I - EXECUTIVE SUMMARY

This synthesis, based on inputs received from all the participants, was consolidated by Gerhard Herndl in the aftermath of the workshop.

TRANSFORMATION AND PERSISTENCE OF DOC

Introduction

The transformations and fate of dissolved organic molecules in marine systems have been the subject of numerous studies, yet clearly there are still important gaps in our understanding of this issue. Generally, more attention has been paid toward understanding biological processes, like microbial degradation and utilization, than abiotic processes, like photochemical degradation. Our view on the general reactivity of dissolved organic matter (DOM) in the ocean has changed dramatically over the past decade. It has been shown that the bulk of the oceanic DOM is below 1000 Da and this low molecular weight DOM is largely refractory to microbial degradation (Amon and Benner, 1996). Conversely, the DOM pool larger than 1000 Da consists of labile and semi-labile molecules preferentially utilized by prokaryotes, largely bacteria, although archaea have been shown to take up at least fractions of the DOM pool as well (Ouverney and Fuhrman, 2000; Herndl *et al.,* 2005).

Biological uptake of DOM by heterotrophic microorganisms, ultimately converts organic molecules to carbon dioxide and water. When molecules are too large (typically greater than 600 Da) to pass across bacterial cell membranes, they have to be first hydrolyzed by bacterial exoenzymes. The hydrolysis products are then transported into cells and metabolized. Photochemical degradation may lead directly to bioavailable DOM or indirectly affect the ability of organic molecules or their "conversion products" to persist in the water column. Here we consider the biological and abiotic processes that may govern the fate and persistence of dissolved organic compounds in the Mediterranean Sea.

Turnover of DOM in the Mediterranean Sea is likely to differ from that in other marine environments, for several reasons:

1) An essential characteristic of the surface waters of the Mediterranean Sea is that phosphorus (P) is the major nutrient limiting primary production (Krom *et al.,* 2004; Thingstad *et al.,* 2005). The limitation of P has important implications for the biotic processes determining the fate of autochthonous dissolved organic carbon (DOC), as bacterial degradation may be P-limited as well. The unusual N/P ratio in the Mediterranean could be due to the process of nitrogen fixation and/or the contribution of atmospheric deposition of nutrients (Herut *et al.,* 1999, 2002; Ridame and Guieu, 2002; Ridame *et al.,* 2003; Özsoy, 2003). Atmospheric input (Saharan dust) of N and P could cause an imbalance of N/P and contribute to a higher N/P ratio. The lack of significant denitrification also contributes to a high N/P ratio and to the highly unusual P limitation of the primary productivity in the eastern Mediterranean (Krom *et al.,* 2004). Moreover, atmospheric input can influence primary production (Bonnet and Guieu, 2004) and are important for the iron cycle in the Mediterranean Sea (Guieu *et al.,* 2002) and globally (Jickells *et al.,* 2005). The deposition of Saharan dust provides iron, which impacts succession and bloom formation and composition.

2) A distinct characteristic of the deep waters in the Mediterranean Sea is their relatively high temperature of 12°C, while deep waters in other oceanic basins generally have much lower temperatures, commonly between 2-4°C. The high temperatures in deep waters of the Mediterranean Sea likely increase the biological degradation of DOC by heterotrophic prokaryotes.

3) Atmospheric deposition, especially of Saharan dust, into the Mediterranean is high, and includes particles from natural and anthropogenic sources. It is an important source of various microelements and nutrients that might influence microbial community structure and activity. Because of a lack in our knowledge and understanding of these complex interactions and their impact on the marine biogeochemistry, biology and climate there is a need for more intense research in this area.

Seasonal accumulation of autochthonous DOC in the Mediterranean Sea

The accumulation of DOC in surface waters is a frequently observed phenomenon and it has been suggested that the lack of P inhibits the efficient biological degradation of DOC (Copin-Montegut and Avril, 1993; Thingstad *et al.,* 1997). In the North-Western Mediterranean Sea (DYFAMED-Site), the major part of autochthonously produced DOC is transported to deep waters. Copin-Montegut and Avril (1993) estimated that the input of surface DOC to deep waters is roughly 2-fold higher than the input of POC and represents therefore the principal export term. Thus, deep waters receive a substantial input of DOC that presumably is highly bioreactive.

The fate of this DOC in deep waters has not been studied so far. The biological degradation of DOC originating from the surface within the deep Mediterranean basin is probably enhanced by the mixing of the P-depleted surface waters with nutrient-rich deep waters. Differences in the prokaryotic community could support or retard the transformation and mineralization of surface DOC in deep waters of the Mediterranean Sea. A recent study from the Eastern Mediterranean Sea indicates that distinctly different prokaryotic communities, as determined by 16S rRNA fingerprints, are present in the surface, the intermediate and the deep water of the Eastern Mediterranean Sea (Moeseneder *et al.,* 2001). In the Sargasso Sea, DOC that accumulated during summer was shown to be degradable by a prokaryotic community originating from deep waters (Carlson *et al.,* 2004). This raises the question whether deep water prokaryotes have different functional capabilities for the degradation of DOC than prokaryotes from near-surface waters. The injection of surface DOC to deep waters could enhance the degradation of bio-resistant deep water DOC due to co-metabolism. This pathway, however, is poorly understood.

Allochthonous sources of DOC to the Mediterranean Sea

The Mediterranean Sea receives DOC from the Atlantic and the Black Sea, and of riverine and atmospheric origin (Figure 1). The only export of Mediterranean waters occurs through the Strait of Gibraltar. The DOC concentration of deep waters leaving the Mediterranean basin is lower than that of incoming Atlantic waters (Dafner *et al.,* 2001a), thus the net inflow of DOC from the neighboring oceans is larger than the outflow.

DOC also enters the Mediterranean Sea via river inflow but, as in other regions, some riverine DOC is removed by physical (see below), biological and photochemical processes before it reaches the Mediterranean Sea. Gross estimates indicate that 3.3-13.5 x 10^{10} mol C a^{-1} of labile (susceptible to rapid bacterial degradation) DOC is exported from European rivers to the entire Mediterranean Sea (Sempéré *et al.,* 2000). The labile fraction defined as the carbon equivalent of measured amino acids and neutral sugars is relatively higher during periods of low sediment load whereas biodegraded organic substances dominate at high discharge rates. Photochemical oxidation reactions may change the bioavailability of riverine dissolved organic compounds (Mopper *et al.,* 1991). Recent studies showed that in summer, high solar radiation in concert with high NO_3^-/NO_2^- concentrations in coastal northwestern Mediterranean Sea may induce substantial production of OH radicals which can in turn oxidize labile organic molecules (Tedetti *et al.,* unpubl. data). Similarly, aggregation of dissolved organic compounds in the colloidal size range to particles may enhance sedimentation in estuarine environments and accumulate near the bottom, forming distinct nepheloid layers (Sempéré *et al.,* 1994).

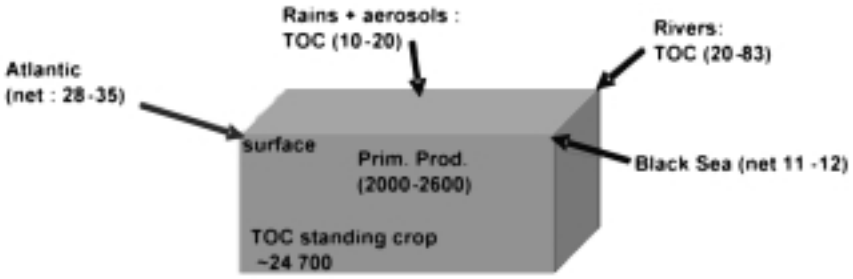

Fig. 1. Summary of estimated sources, standing crop and residence time of dissolved organic carbon in the Mediterranean Sea (from Sempéré *et al.,* 2000 and references therein). *Units* 10^{10} *mol C* a^{-1}.

Atmospheric input is another important pathway for the transport of natural and anthropogenic material from continents to the ocean (Duce *et al.,* 1991; Jickells, 1995). Organic carbon from the atmosphere (e.g., black carbon) enters the marine environment through the sea surface microlayer, where it resides for varying periods of time. Photochemical and biological processes have a profound impact on the concentration and characterization of this organic carbon at the air-sea interface. This deposition seems to be very important for oligotrophic areas and semi-enclosed seas such as the Mediterranean Sea (Bergametti *et al.,* 1989, 1992; Guerzoni *et al.,* 1999). However, although all these different sources import DOC into the Mediterranean Sea, the Mediterranean Sea represents a net sink of organic carbon, suggesting that loss processes must be high.

Removal of DOC in the Mediterranean Sea

As noted above, P limitation in surface waters may lead to low microbial degradation rates compared to other marine areas.

Organically enriched muds (sapropels) in the eastern Mediterranean basin could represent a sink of DOC. However, their importance for the fate of DOC is unknown thus far.
High intensity solar radiation will render photochemical processes an important removal pathway of DOC, especially for DOC of riverine origin. Even though riverine DOC is already substantially photochemically altered when it reaches marine systems (Miller and Zepp, 1995; Graneli *et al.,* 1996; Blough and Del Vecchio, 2002; Obernosterer and Benner, 2004), the interplay between photochemical and biological processes will also have important implications on the further fate of riverine DOC once it enters the Mediterranean Sea. A number of studies have shown that the photochemical transformation of DOC results in increases in heterotrophic prokaryotic production and respiration (e.g., Miller and Moran, 1997; Obernosterer and Herndl, 2000; Smith and Benner, 2005). It has been concluded from these studies that the interaction of photochemical and biological processes can enhance the rates of DOC degradation.

In the Mediterranean Sea, the input from the atmosphere and rivers provides particulate matter, which acts as surface for sorption of DOC. Most dissolved organic compounds are particle-reactive in seawater. Adsorption of DOC onto non-living particles leads to an overall enhanced concentration of organic matter on the particle as compared to the ambient water. Such particles may be biogenic (opal, calcium carbonate, or detritus) or entirely abiotic (clays, iron oxyhydroxides). As such, non-living particles exhibit a broad size spectrum; they—and their adsorbed organic compounds—will be characterized as "particulate" (i.e., larger than the cut-off used to delineate particulate from dissolved matter) or "dissolved" (smaller than the cut-off). Thus, DOC can be "transferred" to the POC pool simply by sorption to larger particles, or by sorption to smaller particles which form larger particles via aggregation.

Depending on their characteristics, the particles may affect the adsorbed organic substances via oxidation or reductive processes. The particles may be important for the preservation of organic molecules or may play an important catalytic role in photochemical processes. It is well known that some dissolved organic (e.g., humics) and inorganic substances (e.g., nitrate, ferric ions), and suspended minerals (clays, zeolithes, TiO_2, ZnO) may accelerate the photochemical degradation of different organic compounds in natural waters (Bajt *et al.,* 1993; Richard and Boule, 1994).

Due to the high content of different metal oxides in natural sediments and suspended matter (Wgrzynek *et al.,* 1997), these processes might be particularly important in shallow coastal Mediterranean waters.

The availability of an organic substance for microbial uptake may also be altered significantly once it is associated with particles. For example, the bioavailability of a molecule may be reduced if its sorption to a particle alters its tertiary structure or changes the nature or concentration of its dominant functional groups. Such changes may make the compound unrecognizable for exoenzymes that would have been able to hydrolyze the compound in its free (i.e., not adsorbed) form. If the sorption of organic molecules to particles reduces their bioavailability (a testable assumption for a range of inorganic and organic particles and a broad spectrum of organic molecules), then the conversion of its "labile" to "refractory" (or at least relatively persistent) status may reflect in part the time needed for an organic molecule to adsorb to particles. Formation of particles due to scavenging and aggregation of DOC by bubbles, however, increases the bacterial utilization of the DOC (Kepkay and Johnson, 1989). At present we can only speculate about most of these processes, but they are quantifiable in controlled experiments.

Mucilage phenomena

In some coastal areas, the accumulation of autochthonous DOC results in the formation of macroaggregates. This so-called mucilage formation is of particular importance in the Northern Adriatic Sea where it has been studied over the past 10 years. Even though not all responsible mechanisms have been identified thus far, there is evidence from *in situ* and laboratory studies that the high N/P ratio in surface waters of the northern Adriatic Sea accounts to some extent to the development of this phenomenon (Kaltenböck and Herndl, 1992; Obernosterer and Herndl, 1995; Smodlaka *et al.,* 2004).

Eutrophication of the Northern Adriatic (Vollenweider *et al.,* 1992; Vollenweider and Rinaldi, 1995 and references therein), due to the run-off from the Po and other rivers, causes hyper-production of phytoplankton during spring/summer at rates that far exceed the grazing potential of herbivores or rate of decomposition by bacteria. Consequently, large standing stocks of phytoplankton build up and extracellular polymers, mainly polysaccharides, accumulate in the water column, especially in the euphotic layer above the thermocline. The hallmark of the mucilage phenomenon is the rapid (1-100 h) appearance of enormous amounts of gelatinous organic matter that can be generally defined as macrogels (Figure 2). While a large number of

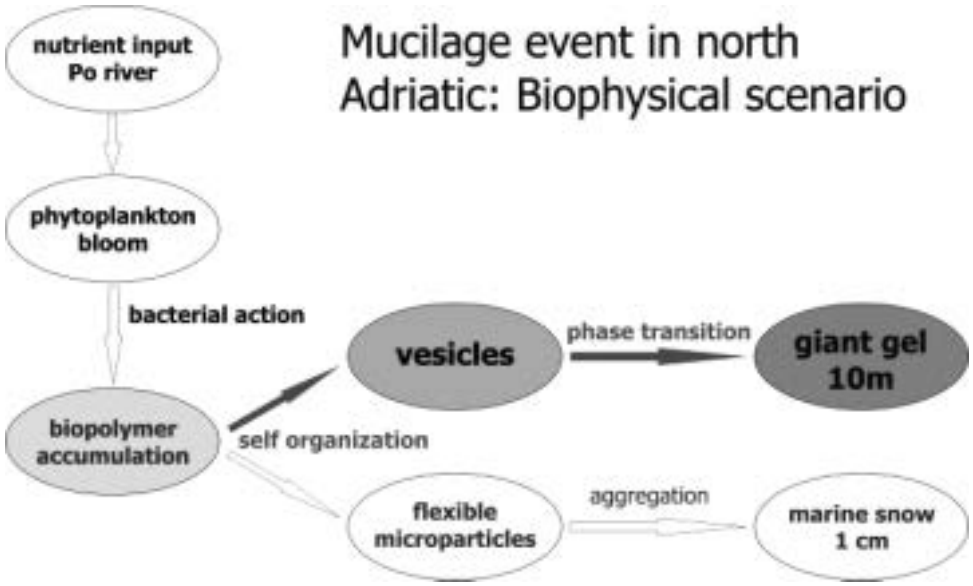

Fig. 2. Biophysical scenario of giant gel formation in the Northern Adriatic.

hypotheses on the cause and mechanism of massive mucilage events have been presented (see Vollenweider *et al.,* 1992; Vollenweider and Rinaldi, 1995; Funari *et al.,* 1999 and references therein), the mechanism of the transformation of DOC to macroaggregates is still not well understood. The formation of such large mucus aggregates at such high rates (within a few hours) cannot be explained by biological process (Žutić *et al.,* 2004). There is a high probability that mucilage formation is preceded by the accumulation of a precursor pool of organic matter until a critical concentration is reached (Žutić and Svetlicic, 2000). The abiotic formation of gel particles from dissolved precursors has been shown (Passow, 2000). It seems that the imbalance of the N/P ratio is causing an increasing phytoplankton extracellular production which is essential for the development of this phenomenon (Smodlaka *et al.,* 2004). The macrogel formation involves the transformation of macromolecular DOM into colloidal organic matter (COM) and further into macrogel by aggregation process (Kovac *et al.,* 2004). In addition, a biophysical scenario was proposed that features self-organization of extracellular polymers into presursor particles that transform into macrogels by first order phase transition (Žutić and Svetlicic, 2000; Žutić *et al.,* 2004; Svetlicic *et al.,* 2005c) (Figure 2).

The chemical characteristics of northern Adriatic macroaggregates, as indicated by spectroscopic studies, are similar to marine high molecular weight DOM (>1 kDa) and to phytoplankton exudates. The structure mainly reflects the primary composition of phytoplankton material including proteins, carbohydrates and lipids (Hedges *et al.,* 2002; Nguyen *et al.,* 2003). The general chemical structure of macroaggregates includes four major classes of organic structural elements: carbohydrates, ester and amide functional groups, aliphatic, and organo-silicon components (Kovac *et al.,* 2002, 2004). The aromatic C moities are much lower then those observed in riverine (Hedges *et al.,* 1992) and estuarine HMW-DOM (van Heemst *et al.,* 2000), and is similar to oceanic HMW-DOM (Benner *et al.,* 1992). Thus, macroaggregates probably originate from a high concentration of dissolved macromolecules of phytoplankton exudates (Kovac *et al.,* 2004). The macroaggregate gel is stabilized by interactions with particles such as calcite, quartz, clay minerals, phytoplankton cells and their remains (Kovac *et al.,* 2004, 2005).

The phenomenon referred to as «mucilage of the Northern Adriatic» has been observed infrequently over the past three centuries but its intensity and frequency of appearance have increased since the major event of 1989 (see Figure 1 in Žutić, this volume). Similar events were noted in recent years in other coastal areas of Mediterranean: along the Dalmatian coast (Stachowitsch *et al.,* 1990), in Greece (Gotsis-Skretas, 1995), Sicily (Calvo *et al.,* 1995), along the Tyrrhenian coast (Innamorati, 1995; Mecozzi *et al.,* 2001), and the Black Sea (Moncheva, pers. comm.), but at such a massive scale the phenomenon has been unique to the Northern Adriatic.

Persistence of DOC: Mediterranean vs. Atlantic

It is possible to estimate the turnover rates of DOC in ocean basins if one assumes that the overall DOC concentration does not change over time (i.e., there is a steady state between production and loss). Then, one can calculate the turnover rates of DOC by combining the average age of the DOC (^{14}C measurements) with the DOC production rate, calculated as the fraction of total annual primary production released as DOC (on an ocean basin scale). If the calculated loss rate of DOC for the Atlantic is not appreciably different from that of the Mediterranean, then one can assume that the unique features of the Mediterranean (warm temperature, short residence time of the water, high N/P ratio) do not affect the long-term fate of the DOC. If the calculated turnover rates do differ appreciably between the two water bodies, then the Mediterranean could become an important "natural laboratory" to explore reasons for these differences. The initial focus should be on the factors that are obviously different between the Mediterranean and the Atlantic. Assuming a steady state, a DOC concentration of 70 µM in the layer 0-200 m and a concentration of 54 µM below, the residence time of DOC in the Mediterranean Sea was estimated (by dividing the DOC reservoir by the overall input) to range between 103 and 149 years (Sempéré *et al.,* 2000).

