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Proportion of marine organic carbon present in self-assembled gels along the subtropical front and its increase in response to reduced pH

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ABSTRACT

Dissolved organic matter (DOM), the largest marine reservoir of reduced carbon, which contains a complex mix of polydisperse biopolymers and particularly small molecules in solution. However, we now know that about 10% of these dissolved moieties can spontaneously aggregate forming a huge mass of highly bioreactive microscopic particles by forming self-assembled gels (SAG) with a significant role in global carbon cycling. However, the effect of varying marine environments on DOM self-assembly remains virtually unknown. Here we report variations of the fraction of DOM that self-assemble forming gels (%SAG) measured in seawater samples collected during two different periods — before and during the autumn bloom— along the Subtropical Frontal Zone off New Zealand; an area characterized by strong spatiotemporal physicochemical and biological variability. Results show that %SAG varies in time and space. Measurements of %SAG ranged between 9–28% (14.5 ± 5, mean ± SD), and this broad variability could be partly associated to changes in phytoplankton biomass and/or UV radiation. Additional studies on the effect of a pH on the %SAG, reveal that small pH reductions of 0.3 units can virtually double the %SAG from 14.5 ± 5 to 27 ± 7% (12.5 ± 4%, mean ± SD). The observed increase in the %SAG under reduced pH was observed in all samples, irrespectively of their origin. This outcome suggests that pH might be a critical parameter controlling the formation of gels in marine environments. Decreased pH increases the SW concentration of free ionized Ca ($[Ca^{+2}]$), the prime DOM crosslinker. Rising $[Ca^{+2}]$ is likely responsible for the observed %SAG increase. The outcome that %SAG can be strongly affected by small changes in seawater properties, suggest that DOM self-assembly is remarkably responsive to subtle variations of environmental conditions. SAG are a leading source of bacterial nutrition and these observations have potentially strong ramifications in the ecology and biogeochemical cycles of the oceans.

1. Introduction

The photosynthetic machinery of the ocean plays a critical role in the biogeochemical cycling of carbon on our planet. This gigantic photo-reactor feeds the ocean with one of the largest stocks of organic carbon on Earth (Hedges, 1992). Two parallel biological systems linked by a polymer system interact in the ocean. A fuel source bioreactor is fed by solar energy and a sink bioreactor burns this fuel generating new biomass that is then recycled as new fuel. Organic fuels released by these marine bioreactors are polymeric in nature, and the energy pipeline between source and sink is a dynamic transport system consisting of marine biopolymers. Biopolymers make up the bulk of the 10^{12} tons of dissolved organic carbon (DOC) found in the ocean comprising about one third of the whole organic carbon stock found in our planet (Hedges, 1992). This pool is equivalent to the carbon present in atmospheric CO_2, in terrestrial biomass, and soil humus (Hedges, 1992). However, prior to complete intracellular metabolization and entry into the carbon cycle, biomolecules must be cleaved to ≤600 Da to cross prokaryotes cell walls, i.e., polysaccharides and proteins from cellular detritus or from secreted exopolymers must be broken down to small oligosaccharides and oligopeptides. In the ocean, much of this molecular dismantling is accomplished by prokaryotic extracellular enzymes (Chrost, 1990; Smith et al., 1992). Nutrients carried through this "bacterial loop" become available for conversion back to living particulate form either through photosynthesis or via transfer of bacterial production up food webs through protists and zooplankton. The continuous transfer of dissolved moieties to self-assembled gels (SAG) and subsequent biologically mediated cycling between SAG and microorganisms is critical on a larger scale to the transfer and fate of nutrients because only living particles can sink to selectively transport bioactive elements from the

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lighted surface ocean into deep storage below the thermocline ("biological pump mechanism"). Dissolved molecules that for some reason get "stranded" between the living and assimilable size extremes (~1000–1 nm), comprise an abundant form of remnant biochemical in the ocean, offsetting the total living biomass by a factor of roughly 200 (Hedges and Oades, 1997). However, the bulk of DOM (~77%) has a molecular mass of <600 Da (Kaiser and Benner, 2009) and while readily assimilable owing to their small size, at the low micromolar concentration found in SW these molecules are not readily accessible to bacteria; in fact the bulk of refractory DOM is found in this small size pool of DOM. A corollary to Hedges argument is that – regardless of the complex and abundant taxonomy of susceptible chemical compounds and corresponding bacterial genes that code for enzymes to cleave specific chemical bonds (Moran et al., 2016) – if DOM self-assembled gels were a main access pathway to microbial nutrition and degradation of DOM, the most abundant residues left behind should be either oligomers too large to be incorporated by bacteria but too small to self-assemble forming gels. In the bulk SW however, these monomers reach only micromolar concentrations which drastically limit their incorporation to bacteria. Consistent with Hedges argument these small oligomers and monomers should progressively increase in time; presently they are among the most abundant moieties in the pool of refractory old organic matter particularly found in the deep ocean, where ~70% of the carbon sequestered in DOM is made out of smaller moieties comprising a major reservoir in the global carbon cycle (Hedges, 1992).

