanks at a concentration of 5 mg L^{-1}. The control treatments included axenic

bacterial cultures and kaolinite clay alone, both at the same concentration as in the treatment tanks. Triplicate tanks for each treatment and duplicate tanks for each control were placed onto a rolling platform and rotated at 3.5 rotations per minute at 25 ± 1°C for seven days. Each tank was observed daily to note aggregate formation until the end of the session. Samples of the cultures were taken at the beginning and end of the rolling period to determine cell abundance, microaggregate formation, and TEP production. At the end of the rolling period for the roller tanks, the number of macroaggregates (ca. >0.1 mm) in each tank was counted following a 15-minute settling period. These aggregates were then carefully removed from the tanks using a syringe and plastic tubing while exerting extreme caution to minimize breaking the aggregates. The macroaggregates were then photographed with an Axiocam 105 color camera and a Discovery V20 stereo microscope to determine the diameter using ImageJ image analysis software. Additionally, the aggregates were visually investigated for the presence of any fibers contained in the aggregates. Any aggregates that contained fibers were not included in the analysis and the treatment with controls was repeated. The sinking speed of the macroaggregates was determined by transferring the aggregates to a 1 L graduated glass cylinder filled with artificial seawater, wherein the aggregates were released 1 cm under the surface to measure the sinking speed. Each bacterial set was composed of clay tanks containing 5 mg L^{-1} kaolinite clay (n=3) and control tanks with no clay (n=2). The first experimental run with *M. adhaerens* HP15 resulted in macroaggregates contaminated with fibers, prompting a second run of the same setup. *M. adhaerens* HP15 and *M adhaerens* HP15* indicates the first and second run of this experiment respectively.

Statistical analysis

To determine whether the differences of growth rates, TEP concentration, and aggregation of the different bacterial cultures were statistically significant, I applied a two-tailed T-test of unpaired values. The Kruskal-Wallis test with a Dunn's post-hoc test was applied to determine statistical significance of roller tanks.

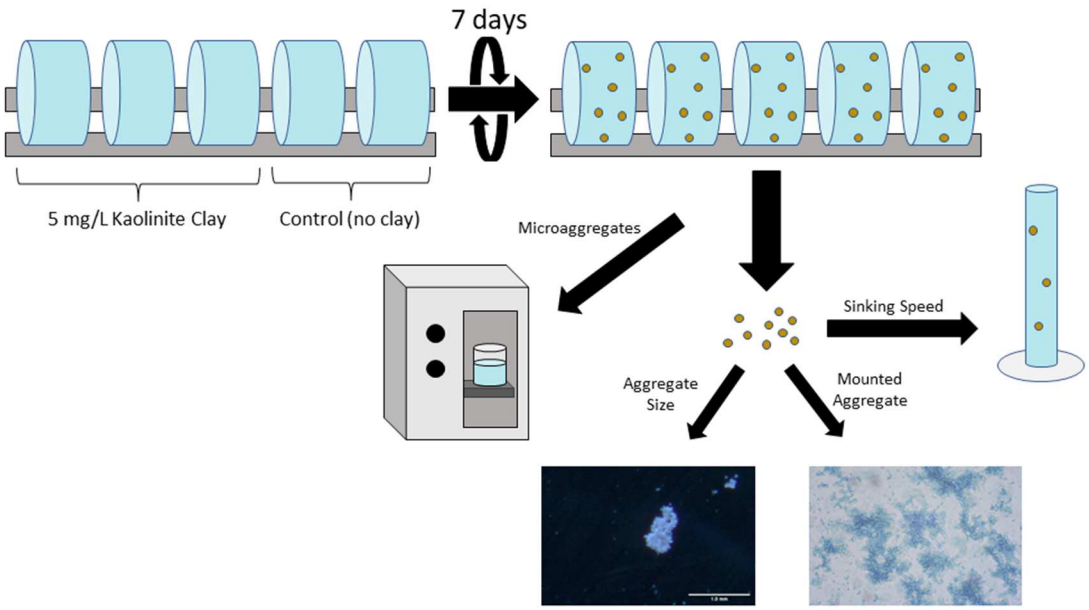

Figure 1. Schematic of the experimental setup using roller tanks and sample processing. Microaggregate formation and volume was determined with a particle counter; macroaggregate formation and size were assessed by microscopy and ImageJ analysis. Mounted aggregates were used to observe qualitative TEP exudation through microscopy. The aggregate sinking velocity was determined by measuring settling times in a graduated cylinder.

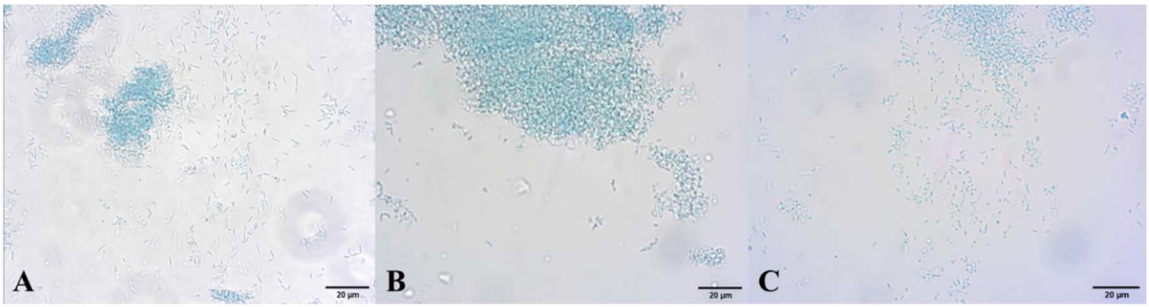

Figure 2. Alcian Blue stained brightfield micrographs (100x magnification) of the axenic bacteria, A) *M. adhaerens* HP15, B) *P. carrageenovora*, and C) *V. thalassae.* Contrast of the micrographs was increased to better visualize cells. Blue coloring indicates the presence of TEP. Scale bar represents 0.02 mm.

Results

Growth Experiments

All bacterial cultures grew exponentially from day 0 to day 2 of incubation (Fig. 3A). *V. thalassae* and *M. adhaerens* HP15 exhibited stationary phase from days 2-8 and showed a decline in abundance from days 8-10. *P. carrageenovora* began the stationary phase at a similar time to the other bacteria but reached the decline phase earlier (2-6 d). *V. thalassae* and *M. adhaerens* HP15 differed significantly over the stationary and declining growth phase with *M. adhaerens* HP15 having a significantly lower cell abundance. The growth rate of *P. carrageenovora* and *V. thalassae* were similar to each other during the exponential phase (2.73 and 2.79 d^{-1}, respectively), while *M. adhaerens* HP15 reached a slower rate with 1.27 d^{-1} (Table 1).

All three bacterial species were found to have TEP adhered to the cells (Fig. 2). Axenic *Prochlorococcus* was used as a negative control and xenic *Synechococcus* used as a positive control (images not shown).

It is notable that the pre-culture of *V. thalassae* was noticeably more viscous than that of the other bacteria. This was not observed during its growth in the L1+1x mixed carbon media. This is likely due to the lower cell concentration in the experimental media compared to the pre-culture ($7.8*10^8$ cells mL^{-1} before inoculation vs a maximum of $2.5*10^7$ cells mL^{-1}).

The TEP concentration of *P. carrageenovora* exhibited little change over the period of incubation while that in the *V. thalassae* cultures increased to a peak by day 4 before slowly declining over the rest of the sampling period to day 10 (Fig. 3B). *M. adhaerens*

HP15 maintained a constant TEP concentration until increasing towards the end of the stationary phase (days 4-6) and then remaining constant once again. The TEP concentration of all bacteria was statistically different from each other over the exponential, stationary, and declining phase of growth with one exception. *P. carrageenovora* and *V. thalassae* were not significantly different from each other during the exponential phase. The cell-normalized TEP production of *P.* carrageenovora and *V. thalassae* increased during the stationary phase with *V. thalassae* experiencing an additional increase during the declining phase (Table 1). *M. adhaerens* HP15 had a small increase in cell-normalized TEP production during the stationary phase, although less than the other bacteria. The increase in cell-normalized TEP production from the exponential to stationary phase of growth was not significant for any of the bacteria due to the variation between the replicates (Table 1). None of the bacteria were statistically different from one another in terms of cell-normalized TEP production over any phase of growth. *M. adhaerens* HP15 was close to being significantly lower (using the $p< 0.05$ criterium) in cell-normalized TEP production than *V. thalassae* and *P. carrageenovora* ($p = 0.059$ and 0.068, respectively, Table 1).

The microaggregate formation of *V. thalassae* and *P. carrageenovora* was similar in reaching a peak towards the end of the exponential phase before slowly declining over the rest of the incubation period (Fig. 4A). *M. adhaerens* HP15 did not achieve a peak in microaggregate formation until day 6, later than the other bacteria and during the end of the stationary phase, before declining over the rest of the incubation period. *P. carrageenovora* and *V. thalassae* were not statistically different from each other but were statistically different from *M. adhaerens* HP15 over the stationary and declining phase in

terms of microaggregate formation. Likewise, *V. thalassae* and *P. carrageenovora* experienced a peak in microaggregate volume towards the end of the exponential phase before declining, thus following the same pattern as microaggregate formation (Fig. 4AB). However, while *M. adhaerens* HP15 peaked slightly around day 6, following the trend of microaggregate formation, the volume decreased (6-8 d) before increasing to a similar peak by day 10 (Fig. 4B). Similar to microaggregate formation, *P. carrageenovora* and *V. thalassae* were not statistically different from each other but were statistically different from *M. adhaerens* HP15 over the stationary and declining phase. The cell-normalized microaggregate volume indicated a maximum on the day of inoculation before decreasing over the rest of the sampling period with the exception of *P. carrageenovora* that experienced a small increase on day 4 (Table 1).