RECOMMENDATIONS FOR FUTURE RESEARCH

1) A number of previous studies indicate that primarily P limits the biological degradation of surface water DOC in the Mediterranean Sea. However, P-limitation occurs even at relatively high concentrations of dissolved organic phosphorus (DOP) (Rassoulzadegan and Thingstad, this volume). This raises the question of the bioreactivity of the DOP pool in the surface waters of the Mediterranean Sea.

2) As a consequence of the accumulation of DOC during summer in the surface waters of the Mediterranean Sea, the DOC is exposed to relatively high intensities of solar radiation over a prolonged period of time. It has been shown recently that exposure of 'young', phytoplankton-derived DOC to solar radiation results in a decrease in its biological reactivity (Benner and Biddanda, 1998; Obernosterer *et al.,* 1999). This decrease has been attributed to phototransformation and photomineralization of the bioreactive DOC component (Obernosterer and Benner, 2004). To better understand the fate of surface DOC transported to deeper waters during winter convective overturn, the impact of photochemical processes needs to be addressed more intensively.

3) A broader interest in the mechanistic basis of the mucilage phenomenon is to prognosticate whether such uncoupling between production and degradation in the carbon cycle may be expected in the future elsewhere in the ocean as well, e.g., due to eutrophication coupled with specific trophodynamic and environmental conditions. Further research on the mechanism of macrogel formation and its fate is important for the more general understanding of abiotic processes in the transformation of DOM and their role in the Mediterranean Sea. Additionally, the macroaggregates in the Northern Adriatic offer a rare opportunity to study the assembling of macromolecular DOM into macrogels.

4) Few studies exist on the prokaryotic growth efficiency in the Mediterranean Sea. How P-limitation impacts prokaryotic growth efficiency is important for the understanding of the cycling of DOC in the Mediterranean Sea.

5) Several studies on the atmospheric input of biogenic and anthropogenic compounds into the Mediterranean Sea have already been conducted in its western part (Bergametti *et al.,* 1989, 1992; Remoudaki *et al.,* 1991; Molinaroli *et al.,* 1999; Rodríguez *et al.,* 2002; Migon *et al.,* 2002; Ridame and Guieu, 2002) and also in the Eastern Mediterranean region (Levin and Ganor, 1996; Ganor and Foner, 1996; Mihalopoulos *et al.,* 1997; Herut *et al.,* 1999, 2002; Markaki *et al.,* 2003; Krom *et al.,* 2004). However, additional research is needed to elucidate the relation between the atmospheric inputs and the biological and biogeochemical consequences for the Mediterranean Sea.

6) Atmospheric input could potentially increase P limitation, because N rather than P is delivered through the atmosphere. However, this hypothesis is controversial.

7) Determination of the age of Mediterranean DOC through ^{14}C analytical techniques and the link between the overall hydrographic regime of the main water masses and the age of the DOM and its transformation.

Dissolved Organic Matter and planktonic engineering

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Se volete credermi, bene. Ora dirò como è fatta Ottavia, città-ragnatela (Italo Calvino)

ABSTRACT

Colloidal materials in seawater, and aggregates and flakes largely of colloidal origin, are grouped into autogenic, derived, and allogenic categories. Some potential functions of autogenic colloids (exopolymers) produced by plankton are outlined, and it is suggested that they act either as engineering tools to modify physical and chemical features of the local environment, or to better exploit those features. Their potential to modify turbulent dissipation, to consolidate stratification, and in information retrieval and processing are briefly considered, and it is suggested that comparisons with metazoan tissues and intracellular metabolism may provide useful insights into their roles in plankton ecology.

INTRODUCTION

The lowest rung of Aristotle's *scala naturae* consists of a primaeval generative slime, and some similar undifferentiated quasi-protoplasmic material has lain at the roots of most theories of abiogenesis. Following Lorenz Oken, many writers have called this *Urschleim*. In Cairns-Smith's (1982) conjectures concerning early evolution, more precisely specified but analogous substances form the missing links between hypothetical life-forms based on colloidal clays and later forms in which DNA has taken over. Here we are concerned with the modern analogues of Urschleim, particularly the kinds which are secreted by plankton, and which make an important contribution to the stock of dissolved organic matter (DOM) in the sea. Much attention has been devoted to the fate of DOM in recent years following its recognition as a component of the carbon pump and other biogeochemical processes, but much less to the roles it has in the lives of the organisms which produce it.

Physiologists have long been familiar with the fact that most biological fluids behave as *non-newtonian* fluids. Rheologists use this term for liquids whose behaviour cannot be described by the Navier-Stokes equations (Barnes *et al.,* 1989). Only two common ones, pulmonary gases and urine, do so, and medical students learn early to detect the non-newtonian properties of the latter which can provide clues to the identity of renal malfunction. Blood is one of the best studied body fluids, and while its behaviour is close to newtonian at high shear rates, its dynamic viscosity increases greatly at low shear rates (below about 10 s^{-1}), and its flow behavior is strongly influenced by the volume percentage of red blood cells (haematocrit) and by the protein composition of the plasma. At low shear rates, red blood cells pile up into columns or *rouleaux* and other kinds of aggregates under the influence of the globulins in the plasma, and the blood is

described as *lumpy* (Lightfoot, 1974). Bile contains a mixture of spherical vesicles and micelles; disturbance of the equilibrium between the two kinds of particles can lead to lumpiness and formation of gallstones. Other body fluids such as the synovial fluid which lubricates the joints also show marked departures from newtonian behavior and are strongly viscoelastic. These fluids, or tissues, thus lie between the idealized extremes of the materials of classical physics, between newtonian fluids and hookean solids.

Little attention has been paid by biologists to the non-newtonian properties of the fluids which lie outside the bodies of aquatic organisms. But seawater too has been described as lumpy (Jenkinson, 1986), and shown to behave in unexpected ways at the low shear rates which characterize most of the ocean interior[1]. Some of this behaviour may be due to the organisms themselves when they reach adequate concentrations (Wyatt *et al.,* 1993), but perhaps a greater part is due to biopolymers secreted by organisms into the surrounding medium, and to materials derived from these secretions.

It is well known that special biopolymers are synthesized to regulate the solution properties of biological fluids. Production of these molecules must be preceded by *design* (by natural selection) since slight modifications of molecular structure can have large consequences, and proceeds by precise placement of these materials in surrounding space (via morphological and behavioural adaptations); it is an active process (Domozych and Domozych, 1993; Giroldo and Vieira, 2002). Physiological processes in these fluids take place mainly at *interfaces,* both those between the continuous and disperse phases of the fluid itself, and between the fluid and some confining boundary. Can we turn this picture around and ask, does the presence of actively secreted biopolymers into an *external* fluid indicate that the latter is physiological, i.e., a special kind of tissue, even though it is *indeterminate,* without an obviously defined outer boundary?

If the answer to this question is yes[2], then some of the *outside* world structured by dissolved organic matter (DOM) is biomass rather than part of the environment, and Jonathan Sharp's provocative question, "Do healthy cells do it?" (Sharp, 1977) is cast in a different light; do healthy cells have *external* tissues? In the context of algal blooms, this general theme was earlier referred to as "environmental management" (Jenkinson and Wyatt, 1995). In terrestrial ecology, parallel views have emerged, and been called "ecosystem engineering" (Jones *et al.,* 1994), or "niche-construction" (Odling-Smee *et al.,* 2003). Turner (2000) argues that these structures are external organs with physiological roles, extensions of the phenotype; he equates them with Dawkins' extended phenotype, and asks "how it alters the flow of matter, energy, and information through the organism and between the organism and its environment". Turner's answer to Sharp's reworded question is another one, "If an organism modifies its environment for adaptive purposes, is it fair to say that in doing so it confers a degree of livingness to its apparently inanimate surroundings?"

The capsular polymers of many bacteria and some phytoplankton might then be compared with the mucociliary system in pulmonary alveoli, with their surfactant component which lowers surface tension, and the more extended networks of some diatom colonies like *Odontella sinensis* (Pickett-Heaps *et al.,* 1986) or *Thalassiosira* (Hasle, 1983) with webs of fibres resembling spiders' gossamer. *En passant*, it can be noted that alveolar surfactants have a half-life of only about ten hours, indicating that there is no obvious relation between the durability of the matrix and the life span of the emperor[3]. Sometimes, of course, the clothes have no emperor inside since he has been eaten or otherwise disposed of, and left his biopolymers behind as a signature, which

1 In *Ti con zero*, Italo Calvino imagines a world turned inside out, where the sunlit water has become our blood and we continue to swim in the same warm sea, but now in darkness; as is well known, blood and seawater share some common features (Macallum, 1910).

2 Fox (1957) went further: he wrote "The whole body of the ocean may be likened to a vast, loosely organized but actively metabolizing cell ...". In contrast, " ... the only bit of an organism that is unambiguously not part of the environment is the bit that is static – the genotype", and "in the end it is the environment that the genetic instructions instruct" (Cairns-Smith, 1982).

3 Williams (1984) uses the analogy of the emperor's clothes in writing of bacterial production in the sea. The point is that most of this matrix material is invisible to the unaided eye, hence Azam's (1998) "oceanic dark matter".

soon becomes a resource for successional change. The half-lives of planktonic secretions may be of the same order of magnitude as alveolar surfactants, and thus similar to the characteristic times of temperature inversions identified by oceanographers. This component of DOM thus has a potential bearing on the production and persistence of thin layers in the water column.

COLLOIDS AND PARTICLES

The word *colloid* was originally used in pathology to describe degenerated tissues of glue-like consistency, and later by chemists for a solid state contrasted with *crystalloid*, with the distinction based on diffusibility. More recent usage of the word often refers to a filter-passing (i.e., "dissolved") fraction, and thus excludes particles above some size threshold. Here the word is used both in this sense, and more inclusively to refer also to filterable particles derived from the dissolved fraction. Colloids then are an operational subset of the total oceanic pool of organic carbon, and account for a large proportion, up to 50%, of the nominally *dissolved* fraction, as well as particles formed by coagulation, flocculation, and other complex processes, some of which may be biologically mediated. What are the origins of these materials? Looking at the whole spectrum of colloidal materials in seawater, and including particulates formed from them, we can distinguish several categories;

i) secretions of organisms, especially phytoplankton, zooplankton, and bacteria, ***autogenic*** purpose-built polymers which are in active use by their producers, and which do not necessarily involve the activities of associated species;

ii) derivatives of these, and aggregated mixtures of them with other things floating around, which are colonized by various organisms, and in which symbioses and other inter-species processes take place; this category includes materials released by the breakdown of organisms, by viral lysis, sloppy feeding, etc, of faecal pellets, and of larger aggregates by shearing forces;

iii) ***allogenic*** materials from other sources aggregated by processes such as tangling and adhesion; in many nearshore waters, sewage discharges and other effluents contribute to this category;

iv) some of the organisms themselves, particularly viruses (see below for discussion of operational definition of *colloidal*) are small enough to belong to the colloidal part of the size spectrum.

Most ecological research in this area to date has concentrated on the derivative category (ii), following the pioneering studies of Alldredge and her colleagues (Alldredge and Silver, 1988; Alldredge and Gotschalk, 1989, 1990; Alldredge *et al.,* 1993) and Azam *et al*. (1993). Here we focus mainly on the autogenic components. The important distinction between autogenic and allogenic polymeric particles in seawater has not always been clearly made, but a variety of terms has been devised to characterize the morphological variation of aggregated (hence particulate) colloids, especially with respect to size and form of extension. As well as the generic terms *aggregate* (formed *in situ*) and *flake* (partly derived from bubbles), these materials have been called veils, lumps, stringers, flocs and macroflocs, gels (nano-, micro-, macro-, giga-), fluff, snow, EPS (extracellular polymeric substances), TEP (transparent exopolymeric particles), or simply mucus. A tentative exploration of the taxonomy of these materials is provided by Verdugo *et al*. (2004). In surface oceanic waters, the approximate numerical ratio of aggregates to flakes is 3:1 to 4:1 (Gordon *et al.,* 1970). The general theme of several of these terms is that they are bits of seawater with enough yield stress to remain functionally solid, but they are not all synonyms. Some of the terms refer to quite varied materials; e.g., both hydrated mucilaginous and rigid crystalline materials are referred to as EPS. Nor is this list exhaustive; we should certainly add bubbles (before they burst), probably also surface foams and scums, and indeed spindrift.

If we proceed beyond characterization and consider functions, we shall see that some *external* parts of organisms, such as spines and other features broken away from larger particles by sampling, should also be added at the particulate end of the continuum. The colloidal part of the size spectrum is usually defined operationally as having a lower size limit, of say ~1000 Da (see

below). A more appropriate lower bound might be ~500 Da, the largest particle size which can pass through bacterial membranes. As an upper limit, we can suggest the size at which the magnitude of gravitational settling starts to exceed brownian movement, the Brownian-Stokesian boundary[4], somewhere between 10^0 μm and 10^1 μm; this is beyond the point at which we would normally place a boundary between dissolved and particulate.

The distinction between particles and dissolved materials is clearly somewhat arbitrary. Thus material which is not retained by a nominal pore size is regarded as dissolved. If freely suspended in the medium, and depending on the pore size of the filter selected, individual viruses, some procaryotes, and even the smallest eucaryotes, could be considered to be "dissolved". With a pore size of 0.5 µm as criterion, the amounts of particulate organic matter (POC) in surface waters in carbon units generally range from about 5 to 120 µg C l^{-1}, and in deeper waters down to 500 m from about 2 to 20 µg C l^{-1} (e.g., Boyd *et al.,* 1999). Roughly half of this amount is contained in living organisms. The amounts of DOM are between ten and a hundred times greater (range from about 500 µg C l^{-1} to about 1800 µg C l^{-1}). These figures will change if we move "dissolved" organisms to the particulate fraction, but this will not alter the fact that planktonic organisms live in a *complex fluid*[5], and not in seawater as commonly perceived.

As well as the categories listed above, we can recognize other kinds of exopolymeric structures in the sea which form extended microlayers. The best known of these are the sea surface films, and slicks associated with internal waves and the Langmuir circulation, and with algal blooms, and biofilms at the water-sediment interface. But there are also planar structures within the water column itself, though they have received little attention. An example of an *internal* slick in an Adriatic estuary is described by Žutić and Legović (1987), which appears when viewed from below as a "silvery-grey sheen", at the interface between the fresh water and seawater. This sheen suggests that spacing in the film is comparable to optical wavelengths leading to interference patterns.

PRIMARY AUTOGENIC COLLOIDS

Phytoplanktonic and macroalgal exudates have been characterized as "the ubiquitous algal attribute" (Boney, 1981). It is possible that some low molecular weight exudates are simply excretory products (Fogg, 1983), or play a role in protecting the photosynthetic machinery in circumstances where supplies of light and nutrients are uncoupled (Wood and van Valen, 1990). But high molecular weight compounds are adaptive tools evolved to gain some measure of direct or indirect control of the local environment. This is clear since these substances are produced in special organelles within cells (e.g., mucocysts in Euglenophyceae), and secreted through specialized structures (e.g., labiate processes in diatoms). They are therefore autogenic, and their main advantage, initially anyway, must be to the species which synthesize them. In many species (but not all) they are secreted during the exponential growth phase.

The first step anything leaving a cell must make, whether an ion or a large polymer molecule, is to break water-water bonds so as to make a hole into which it will fit. Water adjacent to the polymer must then reorient itself as it interacts with the chain, so that one effect of autogenic secretions is to restructure the local environment; this effect may extend to tens of nanometers from the polymer surface in response to the anionic or cationic groups of the polymer. In the presence of solutes, further structuring occurs as microdomains of high and low density form adjacent to the polymer surface (Wiggins, 2002). It is thus the combined and interactive properties of the polymers, water, and salts acting together as partners which define the potential for environmental engineering.

Chaetoceros socialis or *Phaeocystis* colonies are formed with aid of mucilage, as are the chains of *Thalassiosira* and other diatoms. The secretions which structure megaflocs and cause *mare*

4 Defined by the Péclet number; brownian and stokesian forces are approximately equal for particles with ESD values of 1000 to 2000 nm (1-2 mm), but vorticity also enters the equation.

5 Gelbart and Ben-Shaul (1996) "suggest that the common, defining feature of a complex fluid is *the presence of a mesoscopic length scale* which necessarily plays a key role in determining the properties of the system."

sporco[6] in the Adriatic may come largely from *Amphora coffeaeformis* and *Cylindrotheca fusiformis* (DeAngelis *et al.,* 1993), though their origin is not yet fully settled. In tissues, exopolymers are involved in a number of identifying and locating devices such as cell communication and adhesion, and it is thought that matrix signals may be as important in regulating cellular activity as soluble mediators such as hormones; these are likely to be among the functions of external tissues too, at least in colonial forms. Polysaccharides in the capsular antigens of pathogens like *Streptococcus* and *Salmonella* are well studied, and related to cellular recognition and adhesion. These functions offer further clues to their potential ecological importance. In the planktonic world, mechanisms which bring members of the same species together are important in a number of ways, to overcome the Allee effect, to allow quorum sensing, to keep the family together (Jenkinson and Wyatt, 1992). Secretion during the stationary rather than the exponential phase may indicate that distinct functions are catered for, such as a need to establish microbial consortia, or as a prelude to sexual activity.

Exopolymeric colloids can be classified according to the manner in which they are related to their parent cells. Decho (1990) recognizes a gradation from condensed gels which closely invest the cells through less compact slimes to colloidal solutions. These distinctions are based on microscopical and histochemical observations. Chemists classify colloidal macromolecules on the basis of their molecular conformations into globular, random coils, and rods. There is no precise correspondence between these two schemes since they are based on structures seen at different scales, and Decho's scheme is based on naturally occurring colloids, of diverse chemical composition. But for chemically well defined colloids, molecular conformations are determined primarily by their chemical structure, and in solution by interactions between the polymer chain, ions (cations, H^+, anions), water, and solvent molecules. The conformations in turn exert control over the physical properties. Since these colloids in aggregates can flow when sheared, and in doing so acquire some of the orderliness of crystals, there can in principle be circumstances where they behave as *liquid crystals,* with potential roles based on their optical and other properties.

