

Estimating rates of gross TEP production and heterotrophic consumption from natural
assemblages

by

PATRICK I. DUFFY

(Under the Direction of Elizabeth Harvey)

ABSTRACT

Aggregation aided by transparent exopolymer particles (TEP) is the most common mechanism facilitating marine carbon export. However, the mechanisms that mediate TEP accumulation difficult to parameterize. Here, we present a novel adaptation the dilution method, to quantify *in situ* rates of biological community (< 200 μm) instantaneous gross TEP production, TEP consumption, and net TEP production. We examined TEP under two different nutrient regimes in a mesocosm experiment in Bergen, Norway. TEP concentrations ranged from $37.7 \pm 6.6 - 247 \pm 22 \mu\text{g XG eq. L}^{-1}$ under replete conditions and $37.8 \pm 12 - 195 \pm 27 \mu\text{g XG eq. L}^{-1}$ under P-limited conditions. We observed ‘decoupling’ of TEP and chlorophyll-*a*, with 3-4x increases in magnitude of TEP:Chl*a* ratio coinciding with shifts in measured rates of TEP consumption and production. This research reinforces the idea that variation in rates of TEP production and consumption alter TEP accumulation, potentially impacting biological pump efficiency.

INDEX WORDS: TEP, phytoplankton, transparent exopolymer particles

Estimating rates of gross TEP production and heterotrophic consumption from natural
assemblages

by

PATRICK I. DUFFY

BS, University of Delaware, 2016

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment
of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2019

© 2019

Patrick I. Duffy

All Rights Reserved

Estimating rates of gross TEP production and heterotrophic consumption from natural
assemblages

by

PATRICK I. DUFFY

Major Professor:	Elizabeth Harvey
Committee:	Adrian Burd
	Daniel Ohnemus

Electronic Version Approved:

Suzanne Barbour
Dean of the Graduate School
The University of Georgia
May 2019

ACKNOWLEDGEMENTS

I would first like to thank my thesis advisor Dr. Elizabeth Harvey for always providing help and guidance. I am thankful to have had time to be a member of her lab and know I am a more confident researcher with improved skills from their positive influences.

I would also like to acknowledge the members of my thesis committee for their suggestions on my writing and research: Dr. Adrian Burd and Dr. Daniel Ohnemus. I am grateful for their time and comments on this thesis.

Finally, I am profoundly gratefully for the eternal support of my parents.

TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	iv
LIST OF TABLES	vi
LIST OF FIGURES	vii
CHAPTERS	
1 Introduction	1
Methods	12
Results	20
Discussion	24
Future Directions and Conclusion	37
REFERENCES	48
APPENDICES	
A Supplemental Information	64

LIST OF TABLES

	Page
Table 1: Comparison of dilution-rates to changes in environmental TEP	41

LIST OF FIGURES

	Page
Figure 1: Conceptual representation of dilution-series TEP aggregation rates.	42
Figure 2: Assessment of experimental methods	43
Figure 3: Nutrient concentrations during bloom experiment.....	44
Figure 4: TEP and Chlorophyll- <i>a</i> concentrations over observation period	45
Figure 5: Comparing TEP and Chlorophyll- <i>a</i> concentrations	46
Figure 6: TEP aggregation dynamics rates from dilution-series	47

CHAPTER 1

INTRODUCTION

Phytoplankton serve as the base of marine trophic systems, account for at least half of the Earth's primary production, and play a crucial role in the sequestration of atmospheric carbon dioxide (CO₂) into the deep ocean via the biological carbon pump. Marine carbon sequestration is ultimately driven by the flux of phytoplankton-derived particulate organic matter (POM) to the deep ocean, most significantly by coagulation of single particles into larger, rapidly sinking aggregates (Shanks & Trent 1980; Asper et al. 1992; Ducklow et al. 2011). The 'efficiency' of the biological pump is defined as the fraction of primary production produced versus exported from the euphotic zone. Currently, estimates of global carbon export flux are highly variable with estimates spanning from 5 to >15 Pg C y⁻¹ (Falkowski et al 2011; Boyd and Trull 2007; Henson et al. 2011; Henson et al. 2015). A significant source of this variable can be attributed to uncertain elemental ratios of phytoplankton and POM which can vary widely depending on nutrient status and environmental conditions (Geider and LaRoche 2002; Finkel et al. 2010). Thus, how the biological carbon pump will respond to future anthropogenic climate change is uncertain (Riebesell et al. 2009; Wohlers et al. 2009; Passow and Carlson 2012).

Increasing cellular quotas of carbon to nitrogen in phytoplankton have been observed for a variety of species under nutrient limitation (Harrison et al. 1977; Goldman et al. 1992) as well as increased temperatures (Thompson et al. 1992; Berges et al. 2002; Taucher et al. 2012). Toggweiler (1993) termed this phenomenon of excess uptake of dissolved inorganic carbon (DIC) over inorganic nitrogen as 'carbon overconsumption'. It was commonly assumed that this

excess carbon is exuded in the form of dissolved organic carbon (DOC; Kähler & Koeve 2001), however, an increasing number of studies have reported decoupling of carbon and nitrogen dynamics in phytoplankton blooms, with an associated increase of particulate organic carbon to particulate organic nitrogen (POC:PON) ratio (Wetz and Wheeler 2003; Biddanda and Benner 1997; Engel et al. 2002; Taucher et al. 2012). Engel et al (2002) found a significant portion of excess carbon fixation to be channeled into the pool of transparent exopolymer particles (TEP; Alldredge et al. 1993), a vague category of colloidal POM with elevated C:N ratios (i.e. mean value > 20; Engel & Passow 2001; Mari et al. 2001). The partitioning of organic carbon between dissolved and particulate forms, and their differing elemental compositions, likely influences the balance between autotrophic and heterotrophic processes (i.e. the production and consumption of organic matter) and biological pump efficiency.

Phytoplankton-derived organic carbon in the surface ocean is partitioned into a spectrum of sizes, compositions, and fractal-dimensions (Logan & Wilkenson 1990; Jiang & Logan 1991; Li & Logan 1995; Burd & Jackson 1997; Jackson et al. 1997), from dissolved organic carbon (DOC) to POM hundreds of microns long, which are subject to differing export mechanisms. In oligotrophic environments, mixing-controlled export of DOC is likely a crucial process, contributing up to 20% of global export production (Roshan and DeVries 2017). This leaves the remaining majority of phytoplankton-derived carbon to eventually need to become associated with sinking POM in order to be exported. However, POM-export is often considered to be explicitly particulate in composition accompanied with consistent size-dependent sinking velocities and remineralization rates (Siegel et al. 2014; Stukel et al. 2015). Aggregation aided by transparent exopolymer particles (TEP) is one the most common mechanisms that facilitates POM export and alters sinking velocities (Alldredge et al 1993; Engel et al 2004), outside of

mesozooplankton fecal pellet ‘packaging’ (Stemman & Boss 2012; Stukel et al. 2013). TEP are operationally defined as organic particles ($> 4 \mu\text{m}$) that can be stained with Alcian Blue dye (Alldredge et al. 1993; Passow & Alldredge 1995), a copper-based dye which targets the acidic polysaccharides which contribute to TEP’s ‘sticky’ nature. TEP disappears with addition of glucosidase (Smith et al. 1995), experimentally confirming they predominantly consist of sugars. TEP has been found to consist of highly surface-reactive (e.g. ‘sticky’) materials, enriched in fucose and rhamnose and depleted in glucose and galactose (Mopper et al. 1995; Zhou et al. 1998). The acidic molecular characteristic predominantly results from the presence of half-ester (R-OSO_3^-) groups (Zhou et al. 1998). The chemical composition of TEP heavily suggests that they are formed from excreted materials from phytoplankton which are released into the surrounding water (as DOC) to maintain internal stoichiometry balance.

TEP are formed both abiotically and biotically in separate steps. Primarily, TEP are formed spontaneously by coagulation of precursor polysaccharides which creates a linkage between dissolved organic carbon (DOC) and particulate organic carbon (POC) pools (Passow 2000). These DOC precursors can be produced (e.g. extracellular release) in copious amounts relative to POC (e.g. biomass) by phytoplankton especially under nutrient limiting conditions (Obernosterer & Herndl 1995). However, as these precursors are created and released by aquatic organisms, their abundance and chemical composition directly are related not only to the composition of the biotic community but also the physiological status of individual organisms (Passow 2002). Often, exopolymers exuded by phytoplankton consist of high C:N molar ratio (Biddanda & Benner 1997; Kepkay et al. 1997) carbon-rich polysaccharides. Under certain situations, this may result in a buildup of DOM which is not locked to typical Redfield C:N

ratios with the potential to drastically alter ecosystem export efficiency both positively and negatively as a function of aggregate retention time (Mari et al. 2017).

Carbon Export Modelling – Combining Physical and Biological Data

While it is broadly understood that the export of organic carbon to depth is mediated by microbial diversity and food web structure (Turner 2015), the finer mechanistic links between microbial community interactions and carbon export efficiency remain uncertain. The biological pump is generally relatively inefficient. Generally, only about 5-25% of net primary production is exported from the euphotic zone (De La Rocha & Passow 2007); the majority of this is remineralized in the epipelagic zone (100-200 m). Usually only <3% of net primary production reaches the bathypelagic zones (>1000 m) considered as deep sea (De La Rocha & Passow 2007, and references therein). Of the remaining >97% of net primary production which does not reach the deep sea, typically the majority (30-70%) is ‘grazed’ by microzooplankton 20-200 μm in size (Calbet and Landry 2004), 20-35% is consumed by mesozooplankton >200 μm (Hernández-León & Ikeda 2005), and the remaining ~15% subject to bacterial consumption (Ducklow 2000). However, exceptions to these patterns may occur during episodic pulses of carbon export (30-100% export efficiency) which are found during blooms at high latitudes and accompanying spring blooms at midlatitudes (Buesseler 1998). At present, these high-export events are not well understood and are poorly represented in current biogeochemical models which tend to favor strict stoichiometry correlations and steady-state export efficiency for their extrapolation to global carbon cycles.

The carbon export model by Siegel et al. (2014) serves as an example of methods to synthesize food-web dynamics and satellite data. The authors utilized satellite observations of

net primary production, particle sizes, and phytoplankton carbon (using particulate backscattering) to estimate size-fractionated phytoplankton carbon budgets and to model export in a simplified food web (i.e. sinking is either large phytoplankton-POC or fecal materials). The modelled results correlated well with available particle export measurements over a range of environmental export efficiencies ($r^2 = 0.75$ vs. available, regional-scale ^{234}Th determination of export) and predicted global carbon export to be $\sim 6 \text{ Pg C yr}^{-1}$ with $\sim 20\%$ uncertainty. However, there remain significant processes missing from the Siegel et al. (2014) data synthesis. Burd et al. (2000) recognized that the lack of an explicit biological component in particle modelling limits their applicability to steady-state calculations. First, the Siegel et al. (2014) model focuses on sinking particle export solely it does not address pathways of carbon export due to physical mixing or the impacts of zooplankton beyond fecal material production. Further, the analysis does not account for food-web model response to changes in environmental conditions or the plankton community structure (as in Michaels & Silver 1988; Boyd & Stevens 2002). Finally, they made the implicit assumption that all fecal pellets produced by mesozooplankton were exported out of the euphotic zone. While fecal pellets typically have the high sinking velocities (Turner 2015, and references therein), the model by Siegel et al. (2014) does not account for remineralization within the euphotic zone. Protists and bacteria can colonize fecal pellets and POC aggregates (Paulsen & Iversen 2008) which can then be further consumed again or broken apart by mesozooplankton (Paffenhöfer & Strickland 1970; Lampitt et al. 1990). Therefore, it's likely Siegel et al. (2014) overestimates the flux of fecal material and thus total carbon export.

By modifying the Siegel et al. (2014) algorithms to better match regional *in situ* grazing rates, Stukel et al (2015) achieved better carbon export measurements. The authors of Stukel et al. (2015) measured protozoan grazing rates daily at 8 depths using a modified two-point dilution

method (Landry & Hassett 1984; Selph et al. 2015) as well as mesozooplankton grazing rates from gut-pigment contents (Landry et al. 2009; Décima et al. 2015). Synthesizing these measurements, Stukel et al. (2015) created a POC-remineralization term to account for remineralization which occurred between depth of particle generation and the base of the euphotic zone which likely responds to regional and temporal variability in the structure and dynamics of planktonic communities. Incorporation of this remineralization term into the Siegel et al. (2014) algorithm provides improved agreement between model and measured export values with a model-data mismatch within one standard error of zero for 41% of the measurements and within two standard errors for 62% of the measurements (Stukel et al. 2015). This then implies grazers may account for greater phytoplankton mortality than is generally assumed in previous model simulations and that further parameterization of protist-scale processes into carbon export algorithms will increase model accuracy.

The Role(s) of TEP in Carbon Export

Net community productivity and carbon export should, by definition, be equal when integrated over sufficiently large spatiotemporal scales (Brix et al. 2006). Surface-water accumulation of organic matter could be a consequence of high uptake rates of inorganic carbon relative to inorganic nitrogen, i.e. ‘carbon overconsumption’ (Toggweiler 1993) which is explained previously. Accumulation of organic matter has been observed in nutrient limiting conditions from spring to late summer both in dissolved phase as carbohydrate-rich DOM (Copin-Montégut & Avril 1993; Carlson et al. 1995; Williams 1995; Jones et al. 2013) as well as particulate phase as extracellular particulate carbohydrates such as TEP (Mari & Burd 1998; Mari et al. 2001). It has been theorized that TEP are required in surface waters to cause

phytoplankton blooms to sink (Logan et al. 1995; Passow et al. 2001), potentially creating massive-flux events with extremely high ecosystem export efficiency (e.g. > 50%) during blooms at mid to high latitudes (e.g. Buessler 1998). It is generally assumed that the principle fate of TEP is to aggregate with other suspended particles, forming marine snow which then sinks from the euphotic zone (Engel 2004). However, TEP vertical flux may be more complex than downward export alone. Due to TEP's uniqueness in nonconformity to strict C:N stoichiometry it exhibits lesser recognized properties. Asetszu-Scott and Passow (2004) demonstrated TEP have densities much lower than that of seawater (i.e. 700-860 vs. 1020-1030 kg m⁻³) and as a consequence may rise when not ballasted with 'heavy' POC. Mari et al. (2017) hypothesized that *in situ* TEP-rich organic aggregates which linger in surface waters may form frequently and that sinking occurs only when the ratio of TEP to solid particles changes. Such a change would likely be driven by either increased production of particles denser than seawater or preferential degradation of TEP (e.g. when the ratio of TEP production to TEP heterotrophic consumption changes). Reports of non-sinking or ascending pools of particulate organic matter in surface water exist in literature dating back more than two decades and it is now increasingly accepted TEP make up a C-rich 'POC' (e.g. colloidal) pool in surface waters that does not readily sink on its own. From a holistic context, primary production thus encompasses both production of phytoplankton biomass (e.g. POC) and the production of TEP (e.g. DOM extracellular release).

Given the unique characteristics of TEP and its influence on carbon export, a great deal of previous work has focused on how various environmental parameters mediate TEP production and consumption. It has been reported that cell physiological status mediates TEP-precursor release (Passow 2002a) and that nutrient limitation increases phytoplankton TEP production

(Corzo et al. 2000; Radic et al 2006; Berman-Frank et al. 2016; Deng et al. 2016) although the extent of this influence is variable across phytoplankton groups. Substantial extracellular release of TEP precursors and elevated TEP concentrations (2-5x higher concentrations than base environmental levels) have been observed during phytoplankton bloom senescence and associated viral lysis of cells (Van Boeckel et al. 1992; Passow 2002, and references therein; Vardi et al. 2012; Laber et al. 2018). Further studies have focused on the abiotic influences on TEP aggregation dynamics and have observed significant changes in TEP production as a function of environmental parameters such as temperature (Engel et al. 2010), light (Claquin et al. 2008), pH (Bourdin et al. 2017), and gas-exchange at the sea-surface microlayer (Cunliffe et al. 2009). TEP can also be consumed from marine systems just as readily as it can be produced. As a form of POC, TEP profiles have been observed to decrease with increasing depth (Engel 2004), akin to a traditional Martin Curve, presumably due to consumption and degradation by heterotrophic processes.

