

**Transparent exopolymer particle (TEP) production under CO₂ enrichment: a mesocosm
experiment**

Daneil Newcomb

2013 Ocean Acidification Apprenticeship, Friday Harbor Laboratories, Friday Harbor, WA.

Department of Oceanography, University of Washington, Seattle, WA.

Contact Information

Email: djn3@uw.edu

Abstract

Transparent exopolymer particles (TEP) are abiotically formed aggregates within the water column from physiological stress causing polysaccharides to be exuded by phytoplankton and some bacteria. They are considered a possible mechanism for carbon export from surface waters due to their high stickiness and thus ability to cause large aggregates to form. Ocean acidification is a process which may greatly influence the production and cycling of TEP within the water column. The San Juan Islands experience both anthropogenic and natural acidification of their surrounding waters. The influence of ocean acidification on TEP production within Friday Harbor waters has not yet been studied. To study TEP production under ocean acidification nine mesocosms with three treatments were utilized. Though no difference between the control and high treatments were found, possible evidence was seen for CO₂ effecting TEP production; The drift treatment was significant different from the maintained high and control treatments, suggesting repetitive addition of CO₂ to the environment does have an effect on TEP production within the mesocosms.

Introduction

Transparent exopolymer particles (TEP) are discrete, non-living particles composed of carbohydrate-rich acidic polysaccharides excreted from phytoplankton and bacteria (Passow 2002). The exudation of these polysaccharides may be a response to physiological stress (Kahl 2008). TEP form abiotically, and are found in high concentrations during phytoplankton blooms (Passow et. al 2001). TEP vary in size from submicron to several hundred microns, and can be as abundant as thousands of particles per milliliter (Logan et al. 1995). TEP may cause aggregation of smaller particles suspended in the water column, which could ultimately lead to increased export of carbon from the eutrophic zone (Logan et al. 1995).

Previous studies have shown both biotic and abiotic factors can affect the production of TEP. Variations in size have been correlated with differences in turbulence and nutrient regimes. Turbulent-sheared environments are correlated to increased production of TEP by both bacteria (Passow 2002, Stoderegger and Herndl 1998) and phytoplankton (Logan et al. 1995). TEP production by phytoplankton is enhanced by both nutrient limitation (Engel et al. 2002) and high concentrations of nutrients (Perdotti et al. 2010), and production of TEP under various conditions is in many cases species-specific (Passow 2002). In addition, past research has drawn correlation between TEP production and chlorophyll-a as a proxy of phytoplankton biomass (Passow 2002) and bacterial production (Ortega-Retuerta 2010), with the presence or absence of a phytoplankton bloom appearing to greatly influence the strength of particular correlations (Wurl 2011). Many of these factors undergo a series of complex interactions, some of which are not yet fully understood, making the study of TEP cycling challenging.

The addition of anthropogenic carbon dioxide (CO₂) to the atmosphere, mainly from the burning of fossil fuels, is transferred to the ocean by air-sea gas exchange resulting in an increased concentration of CO₂ in surface waters, which may then be mixed down through physical processes. Excess CO₂ added to the surface waters reacts to form carbonic acid, which leads to a decrease in pH and speciation shifts within the carbonate system (Doney et al. 2009). This process is known as ocean acidification. Seawater surrounding the San Juan Islands, including Friday Harbor, is highly susceptible to ocean acidification conditions from both natural and anthropogenic forcing. When there are northerly winds, naturally CO₂-rich waters upwell along the Pacific Northwest coast, and is transported through the Strait of Juan de Fuca (Feely et al. 2008) to local waters such as those found at Friday Harbor Laboratories.

Recent focus has been to study the effects of ocean acidification on TEP production and utilization as increased levels of CO₂ may cause physiological stress to occur in microorganisms (CITE). Ocean acidification has been known to have many consequences, including negative influence on the calcification rates of marine biota (Doney et al. 2009) and alteration to the mechanism of carbon transport called biological pump (Passow and Carlson 2012). Because TEP are formed from carbon rich biological exudates, their production is closely linked to the biological pump, therefore, the production and

utilization of TEP may be affected by ocean acidification as well. Though some studies have given focus to TEP production under ocean acidification conditions, similar studies have not been completed for the San Juan Islands. As the San Juan Islands ambient ocean CO₂ levels are higher than many previously studied areas, microorganisms present in Friday Harbor waters may offer insight concerning predicted levels of CO₂ during the next century.