The notion that the bulk of free DOC polymers are not directly available to the marine microbial loop is now well established. Work by several groups shows that prokaryotes feed principally on colloidal-nanometer and micron-size polymer gels (Amon and Benner, 1994; Johnson and Kepkay, 1992; Orellana et al., 2000; Skoog et al., 1999). A critical source-sink mass transfer mechanism whereby free DOC polymers self-assemble forming discrete gels of high substrate concentration that become available to microorganisms has been shown to be present in the ocean. Laboratory results first demonstrated that free DOC polymers can spontaneously assemble forming colloidal nanogels and microgels. This process is reversible, and at equilibrium about 10% (~10^{11} tons of organic carbon) of the massive stock of DOC polymers could potentially self-assemble in seawater (Chin et al., 1998; Ding et al., 2007; Orellana et al., 2011; Verdugo et al., 2008). Laboratory and field studies indicate that highly bioreactive SAG can be readily colonized by bacteria (Nagata et al., 2010; Verdugo, 2012; Verdugo et al., 2008; Verdugo and Santschi, 2010). These findings have fundamentally changed the way that oceanographers think about processes linking the microbial loop and biological pump to the rest of the biosphere and the geosphere (Wells, 1998).

In agreement with previous data of DOM self-assembly (Chin et al., 1998) and with rates of ^{234}Th pumping from colloidal to particulate size (Guo et al., 2002; Moran and Buesseler, 1992), profiles from 4000 m to surface in the open Atlantic and Pacific Oceans and in coastal waters of the Puget Sound have reported SAG concentrations from ~1×10^6 to ~3×10^{12} SAGL^{-1} (Ding et al., 2007; Verdugo et al., 2008). These results suggest that up to 7×10^{16} g of organic carbon may be present as SAG in the world oceans. These figures amount to a mass ~10 to 50 times bigger than the global biomass of marine organisms (Begon et al., 1996). Considering that gel formation increases bioreactivity (Amon and Benner, 1994; Kirchman et al., 1991; Moon et al., 2007; Orellana et al., 2000), SAG could well be among the richest pools of bio-reactive carbon on our planet. Changes in the %SAG yield may have ramifications scaling from the microbial loop to the biogeochemical cycles and global climate (Verdugo et al., 2004; Wells, 1998).

DOC self-assembly follows standard mass law whereby biopolymers remain in a dynamic and reversible assembly-dispersion equilibrium. Assembled polymers are held together by Ca^{2+} crosslink. They disassemble and disperse in Ca^{2+}-free seawater. Like other polymer gels (De Gennes and Leger, 1982; Dušek and Patterson, 1968; Tanaka, 1981) minute changes in physical or chemical parameters cause abrupt microgel volume phase transitions (Chin et al., 1998; De Gennes and Leger, 1982). However, despite SAG comprising a huge mass of marine organic carbon and playing a critical role in global carbon cycling, field studies addressing the effect of physical and chemical environmental fluctuations on DOC self-assembly and corresponding spatiotemporal variability of %SAG have received very little attention. Based on the hypothesis that fluctuations of environmental parameter may induce corresponding changes in the %SAG, here we studied fluctuations of %SAG along a transect characterized by strong spatial and temporal changes in physicochemical and biological parameters (e.g. temperature, nutrients, pH, chlorophyll-*a*).

2. Material and methods

2.1. Study site and sampling

Samples were collected along the Munida Time Series Transect (off Otago, New Zealand) (Fig. 1). This transect crosses the Southland Front, the coastal manifestation of the Subtropical Frontal Zone (Butler et al., 1992; Currie et al., 2011; Hawke, 1989; Heath, 1985; Jillett, 1969; Sutton, 2003). This transects gives us access to three contrasting water masses (i.e., modified subtropical waters, sub-Antarctic waters and near coastal neritic waters) in a very short distance offshore. This site has been continuously sampled for physicochemical parameters for almost two decades (Currie and Hunter, 1999; Currie et al., 2011). Samples for this study were collected in two single-day cruises, on the 10th of March and the 21st of April of 2015, on board the RV Polaris II, and targeted 8 stations matching with previous research (Baltar et al., 2016; Baltar et al., 2015; Currie et al., 2011; Gault-Ringold et al., 2012; Jones et al., 2013). Continuous surface temperature and salinity measurements were collected along the transect using a Sea Bird SBE45 thermosalinograph and associated SBE38 remote temperature sensor, with position information appended from the vessel GPS system. At each station, surface samples were collected in 5 L acid-rinsed plastic bottles by means of an onboard continuous pump with the inlet located approximately 2 m below the surface. During each of the cruises, samples were also collected along a depth profile (from surface to 500 m deep) at the deepest station (Station 1) using a SBE 25s V2 SEACAT Profiler CTD with a rosette configuration of four to six 12 L Niskin Bottles.