Macroaggregates formed in the roller tanks were light colored and varied in appearance from fluffy to dense (Fig. 5A,B,C). Macroaggregates only formed in tanks treated with clay and *P. carrageenovora* produced the highest overall number of macroaggregates (Fig. 6A, Table 2). There was no significant difference in macroaggregate formation or diameter between the bacteria (Fig. 6A,B). *V. thalassae* had a significantly slower sinking velocity (308 ± 11 m d^{-1}) than either *P. carrageenovora* or *M. adhaerens* HP15* (695 ± 54 m d^{-1} and 712 ± 71 m d^{-1}, respectively) (Fig. 6C, Table 2).

Microaggregates abundance and volume decreased significantly during the rolling period in *P. carrageenovora, V. thalassae,* and *M. adhaerens* HP15 replicates with clay (Fig. 7C,D) but not in the control tanks (Fig. 7A,B). However, the second experiment with *M.*

adhaerens HP15 (indicated with asterisk)* with clay did not show a significant decline in microaggregate number and volume during the incubation (Fig. 7C,D).

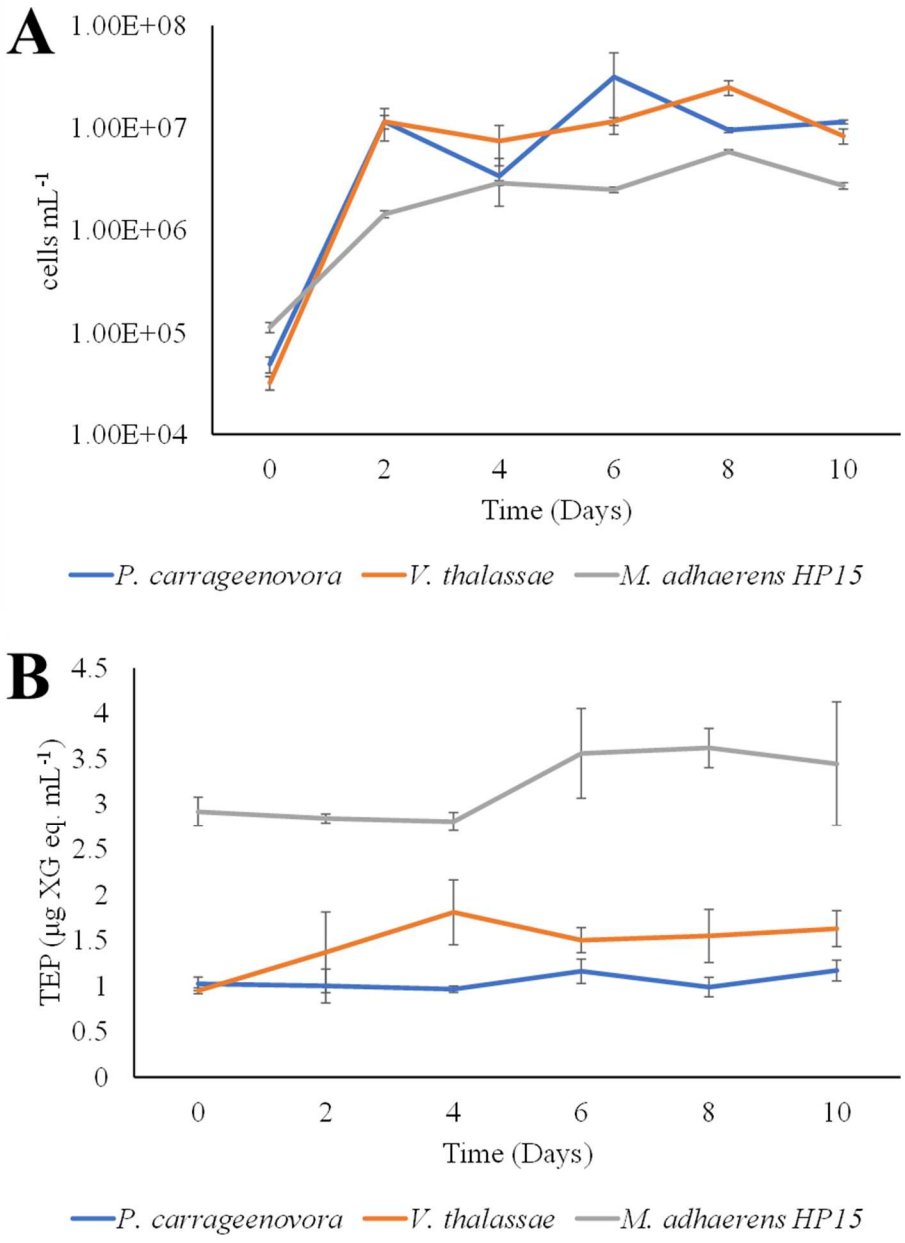

Figure 3. Cell abundance (A) and TEP concentration (B) of *P. carrageenovora*, *V. thalassae*, and *M. adhaerens* HP15 throughout the 10-day incubations. Error bars denote standard error of the mean for triplicate cultures.

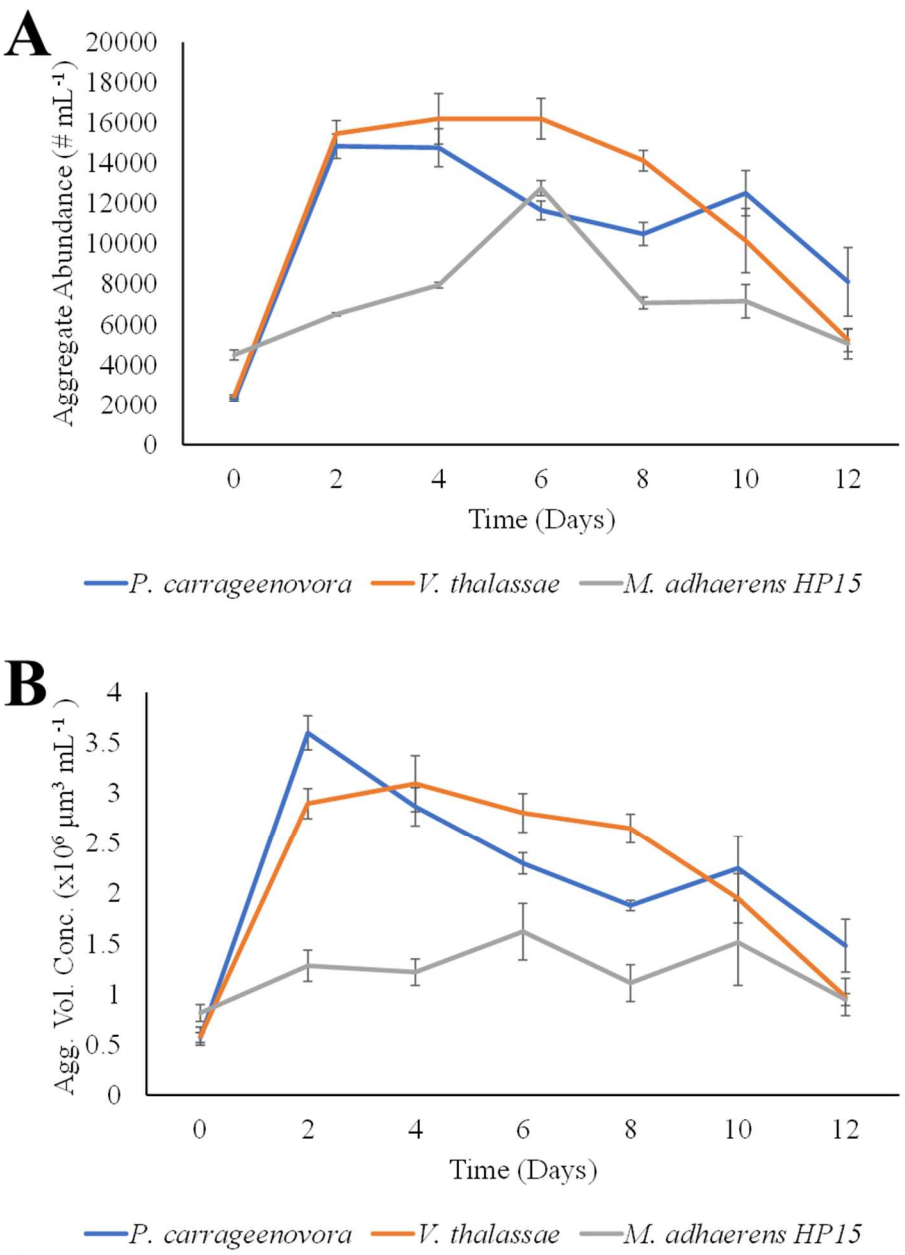

Figure 4. Microaggregate formation (A) and volume (B) for *P. carrageenovora*, *V. thalassae*, and *M. adhaerens* HP15 throughout the 10-day incubations. Error bars denote standard error of the mean for triplicate cultures.

Table 1. Average cell abundances at inoculation, growth rates, formation of microaggregates (both abundance and volume), TEP concentration and cell-normalized TEP production of axenic *P. carrageenovora, V. thalassae,* and *M. adhaerens* HP15 over phases of growth. Values are the mean ± standard error of the mean.