Mesocosm experiments offer a unique opportunity to study potential perturbations to an ecosystem without altering the ecosystem itself. When completed within in situ conditions, these experiments allow for manipulation of variables while maintaining many of the ambient conditions. This allows CO₂ concentrations to be enhanced for enrichment treatments, while experiencing the same conditions as control treatments. Mesocosms also provide an opportunity to study large-scale community interactions which are not possible to observe in isolated, small scale lab experiments. Therefore, mesocosm studies enable examination of elevated CO₂ levels to be expanded from the focus on individual organisms to the entire community. Mesocosm studies by Riebesell et al. (2007) showed a fourfold increase in TEP production between ambient and enriched CO₂ levels.

Because the presence of TEP in the water column enhances particle aggregation, and thus has the ability to increase the export of carbon to depth, studies focusing on the differences between TEP cycling during *in situ* conditions versus elevated CO₂ conditions are crucial. This study focused on how enrichment of waters with CO₂ affects the biological activity of naturally occurring communities of microorganisms within Friday Harbor, and how TEP production differs between ambient and consistently enriched waters. The following biotic factors were investigated: production of TEP by phytoplankton and bacteria, grazing of phytoplankton by protists, the modification and/or degradation of TEP by bacteria.

Methods and Materials

This experiment utilized nine 3500-L polyurethane mesocosm bags, supported by a metal frame with flotations and tethered to a dock in the coastal waters of Friday Harbor, WA (48 N 55', 123W 01'). Mesocosm treatments were as follows: three controlled ambient pCO₂ (690 ppm), three controlled

elevated pCO₂ (1250 ppm), and three drifting elevated pCO₂ (initially 1250 ppm). Filtered seawater equilibrated with 1 atm CO₂ was added as necessary to individual mesocosms (via an apparatus called “the spider”— tubing with a series of holes and miniature hoses attached) to maintain the concentration of CO₂ within the controlled mesocosms, while the drift were initially elevated but no additional CO₂ enriched water was added throughout the experiment.

Mesocosms were filled with seawater pre-filtered to exclude zooplankton grazers and nekton larger than 0.5 mm. Seawater was pumped into a reservoir from the harbor using a biologically safe pump, then distributed to the mesocosms simultaneously through tubing running from the reservoir. This process spanned three days (50 hours), and mesocosms were monitored throughout to ensure uniform filling and to prevent contamination. Following filling, each mesocosm was covered with a black mesh bag and top covering, decreasing the irradiance within the bag - by 55% initially - to slow biological production, and capped with a plastic dome when not sampling to prevent contamination.

Measurements took place from 9 April 2013 to 30 April 2013. Light levels in the mesocosm and off the dock were measured using a light meter (Li-Cor) and temperature and salinity were measured using a CTD (Sea-Bird 375MP). All mesocosms were manually stirred using a hydrodynamic disk prior to sampling. Samples were drawn from the mesocosms using a three meter long depth-integrated sampler, and placed in a 10 L acid washed polycarbonate carboy. 250 mL samples were taken from this carboy for each mesocosm and the dock and stored in a cooler with an ice pack to be transported back to the lab for processing. To determine bulk TEP within each mesocosm, duplicate 100 mL samples were vacuum filtered onto 0.4 µm polycarbonate filters (Milipore HTPP25) then analyzed using the Alcian Blue dye-binding assay method (Passow and Alldredge 1995)(Fig. 1). Chlorophyll-a was determined as described in Porcino 2013, bacterial abundance and in Apple 2013, and biogenic silica as in Shutt 2013.

Experimental results were analyzed using the statistical analysis program IBM SPSS Statistics. Friedman tests were completed to determine statistical differences between treatments, and Person’s Correlation Coefficients were determined for relationships between biotic factors.