In the treatment of viscosity due to Einstein, if the volume fraction, Φ, is assumed to be $<< 0.1$, so that interparticle interactions do not occur; then the viscosity of a suspension is independent of particle size ($\eta = \eta_0 [1 + k_1 \Phi]$, where η is suspension viscosity, and η_0 is solvent viscosity). In this case, the model can be applied as a first order approach to the viscosity of phytoplankton suspensions as well as to colloidal suspensions. But for cell concentrations on their own to significantly impact viscosity, special pleading is necessary, such as that they are confined to thin layers, that the real concentrations (expressed as Φ) are much higher than bulk estimates indicate (Wyatt *et al.,* 1993); but this is certainly the case in some blooms.

Einstein's expression for suspension viscosity also assumes that particles are spherical and rigid. These constraints are patently not obeyed by phytoplankton, so that cell shape and colony form must be considered in this context. An account of the possibilities will lead us a long way from DOM, so it is simply noted here that the rationale for some of the *life-forms* of phytoplankton might be found in the same terms as colloidal molecules, and useful parallels might be drawn between the rigid or flexible, straight, curved or spiral chains of diatoms and the similar forms of different polymer species, and between colloidal aggregates and the colonies of such species as *Thallasiothrix subtilis* and *Chaetoceros socialis*. Relations between turbulence and cell morphology, colony form, and the prominence of exopolymeric structures have been noted a number of times (e.g., Karsten, 1898; Mangin, 1908; Castellvì, 1971). Chain forming species can span the Brownian-Stokesian divide (Davey and Crawford, 1986).

ENVIRONMENTAL ENGINEERING BY PHYTOPLANKTON

As Wyatt and Jenkinson (1993) noted, phytoplankton are too often regarded as purely passive victims of circumstances. In Malthusian models, they are victims of their own fecundity, in the logistic, victims of their own greed, in Volterra systems of their enemies. While all these

6 "Dirt's a bit more fun when you add water" (Hank Ketcham's Dennis the Menace).

approaches clearly contain elements of reality, they do not specifically address the extent to which phytoplankton populations can avoid the models' implications, nor do they ask what it is that algae really set out to achieve. The assumed aim is often maximization of biomass, but fecundity does not normally run riot, and is controlled by sophisticated mechanisms involving clocks, pheromones, signal transduction, and so on; vegetative growth in nature rarely reaches the limits set by nutrient resources before life-history imperatives intervene to render such limits irrelevant, and a variety of adaptations have evolved to reduce the mortal impact of herbivores and other enemies to tolerable levels. Organisms require the right amounts of the appropriate resources in the right places at the right times; some adaptations which lead to darwinian success rather than malthusian surfeit are reviewed by Jenkinson and Wyatt (1992, 1995) and Zingone and Wyatt (2005); here we revisit some options and note others. We ask the question, *why* do healthy cells do it?

A brief review follows of some physical processes that *can* be modified or controlled by phytoplankton by means of exopolymers. For such modifications to be considered as engineering tools, it is also necessary to consider their putative functions. We consider the effects of algal biopolymers on i) viscosity, ii) stratification, and iii) information retrieval and processing. These by no means exhaust the many roles to which exopolymers contribute, such as the motility of raphe-bearing diatoms, adhesion to and stabilization of sediments, protection from dessication in temporary waters or the intertidal zone, or the stimulation of bacterial growth leading to local nutrient regeneration (Hoagland *et al*., 1993).

i) viscosity : Water is amazingly complicated stuff, as Douglas Adams[7] might have said. Even 'pure' water – from which almost everything of interest to biologists has been removed - exists in a sort of transition zone between molecular and liquid. This is because water molecules are polar, and can link up to form *clusters* by means of hydrogen bonds. It is this clustering and its effect on flow that manifests itself as a resistance or viscosity. Newton proposed that in a fluid in which one layer moves parallel to another, resistance to flow is proportional to the relative movement. A stress or shear force, τ, is required to generate the movement which continues as long as the force is maintained. The velocity gradient between the two layers is the shear rate, dv/dr, and the constant of proportionality is the viscosity, η. Thus $\tau = \eta\ dv/dr$, expresses linear viscous flow. This implies that the viscosity is constant in time, does not change with shear rate, and that stress in the fluid is zero when the shear force is zero. The viscosity of *pure* water is influenced by temperature and the presence of electrolytes. Increase in temperature lowers viscosity, by about 2.5% for each degree. Ions act in different ways traditionally thought to depend on their impact on hydrogen bonding (but see Omta *et al*., 2003); for example, the addition of 25% by weight of NaCl to water at 20° C doubles its viscosity.

The addition of certain polymers to water also influences viscosity, the effect depending on concentration and molecular weight, chain configuration, and the concentration of electrolytes. Molecular weights less than about 105 are generally ineffective at the concentrations of interest here. Linear (unbranched) chains in the absence of shear tend to form coils with spherical envelopes, with the diameter of the envelope dictated by ionic concentrations. More salt tends to reduce diameters (radii of gyration) and decrease viscosity, other things being equal. At adequate concentrations, these chains form tangles which enhance viscosity; if shear is then applied, the chains become aligned rather than tangled, so that viscosity declines as shear rate increases. Rheologists call this *shear thinning*. Branched polymers can form space-filling networks, and at sometimes very low concentrations (< 0.5%) form high viscosity cross-linked gels. All these effects depend on hydrodynamic forces acting on the surfaces of the suspended colloidal particles, so that, as in soil science, phase volume (Φ) is the appropriate measure of concentration rather than weight. The time constants of shearing processes are enshrined in the Deborah number, *De,* the ratio of the time constant of the material, τ, to that of the deforming force, T. $D_e = \tau/T$, where τ is 'zero' (10^{-12} s) for a Newtonian fluid and infinite for a Hookean solid. The potential significance of the Deborah number in plankton ecology is explored and extended by Jenkinson and Wyatt (1992).

7 Author of *The Hitch-Hikers Guide to the Galaxy.*

The particle dynamics of colloidal suspensions are influenced by van der Waals forces which are largely attractive, and electrostatic forces which are repulsive. When the former dominate, or when the latter are reduced by the addition of salt, flocculation takes place, as for example when rivers carry suspended clay particles into estuaries. In general, flocculation increases settlement rates, but if it leads to the formation of chains or long fibres rather than globules, these rates may slow. These various effects indicate some of the design requirements of a polymer if its biological function is to alter viscosity (create lumpiness, say) in the immediate environment of the cell which secretes it. We can therefore ask whether the chemical properties of polymers naturally secreted during phytoplankton growth match these requirements, and what advantages accrue to the species responsible for their production. At present we lack detailed information about most phytoplanktonic exopolymers required to pursue these questions, but some is beginning to emerge (e.g., Solomon *et al*., 2003; Orellana *et al*., 2003).

Eckart (1948) asks us to consider what happens when we add cream to coffee. Initially the cream forms white streaks at the surface of the cup, separated by the black coffee. This is *stirring*, and increases the surface area of the interface between cream and coffee; the cup's contents becomes occupied by a surface of great extent and complexity, and if this surface were truly two-dimensional, the surface/volume ratio would tend towards infinity. Ottino *et al*. (1988) use the image of flaky pastry for the same process. So if a passive scalar is introduced into a stirred fluid, the scalar marked region has a sharp boundary, and could potentially act like an investment. Real surfaces of course have some thickness as we see when the cream and coffee start to blend or *mix*, and the black and white streaks disappear to be replaced by an even brown colour (due to molecular diffusion), and biological surfaces have some finite thickness due to their structural components. At some stage in the trend from stirring to mixing the scales and rhythms of turbulent decay, different for each species and life-history stage, become compatible with the scales and rhythms of exchanges between the medium and the organisms, and hence provide the *auxiliary energy* of Legendre and Demers (1984).

Thus the control of turbulent deformation and dissipation rates are ways in which autogenic exopolymers may modify local conditions around phytoplankton cells to create improved microenvironments. The polymers provide the tools with which the cells can modify spatial and temporal features of their local environment, especially those circumscribed by the Kolmogorov and Batchelor scales, and thus facilitate the traffic of nutrients, signal molecules, excretory products. By slowing the transition from stirring to mixing, more interface becomes available as surface/volume ratios increase, and spatially restricted and chemically controlled environments with microscopic dimensions emerge, where molecular diffusion is under closer control as in lung alveoli, muscle fibres, and so on. Inside a cell, "... the entire population of reacting and interacting molecules can be viewed as a highly connected dynamic molecular network" (Stange *et al*., 1998). Though more dilute, something of this model must be retained in the space structured by exopolymers surrounding phytoplankton cells. The technology of micro- and nano-fluidic devices ("lab on a chip") is learning how to exploit these same processes.

ii) stratification: Phytoplankton when sufficiently abundant controls the absorption, reflection and scattering of light (Poole and Atkins, 1929). Grindley and Taylor (1971) noted marked increases in surface temperature in *Noctiluca* patches with mucus. Lewis *et al*. (1983) predicted layered populations of plankton would, by absorption of light, change the thermal stratification field. Sathyendranath *et al*. (1991) showed that surface temperature of Arabian Sea could be increased up to 4º C by phytoplankton absorbance of sunlight. While the pigments in plankton patches can act alone to increase absorption and raise temperature locally, the layers so formed would be more persistent in synergy with a stratification mechanism, i.e., vertical migration to a common depth, and by reduction of the mixing rate by appropriate exopolymers to reduce the rate of heat dispersal.

iii) information retrieval and processing: The intensity and spectral quality of light reaching phytoplankton controls photosynthesis, but light also carries information which can modulate cell activities such as vertical migration, circadian rhythms, and life-cycle transitions (e.g., Ragni and Ribera d'Alcalà, 2004). The light received by the photosynthetic apparatus and other receptors within the cells can be modified in various ways by the optical properties of any DOM which

intersects the light path before it reaches them. DOM contains chromophores (CDOM), which absorb preferentially in the blue region of the spectrum; therefore a modification in band ratios of the radiation reaching the cell might occur so that its absorption and emission spectra may be affected, along with signalling. Absorption of UV and blue are important aspects of this transformation. But the optical properties of DOM can also depend on the atomic architecture in the absence of chromophores, as in liquid crystals.

ENVIRONMENTAL ENGINEERING BY ZOOPLANKTON

Appendicularian houses: The houses of appendicularians like *Oikopleura* are built entirely of mucus secreted by small areas of ectodermal cells lying around and inside the mouth; once secreted, the house must be unfolded like a piece of origami. An individual may secrete and discard as many as 20 such houses a day. When *O. dioica* is actively growing and reproducing, it can reach concentrations as high as 15.10^{3} m^{-3}, so at these times of high population abundance, literally millions of houses are discarded by each generation under each square meter of sea. The larger *Oikopleura vanhoeffeni* of boreal waters reaches concentrations greater than 2.10^{3} m^{-3}. Deep water appendicularians are less well known, but a large species of *Bathychordeus* has been studied by Hamner and Robison (1992) in Monterey Bay, California. This species has a body 30 to 50 mm long, about ten times longer than the *Oikopleura* species mentioned. It secretes mucus continuously from cells near the spiracle. This mucus eventually comes to form an umbrella-shaped canopy sometimes resembling a parawing, which envelops the body and filter, and which can in some specimens extend more than 2 m across, though most of those seen are less than 1m. Hamner and Robison estimated that the umbrella increases in size at a rate of 1000 $cm^2 d^{-1}$, so that a canopy 1 m across might have an age of 30 days.

Byssus drifters: The gossamer of spiders consists of scleroproteins which harden when drawn. Juveniles of various species employ gossamer for dispersal. A young spider prepares for an aerial voyage by first facing the wind; it holds itself down with its front legs, raises its abdomen and secretes a thread of gossamer which is entrained in the air flow. When tension on the thread is sufficient, the spider releases its grip, and sails away. In the orb-weaver *Argiope trifasciata,* a wind speed of about 0.5 m sec^{-1} is preferred, and when the speed exceeds 0.9 m sec^{-1}, dispersal does not take place. Threads as long as 3 m have been recorded in some species. Some wolf-spiders secrete six to eight threads which reach lengths of 70 cm or more before the spiderlings take off, and require higher wind speeds, 0.35 to 1.7 m sec^{-1}. If during flight the threads should prove too long, they can be reefed or shortened. With a few reasonable assumptions, it is possible to calculate the drag which these drogues generate, and hence their lifting power.

The post-larval stages of some benthic invertebrates have evolved dispersal methods analogous to those of aerial spiders, and secrete byssus threads composed of acid muco-polysaccharides. Sigurdsson *et al*. (1976) draw attention to the parallels between the dispersal techniques of spiders and those of some bivalve molluscs. In the blue mussel, *Mytilus edulis,* the threads are up to 7 cm in length and 1-3 µm in diameter for spat with shells 750 µm long (Lane *et al.,* 1985), and are secreted by special stem glands on the foot. Lane *et al*. (1982) observed that the threads could be discarded and re-secreted frequently, and that similar threads are found in scallop and oyster larvae. In the laboratory, these threads lead to considerable reductions in sinking rates. There is also a parallel to early observation of reefing by spiders. Beaumont and Barnes (1992) found that the spat of two pectinids, *Pecten maximus* and *Aequipecten opercularis,* can change their sinking rates rapidly, and suggested this ability "might be due to spat actively climbing back up the thread and so reducing thread length".[8]

AGGREGATES DERIVED FROM AUTOGENIC DOM

The flocculation and adhesion of exudates and other categories of DOM lead to the production of aggregates. Whether this happens before, during, or after the exudates have served the primary

8 Nor is it simply a question of deploying a drogue to become waterborne and reefing when the current becomes too strong. *Macoma balthica* spat which disperse by this means only do so on the ebb tide and thus move from the Wadden Sea to the North Sea (Beukema and de Vlas, 1989). In this way, they achieve a measure of control over both space and time.

functions for which natural selection designed them is not resolved, nor is it known to what extent the physical and chemical characteristics of the aggregates, or the processes which lead to their formation, are controlled by the communities which exploit them, or by the inanimate processes of polymer physics. These aggregates are perhaps aquatic analogues of humus in soil, made by the community and not by individual species[9]; the hydration properties of aggregates and humus have some features in common (Benedetti *et al.,* 1996; Chin *et al.,* 1998). The multispecies nature of aggregate colonists also invites comparison with systems like the rhizosphere, the zone around plant roots in which the plant, microorganisms and substrate interact, where, for example, differential uptake of nitrate and ammonium control the fluxes of hydogen and hydroxyl ions, hence the local pH. The rhizosphere, like many phytoplankton colonies as well as aggregates, is rich in exudates, which can account for up to 40% of the organic carbon production of the parent tree. In marine aggregates, as in the rhizosphere, the metabolites of interacting species (phytoplankton, bacteria, etc.) comprise the chemical environment which activates or inhibits the genes responsible for the control of metabolic and signaling pathways, and interspecific interactions *(cross talk)*. Aggregation is a prelude amongst other processes to molecular deconstruction by bacterial exoenzymes (Smith *et al.,* 1992) and other *invisible* processes of the microbial loop which return some of the POM to the DOM.

pH/redox boundary: The Archaean ocean was largely anoxic, but probably contained aggregates or other structures with oxygenated cores. In the modern ocean, this pattern is reversed, the water is mainly oxygenated, but there are suboxic and anoxic micro-environments, in the centres of aggregates, and in such structures as *Trichodesmium* mats. Anoxic zones can only exist in places where the accumulation and degradation of organic matter proceeds at a faster rate than that at which oxygen is supplied. It is in such environments that anaerobic processes like methanogenesis and fermentation can take place. In the absence of barriers to oxygen diffusion, the characteristic time scales of such zones are determined by molecular diffusion, say about 2.10^{-5} cm^2 s^{-1}, which means about 4 minutes for a sphere of radius 1 mm, 7 hours for one of radius 1 cm. Obviously the larger particles provide more opportunity to anaerobic consortia unless barriers to diffusion are somehow erected. The minimum size of an anaerobic habitat embedded in bulk aerobic phase is about 1 mm, the size of a termite's hindgut.

Films as resources: It is now recognised that the sea surface microlayer (SML) is of fundamental importance in governing exchanges between the atmosphere and the ocean, and that biological and chemical activities here are very distinct from those in the water just below. Specialized bacteria, phytoplankton, and zooplankton, collectively the neuston, inhabit the SML, and are one to three orders of magnitude more abundant there than in subsurface waters. The DOM here is derived from buoyant material from below, especially during algal blooms, from bubble transport, and from the atmosphere, as well as from the activities of the neuston.

Bubbles and flakes: Bubbles driven into the sea by breaking waves rapidly acquire coatings of organic matter from the water by adsorption, or by carrying surface film material into the water as they are formed. These colloidal coatings might stiffen the bubbles and prolong their existence by resisting the force of surface tension which would otherwise lead to their collapse as their contained gases diffuse outwards; or the bubbles might collapse and leave residues of organic material as flakes[10]. When they rise to the surface and burst, the coatings remain behind to contribute to the surface film again (Baylor and Sutcliffe, 1963). The half lives of wind-generated bubbles are short (minutes), so their importance lies in their scavenging and transporting of DOM and its transformation into particles. The resulting surfaces are extensive. For example, a bubble population of ~5×10^5 m^{-3} with radii 25 µm has a surface area of ~800 mm^2 ml^{-1}, much larger than the equivalent figure for phytoplankton, for which Kahru *et al*. (1991) provide a figure of 1.5 mm^2 ml^{-1}.

9 "...the accumulation of humus ... is a direct reaction of the plant community itself upon its habitat" (Tansley, 1946).

10 This route from DOM to POM was hailed in the 1960s as a solution of the conundrum posed by Pütter concerning the potential of DOM to support zooplankton metabolism.

DISCUSSION

If we take 150 µmol/l as carbon as an upper limit of *in situ* DOM concentration, and assume different average molecular weights for DOM as carbon, say 30, 100, 500, with the first being glycerol, glycolate etc. and the last being a polymer, then the abundance of molecules would range from approximately 3 molecules per million water molecules to 16 molecules for each billion water molecules. If we consider only the large colloids (e.g., MW>500 Da) which should correspond very roughly to molecules with at least 15 carbon atoms, the range will be from 1/1,000,000 to, say 5/100,000,000. These (bulk) concentrations may seem very low, but indicate that there are more organic molecules than nutrient ions in a given volume of water. From another perspective, there will be 300 billion very small molecules for each bacterial cell (assuming an abundance of 10^{8} cells l^{-1}) or 2 billion large molecules for each cell.