Growth Experiment							
Treatment	Cell abundance at Day 0 ($x10^5$ mL^{-1})	Growth Phase (d)	Growth Rate (d^{-1})	Microaggregate formation (particles mL^{-1} day^{-1})	Microaggregate volume concentration ($x10^6$ μm^3 mL^{-1})	TEP Concentration (10^{-6} µg XG eq. $cell^{-1}$)	Cell-normalized TEP production ($x10^{-9}$ µg XG eq. $cell^{-1}$ day^{-1})
P. carrageenovora, axenic	0.50 ± 0.08	Exponential (0-2)	2.73 ± 0.17	6304 ± 475	2.08 ± 0.06	1.01 ± 0.08	-
		Stationary (2-6)	0.12 ± 0.17	-797 ± 59	2.92 ± 0.06	1.03 ± 0.07	3.01 ± 6.11
		Decline (6-10)	-0.11 ± 0.18	214 ± 193	2.14 ± 0.14	1.08 ± 0.11	-
V. thalassae, axenic	0.33 ± 0.05	Exponential (0-2)	2.79 ± 0.33	6506 ± 145	1.74 ± 0.06	1.16 ± 0.24	-
		Stationary (2-8)	0.17 ± 0.08	-222 ± 62	2.86 ± 0.07	1.56 ± 0.30	3.24 ± 2.84
		Decline (8-10)	-0.54 ± 0.02	-1979 ± 459	2.30 ± 0.18	1.59 ± 0.24	-
M. adhaerens HP15, axenic	1.14 ± 0.10	Exponential (0-2)	1.27 ± 0.04	1010 ± 526	1.05 ± 0.09	2.88 ± 0.10	-
		Stationary (2-8)	0.23 ± 0.01	93 ± 44	1.31 ± 0.07	3.21 ± 0.20	0.42 ± 0.15
		Decline (8-10)	0.04 ± 0.00	45 ± 940	1.31 ± 0.30	3.53 ± 0.44	-

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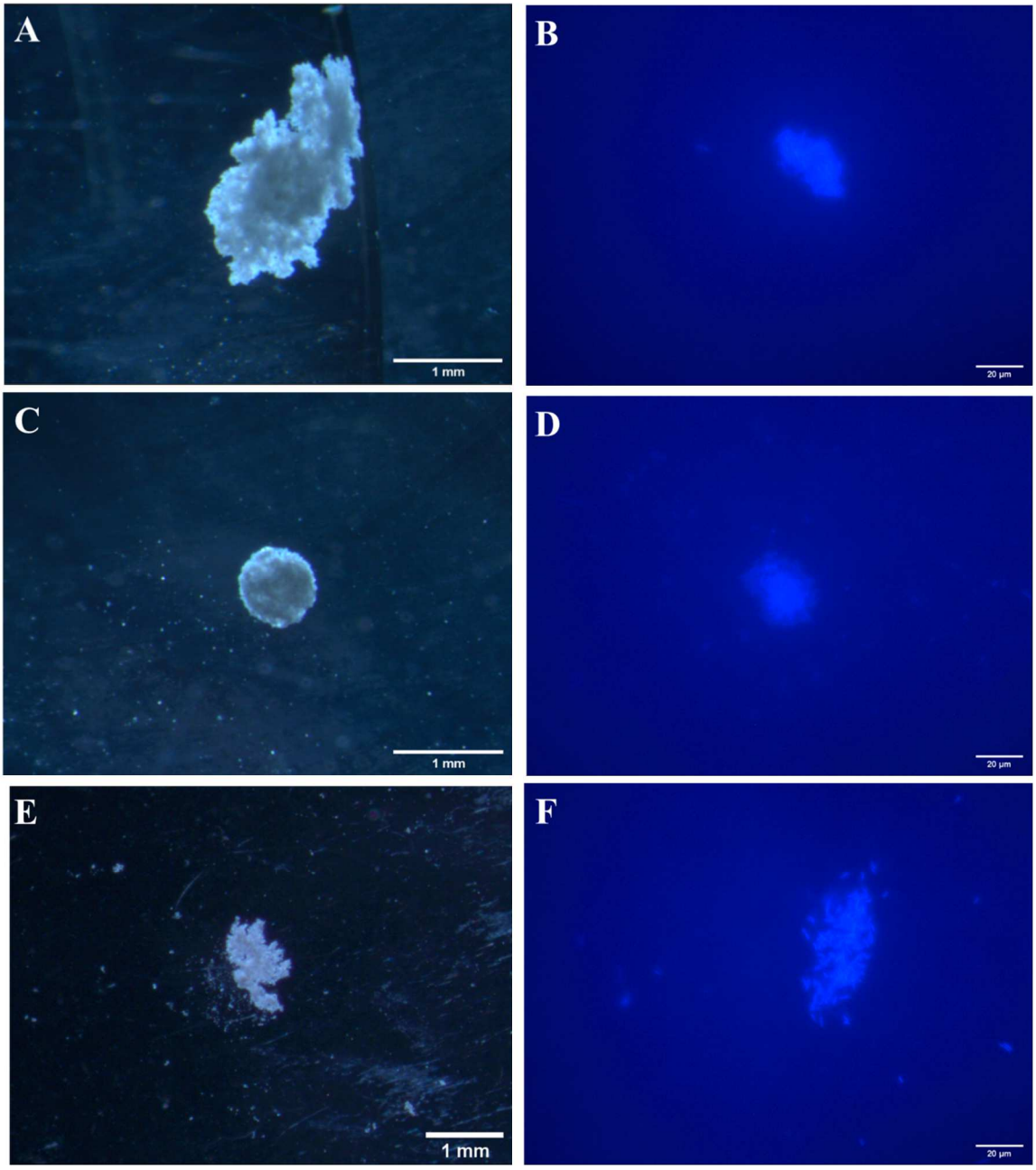

Figure 5. Micrographs of macro- and microaggregates of *P. carrageenovora* (A, B), *M. adhaerens* HP15 (C, D), and *V. thalassae* (E, F) respectively taken with a dissecting scope and epifluorescent microscope. Scale bars represent 1.0 mm for macroaggregates and 20 µm for microaggregates.

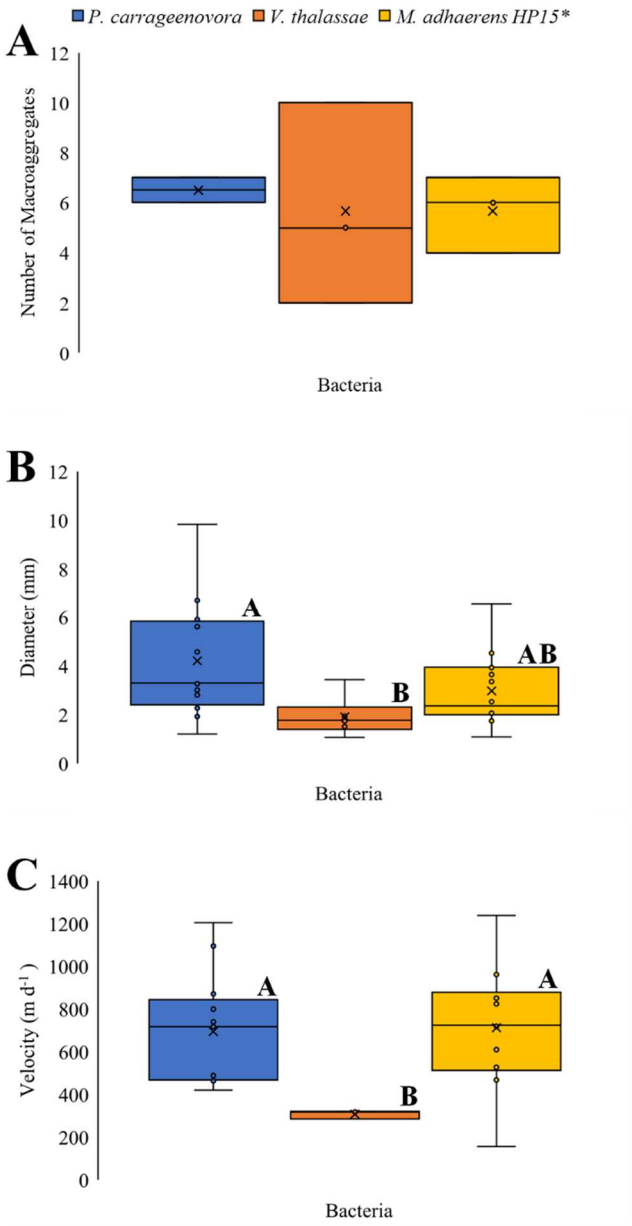

Figure 6. Abundance (A), diameter (B), and sinking velocity (C), of macroaggregates formed in roller tanks of *P. carrageenovora*, *V. thalassae*, and *M. adhaerens* HP15* with 5 mg L^{-1} kaolinite clay. Uppercase letters indicate significant difference between the bacteria (Kruskal-Wallis and post-hoc Dunn's test, $p < 0.05$). Box and whisker plot composed from five-number summary: minimum, lower quartile, median, upper quartile, and maximum.