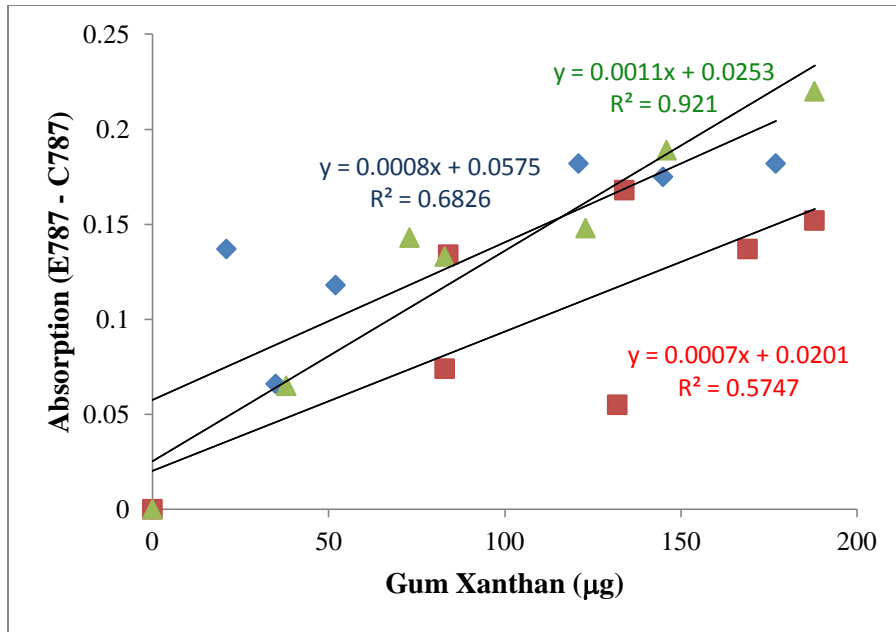


Figure 1. Calibration curve used to convert absorption values received from the spectrophotometer to weight values using a Gum Xanthan Equivalent. Linear regression statistics were run in Microsoft Excel, and the slope of the line with the highest R^2 value was chosen to calculate the conversion factor.

Results

Initial TEP concentrations within the mesocosms ranged from 77.3 to 190.9 $\mu\text{g L}^{-1}$, with median values for control, high, and drift being 118.2 $\mu\text{g L}^{-1}$, 100.0 $\mu\text{g L}^{-1}$, and 118.2 $\mu\text{g L}^{-1}$, respectively (Fig. 2). Initial TEP concentration for the dock was 95.5 $\mu\text{g L}^{-1}$. TEP concentrations generally increased over time, with the control, high, and drift reaching median maxima of 713.6 $\mu\text{g L}^{-1}$, 581.8 $\mu\text{g L}^{-1}$, and 472.7 $\mu\text{g L}^{-1}$, respectively. This is a 33% difference in TEP production from the drift to the control, and a 9% difference in TEP production from the drift to the high. The dock reached a maximum of 345.5 $\mu\text{g L}^{-1}$. All maxima occurred on the final sampling day with the exception of the high treatment. Significant differences were found between control and drift treatments ($F = 0.879$, $p < 0.01$), and high and drift treatments ($F = 0.848$, $p = 0.01$), but not between control and high treatments ($F = 0.030$, $p = 0.902$).

Significant correlations were found between TEP concentrations and Chlorophyll a (Table 1), Bacterial Abundance (Table 2), and Biogenic Silica (Table 3). Both Chlorophyll-a and Bacterial Abundance exhibit similar trends: low values until day ten, followed by dramatic increase. The correlation between TEP concentration and microzooplankton grazing rate was not significant.

Initial median Chlorophyll a concentrations for the control, high, and drift were 0.70 mg L^{-1} , 0.80 mg L^{-1} , and 0.80 mg L^{-1} respectively. Chlorophyll a concentrations reached their maxima on the final sampling day in all treatments, and were 23.8 mg L^{-1} , 20.7 mg L^{-1} , and 14.9 mg L^{-1} for the control, high, and drift, respectively. Significant differences were found between control and high treatments, and control and drift treatments, but not high and drift treatments. (Porcino 2013)

Initial median Bacterial Abundances for the control, high, and drift were $9.46 \times 10^5 \text{ cells mL}^{-1}$, $9.72 \times 10^5 \text{ cells mL}^{-1}$, and $1.02 \times 10^6 \text{ cells mL}^{-1}$, respectively. The median maxima for bacterial abundance occurred between days fourteen and eighteen, and were $3.19 \times 10^6 \text{ cells mL}^{-1}$, $2.10 \times 10^6 \text{ cells mL}^{-1}$, and $1.97 \times 10^6 \text{ cells mL}^{-1}$ for the control, high, and drift, respectively. Significant differences were found between all three treatments (Apple 2013)

Biogenic Silica was initially at $3.28 \text{ } \mu\text{mol L}^{-1}$, $3.33 \text{ } \mu\text{mol L}^{-1}$, and $3.58 \text{ } \mu\text{mol L}^{-1}$ for the control, high and drift, respectively, but then decreased with time. Values were high from the first sampling day until sampling day ten. There were no significant differences between treatments. (Shutt 2013)