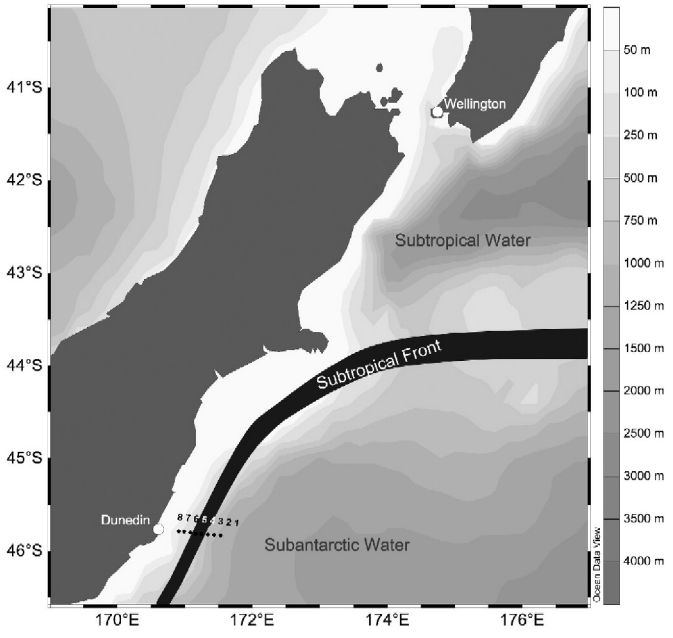

Fig. 1. Map showing the oceanographic settings around the Southern Island of New Zealand and the position of the transect and sampling stations. Note that Station 1 is the farthest from the coast.

2.2. Inorganic nutrients

Water samples for nitrogen-nitrate (hereafter referred to as nitrate), dissolved reactive phosphorous (phosphate), and dissolved reactive silica (silicate) analysis were collected from the CTD bottle casts and surface supply intake system using the Joint Global Ocean Flux Study (JGOFS) recommended procedures (Knap et al., 1996). Samples were colourimetrically-analyzed for nutrient concentrations using flow-injection analysis on a Lachat Auto-analyzer according to the methods of (Parsons et al., 1984).

2.3. Chlorophyll-a concentration

Samples for chlorophyll-a (chl-*a*) concentration were filtered on Whatman GF/F 0.7 μm filters on-board, using low vacuum (e.g. <200 mm Hg) to prevent breaking of algal cells. Following filtration, these filters were folded and placed into pre-labeled 15 mL polypropylene centrifuge tubes and were frozen in preparation for analysis. Chl-*a* filters were extracted in 90% acetone for approximately 16–24 h and measured using a pre-calibrated Turner Designs Fluorometer following the procedures outlined by (Parsons et al., 1984).

2.4. Proportion of organic carbon present in self-assembled gels (%SAG) in seawater

DOC self-assembly is a reversible process that can eventually vary depending upon environmental conditions. The method introduced by Ding et al. (2007) yields precise ratios of assembled to free DOC. The accuracy of this technique was validated against measurements of SAG concentration using flow cytometry, dynamic laser scattering spectroscopy, and measurements of total organic carbon carried out by high temperature (680 °C) oxidation using a Shimadzu TOC-V carbon analyzer (Ding et al. (2007)). In the present work, the focus is not to establish absolute values of assembled versus free DOC budgets but in finding if environmental conditions can indeed affect the equilibrium between self-assembled DOC and free DOC, and this is exactly what our results indicate. The method is based on Chlortetracycline (CTC) labeling which can be used as a tracer of bound Ca (Rudolf et al., 2003), and we previously validated its application to label SAG (Chin et al., 1998). This reagent selectively fluoresces (λ_{ex} = 374 nm, λ_{em} = 560 nm) when complexed with Ca bound to free DOC, or to DOC assembled in the matrix of microgels (Chin et al., 1998). However, fluorescence of CTC bound to SAG is significantly compared to CTC bound to free DOC.

Briefly, 40 mL samples of seawater were collected in 50 mL Eppendorf tubes and treated with 5.4 mM NaN_3 to inhibit microbial activity. Samples were refrigerated at 4 °C and processing continued on the following day. Each sample was gently mixed and gravity filtered through an 8 μm filter. Seawater samples were labeled with 10 μm CTC. Each sample was divided into six aliquots of 5 mL each; three were exposed to ethylenediaminetetraacetic acid (EDTA, 20 mM, pre-titrated at pH 8.2) to chelate Ca^{2+} and disperse gels, the other three were controls that, instead of EDTA, were supplied an equivalent volume of 0.22 μm filtered sample water. As mentioned in the original protocol (Ding et al., 2007), chelation of Ca^{2+} results in a decrease of pH (due to the release of H^+ from EDTA) that was carefully titrated back to its original value using NaOH. CTC fluorescence was excited at λ = 380 nm and its emission was collected at λ = 522 nm using a SpectraMax M3 (Molecular Devices, Sunnyvale, CA) spectrofluorometer at room temperature. The proportion of organic carbon present in self-assembled gels was calculated as the 'quenching fraction' (QF) from the increase in CTC emission between paired samples treated and not treated with EDTA, using the following expression:

$$QF = (DQCTCF - QCTCF)/DQCTCF \times 100) \quad (1)$$

where DQCTCF = CTC emission after EDTA chelation of Ca2 +; QCTCF = quenched CTC emission before EDTA chelation of Ca2 + .