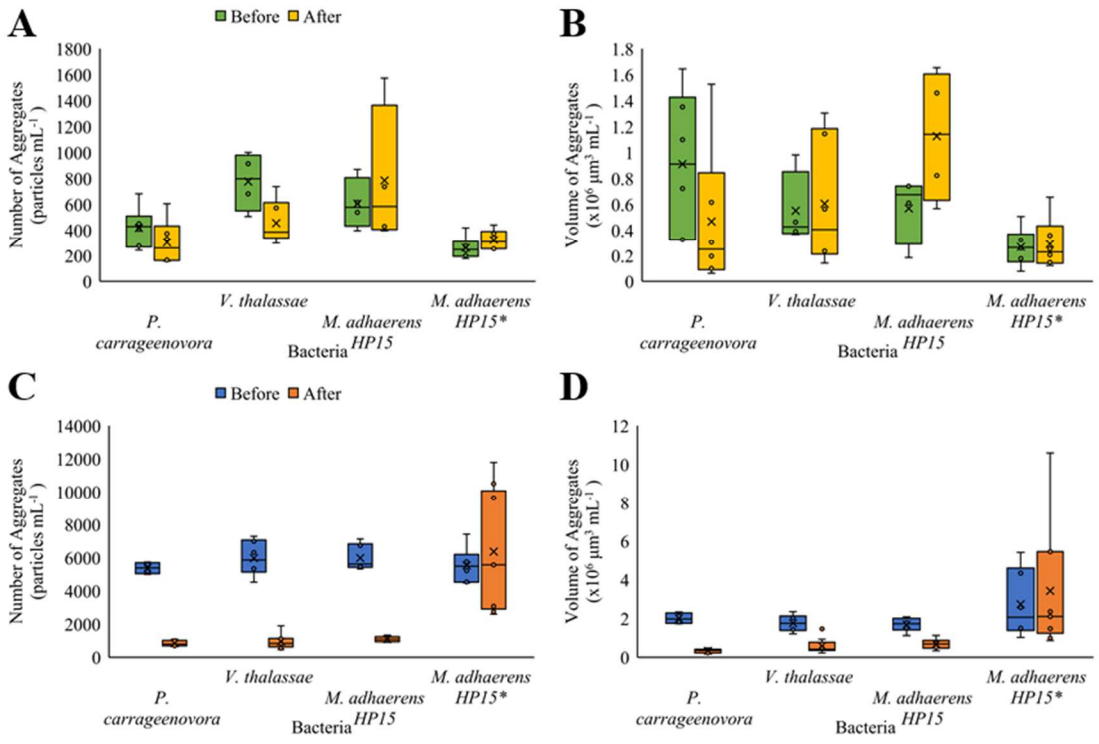

Figure 7. Microaggregate formation (A) and volume (B) in roller tanks of *P. carrageenovora*, *V. thalassae*, and *M. adhaerens* HP15 without clay. Microaggregate formation (C) and volume (D) in roller tanks of *P. carrageenovora*, *V. thalassae*, and *M. adhaerens* HP15 with clay. Box and whisker plot composed from five-number summary: minimum, lower quartile, median, upper quartile, and maximum.

Table 2. Cell and aggregate abundances before and after rotation and diameter and sinking velocity of aggregates that formed in the roller tanks. Values are the mean ± standard error of the mean of $n = 2$ (control tanks) and $n = 3$ (clay treatment tanks).

Roller Tank Experiments										
Treatment	Background cell abundance ($x10^5$ mL^{-1})				Aggregate abundance (per tank)		Diameter (mm)		Sinking velocity ($m\ d^{-1}$)	
	Before rotation		After rotation		After rotation		After rotation		After rotation	
	Control	5.0 mg L^{-1}	Control	5.0 mg L^{-1}	Control	5.0 mg L^{-1}	Control	5.0 mg L^{-1}	Control	5.0 mg L^{-1}
V. thalassae	0.34 ± 0.02	0.29 ± 0.01	0.14 ± 0.01	0.13 ± 0.01	0	6 ± 2	-	1.91 ± 0.46	-	308 ± 11
P. carrageenovora	0.9 ± 0.06	0.57 ± 0.04	0.31 ± 0.04	0.15 ± 0	0	7 ± 0	-	4.2 ± 0.51	-	695 ± 54
M. adhaerens HP15	0.29 ± 0.02	0.22 ± 0.02	0.34 ± 0	0.38 ± 0.03	0	-	-	-	-	-
M. adhaerens HP15*	1.21 ± 0.18	0.86 ± 0.07	0.4 ± 0.03	0.31 ± 0.03	0	6 ± 1	-	2.97 ± 0.39	-	712 ± 71

- : No data

* : Secondary run of an experimental set

Discussion

My main goal was to characterize growth rate, TEP production, and aggregation of heterotrophic marine bacteria found to be associated with marine particles. My first hypothesis, that *M. adhaerens* HP15 would produce the highest TEP concentration and therefore the higher microaggregate volume concentrations among the three bacterial species, was not entirely supported by my findings. *M. adhaerens* HP15 had the highest TEP concentration but no significant increase in microaggregate volume. My second hypothesis, that all bacteria form macroaggregates in the presence of kaolinite clay acting as a ballasting material, was supported by my results. All bacterial species formed macroaggregates with kaolinite clay as a ballasting material. The second roller tank set of *M. adhaerens* HP15 exhibited high levels of variability among the triplicates. To my knowledge, I am the first to report on TEP production and micro- and macroaggregate formation of particle-associated bacteria.

Growth of marine bacteria

The significantly slower growth rate of *M. adhaerens* HP15 may lie in its ability to only use succinic and pyruvic acid from the mixed carbon in the medium as the sole carbon source because it lacks the appropriate carbohydrate-utilizing genes necessary to utilize the other carbon sources (Stahl and Ullrich 2016). This differs from *P. carrageenovora* and *V. thalassae* that are known to utilize a broader spectrum of carbon sources (Kaeppel et al. 2012, Akagawa-Matsushita et al. 1992, and Tarazona et al. 2014). The reduced availability of usable carbon sources could be limiting the growth of *M. adhaerens* HP15. Furthermore, Stahl and Ullrich (2016) found that *Flavobacterium johnsoniae*, a

freshwater and marine gammaproteobacterium, possessed almost quadruple the number of carbohydrate-utilizing genes as *M. adhaerens* HP15 (5.41% vs 1.43% of total genes, respectively). Another reason could be that as a slight thermophile *M. adhaerens* HP15 has a higher optimal growth temperature (34-38□) than the other bacteria and my incubation temperature of 24°C was below its optimal range (Kaeppel et al. 2012).

There have been a few studies that studied the growth of *M. adhaerens* HP15. Stahl and Ullrich (2016) found a growth rate of 0.53 d^{-1} from day 0-2 in f/2 media supplemented with *Thalassiosira weissflogii* supernatant. The supernatant likely provided a source of carbon for the bacteria through the exudation of exopolymeric substances and other carbohydrates by the diatom. Gärdes et al. (2012) also grew *M. adhaerens* HP15 in f/2 media, but without a carbon source, and found that the bacteria declined (-0.10 d^{-1}) from day 0 to 4 of the incubation. In my experiment, *M adhaerens* HP15 had a growth rate of 1.27 d^{-1} from day 0-2 and 0.81 d^{-1} from day 0-4 (Fig. 3B), therefore the growth rate of *M. adhaerens* HP15 was most comparable to that found by Stahl and Ullrich (2016). This similarity is reasonable as both media had an added a carbon source in contrast to Gärdes et al. (2012). Kavita et al. (2013) grew *Pseudoalteromonas* sp. and three *Vibrio* species in Zobell 2216 media and measured growth using absorbance. *Pseudoalteromonas* sp. and *Vibrio* spp. exhibited a similar level of absorbance of approximately 0.45-0.6 after 1 day of growth, growth rates could not be determined due to lack of information. The lack of research prevents further comparison of growth rates for these bacteria. Cho and Giovannoni (2004) cultivated various strains of Gammaproteobacteria using Low Nutrient Heterotrophic Media (LNHM) with various concentrations, including 1x, of the

same mixed carbon addition used in my experiments. Of the strains grown in LNHM+1x mixed carbon the growth rate during the exponential phase ranged from 1.57 to 2.32 d^{-1} with a maximum cell concentration of 0.87-15.7*10^6 cells mL^{-1}. The growth rates for all my bacteria are either slower (1.27 d^{-1} for *M. adhaerens* HP15) or higher (2.73 d^{-1} for *P. carrageenovora* and 2.79 d^{-1} for *V. thalassae*). Additionally, despite the lower availability of nitrogen and phosphorous in the LNHM media compared to L1 and the low inoculation of 8.0*10^2 cells mL^{-1}, the strains used by Cho and Giovannoni (2004) managed to grow to a comparable cell concentration to my own bacteria. Therefore, despite the similar carbon sources compared to my experiments there was still a difference in growth rate, potentially due to the difference in nitrogen and phosphorous availability.

TEP production and microaggregation

My first hypothesis, that *M. adhaerens* HP15 would produce the highest TEP concentration and therefore the higher microaggregate volume concentrations among the three bacterial species, was not entirely supported by my findings. I found that *M. adhaerens* HP15 possessed the highest TEP concentration among the bacteria but also exhibited a slower increase in microaggregate formation and little to no change in aggregate volume (Fig. 3B, 4B, Table 1). An increase in TEP has previously been linked to an increase in microaggregate formation and volume as the sticky TEP holds together particles (Passow 2002). Meanwhile, despite the lower TEP concentration found in *P. carrageenovora* and *V. thalassae,* these bacteria exhibited an increase in microaggregate formation and volume. This disconnect between TEP and microaggregation indicates that

aggregation could have been influenced by other factors, such as exudates besides TEP, as suggested by Cruz and Neuer (2022), or that TEP served as a carbon source and hence was consumed during the growth period. Furthermore, the high TEP concentration of *M. adhaerens* HP15 could be attributed to the acidic polysaccharides within the media coagulating over time and forming TEP, which was an unexpected result of my experiments. Due to contamination within the first set of *M. adhaerens* HP15, the experimental set was repeated. The media prepared for the first set of experiments was composed the day of experiments while the media used for the second set was prepared approximately three days in advance. Cho et al. (2004), the first to propose the mixed carbon source for bacterial media, used the viscosity of their media to determine TEP. This technique does not depend on staining TEP, or potentially coagulated acidic polysaccharides, and therefore sidestepped this issue. Few other studies used the mixed carbon addition and of the ones that did (Cho and Giovannoni 2004), TEP was not investigated.