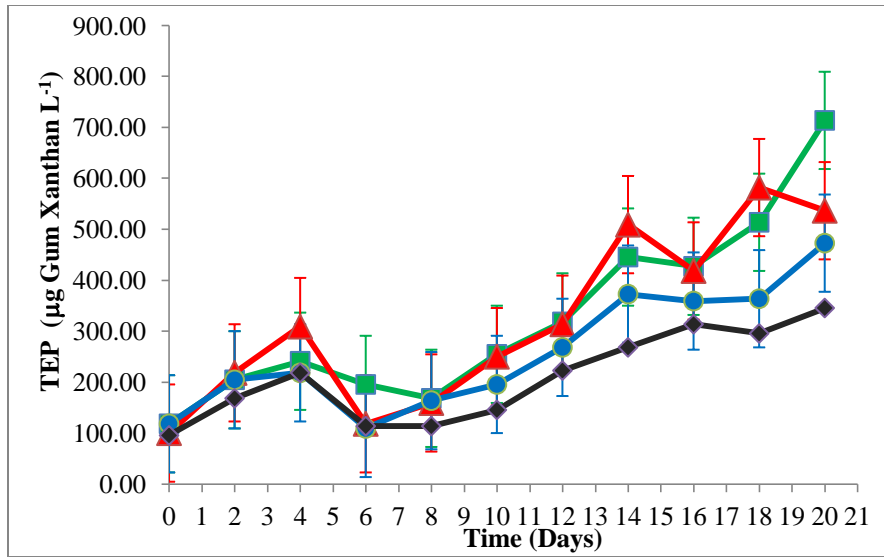


Figure 2. Time series plot of median transparent exopolymer particle production. High treatment shown in red, control in green, drift in blue, and dock in black. Significant differences were found between control and drift treatments ($F = 0.879$, $p < 0.01$), and high and drift treatments ($F = 0.848$, $p = 0.01$), but not between control and high treatments ($F = 0.030$, $p = 0.902$). Error bars show the median deviation/

Table 1. Person's Correlation Coefficients for TEP and Chlorophyll a (Porcino 2013).

Treatment	ρ
Control	0.837
High	0.851
Drift	0.794

Table 2. Person's Correlation Coefficients for TEP and Bacterial Abundance (Apple 2013).

Treatment	ρ
Control	0.837
High	0.851
Drift	0.794

Table 1. Person's Correlation Coefficients for TEP and Biogenic Silica (Shutt 2013).

Treatment	ρ
Control	0.871
High	0.794
Drift	0.687

Discussion

The high correlation between chlorophyll a, as a proxy for phytoplankton, and TEP suggests a significant proportion of TEP may have been produced by phytoplankton. This corresponds to the high correlation to biogenic silica, which is a proxy from diatoms within the water column, which were the most abundant phytoplankton. Studies have shown diatom blooms are often associated with high production of TEP (Passow and Alldredge 1995, Perdotti et al. 2010), therefore this result reinforces previous research.

High correlations of TEP to bacterial abundance could be explained by multiple biotic interactions. One possibility is bacterial production of TEP. Previous studies have suggested bacteria may be responsible for up to 25% of polysaccharides within the water column (Ortega-Retuerta et. al 2010), though there is currently not a means of differentiating bacteria-produced TEP from phytoplankton-produced TEP. Further focus would need to be given to individual organisms to attempt differentiation. Alternatively, bacterial colonization and remineralization of TEP may be a significant process occurring within the mesocosms. Pointek et. al (2010) found increased degradation of TEP under high CO₂ conditions (2010). Identification of bacterial species that were present in the mesocosms could allow for identification of surface dwelling, or attached, bacterial species often associated with TEP.

The lack of correlation between microzooplankton grazing and TEP within the mesocosms suggests little TEP was produced as a result of grazing by microzooplankton on phytoplankton.