In aquatic systems, bacterial uptake of organic carbon is assumed to be a major pathway where DOM is converted into POM. Since TEP is predominantly comprised of labile, readily accessible carbon, bacteria are closely associated with TEP aggregates (Alldredge et al. 1993; Passow & Alldredge 1994; Mari & Kiørboe 1996). The influence of bacteria on TEP and their relevance in TEP aggregation and accumulation remains a topic of debate. Colonization can decrease aggregation through remineralization of the labile organic carbon contained in TEP molecules (Rochelle-Newall and Fisher 2002; Grossart et al. 2006; Gärdes et al. 2012). They may also promote TEP aggregation by increasing ‘stickiness’ through hydrolysis of mucus from the cell-surface of phytoplankton (Smith et al. 1995; Rochelle-Newall et al. 2010) or directly releasing precursors themselves as capsular materials (Sugimoto et al. 2007; Koch et al. 2014).

Mesozooplankton such as calanoid copepods (Ling & Alldredge 2003) and euphausiids (Passow & Alldredge 1999) have been observed to consume TEP particles as well as the particles entrapped and adhering to the surface of aggregates. Through aggregation, TEP can combine individual small particles (e.g. bacteria, small cells, detrital material), potentially ‘packaging’ previously inefficiently-acquired (i.e. energetic cost to consumer is greater than energy gain) carbon sources into a larger, more accessible form. As a result, energy from remineralization in the microbial loop would instead be shunted directly to higher trophic levels creating a ‘trophic elevator’ (Mari et al. 2004) which circumvents the relatively low efficiency of a shallow microbial food web. Protist microzooplankton may also directly consume TEP. Tranvik et al. (1993) demonstrated that heterotrophic flagellates were able to ingest colloidal DOM as small as 8 kDa. Furthermore, when provided with colloidal DOM >2,000 kDa they observed increased flagellate biomass unexplainable by consumption of bacteria alone. This would then imply that not only can microzooplankton consume colloidal DOM directly but also that they may actively utilize it for biomass growth. In summary, uptake of DOM by zooplankton may also be a pathway by which DOM is converted to POM that is lesser explored experimentally in literature.

It is evident that TEP aggregation dynamics and their influence on both nutrient-uptake, remineralization, and carbon export is a complex phenomenon that are closely linked to ecosystem-level variations in biological pump efficiency and total ecosystem export efficiency. Due to the complexity of TEP, as outlined previously, and its interactions with other forms of organic carbon there is debate on its specific influences in carbon export including how to parameterize TEP coagulation and TEP aggregate accumulation (i.e. gel-formation versus particle aggregation). In theory, TEP-precursory DOM first form gels (e.g. their volume to mass

ratio is inconsistent) according to coagulation theory (Passow 2000); it is known that particle aggregation is a second-order process (Pruppacher & Klett 1980). However, the flow from suspended to settling particles by aggregation has long been considered a first-order process (Burd et al. 2000). In reality, TEP aggregation likely most closely manifests as a pseudo-first order process in most field-samples because of vastly different magnitudes of reactant concentrations. The size distributions of TEP follow a power law distribution (Passow & Alldredge 1994; Mari & Kiørboe 1996; Kiørboe et al. 1998; Mari & Burd 1998; Worm & Søndergaard 1998) therefore estimates of numerical abundance depend on the smallest size classes. However, with respect to total volume estimates, TEP are dominated by contributions of few, large particles the contributions of small particles are virtually negligible (Passow 2002). Therefore, since the majority of TEP exists in precursory forms ($< 2 \mu\text{m}$) and we are measuring rates of change in the largest size-categories of TEP ($> 4 \mu\text{m}$), we assert that for practical measurements of field TEP aggregation the second-order rate equation is reduced to a pseudo-first order rate which makes treatment to obtain TEP production rates much easier.

Singular *in situ* TEP concentration measurements provide only limited information about the underlying processes occurring in the euphotic zone. Mari et al. (2017) highlights that TEP accumulation is the end result of concurrent production and degradation processes. A novel method to parameterize biologically mediated gross TEP production and consumption rates *in situ* would result in an enhanced mechanistic understanding of TEP aggregation dynamics in the ocean for incorporation into carbon export models. TEP is particularly crucial to better understand in this context for parameterization of non-fixed C:N organic material. By treating TEP production as a pseudo-first order process, utilization of similar research techniques which are already used to parameterize the biological influence on phytoplankton standing stocks is

possible. This method would produce biologically-mediated TEP production and consumption parameters which can be pieced into the carbon export models reviewed previously via similar remineralization terms as that of POC.

The dilution method (Landry & Hassett 1982) is commonly used to measure instantaneous rates of phytoplankton growth, mortality by microzooplankton grazing, has been modified to estimate rates of viral lysis (Evans et al. 2012), and it has been utilized by Stukel et al. (2015) to incorporate protozoan trophic-interactions into carbon export models. Traditionally, the dilution method involves setting up a series of dilution treatments to create a gradient in encounter rates between grazers and prey based on first-order processes of phytoplankton growth. As TEP formation has been extensively linked to phytoplankton and bacterial production, the biotic influences on net TEP production likely can be estimated using similar dilutions. In this study, large volume mesocosms were used to induce phytoplankton blooms under Redfield ratio and phosphorous-replete nutrient regimes. Over the course of the observed blooms, a series of dilution experiments were performed to measure instantaneous net TEP production rates. The dilution method was then utilized to estimate net biotic TEP production and loss rates, which are then compared to differences in nutrient status and bloom state.

CHAPTER 2

METHODS

Study site, mesocosm description, and sampling strategy

Six large-volume (20,000 L) mesocosms were filled with unfiltered fjord water and deployed at the University of Bergen Espeland Marine Biological Station near Bergen, Norway (60°22.1'N, 5°28.1'E) from 14 May 2017 to 31 May 2017, numbered hereafter as Days 1 through 18. Two environmentally-relevant experimental conditions were created in triplicate: nutrient-replete conditions (goal N:P = 16:1) and phosphate-limited conditions (hereafter referred to as P-limited; goal N:P = 60:1). Additions of nitrate (4 μ M) and phosphate (0.25 μ M Replete; 0.06 μ M P-limited) in their respected desired ratios were added to each nutrient condition on the first two days, followed by the mesocosms being gently mixed by bubbling with ambient air for two days. For all dilution experiments described here, numbered as Exp-1 (beginning 14 May) through Exp-9 (beginning 30 May), equal volumes of water were collected from each mesocosm and were pooled by treatment into a single dilution experiment which took 2 total days to complete. On Day-9, one of the P-limited mesocosms became compromised and thereafter water was only sampled from the remaining duplicate P-limited mesocosms. TEP dynamics (net TEP production, gross TEP production, TEP consumption/degradation) were measured by dilution experiments (N = 9) conducted every other day over the course of the mesocosm deployment following the general procedure outlined below.

Theoretical Considerations

The dilution-method (Landry & Hassett 1982) has been key in establishing the role of protist-grazing in marine trophic webs. Landry and Hassett (1982) developed the dilution method because a “true” control treatment devoid of predation pressure by protist predators is not achievable when using nature field samples since predators and prey occupy similar size spectrums. Conceptually, the dilution method is simple in that diluting samples reduces encounter rate between predator and prey. Mathematically, this is calculated based off the first-order process of changes in phytoplankton density based off changes in chl a:

$$P_t = P_0 e^{(k-g)t},$$

where t is incubation duration (units of days), P_0 is chl a concentration at the beginning of the experiment, P_t is chl a concentration at the end after time t , and k and g instantaneous coefficients of phytoplankton population growth and grazing mortality, respectively. The coefficients k and g may vary with time of day without affecting the comparison of growth rates of natural phytoplankton stocks in dilutions over a fixed period of incubation (Landry & Hassett 1982), providing a ‘snapshot in time’ of the rate processes of the standing stock at the time of initial sampling. Using this first-order equation, observed rate of change of phytoplankton density at different dilutions is linearly related to the dilution-fraction (i.e. decimal fraction of unfiltered seawater), creating a linear relationship between dilutions where the negative slope of the relationship is the grazing coefficient g and the Y-axis intercept is the inherent phytoplankton growth rate k (i.e. growth without loss; Landry & Hassett 1982). Although TEP does not ‘grow’ using this equation, DOC release by phytoplankton is undoubtedly related to their growth rates; therefore, in the following sections we base our TEP-rate calculations off this concept.

To balance sampling frequency with accuracy, we followed the two-point dilution method approach using one low dilution level [20% unfiltered] and an undiluted treatment [100% unfiltered] (Landry et al. 2008; Morison and Menden-Deuer 2015, 2017; Anderson et al. 2018). For growth and grazing rate estimates, the two-point approach has been found to be simultaneously conservative (Worden and Binder 2003; Lawrence and Menden-Deuer 2012), accurate (Morison and Menden-Deuer 2017), and reliably estimates situations of nonlinearity in grazing responses (Chen et al. 2015; Morison and Menden-Deuer 2017). Therefore, we assert that a two-point dilution experiment has the potential to be applied to biotically-influenced TEP production dynamics even if TEP-aggregation response is a non-first order process (e.g. gel-coagulation is a second-order process) at extreme dilution fractions (e.g. abiotic precursor coagulation only with no biological influence). Our reasoning is that, broadly, biotic influence can become the rate-determining processes over a suitable temporal scale (i.e. 24 hours) which the two-point dilution method uses or in non-steady-state situations such a phytoplankton bloom. Furthermore, as we assume that TEP aggregation in the scale of dilution-incubations manifests as a pseudo-first order process, we are implicitly assuming that the concentration of existing precursory materials is several orders of magnitude greater than the concentration of TEP-aggregates. The result of diluting the biological community would then fractionate the biotic-community influence on large ($> 0.4 \mu\text{m}$) TEP production creating a negative linear regression term, g , akin to the grazing-mortality coefficient. However, it is questionable what exactly the Y-axis intercept value would be estimating as at the lowest dilution fractions the pseudo-first order process begins to manifest more as a “true” second-order process.

In accordance with the assumptions of the traditional dilution method (Landry & Hassett 1982; Fig. 1), similar assumptions regarding the interactions between TEP and the planktonic

community (bacteria, phytoplankton, and micro-zooplankton) were made. **(1)** First, it was assumed that a reduction in the density of TEP particles (via dilution) will not directly cause a change in the rate of abiotic TEP aggregation (i.e. aggregation in the diluted fraction is independent of undiluted TEP concentration). **(2)** Second, it was assumed that the probability of individual TEP-particle consumption is a direct function of rate of encounter with potential consumers (bacteria and micro-zooplankton).

Verification of methods

To experimentally test our assumptions of TEP's behavior compared to the traditional dilution method (Landry & Hassett 1982) an experiment was designed to test the first assumption listed previously, that changing TEP density (by filtration) does not alter TEP formation rates. It has been previously reported that large amounts of freshly formed TEP-precursors are fibrillar or colloidal (< 8 kDa) and reform TEP with rates of hours to days (Passow 2000) when the largest size-fraction of TEP are removed. This would mean that achieving 'production in the absence of biotic loss' may be experimentally possible through diluting the planktonic community by filtration to compare with predicted rates from dilutions. Furthermore, testing the theoretical Y-axis (e.g. gross TEP production) value will allow us to experimentally test the extent to which TEP production adheres to a pseudo-first order process. If TEP, in a natural seawater sample, behaves as second-order instead, an incubation consisting solely of TEP-precursors would have a net production rate much higher than that of an incubation influenced by a biotic community. In order to examine this possibility in our experiments, a dilution experiment was set up as detailed above using Skidaway River Estuary water, a coastal ecosystem rich in TEP ($>750 \mu\text{g XG eq. L}^{-1}$). In addition, a triplicate set of

incubation bottles were prepared targeting a 0% WSW dilution level by filtering natural seawater samples using tangential flow filtration with an effective nominal pore size of 30 kDa (Millipore).

TEP rate calculations

For each dilution level in each experiment the daily net change in TEP (TEP_k ; day^{-1}) was calculated by the equation using bulk changes in TEP concentration normalized to initial concentration (Egge et al. 2009; MacGilchrist et al. 2014; Passow 2012; Iuculano et al. 2017):

$$(1) TEP_k = \frac{(TEP_{24} - TEP_0)}{TEP_0}$$

where TEP_{24} and TEP_0 are the TEP concentrations measured at each time point. The TEP consumption rate (TEP_g ; day^{-1}) was then calculated using the equation:

$$(2) TEP_g = \frac{(TEP_{kd} - TEP_{k1})}{(1-x)}$$

where TEP_{kd} and TEP_{k1} are the net change in TEP concentrations over the 24 h incubation in the diluted (TEP_{kd}) or undiluted 100% WSW (TEP_{k1}) treatments, and x is the corresponding fraction of WSW dilution. Similar to the calculations for the dilution experiments (Strom and Fredrickson 2008), when TEP_g was calculated to not be significantly different from zero ($p < 0.05$), TEP_g (day^{-1}) was set to 0. Gross production rates were then determined by the relationship:

$$(3) TEP_{\mu} = TEP_g + TEP_{k1}$$

Dilution Experiment Procedure

Due to time constraints, the diluent was always prepared on the day prior to the experiment day. Surface seawater (1 m) was collected via a 5 L Niskin bottle and screened through 200 μm mesh to remove mesozooplankton. The whole-seawater (WSW) was then immediately transferred to the lab for diluent preparation and gravity filtered through a 0.45 μm

filter (PALL AcropakTM Supor® membrane capsule) into a clean carboy. The water was then filtered through a 30 kDa filter via a tangential flow filtration system (TFF; Millipore), creating the TFF-diluent. The diluent was kept overnight at 18°C in the dark and was acclimated to room temperature prior to dilution experiments.

The next day ($T=0$), fresh WSW was collected from the mesocosms and added to TFF-diluent to a proportion of 20% WSW. The 20% dilution and 100% WSW were siphoned into triplicate, 1.2L bottles and closed without air bubbles. Bottles (six per experimental treatment) were incubated for 24 h in a flow-through incubation tank covered with mesh to simulate ambient depth-adjusted irradiance. Aliquots were taken for measurement of TEP and chlorophyll *a* at the start (T_0) and end (T_{24}) of the incubation period. TEP rate dynamics were then calculated following the equations outlined below.

TEP quantification

Water samples (30 to 150 mL) were gently (< 150 mbar) filtered through 25 mm, 0.40µm polycarbonate filters (Millipore, Isopore, HTTP025000). Post-filtration, filters were stained with 500 µm of a 0.02% Alcian Blue (AB) solution buffered with 0.06% acetic acid (pH 2.5) and frozen at -20°C for later processing. For extraction, filters were immersed in sulfuric acid (80%) for at least 2 h and then measured spectrophotometrically for absorbance (Agilent 8453 UV-visible spectrophotometer) at a fixed wavelength of 787 nm. The recently updated method for calibration of TEP-measurements described in Bittar et al. (2018) was used to generate xanthan gum (XG) calibration curves. The updated method is used since the chemical composition of commercially available XG powder today has higher solubility and forms negligible amounts of gel particles compared to that of the original calibration method (Passow & Alldredge 1995;

Bittar et al. 2018). Briefly, AB-dye was calibrated to XG equivalence by staining a dilution-series of known concentrations of XG. Two separate AB solutions (f factors of 169 and 129) were used during the mesocosm experiments, the first from Day-2 to Day-13 and the second from Day-14 to the end. TEP concentrations ($\mu\text{g XG eq. L}^{-1}$) were calculated according to Passow and Alldredge (1995).

Chlorophyll and nutrient analysis

Chlorophyll *a* (Chl *a*) was determined by filtering water samples (100-150 mL under low vacuum onto 25 mm GF/F filters (nominal pore size $0.7\mu\text{m}$). Chl *a* was extracted in the dark for 12 h in 95% ethanol, and then measured on a Turner AU10 fluorometer. Daily phytoplankton growth and mortality rates were determined from changes in total extracted chlorophyll in incubation bottles over 24 h (see Anderson et al. 2018 for details). Ethanol blanks were included, and all samples were corrected for phaeophytin (Jespersen and Christoffersen 1987; Graff and Rynearson 2011).

Samples for nutrient analysis (N and P) were filtered through a pre-combusted GF/F filter and stored at -20°C for future analysis. Nutrients were quantified using a Lachat QuickChem8500 Nutrient Analyzer Flow Injection Analysis System (Rutgers Nutrient Analysis Facility).