TEP production is used to examine the rate at which TEP concentration changes over time. Cell-normalized TEP production takes into consideration the change in cell abundance in addition to the growth rate. The cell-normalized TEP production for all of my bacteria across all growth stages was not significantly different (Table 1). However, there was still an increase in cell-normalized TEP production within the stationary phase for all bacteria, although *M. adhaerens* HP15 experienced the lowest increase. The TEP production of *Lentisphaera araneosa*, a marine bacterium isolated from the coast of Oregon, was found to increase over the stationary growth phase (Cho et al. 2004). The

experiment used media with a similar level of carbon but a lower initial cell concentration than those used in my experiments. Mancuso Nichols et al. (2003) investigated the *Pseudoalteromonas* strains CAM025 and CAM036, and both produced measurable levels of EPS by the end of their growth period in marine broth, but TEP was not determined. Li et al. (2016) grew strains of *Pseudoalteromonas* in marine broth and used spectrophotometry to determine TEP concentrations. They found that the TEP concentration of *Pseudoalteromonas atlantica* and *Pseudoalteromonas homiensis* increased over the stationary phase of growth. Additionally, *P. atlantica* produced significantly more TEP than *P. homiensis*, implying that TEP production varies at the species level. There was no significant difference between the stages of growth for TEP concentration in any of my incubations. One explanation for this is that none of the bacteria used in the experiment produced measurable levels of TEP. However, that would contradict my observation that all bacteria stained positively for TEP using Alcian Blue (Fig. 2). Furthermore, while not significant, an increase in cell-normalized TEP production can be observed from the exponential to stationary phase of growth for all my bacteria (Table 1). Another explanation could be that the bacteria are consuming and producing TEP at a nearly-equivalent rate. This would result in little to no change in the TEP concentration as seen in my experiments, as any TEP produced is being consumed at a similar rate. (Fig. 3B, and Table 1).

To compare my TEP results with those from other studies, I converted my results into cell volume-normalized TEP concentration. I found that the mean cell volume-normalized TEP concentration of *M. adhaerens* HP15 was most comparable to

Synechococcus sp., both xenic and axenic cultures (Table 4). *P. carrageenovora* and *V. thalassae* were comparable to *M. polymorphus* along with the co-cultures of *M. polymorphus* and bacteria. However, since the comparison takes into consideration the TEP concentration, the previously mentioned coagulation of acidic polysaccharides within the media could have also artificially inflated these results.

Macroaggregate formation

My second hypothesis, that all bacteria will form macroaggregates in the presence of kaolinite clay acting as a ballasting material, was supported by my results. For the first time, I report that axenic cultures of heterotrophic marine bacteria with ballasting material can form macroaggregates. The diameter and sinking velocity of *P. carrageenovora* macroaggregates were significantly greater than those of *V. thalassae*. Aggregation depends on the number of particles and the stickiness of those particles (Jackson 1990). If particles were not sticky, through such means as TEP or other exudates, then colliding particles would not stick to each other. As the size, and thus surface area, of a particle increases as they are aggregating with the addition of cells or lithogenic material, the probability of colliding with other particles increases as well. Cruz and Neuer (2022) inoculated roller tanks with 10^3 cells mL^{-1} and 10^5 cells mL^{-1} of the same bacteria, but no macroaggregates formed. These authors did not add clay, but added 2.5 μm silica beads at a concentration of 10^2 beads mL^{-1}; those beads did not lead to aggregation. Deng et al. (2015) found that *Synechococcus*, a cyanobacterium, formed macroaggregates with and without the presence of clay at various concentrations, but this

may have been due to the precipitation of minerals during the preparation of artificial seawater, acting as a ballasting material similar to kaolinite clay (Cruz and Neuer 2019). In my experiments, the nutrient-limited environment of the roller tank likely increased TEP exudation by the bacterial cells, as found during the stationary growth phase in my growth experiments, thus increasing the stickiness of the particles (Bhaskar and Bhosle 2005). Hence, the formation of macroaggregates is likely due to a combination of an increase in stickiness and the addition of ballasting material increasing the potential of collision between particles. These results indicate that these bacteria can form fast sinking aggregates (300-700 m d^{-1}) that sink with a similar speed to macroaggregates composed of diatoms or cyanobacteria (Cruz and Neuer 2019, Engel et al. 1999, Iversen et al. 2010).

Various studies have utilized clay as a ballasting material but the increase in particle concentration as a result has not been closely investigated (Deng et al. 2015, Cruz and Neuer 2022, Iversen and Robert 2015, and van der Jagt et al. 2018). A concentration of 5 mg L^{-1} of kaolinite clay, corresponding to $5.9 \pm 2.1*10^3$ particles mL^{-1}, increases the total particle concentration within the tanks by approximately $0.06*10^5$ particles mL^{-1} (Table 2). Although this increase in particle concentration is within the standard error of the mean for all cell abundances, the size of the clay particles may also contribute to aggregation. Microscopic analysis shows that the kaolinite clay particles I used range in size from 2-45 μm, providing a large surface area for the collision of particles.

The microaggregate abundance and volume of *P. carrageenovora, V. thalassae*, and *M. adhaerens* HP15 decreased significantly in the tanks with clay. There are no previous studies that determined the microaggregate abundance and volume of heterotrophic bacteria inoculated in roller tanks. However, Deng et al (2015) found that the microaggregate volume concentration of *Synechococcus* with a similar clay concentration to my experiments increased after a 7 day rolling period. My results are not in agreement with these findings as the volume of microaggregates for all bacteria decreased after rolling. A potential reason for this is that while *Synechococcus* formed more macroaggregates, the bacteria used in my experiments formed larger macroaggregates that incorporated more single cells, microaggregates, and kaolinite clay. Furthermore, as macroaggregates are formed, the available ballasting material within the roller tanks and the possibility of further collisions decreases.

Unique characteristics of the bacteria

M. adhaerens HP15, despite being used in a variety of studies focused on the aggregation of large bloom-forming diatoms with great success (Gärdes et al. 2010, Gärdes et al. 2012, Baker et al. 2016, Lupette et al. 2016, and Cruz and Neuer 2022), did not perform as expected in my experiments. Previous experiments performed with *M. adhaerens* HP15 found that the bacterium does not utilize any sugar found to be secreted by *T. weissflogii* as a sole carbon source. Instead, *M. adhaerens* HP15 has been shown to use amino acids as their preferred carbon source (Stahl and Ullrich 2016). Therefore, the carbon sources in the media used in my experiments may not be the ones preferred by *M. adhaerens* HP15 and would further explain the low performance of this bacterium in

terms of TEP production and aggregation compared to *P. carrageenovora* and *V. thalassae*. *M adhaerens* HP15 could potentially be modifying the exudates from phytoplankton by enzymatic hydrolysis (Sonnenschein et al. 2012, Passow 2002, and Passow 2002a).

P. carrageenovora is the only strain of *Pseudoalteromonas* and the only bacterium used in my experiments capable of degrading gamma-carrageenan, sulfated galactans, by using a depolymerizing enzyme (Guibet et al. 2007). Through horizontal gene transfer, *P. carrageenovora* obtained a plasmid that contains all genes associated with carrageenan degradation (Gobet et al. 2018). Additionally, *P. carrageenovora* can degrade sucrose and then convert the resulting fructose into levan, a branching exopolysaccharide that could act as a structural polysaccharide in biofilms (Gobet et al. 2018). These abilities of *P. carrageenovora* along with the large number of carbohydrate-active enzymes (CAZymes) could give it an advantage over the other bacteria due to the additional ways in which sugars can be processed. However, the CAZymes of *P. carrageenovora* evolved to utilize polysaccharide exudates by phytoplankton, therefore in experiments that provide only limited carbon sources such as in my experiments, *P. carrageenovora* did not perform differently from *Vibrio*, which is not known to utilize these enzymes.

Role of lithogenic ballasting material

The addition of ballasting materials has been found to influence the formation of macroaggregates and sinking velocity both *in situ* and in laboratory settings (Hamm 2002, Passow and De La Rocha 2006, Deng et al. 2015, Ploug et al. 2008, and van der

Jagt et al. 2018). Additionally, as the concentration of lithogenic clays increases the size of macroaggregates decreases and the density increases (Deng et al. 2015). Kaolinite is a common lithogenic material used in aggregation experiments as it is sourced from aeolian dust (Deng et al. 2015, Cruz and Neuer 2019, Passow and De La Rocha 2006, Hamm 2002, De La Rocha and Passow 2008). The cation exchange capacity (CEC) of kaolinite (30-150 meq kg^{-1}) combined with the acidic polysaccharides exudated by cells can assist in the formation of aggregates through cation bridging (Verspagen et al. 2006). While kaolinite is considered less efficient than other clays such as bentonite, due to a lower surface area and cation exchange capacity, it has been shown that kaolinite particles incorporated into aggregates at higher rates than bentonite (Deng et al. 2015).