To study the effect of pH reduction on the proportion of organic carbon found in self-assembled gels, the pH of control and the EDTA-containing subsamples was titrated down by 0.3 pH units using HCl. Previous reports have indicated that turnover of calcium carbonate in the shell of several species is affected differently when SW pH is titrated with acid as compared by lowering SW pH by increasing carbon dioxide (Iglesias-Rodriguez et al., 2008). Unfortunately, while bubbling of CO_2 can indeed accomplish SW acidification it also results in massive coagulation and formation of macrogels (Kepkay, 1994; Kepkay, 2000; Mopper et al., 1995). Notwithstanding potential constrains, our results show that lower SW pH increases SAG formation, and as pointed later, this outcome is consistent with increased ionic Ca at low SW pH. Although the bubbling with CO_2 gas seems to more accurately simulates future ocean acidification chemistry, the HCl-addition method has been also suggested to be an appropriate method for ocean acidification studies (Andersson and Mackenzie, 2012). Upon reaching the desired pH (i.e. in situ pH −0.3), the fluorescence of subsamples was again measured following the same protocol described above. The pH probe response was checked each day following standard procedures (Dickson et al., 2007).

2.5. Statistical analyses

The relations between biological variables were examined by means of correlation analysis computing Pearson pairwise statistics. Data were log transformed to equalize variance, and normality was checked with a Shapiro-Wilks test before Pearson correlations were calculated.

3. Results and discussion

3.1. Regional oceanographic settings

A strong variability was found in temperature, salinity and nutrients along the transect (Fig. 2), revealing the presence of the main water masses found in this region (i.e., neritic waters [NW], subtropical waters [STW] and subantarctic waters [SAW]) (Jillett, 1969). A sharp decrease in salinity in the coastal waters delimited the NW; which are characterized by low, and variable, salinity as well as variable seasonal temperatures due to the influence of riverine dilution and coastal processes (Croot and Hunter, 1998; Jillett, 1969). The coastal NW are adjacent to and mixed with the STW, which are characterized by the highest salinity values (Fig. 2B). Then, separating the STW and SAW, there is a strong decrease in temperature and salinity delimiting the position of the Subtropical Frontal Zone (Fig. 2A, B). The boundary positions between the different water masses determined in this study are consistent with previous oceanographic surveys (Baltar et al., 2016; Baltar et al., 2015; Currie et al., 2011; Jones et al., 2013; Van Hale and Frew, 2010). The location of the front is known to oscillate seasonally, being furthest inshore in the summer (ranging from 28–40 km offshore) and farthest offshore in the winter, ranging from 35 to 50 km (Jillett, 1969; Jones et al., 2013). However, the position of the front in this study, located between Station 6–4, did not change significantly between the two cruises due to short duration of this study (i.e., from the beginning of March to the end of April).

At this front, the macronutrient-limited (e.g. N, P) STW encounter the micronutrient-limited (e.g. Fe) SAW. This explains why the nutrient concentration increased by 3–8 times oceanward (Fig. 2D, E, F). The degree of macronutrient limitation in STW was more pronounced in March than in April, as shown by the lower nitrate and phosphate concentrations in March (Fig. 2). In this region, austral spring and summer seasons (September to February) coincide with the classic spring phytoplankton bloom period, and the late austral autumn (April–May) coincide with the autumn bloom (Jones et al., 2013). This can explain why the concentration of macronutrients was lower at the beginning of

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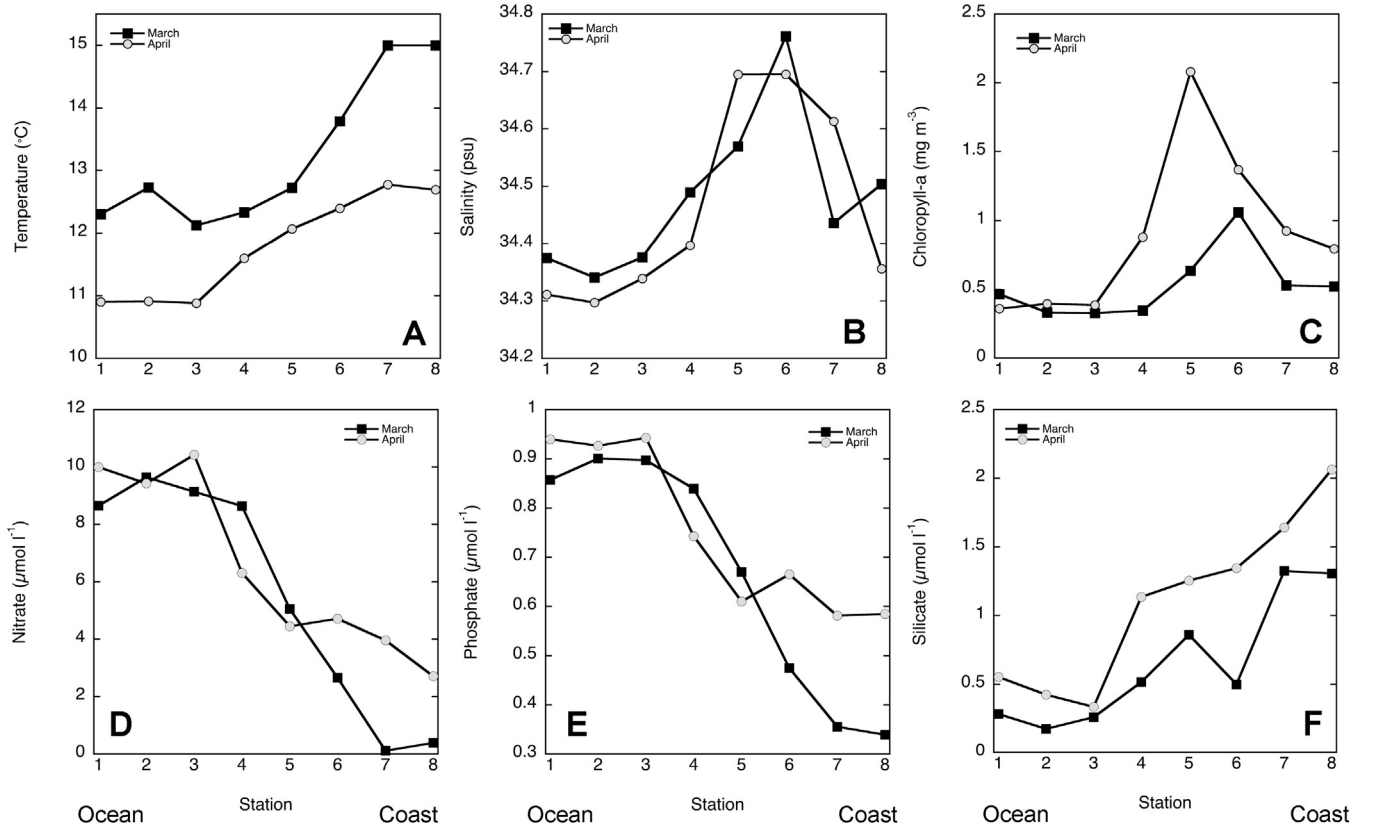