Statistical analysis

Statistical analysis was performed using GraphPad Prism V7.0 (GraphPad Software). Initially, paired t -tests were used to test if instantaneous net TEP production rates in the diluted (k_d) and non-diluted (k_l) treatments were significantly different ($p < 0.05$). When TEP_k rates

between the two dilution levels were not significantly different, consumption rate was set to zero for future analysis as it is implied there was no discernable negative influence from the biotic community present. Two-way ANOVA with Sidak's multiple comparisons test were used to test for significant difference ($p < 0.05$) in same-day rates of grazing, growth, and accumulation between nutrient treatments. Changes in rates over time were assessed using two-way ANOVA with repeated measures and Sidak's multiple comparisons test. Model-II linear regressions were used to estimate direct relationships between environmental variables and TEP concentrations to determine potential covariance over time.

CHAPTER 3

RESULTS

Methods examination

Assessing the impact of the filtration methods on TEP production rates was accomplished in two ways (Figure 2). First, from the TEP-dilution experiment performed using Skidaway estuary water we observed a significant difference in measured TEP production rates between 20% and 100% WSW-fraction dilution levels ($p < 0.01$; Figure 2a) compared using linear regression. Using this data, a negative slope of $-0.576 \pm 0.12 \text{ d}^{-1}$ was calculated, indicating that TEP consumption occurred. The theoretical gross TEP production (i.e. production without biological losses) was calculated as a Y-intercept value of $0.455 \pm 0.09 \text{ d}^{-1}$. In the experimental 0% WSW-fraction incubations consisting solely of TFF-treated seawater we measured a TEP production rate of $0.503 \pm 0.30 \text{ d}^{-1}$. There was no significant difference (unpaired t-test; $p = 0.886$) between measured TEP production in the 0% incubations and that predicted by TEP-dilution experiment rates. Therefore, at the scales of our incubations (1.2L bottles, 24-h periods) TEP behaved similar to a pseudo-first order process, with the process of TFF-filtration not stimulating artificially high TEP concentrations.

Second, from the field experiments conducted in Bergen, Norway the initial TEP concentration prior to TFF filtration was compared to the change in TEP in diluted-fraction incubations. This tested the idea that having ‘more TEP’ initially implies greater concentrations of precursors, which would then result in artificially enhanced TEP production rates in diluted incubation treatments. No such trend was observed across the mesocosm dilution experiments (N

= 9) in either replete ($R^2 = 0.098$) or P-limited ($R^2 = 0.124$) nutrient treatments (Figure 2b.).

Further, as we measured varied rates from dilution-experiments the experiments didn't always 'work'; in 11 of 18 total dilution experiments there was no significant difference between normalized TEP production in undiluted vs. diluted triplicates therefore the slope of the regression was not significantly different from zero.

Given the results of these two tests the assumption that abiotic forces are similar regardless of dilution level or initial concentration of TEP is validated, that our TEP-dilutions measured changes in biological-community based influences, and that TEP rate results presented here are not artifacts of experimental procedure.

General mesocosm observations

A dense and diverse bloom of phytoplankton, dominated by pico- and nano- eukaryotes, was generated in all mesocosms of both treatments. Nitrate+nitrite (N+N) concentrations reached peak concentrations of around $\sim 15 \mu\text{M L}^{-1}$ and gradually decreased to concentrations below detection [$< \mu\text{M L}^{-1}$] post-bloom on Day-15 (Figure 3a). In LOP treatments, orthophosphate concentrations peaked at $0.49 \pm 0.10 \mu\text{M L}^{-1}$ and decreased below detection [$< 0.01 \mu\text{M L}^{-1}$] on Day-14 (Figure 3b). Orthophosphate concentrations were roughly 3.5x higher in replete treatments, peaking at $1.69 \pm 0.36 \mu\text{M L}^{-1}$ and decreasing to $< 0.5 \mu\text{M L}^{-1}$ during the post-bloom period. Chlorophyll *a* concentrations ranged from 0.73 ± 0.15 to $4.2 \pm 0.34 \mu\text{g L}^{-1}$ under replete conditions and 0.82 ± 0.07 to $2.7 \pm 0.06 \mu\text{g L}^{-1}$ under LOP conditions. Maximum chlorophyll concentrations occurred on Day-13 for replete and Day-14 for LOP (Fig. 4). Observational periods are designated by pre-bloom, bloom, and post-bloom groupings of 6 days.

In general, chlorophyll in the replete treatment was observed to have a sudden and pronounced increase and crash (Fig 4a), while chlorophyll in the LOP treatment increased to a lower maximum and crashed more gradually (Fig 4b). Throughout the mesocosm experiment, TEP concentrations ranged from $37.7 \pm 6.6 - 247 \pm 22 \mu\text{g XG eq. L}^{-1}$ under replete conditions and $37.8 \pm 12 - 195 \pm 27 \mu\text{g XG eq. L}^{-1}$ under LOP conditions. Maximum TEP concentrations occurred on May 28th under replete conditions and on Day-12 in LOP mesocosms. Average TEP concentrations between the two treatments were not significantly different from one another until Day-16 when replete mesocosms showed significantly higher TEP concentrations ($p = 0.0023$).

Average *in situ* TEP to chlorophyll *a* ratios (TEP:Chl*a*; $\mu\text{g L}^{-1}$) for each observational period were compared using a Holm Sidak's multiple comparisons test and were not different between treatments during pre-bloom or bloom. However, post-bloom, the TEP:Chl*a* ratio significantly differed between nutrient regimes (Fig 5a, $p < 0.001$) with $143 \pm 26\%$ higher TEP:Chl*a* ratio observed in the replete mesocosm relative to the ratio observed in the LOP treatment. Log-transformed TEP and chlorophyll concentrations were positively linearly (Model II) correlated in both nutrient treatments replete and LOP ($p < 0.007$) during the growth phases of the bloom. However, in the post-bloom period, the relationship between log-transformed TEP and chlorophyll became decoupled from the prior correlation (Fig. 5b) in both treatments.

TEP consumption and gross production estimates

Variations in TEP consumption rate (TEP_g) and production rate (TEP_μ) were observed over the sampling period under both replete (TEP_g = 0.0 to 1.77 d^{-1} ; TEP_μ = 0.0 to 0.99 d^{-1}) and LOP nutrient conditions (TEP_g = 0.0 to 1.13 d^{-1} ; TEP_μ = 0.0 to 1.83 d^{-1} ; Figure 5). Gross production rates differed and were significantly higher under replete conditions at three

timepoints ($p < 0.03$; Fig. 6a). TEP consumption rates were significantly higher under replete conditions at four of nine timepoints ($p < 0.005$; Table S1); no TEP consumption was detected at all at three timepoints (Fig 6b). Measurable TEP consumption occurred simultaneously in both nutrient treatments during only one timepoint during Exp-7, coinciding with early observed bloom senescence. Net TEP production rates were similar between nutrient conditions during the pre-bloom period, were significantly greater in replete conditions during bloom growth ($p < 0.009$) and were significantly greater two of three post-bloom experiments under LOP conditions ($p < 0.0001$; Fig 6c). Measured TEP_k rates were similar to observed variation in environmental concentrations of TEP during early phases of the bloom (Table 1).

Variation in TEP gross production rates over the observational period were not explained by nutrient condition ($p = 0.944$; Table S1), nor were they related to initial chlorophyll-normalized TEP concentrations (replete: $R^2 = 0.437$, $p = 0.053$; P-limited: $R^2 = 0.065$, $p = 0.507$). Similarly, TEP consumption rates were not significantly correlated to concentrations of chlorophyll-normalized TEP in all treatments (replete: $R^2 = 0.011$, $p = 0.788$; P-limited: $R^2 = 0.061$, $p = 0.522$), nor to total POC concentrations ($R^2 < 0.0001$; $p > 0.95$).

CHAPTER 4

DISCUSSION

Assessing the Usage of the Dilution Method on TEP rate dynamics

Understanding and accurately parameterizing TEP aggregation dynamics is critical for improving predictions of oceanic carbon production and particle export. Particle aggregation has often been examined from a physical-perspective using gel-coagulation theory, which predicts the changes in particle concentration and size as a result of particles colliding and sticking via second-order rate kinetics (Jackson & Lochman 1992; Ruiz 1997; Burd et al. 2000; Jackson 2001; Burd & Jackson 2009), yet the specific role of TEP's influence remains largely a mystery. A great deal of previous research has attempted to understand the biological mechanisms that mediate TEP-precursor release (Passow 2002b, and references therein), often by using bottle incubations to estimate net TEP production rates and relating these rates to key environmental factors and POC export (Egge et al. 2007; MacGilchrist et al. 2014; Passow 2012; Iuculano et al. 2017). These experiments have helped show that changes in TEP are closely, but not always, related to biological community structure. However, due to the semi-quantitative method of measuring TEP, these studies were only able to examine changes in TEP as bulk concentrations.

Mari et al (2017) identified that two main parameters likely affect the contribution of TEP to POC cycling (e.g. carbon export): TEP stickiness, and the balance between TEP production and degradation rates. Work by Mari et al. (2007) found freshly produced organic material to be more sticky than older organic matter, however, Rochelle-Newall et al. (2010) observed stickiness to increase with age of organic material in incubations of seawater from the

same system. These contradictory observations of TEP stickiness imply the biotic community (e.g. bacteria) potentially impacts the physical properties of TEP through heterotrophic activities. Rochelle-Newall et al. (2010) proposed the contradictory TEP-stickiness observations were due to the existence of two types of 'sticky-TEP'. The primary type formed as a by-product of primary production (Engel et al. 2000; Mari et al. 2007) which is highly bioavailable with a relatively short half-life (Engel 2000; Grossart & Ploug 2000; Wild et al. 2004). This autotrophically produced TEP is then heterotrophically processed through bacterial transformation of the existing DOM, similar to accumulations of bacterially produced CDOM in batch cultures (Rochelle-Newall & Fisher 2002). As active bacteria produce mucopolysaccharidic capsular material (Luft 1971; Stoderegger & Herndl 1998) which can represent up to 25% of bacterial respiration in terms of carbon (Stoderegger & Herndl 1998), this material potentially contributes to aggregate formation and due to its acidic polysaccharide content is stained by Alcian Blue and therefore included in the bulk TEP pool (Rochelle-Newall et al. 2010). Furthermore, even in the absence of 'fresh' autotrophic byproducts, bacteria continue to produce capsular materials which can accumulate in the water column due to its relatively recalcitrant nature (Stoderegger & Herndl 1998). Together, these studies emphasize the lack of information on the underlying processes of TEP accumulation provided by ordinary incubation techniques due to likely existence of multifaceted influences on TEP production and degradation. TEP production may not only be positively correlated to autotrophic activity but also to heterotrophic activity as well through rapid (<24 hour) bacterial DOM production. In summary, the influence of the biotic community alters TEP production in ways which make teasing apart abiotic and biotic influences difficult. It is our hope that by using the dilution experiment setup, we can juxtapose these influences against one another by removing the biotic community in

large-fractions and thus compare TEP-production as a function of the seawater's life history and as a function of the complete biological community ($< 200 \mu\text{m}$).

To our knowledge, no studies exist which attempt to experimentally parameterize *in situ* rates of TEP production and biotic degradation concurrently. Here, we used modified dilution experiments (Landry & Hassett 1982; Evans et al. 2012) to estimate TEP production dynamics of phytoplankton blooms stimulated under field-relevant nutrient conditions within large volume mesocosms. This technique offers a novel way to holistically parameterize (e.g. rates of net production, gross production, and consumption) TEP dynamics *in situ* and to provide rate data critical for improved estimates of carbon cycling in the ocean. The results from our comparison of TEP-dynamics to a traditional dilution experiment through linear regression imply that the production rates of this largest-size fraction likely is the rate determining step in TEP particle ($> 0.4 \mu\text{m}$) formation. Although the coagulation of dissolved precursors into gels and then into colloidal TEP is a second-order process (Pruppacher & Klett 1980; Burd et al. 2000; Burd & Jackson 2009), the concentrations of these smallest size-fraction of TEP-related materials is several orders of magnitude greater than the TEP 'particles' ($> 0.4 \mu\text{m}$) following a power-law in the aggregate size-spectrum (Sheldon et al. 1972; Hunt 1980; Jiang & Logan 1991; Burd & Jackson 2009). As the method presented here solely, semi-quantitatively measures the largest size-fraction of TEP 'particles' ($> 0.4 \mu\text{m}$), this culminates in an apparent pseudo-first order process due to TEP-particle production's direct influences from the biotic community via autotrophic, heterotrophic, and physical (e.g. breaking up or 'freeing' TEP materials) processes. From fractionating out the biological community influences by tangential flow filtration and performing a dilution experiment, the apparent rates of TEP production without biotic influence and rates of biotic-community related degradation were calculated.

No correlations between the initial TEP concentration and measured TEP production rates in the diluted-fraction incubations were observed (Figure 2b). This further reinforces the assumption the biological influences manifest as the rate determining steps in production of TEP particles ($> 0.4 \mu\text{m}$) because, following second-order rate kinetics, greater initial TEP concentrations should have caused a noticeable increase in measured TEP production in the highly diluted fraction. In reality, collision occurs between all TEP-related materials (i.e. dissolved, gels, colloids, ‘particles’) and rates of TEP coagulation are heavily related to the available surface area (e.g. fractal dimension; Hunt 1980, Jiang & Logan 1991); these interactions then follow second-order kinetics (Burd & Jackson 2009). However, these processes likely occur very rapidly as TEP has been demonstrated to rapidly reform even after filtration by dialysis ($< 8 \text{ kDa}$; Passow 2000). Therefore, as demonstrated by this methods assessment, over the temporal period of a 24-hour incubation involved in the dilution experiment the overall TEP production rate appeared to be directly related to the biological community influence which can then be calculated from the perspective of a pseudo-first order process.

It is crucial to reiterate that current TEP measurement techniques are semi-quantitative (Passow & Alldredge 1995; Bittar et al. 2018), only providing information on bulk concentrations. Therefore, the implications of our TEP-dilutions in broader environmental context (e.g. carbon-flux) still need to be paired with other flux-related measurements, specifically POC, in order to establish a more complete picture of the vertical transport of carbon in any given system. The sinking velocity of aggregates varies wildly as a function of size, porosity (Iversen & Ploug 2010), and density (Alldredge & Gotschalk 1988; De La Rocha & Passow 2007). TEP’s influence on aggregate sinking velocity is most noticeable in the latter, as density appears to vary as a function of TEP to solid particles (Asper 1987; Azetsu-Scott &

Passow 2004). Owing to the low density of TEP, changes in the TEP to solid particle ratio may reduce sinking velocity (Engel & Schartau 1999; Azetsu-Scott & Passow 2004; Mari 2008), potentially even leading to upward flux (Mari et al. 2017). Changes to this ratio can occur in several ways with varying impacts on aggregate sinking velocity. High levels TEP production may lead to POC aggregation with rapid sinking velocities following the traditional perspective of TEP's influence (Passow et al. 1995; Azetsu-Scott & Passow 2004; Engel 2004; Cunliffe et al. 2013), however, as discussed previously high TEP production may also create upward flux depending on the system's current conditions (Engel & Schartau 1999; Azetsu-Scott & Passow 2004; Mari et al. 2017). In addition, TEP-dilutions offer a method to parameterize rates of TEP remineralization providing another missing piece of the puzzle that is carbon aggregate flux. The preferential remineralization and solubilization of TEP in aggregates (Ploug & Passow 2007) results in a decrease in the TEP:POC ratio and an increase in vertical sinking velocity (Ploug et al. 2008). Furthermore, bacterially modified TEP-materials would remain in the dissolved (or low-buoyancy colloidal and gel pools) in surface waters potentially temporally disconnected from POC produced by primary production, referred to in literature as 'decoupling' between primary production and downward export (Asper & Smith 1999; Nodder & Waite 2001; Buesseler et al. 2003; Plattner et al. 2005; Ortega-Retuerta et al. 2009; Estapa et al. 2015; Mari et al. 2017). Therefore, TEP-dilutions potentially offer a missing piece to the puzzle that is carbon aggregate flux.