In normal conditions (DOM concentration of 100 µmol/l as carbon) with an intermediate value for molecular size of 100 C atoms/molecule (which could amount to about 2500 Da), again very roughly, there is still approximately one such DOM molecule for each nitrate ion, assuming homogeneity. It is only at depth that there will be 50 nitrate ions for each DOM molecule. But there will still be an overwhelming abundance of molecules for each cell (molecules decrease by less than an order of magnitude while cells decrease by two to three orders of magnitude). These numbers suggest that ionic substrates very frequently interact with DOM; so, while we know that DOM can modify the physico-chemical properties of water, the interactions between ions, DOM, and cells may also be critical, although they remain largely unknown.

Chemical activities inside cells are often framed in terms of Michaelis-Menton kinetics, but the end results of this activity are much more certain than such mass action models imply because they are programmed and highly coordinated; reactions occur sequentially, each step at the proper place and time. In addition, the cytoplasm is *crowded* with macromolecules which provide barriers to the free diffusion of molecules; such controls over diffusion reduce diffusion-limited reaction rates there, which leads to microzones of concentration different from those *in vitro*. Reaction rates in the cell are therefore not the same as *in vitro* where concentrations of reactants are generally much lower. Crowding leads to exclusion of reactants from parts of the cytoplasm, and to their segregation when they are present (Schnell and Turner, 2004)[11]. The cytoplasm of the cell is contained in the interstices of the cytoskeleton. We can view the extended exopolymers *outside* the cell as a kind of periskeleton; its architecture must be determined partly by the chemistry of the polymers comprising it and by their interactions with the local medium, and probably also by the spines and other features of the cells themselves which secrete them, acting in ways analogous to the armature of reinforced concrete. We can then ask in what ways this structure constrains chemical concentrations and reactions in the spaces it defines. Macromolecules occupy from 5% to 40% of intracellular space; for extracellular space to be equally crowded, hence equally effective in overcoming the drawbacks of mass action, the DOM would need to be restricted to between 4% and 0.5% of the water volume, i.e., about an order of magnitude more abundant than bulk measurements indicate. Since studies indicate that phytoplankton numbers in thin layers a few centimeters to <1 m in thickness can be several orders of magnitude higher than above or below them, to the extent that the exopolymers are autogenic, it seems reasonable to expect them to be crowded when blooms of healthy cells do it. Further concentration must result from aggregation and phase separation.

A tissue is usually thought of as a layer or mass of cells that performs a specific function within an organism, but the word is also used for woven fabrics, and figuratively for webs or textures. The essence of connective tissue, for example, is a weave of elastic and reticulated collagen fibres. This second meaning matches the polymeric networks of autogenic polymers secreted by some plankton. Or we can imagine it as engineering or architecture as already outlined. “Architecture is not only about domesticating space”, writes Karsten Harries, “It is also a deep defence against the terror of time.” But unlike man-made tools and buildings, tissues are dynamic entities, contextual and responsive. The thin layers in the ocean where much of the phytoplankton biomass congregates may be established by physical processes, but by means of

11 The reactants thus face a problem analogous to that of dispersal stages in fragmented environments.

rheological tools such as DOM, as well as other adaptations, the organisms can domesticate them and prolong their usefulness.

Then the functions of these tissues in general terms may be to act as *adaptors* (in the sense coined by Francis Crick) between cells and the surrounding medium, that is to perform two independent recognition processes (Barbieri, 2003); and to manipulate the dictates of physical and chemical laws, e.g., by uncoupling solute dispersion from energy dissipation, or by controlling redox gradients. Calvino's city of *Ottavia* is suspended over a chasm, "bound to the crests on either side by ropes and chains and catwalks. You walk on the little wooden ties, careful not to set your foot in the open spaces, or you cling to the hempen strands. Below there is nothing for hundreds and hundreds of feet: a few clouds glide past. This is the foundation of the city: a net which serves as passage and as support ... Suspended above the abyss, the life of Octavia's inhabitants is less uncertain than in other cities. They know the net will last only so long." [12]

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12 From William Weaver's translation.

Food web driven dynamics of DOM in the Mediterranean Sea

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Marine heterotrophic bacteria have for a long time been recognised as central partners in the pelagic carbon cycle (Pomeroy, 1974; Williams, 1981; Azam *et al.,* 1983). There is therefore a need to understand the extent of such a role in Particulate Organic Matter (POM)<– – –> Dissolved Organic Matter (DOM) global dynamics within pelagic ecosystems.

Mechanisms underlying the production and fate of DOM in oligotrophic marine environments seem to intimately be linked with the balance between inorganic macronutrients, as mainly "viewed" by the food web's microbial actors (Figure 1). This feature becomes more effective when the latter undergo a limitation and/or co-limitation by the above nutrients.

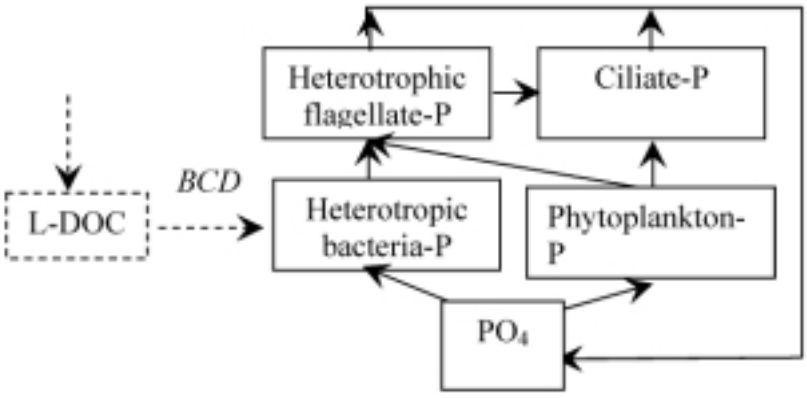

Fig. 1. Idealized food web used for analysis of steady state P-flow. Labile dissolved organic carbon (L-DOC) is assumed to be in excess of bacterial carbon demand (BCD).

In such a framework, complex microbial recycling, uptake, incorporation and transfer of both inorganic and organic dissolved matters play a central role. As a system, a clear example for such a situation is a large part of the eastern basin of the Mediterranean Sea (Thingstad and Rassoulzadegan, 1999).

Considering the two basic compartments of the marine food webs, because of their small size, bacteria exhibit a higher affinity for inorganic nutrients than phytoplankton (Figure 2), mainly when such nutrients are at low concentrations (Tanaka *et al*., 2003, 2004).

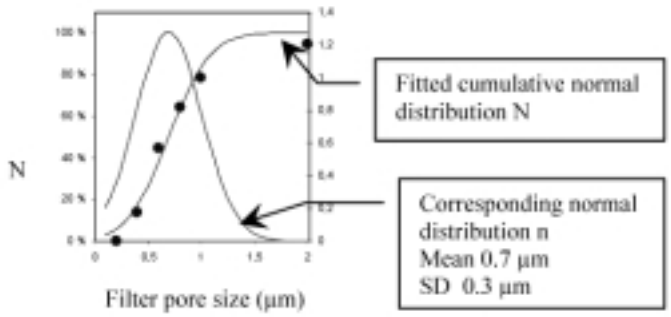

Fig. 2. Dominance of Phosphate uptake by small organisms in oligotrophic areas: Mediterranean Sea.

The eastern Mediterranean is a typical low-nutrient-low-chlorophyll (LNLC) area with chlorophyll levels < 20 µg m^{-3} and free mineral forms of both phosphorous and nitrogen at very low concentrations in the stable summer situation (Krom *et al*., 1993). High nitrate:phosphate-ratios (Krom *et al*., 1991) and bioassay experiments for phytoplankton (Bonin *et al*., 1989) and heterotrophic bacteria (Zohary and Robarts, 1998) have suggested growth of these osmotrophs to be P-limited. An earlier model has been proposed as a generic framework for the microbial food web in such a situation (Thingstad and Rassoulzadegan, 1995).

A more recent model predicted that DOC would accumulate in Mediterranean surface waters during the productive season (Figure 3). This is consistent with observations from NW Mediterranean (Copin-Montegut and Avril, 1993).

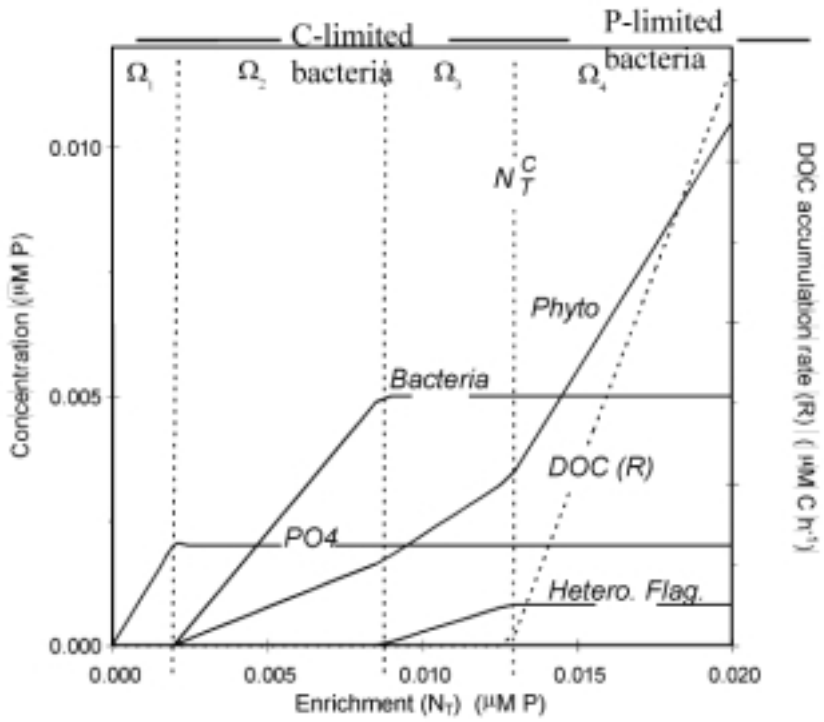

Fig. 3. Model prediction for extreme oligotrophy: high bacteria:phytoplankton biomass at low total-P concentrations, leading to C-limited bacteria when phytoplankton biomass becomes *sufficiently* low (after Thingstad *et al.,* 1997, modified).

Such a feature has been related to a "malfunctioning" of the microbial loop with severely nutrient limited bacteria (Thingstad *et al.,* 1997). Some of our results on the eastern Mediterranean, have shown also that in such conditions the major part of the phosphorus can be represented by the dissolved organic phosphorus (DOP) pool (Figure 4). In this work, we try to explain the possible pathways to such a situation. Assuming a steady state with P-limited bacteria, we conceive a system with a production, or an accumulated reservoir, of labile organic substrates sufficient to support the bacterial carbon demand.

Such a system is mainly based on the dominance of the dissolved primary production (exceeding by more than one order of magnitude the particulate primary production), situation occurring under severe P-starvation (e.g. Dubinsky and Berman-Frank, 2001). One of us (Thingstad, 2005) recently proposed a model conceptualizing the functional response of such P-starved ecosystems to a Lagrangian P-addition experiment in eastern Mediterranean (Thingstad *et al.,* 2005).

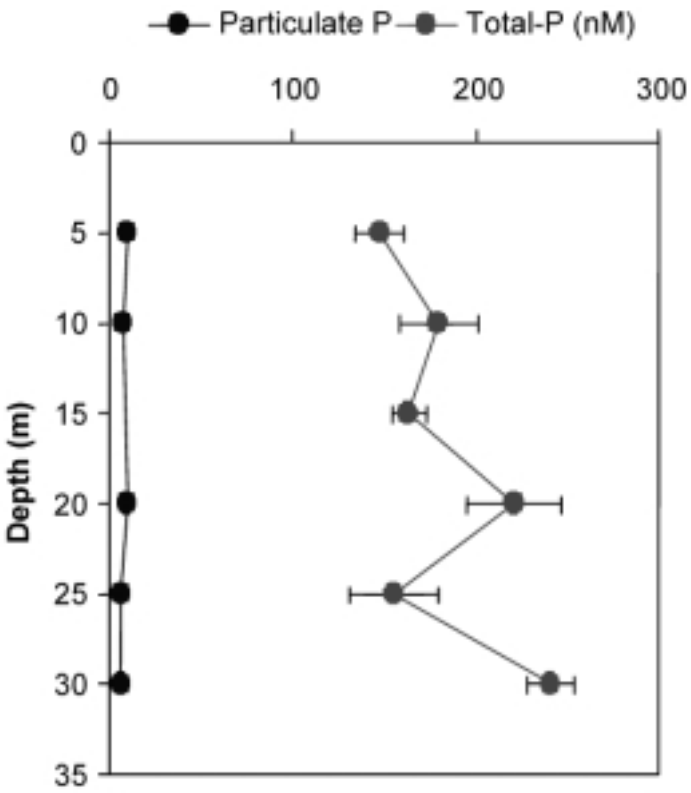

Fig. 4. Results from the eastern Mediterranean Sea showing that most of P in the system seems to be in dissolved organic forms, the dissolved inorganic PO_4 being << 20 nM.

A geochemical approach to the origin and fate of dissolved organic matter in the Mediterranean Sea

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ABSTRACT

Application of water masses/transport inverse methods, successfully applied to other ocean areas, is suggested to study the basin scale/long term and local scale/short term physical mixing and biogeochemical processes responsible for the spatial and temporal changes observed in the distribution of dissolved organic matter (DOM) in the Mediterranean Sea.

RATIONALE

The study of the role played by dissolved organic matter (DOM) in the oceans in general, and in the Mediterranean Sea in particular, can be approached from a (micro) biological or a geochemical perspective. These two approaches use different techniques *(in vitro* tests *versus* water masses/transport inverse methods), apply to different temporal and spatial scales (10^{-1}–10^{1} days/10^{2}–10^{3} m *versus* 10^{2}–10^{5} days/10^{4}–10^{6} m) and are rarely combined within the same study.

The topography, hydrography and dynamics of the Mediterranean Sea are quite suitable for the application of water masses/transport inverse methods. In this sense, the Mediterranean Sea is characterized by an intricate topography, with two nearly equal size basins (Eastern and Western) connected by the Strait of Sicily, and diverse sub basins. The circulation of the Mediterranean Sea, shaped by the complexity of the topography and by the variety of intermediate and deep water formation areas, is naturally also complex. It is forced by water exchange through the various straits, wind stress and freshwater discharge.

Water masses inverse methods could be applied to the Mediterranean Sea to study the currently unknown:

1) Physical and biogeochemical components of the bulk dissolved organic carbon (DOC), nitrogen (DON) and phosphorus (DOP) by defining C/N/P water types and C/N/P biogeochemical anomalies. Few studies, with a limited number of sampling points, have determined the DOC concentration of the different water masses present in the Gibraltar strait (Dafner *et al.,* 2001b, c), the Dardanelles strait (Polat and Tugrul, 1996), the Catalan Sea (Doval *et al.,* 1999; Lucea *et al.,* 2003), the Ligurian Sea (Copin–Montégut and Avril, 1993; Avril, 2002) the Tyrrhenian Sea (Santinelli *et al.,* 2002), the Ionian Sea (Seritti *et al.,* 2003) and the Aegean Sea (Sempéré *et al.,* 2000). Times series of full–depth DOC profiles are only available for the DYFAMED site in the Ligurian Sea (Avril, 2002). A seasonal cycle of DON is also available at the DYFAMED site (Pujo–Pay and Conan, 2003) and the only available

data on DOP are from the Gulf of Lions continental shelf (Durrieu de Madron *et al.,* 2003) and the Catalan Sea (Lucea *et al.,* 2003). However, an objective separation of the physical and biogeochemical components of the bulk DOM concentrations and C/N/P stoichiometry over the entire Mediterranean Sea has not yet been performed.

2) Relative contribution of DOM to the oxygen demand of the surface (ASW, BSSW), intermediate (LIM) and deep waters (WMDW, EMDW, LDW) of the Mediterranean Sea by comparing inorganic and dissolved organic carbon, nitrogen and phosphorus water types (long term /basins scale processes) and biogeochemical anomalies (short term/local processes). A few studies have related the apparent oxygen utilization with the DOC changes in the different water masses of the Ligurian Sea (Copin–Montégut and Avril, 1993; Avril, 2002), the Tyrrhenian Sea (Santinelli *et al.,* 2002) and the Ionian Sea (Seritti *et al.,* 2003). Once again, the objective separation of physical and biogeochemical components of DOC–ΣCO_2, DON–NO_3 and DOP–PO_4 would allow to quantify the contribution of DOM to the nutrient mineralization in the different water masses of the Mediterranean Sea, at different sites (formation areas, mid basins, straits) and times (seasonal, decadal).

3) Long term/basin scale and short term/local formation of coloured dissolved organic matter (CDOM) as a by–product of the oxidation of DOM at surface, intermediate and deep layers of the Mediterranean Sea. Several studies have used the absorbance/fluorescence of DOM to trace the circulation and, to a lesser extent, the descriptive biogeochemistry (mainly photo-degradation) of the epipelagic Mediterranean Sea (Momzikoff *et al.,* 1992; 1994; Seritti *et al.,* 1998; Obernosterer *et al.,* 1999; Ferrari, 2000; Claustre *et al.,* 2000; Babin *et al.,* 2003; Gaines *et al.,* 2004; Vignudelli *et al.,* 2004). However, CDOM has been never used to trace mineralization processes in the meso– and batipelagic Mediterranean Sea. CDOM could be used to tracer the penetration of the deep waters of the Eastern Mediterranean (80–120 years old; with relatively low oxygen utilization rates, OURs) into the deep Western Mediterranean (20–40 years old; with relatively high OURs) through the Strait of Sicily (Stratford *et al.,* 1998).