Fig. 2. Spatial variability along the surface transect in early March and late April of A) temperature (°C), B) salinity (psu), C) chlorophyll-*a* (mg m^{-3}), D) nitrate (μmol L^{-1}), E) phosphate (μmol L^{-1}), and F) silicate concentration (μmol L^{-1}). Note that the variability of the %SAG along the surface transect is shown in Fig. 4 (A, C).

March (after the end of the spring bloom) than in April (late autumn bloom was taking place). This is supported by the higher chlorophyll-*a* concentration found in April than in March (Fig. 2C). The temperature, salinity and nutrients patterns observed along the depth profiles were very similar in March and April (Fig. 3). Temperature decreased and salinity and nutrients increased with depth, a common pattern commonly found in deep ocean studies. Nutrient concentrations increased by 2–10 fold from surface to 200 m depth (Fig. 3 C, D, E). Overall, a strong variability in temperature, salinity, nutrients and chlorophyll-*a* were found in the region of study, with changes of up to one order of magnitude in nutrient concentration.

3.2. Proportion of organic carbon present in self-assembled gels at in situ pH

The in situ percentage of organic carbon present in self-assembled gels (%SAG) ranged from 9–28% (Fig. 4). These numbers are within the ranges found in the few available studies where the %SAG has been reported. A 10% yield of DOM assembly into SAG was found in the laboratory using 0.2-μm filtered seawater (Chin et al., 1998), a 6–13% yield was reported in a depth profile (from surface to bathypelagic waters) at station ALOHA (Ding et al., 2007; Verdugo et al., 2008), and an average yield of 32% (±15%; SD) was found in the surface waters of the high Arctic (Orellana et al., 2011). These numbers are also consistent with the 10–30% of ultra-filterable, colloidal DOC in the ocean (Baskaran et al., 1992; Guo and Santschi, 1977; Moran and Buesseler, 1992; Verdugo and Santschi, 2010).

This is the first study, to our knowledge, in which the %SAG has been quantified along a spatial transect, and also in the same stations but at different times. The region of study was selected due to its known strong spatial and temporal variability in physicochemical and biological parameters. This allows us to shed light on the degree of spatial and temporal variability of the %SAG. The %SAG variability in the surface waters along the transect was more pronounced in March (9.3–28.2%) than in April (9.7–14.6%), revealing, for the first time, that the %SAG shows temporal changes (Fig. 4). The highest %SAG was found in the coastal waters during March. No correlation was found between the %SAG and the physicochemical parameters analyzed in this study in March. The only significant correlation between %SAG and other parameters measured was in April, during the autumn bloom, when SW samples showed increased chlorophyll-*a* concentration (Pearson's $r = 0.70$, $N = 8$, $p < 0.005$). This positive relationship between phytoplankton biomass and the %SAG suggests an important role of the biology (phytoplankton) on the in situ formation and yield of SAG. The assembly of marine biopolymers depends on their concentration as it follows characteristic mass law. This is particularly significant for exopolymer substances (EPS) released by bacteria and phytoplankton. These biopolymers contain polyanionic polysaccharides and amphiphilic detergent-like moieties that can form both electrostatic Ca-crosslinks and hydrophobic bonds (Verdugo, 2012) that are critical in SAG formation (Ding et al., 2008; Engel et al., 2002; Passow, 2002; Verdugo, 2012; Wingender et al., 2012). Although field studies on the role of phytoplankton on DOC polymer assembly has not been explored, EPS exocytosed by phytoplankton are known to be an important source of freshly released undegraded polymers that can readily assemble forming marine gels (Chin et al., 2004; Quesada et al., 2006; Verdugo and Santschi, 2010). These observations, together with our results, confirm that phytoplankton are probably a major player in the formation of marine macro and microgels.