Decoupling of TEP and Chlorophyll a

Under both replete and P-limited nutrient treatments, algal blooms were initiated, peaked, and declined all within the experimental time period of 18 days. This provided a platform to test

TEP-dilutions and examine TEP dynamics over a gradient of algal densities and physiologies under differing field-relevant nutrient regimes. High concentrations of TEP ($> 4\times$ typical TEP concentrations for the system; $>1000\ \mu\text{g XG eq. L}^{-1}$) have predominantly been observed during blooms dominated by diatoms (Passow & Alldredge 1994; Passow et al. 1994, 2001; Mari & Kiørboe 1996; Mari & Burd 1998; Mari 1999; Ortega-Retuerta et al. 2018), *Phaeocystis* spp. (Riebesell et al. 1995; Hong et al. 1997), or coccolithophorids (Engel et al. 2004; Van Oostende et al. 2013). In the present study, blooms in both nutrient treatments were dominated by pico- and nano- eukaryotes, and while these groups can still produce TEP (Berman-Frank et al. 2016; Deng et al. 2016; Iuculano et al. 2017), concentrations are often lower relative to diatom production (Bar-Zeev et al. 2009). Consequently, maximum TEP concentrations observed in the present study ($\sim 200\text{-}250\ \mu\text{g XG eq. L}^{-1}$) were lower than those reported for diatom-bloom observational and mesocosm studies in similar systems which are commonly around $1000\ \mu\text{g XG eq. L}^{-1}$ (Passow 2002b, and references therein; Cunliffe et al. 2009), but were within the $100\text{-}300\ \mu\text{g XG eq. L}^{-1}$ range of reported values for sub-surface ($\sim 1\text{ m}$ depth) waters in Norwegian fjords at a similar stage in the annual cycle (spring) (Riebesell et al. 1995; Passow & Alldredge 1995). Studies which reported higher ($750\text{--}1000\ \mu\text{g XG eq. L}^{-1}$) TEP concentrations in the same system typically observed blooms more dominated by larger nanophytoplankton $> 20\ \mu\text{m}$.

Consistent with previous literature, maximum TEP concentrations in each treatment were observed during the decline and post-bloom periods regardless of nutrient regime (Passow 2002b; Engel et al. 2004; Cunliffe et al. 2009; Vardi et al. 2012). DOC-exudation, specifically labile polysaccharides, has been found to increase during the later stages of phytoplankton blooms due to the declining nutritional-state of phytoplankton cells and direct release associated with autocatalytic and viral lysis (Corzo et al. 2000; Engel 2002; Berman-Frank et al. 2007;

Cunliffe et al. 2009; Vardi et al. 2012; Laber et al. 2018; Nissimov et al. 2018). Maximum TEP concentrations thus coincide with stationary phase of the cells (Deng et al. 2016; Iuculano et al. 2017) and can remain high in the following days (Cunliffe et al. 2009) despite low chlorophyll concentrations as we observed in mesocosms under both nutrient regimes. Nutrient imbalance has been demonstrated to stimulate carbohydrate release, specifically when algae are P-limited (Magaletti et al. 2004), so we expected potentially greater net TEP production in P-limited mesocosms compared to replete conditions after normalization to biomass (using chl *a* as proxy).

Both experimental and *in situ* studies investigating TEP production dynamics often use chlorophyll concentrations as a proxy of algal biomass. As TEP-precursors are produced by phytoplankton, a positive linear relationship between TEP and chlorophyll concentrations is often, but not always, observed (Beauvis et al. 2003; Ortega-Retuerta et al. 2009; 2018; Wurl et al. 2011; Ebersbach et al. 2014). When bulk concentrations of TEP and chl *a* were log-transformed, the ratio of *in situ* TEP to chlorophyll *a* concentration was slightly higher under P-limited conditions compared to replete as expected from previous studies (Magaletti et al. 2004). In both nutrient treatments a linear relationship between log-transformed TEP and chl *a* during bloom initiation and growth was observed. This implies that during phytoplankton growth phases the ratio of primary production utilized for cellular-growth and multiplication to that released as extracellular materials is relatively consistent. However, during bloom termination a decoupling of the prior correlations in both nutrient treatments with substantially higher ratios of TEP per unit chlorophyll *a* was observed. Estapa et al. (2015) suggested that even when there is no explicit link between TEP and chl *a* or ^{234}Th , their spatial variability is similar which suggests control by similarly scaled physical and ecosystem processes with differing temporal variability. In context of carbon-modelling, our observations imply that during steady-state conditions

consistent rates of TEP production occur relative to levels of chl *a*, which are observable from satellite data. Only when the system is undergoing regime-shifts (i.e. bloom termination and system ‘resets’) does this ratio become decoupled and TEP may behave as its own, separate carbon pool.

This decoupling and occurrences of high TEP formation in relatively low-productivity environments have been described previously (Passow 2002a; Ortega-Retuerta et al. 2009 and references therein; Mari et al. 2017), and likely results from bottom-up influences in the form of increased extracellular material release relative to new biomass production (e.g. growth). The percentage of extracellular release from phytoplankton varies from <5% to >50% of primary production (Thomas 1971; Fogg 1983; Baines & Pace 1991; Fernandez et al. 1994; Biddanda & Benner 1997; Karl et al. 1998; Teira et al. 2001; Marañón et al. 2004; Pugnetti et al. 2005; Alonso-Saez et al. 2008; Lopez-Sandoval et al. 2011) and can reach upwards of >80% during bloom senescence (Nagata 2000). Mari et al. (2017) highlighted the importance of production and degradation of TEP using models which demonstrated that small changes in ratio of TEP production to biomass production potentially greatly impact the export efficiency of a modeled biological pump. When increase release extracellular materials (e.g. TEP precursors) relative to new biomass production, ‘TEP-rich’ organic aggregates may form. The role of TEP in vertical POC export is not solely linked to their ability to promote aggregation but also to their relative contribution to the buoyancy of POC (Engel & Schartau 1999; Chow et al. 2015). Previously it was assumed that TEP was produced by phytoplankton and acts as biological glue, creating ‘sticky’ aggregates which drive downward flux of POM (Engel 2004). However, modern understanding of TEP’s influence to aggregate transport emphasizes the need to better

understand the fate of TEP as a carbon pool subject to different temporally-scaled ecosystem processes (Estapa et al. 2015) than direct relation to chl *a*.

TEP Rate Dynamics

Fundamentally, TEP aggregation dynamics rely on abiotic coagulation of precursor polysaccharides originating from the release of either new organic materials or modification of existing ones.

The rates referred to as net TEP production (TEP_k) are comparable to the change in TEP which would be observed over a traditional 24-hour incubation of unfiltered, whole seawater (< 200 μm). The choice was made to use the equation normalizing this change to the starting concentration (Egge et al. 2009; MacGilchrist et al. 2014; Passow 2012; Iuculano et al. 2017) for two reasons: primarily, by normalizing this to the initial TEP concentration, (thus also initial DOC and precursor pools assuming a power-law size spectrum existed) further differentiation between physical influences from biological-community related ones would be possible. In addition, this method allowed for the calculation of rates of net TEP production which were directly comparable across experiments and to existing reported values. Excluding the final P-limited experiment, calculated TEP_k rates fell between -1 and 1, or roughly potential halving or doubling of the standing stock per day, comparable to reported values for other natural systems or bottle incubations (Passow 2012; Iuculano et al. 2017). By comparing these rates to observed changes in environmental TEP, one can see a clear disconnect emerge in the change in bulk TEP concentration between incubations and *in situ* during the later phases of bloom development, specifically during the transitional period from bloom peak to post-bloom. From field measurements, an obvious increase in environmental TEP occurs which can be largely associated

with increasing nutrient limitation and decaying physiological or growth status of the majority of phytoplankton standing stock (Obernosterer & Herndl 1995; Corzo et al. 2000; Passow 2002a; Berman-Frank et al. 2016; Deng et al. 2016) this effect is further enhanced by viral lysis (Van Boeckel et al. 1992; Passow 2002b; Vardi et al. 2012; Laber et al. 2018). However, rates of net TEP production from what would be a traditional bottle incubation results in negative values of TEP_k . Together this information provides an incomplete picture of what is occurring to TEP ‘particles’ ($> 0.4 \mu\text{m}$) in the sub-surface layer ($\sim 1 \text{ m}$ depth), potentially leading one to hypothesize that large aggregates were trapped inside bottle incubations which would have otherwise sunk quickly. However, the calculations of gross TEP production and heterotrophic TEP consumption tell a different potential story.

Gross TEP production (TEP_u) by definition is the production of TEP in the absence of biological loss processes. Therefore, in context of a dilution experiment would be a function of the physio-chemical properties of TEP-precursors and thus depend on their history and specific formation conditions (Kloareg & Quatrano 1988) also referable to as the seawater’s life history (Passow 2000; Passow 2002b). It is debatable how accurate the calculation would be at a theoretical y-intercept value since we assume TEP ‘particle’ formation manifests as a pseudo-first order reaction when in reality coagulation of gels is a second order process (Pruppacher & Klett 1980; Burd et al. 2000; Burd & Jackson 2009). Regardless, in a natural system this theoretical ‘no biotic influence’ likely largely does not occur especially over the temporal scales of particle aggregation as bacteria have been demonstrated to rapidly (scale of hours) colonize TEP aggregates (Passow 2002a; Poulsen & Iversen 2008), even in the sub-surface microlayer (Cunliffe et al. 2009a; Wurl et al. 2011). Taking potential under-estimation of TEP production into account (as a second-order process would generate higher TEP_u values at the lowest dilution

fractions), our calculated TEP_u values appear to agree with the environmental processes predicted in literature. As rates of photodegradation are typically considered consistent day-to-day, Mari et al. (2017) describes that in order for surface accumulation of TEP to occur the rate of production must be temporarily higher than the rate of degradation by heterotrophic process. From our dilution-experiments in both nutrient regimes, the highest rates of TEP_u (excluding the final P-limited experiment) were observed during the build-up period of bloom growth before peak chl *a* concentrations, in agreement with Mari et al. (2017)'s assumptions on the drivers of TEP accumulation. Furthermore, positive gross TEP production during the bloom crash was calculated; compared to negative TEP_k during these experiments, this indicates that TEP was still forming from precursors and that the negative net change in TEP was due to the diluted community influence. In combination with the results implying TEP_{kd} is not correlated with starting TEP concentration, this would imply that negative TEP_k were due to largely biotic and not abiotic influence.

TEP_g consumption (TEP_g) was measured sporadically throughout the mesocosm experiment, with significant rates ($p < 0.01$; Figure 6c) observed during 4 dilution-experiments from replete conditions and 3 from the P-limited treatment. TEP consumption was measured during the same experiment in both treatments once, during experiment-7 at the transitional period between bloom peak and post-bloom conditions. The measurements taken in these experiments did not allow for differentiating of heterotrophic consumption between protist-grazers and bacterial-remineralization, therefore, the trophic-fate of TEP-related carbon across the experiments cannot be directly elucidated. However, in the future this could be done by monitoring concentrations of metabolic by-products of their different processes. Poulsen & Iversen (2008) examined *in situ* fecal pellet (POC) degradation and demonstrated that bacterial

influence was minimal but highly variable, and the majority (15-50 %) of degradation was due to protists 20 – 100 μm in size, likely mainly heterotrophic flagellates. While there has been little work investigating the role of microzooplankton in consuming TEP, microzooplankton are voracious consumers, ingesting a significant proportion of primary production daily (Calbet and Landry, 2004). TEP often contains lipids (Bar-Zeev et al. 2015; Li et al. 2016) and nitrogen-rich substances like amino acids (Wurl & Cunliffe 2016) that would make TEP particularly palatable for microzooplankton. In laboratory settings, Tranvik et al. (1993) found marine heterotrophic flagellates to actively ingest colloidal DOM with molecular weights between 55 to 2000 kDa. Furthermore, they observed biomass increases in their cultured flagellates unexplainable by bacterivory alone when given 10mg of 2000 kDa dextran L^{-1} . On the basis of the results of these studies it is suggested that protists not only have the ability to ingest colloids spanning the size-range of bacteria, phytoplankton, and colloids, but also may actively utilize colloidal macromolecules (which includes TEP-related materials) for biomass growth.

Bacteria are generally considered to be the only pelagic organisms which utilize DOM. Harvey et al. (1995) showed that extracellular particular carbohydrates released by phytoplankton (i.e. largely, but not all, TEP) have biological turnover rates due to bacterial remineralization of 0.53 d^{-1} , which is higher than that of POC ($< 0.25 \text{ d}^{-1}$; Poulsen & Iversen 2008). The preferential degradation of the more labile TEP-C compared to POC is further demonstrated by Hamanaka et al. (2002) who found similar rates of degradation between TEP-C and POC. However, it is often less recognized that bacterial activity can induce TEP formation as well (Mari et al. 2017). Bacteria frequently produce extracellular polymeric substances (Decho 1990) that can coagulate to form ‘new’ TEP (Schuster & Herndl 1995; Stoderegger & Herndl 1999; Passow 2002b; Sugimoto et al. 2007; Koch et al. 2014). This release can be

enhanced by UV-radiation, suggested as a ‘sunblock’ mechanism (Wurl et al. 2011). Together, the net effect of bacteria on TEP accumulation in surface waters remains a mystery as bacteria have both positive and negative influences which may occur at similar rates and scales depending on the specific local environmental conditions. In the context of the rates of TEP consumption obtained by the dilution method, this would then imply that high ($> 0.5 \text{ d}^{-1}$) rates of TEP_g might be largely due to protists.

CHAPTER 5

FUTURE DIRECTIONS/CONCLUSION

Considerations for Future TEP Dilutions

While the size-spectra of TEP was not measured in this study, it is possible that the disconnect between observed and calculated net TEP production (Table 1) was due to large-rapidly sinking aggregates that were contained in a bottle incubation experiment, but quickly sunk out of the surface waters in the mesocosm experiments. These large aggregates would be subject to increased levels of UV-radiation compared to one which was sinking and might result in increased degradation into low molecular weight organic matter (LMW-OM). Alternatively, the lingering standing stock of TEP observed *in situ* post-bloom could have been supported by large quantities of ascending TEP-precursors and continual modification of existing TEP materials at varying spatial scales. This can potentially explain the anomalously high ($1.83 \pm 0.13 \text{ d}^{-1}$) TEP_μ rate calculated during the final P-limited dilution experiment. Thus, the lack of vertical structure in the dilution experiments (and any bottle incubation) may potentially underestimate both overall production of TEP at the system level due to controlled spatial scales. To investigate these potential effects, it would be informative to perform TEP dilution experiments using water from multiple depths (1, 5, and 10 m for example) to compare TEP_g and TEP_μ rates.

In the field, presence of TEP is thought to impact the microbial food web by enhancing the activity of specific prokaryotic extracellular enzymes (Ortega-Retuerta et al. 2018) by serving as a starting substrate for microbial communities. Performing dilution-based investigations of TEP dynamics while monitoring key enzymatic activities (i.e. β -glucosidase,

esterase and alkaline phosphatase) would better elucidate the molecular byproducts of TEP consumption and degradation. Further, heterotrophic activity may impact the abiotic degradation (e.g. UV photodegradation) of TEP as generally the most labile organic molecules are the most energetically efficient to utilize, vice-versa UV-radiation can stimulate heterotrophic activity by increasing the concentrations of LMW-OM. Future efforts using TEP dilutions could monitor DIC, LMW-OM, or protist lipid content to attempt to differentiate between remineralization, 'breakdown' of TEP into smaller organic molecules (not measured by the TEP semi-quantitative methods), or carbon-acquisition by grazers.

Models and field data can be utilized together in many ways. Often, field data is used to calibrate models to particular environmental conditions and geographical regions (Clegg & Whitfield 1993). Field data and models can also be combined to parameterize important processes which are difficult to measure or have potentially high variability in rates (Murnane et al. 1996). In this work, the *in situ* TEP rate dynamics measure by dilution experiments have the potential to be used in both efforts once the pathway of heterotrophy can be differentiated between bacterial remineralization and protist grazing. As it stands now, TEP consumption rates calculated by the dilution method are a general 'degradation' of TEP particles ($> 0.4 \mu\text{m}$) term. This term still has importance, however, in advancing understanding of carbon aggregate sinking processes. TEP degradation from biotic influence implies TEP-C is remaining in the euphotic zone instead of being exported in rapidly sinking aggregates. Further, this process potentially alters the sinking velocity of carbon aggregates themselves by decreasing the TEP:POC ratio which may increase short-term carbon export during massive flux events. TEP's influence on carbon vertical transport during non-steady-state conditions is undoubtedly important and further

studies investigating rates of these processes is crucial to better estimate carbon flux in the oceans.