Aggregation potential of particle-associated picoplankton in the ocean

For the first time, I report on the role of heterotrophic marine bacteria found to be associated with sinking particles in aggregate formation. All bacteria formed visible macroaggregates with ballasting clay with sinking velocities (150-1200 m d-1) that are comparable to those of macroaggregates of phytoplankton. These findings expand our understanding of how heterotrophic bacteria can contribute to export flux in the ocean. Cruz et al. (2019) found that the POC flux at 150 m is near 200 mg C m^{-2} d^{-1} during the spring. I calculated the potential contribution of bacteria to POC flux in the following way: I assumed that there is a concentration of 10^6 bacterial cells mL^{-1} and an average of 11 fg C $cell^{-1}$ (Pasulka et al. 2015, Garrison et al. 2000), resulting in a bacterial biomass of 3.3 μg C L^{-1} , a value which is comparable to that found by Cho and Azam (1990) who

found 6 μg C L^{-1} in bacterial biomass, contributing 40% to total POC in oligotrophic ocean regions. I further assumed a bacterial growth rate of one doubling per day, and that approximately 20% of bacterial production are lysed by viruses and 50% are grazed (Suttle 2007, Landry et al. 2010). The remaining 30% of bacterial production would be available to sink below the euphotic zone. Based on my calculations, this amount is approximately 495 mg C m^{-2} d^{-1} , twice the total POC flux reported by Cruz et al. (2019). This result shows that the flux of bacteria must be far lower, however, they can contribute notably to POC flux and the biological carbon pump. Future efforts could focus on the interactions between bacterial strains in co-cultures, experiments with phytoplankton exudates as carbon sources, and further research on other factors that could influence aggregation.

Table 3. Aggregate abundance, size and sinking velocity found in this study compared to other studies.

Species	Initial Concentration (cells mL^{-1})	Ballasting Materials		Aggregate Abundance	Size (mm)	Sinking Velocity ($m\ d^{-1}$)	Study
		Material	Concentration ($mg\ L^{-1}$)				
Prochlorococcus, axenic	10^6	Kaolinite	5	0	-	-	Cruz and Neuer 2019
Synecoccus, axenic	10^6	Kaolinite	5	4.4 ± 3.6	1.5 ± 0.3	743 ± 74	
Minutocellus polymorphus, axenic	10^5	-	-	1.67 ± 2.89	1.9 ± 0.25	304 ± 105	Cruz and Neuer 2022
M. polymorphus + *M. adhaerens* HP15	Diatom: 10^2 Bacteria: 10^3	-	-	13.70 ± 4.04	-	-	
M. polymorphus + *P. carrageenovora*	Diatom: 10^2 Bacteria: 10^3	-	-	0	-	-	
M. polymorphus + *V. thalassae*	Diatom: 10^2 Bacteria: 10^3	-	-	0	-	-	
M. adhaerens HP15, axenic	10^5	-	-	0	-	-	
P. carrageenovora, axenic	10^3	-	-	0	-	-	
V. thalassae, axenic	10^3	-	-	0	-	-	
Emiliania huxleyi	10^5	-	-	12	1.67 ± 0.68	246 ± 41	Iversen and Ploug 2010
Skeletonema costatum	10^5	-	-	26	2.51 ± 0.83	113 ± 42	
Thalassiosira weissflogii	-	Illite	0.01-50	~5-1000	-	-	Passow and De La Rocha 2005
		Calcium carbonate	0.07-37	~5-1000	-	-	
Collected Seawater	-	High dust	4.2	16.87 ± 9.21	0.62 ± 0.51	430 ± 280	van der Jagt et al. 2018
		Low dust	1.4	23.04 ± 6.60	0.75 ± 0.61	109 ± 42	
P. carrageenovora, axenic	10^5	Kaolinite	5	6.5 ± 0.41	4.21 ± 0.51	695 ± 54	This study
M. adhaerens HP15, axenic	10^5	Kaolinite	5	5.67 ± 0.88	2.97 ± 0.39	712 ± 71	
V. thalassae, axenic	10^5	Kaolinite	5	5.67 ± 2.33	1.91 ± 0.47	308 ± 11	

- : No data

Table 4. Comparison of cell volume-normalized TEP concentrations from literature. All data are from the exponential growth phase. Values from this study are the mean ± standard deviations of triplicate cultures.

Species	Cell volume-normalized TEP concentration ($x10^{-9}$ µg XG eq. $µm^{-3}$)		Study
	Xenic	Axenic	
Chaetoceros affinis (CCMP 159)	22	-	Passow 2002
Coscinodiscus granii	-	341.6 ± 56.33	Fukao et al. 2010
Rhizosolenia setigera	-	74.7 ± 8.63	
Skeletonema sp.	-	68.4 ± 3.28	
Synechococcus sp.	1758 ± 278	1028 ± 337	Cruz and Neuer 2019
Prochlorococcus marinus (MED4)	28 ± 3	<1	
M. polymorphus	-	116 ± 37	Cruz and Neuer 2022
M. polymorphus + *M. adhaerens*	181 ± 18	-	
M. polymorphus + *V. thalassae*	118 ± 46	-	
M. polymorphus + *P. carrageenovora*	160 ± 58	-	
M. adhaerens HP15	-	2516 ± 284	This study
V. thalassae	-	108 ± 8	
P. carrageenovora	-	239 ± 191	

- : No data

REFERENCES

Akagawa-Matsushita, M., Matsuo, M., Koga, Y., and Yamasato, K. (1992). *Alteromonas atlantica* sp. nov., bacteria that decompose algal polysaccharides. *International Journal of Systematic Bacteriology*. 42(4): 621-627. doi:10.1099/00207713-42-4-621

Alldredge, A. L., Passow, U., and Logan, B. E. (1993). The abundance and significance of a class of large, transparent organic particles in the ocean. *Deep-Sea Research Part I: Oceanographic Research Papers*. 40: 1131–1140. doi:10.1016/0967-0637(93)90129-Q

Alldredge, A. L., and Silver, M. W. (1988). Characteristics, dynamics and significance of marine snow. *Progress in Oceanography*. 20(1): 41–82. doi:10.1016/0079-6611(88)90053-5

Benoiston, A. -S., Ibarbalz, F., Bittner, L., Guidi, L., Jahn, O., Dutkiewicz, S., and Bowler, C. (2017). The evolution of diatoms and their biogeochemical functions. *Philosophical Transactions of the Royal Society B: Biological Sciences*. 372(1728): 20160397–20160397. doi:10.1098/rstb.2016.0397

Bhaskar, P. V., and Bhosle, N. B. (2005). Microbial extracellular polymeric substances in marine biogeochemical processes. *Current Science*. 88(1): 45–53.

Bhaskar, P. V., Grossart, H., Bhosle, N. B., and Simon, M. (2005). Production of macroaggregates from dissolved exopolymeric substances (EPS) of bacterial and diatom origin. *FEMS Microbial Ecology*. 53(2): 255–264. doi:10.1016/j.femsec.2004.12.013

Bittar, T. B., Passow, U., Hamaraty, L., Bidle, K. D., and Harvey, E. L. (2018). An updated method for the calibration of transparent exopolymer particle measurements. *Limnology Oceanography Methods*. 16(10): 621–628. doi:10.1002/lom3.10268

Burd, A. B., and Jackson, G. A. (2009). Particle aggregation. *Annual Review of Marine Science*. 1(1): 65–90. doi:10.1146/annurev.marine.010908.163904

Cho, B., and Azam, F. (1990). Biogeochemical significance of bacterial biomass in the ocean's euphotic zone. *Marine Ecology Progress Series*. 63: 253–259. doi:10.3354/meps063253

Cho, J. -C., and Giovannoni, S. J. (2004). Cultivation and growth characteristics of a diverse group of oligotrophic marine gammaproteobacteria. *Applied and Environmental Microbiology*. 70(1): 432–440. doi:10.1128/AEM.70.1.432-440.2004

Cho, J. -C., Vergin, K. L., Morris, R. M., and Giovannoni, S. J. (2004). *Lentisphaera araneosa* gen. nov., sp. nov, a transparent exopolymer producing marine bacterium, and the description of a novel bacterial phylum, *Lentisphaerae*. *Environmental Microbiology*. 6(6): 611–621. doi:10.1111/j.1462-2920.2004.00614.x

Cisternas-Novoa, C., Lee, C., and Engel, A. (2015). Transparent exopolymer particles (TEP) and Coomassie stainable particles (CSP): Differences between their origin and vertical distributions in the ocean. *Marine Chemistry*. 175: 56–71. doi:10.1016/j.marchem.2015.03.009

Cruz, B. N., and Neuer, S. (2019). Heterotrophic bacteria enhance the aggregation of the marine picocyanobacteria *Prochlorococcus* and *Synechococcus*. *Frontiers in Microbiology*. 10: 1864. doi:10.3389/fmicb.2019.01864

Cruz, B. N., and Neuer, S. (2022). Particle-associated bacteria differentially influence the aggregation of the marine diatom *Minutocellus polymorphus*. *ISME Communications*. 2(1): 1-11. doi:10.1038/s43705-022-00146-z

De La Rocha, C. L., Nowald, N., and Passow, U. (2007). Factors influencing the sinking of POC and the efficiency of the biological carbon pump. *Deep-Sea Research Part II-Topical Studies in Oceanography*. 54(5): 639–658. doi:10.1016/j.dsr2.2007.01.004

De La Rocha, C. L., Nowald, N., and Passow, U. (2008). Interactions between diatom aggregates, minerals, particulate organic carbon, and dissolved organic matter: Further implications for the ballast hypothesis. *Global Biogeochemical Cycles*. 22(4). doi:10.1029/2007GB003156

Decho, A. W. (1990). Microbial exopolymer secretions in ocean environments: their role(s) in food webs and marine processes. *Oceanography Marine Biology Annual Review*. 28: 73–154.