4) Net consumption rates of DOC, DON, DOP and net production rate of CDOM in surface, intermediate and deep layers of the Mediterranean Sea combining water types and anomalies of these DOM tracers with age estimates from a transient tracer such as tritium, helium or CFCs. The good agreement between ΣCO_2 oxidation rates measured from ETS (local/short term) and the Mediterranean Sea water masses (La Ferla *et al.,* 1999; 2003; La Ferla and Azzaro, 2001; 2004) should be compared with those obtained with this geochemical approach. Increased OURs in deep layers of the Eastern Mediterranean after the Eastern Mediterranean Transit (EMT) could be related to the introduction of DOM–rich younger waters recently formed in the Aegean Sea (La Ferla and Azzaro, 2001; Klein *et al.,* 2003; La Ferla *et al.,* 2003; Kress *et al.,* 2003). In agreement with this hypothesis, bacterial activity measurements in the Ionian Sea suggest that deep waters carry a larger amount of labile DOM because of their young age (Zaccone *et al.,* 2003).

The Mediterranean Sea is also suitable for the application of water/property transport inverse methods to study the (relatively) unknown:

5) DOC, DON, DOP and CDOM transports by the surface, intermediate and deep waters of the Mediterranean Sea through the most relevant straits (Dardanelles, Otranto, Sicily and Gibraltar). There is currently some information about the Strait of Gibraltar (Dafner *et al.,* 2001a; 2001b), but the results are surprising because the Mediterranean Sea seems to export to the Atlantic Ocean one order of magnitude more ΣCO_2 than the organic carbon imported from the Atlantic Ocean and the continental inputs. Can the atmospheric inputs of organic carbon close the balance? Or is the Mediterranean Sea consuming reserves of organic carbon accumulated in the previous decades (non steady state)? Although water fluxes (seasonal and annual) across the other major straits of the Mediterranean Sea are well known (e.g. Kovačević *et al.,* 1999; Briand, 1996; Beşiktepe, 2003; Béranger *et al.,* 2003, 2005), organic matter fluxes have not been evaluated yet although they have been identified as important (e.g. Otranto strait; Civitarese *et al.,* 1998). The approach of Ribera d'Alcala *et al.* (2003) is

exemplary on how these inverse methods can be used to study the contrasting nutrient stoichiometry of the Eastern and Western Mediterranean basins.

6) DOC, DON, DOP and CDOM balances for the whole Mediterranean Sea and for the subbasins. This will require a quantitative knowledge of the seasonal variability of the continental and atmospheric entries of DOM to each basin/sub basin, which is well known for inorganic nutrients (Herut *et al.,* 1999; Migon *et al.,* 2001; Durrieu de Madron *et al.,* 2003; Markaki *et al.,* 2003; Krom *et al.,* 2004) but very limited for DOM (Cauwet *et al.,* 1990; Leveau *et al.,* 1990; Guerzoni *et al.,* 1997; Sempéré *et al.,* 2000; Mace *et al.,* 2003; Durrieu de Madron *et al.,* 2003; Vignudelli *et al.,* 2004). In this sense, Sempéré *et al*. (2000) produced a tentative budget of organic carbon sources to the Mediterranean Sea on the basis of limited available datasets, estimating that 20-83 x 10^{10} mol C y^{-1} came from the rivers, 10-20 x 10^{10} mol C y^{-1} from the atmosphere, 11–12 x 10^{10} mol C y^{-1} from the Black Sea and 125 10^{10} mol C y^{-1} from the Atlantic Ocean.

Temporal changes are also relevant for each of the six previous issues/questions, either at the seasonal (mainly in the surface waters domain) or decadal (mainly in the intermediate and deep waters domains; EMT) scales.

The answer to these key questions should be achieved by a combination of:

1) A proper re-analysis of existing data on water column profiles, and freshwater and atmospheric inputs of DOM (DOC, DON, DOP and CDOM).

2) Time series stations at the site of formation of intermediate and deep waters of the Mediterranean Sea (monthly frequency optimum). Currently, the French JGOFS time series station DYFAMED is operative in the Ligurian Sea and is accompanied by an atmospheric station at Cap Ferrat (http://www.obs–vlfr.fr/sodyf).

3) Time series (high spatial resolution) transects across the Dardanelles, Otranto, Sicily and Gibraltar straits. Water and property transport by straits are specially sensitive to natural/anthropogenic environmental changes. An effort is currently been done in the Gibraltar Strait within the framework of the EU Integrated Project CARBOCEANS (http://www.carboocean.org/).

4) Coupled Mediterranean biogeochemical models. Some models of the Mediterranean Sea have already incorporated DOM in their biogeochemical modules (Levy *et al.,* 1998; Tusseau–Vuillemin *et al.,* 1998; Crispi *et al.,* 2001; Anderson and Turley, 2003; Vichi *et al.,* 2003; Raick *et al.,* 2005).

CDOM AS A TRACER FOR BIOGEOCHEMICAL PROCESSES

A recent study by Nieto–Cid *et al*. (2005) demonstrated that significant changes occurred in CDOM after 24 h light and dark incubations under a wide variety of oceanographic and trophic conditions. Respiration rates were positively correlated with the net production of CDOM in the dark (r = +0.73, $n = 79$, $p < 0.001$; Figure 1) at a net rate of 0.027±0.003 ppb Quinine Sulphate (µmol kg^{-1} $O_2)^{-1}$, suggesting a daily synthesis of marine humics as a by–product of the bacterial respiration of DOM.

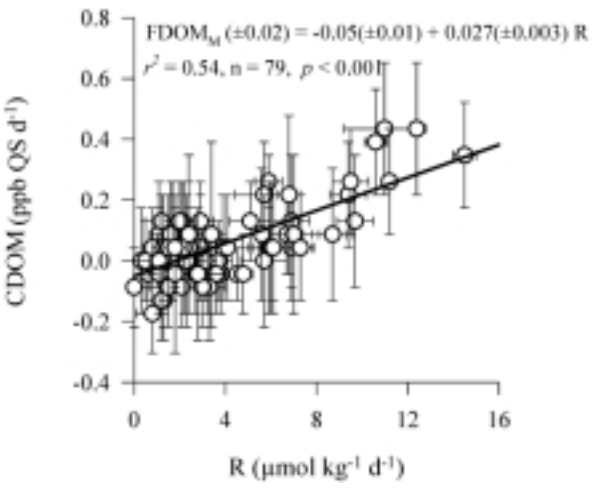


Fig. 1. X–Y plot of net CDOM production vs. dissolved oxygen respiration after 24h dark incubations. Solid lines represent the corresponding linear regression lines. Taken from Nieto–Cid *et al.* (2005).

WATER MASSES INVERSE METHODS

WMIM can be used to trace mixing and biogeochemical processes in time series stations (allowing to resolve the seasonal and decadal variability) and/or hydrographic sections (allowing to resolve the spatial variability). The theoretical basis of a water mass inverse model is that, for a given sample,

$$\sum_{i=1}^{n} x_{ij} = 1 ,$$

where x_{ij} is the proportion of the water mass i in sample j and n is the number of water masses identified in the study area. NO_3, DOC, DON and CDOM water types, can be obtained by inverting the system of n linear mixing equations

$$N_j = \sum_{i=1}^{n} x_{ij} \cdot N_i ,$$

where N_j is the actual concentration of the chemical variable in sample j (known) and N_i is the concentration of the chemical variable in water type i (unknown). Since the number of samples greatly exceeds the number of equations, an over determined system has to be solved for each chemical variable by minimising the residuals of the equations in a Non–Negative Least Squares sense, where N_i must be positive (Álvarez *et al*., 2004). A geochemical parameter anomaly (ΔN_j) can be defined for each sample

$$\Delta N_j = N_j - \sum_{i=1}^{n} x_{ij} \cdot N_i . \quad \text{Whereas} \quad \sum_{i=1}^{n} x_{ij} \cdot N_i$$

accounts for the physical mixing and long term/basin scale geochemical processes, ΔN_j accounts for the short term/local geochemical processes.

Together with C. Castro, we recently applied a water mass inverse method to a time series in order to study the seasonal mineralization patterns in the mesopelagic layer off NW Spain. The variability of the chemical parameters not explained by the thermohaline properties corresponds to the aging that Eastern North Atlantic Central Water (ENACW) experienced due to biogeochemical or ventilation processes. Figure 2 shows the seasonal evolution of the temperature, dissolved oxygen and CDOM concentrations and geochemical anomalies on ENACW waters for the study period. Negative (positive) oxygen (CDOM) anomalies are associated with enrichment due to mineralization of organic matter and the opposite is associated with ventilation processes. The slope of the linear relationship between ΔCDOM and ΔO_2 for the shallowest ENACW, $\sigma_0 = < 26.95$ ($r^2 = 0.78$, $p < 0.001$) was 0.019±0.007 ppb QS (µmol/kg O_2)$^{-1}$, which is not significantly different from the slope of 0.027±0.003 ppb QS (µmol/kg O_2)$^{-1}$ obtained from the in vitro experiments of Figure 1.

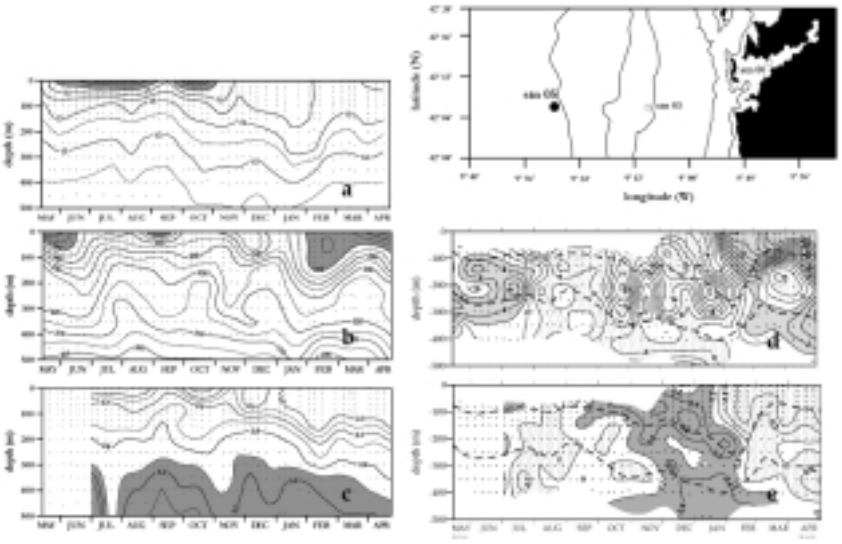


Fig. 2. Seasonal cycle of temperature (**a**), dissolved oxygen, O_2 (**b**), CDOM (**c**), ΔO2 (**d**) and ΔCDOM profiles (**e**) at station 05 located at the shelf break of the NW coast of Spain. From Castro *et al.* (submitted).

Our group applied a water mass inverse method to a hydrographic section, in the meridional overturning circulation of the North Atlantic in order to study the metabolism of DOM. We obtained for the first time DON, C/N ratios of DOM, and CDOM types for the different water masses that mix in the North Eastern North Atlantic (Figure 3).

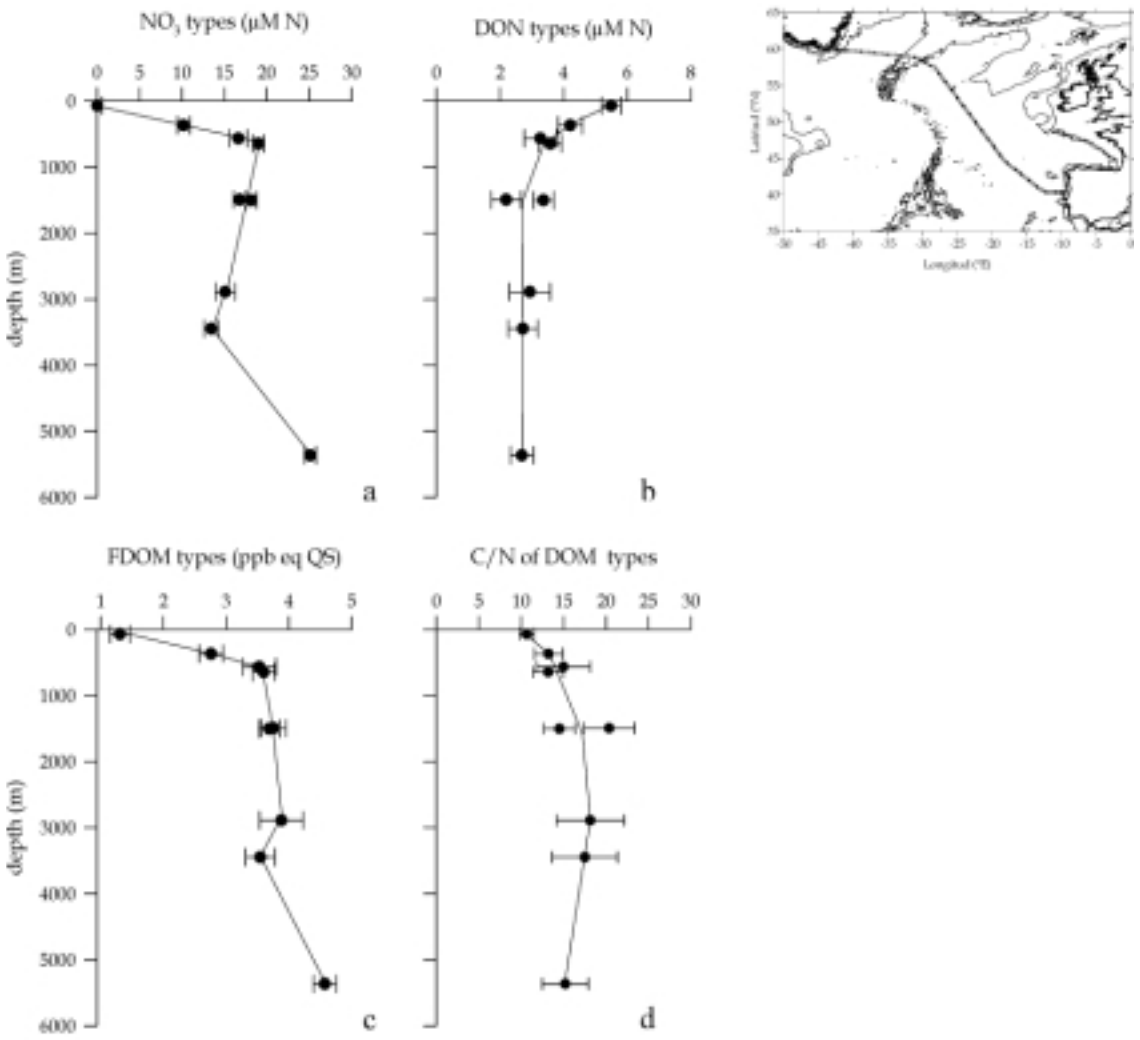
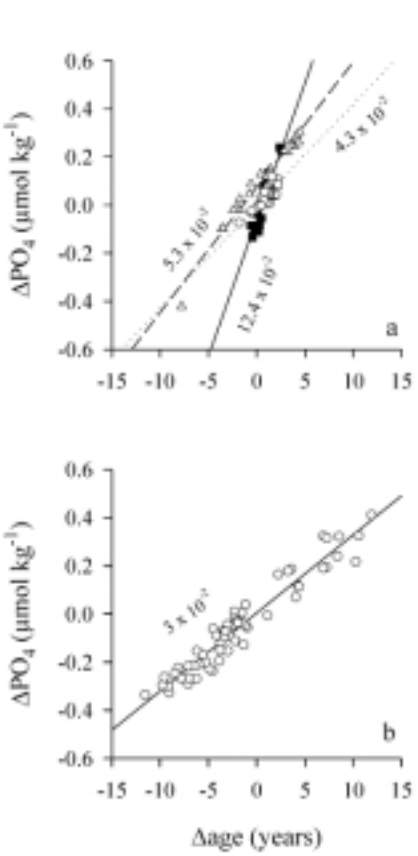


Fig. 3. Profiles of NO_3 (**a**), DON (**b**), FDOM (**c**) and C/N molar ratio of DOM (**d**) types for the end members mixing in the Eastern North Atlantic. From Álvarez–Salgado *et al.* (submitted).

The anomalies of these chemical parameters revealed that from 23±6% in central waters (100–500 m) to 88±27% in deep waters (3000–5500 m) of NO_3 mineralized locally comes from the oxidation of dissolved organic nitrogen DON. Concomitantly, the quality of the mineralized material (C/N molar ratio) improves from the central (8±1 mol C mol N^{-1}) to the deep water (5±2 mol C mol N^{-1}) domains. The significant ($p > 0.001$) covariance between CDOM and NO_3 points to the bacterial oxidation of DON as a relevant source of NO_3 in the North Eastern North Atlantic, one of the main areas of central, intermediate and deep waters formation of the world ocean.

Finally, a good example of a water mass inverse methods applied to obtain oxygen and nutrient mineralization rates has been recently provided by Brea *et al*. (2004), who estimated the spatial variability of PO_4 mineralization rates along different provinces (from subantarctic to

subequatorial) of the Eastern South Atlantic Ocean at the central and Antarctic Intermediate Water (AAIW) domains. They developed a new method to estimate mineralization rates and found significant differences between provinces depth ranges (Figure 4).



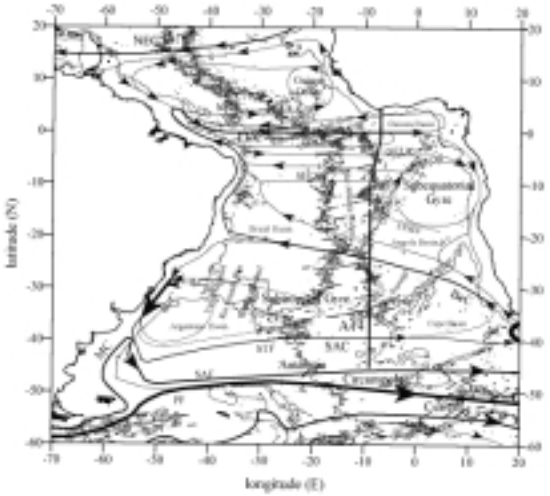


Fig. 4. PO_4 versus CFC–age anomalies along (**a**) the South Atlantic Central Water (SACW) and (**b**) Antarctic Intermediate Water (AAIW) domains of the Eastern South Atlantic. The slopes of the regression lines indicate the apparent PO_4 mineralization rates. Taken from Brea *et al.* (2004).