Conclusions

We found substantial *in situ* TEP formation during late stationary and senescent phases of mesocosm blooms dominated by pico- and nano- eukaryotes under both nutrient replete (Redfield) and phosphorous-limited conditions. We present further evidence that TEP accumulations results from a combination of factors rather than variation of a single predictor variable. We demonstrated that TEP production was higher than rates of TEP degradation in the period leading up to *in situ* TEP accumulation, in agreement with Mari et al. (2017)'s prediction. Gross TEP production rates were not significantly different across the two nutrient treatments, suggesting that underlying factors such as individual cell growth stage were the predominant influence driving changes in cell-mediated gross TEP production and subsequent TEP accumulation. Maximum TEP consumption rates coincided with stationary and decline phases of bloom growth in both nutrient treatments, implying that heterotrophic TEP consumption helps drive remineralization and fuels the microbial loop. In turn, this would presumably impact late-bloom carbon cycling dynamics during massive-flux events, although the specific effects (increased vs. decreased sinking velocity) remain unclear. In the field, changes in the efficiency of carbon remineralization and export, especially labile carbon such as TEP, are key controls over the efficiency of the biological carbon pump. Incorporating gross TEP production and consumption rates from dilution-experiments with models of TEP aggregation and sinking would be an advantageous way to utilize the data generated from dilution experiments. Better understanding of TEP dynamics, as elucidated via measurements of specific production and

consumption rates, will improve our predictions of oceanic carbon export and allow more accurate parameterization of positive and negative influences on net TEP accumulation and subsequent POC export.

Table 1: Comparison of dilution-rates to changes in environmental TEP. Net TEP

production rates from dilution experiments are compared to changes in environmental TEP concentrations during each phase of the bloom. Significant differences (paired t-test, $p < 0.05$) are highlighted with bold text.

		Replete		LOP	
		<i>In situ</i>	Dilution	<i>In situ</i>	Dilution
Pre-Bloom	Range	-0.19±0.07 - 0.13±0.12	-0.34±0.34 - 0.55±0.01	-0.12±0.18 - 0.08±0.21	-0.27±0.07 - 0.65±0.22
	Avg	0.02±0.06	0.09±0.02	-0.05±0.11	0.07±0.08
	<i>p</i> value	0.1816		0.2142	
Bloom	Range	-0.07±0.09 - 1.9±0.33	0.36±0.12 - 1.0±0.07	-0.07±0.09 - 0.92±0.37	-0.29±0.09 - 0.20±0.12
	Avg	0.65±0.12	0.71±0.05	0.38±0.16	-0.03±0.05
	<i>p</i> value	0.4622		*0.0263	
Post-Bloom	Range	-0.26±0.05 - 0.22±0.11	-0.78±0.12 - 0.01±0.08	0.27±0.08 - 0.07±0.10	-0.69±0.04 - 1.83±0.13
	Avg	-0.02±0.06	-0.35±0.05	-0.10±0.06	0.52±0.08
	<i>p</i> value	*0.0055		*0.0016	

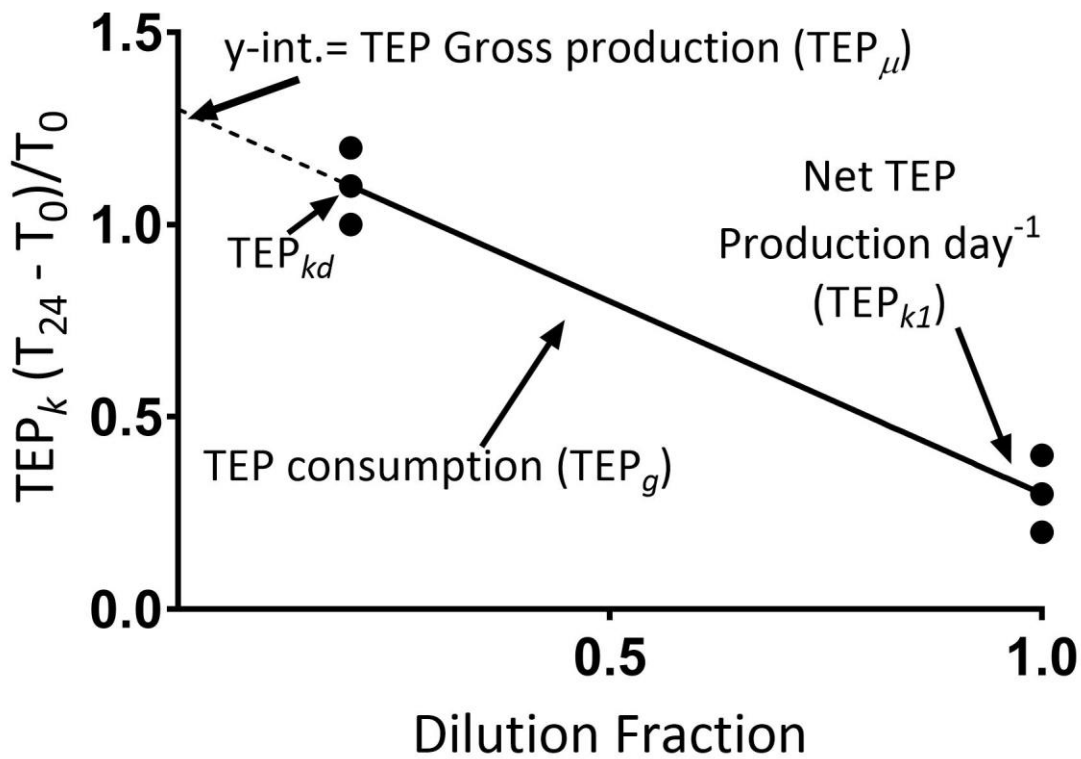


Figure 1. Conceptual representation of dilution-series TEP aggregation rates. TEP gross production (TEP_{μ}), TEP consumption (TEP_g), and net TEP production (TEP_{k1}) are related via a linear relationship along a created dilution gradient, similar to measurements performed in a traditional dilution experimental (Landry & Hassett 1982).

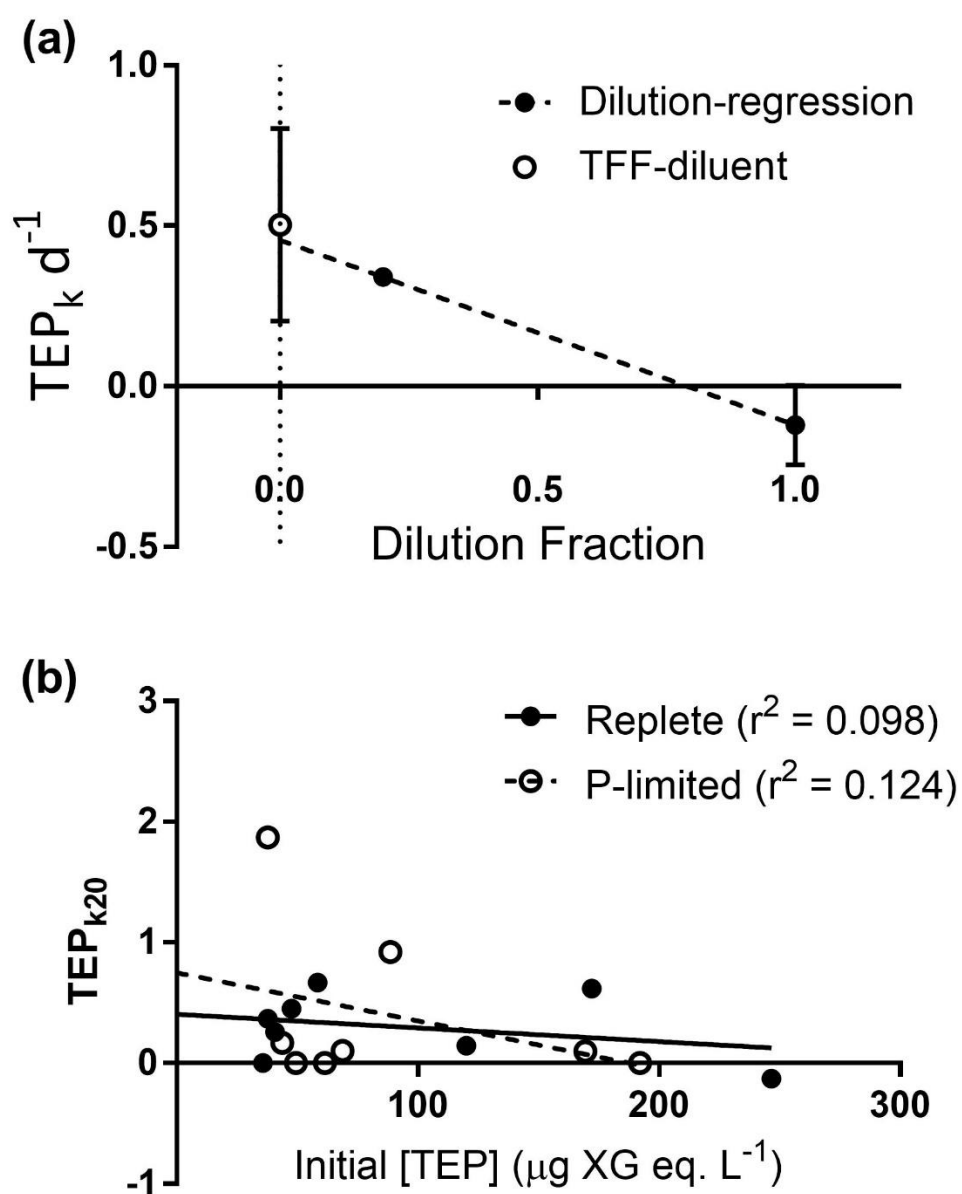


Figure 2: Assessment of experimental methods. (a) An example of relationship between net TEP production rates measured experimentally from a TEP dilution and an incubation consisting wholly of tangential-flow filtrated (TFF) diluent. (b) Comparison of net TEP production rates from diluted-fraction incubations to initial TEP concentration demonstrating no apparent effect.

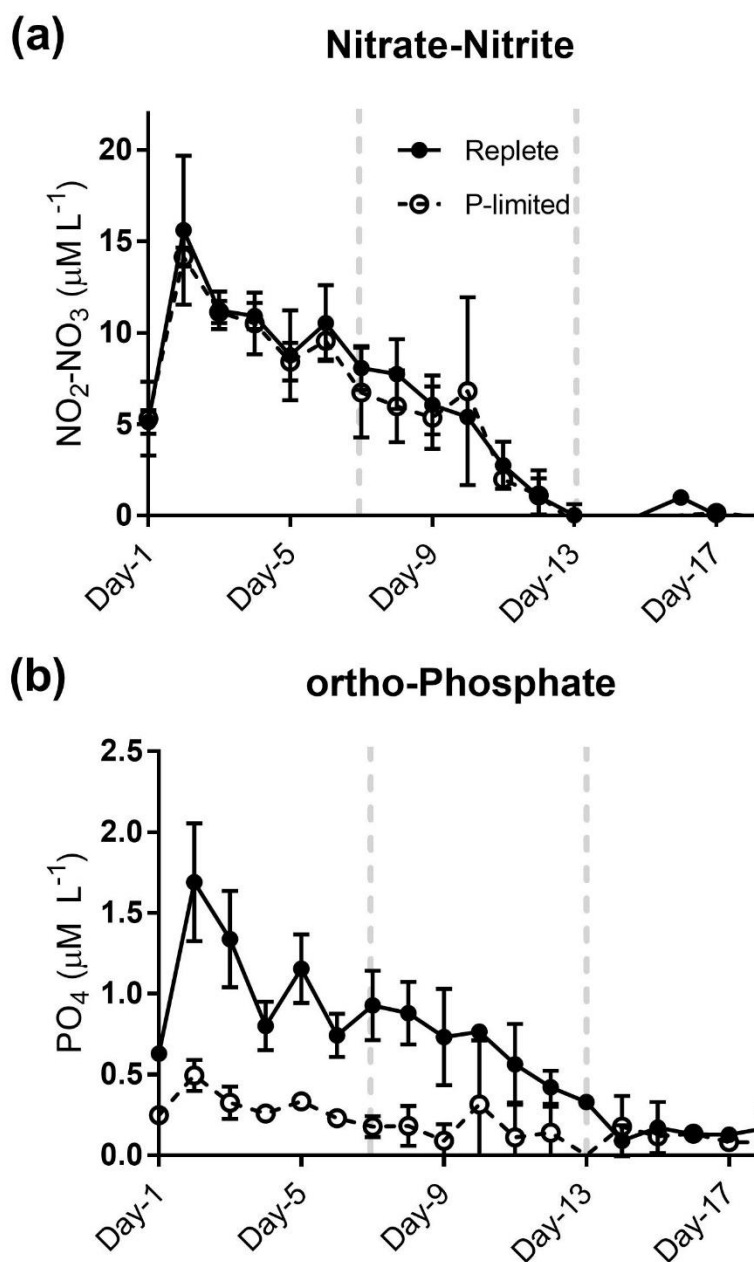


Figure 3: Nutrient concentrations during bloom-experiment. (a) Nitrite-nitrate (N+N) and (b) orthophosphate ($\mu\text{M L}^{-1}$) over the sampling period (N = 18 days) in nutrient-treatment replete (black, solid) and P-limited (grey-dashed, hollow) mesocosms.

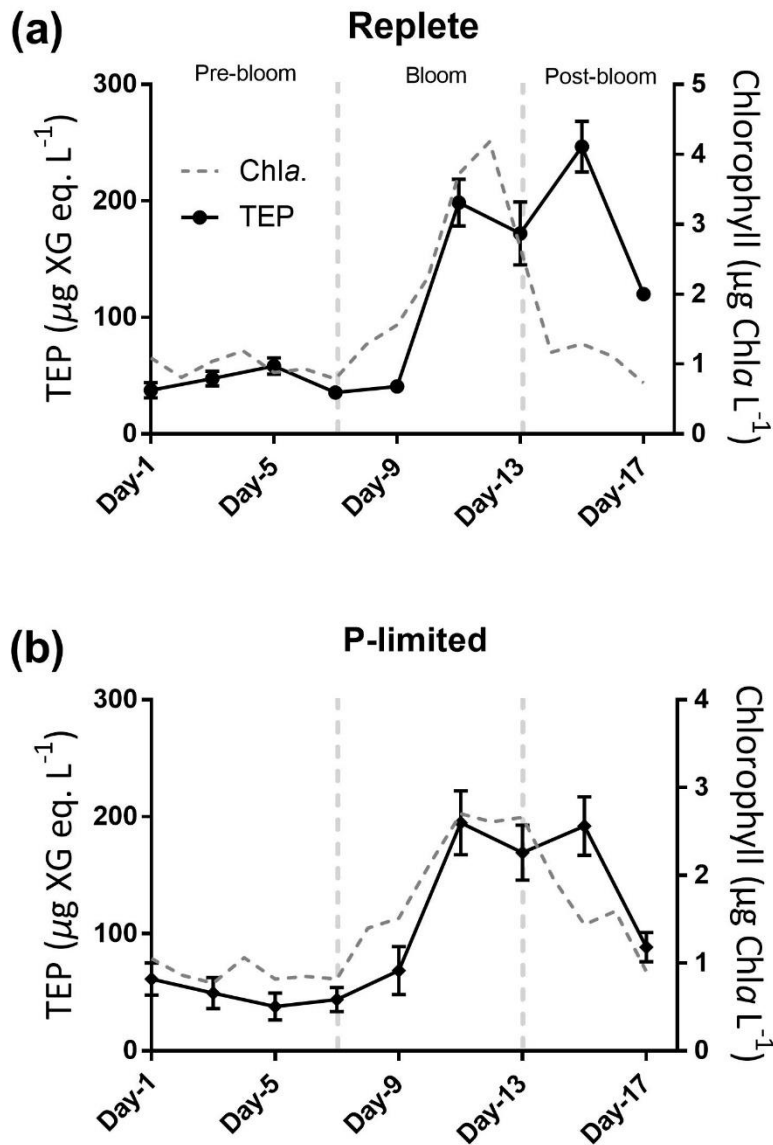


Figure 4: TEP and Chlorophyll-*a* concentrations over observational period. TEP ($\mu\text{g XG eq. L}^{-1}$; black, solid; $N = 9$) and chlorophyll-*a* ($\mu\text{g L}^{-1}$; grey, dashed; $N = 18$) concentrations over the sampling period of 18 Days in nutrient treatments **(a)** Replete ($N:P = 16:1$) and **(b)** Phosphorous-limited (P-limited; $N:P = 60:1$). Error bars represent one standard deviation from the mean of triplicate observations; dashed, vertical lines represent bloom-stages of 6 days each.