Decho, A. W., and Gutierrez, T. (2017). Microbial extracellular polymeric substances (EPSs) in ocean systems. *Frontiers in Microbio*logy. 8: 922. doi:10.3389/fmicb.2017.00922

DeLong, E. F., Franks, D. G., and Alldredge, A. L. (1993). Phylogenetic diversity of aggregate□attached vs. free□living marine bacterial assemblages. *Limnology and Oceanography*. 38(5): 924–934. doi:10.4319/lo.1993.38.5.0924

Deng, W., Cruz, B. N., and Neuer, S. (2016). Effects of nutrient limitation on the growth, TEP production and aggregate formation of marine *Synechococcus*. *Aquatic Microbial Ecology: International Journal*. 78(1): 39–49. doi:10.3354/ame01803

Deng, W., Monks, L., and Neuer, S. (2015). Effects of clay minerals on the aggregation and subsequent settling of marine *Synechococcus*. *Limnology and Oceanography*. 60(3): 805–816. doi:10.1002/lno.10059

DuRand, M. D., Olson, R. J., and Chisholm, S. W. (2001). Phytoplankton population dynamics at the Bermuda Atlantic Time-series station in the Sargasso Sea. *Deep Sea Research Part II: Topical Studies in Oceanography*. 48(8): 1983–2003. doi:10.1016/S0967-0645(00)00166-1

Durkin, C. A., Estapa, M. L., and Buesseler, K. O. (2015). Observations of carbon export by small sinking particles in the upper mesopelagic. *Marine Chemistry*. 175: 72–81. doi:10.1016/j.marchem.2015.02.011

Engel, A., and Schartau, M. (1999). Influence of transparent exopolymer particles (TEP) on sinking velocity of *Nitzschia closterium* aggregates. *Marine Ecology Progress Series*. 182: 69–76. doi:10.3354/meps182069

Fowler, S. W., and Knauer, G. A. (1986). Role of large particles in the transport of elements and organic compounds through the oceanic water column. *Progress in Oceanography*. 16(3): 147–194. doi:10.1016/0079-6611(86)90032-7

Fukao, T., Kimoto, K., and Kotani, Y. (2012). Effect of temperature on cell growth and production of transparent exopolymer particles by the diatom *Coscinodiscus granii* isolated from marine mucilage. *Journal of Applied Phycology*. 24(2): 181–186. doi:10.1007/s10811-011-9666-3

Gärdes, A., Iversen, M. H., Grossart, H. -P., Passow, U., and Ullrich, M. S. (2011). Diatom-associated bacteria are required for aggregation of *Thalassiosira weissflogii*. *The ISME Journal*. 5(3): 436–45. doi:10.1038/ismej.2010.145

Gärdes, A., Ramaye, Y., Grossart, H. -P., Passow, U., and Ullrich, M. S. (2012). Effects of *Marinobacter adhaerens* HP15 on polymer exudation by *Thalassiosira weissflogii* at different N: P ratios. *Marine Ecology Progress Series*. 461: 1–14. doi:10.3354/meps09894

Garrison, D. L., Gowing, M. M., Hughes, M. P., Campbell, L., Caron, D. A., Dennett, M. R., Shalapyonok, A., Olson, R. J., Landry, M. R., Brown, S. L., Liu, H.-B., Azam, F., Steward, G. F., Ducklow, H. W., and Smith, D. C. (2000). Microbial food web structure in the Arabian Sea: A US JGOFS study. *Deep Sea Research Part II: Topical Studies in Oceanography*. 47: 1387–1422. doi:10.1016/S0967-0645(99)00148-4

Gobet, A., Barbeyron, T., Matard-Mann, M., Magdelenat, G., Vallenet, D., Duchaud, E., and Michel, G. (2018). Evolutionary evidence of algal polysaccharide degradation acquisition by *Pseudoalteromonas carrageenovora* 9T to adapt to macroalgal niches. *Frontiers in Microbiology*. 9: 2740. doi:10.3389/fmicb.2018.02740

Grossart, H. -P., Czub, G., and Simon, M. (2006). Algae-bacteria interactions and their effects on aggregation and organic matter flux in the sea. *Environmental Microbiology*. 8(6): 1074–1084. doi:10.1111/j.1462-2920.2006.00999.x

Guidi, L., Chaffron, S., Bittner, L., Eveillard, D., Larhlimi, A., Roux, S., et al. (2016). Plankton networks driving carbon export in the oligotrophic ocean. *Nature*. 532: 465–470. doi:10.1038/nature16942

Guillard, R. R. L., and Hargraves, P. E. (1993). *Stichochrysis immobilis* is a diatom, not a chrysophyte. *Phycologia*. 32(3): 234–236. doi:10.2216/i0031-8884-32-3-234.1

Hamm, C. E. (2002). Interactive aggregation and sedimentation of diatoms and clay-sized lithogenic material. *Limnology and Oceanography*. 47(6): 1790-1795. doi:10.4319/lo.2002.47.6.1790

Hoagland, K. D., Rosowski, J. R., Gretz, M. R., and Roemer, S. C. (1993). Diatom extracellular polymeric substances: function, fine structure, chemistry, and physiology. *Journal of Phycology*. 29(5): 537–566. doi:10.1111/j.0022-3646.1993.00537.x

Iuculano, F., Mazuecos, I. P., Reche, I., and Agustí, S. (2017). *Prochlorococcus* as a possible source for transparent exopolymer particles (TEP). *Frontiers in Microbiology*. 8: 709. doi:10.3389/fmicb.2017.00709

Iversen, M. H., and Ploug, H. (2010). Ballast minerals and the sinking carbon flux in the ocean: carbon-specific respiration rates and sinking velocity of marine snow aggregates. *Biogeosciences*. 7(9): 2613–2624. doi:10.5194/bg-7-2613-2010

Iversen, M. H., and Robert, M. L. (2015). Ballasting effects of smectite on aggregate formation and export from a natural plankton community. *Marine Chemistry*. 175: 18–27. doi:10.1016/j.marchem.2015.04.009

Jackson, G. A. (1990). A model of the formation of marine algal flocs by physical coagulation processes. *Deep-Sea Research. Part A. Oceanographic Research Papers*. 37(8): 1197–1211. doi:10.1016/0198-0149(90)90038-W

Jickells, T. D., Dorling, S., Deuser, W. G., Church, T. M., Arimoto, R., and Prospero, J. M. (1998). Air-borne dust fluxes to a deep water sediment trap in the Sargasso Sea. *Global Biogeochemical Cycles*. 12(2): 311–320. doi:10.1029/97GB03368

Kaeppel, E. C., Gärdes, A., Seebah, S., Grossart, H. -P., and Ullrich, M. S. (2012). *Marinobacter adhaerens* sp. nov., isolated from marine aggregates formed with the diatom *Thalassiosira weissflogii*. *International Journal of Systematic and Evolutionary Microbiology*: 62(1), 124–128. doi:10.1099/ijs.0.030189-0

Kavita, K., Mishra, A., and Jha, B. (2013). Extracellular polymeric substances from two biofilm forming *Vibrio* species: Characterization and applications. *Carbohydrate Polymers*. 94(2): 882–888. doi:10.1016/j.carbpol.2013.02.010

Kester, D., Duedall, I., Connors, D., and Phytkowicz, R. (1967). Preparation of artificial seawater. *Limnology and Oceanograph*y. 12(1):176-179. doi:10.4319/lo.1967.12.1.0176

Landry, M. R., Selph, K. E., Taylor, A. G., Décima, M., Balch, W. M., and Bidigare, R. R. (2011). Phytoplankton growth, grazing and production balances in the HNLC equatorial Pacific. *Deep Sea Research Part II: Topical Studies in Oceanography*. 58: 524–535. doi:10.1016/j.dsr2.2010.08.011

Li, S., Winters, H., Jeong, S., Emwas, A., Vigneswaran, S., and Amy, G. L. (2016). Marine bacterial transparent exopolymer particles (TEP) and TEP precursors: Characterization and RO fouling potential. *Desalination*. 379: 68–74. doi:10.1016/j.desal.2015.10.005

Li, S., Winters, H., Villacorte, L. O., Ekowati, Y., Emwas, A. -H., Kennedy, M. D., and Amy, G. L. (2015). Compositional similarities and differences between transparent exopolymer particles (TEPs) from two marine bacteria and two marine algae: Significance to surface biofouling. *Marine Chemistry.* 174: 131-140. doi:10.1016/j.marchem.2015.06.009

Logan, B. E., Passow, U., Alldredge, A. L., Grossart, H. -P., and Simon, M. (1995). Rapid formation and sedimentation of large aggregates is predictable from coagulation rates (half-lives) of transparent exopolymer particles (TEP). *Deep-Sea Research Part II: Topical Studies in Oceanography*. 42(1): 203–214. doi:10.1016/0967-0645(95)00012-F

Lomas, M. W., Bates, N. R., Johnson, R. J., Knap, A. H., Steinberg, D. K., and Carlson, C. A. (2013). Two decades and counting: 24-years of sustained open ocean biogeochemical measurements in the Sargasso Sea. *Deep-Sea Research Part II: Topical Studies in Oceanography*. 93:16–32. doi:10.1016/j.dsr2.2013.01.008

Lomas, M. W., and Moran, S. B. (2011). Evidence for aggregation and export of cyanobacteria and nano-eukaryotes from the Sargasso Sea euphotic zone. *Biogeosciences*. 8(1): 203–216. doi:10.5194/bg-8-203-2011

Majumdar, I., D'Souza, F., and Bhosle, N. B. (1999). Microbial exopolysaccharides: effect on corrosion and partial chemical characterization. *Journal of the Indian Institute of Science*. 79(6): 539–550.

Mancuso Nichols, C. A., Garon, S., Bowman, J.P., Raguénès, G., and Guézennec, J. (2003). Production of exopolysaccharides by Antarctic marine bacterial isolates.