WATER/PROPERTY TRANSPORTS INVERSE METHODS

Water/property transport inverse methods can be used to study DOM exchange fluxes across straits (e.g. Dafner *et al*., 2001a, 2001b in the Strait of Gibraltar) or enclosed sections as well as to perform balances considering all inputs (including oceanic, riverine and atmospheric) and outputs to the volume of the Mediterranean Sea delimited by a given hydrographic section. Relevant volumes to study from a DOM mass balance perspective are the Gulf of Lions, the Adriatic Sea, the Aegean Sea and the Levantine basin. Here we show an example of an application of a WTIM to the North Atlantic from 24.5 to 72ºN (Hansell *et al*., 2004). Dissolved inorganic and organic carbon transports and balances for the volume studied (Figure 5) suggest that the net mineralization of DOC with the basin (at 9.2 Tmol C y^{-1}) account for only 10% of the net inorganic production.

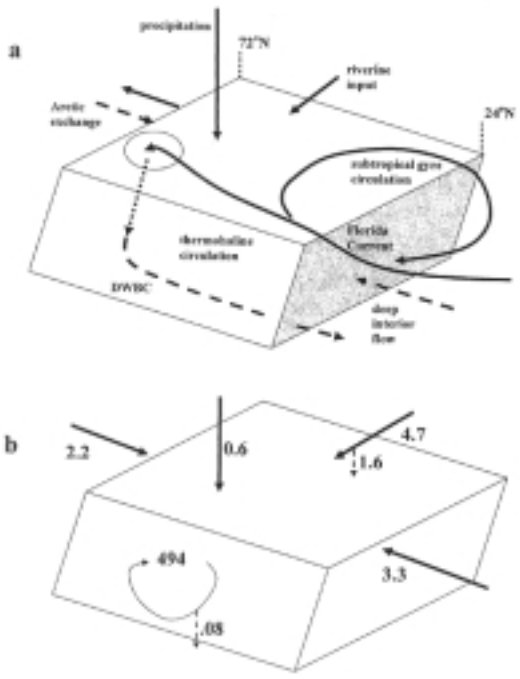


Fig. 5. (**a**) Important basin boundary exchanges and circulations in the North Atlantic. The circle represents the area of the NADW formation. (**b**) Net transports (Tmol C y^{-1}) of DOC in the North Atlantic (24.5–72ºN). Burial of fluvial POC as well as that produced by *in situ* primary production are shown as dashed arrows. Taken from Hansell *et al.* (2004).

Dying plankton as a source of Dissolved Organic Matter

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ABSTRACT

The rates of release of organic compounds from decomposing planktonic debris are presented. Carbon loss rates from decomposing copepod carcasses are greater than from decomposing phytoplankton cells, which in turn are greater than from zooplankton fecal pellets. Generally, carbon loss rates decrease with temperature and decrease rapidly and non-linearly after the first few days of decomposition; leaching is a major route of carbon loss, typically accounting for about half of the loss of POC to DOC. The presence of bacteria roughly doubles the rates of carbon loss from planktonic debris and sinking increases these rates by about 20%.

What happens to a pelagic organism when it dies? In the case of large animals, sinking of carcasses to deep waters or sediments ensues, but most organisms in surface waters are smaller than a millimeter, and their sinking rates are far lower. Thus, they tend to decompose in the upper regions of the oceanic water column. Clearly, decomposition is a major source of dissolved organic matter and this occurs both in surface waters for planktonic organisms and, to a lesser degree, in the "twilight zone" for some sinking biogenic debris. The sinking debris is largely of planktonic origin (Angel, 1984; Fowler and Knauer, 1986), and most sinking debris decomposes in the upper 1000 m of the water column (Walsh, 1983; Longhurst and Harrison, 1989). This process probably accounts for nearly all of the labile dissolved organic matter in surface waters and much of the refractory material as well. Thus, to understand the cycling and distributions of dissolved organic compounds in the sea, it is necessary to assess the sources of these compounds and their rates of production.

While some studies have identified biochemical components of cells that may be released into the water and ultimately remineralized (Wakeham *et al.,* 1997), the rates of decomposition of different planktonic organisms upon death are still not well known, nor are the processes involved. A series of experimental studies quantified the rates of carbon loss from dying phytoplankton and zooplankton (Lee and Fisher, 1992a,b, 1993; Fisher and Wente, 1993; Reinfelder *et al.,* 1993). The findings from these studies shed some light on this problem; they are consistent with field measurements of dissolved organic carbon concentrations in surface seawater and help explain observed patterns of DOC concentrations at different depths. From these studies the following conclusions can be drawn:

1) Carbon loss from dying phytoplankton cells occurs as a result of a number of processes, including leaching and bacterial decomposition. As expected, loss rates decrease with decreasing temperature. Further, initial rates of carbon loss from different species maintained under identical conditions can vary up to 3-fold, with values from all experiments typically ranging from about 3% to 11% d^{-1} at 18 °C. Loss rates at 4 °C are about 2-3-fold lower.

Initial loss rates of particulate nitrogen from dying cells also vary with species and were found to range from about 1% to 9% d^{-1} at 18 °C. As with particulate carbon loss, protein loss is 2-3-fold lower at 4 °C (Table 1).

Table 1. Rates of decomposition of marine diatoms and calanoid copepods. Instantaneous release rates (% d^{-1}) of carbon (C) and protein (P) from decomposing diatoms (*Thalassiosira pseudonana*) held in filtered seawater with or without added microorganisms (< 3 μm) over a 30-day period at 18 °C or 4 °C. nd: not determined. Also shown are carbon loss rates from decomposing copepod (*Acartia tonsa*) carcasses and fecal pellets held at 18 °C or 2 °C, from animals fed on diatoms. From Lee and Fisher (1992b, 1993).

	1 d	2 d	3 d	4 d	6 d	10 d	20 d	30 d
Diatoms 18 °C, control	11.0 C 4.0 P	6.6 C 2.6 P	4.6 C 1.9 P	3.5 C 1.5 P	2.3 C 1.0 P	1.3 C 0.6 P	0.6 C 0.3 P	0.3 C 0.2 P
Diatoms 18 °C, microorganisms added	17.5 C 9.2 P	9.5 C 5.6 P	6.2 C 4.0 P	4.5 C 3.0 P	2.7 C 2.0 P	1.4 C 1.2 P	0.5 C 0.5 P	0.3 C 0.3 P
Diatoms 4 °C, control	4.5 C nd P	2.9 C nd P	2.1 C nd P	1.6 C nd P	1.1 C nd P	0.7 C nd P	0.3 C nd P	0.2 C nd P
Diatoms 4 °C, microorganisms added	5.3 C 1.9 P	3.3 C 1.2 P	2.4 C 0.9 P	1.9 C 0.7 P	1.3 C 1.5 P	0.8 C 0.3 P	0.4 C 0.2 P	0.2 C 0.1 P
Copepods 18°C, carcasses	21.3 C	10.7 C	6.6 C	4.6 C	2.1 C	1.2 C	0.4 C	0.2 C
Copepods 2 °C, carcasses	13.6 C	7.9 C	5.3 C	4.0 C	2.1 C	1.4 C	0.6 C	0.3 C
Copepods 18°C, fecal pellets	10.1 C	6.1 C	4.3 C	3.3 C	1.8 C	1.2 C	0.5 C	0.3 C
Copepods 2°C, fecal pellets	3.6 C	2.3 C	1.7 C	1.3 C	0.8 C	0.6 C	0.3 C	0.2 C

2) Initial rates of carbon loss from decomposing copepod carcasses are faster than loss from phytoplankton debris, with values from all experiments ranging from 14% d^{-1} at 2 °C to 22% d^{-1} at 18 °C. Initial rates of protein loss from decomposing copepod carcasses are also faster than loss from phytoplankton debris, with values ranging from 24% d^{-1} at 2 °C to 63% d^{-1} at 15 °C. However, loss rates of carbon from copepod fecal pellets were slower than from phytoplankton cells. Thus, only 20% of fecal carbon is lost after 30 days at 2 °C. Table 1 shows some representative data on carbon loss from decomposing copepod carcasses and fecal pellets. Leaching of carbon accounts for about half of the loss of particulate carbon to DOC, even in the absence of microbial activity. A greater fraction of the carbon that is released from carcasses is readily oxidized by microorganisms to CO_2 than from fecal pellets.

3) The presence of bacteria in seawater almost doubles the rate of carbon loss from decomposing planktonic debris at 18 °C, but increases it by only 16% at 4 °C, at which microbial metabolic rates are slower.

4) The loss rates of particulate carbon and protein from decomposing cells decrease sharply and non-linearly with time, as measured over a one-month period. Averaged over the entire period, carbon loss rates from dying diatoms are about 3% d^{-1} at 18 °C and 1.0% d^{-1} at 4°C. However, instantaneous rates of loss decrease from 18% d^{-1} after 1 day to 0.3% d^{-1} after 30 day at 18 °C, and from 5% d^{-1} after 1 day to 0.2% d^{-1} after 30 day at 4 °C. Thus, labile material is lost the quickest, followed by loss of more refractory material. The presence of bacteria

does not greatly affect the loss of refractory material from planktonic debris. Table 1 shows representative values for instantaneous rates of carbon loss from decomposing diatoms.

5) Sinking increases the rates of carbon loss from planktonic debris (e.g., copepod carcasses, fecal pellets) by about 20%, and copepod grazing on phytoplankton has a modest effect on carbon remineralization rates.

Future studies should measure the rates of release of specific organic compounds from decomposing planktonic debris and the rates of microbial oxidation of these compounds in surface and "twilight zone" depth waters.

Degradation of complex polysaccharides in marine pelagic communities

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ABSTRACT

The mucus that is frequently observed to occur in the Mediterranean Sea consists largely of chemical complex polysaccharides, with acidic polysaccharides contributing a large fraction. Microbial remineralization of such molecules begins with extracellular enzymatic hydrolysis, and only thereafter can these substances be taken up by bacteria. During the last few years the potential for extracellular hydrolysis of specific complex polysaccharides like chondroitin sulfate, fucoidan, laminarin and xylan in pelagic marine environments has been studied. Here, we review the range of potential hydrolysis rates of several complex polysaccharides over a variety of spatial and temporal scales and as a function of dominating phytoplankton. The patterns of hydrolysis rates of different substrates differ distinctly from each other and over time and cannot be explained simply by correlations with other microbial parameters, such as bacterial cell concentration, growth or protein production rate. The observed patterns of hydrolytic activity over time differ as well between specific substrates, suggesting that the presence or absence of an extracellular hydrolytic enzyme is controlled by a tight interaction between environmental conditions and specific members of microbial communities.

INTRODUCTION

Large accumulations of mucus which impact the local tourism and fishing industries occur commonly in the Mediterranean Sea. While the accumulation of such large amounts of mucus is unique for the Mediterranean Sea the formation of mucus from phytoplankton exudates is known from all oceans (Verdugo *et al*., 2004). The accumulation of this material in the Mediterranean is thought to be the consequence of the prevailing currents as well as of a temporal disequilibrium between production and degradation. The fate of these polysaccharides is also important with respect to climate research. As these polysaccharides are characterized by a high C: N ratio (Engel and Passow, 2001), an increase in their production with rising CO_2 concentrations (Engel, 2002) would strengthen the biological pump, if this material is not degraded in the surface ocean, but instead sediments to the deep ocean.

This mucous material largely consists of chemical complex polysaccharides, with acidic polysaccharides contributing a large fraction (Mopper *et al*., 1995; Verdugo *et al*., 2004). Large molecules (> 600 Da) have to be hydrolyzed before uptake by bacteria is possible. Microbial remineralization of such molecules therefore begins with extracellular enzymatic hydrolysis. The rates of extracellular hydrolysis thus determine the potential for the degradation of a specific

substance. However, different prokaryotes express completely different levels of extracellular enzymatic activity under a given set of conditions and only a few strains are responsible for the majority of the activity (Arrieta and Herndl, this volume).

Extracellular enzyme activity of carbohydrates is typically assessed using substrate proxies such as MUF-α-glucose or MUF-β-glucose (Hoppe, 1983). However, these simple, low molecular substrates cannot represent the complexity and tertiary structure of the high molecular polysaccharides responsible for mucus formation. Furthermore, the MUF-dimers are the target of enzymes that cleave monomeric units from the end of a polymer (exo-acting extracellular enzymes), whereas endo-acting extracellular enzymes, which cleave polymers mid-chain, may be more important for the degradation of complex polysaccharides.

Recently a method has been developed to investigate the potential for extracellular hydrolysis of specific complex polysaccharides (Arnosti, 1995; Arnosti, 1996). The composition and structure of the polysaccharides responsible for mucus formation still remain to be determined, but by investigating the dynamics and hydrolysis rates of polysaccharides known to occur in marine systems, the enzymatic potential and activity of complex microbial communities can be probed directly. A set of fluorescently labeled polysaccharides found in marine systems and assumed to be important for mucus formation was chosen for the determination of enzymatic activity. Microbial community composition will most likely change during the incubation time of five to six days for these measurements. Measured enzymatic activity thus represents the potential of the population to generate the extracellular enzymes responsible for the degradation of the specific substrate. This may be accomplished by the selective growth of one fraction of the population causing a shift in the population composition or by induction of a suite of enzymes (selective gene expression) within a stable population, or a combination of both. The hydrolysis rates consequently express the potential metabolic capability of the population (Keith and Arnosti, 2001). Rates discussed here should also be regarded as 'potential hydrolysis rates', since substrates added to a system are in competition with naturally-occurring substrates (of unknown concentration) for enzyme active sites.

Here we present an overview of the enzymatic activity of four complex polysaccharides in a variety of different environments (Arnosti *et al.,* 2005; Keith and Arnosti, 2001; Passow *et al.,* unplubl.; Arnosti, unpubl.). The range in potential hydrolysis rates of chondroitin sulfate, fucoidan, laminarin and xylan in different pelagic environments and their temporal or spatial variability will be discussed. Molecular methods can provide a picture of compositional diversity in marine microbial communities; the extent of functional diversity among these communities is unknown. The data reviewed and compared here may give us indications whether functionally a microbial community is stable within a geographical area or over time or if large changes are evident between freshwater and marine systems or are induced by exudation products of phytoplankton.

METHOD

Ranges of potential hydrolysis rates of four specific polysaccharides and their variability on different spatial and temporal scales are compared. Samples stemming from four stations in the eastern Pacific and Arctic Ocean are used to assess large scale spatial variability (Arnosti *et al.,* 2005). Samples collected at four stations along a transect reaching from the Delaware River through Delaware Bay to the shelf are used to look at specific hydrolysis rates along a salinity gradient (Keith and Arnosti, 2001). Three of these four stations were sampled during three different seasons (winter, early and late summer) and these data are used to look at seasonal variability at the three stations. Samples collected from two diatom blooms grown in 20 m3 mesocosms (M1 and M7) were used to assess the range and temporal variability in hydrolysis rates on the time scale of blooms (Passow *et al.,* unpubl.). Both mesocosms were filled with identical water, but the atmospheric CO_2 concentration differed, mimicking future (M1) and glacial (M7) CO_2 conditions (Grossart *et al.,* in press). Diatom dominated, mixed phytoplankton blooms developed in both mesocosms. Data from a different diatom bloom grown in a 1 m^3 mesocosm are also used to look at the temporal variability on the time scale of a bloom (Arnosti unpubl.). Lastly, the effect of phytoplankton exudation (and microbial community shifts) on

potential hydrolysis rates is investigated by comparing data of three 1 m^3 mesocosms harboring different phytoplankton communities (Arnosti unpubl.). The three mesocosms were inoculated with the same natural microbial community on day 0, but diatoms and *Phaeocystis* were added to the *Diatom* and *Phaeocytis* tanks, respectively, whereas the *no-addition* tank was left unperturbed (Grossart *et al.,* unpubl.; Passow *et al.,* unpubl.).

Four polysaccharides of different structure and composition were chosen to asses the functional diversity of microbial extracellular enzymes present in the different planktonic microbial communities. Chondroitin sulfate is a sulfated polymer of N-acetyl galactoseamine and glucuronic acid with a MW estimated at 150 kDa. Fucoidan is a sulfated fucose polysaccharide (MW> 50 kDa) produced by phaeophytes. Laminarin is a storage product of bacillariophytes, consisting of β-(1,3) glucose (MW≈ 6 kDa). Xylan, a polymer of β-(1, 4) xylose with a MW of about 8 kDa, is produced by rhodophytes and chlorophytes, and also occurs in terrestrial plants. These neutral and charged polysaccharides are common in marine systems. The behavior of sulfated polysaccharides may be particularly relevant to questions of gel formation and dynamics, as sulfated deoxysugar containing carbohydrates have been found to be significant components of gel particles (Mopper *et al.,* 1995; Zhou *et al.,* 1998).
For each experiment, two or three replicate 10 ml samples plus one killed control (either formalin at 10% end concentration, or mercuric chloride) were prepared for each substrate and each time step. Samples were inoculated with one of the fluorescently labeled substrates and incubated at *in situ* light and temperature. After the incubation time (5 or 6 days for the results presented here) subsamples of 1.5 ml were taken from each of labeled samples for analysis, filtered through 0.2 µm pore size syringe filter and stored frozen until analysis. The fluorescently labeled polysaccharides and their hydrolysis products were separated chromatographically using a gel permeation chromatography system connected to a fluorescence detector as described in Arnosti (2000). Hydrolysis rates are based on integration of these chromatograms, calculated as described in Arnosti (1995; 2000).

The polysaccharides were all obtained from Sigma or Fluka and labeled with fluoresceineamine (isomer II, Sigma) using the procedure of Glabe *et al*. (1983) as modified by Arnosti (1995). Equivalent monomer concentrations of each substrate were added to the samples to keep carbon addition levels consistent among experiments and to provide a measure for the hydrolysis that is independent of the rate calculation (Arnosti *et al.,* 2005).

Results and Discussion

Hydrolysis rates

The range of potential hydrolysis rates of the four substrates was investigated over a variety of spatial and temporal scales and as a function of dominating phytoplankton (all data shown in Table 1). Hydrolysis rates of fucoidan were undetectable in all samples, with the exception of day 15 of the diatom bloom in M 1, where a hydrolysis rate of 17 nM h^{-1} was measured (Table 1). No fucoidan hydrolysis was detected during the same time series of a diatom bloom in M 7. The absence of significant hydrolysis of fucoidan in most pelagic environments measured suggests that this substrate is often semi-resistant in the pelagic environment. Substances similar to fucoidan, e.g. sulfated deoxy-sugars, are thought to be enriched in transparent exopolymer particles (TEP) which are mucus particles that are abundant in all oceans (Passow, 2002). Retarded degradation of TEP has been suggested (Passow, 2002). Fucoidan is hydrolyzed, albeit slowly, in sediment samples (Arnosti, 2000) and in a very few pelagic locations investigated to date, most notably inshore waters of the Gulf of Mexico (Arnosti, unpubl. data).