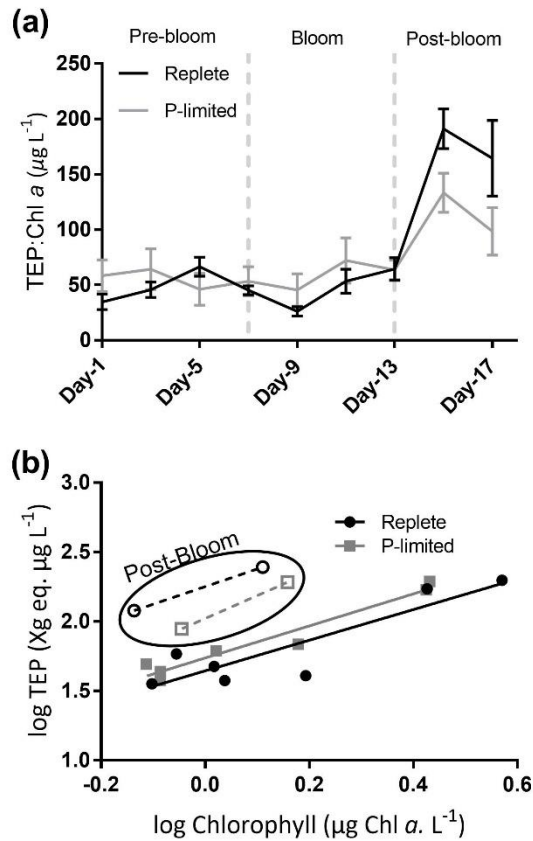


Figure 5: Comparing TEP and Chlorophyll-*a* concentrations. (a) ratio of TEP per unit chlorophyll ($\mu\text{g L}^{-1}$) in nutrient treatments replete (black) and P-limited (grey). Vertical, dashed lines represent bloom-phases of 6-days each. (b) log normalized chlorophyll-*a* and TEP concentrations compared using type II linear regression; plotted points are divided into two groupings, one prior to bloom termination (Day-1 through Day-14; solid points) and one of post-bloom observations (N = 2; dashed, hollow points)

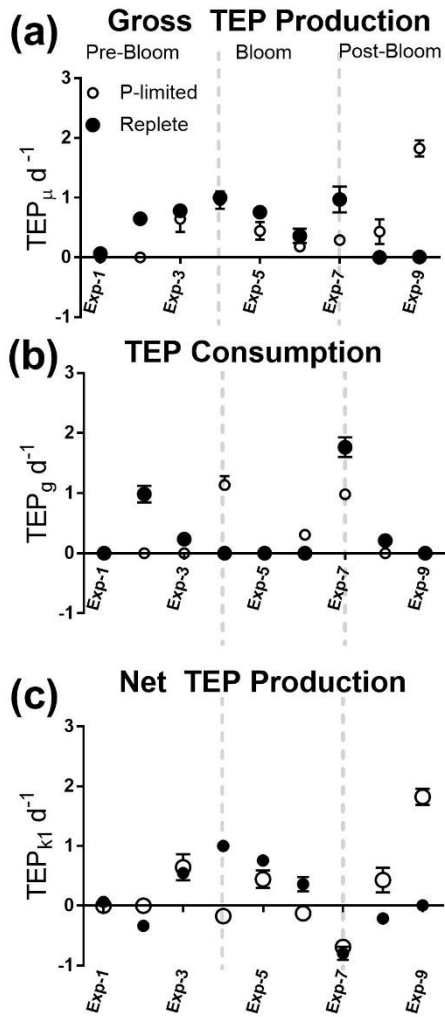


Figure 6: TEP aggregation dynamics rates from dilution-series. Rates of (a) instantaneous gross TEP production (TEP_{μ}) (b) biotic-community based TEP consumption and/or degradation rates (TEP_g) and (c) normalized net TEP production (TEP_k) over the course of the mesocosm-bloom experiment in nutrient treatments replete (solid) and P-limited (hollow). Bloom stages of 6-days each are superimposed as vertical dashed, grey lines. Error bars represent one standard deviation from the mean of triplicate samples; small error is contained within sample points.

REFERENCES

- Allredge, A. L., & Gotschalk, C. (1988). In situ settling behavior of marine snow 1. *Limnology and Oceanography*, 33(3), 339-351.
- Allredge, A. L., Passow, U., & 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(6), 1131-1140.
- Alonso-Saez, L., Vazquez-Dominguez, E., Cardelus, C., Pinhassi, J., Sala, M., Lekunberri, I., Balague, V., Vila-Costa, M., Unrein, F., Massana, R., Simo, R., Gasol, J.M., 2008. Factors controlling the year-round variability in carbon flux through bacteria in a coastal marine system. *Ecosystems* 11(3), 397-409, doi:10.1007/s10021-008-9129-0.
- Anderson, S. R., Diou-Cass, Q. P., & Harvey, E. L. (2018). Short-term estimates of phytoplankton growth and mortality in a tidal estuary. *Limnology and Oceanography*, 63(6), 2411-2422.
- Asper, V. L. (1987). Measuring the flux and sinking speed of marine snow aggregates. *Deep Sea Research Part A. Oceanographic Research Papers*, 34(1), 1-17.
- Asper, V. L., Deuser, W. G., Knauer, G. A., & Lohrenz, S. E. (1992). Rapid coupling of sinking particle fluxes between surface and deep ocean waters. *Nature*, 357(6380), 670.
- Asper, V.L., Smith, Jr. W.O., 1999. Particle fluxes during austral spring and summer in the southern Ross Sea, Antarctica. *Journal of Geophysical Research* 104(C3), 5345-5359, doi:10.1029/1998JC900067.
- Azam, F., Fenchel, T., Field, J. G., Gray, J. S., Meyer-Reil, L. A., & Thingstad, F. (1983). The ecological role of water-column microbes in the sea. *Marine ecology progress series*, 257-263.
- Azetsu-Scott, K., & Passow, U. (2004). Ascending marine particles: Significance of transparent exopolymer particles (TEP) in the upper ocean. *Limnology and Oceanography*, 49(3), 741–748. <https://doi.org/10.4319/lo.2004.49.3.0741>
- Baines, S.B., Pace, M.L., 1991. The production of dissolved organic matter by phytoplankton and its importance to bacteria: Patterns across marine and freshwater systems. *Limnology and Oceanography* 36(6), 1078-1090, doi:10.4319/lo.1991.36.6.1078.

- Bar-Zeev, E., Berman-Frank, I., Stambler, N., Domínguez, E. V., Zohary, T., Capuzzo, E., ... & Berman, T. (2009). Transparent exopolymer particles (TEP) link phytoplankton and bacterial production in the Gulf of Aqaba. *Aquatic Microbial Ecology*, 56(2-3), 217-225.
- Bar-Zeev, E., Passow, U., Romero-Vargas Castrillón, S., & Elimelech, M. (2015). Transparent exopolymer particles: from aquatic environments and engineered systems to membrane biofouling. *Environmental Science & Technology*, 49(2), 691-707.
- Beauvais, S., Pedrotti, M. L., Villa, E., & Lemée, R. (2003). Transparent exopolymer particle (TEP) dynamics in relation to trophic and hydrological conditions in the NW Mediterranean Sea. *Marine Ecology Progress Series*, 262, 97-109.
- Berges, J. A., Varela, D. E., & Harrison, P. J. (2002). Effects of temperature on growth rate, cell composition and nitrogen metabolism in the marine diatom *Thalassiosira pseudonana* (Bacillariophyceae). *Marine Ecology Progress Series*, 225, 139-146.
- Berman-Frank, I., Rosenberg, G., Levitan, O., Haramaty, L., & Mari, X. (2007). Coupling between autocatalytic cell death and transparent exopolymeric particle production in the marine cyanobacterium *Trichodesmium*. *Environmental Microbiology*, 9(6), 1415–1422. <https://doi.org/10.1111/j.1462-2920.2007.01257.x>
- Berman-Frank, I., Spungin, D., Rahav, E., Van Wambeke, F., Turk-Kubo, K., & Moutin, T. (2016). Dynamics of transparent exopolymer particles (TEP) during the VAHINE mesocosm experiment in the New Caledonian lagoon. *Biogeosciences*, 13(12), 3793–3805. <https://doi.org/10.5194/bg-13-3793-2016>
- Biddanda, B., & Benner, R. (1997). Carbon, nitrogen, and carbohydrate fluxes during the production of particulate and dissolved organic matter by marine phytoplankton. *Limnology and Oceanography*, 42(3), 506-518.
- Bittar, T. B., Passow, U., Haramaty, L., Bidle, K. D., & Harvey, E. L. (2018). An updated method for the calibration of transparent exopolymer particle measurements. *Limnology and Oceanography: Methods*, 16(10), 621-628.
- Bourdin, G., Gazeau, F., Kerros, M. E., Marro, S., & Pedrotti, M. L. (2017). Dynamics of transparent exopolymeric particles and their precursors during a mesocosm experiment: Impact of ocean acidification. *Estuarine, Coastal and Shelf Science*, 186, 112–124. <https://doi.org/10.1016/j.ecss.2016.02.007>
- Boyd, P. W., & Stevens, C. L. (2002). Modelling particle transformations and the downward organic carbon flux in the NE Atlantic Ocean. *Progress in Oceanography*, 52(1), 1-29.
- Boyd, P. W., & Trull, T. W. (2007). Understanding the export of biogenic particles in oceanic waters: Is there consensus? *Progress in Oceanography*, 72(4), 276–312. <https://doi.org/10.1016/j.pocean.2006.10.007>

- Buesseler, K. O. (1998). The decoupling of production and particulate export in the surface ocean. *Global Biogeochemical Cycles*, 12(2), 297-310.
- Buesseler, K.O., Barber, R.T., Dickson, M.-L., Hiscock, M.R., Moore, J.K., Sambrotto, R., 2003. The effect of marginal ice-edge dynamics on production and export in the Southern Ocean along 170°W. *Deep-Sea Research II* 50(3-4), 579-603, doi:10.1016/S0967-0645(02)00585-4.
- Burd, A.B., Jackson, G.A., 1997. Predicting particle coagulation and sedimentation rates for a pulsed input. *Journal of Geophysical Research* 102, 10545-10561.
- Burd, A. B., Moran, S. B., & Jackson, G. A. (2000). A coupled adsorption–aggregation model of the POC/234Th ratio of marine particles. *Deep Sea Research Part I: Oceanographic Research Papers*, 47(1), 103-120.
- Burd, A. B., & Jackson, G. A. (2009). Particle aggregation. *Annual review of marine science*, 1, 65-90.
- Brix, H., Gruber, N., Karl, D. M., & Bates, N. R. (2006). On the relationships between primary, net community, and export production in subtropical gyres. *Deep Sea Research Part II: Topical Studies in Oceanography*, 53(5-7), 698-717.
- Calbet, A., & Landry, M. R. (2004). Phytoplankton growth, microzooplankton grazing, and carbon cycling in marine systems. *Limnology and Oceanography*, 49(1), 51-57.
- Carlson, C. A., & Ducklow, H. W. (1995). Dissolved organic carbon in the upper ocean of the central equatorial Pacific Ocean, 1992: Daily and finescale vertical variations. *Deep Sea Research Part II: Topical Studies in Oceanography*, 42(2-3), 639-656.
- Chen, J., & Thornton, D. C. O. (2015). Transparent exopolymer particle production and aggregation by a marine planktonic diatom (*Thalassiosira weissflogii*) at different growth rates. *Journal of Phycology*, 51(2), 381–393. <https://doi.org/10.1111/jpy.12285>
- Chow, J. S., Lee, C., & Engel, A. (2015). The influence of extracellular polysaccharides, growth rate, and free coccoliths on the coagulation efficiency of *Emiliania huxleyi*. *Marine Chemistry*, 175, 5-17.
- Christina, L., & 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(5-7), 639-658.
- Claquin, P., Probert, I., Lefebvre, S., & Veron, B. (2008). Effects of temperature on photosynthetic parameters and TEP production in eight species of marine microalgae. *Aquatic Microbial Ecology*, 51(1), 1–11. <https://doi.org/10.3354/ame01187>
- Clegg SL, Whitfield M. 1990. A generalized model for the scavenging of trace metals in the open ocean—I. Particle cycling. *Deep-Sea Res.* 37:809–832

- Copin-Montégut, G., & Avril, B. (1993). Vertical distribution and temporal variation of dissolved organic carbon in the North-Western Mediterranean Sea. *Deep Sea Research Part I: Oceanographic Research Papers*, 40(10), 1963-1972.
- Corzo, A., Morillo, J. A., & Rodríguez, S. (2000). Production of transparent exopolymer particles (TEP) in cultures of *Chaetoceros calcitrans* under nitrogen limitation. *Aquatic Microbial Ecology*, 23(1), 63-72.
- Cunliffe, M., Salter, M., Mann, P. J., Whiteley, A. S., Upstill-Goddard, R. C., & Murrell, J. C. (2009). Dissolved organic carbon and bacterial populations in the gelatinous surface microlayer of a Norwegian fjord mesocosm. *FEMS Microbiology Letters*, 299(2), 248–254. <https://doi.org/10.1111/j.1574-6968.2009.01751.x>
- Cunliffe, M., Engel, A., Frka, S., Gašparović, B. Ž., Guitart, C., Murrell, J. C., ... Wurl, O. (2013). Sea surface microlayers: A unified physicochemical and biological perspective of the air-ocean interface. *Progress in Oceanography*, 109, 104–116. <https://doi.org/10.1016/j.pocean.2012.08.004>
- Décima, M., Landry, M. R., Stukel, M. R., Lopez-Lopez, L., & Krause, J. W. (2015). Mesozooplankton biomass and grazing in the Costa Rica Dome: amplifying variability through the plankton food web. *Journal of plankton research*, 38(2), 317-330.
- Decho, A.W., 1990. Microbial exopolymer secretions in ocean environments: their role(s) in food webs and marine processes. *Oceanography and Marine Biology* 28, 73-153.
- Deng, W., Cruz, B. N., & Neuer, S. (2016). Effects of nutrient limitation on cell growth, TEP production and aggregate formation of marine *Synechococcus*. *Aquatic Microbial Ecology*, 78(1), 39–49. <https://doi.org/10.3354/ame01803>
- Ducklow, H. (2000). Bacterial production and biomass in the oceans. *Microbial ecology of the oceans*, 1, 85-120.
- Ducklow, H. W., Myers, K. M., Erickson, M., Ghiglione, J. F., & Murray, A. E. (2011). Response of a summertime Antarctic marine bacterial community to glucose and ammonium enrichment. *Aquatic Microbial Ecology*, 64(3), 205-220.
- Ebersbach, F., Assmy, P., Martin, P., Schulz, I., Wolzenburg, S., & Nöthig, E. M. (2014). Particle flux characterisation and sedimentation patterns of protistan plankton during the iron fertilisation experiment LOHAFEX in the Southern Ocean. *Deep-Sea Research Part I: Oceanographic Research Papers*, 89, 94–100. <https://doi.org/10.1016/j.dsr.2014.04.007>
- Egge, J. K., Thingstad, T. F., Engel, A., Bellerby, R. G. J., Riebesell, U., Egge, J. K., ... Primary, U. R. (2007). Primary production during nutrient-induced blooms at elevated CO₂ concentrations To cite this version : HAL Id : hal-00297945 Primary production during nutrient-induced blooms at elevated CO₂ concentrations.