Amphiphilic exopolymers from bacteria are likely to play an important role in the formation of marine gels (Ding et al., 2008). Conversely however, bacteria are probably the main agent in marine gel degradation. Observations that bacteria are found in hot spots (Azam, 1998) agree with reports of bacteria clustering in marine gels (DeLong et al., 1993) suggesting that gels may comprise a rich source of marine microbial nutrients. In fact, while the nutrient concentration of DOC in SW is only ~10^{-3} g L^{-1} of SW, SAG contain ~10 g L^{-1} of gel of DOC substrate.

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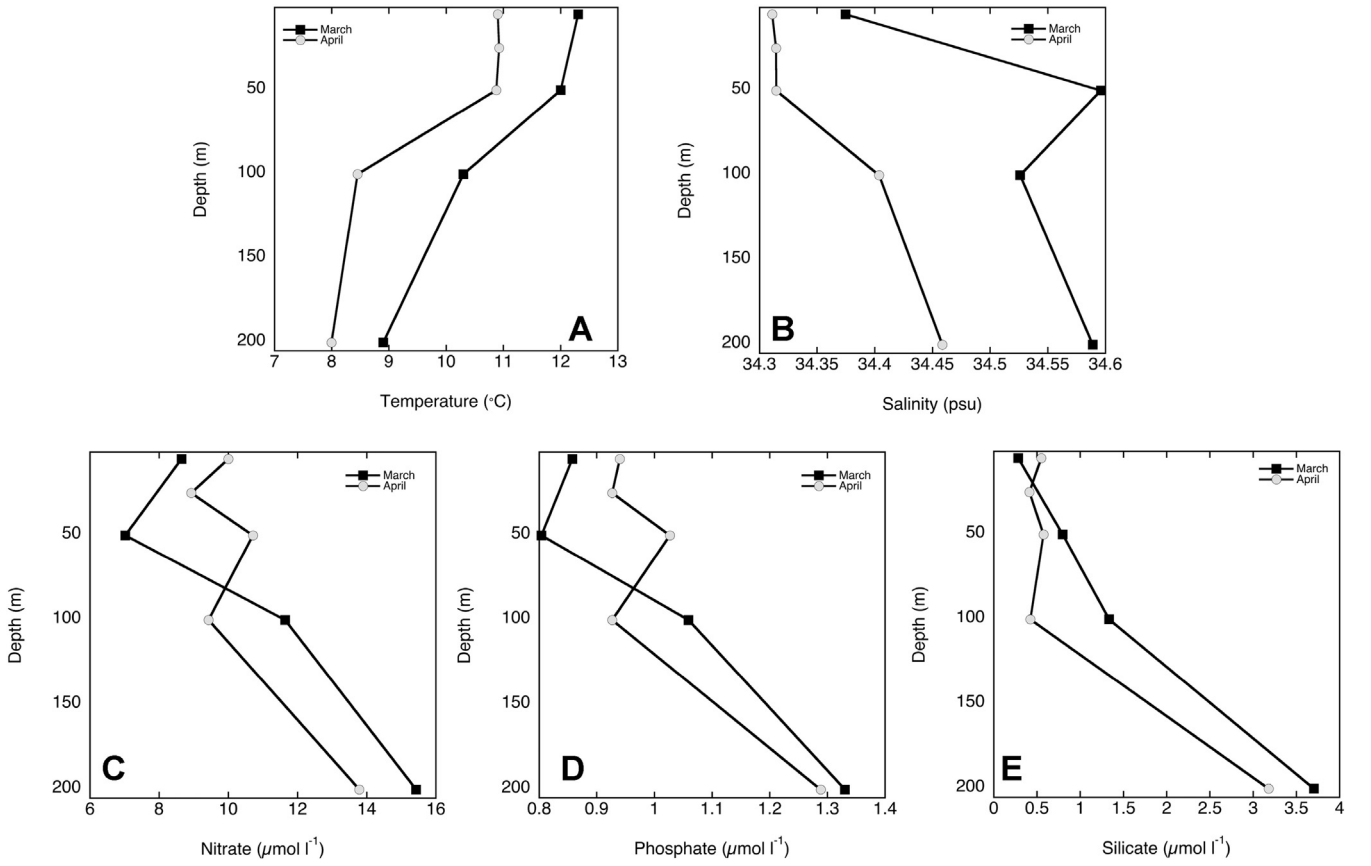

Fig. 3. Depth profile at the deepest station (Station 1) in early March and late April of A) temperature (°C), B) salinity (psu), C) nitrate (μmol l^{-1}), D) phosphate (μmol l^{-1}), and E) silicate concentration (μmol l^{-1}). Note that the depth profile at Station 1 of the %SAG along the surface transect is shown in Fig. 4 (B, D).