Many differences can be seen between experimental results of this year's experiment to that of Chong (2012) from the previous year. Conversion of Chong's values of TEP Absorbance to $\mu\text{g Gum Xanthan Equivalent L}^{-1}$ by the same factor as was used for this study demonstrates notable differences. Doing so results in TEP concentration ranging from roughly 200 to 3000 $\text{mg Gum Xanthan L}^{-1}$, suggesting three times more TEP was produced the previous year. However, no significant difference was observed between any treatments during the previous study. Dominant species were the same in the previous year, suggesting other factors must be responsible for differing results. Methods of CO₂ addition were vastly different and could have caused production differences between experiments as well as the lack of significant difference between treatments. Light was not limited prior to this year, resulting in an immediate bloom that masked the effects of CO₂. The differential light source may also explain the large difference in TEP production. Studies have shown intense UVB radiation can lead to increased TEP production (Ortega- Retuerta et al. 2009), thus production could have been effected by the initially low light conditions for this experiment.

Previous studies focusing on *Emiliana huxleyi* found daily concentrations ranging from roughly 100 to 900 $\mu\text{g L}^{-1}$ of TEP (Engel et. al 2004). Significant difference was observed between high and

control treatment, which was not seen within this study. Experimental set up differed in the number of CO₂ additions made, as well as the levels of CO₂ used: the CO₂ of the control treatment for this experiment was roughly equivalent to the CO₂ level of Engel et al.'s high treatment, and no further CO₂ additions were made following the initial. Community structure and population were also highly different between experiments. Seawater was pre-filtered in this study, and the population was diatom-dominated, whereas Engel et al. utilized ambient seawater without filtration and initially found diatoms, followed by coccolithophores. Because TEP production is thought to be species- and taxon-specific, differences between experimental results observed here and those of Engel et. al are most likely influenced by biological activity as well as differences in CO₂.

Though this study exhibited no significant difference between high and control treatments, there may still be a visible CO₂ effect within our results; the drift treatment was significantly different from the other two treatments. Most abiotic factors were consistent throughout all mesocosms. For the experiment, temperature trends followed the same pattern for all mesocosms and the dock. Though variations in salinity occurred over time for the dock sample, salinity within the mesocosms remained constant and higher than the dock. Initial nutrient concentrations for nitrate, nitrite, ammonia, phosphate, silicate, and oxygen were the same for all mesocosms and the dock. Thus, in looking at other abiotic factors, significant differences cannot be seen between treatments, suggesting manipulation of CO₂ as the only factor differentially influencing TEP production by microbiota. The drift mesocosms experienced a single CO₂ addition, while the control and high had multiple CO₂ addition. It is possible that maintaining CO₂ levels influenced physiological responses of TEP producers within the mesocosms, resulting in the observed difference.

During natural bloom conditions, nutrients required for photosynthesis are utilized in particular ratios, resulting in decreasing nutrient concentrations. Because we maintained the levels of CO₂ producers were exposed to, it is possible the cells encountered more CO₂ than possible during a normal bloom period, resulting in carbon overflow (Engel et. al 2004) and the exudation of TEP in response to cell stress.

Conclusions

This study confirms previously observed correlations between TEP and the biotic factors Chlorophyll a and Bacterial Abundance. The high significant correlations between multiple factors suggest interactions within the mesocosm are complex and not easily separated from each other, making in depth analysis of these factors necessary to better understand TEP cycling within the oceans. Repetitive additions of CO₂ may act as a nutrient enrichment, altering the ratio phytoplankton usually experience to one that promotes cellular carbon overflow. Thus, exudation of TEP occurs in significantly larger quantities under maintained CO₂ conditions than drifting conditions.

Acknowledgements

I would like to offer thanks to all of the Ocean Acidification Apprenticeship instructors, technicians, and apprentices for their company and hard work. Additional thanks to James Murray for advising me throughout my thesis, Evelyn Lessard for being an excellent reference, and Kelsey Gaessner for her advice. Thank you to Friday Harbor Laboratories for providing me not only a workspace, but a quarter filled with natural beauty, to Steve Emerson's lab for use of their oven, and to Kathy Kroglund's lab for use of their microgram balance. Thank you to all of the sources of funding received for this quarter: Educational Foundation of America and the National Science Fund for funding the project and Mary Gates Endowment Fund for providing my housing. Special thanks to Andrea G. Reister, and Lowell K. and Alice M. Barger for funding the past two years of my education through the UW Oceanography Departments Scholarships. Finally, thank you to my family, partner, and friends – especially my roommate Collin – for supporting me achieve all of my educational goals.