- Engel, A., & Schartau, M. (1999). Influence of transparent exopolymer particles (TEP) on sinking velocity of *Nitzschia closterium* aggregates. *Marine Ecology Progress Series*, 182, 69-76.
- Engel, A., & Passow, U. (2001). Carbon and nitrogen content of transparent exopolymer particles (TEP) in relation to their Alcian Blue adsorption. *Marine Ecology Progress Series*, 219(1977), 1–10. <https://doi.org/10.3354/meps219001>
- Engel, A. (2002). Direct relationship between CO₂ uptake and transparent exopolymer particles production in natural phytoplankton. *Journal of Plankton Research*, 24(1), 49-53.
- Engel, A., Goldthwait, S., Passow, U., & Alldredge, A. (2002). Temporal decoupling of carbon and nitrogen dynamics in a mesocosm diatom bloom. *Limnology and Oceanography*, 47(3), 753-761.
- Engel, A. (2004). Distribution of transparent exopolymer particles (TEP) in the northeast Atlantic Ocean and their potential significance for aggregation processes. *Deep-Sea Research Part I: Oceanographic Research Papers*, 51(1), 83–92. <https://doi.org/10.1016/j.dsr.2003.09.001>
- Engel, A., Delille, B., Jacquet, S., Riebesell, U., Rochelle-Newall, E., Terbruggen, A., & Zondervan, I. (2004). TEP and DOC production by *Emiliana huxleyi* exposed to different CO₂ concentrations: A mesocosm experiment. *Aquatic Microbiology and Ecology*, 34(Dic), 93–104. <https://doi.org/10.1109/DAC.2005.193796>
- Engel, A., Händel, N., Wohlers, J., Lunau, M., Grossart, H. P., Sommer, U., & Riebesell, U. (2010). Effects of sea surface warming on the production and composition of dissolved organic matter during phytoplankton blooms: results from a mesocosm study. *Journal of Plankton Research*, 33(3), 357-372.
- Estapa, M. L., Siegel, D. A., Buesseler, K. O., Stanley, R. H., Lomas, M. W., & Nelson, N. B. (2015). Decoupling of net community and export production on submesoscales in the Sargasso Sea. *Global biogeochemical cycles*, 29(8), 1266-1282.
- Evans, C., & Brussaard, C. P. D. (2012). Viral lysis and microzooplankton grazing of phytoplankton throughout the Southern Ocean. *Limnology and Oceanography*, 57(6), 1826–1837. <https://doi.org/10.4319/lo.2012.57.6.1826>
- Falkowski, P., Scholes, R. J., Boyle, E., Canadell, J., Canfield, D., ... Hibbard, K. (2011). The Global Carbon Cycle : A Test of Our Knowledge of Earth as a System. *Science*, 291(2000), 290–296. <https://doi.org/10.1126/science.290.5490.291>
- Fernandez, M., Bianchi, M., Van Wambeke, F., 1994. Bacterial biomass, heterotrophic production and utilization of dissolved organic matter photosynthetically produced in the Almeria-Oran front. *Journal of Marine Systems* 5, 313-325, doi:10.1016/0924-7963(94)90053-1.

- Finkel, Z. V., Matheson, K. A., Regan, K. S., & Irwin, A. J. (2010). Genotypic and phenotypic variation in diatom silicification under paleo-oceanographic conditions. *Geobiology*, 8(5), 433-445.
- Fogg, G.E., 1983. The ecological significance of extracellular products of phytoplankton photosynthesis. *Botanica Marina* 26(1), 3-14, doi:10.1515/botm.1983.26.1.3.
- Gärdes, A., Ramaye, Y., Grossart, H. P., Passow, U., & 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(Stal 2003), 1–14. <https://doi.org/10.3354/meps09894>
- Geider, R. J., & La Roche, J. (2002). Redfield revisited: variability of C [ratio] N [ratio] P in marine microalgae and its biochemical basis. *European Journal of Phycology*, 37(1), 1-17.
- Goldman, J.C., D.A Hansell and M.R. Dennett, 1992. Chemical characterization of three large oceanic diatoms: potential impact on water column chemistry. *Mar. Ecol Prog. Ser.*, 88: 257-270.
- Graff, J. R., & Ryneanson, T. A. (2011). Extraction method influences the recovery of phytoplankton pigments from natural assemblages. *Limnology and Oceanography: Methods*, 9(4), 129-139.
- Grossart, H. P., & Ploug, H. (2000). Bacterial production and growth efficiencies: Direct measurements on riverine aggregates. *Limnology and Oceanography*, 45(2), 436-445.
- Grossart, H. P., Czub, G., & Simon, M. (2006). Algae-bacteria interactions and their effects on aggregation and organic matter flux in the sea. *Environmental Microbiology*, 8(6), 1074–1084. <https://doi.org/10.1111/j.1462-2920.2006.00999.x>
- Hamanaka, J., Tanoue, E., Hama, T., Handa, N., 2002. Production and export of particulate fatty acids, carbohydrates and combined amino acids in the euphotic zone. *Marine Chemistry* 77(1), 55-69, doi:10.1016/S0304-4203(01)00075-5.
- Harrison, P.J., Conway, H.L., Holmes, R.W. et al. *Mar. Biol.* (1977) 43: 19. <https://doi.org/10.1007/BF00392568>
- Harvey, H.R., Tuttle, J.H., Bell, J.T., 1995. Kinetics of phytoplankton decay during simulated sedimentation: changes in biochemical composition and microbial activity under oxic and anoxic conditions. *Geochimica et Cosmochimica Acta* 59(16), 3367-3377, doi:10.1016/0016-7037(95)00217-N.

- Henson, S. A., Sanders, R., Madsen, E., Morris, P. J., Le Moigne, F., & Quartly, G. D. (2011). A reduced estimate of the strength of the ocean's biological carbon pump. *Geophysical Research Letters*, 38(4).
- Henson, S. (2015). Variability in efficiency of particulate organic carbon export: A model study. *Global Biogeochemical Cycles*, 29(1), 33.
- Hernández-León, S., & Ikeda, T. (2005). A global assessment of mesozooplankton respiration in the ocean. *Journal of Plankton Research*, 27(2), 153-158.
- Hong, Y., Smith Jr, W. O., & White, A. M. (1997). STUDIES ON TRANSPARENT EXOPOLYMER PARTICLES (TEP) PRODUCED IN THE ROSS SEA (ANTARCTICA) AND BY PHAEOCYSTIS ANTARCTICA (PRYMNESIOPHYCEAE) 1. *Journal of Phycology*, 33(3), 368-376.
- Hunt JR. 1980. Prediction of oceanic particle size distributions from coagulation and sedimentation mechanisms. In *Particulates in Water: Characterization, Fate, Effects, and Removal*, ed. M.C. Kavanaugh, J.O. Leckie, pp. 243–257. *Adv. Chem. Ser. No. 189*. Washington, DC: Am. Chem. Soc.
- Iuculano, F., Mazuecos, I. P., Reche, I., & Agustí, S. (2017). Prochlorococcus as a possible source for transparent exopolymer particles (TEP). *Frontiers in Microbiology*, 8(APR), 1–11. <https://doi.org/10.3389/fmicb.2017.00709>
- Iversen, M., & 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:, 2613-2624.
- Jackson GA. 2001. Effect of coagulation on a model planktonic food web. *Deep-Sea Res. I* 48:95–123
- Jackson GA, Lochman SE. 1992. Effect of coagulation on nutrient and light limitation of an algal bloom. *Limnol. Oceanogr.* 37:77–89
- Jackson, G.A., Maffone, R., Costello, D.K., Alldredge, A.L., Logan, B.E., Dam, H.G., 1997. Particle size spectra between 1 μm and 1 cm at Monterey Bay determined using multiple instruments. *Deep-Sea Research I* 44, 1739-1767.
- Jackson, G. A., & Burd, A. B. (2015). Simulating aggregate dynamics in ocean biogeochemical models. *Progress in Oceanography*, 133, 55-65.
- Jespersen, A. M., & Christoffersen, K. (1987). Measurements of chlorophyll a from phytoplankton using ethanol as extraction solvent. *Archiv für Hydrobiologie* 109: 445-454.
- Kvam, O.V. & O.T. Kleiven, 1995. Diel horizontal migration and swarm formation in *Daphnia* in response to *Chaoborus*. *Hydrobiologia*, 307, 177-184.

- Jiang, Q., Logan, B.E., 1991. Fractal dimensions of aggregates determined from steady-state size distributions. *Environmental Science and Technology* 25, 2031-2038.
- Jokulsdottir, T., & Archer, D. (2016). A stochastic, Lagrangian model of sinking biogenic aggregates in the ocean (SLAMS 1.0): model formulation, validation and sensitivity. *Geoscientific Model Development*, 9(4), 1455-1476.
- Jones, V., Meador, T.B., Gogou, A., Migon, C., Penkman, K.E.H., Collins, M.J., Repeta, D.J., 2013. Characterisation and dynamics of dissolved organic matter in the Northwestern Mediterranean Sea. *Progress in Oceanography* 119, 78-89, doi:10.1016/j.pocean.2013.06.007.
- Kähler, P., & Koeve, W. (2001). Marine dissolved organic matter: can its C: N ratio explain carbon overconsumption?. *Deep Sea Research Part I: Oceanographic Research Papers*, 48(1), 49-62.
- Karl, D.M., Hebel, D.V., Bjorkman, K., Letelier, R.M., 1998. The role of dissolved organic matter release in the productivity of the oligotrophic North Pacific Ocean. *Limnology and Oceanography* 43(6), 1270-1286.
- Kepkay, P. E., Niven, S. E. H., & Jellett, J. F. (1997). Colloidal organic carbon and phytoplankton speciation during a coastal bloom. *Journal of Plankton Research*, 19, 369–389.
- Koch, B., Kattner, G., Witt, M., & Passow, U. (2014). Molecular insights into the microbial formation of marine dissolved organic matter: recalcitrant or labile?. *Biogeosciences*, 11(15), 4173-4190.
- Laber, C. P., Hunter, J. E., Carvalho, F., Collins, J. R., Hunter, E. J., Schieler, B. M., ... Bidle, K. D. (2018). Coccolithovirus facilitation of carbon export in the North Atlantic. *Nature Microbiology*, 3(5), 537–547. <https://doi.org/10.1038/s41564-018-0128-4>
- Lampitt, R. S., Noji, T., & Von Bodungen, B. (1990). What happens to zooplankton faecal pellets? Implications for material flux. *Marine Biology*, 104(1), 15-23.
- Landry, M. R., & Hassett, R. P. (1982). Estimating the grazing impact of marine micro-zooplankton. *Marine Biology*, 67(3), 283–288. <https://doi.org/10.1007/BF00397668>
- Landry, M. R., Decima, M., Simmons, M. P., Hannides, C. C., & Daniels, E. (2008). Mesozooplankton biomass and grazing responses to Cyclone Opal, a subtropical mesoscale eddy. *Deep Sea Research Part II: Topical Studies in Oceanography*, 55(10-13), 1378-1388.
- Landry, M. E., Wagner, E., Jones, R., Forton, C., & Khoo, L. (2009). U.S. Patent No. 7,491,218. Washington, DC: U.S. Patent and Trademark Office.

- Lawrence, C., & Menden-Deuer, S. (2012). Drivers of protistan grazing pressure: seasonal signals of plankton community composition and environmental conditions. *Marine Ecology Progress Series*, 459, 39-52.
- Li, X., Logan, B.E., 1995. Size distributions and fractal properties of particles during a simulated phytoplankton bloom in a mesocosm. *Deep-Sea Research II* 42, 125-138.
- Li, S., Winters, H., Jeong, S., Emwas, A. H., Vigneswaran, S., & Amy, G. L. (2016). Marine bacterial transparent exopolymer particles (TEP) and TEP precursors: Characterization and RO fouling potential. *Desalination*, 379, 68-74.
- Ling, S. C., & Alldredge, A. L. (2003). Does the marine copepod *Calanus pacificus* consume transparent exopolymer particles (TEP)? *Journal of Plankton Research*, 25(5), 507–515. <https://doi.org/10.1093/plankt/25.5.507>
- Logan, B.E., Wilkinson, D.B., 1990. Fractal geometry of marine snow and other biological aggregates. *Limnology and Oceanography* 35, 130-136.
- Logan, B. E., Passow, U., Alldredge, A. L., Grossartt, H. P., & Simont, 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.
- Lopez-Sandoval, D.C., Fernandez, A., Maranon, E., 2011. Dissolved and particulate primary production along a longitudinal gradient in the Mediterranean Sea. *Biogeosciences* 8(3), 815-825, doi:10.5194/bg-8-815-2011.
- Luft, J. H. (1971) Ruthenium red and violet. 1. Chemistry, purification, methods of use for electron microscopy and mechanism of action. *Anat. Rec.*, 171, 347.
- MacGilchrist, G. A., Shi, T., Tyrrell, T., Richier, S., Moore, C. M., Dumousseaud, C., & Achterberg, E. P. (2014). Effect of enhanced pCO₂ levels on the production of dissolved organic carbon and transparent exopolymer particles in short-term bioassay experiments. *Biogeosciences*, 11(13), 3695–3706. <https://doi.org/10.5194/bg-11-3695-2014>
- Magaletti, E., Urbani, R., Sist, P., Ferrari, C. R., & Cicero, A. M. (2004). Abundance and chemical characterization of extracellular carbohydrates released by the marine diatom *Cylindrotheca fusiformis* under N- and P-limitation. *European Journal of Phycology*, 39(2), 133–142. <https://doi.org/10.1080/0967026042000202118>
- Marañón, E., Cermeno, P., Fernandez, E., Rodriguez, J., Zabala, L., 2004. Significance and mechanisms of photosynthetic production of dissolved organic carbon in a coastal eutrophic ecosystem. *Limnology and Oceanography* 49(5), 1652-1666.
- Mari, X. (1999). Carbon content and C: N ratio of transparent exopolymeric particles (TEP) produced by bubbling exudates of diatoms. *Marine Ecology Progress Series*, 183, 59-71.

- Mari, X. (2008). Does ocean acidification induce an upward flux of marine aggregates?. *Biogeosciences Discussions*, 5(2), 1631-1654.
- Mari, X., & Kiørboe, T. (1996). Abundance, size distribution and bacterial colonization of transparent exopolymeric particles (TEP) during spring in the Kattegat. *Journal of Plankton Research*, 18(6), 969-986.
- Mari, X., & Burd, A. (1998). Seasonal size spectra of transparent exopolymeric particles (TEP) in a coastal sea and comparison with those predicted using coagulation theory. *Marine Ecology Progress Series*, 163, 63–76. <https://doi.org/10.3354/meps163063>
- Mari, X., Beauvais, S., Lemée, R., & Pedrotti, M. L. (2001). Non-Redfield C: N ratio of transparent exopolymeric particles in the northwestern Mediterranean Sea. *Limnology and Oceanography*, 46(7), 1831-1836.
- Mari, X., Rassoulzadegan, F., & Brussaard, C. P. (2004). Role of TEP in the microbial food web structure. II. Influence on the ciliate community structure. *Marine Ecology Progress Series*, 279, 23-32.
- Mari, X., Rassoulzadegan, F., Brussaard, C. P., & Wassmann, P. (2005). Dynamics of transparent exopolymeric particles (TEP) production by *Phaeocystis globosa* under N-or P-limitation: a controlling factor of the retention/export balance. *Harmful algae*, 4(5), 895-914.
- Mari, X., Passow, U., Migon, C., Burd, A. B., & Legendre, L. (2017). Transparent exopolymer particles: Effects on carbon cycling in the ocean. *Progress in Oceanography*, 151, 13–37. <https://doi.org/10.1016/j.pocean.2016.11.002>
- Martin, P., Lampitt, R. S., Jane Perry, M., Sanders, R., Lee, C., & D'Asaro, E. (2011). Export and mesopelagic particle flux during a North Atlantic spring diatom bloom. *Deep-Sea Research Part I: Oceanographic Research Papers*, 58(4), 338–349. <https://doi.org/10.1016/j.dsr.2011.01.006>
- Michaels, A. F., & Silver, M. W. (1988). Primary production, sinking fluxes and the microbial food web. *Deep Sea Research Part A. Oceanographic Research Papers*, 35(4), 473-490.
- Mopper, K., Zhou, J., Ramana, K. S., Passow, U., Dam, H. G., & Drapeau, D. T. (1995). The role of surface-active carbohydrates in the flocculation of a diatom bloom in a mesocosm. *Deep-Sea Research II*, 42, 47–73.
- Morison, F., & Menden-Deuer, S. (2015). Early spring phytoplankton dynamics in the subpolar North Atlantic: The influence of protistan herbivory. *Limnology and Oceanography*, 60(4), 1298-1313.