SAG are discrete spots containing high nutrient supply where bacteria can readily settle and use their exoenzymes to harvest and metabolize a rich substrate of cleaved oligomeric residues (Verdugo et al., 2008; Verdugo and Santschi, 2010). Confocal optical tomography of SAG collected by filtration from SW of the Puget Sound shows that bacteria inside microgels can reach ~8×10^8 bacteria mL^{-1} of gel while the concentration of bacteria in these SW samples was only ~1.2×10^6 bacteria L^{-1}; i.e., bacteria can probably reach concentrations 2–3 orders of magnitude higher inside gels than in bulk SW (Moon et al., 2007; Verdugo, 2012). The critical role of SAG as a source of bacterial substrate is further supported by findings that bacterial growth measured by thymidine incorporation is four times higher in SW containing SAG than in SW lacking SAG (Orellana et al., 2000). SAG can be also consumed by protists and larger organisms potentially bypassing the microbial loop (Orellana et al., 2003). These observations substantiate the notion that DOC self-assembly and SAG forming can virtually change established concepts about spatial and temporal dynamics of nutrients and organisms that sustain the microbial loop (Wells, 1998).

As observed in the surface transect, the strongest %SAG variability along the water column was observed in March. These results reveal, for the first time, temporal changes in the %SAG occurring also in the deep waters (mesopelagic). The lowest %SAG along the water column, in both cruises, were found in the surface waters (Fig. 4). In the only published %SAG depth profile, the lowest %SAG (around 6%) were also found at the surface waters (and at 500 m depth), but were higher (ranging from 9–13%) at all the other sampled depths (i.e., 50, 100, 200, 500, 1000 and 4000 m) (Ding et al., 2007; Verdugo et al., 2008). Lowest %SAG in surface waters is consistent with the strong effect of UV on SAG formation observed in laboratory experiments (Orellana and Verdugo, 2003). These authors found that exposure of DOC to short wave UV-B (at natural fluxes) results in extensive DOM polymer cracking, thereby changing the polydispersity of DOC to shorter chain length, and resulting in decreased yield of assembly, longer assembly times, and smaller SAG size. This is consistent with polymer networks theory predictions that assembly/dispersion dynamics of self-assembled tangle networks and the equilibrium size of the resulting gels depends on the second power of the length of the assembling polymers (Edwards, 1974; Edwards, 1985; Verdugo, 2012). Atomic Force Microscopy imaging of marine colloidal organic revealed abundant fibrillar biopolymers of modern radiocarbon age in the surface waters of the North Atlantic that were not evident at 550 m (Santschi et al., 1998), suggesting a potentially lower gel formation in the mesopelagic waters. However, as found in this and in the previous available study, the %SAG in the deep can be in the same order or greater than in the surface waters. Studies of DOM concentration through the water column are well established (Hansell and Carlson, 2001; Hansell and Carlson, 2002; Hopkinson and Vallino, 2005). However, changes of polymer size of marine biopolymers through the water column that may play a critical role in polymer self-assembly have not been investigated. Our observations are consistent with the idea that marine polymer size might change with depth. Surface water SAG formation might be more dependent on short chain DOM due to the effect of UV polymer cracking. Small moieties like those described by Santschi et al., 1998, might compose the stock present in small micro and nanogels recently found to readily migrate to the atmosphere (Orellana et al., 2011); whereas in deep waters not affected by UV radiation, larger biopolymers chains might form relatively bigger and more stable microgels. Biopolymer concentration and polymer size controlled by phytoplankton secretion on one hand and by microbial degradation and UV fluxes on the other, are likely to be important determining factors on control the self-assembly of DOM polymers and %SAG at different depths.

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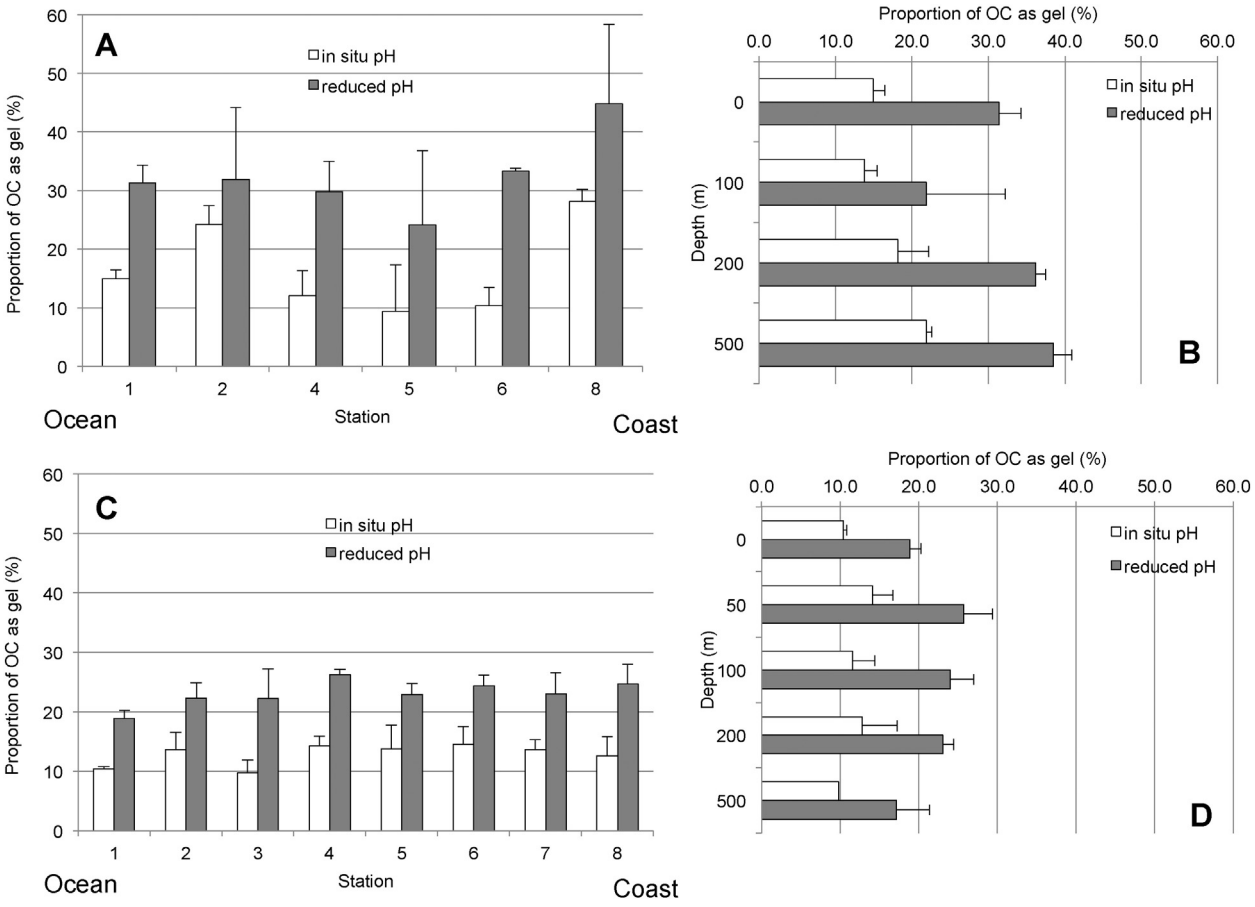