Peak hydrolysis rates of laminarin and xylan > 40 and 70 nM h^{-1}, respectively were observed during the river – shelf transect, with highest hydrolysis rates of both substrates at the fresh water station in September. Maximal values of laminarin hydrolysis measured in the mesocosms during the progressions of the diatom blooms were appreciably lower. Laminarin is a storage product of diatoms, thus at first sight the low hydrolysis rates during diatom blooms appear surprising. However, storage products are not identical to exudation products, and laminarin may be protected within the healthy cell from hydrolysis. Possibly laminarin concentrations are only high during senescence of diatoms, when cells lyse. Laminarin hydrolysis rates were always

Table 1. Variability in hydrolysis rates after 5 to 6 days incubation time of four complex polysaccharides over different spatial and temporal scales or as a function of dominating phytoplankton community. All samples from < 10m depths.

Variable	Chondroitin sulfate		Fucoidan		Laminarin		Xylan		n	comments
	Range (nM h^{-1})	std/avg (%)	Range (nM h^{-1})	std/avg (%)	Range (nM h^{-1})	std/avg (%)	Range (nM h^{-1})	std/avg (%)		
Spatial										
Ocean	0.0-2.3	1.2	0.0-0.0	-	0.1-14.6	1.0	0.0-17.0	1.6	4	St J, T33, T15s, T3 $
River-shelf Transect	nd	nd	nd	nd	2.4-27.9	1.0	0.4-60.0	1.3	4	Jan 1998: freshwater to shelf #
Temporal										
Seasonal: Freshwater	nd	nd	nd	nd	6.4-40.2	1.0	50.7-72.2	0.2	3	Jun 97, Sep 97, Jan 98 #
Seasonal: Midbay	nd	nd	nd	nd	4.3-23.5	0.9	3.8-54.9	0.8	3	Jun 97, Sep 97, Jan 98 #
Seasonal: Plume	nd	nd	nd	nd	2.4-23.4	0.8	0.4-45.6	0.9	3	Jun 97, Sep 97, Jan 98 #
Diatom bloom: 15 days	4.7-42.6	0.7	0.0-17.0	1.6	0.4-0.9	0.3	0.0-2.6	1.1	3	Mesocosm 1 & days 0; 7; 15
Diatom bloom: 15 days	2.8-38.4	1.0	0.0-0.0	-	0.6-2.5	0.5	0.1-2.4	0.7	3	Mesocosm 7 & days 0; 7; 15
Diatom bloom: 20 days	2.9-21.0	0.7	0.0-0.0	-	3.5-8.2	0.2	0.0-15.7	0.8	9	*Diatom* mesocosm * days 4, 6, 8, 10, 12, 14, 16, 18, 20
Phytoplankton										
Day 8	3.8-4.6	0.1	0.0-0.0	-	5.6-6.3	0.1	13.3-14.3	0.0	3	*No-addition*-, *Phaeocystis*-, *Diatom*-Mesocosm *
Day 12	6.1-6.7	0.1	0.0-0.0	-	5.4-7.0	0.1	10.4-12.3	0.1	3	*No-addition*-, *Phaeocystis*-, *Diatom*-Mesocosm *
Day 16	7.6-11.0	0.2	0.0-0.0	-	4.4-6.1	0.2	0.6-5.4	0.9	3	*No-addition*-, *Phaeocystis*-, *Diatom*-Mesocosm *
Day 20	10.2-21.0	0.4	0.0-0.0	-	5.6-6.5	0.1	0.6-10.5	1.2	3	*No-addition*-, *Phaeocystis*-, *Diatom*-Mesocosm *

$ Arnosti *et al*., 2005; # Keith and Arnosti, 2001; & Passow *et al*., unpubl.; * Arnosti, unpubl..; nd = not determined; not detected was assumed to be zero

detectable, but varied over more than one order of magnitude, whereas those of the other substrates were frequently below the detection limit. Possibly laminarin is a more "universally" present polysaccharide, but nevertheless, the high variations in its hydrolysis rate suggest a large variability in its availability.

Other than at the river – shelf transect, maximal xylan hydrolysis rates were relatively elevated (> 10 nM h^{-1}) during the first half of the investigation of the *no-addition*, the *Phaeocystis* and the *Diatom* tanks, whereas they were appreciably lower during both diatom blooms growing in M 1 and M 7. As the prefiltration of the water while filling the mesocosms (Passow *et al.,* unpubl.) had eliminated larger phytoplankton, chlorophytes (which contain xylan) may have been relatively abundant during this first part of the investigation. The fact that xylan hydrolysis rates decreased substantially during the course of the bloom in the *no-addition*, *Phaeocystis*, and *Diatom* tanks raises questions about the 'lifetime' of extracellular enzymes in pelagic systems. Extracellular enzymes that were active during the initial phase of the bloom somehow became inactivated, perhaps through aggregation, photochemical effects, hydrolysis of the enzymes by proteases, or some other unknown mechanism. Hydrolysis of chondroitin sulfate, which was not determined during the river – shelf transect, reached peak rates of about 40 nM h^{-1} during the diatom blooms M 1 and M 7 and appreciably lower peak values during the second mesocosm study. These maximum rates are the highest measured to date in pelagic systems (Arnosti *et al.,* 2005). Investigations of chondroitin hydrolysis in sediments and seawater frequently show relatively low initial rates, followed by significantly higher hydrolysis rates, suggesting that this enzyme may be induced (Arnosti, 2004; Arnosti *et al.,* 2005). Conditions in the M1 and M7 mesocosms may have been particularly suitable for induction of highly active enzymes.

The patterns of hydrolysis rates of all four substrates differ distinctly from each other and over time. These patterns cannot be explained simply by correlations with other microbial parameters, such as bacterial cell concentration, growth or protein production rate (Arnosti *et al.,* 2005; Keith and Arnosti, 2001). The observed patterns of hydrolytic activity differ as well between specific substrates, suggesting that the presence or absence of an extracellular hydrolytic enzyme is controlled by a tight interaction between environmental conditions and specific members of microbial communities.

Spatial and temporal variability in hydrolysis rates

The variability in the hydrolysis rate of each of the four substrates was estimated by comparing the ratio between one standard deviation and the average of samples integrating over temporal or spatial scales (Table 1). Variability in enzyme activity may reflect seasonal or site-related differences, which stem from differences in the available substrates, the microbial community, or its growth history. Starvation, for example, may induce enhanced enzyme activity (Albertson *et al.,* 1990).

High variability in hydrolysis rates generally went along with high peak values, as low hydrolysis rates were observed in most data sets. This suggests that high hydrolysis rates of any one substrate are short term events, rather than a continuous situation. Variability was clearly not a function of the substrate, as hydrolysis rates of all substrates showed signs of high variability at one time or another.

The spatial variability in hydrolysis rates between four oceanic stations ranging from 79 N to 39 S, as well as the spatial variability between four stations situated along a transect following the salinity gradient from the Delaware river to the shelf waters were high (≥ 1.0) for all substrates. The seasonal variability of laminarin and xylan (fucoidan and chondroitan sulfate were not determined) at each of three stations along the Delaware transect was almost as high (0.8-1.0), with a single exception, suggesting annual variability to be comparable to ocean wide spatial variability.

During the development of three diatom blooms, the temporal variability in each case was also high for all substrates, except for laminarin, where temporal variability during the bloom appeared low (≤ 0.5) for all three blooms. Interestingly, the absolute hydrolysis rate of laminarin was relatively low during all stages of these diatom blooms, although laminarin is known to be a storage product of diatoms (see above).

Variability in hydrolysis rates of all substrates was extremely low (≤ 0.2) during the comparisons between different phytoplankton communities, with the exception of the xylan hydrolysis rates which varied appreciably during the second half of the investigation (days 15 and 20). This suggests that the impact of phytoplankton and their released products on the microbial community was relatively small. As different phytoplankton species or taxa release different exudates, and these exudates can then serve as substrates for microbial communities, large differences in hydrolysis rates of specific substances between the three tanks had been expected. But other data of that study also support the result that the activities of microbial communities in the three mesocosms diverged less than expected, as the observed shift in the community composition was similar in all three tanks (Grossart *et al.,* unpubl.; Passow *et al.,* unpubl.).

Low hydrolysis rates even after six days of incubations, suggest that the presence of a substrate is not necessarily sufficient to trigger the release of extracellular enzymes, able to hydrolyze this substrate. By what means do microbes detect the presence of high molecular weight substrates in order to 'turn on' the production of a specific enzyme? A further question relates to the 'lifetime' and dynamics of an extracellular enzyme: how long is it active in pelagic environments, and what are the primary controls on enzyme 'lifetimes'? Some of the keys to these questions likely can be found in the complexities of microbial communities: if specific members needed to produce a given type of enzyme are either not present or lack a required inducer, a substrate that appears 'labile' in one environment may be 'recalcitrant' in another. Perhaps some bacteria also specialize in degradation of hydrolytic enzymes? Given our limited understanding of the identity (much less the metabolic capabilities) of the vast diversity of marine microbial communities, a great deal of work remains to be carried out.

Production and fate of Dissolved Organic Matter in the Mediterranean: formation and function of giant gels in the northern Adriatic

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ABSTRACT

The DOM concept has served its purpose in global carbon cycles on oceanic and geological scales (Hedges, 1992) and it has been supported by improvements in operational DOC measurement techniques (Hedges and Lee, 1993). However, the DOM concept is questioned when it comes to a particular process of a new photosynthetic production and DOM fate in a given marine ecosystem. Therefore it is of importance to reconsider and redefine the traditional DOM concept for coastal seas.

INTRODUCTION

As we have gone beyond the traditional oceanographic routines in our field studies of characterization of seawater, we have seen that an important fraction of marine organic matter is in the size range of a few nanometers to few micrometers. The structures that this fraction forms in seawater stand apart from the conventional colloidal systems of solid particles and can be described in terms of complex fluids (gels and vesicles) (Žutić and Svetličić, 2000).

We have selected the case of a most intensive primary production and DOM transformation in a Mediterranean basin, the northern part of the Adriatic Sea, as a possible excercise in validating the DOM concept in view of new instrumental developments (Žutić and Svetličić, 2000; Svetličić *et al*., 2005a; Svetličić *et al*., 2005b) understanding of microbiological processes (Azam and Worden, 2004) and interactions of biopolymers at micro and nanoscales (Svetličić *et al*., 2005b; Leppard, 1995).

The knowledge of formation mechanism and structural organization of organic matter at nanoscales and microscales is the clue to resolve the mechanism of oceanic processes that sometimes extend to the kilometer scale. Our biophysical scenario of mucilage event features self-organization of biopolymers into microparticles («marine vesicles») that under specific conditions transform to giant-gel by a fast vesicle to gel transition.

METHODS

We introduced direct electrochemical sensing of microparticles and Atomic Force Microscopy (AFM) to image supramolecular organization of marine gel network.

Direct electrochemical sensing of organic matter in seawater.

Based on the phenomena of molecular adsorption and particle adhesion at the dropping mercury electrode (Žutić *et al*., 1990; Svetličić *et al*., in press) the organic constituents of seawater can be

classified by their electrochemical responses as: (i) dissolved biopolymer molecules and colloids; (ii) surface-active microparticles; and (iii) gel microparticles. While in the chronoamperometric curve the adsorption manifests as a gradual decrease in the oxygen reduction current, the collision of a particle at the mercury interface leaves a fingerprint on the i-t curve of oxygen reduction. The specific electrochemical signals (spikes vs. depressions) of the two classes of microparticles enable their simultaneous detection in seawater samples and monitoring their abundance.

AFM imaging of marine gel on nanoscale.

Atomic force microscopy (AFM) is a member of a family of new microscopic techniques that are referred to as scanning-probe microscopies (SPMs) (Morris *et al.,* 1999; Noelting, 2004). The concept on which all SPMs are based is the generation of images of surfaces by measuring the physical interaction between a sharp tip and the sample rather than by using an incident beam (light or electrons) as in classical microscopy. AFM provides real topographic images of the sample surfaces in three dimensions with a vertical resolution as small as 1 Å, and with lateral resolution superior to 1 nm. The AFM emerges as the method of choice for characterization of marine organic matter on nanoscale (Svetličić *et al.,* 2005a; Svetličić *et al.,* 2005b; Santos and Castanho, 2004). We used drop deposition method on freshly cleaved mica. Images were collected using tapping mode AFM, which is particularly well adapted to soft samples due to a nearly complete reduction of lateral forces.

FORMATION AND FUNCTION OF GIANT GEL NETWORK IN MARINE ECOSYSTEM

The enigmatic gel phase (Figure 1) appears episodically in the northern Adriatic Sea. Several reviews have appeared in recent years (Stachowitsch *et al.,* 1990; Funari *et al.,* 1999) that might be consulted for details. The phenomenon referred as "mucilage of northern Adriatic" has been observed infrequently over the past three centuries but its intensity and frequency of appearance has increased since the "big" event of 1989 (Stachowitsch *et al.,* 1990). The economic cost of these events, through loss of tourism and fisheries, is substantial. For instance, the European Union provided the Adriatic fishery industries with 29 million Euros during the dramatic mucus event in the summer 2000 (www.newton.rcs.it/PrimoPiano/News/2003/08_Agosto/18/Mucillaggini.shtml). Effects of phosphate ban and zeolite introduction (EC/CSTEE, 2003) and global climate change are recognized.

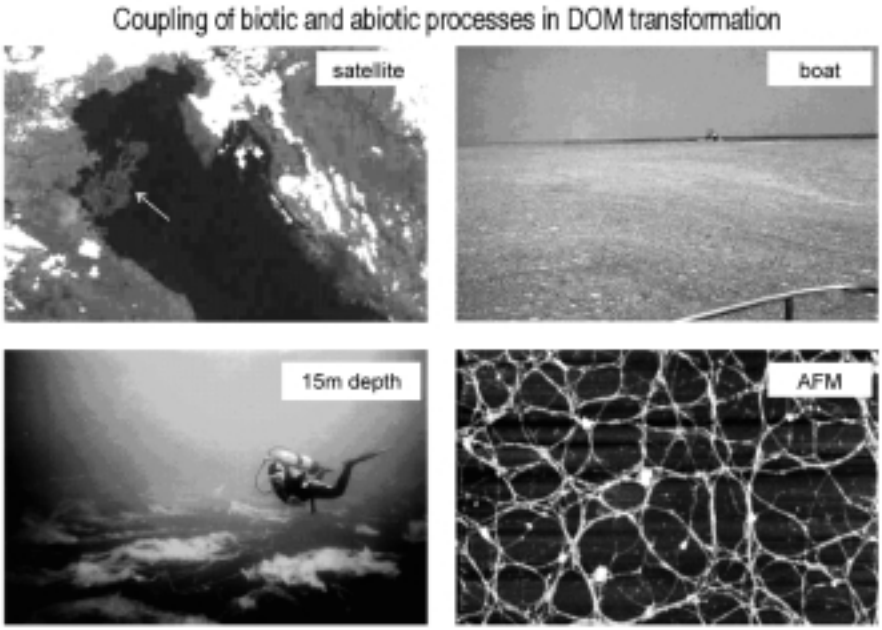

Figure 1. Mucilage phenomenon in the northern Adriatic observed at different scales: remote sensing by satellite (Zambianchi *et al.,* 1992); from the sea surface (Stachowitsch *et al.,* 1990); in the water column by scuba diver (Zutic and Svetlicic, 2000) and seen at the nanoscale by atomic force microscopy (scan size 4.5 μm, total vertical scale 5 nm (Svetlicic *et al.,* 2005c).

Eutrophication of the northern Adriatic(Vollenweider *et al.,* 1992; Degobbis *et al.,* 2000), due to run-off from the Po and other rivers, causes hyper-production of microalgae during spring/summer at rates which far exceed the grazing potential of herbivores or rate of decomposition by bacteria. Consequently, large standing stocks of microalgae build up and extracellular polymers, mainly polysaccharides, accumulate in the euphotic layer above the thermocline. With prolonged residence time the accumulated biopolymers transform by self-organization to vesicles. These particles are considered mucilage precursors that gel at a critical concentration, N_c. Our present estimate (within the mesurable size range 1-100 μm) centers around $N_c \approx 5x10^7$ L^{-1} (Svetličić *et al.,* 2005c; Žutić *et al.,* 2004b).

The hallmark of the mucilage phenomenon is the *rapid* (1-100 hr) appearance of enormous amounts of gelatinous organic matter. No biological process could reasonably be identified that is capable of producing organic matter at such rapid rate. While a large number of hypotheses have been advanced (see Hedges and Lee, 1993; Svetličić *et al.,* 2005b, for a discussion) there is a strong probability that mucilage formation is preceded by the accumulation of a precursor pool of organic matter to some critical concentration (Žutić and Svetličić, 2000; Svetličić *et al.,* 2005c; Žutić *et al.,* 2004b).

Eutrophication of the northern Adriatic (Vollenweider *et al.,* 1992; Degobbis *et al.,* 2000), due to run-off from the Po and other rivers, causes hyper-production of microalgae during spring/summer at rates which far exceed the grazing potential of herbivores or rate of decomposition by bacteria. Consequently, large standing stocks of microalgae build up and extracellular polymers, mainly polysaccharides, accumulate in the euphotic layer above the thermocline. With prolonged residence time the accumulated biopolymers transform by self-organization to vesicles. These particles are considered mucilage precursors that gel at a critical concentration, Nc. Our present estimate (within the measurable size range 1-100μm) centers around $N_c \approx 5x10^7$ L^{-1} (Svetličić *et al.,* 2005c; Žutić *et al.,* 2004b).

The large-scale phase transition from the dispersed to gel state takes place almost instantaneously (see Figure 2).