- Morison, F., & Menden-Deuer, S. (2017). Doing more with less? Balancing sampling resolution and effort in measurements of protistan growth and grazing-rates. *Limnology and Oceanography: Methods*, 15(9), 794–809. <https://doi.org/10.1002/lom3.10200>
- Murnane RJ. 1994. Determination of thorium and particulate matter cycling parameters at station P: a re- analysis and comparison of least squares techniques. *J. Geophys. Res.* 99:3393–405
- Nagata, T., 2000. Production mechanisms of dissolved organic matter. In: Kirchman, D.L. (Ed.), *Microbial Ecology of the Oceans*. Wiley-Liss, Inc., New York, pp. 121-152.
- Neuer, S., Deng, W., Cruz, B. N., & Monks, L. (2016). Marine Synechococcus Aggregation. In American Geophysical Union, Ocean Sciences Meeting 2016, abstract# B24A-0320.
- Nissimov, J. I., Vandzura, R., Johns, C. T., Natale, F., Haramaty, L., & Bidle, K. D. (2018). Dynamics of transparent exopolymer particle production and aggregation during viral infection of the.
- Nodder, S.D., Waite, A.M., 2001. Is Southern Ocean organic carbon and biogenic silica export enhanced by iron-stimulated increases in biological production? Sediment trap results from SOIREE. *Deep-Sea Research II* 48(11-12), 2681-2701, doi:10.1016/S0967-0645(01)00014-5.
- Obernosterer, I., & Herndl, G. J. (1995). Phytoplankton extracellular release and bacterial growth: Dependence on the inorganic N:P ratio. *Marine Ecology Progress Series*, 116, 247–257.
- Oguz, T. (2017). Modeling aggregate dynamics of transparent exopolymer particles (TEP) and their interactions with a pelagic food web. *Marine Ecology Progress Series*, 582, 15-31.
- Ortega-Retuerta, E., Reche, I., Pulido-Villena, E., Agustí, S., & Duarte, C. M. (2009). Uncoupled distributions of transparent exopolymer particles (TEP) and dissolved carbohydrates in the Southern Ocean. *Marine Chemistry*, 115(1–2), 59–65. <https://doi.org/10.1016/j.marchem.2009.06.004>
- Ortega-Retuerta, E., Marrasé, C., Muñoz-Fernández, A., Sala, M. M., Simó, R., & Gasol, J. M. (2018). Seasonal dynamics of transparent exopolymer particles (TEP) and their drivers in the coastal NW Mediterranean Sea. *Science of The Total Environment*, 631, 180-190.
- Paffenhöfer, G. A., & Strickland, J. D. H. (1970). A note on the feeding of *Calanus helgolandicus* on detritus. *Marine Biology*, 5(2), 97-99.
- Passow, U., & Alldredge, A. L. (1994). Distribution, size and bacterial colonization of transparent exopolymer particles (TEP) in the ocean. *Marine Ecology Progress Series*, 185-198.

- Passow, U., Alldredge, A. L., & Logan, B. E. (1994). The role of particulate carbohydrate exudates in the flocculation of diatom blooms. *Deep Sea Research Part I: Oceanographic Research Papers*, 41(2), 335-357.
- Passow, U., & Alldredge, A. L. (1995). A dye-binding assay for the spectrophotometric measurement of transparent exopolymer particles (TEP). *Limnology and Oceanography*, 40(7), 1326–1335.
- Passow, U., Kozlowski, W. E. N. D. Y., & Vernet, M. A. R. I. A. (1995). Palmer LTER: temporal variability of transparent exopolymer particles in Arthur Harbor during the 1994–1995 growth season. *Antarctic Journal of the United States*, 30(5), 265-266.
- Passow, U., & Alldredge, A. L. (1999). Do transparent exopolymer particles (TEP) inhibit grazing by the euphausiid *Euphausia pacifica*? *Journal of Plankton Research*, 21(11), 2203–2217. <https://doi.org/10.1093/plankt/21.11.2203>
- Passow, U. (2000). Formation of transparent exopolymer particles, TEP, from dissolved precursor material. *Marine Ecology Progress Series*, 192, 1-11.
- Passow, U., Shipe, R. F., Murray, A., Pak, D. K., Brzezinski, M. A., & 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. [https://doi.org/10.1016/S0278-4343\(00\)00101-1](https://doi.org/10.1016/S0278-4343(00)00101-1)
- Passow, U. (2002a). Production of transparent exopolymer particles (TEP) by phyto-and bacterioplankton. *Marine Ecology Progress Series*, 236, 1-12.
- Passow, U. (2002b). Transparent exopolymer particles (TEP) in aquatic environments. *Progress in oceanography*, 55(3-4), 287-333.
- Passow, U. (2012). The abiotic formation of TEP under different ocean acidification scenarios. *Marine Chemistry*, 128–129, 72–80. <https://doi.org/10.1016/j.marchem.2011.10.004>
- Passow, U., & Carlson, C. A. (2012). The biological pump in a high CO₂ world. *Marine Ecology Progress Series*, 470, 249-271.
- Plattner, G.-K., Gruber, N., Frenzel, H., McWilliams, J.C., 2005. Decoupling marine export production from new production. *Geophysical Research Letters* 32, L11612, doi:10.1029/2005GL022660.
- Ploug, H., & Passow, U. (2007). Direct measurement of diffusivity within diatom aggregates containing transparent exopolymer particles. *Limnology and oceanography*, 52(1), 1-6.
- Ploug, H., Iversen, M. H., & Fischer, G. (2008). Ballast, sinking velocity, and apparent diffusivity within marine snow and zooplankton fecal pellets: Implications for substrate turnover by attached bacteria. *Limnology and Oceanography*, 53(5), 1878-1886.

- Poulsen, L. K., & Iversen, M. H. (2008). Degradation of copepod fecal pellets: key role of protozooplankton. *Marine Ecology Progress Series*, 367, 1-13.
- Pruppacher, H. R., & Klett, J. D. (2012). *Microphysics of Clouds and Precipitation: Reprinted 1980*. Springer Science & Business Media.
- Pugnetti, A., Armeni, M., Camatti, E., Crevatin, E., Dell'Anno, A., Del Negro, P., Milandri, A., Socal, G., Umani, S.F., Danovaro, R., 2005. Imbalance between phytoplankton production and bacterial carbon demand in relation to mucilage formation in the Northern Adriatic Sea. *Science of the Total Environment* 353(1-3), 162-177, doi:10.1016/j.scitotenv.2005.09.014.
- Radić, T., Ivančić, I., Fuks, D., & Radić, J. (2006). Marine bacterioplankton production of polysaccharidic and proteinaceous particles under different nutrient regimes. *FEMS Microbiology Ecology*, 58(3), 333–342. <https://doi.org/10.1111/j.1574-6941.2006.00176.x>
- Riebesell, U., Reigstad, M., Wassmann, P., Noji, T., & Passow, U. (1995). On the trophic fate of *Phaeocystis pouchetii* (Hariot): VI. Significance of *Phaeocystis*-derived mucus for vertical flux. *Netherlands Journal of Sea Research*, 33(2), 193-203.
- Riebesell, U., Körtzinger, A., & Oschlies, A. (2009). Sensitivities of marine carbon fluxes to ocean change. *Proceedings of the National Academy of Sciences of the United States of America*, 106(49), 20602-20609.
- Rochelle-Newall, E. J., & Fisher, T. R. (2002). Chromophoric dissolved organic matter and dissolved organic carbon in Chesapeake Bay. *Marine Chemistry*, 77(1), 23-41.
- Rochelle-Newall, E. J., Mari, X., & Pringault, O. (2010). Sticking properties of transparent exopolymeric particles (TEP) during aging and biodegradation. *Journal of Plankton Research*, 32(10), 1433–1442. <https://doi.org/10.1093/plankt/fbq060>
- Roshan S., DeVries, T. (2017). Efficient dissolved organic carbon production and export in the oligotrophic ocean. *Nature communications*, 8, Article number 2036.
- Ruiz J. 1997. What generates daily cycles of marine snow? *Deep-Sea Res. I* 44:1105–26
- Schartau, M., Engel, A., Schröter, J., Thoms, S., Völker, C., & Wolf-Gladrow, D. (2007). Modelling carbon overconsumption and the formation of extracellular particulate organic carbon. *Biogeosciences Discussions*, 4(1), 13-67.
- Schuster, S., Herndl, G.J., 1995. Formation and significance of transparent exopolymeric particles in the northern Adriatic Sea. *Marine Ecology Progress Series* 124(1-3), 227-236, doi:10.3354/meps124227.

- Selph, K. E., M. R. Landry, A. G. Taylor, A. Gutierrez-Rodriguez, M. R. Stukel, J. Wokuluk, and A. Pasulka (2015), Phytoplankton production and taxon-specific growth rates in the Costa Rica Dome, *J. Plankton Res.*, doi:10.1093/plankt/fbv063, in press
- Shanks, Alan & Trent, Jonathan. (1980). Marine snow: sinking rates and potential role in vertical flux. *Deep Sea Research Part A. Oceanographic Research Papers*. 27. 137-143. 10.1016/0198-0149(80)90092-8.
- Sheldon RW, Prakash A, Sutcliffe WH Jr. 1972. The size distribution of particles in the ocean. *Limnol. Oceanogr.* 17:327–339
- Siegel, D. A., Buesseler, K. O., Doney, S. C., Sailley, S. F., Behrenfeld, M. J., & Boyd, P. W. (2014). Global assessment of ocean carbon export by combining satellite observations and food-web models. *Global Biogeochemical Cycles*, 28(3), 181-196.
- Smith, D. C., Steward, G. F., Long, R. A., & Azam, F. (1995). Bacterial mediation of carbon fluxes during a diatom bloom in a mesocosm. *Deep-Sea Research II*, 42,75–97.
- Sorensen, J. C., & Siegel, D. A. (2001). Variability of the effective quantum yield for carbon assimilation in the Sargasso Sea. *Deep Sea Research Part II: Topical Studies in Oceanography*, 48(8-9), 2005-2035.
- Stoderegger, K., & Herndl, G. J. (1998). Production and release of bacterial capsular material and its subsequent utilization by marine bacterioplankton. *Limnology and Oceanography*, 43(5), 877-884.
- Stoderegger, K.E., 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.
- Strom, S. L., & Fredrickson, K. A. (2008). Intense stratification leads to phytoplankton nutrient limitation and reduced microzooplankton grazing in the southeastern Bering Sea. *Deep Sea Research Part II: Topical Studies in Oceanography*, 55(16-17), 1761-1774.
- Stukel, M. R., Ohman, M. D., Benitez-Nelson, C. R., & Landry, M. R. (2013). Contributions of mesozooplankton to vertical carbon export in a coastal upwelling system. *Marine Ecology Progress Series*, 491, 47-65.
- Stukel, M. R., Kahru, M., Benitez-Nelson, C. R., Décima, M., Goericke, R., Landry, M. R., et al. (2015). Using Lagrangian-based process studies to test satellite algorithms of vertical carbon flux in the eastern North Pacific Ocean. *J. Geophys. Res. Oceans*. 120, 7208–7222. doi: 10.1002/2015jc011264
- Sugimoto, K., Fukuda, H., Baki, M. A., & Koike, I. (2007). Bacterial contributions to formation of transparent exopolymer particles (TEP) and seasonal trends in coastal waters of

- Sagami Bay, Japan. *Aquatic Microbial Ecology*, 46(1), 31–41.
<https://doi.org/10.3354/ame046031>
- Taucher, J., Schulz, K. G., Dittmar, T., Sommer, U., Oeschlies, A., & Riebesell, U. (2012). Enhanced carbon overconsumption in response to increasing temperatures during a mesocosm experiment. *Biogeosciences (BG)*, 9, 3531–3545.
- Teira, E., Pazo, M.J., Serret, P., Fernandez, E., 2001. Dissolved organic carbon production by microbial populations in the Atlantic Ocean. *Limnology and Oceanography* 46(6), 1370–1377.
- Thomas, J.P., 1971. Release of dissolved organic matter from natural populations of marine phytoplankton. *Marine Biology* 11(4), 311–323, doi:10.1007/BF00352449.
- Thompson, P. A., Guo, M. X., & Harrison, P. J. (1992). EFFECTS OF VARIATION IN TEMPERATURE. I. ON THE BIOCHEMICAL COMPOSITION OF EIGHT SPECIES OF MARINE PHYTOPLANKTON 1. *Journal of Phycology*, 28(4), 481–488.
- Toggweiler, J. R. (1993). Carbon overconsumption. *Nature*, 363(6426), 210.
- Tranvik, L. J., Sherr, E., & Sherr, B. F. (1993). Uptake and utilization of colloidal DOM by heterotrophic flagellates in seawater.
- Turner, J. T. (2015). Zooplankton fecal pellets, marine snow, phytodetritus and the ocean's biological pump. *Progress in Oceanography*, 130, 205–248.
<https://doi.org/10.1016/j.pocean.2014.08.005>
- Van Boekel, W. H. M., Hansen, F. C., Riegman, R., & Bak, R. P. M. (1992). Lysis-induced decline of a *Phaeocystis* spring bloom and coupling with the microbial foodweb. *Marine Ecology Progress Series*, 81(3), 269–276. <https://doi.org/10.3354/meps081269>
- Van Oostende, N., Moerdijk-Poortvliet, T. C., Boschker, H. T., Vyverman, W., & Sabbe, K. (2013). Release of dissolved carbohydrates by *Emiliania huxleyi* and formation of transparent exopolymer particles depend on algal life cycle and bacterial activity. *Environmental microbiology*, 15(5), 1514–1531.
- Vardi, A., Haramaty, L., Van Mooy, B. A. S., Fredricks, H. F., Kimmance, S. A., Larsen, A., & Bidle, K. D. (2012). Host-virus dynamics and subcellular controls of cell fate in a natural coccolithophore population. *Proceedings of the National Academy of Sciences*, 109(47), 19327–19332. <https://doi.org/10.1073/pnas.1208895109>
- Wetz, M. S., & Wheeler, P. A. (2003). Production and partitioning of organic matter during simulated phytoplankton blooms. *Limnology and Oceanography*, 48(5), 1808–1817.
- Wild, C., Huettel, M., Klueter, A. et al. (2004) Coral mucus functions as an energy carrier and particle trap in the reef ecosystem. *Nature*, 428, 66–70.

- Williams, P. J. L. (1995). Evidence for the seasonal accumulation of carbon-rich dissolved organic material, its scale in comparison with changes in particulate material and the consequential effect on net CN assimilation ratios. *Marine Chemistry*, 51(1), 17-29.
- Wohlers, J., Engel, A., Zöllner, E., Breithaupt, P., Jürgens, K., Hoppe, H. G., ... & Riebesell, U. (2009). Changes in biogenic carbon flow in response to sea surface warming. *Proceedings of the national academy of sciences*, 106(17), 7067-7072.
- Worden, A. Z., & Binder, B. J. (2003). Application of dilution experiments for measuring growth and mortality rates among *Prochlorococcus* and *Synechococcus* populations in oligotrophic environments. *Aquatic Microbial Ecology*, 30(2), 159-174.
- Worm, J., & Søndergaard, M. (1998). Alcian blue-stained particles in a eutrophic lake. *Journal of plankton research*, 20(2), 179-186.
- Wurl, O., Wurl, E., Miller, L., Johnson, K., & Vagle, S. (2011). Formation and global distribution of sea-surface microlayers. *Biogeosciences*, 8(1), 121–135.
<https://doi.org/10.5194/bg-8-121-2011>
- Wurl, O., & Cunliffe, M. (2016). Transparent exopolymeric particles: an important EPS component in seawater. *The Perfect Slime: Microbial Extracellular Polymeric Substances (EPS)*, 249.
- Zhou, J., Mopper, K., & Passow, U. (1998). The role of surface-active carbohydrates in the formation of transparent exopolymer particles by bubble adsorption of seawater. *Limnology and Oceanography*, 43, 1860–1871.

SUPPLEMENTAL INFORMATION

Table S1: Examination of environmental variables. Comparisons of variation in gross TEP production and TEP consumption rates to environmental variables such as nutrient condition, chlorophyll-normalized TEP concentration, and particulate organic carbon (POC).

	TEP _{k1}	TEP _g	TEP _μ
Replete vs Time	<0.0001	<0.0001	<0.0001
LOP vs Time	<0.0001	<0.0001	<0.0001
Replete vs LOP	0.006	<0.0001	0.944
14-May	0.0651	>0.9999	0.1397
16-May	0.2393	<0.0001	<0.0001
18-May	0.9388	0.0014	0.8038
20-May	<0.0001	<0.0001	>0.9999
22-May	0.0085	>0.9999	0.0261
24-May	<0.0001	<0.0001	0.4956
26-May	0.8974	<0.0001	<0.0001
28-May	<0.0001	0.0045	<0.0001
30-May	<0.0001	>0.9999	<0.0001