Fig. 4. Spatial variability along the surface transect (A, C) and depth profile at the deepest station (Station 1) (B, D) in early March (A, B) and late April (C, D) of the proportion of organic carbon (OC) found in self-assembled gels (%SAG) at in situ pH (white bars) and at 0.3 units-reduced pH using HCl (white plus grey bars). Error bars denote SD (N = 3). Upon reaching the desired pH, the fluorescence of subsamples (controls and EDTA containing subsamples) was measured. The proportion of organic carbon present in self-assembled gels was calculated as the 'quenching fraction' from the increase in CTC emission between paired samples treated and not treated with EDTA.

3.3. Effect of pH on the proportion of organic carbon present in self-assembled gels

When pH of SW samples was reduced by 0.3 units, the %SAG increased (almost doubled) from a global average of 14.5 ± 5 to 27 ± 7% (mean ± SD) (Fig. 4). Under low pH, the %SAG ranged from 17–45%. The observed increase in the %SAG under reduced pH (12.5 ± 4%, mean ± SD) was relatively conserved among all samples, irrespective of their origin. The mean %SAG increase in response to low pH was 15.5 ± 4.8% and 10.3 ± 1.8% in March and April, respectively. This conserved positive response of the %SAG to the pH reduction suggests that pH might be a critical parameter controlling the formation of gels in marine environments.

Self-assembled DOC polymers forming gels are crosslinked by Ca^{2+} bonds (Chin et al., 1998). The dissociation of calcium salts in seawater (e.g. carbonate, phosphate) increases as the pH decreases with a corresponding increase of free ionized Ca^{2+} available to crosslink free DOC polymers that may explain the increased %SAG yield under reduce pH.

3.4. Conclusions

The world oceans holds the largest pool of reduced carbon of our planet. The stock of DOC is comparable to the mass of carbon in atmospheric CO_2 and in terrestrial biomass and soil humus (Hedges, 1992). The predicted decrease of around 0.3 units in oceanic pH by the end of the century — in response to anthropogenic CO_2 emission (Brewer, 1997; Feely et al., 2009; Haugan and Drange, 1996) — could dramatically increase (double) the proportion of DOC present as assembled gels. This is critical, since marine gels are an important link for mass transfer in the ocean. Self-assembly of marine biopolymers move reduced carbon from a highly diluted DOM pool to form the discrete concentrated porous gels (Verdugo, 2012). Since gel formation increases bioreactivity (Verdugo, 2012; Verdugo et al., 2008), self-assembled gels may be among the richest pools of bioreactive carbon in our planet. Thus, the observed increase in the proportion of DOM that self-assemble forming gels (%SAG) could strongly influence the passage of DOC through the microbial loop with ramifications that may scale to global biogeochemical cycles (Verdugo, 2012; Wells, 1998).

Overall, we have found that the %SAG in surface and deep waters changes in time and space. The main factors controlling the %SAG seemed to be related to the phytoplankton biomass and the UV light. Nevertheless, despite strong changes in physicochemical parameters, the %SAG was constrained between 9–28%. This stability of the %SAG yield is not surprising considering that the concentration of DOC, and the concentration of Ca crosslinker, remain within narrow ranges in seawater. However, a reduction in pH (like the one predicted for the end of this century) could increase free ionized Ca and DOM polymers crosslinking with potential dramatic increase of %SAG and strong ramifications in the ecology and biogeochemical cycles of the oceans.

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