Work Cited

- Apple, J. 2013. Impact of elevated $p\text{CO}_2$ on heterotrophic bacterioplankton: a mesocosm experiment. Unpublished student paper. Friday Harbor Laboratories.
- Chong, M. 2012. Particulate organic C:N ratio and transparent exopolymer particle production in elevated CO_2 concentrations: a mesocosm experiment. Unpublished student paper. Friday Harbor Laboratories.
- Doney, S. C., V. J. Fabry, R. a. Feely, and J. a. Kleypas. 2009. Ocean Acidification: The Other CO_2 Problem. *Annual Review of Marine Science* **1**: 169–192.
- Engel, a. 2002. Direct relationship between CO_2 uptake and transparent exopolymer particles production in natural phytoplankton. *Journal of Plankton Research* **24**: 49–53.
- Feely, R. a, C. L. Sabine, J. M. Hernandez-Ayon, D. Ianson, and B. Hales. 2008. Evidence for upwelling of corrosive “acidified” water onto the continental shelf. *Science* **320**: 1490–2.
- Kahl, L., a Vardi, and O. Schofield. 2008. Effects of phytoplankton physiology on export flux. *Marine Ecology Progress Series* **354**: 3–19.
- Kim, J.-M., K. Lee, K. Shin, E. J. Yang, A. Engel, D. M. Karl, and H.-C. Kim. 2011. Shifts in biogenic carbon flow from particulate to dissolved forms under high carbon dioxide and warm ocean conditions. *Geophysical Research Letters* **38**: n/a–n/a.
- Logan, B. E., U. Passow, A. L. Alldredge, H.-P. Grossartt, and M. Simont. 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**: 203–214.
- Ortega-Retuerta, E., U. Passow, I. Reche, and I. Balears. 2009. Effects of ultraviolet B radiation on (not so) transparent exopolymer particles. *Biogeosciences* **6**: 3071–3080.

- Ortega-Retuerta, E., C. M. Duarte, and I. Reche. 2010. Significance of bacterial activity for the distribution and dynamics of transparent exopolymer particles in the Mediterranean sea. *Microbial ecology* **59**: 808–18.
- Passow, U., and A. L. Alldredge. 1995. Aggregation of a diatom bloom in a mesocosm: The role of transparent exopolymer particles (TEP). *Deep Sea Research Part II: Topical Studies in Oceanography* **42**: 99–109.
- Passow, U., and C. Carlson. 2012. The biological pump in a high CO₂ world. *Marine Ecology Progress Series* **470**: 249–271.
- Passow, U., R. . Shipe, a Murray, D. . Pak, M. . Brzezinski, and a. . Alldredge. 2001. The origin of transparent exopolymer particles (TEP) and their role in the sedimentation of particulate matter. *Continental Shelf Research* **21**: 327–346.
- Passow, U. 2002. Production of transparent exopolymer particles (TEP) by phyto- and bacterioplankton. *Marine Ecology Progress Series* **236**: 1–12.
- Passow, U. 2012. The abiotic formation of TEP under different ocean acidification scenarios. *Marine Chemistry* **128-129**: 72–80.
- Pedrotti, M., F. Peters, S. Beauvais, M. Vidal, J. Egge, a Jacobsen, and C. Marrasé. 2010. Effects of nutrients and turbulence on the production of transparent exopolymer particles: a mesocosm study. *Marine Ecology Progress Series* **419**: 57–69.
- Piontek, J., M. Lunau, N. Händel, C. Borchard, M. Wurst, and a. Engel. 2010. Acidification increases microbial polysaccharide degradation in the ocean. *Biogeosciences* **7**: 1615–1624.
- Porcino, N. 2013. Phytoplankton community changes in a shifting CO₂ environment. Unpublished student paper. Friday Harbor Laboratories.

- Riebesell, U., K. G. Schulz, R. G. J. Bellerby, M. Botros, P. Fritsche, M. Meyerhöfer, C. Neill, G. Nondal, a Oschlies, J. Wohlers, and E. Zöllner. 2007. Enhanced biological carbon consumption in a high CO₂ ocean. *Nature* **450**: 545–8.
- Shutt, K. 2013. Diatom silicification changes in high pCO₂ seawater: a mesocosm experiment at Friday Harbor Laboratories. Unpublished student paper. Friday Harbor Laboratories.
- Stoderegger, K., and G. J. Herndl. 1998. Production and release of bacterial capsular material and its subsequent utilization by marine bacterioplankton. *Limnology And Oceanography* **43**: 877–884.
- Wurl, O., L. Miller, and S. Vagle. 2011. Production and fate of transparent exopolymer particles in the ocean. *Journal of Geophysical Research* **116**: C00H13.