Journal of Applied Microbiology. 96(5): 1057–1066. doi:10.1111/j.1365-2672.2004.02216.x

Morán, X. A. G., López-Urrutia, Á., Calvo-Díaz, A., and Li, W. K. (2010). Increasing importance of small phytoplankton in a warmer ocean. *Global Change Biology*. 16(3): 1137-1144 doi:10.1111/j.1365-2486.2009.01960.x.

Neuer, S., Iversen, M. H., and Fischer, G. (2014). The ocean's biological carbon pump as part of the global carbon cycle. *Limnology and Oceanography e-Lectures*. 4(4): 1-51. doi:10.1002/lob.10083

Ortega-Retuerta, E., Duarte, C. M., and Reche, I. (2010). Significance of bacterial activity for the distribution and dynamics of transparent exopolymer particles in the Mediterranean Sea. *Microbial Ecology*. 59(4): 808–818. doi:10.1007/s00248-010-9640-7

Passow, U. (2000). Formation of transparent exopolymer particles, TEP, from dissolved precursor material. *Marine Ecology Progress Series*. 192: 1–11. doi:10.3354/meps192001

Passow, U. (2002). Production of transparent exopolymer particles (TEP) by phyto- and bacterioplankton. *Marine Ecology Progress Series*. 236: 1–12. doi:10.3354/meps236001

Passow, U. (2002). Transparent exopolymer particles (TEP) in aquatic environments. *Progress in Oceanography*. 55(3-4): 287–333. doi:10.1016/S0079-6611(02)00138-6

Passow, U., and Alldredge, A. L. (1994). Distribution, size, and bacterial colonization of transparent exopolymer particles (TEP) in the ocean. *Marine Ecology Progress Series*. 113: 185–198. doi:10.3354/meps113185

Passow, U., and Alldredge, A. L. (1995). A dye-binding assay for the spectrophotometric measurement of transparent exopolymer particles (TEP). *Limnology and Oceanography*. 40(7): 1326–1335. doi:10.4319/lo.1995.40.7.1326

Passow, U., and De La Rocha. C. L. (2006). Accumulation of mineral ballast on organic aggregates. *Global Biogeochemical Cycles*. 20(1): 1–7. doi:10.1029/2005GB002579

Passow, U., Shipe, R. F., Murray, A., Pak, D. K., Brzezinski, M. A., and Alldredge, A. L. (2001). The origin of transparent exopolymer particles (TEP) and their role in the sedimentation of particulate matter. *Continental Shelf Research*. 21(4): 327–346. doi:10.1016/S0278-4343(00)00101-1

Pasulka, A. L., Samo, T. J., and Landry, M. R. (2015). Grazer and viral impacts on microbial growth and mortality in the southern California Current Ecosystem. *Journal of Plankton Research*. 37(2): 320–336. doi:10.1093/plankt/fbv011

Ploug, H., Terbrüggen, A., Kaufmann, A., Wolf□Gladrow, D., and Passow, U. (2010). A novel method to measure particle sinking velocity in vitro, and its comparison to three other in vitro methods. *Limnology and Oceanography Methods*. 8(8): 386–393. doi:10.4319/lom.2010.8.386

Polovina, J. J., Howell, E. A., and Abecassis, M. (2008). Ocean's least productive waters are expanding. *Geophysical Research Letters*. 35(3): L03618. doi:10.1029/2007GL031745

Radić, T., Ivančić, I., Fuks, D., and Radić, J. (2006). Marine bacterioplankton production of polysaccharidic and proteinaceous particles under different nutrient regimes. *FEMS Microbiology Ecology*. 58(3): 333–342. doi:10.1111/j.1574-6941.2006.00176.x

Richardson, T. L. (2019). Mechanisms and pathways of small-phytoplankton export from the surface ocean. *Annual Review of Marine Science*. 11(1): 57–74. doi:10.1146/annurev-marine-121916-063627

Richardson, T. L., and Jackson, G. A. (2007). Small phytoplankton and carbon export from the surface ocean. *Science*. 315(5813): 838–840. doi:10.1126/science.1133471

Rochelle-Newall, E. J., Mari, X., and Pringault, O. (2010). Sticking properties of transparent exopolymeric particles (TEP) during aging and biodegradation. *Journal of Plankton Research.* 32(10: 1433–1442. doi:10.1093/plankt/fbq060

Roux, P., Siano, R., Collin, K., Bilien, G., Sinquin, C., Marchand, L., Zykwinska, A., Delbarre-Ladrat, C., and Schapira, M. (2021). Bacteria enhance the production of extracellular polymeric substances by the green dinoflagellate *Lepidodinium chlorophorum*. *Scientific Reports*. 11(1): 4795–4795. doi:10.1038/s41598-021-84253-2

Santschi, Xu, C., Schwehr, K. A., Lin, P., Sun, L., Chin, W.-C., Kamalanathan, M., Bacosa, H. P., and Quigg, A. (2020). Can the protein/carbohydrate (P/C) ratio of exopolymeric substances (EPS) be used as a proxy for their "stickiness" and aggregation propensity? *Marine Chemistry*. 218: 103734. doi:10.1016/j.marchem.2019.103734

Shanks, A. L., and Edmondson, E. W. (1989). Laboratory-made artificial marine snow: a biological model of the real thing. *Marine Biology*. 101: 463–470. doi:10.1007/BF00541648

Signorini, S. R., Franz, B. A., and McClain, C. R. (2015). Chlorophyll variability in the oligotrophic gyres: mechanisms, seasonality and trends. *Frontiers in Marine Science*. 2: 1–11. doi:10.3389/fmars.2015.00001

Simon, M., Grossart, H., Schweitzer, B., and Ploug, H. (2002). Microbial ecology of organic aggregates in aquatic ecosystems. *Aquatic Microbial Ecology*. 28(2): 175–211. doi:10.3354/ame028175

Sonnenschein, E. C., Syit, D. A., Grossart, H.-P., and Ullrich, M. S. (2012). Chemotaxis of *Marinobacter adhaerens* and Its Impact on Attachment to the Diatom *Thalassiosira weissflogii*. *Applied and Environmental Microbiology*. 78(19): 6900–6907. doi:10.1128/AEM.01790-12

Stahl, A., and Ullrich, M. S. (2016). Proteomics analysis of the response of the marine bacterium *Marinobacter adhaerens* HP15 to the diatom *Thalassioisira weissflogii*. *Aquatic Microbial Ecology : International Journal*. 78(2): 65–79. doi:10.3354/ame01804

Stoderegger, K. E., and Herndl, G. J. (1999). Production of exopolymer particles by marine bacterioplankton under contrasting turbulence conditions. *Marine Ecology Progress Series*. 189: 9–16. doi:10.3354/meps189009

Suttle, C. A. (2007). Marine viruses—Major players in the global ecosystem. *Nature Reviews Microbiology*. *5*(10): 801–812. doi:10.1038/nrmicro1750

Tang, B.-L., Yang, J., Chen, X.-L., Wang, P., Zhao, H.-L., Su, H.-N., Li, C.-Y., Yu, Y., Zhong, S., Wang, L., Lidbury, I., Ding, H., Wang, M., McMinn, A., Zhang, X.-Y., Chen, Y., and Zhang, Y.-Z. (2020). A predator-prey interaction between a marine *Pseudoalteromonas* sp. and Gram-positive bacteria. *Nature Communications*. 11(1): 285. doi:10.1038/s41467-019-14133-x

Tarazona, E., Lucena, T., Arahal, D. R., Macián, M. C., Ruvira, M. A., and Pujalte, M. J. (2014). Multilocus sequence analysis of putative *Vibrio mediterranei* strains and description of *Vibrio thalassae* sp. nov. *Systematic and Applied Microbiology*. 37(5): 320–328. doi:10.1016/j.syapm.2014.05.005

Thornton, D. C. O., Fejes, E. M., Dimarco, S. F., and Clancy, K. M. (2007). Measurement of acid polysaccharides in marine and freshwater samples using Alcian Blue. *Limnology and Oceanography*. 5(2): 73–87. doi:10.4319/lom.2007.5.73

Tran, N.T., Tamburic, B., Evenhuis, C.R., and Seymour, J.R. (2020). Bacteria-mediated aggregation of the marine phytoplankton *Thalassiosira weissflogii* and *Nannochloropsis oceanica*. *Journal of Applied Phycology*. 32(6): 3735–3748. doi:10.1007/s10811-020-02252-8

van der Jagt, H., Friese, C., Stuut, J. B. W., Fischer, G., and Iversen, M. H. (2018). The ballasting effect of Saharan dust deposition on aggregate dynamics and carbon export: aggregation, settling, and scavenging potential of marine snow. *Limnology and Oceanography*. 63(3): 1386–1394. doi:10.1002/lno.10779

Verspagen, J. M. H., Visser, P. M., and Huisman, J. (2006). Aggregation with clay causes sedimentation of the buoyant cyanobacteria *Microcystis* spp. *Aquatic Microbial Ecology: International Journal*. 44(2): 165–174. doi:10.3354/ame044165

Villacorte, L. O., Y. Ekowati, Calix-Ponce, H. N., Schippers, J. C., Amy, G. L., and Kennedy, M. D. (2015). Improved method for measuring transparent exopolymer particles (TEP) and their precursors in fresh and saline water. *Water Research*. 70: 300–312. doi:10.1016/j.watres.2014.12.012

Yamada, Y., Fukuda, H., Tada, Y., Kogure, K., and Nagata, T. (2016). Bacterial enhancement of gel particle coagulation in seawater. *Aquatic Microbial Ecology*. 77(1): 11–22. doi:10.3354/ame01784