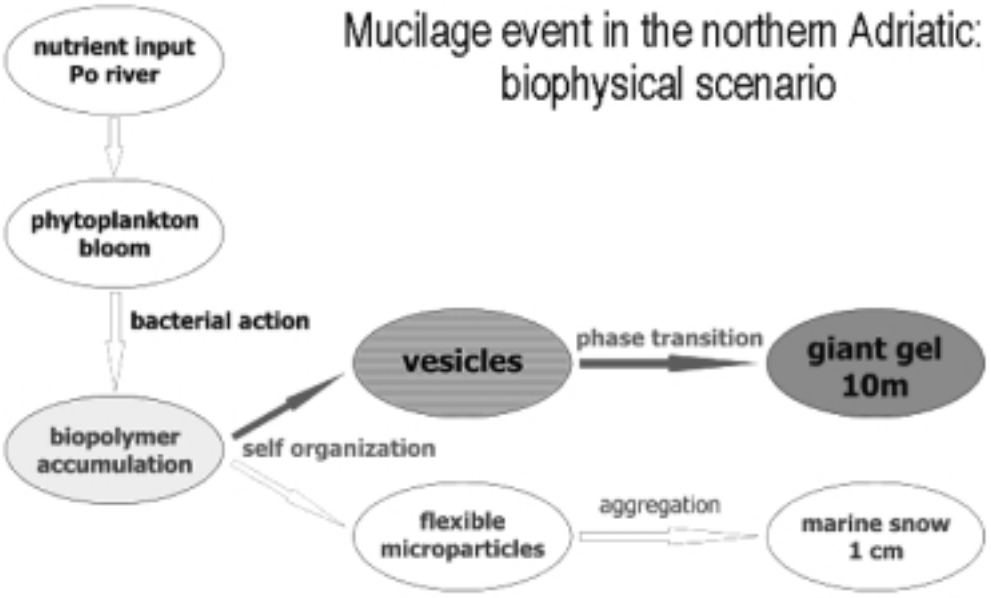

Figure 2. Biophysical scenario of giant gel formation in the northern Adriatic.

On the macroscopic scale the shape and the size of the giant gel formed by the phase transition are dictated by the local hydrodynamics (turbulence and shear) and the proximity of the sea surface and density interfaces in the water column. Buoancy and vertical migration of macroaggregates depend upon the ongoing microbial activity within the gel. The nanoscale imaging of native marine gel demonstrates that an important fraction of specimen consists of

entangled fibrils. The gel structure exhibits a repeating network of solvent cavities (ranging from 150 to 500 nm) between polymeric strands. The fibrils are rigid macromolecules with a typical diameter between 0.6-3 nm and a length of 0.1 to 5 µm. Although it is difficult to determine the nature of the fibril based on shape analysis, there is strong evidence that the fibrils are mainly polysaccharides since AFM images of polysaccharide molecules have demonstrated similar features (Abu-Lail and Camesano, 2003; Brant, 1997) as well as chemical and structural analysis of marine gel (Kovač *et al.,* 2002; Kovač *et al.,* 2004).

CONCLUDING REMARKS

We propose that giant gels (macroaggregates) that are formed episodically in the northern Adriatic during the summer season (Žutić *et al.,* 1990; Svetličić *et al.,* in press), function as efficient bioreactors to remove excess photosynthetic extracellular production from seawater and the sub-basin as a whole, and restore normal microbial populations. With climate change and increasing eutrophication (Morris *et al.,* 1999), the phenomenon is likely to intensify and spread over other Mediterranean sub-basins.

The mechanistic basis of the phenomenon raises the question of whether such uncoupling in carbon cycle might in the future also occur elsewhere in the ocean, e.g. due to eutrophication coupled with specific trophodynamic and environmental conditions.

Production and fate of autochthonous DOM: an experimental approach

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ABSTRACT

The DOM pathway is a dominant recycling process of the production of organic matter in plankton communities under most circumstances and the intensity is most probably related to the nutrient regime and community structure. Newly produced DOM can in most circumstances accumulate, partly due to the chemical nature of the produced material and because part of the new DOM enters a refractory pool almost immediately after its production. The stoichiometry of new DOM can be a function of the nutrient regime. It seems that P-limited plankton systems behave differently from N-limited systems. This might be of special interest for the DOM pathway in the Mediterranean Sea.

THE IMPORTANCE OF DOM

Organic matter in oceans and many freshwaters systems is dominated by dissolved compounds (DOM), i.e. dissolved organic carbon (DOC), nitrogen (DON) and phosphorus (DOP) and the ecological functioning and nutrient sequestration in the sea is intimately linked to the biogeochemistry of DOC, DON and DOP (Hedges, 2002).

In his foreword to a recent book (Hansell and Carlson, 2002), Hugh Ducklow introduced marine DOM as "the soil of the oceans" and rightly so, recognising the many different biotic and abiotic processes involving DOM, its huge quantity and the provision of ecosystem services. Although the bulk of measured DOC cannot be decomposed within timescales of years the recalcitrant background concentration is in the upper ocean often overlaid with a small but dynamic DOC pool susceptible of degradation with time scales from days to months (Søndergaard and Middelboe, 1995; Carlson, 2002). The seasonal accumulation and removal of carbon-rich DOM observed in the euphotic and upper mixed layer during productive periods in many plankton dominated systems (Copin-Montégut and Avril, 1993; Williams, 1995; Carlson *et al.,* 1994; Børsheim and Myklestad, 1997, among others) show that part of the DOM pool participates in the biogeochemical cycling of C, N and P. From an ecological point of view some main questions linked to DOM research are: *How much is there, where does it come from and how does it disappear? What is the role? What are the biotic and environmental factors in control of the production and fate?*

DOM is an operational term linked to chemical measurements of compounds in a sample where particles > 0.2 to 0.7 µm have been removed. Many geochemists have studied specific chemical molecules in DOM (reviewed by Benner, 2002), but the simplest approach is to measure three different compartments: organic carbon in DOC, nitrogen in DON and phosphorus in DOP.

Current knowledge on their dynamics in time and space can safely be set at DOC > DON > DOP judged from the two most recent and very comprehensive reviews of DOM in marine (Hansell and Carlson, 2002) and freshwater environments (Findlay and Sinsabaugh, 2003). The word DOM is an abstraction and cannot be measured chemically and DOM is often used almost synonymously with DOC because carbon is an accepted ecosystem currency. At the moment methodological constraints hamper a detailed scrutiny of DON and DOP compared with DOC (Sharp, 2002). Better knowledge on the DON and DOP dynamics is a pivotal priority area.

The concept of the microbial loop, with bacteria and dissolved organic matter (DOM) taking a key position for the turnover of primary production in planktonic systems, was predicted and evolved by Pomeroy (1974) and Williams (1981) at times when our quantitative knowledge of bacterial production (BP) and bacterial growth efficiency was rather rudimentary. Azam *et al*. (1983) fine-tuned the concept of the microbial loop shortly after it became possible to measure bacterial production and biomass with some certainty. One should however not forget that one of the first predictions of a high quantitative importance of bacterial utilisation of organic matter in the oceans was forwarded by August Krogh in 1934.

Bacterial production is a measurement of a flux of carbon in organic molecules no longer present to be measured chemically. Molecules in high demand are cycled fast and are at steady state only present in very low concentrations. Thus, compounds in rather low demand and/or with low reactivity must in most circumstances dominate the huge pool of DOC in the oceans that we measure chemically.

Measurements of bacterial net production and bacterial respiration has led to the current understanding that often 50% or more of phytoplankton primary production in marine waters and many lakes is routed via a dissolved phase to bacteria (Cole *et al*., 1988; Ducklow and Carlson, 1992; Ducklow, 2000; Williams, 2000). As bacteria can only transport small molecules across the cell membrane we have solid evidence to view DOM (DOC at least) turnover as a metabolic highway in aquatic systems.

ALLOCHTHONOUS AND AUTOCHTHONOUS DOM IN THE OCEANS

Large amounts of DOM of terrestrial origin are transported in rivers or by atmospheric deposition to coastal waters and open seas (Cauwet, 2002). The riverine allochthonous sources seem to be removed in coastal waters, as terrestrial signals are virtually absent in the oceans (Hedges *et al*., 1997; Opsahl and Benner, 1997). It can therefore be concluded that most of the DOM fuelling a high instant bacterial production and creating seasonal cycles in surface concentrations must be from autochthonous sources, ultimately primary production. The distribution in time and space is controlled by food web processes and abiotic reactions.

DOM production by plankton communities is without doubt the most important source in the world ocean. Having said so, we should not forget the high production by seagrasses and macroalgae in shallow areas. Most of the production by larger plants is decomposed via the detrital pathway, i.e. via DOM.

PRODUCTION OF AUTOCHTHONOUS DOM

The production and decomposition of marine DOM has recently been reviewed by Carlson (2002), thus here we shall just provide a brief summary and focus on specific questions concerning environmental and biotic control of the production.

Extracellular release of organic carbon (EOC) by phytoplankton has long been viewed as one of the most important processes producing DOM of a high quality, i.e. a direct link from algae to bacteria (Larsson and Hagström, 1979; Foggn, 1983; Søndergaard *et al*., 1985; Williams, 1990, among others). Although many studies on EOC probably have been biased by insufficient controls/blanks (Sharp, 1977), no doubt, the loss of EOC is a real phenomenon (Mague *et al*., 1980; Williams, 1990). In 1991 Baines and Pace reviewed the literature and reported an average loss of about 13% of the net carbon production for both marine and freshwater phytoplankton, albeit with very large variations among systems. They also observed that the absolute rate of EOC depended on the nutrient regime (see Morán, this volume).

Several studies have tried to elucidate the environmental factors in control of or regulating extracellular loss but the results are somewhat conflicting. Loss rates have most often been related to algal growth at different nutrient regimes and light, with nutrient deficient conditions provoking high relative release. And, there is some evidence to support the suggestion, both from fieldwork (e.g. Søndergaard *et al.,* 1985; Karl *et al.,* 1998) and in algal cultures (Watt, 1966). Most solid evidence comes from results showing increased loss of carbohydrates from diatoms at nutrient deficient conditions (Myklestad, 1977; Ittekkot *et al.,* 1981; Fajon *et al.,* 1999). However, plankton communities dominated by fast growing diatoms at nutrient replete conditions can also produce large amounts of carbohydrates (Søndergaard *et al.,* 2000a). There have also been reported clear effects whether P or N was limiting (Obernosterer and Herndl, 1995; Karl *et al.,* 1998). In *Chaetoceros* cultures P-limitation induced a high loss and the compounds were not utilised efficiently by bacteria (Obernosterer and Herndl, 1995). Under most circumstances the amount of organic carbon released by phytoplankton cannot support bacterial carbon demand (Baines and Pace, 1991).

Jumars *et al*. (1989) suggested that the loss of DOC (and DOM) due to grazing/feeding at all trophic levels (Figure 1) would saturate bacterial carbon demand. Larger or minor parts of the handled and ingested organic material can and will be lost to the DOM pool through faeces, cell sap, excretion or by other means. In a recent review Nagata (2000) reached the conclusion that DOC produced as a function of feeding, and especially protozoan grazing on bacteria, is the quantitatively most important DOC source in marine environments. That might be the case during some period, but probably not all the time.

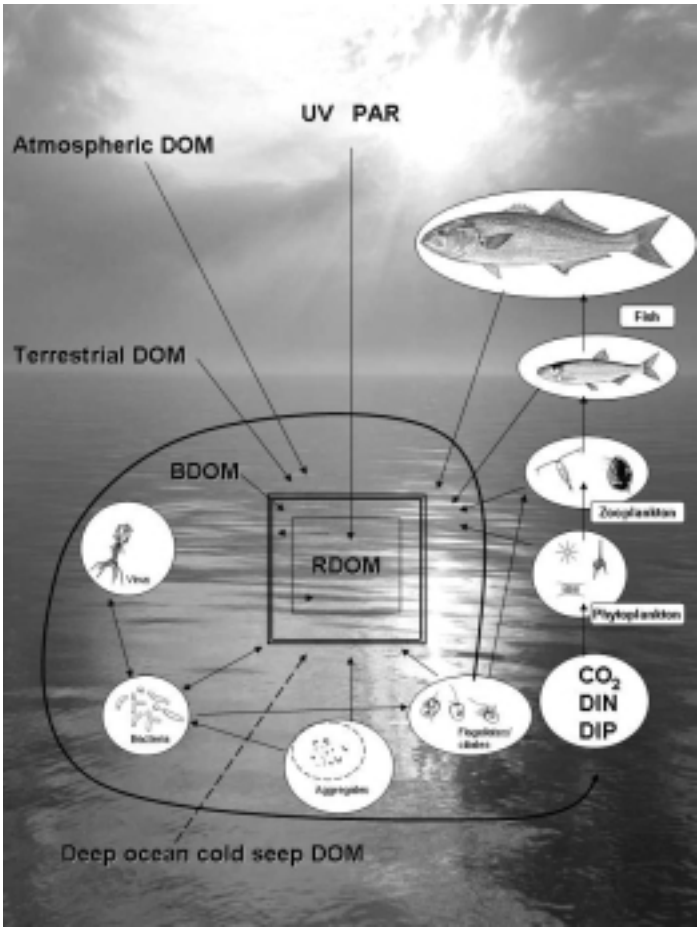

Fig. 1. A conceptual food web model including both the particulate (right side) and the microbial dissolved pathway (middle and left side) can be used to identify the processes and organisms involved in the production and degradation of DOM. BDOM = biodegradable DOM, RDOM = recalcitrant and refractory DOM (Søndergaard and Thomas, 2004).

From the work on DOC production in aquatic systems we must by now have learned at least one lesson; it is very difficult to seek out one single process at any given time and say: "This is the most important DOC producer". Phytoplankton exudation can be important during some periods, grazing at other times, viral induced cell lysis of phytoplankton (Brussard *et al.,* 1995; Baldi *et al.,* 1997) and bacteria (Middelboe *et al.,* 2003) are co-occurring all the time, as is the solubilisation of detrital particles by bacterial ectoenzymes (Smith *et al.,* 1992). To our knowledge partial UV-degradation of particles to DOM compounds has not been reported, but our prediction is that such a process could be added as most likely (see Vähätalo *et al.,* 1998). What is clear is that all these processes (see Figure 1) simultaneously produce DOC and other DOM species. These products are either utilised by bacteria at high rates or can accumulate over seasonal time scales. The produced DOM enters a diagenetic development in interaction between photochemical and microbial processes. And, ultimately a small but important fraction enters the recalcitrant DOM background dominated by small molecules (Hopkinson *et al.,* 2002) with a turnover time counted in hundred and thousands of years.

DOM accumulation during the productive season and episodic plankton blooms (Carlson *et al.,* 1994; Carlson *et al.,* 1998; Williams, 1995) show that production and removal are not balanced in time and space and experimental studies may help to zoom in on the biotic and environmental factors in control.

Two case studies on DOM production

The factors in control of autochthonous DOM production and accumulation during productive seasons and periods have been studied in several large-scale experimental studies over recent years (Norrman *et al.,* 1995; Fajon *et al.,* 1999; Søndergaard *et al.,* 2000a; Meon and Kirchman, 2001, among many).

A summary of the results from an experiment in coastal waters is presented in Figure 2 (from Søndergaard *et al.,* 2000a). By addition of nutrients (N, P and Si) to large (11 m^3) mesocosms the plankton community was moved from a nutrient replete condition in Phase I to nutrient deficiency (Phase II with competition for nutrients, here P) and then taken out of deficiency with a five-fold increase in nutrient additions (Phase III). Each phase lasted about seven days. With concurrent measurements of the chemical environment and biological processes it was possible to evaluate how the organic production was distributed between particles, estimate new accumulating DOC and the DOC immediately routed to bacteria.

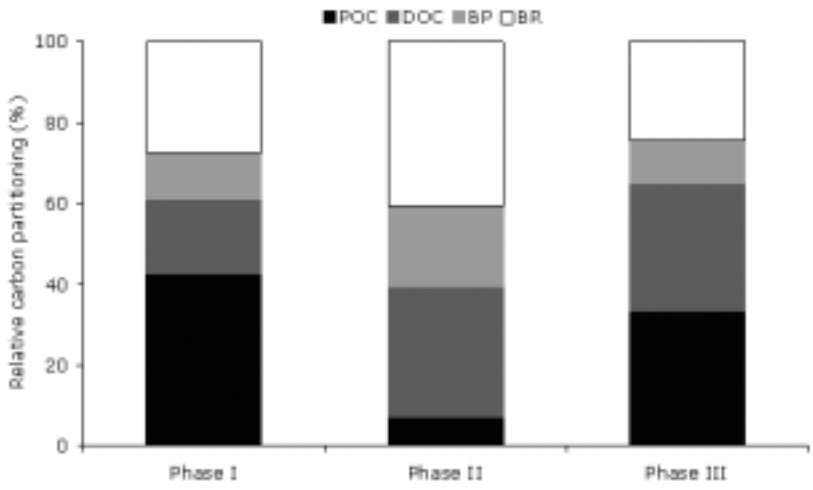

Fig. 2. Relative carbon partitioning in a marine plankton community. Phase I, II & III explained in the text. POC = particulate organic carbon, DOC = dissolved organic carbon, BP = net bacterial production, BR = bacterial respiration calculated from a BGE at 35% (Søndergaard *et al.,* 2000a).

At nutrient replete conditions during Phase I and III about 60% of the total production was either used immediately by bacteria or accumulated as new DOC. Although we do not have a strong handle on how P-deficient the plankton community was, a striking observation is that over 90%

of the total production in Phase II was routed to DOC. The nutrient regimes had a consequence for ecosystem functioning and carbon sequestration. The outcome agrees with results on carbon partitioning in the Sargasso Sea (Carlson *et al*., 1998) and thus adds support to the view that with respect to carbon the DOM route is a highway for the autochthonous production. The dissolved route can be very important both at nutrient poor and nutrient replete conditions. However, as for all *in situ* studies the specific sources cannot be identified.

It was also found that polysaccharides measured by the HBTH-method could explain some 50% of the new DOC. While new DON was produced, the accumulation was apparently not strongly related to the nutrient regime. The new DOM had a DOC:DON ratio between 11 and 20, a range encompassing both surface and deep sea DOM (Benner, 2002). However, some important questions remain unanswered. *What is the fate of the new DOC? Do different nutrient regimes (N or P deficiency) affect the accumulation of new DOM, how much is refractory and is there a time sequence for the production of refractory DOC?*

We once more have used the mesocosm approach to control the inorganic environment and to «contain» the DOM signals. Two treatments were used; (1) mineral N and P were added in molar ratios from 64 to 4 in two sets of each five mesocosms, thus both severe N and P deplete and replete conditions were present and (2) one set of bags was dosed with silicate to promote diatom dominance. Furthermore, the added amount of the limiting nutrient should result in the same biomass in all bags, if Redfield ratios were obeyed (they were not, but that is a different story). Diatoms and green algae dominated the Si dosed bags with 70 and 30% of the biomass, respectively, while five algal groups had an even biomass distribution in the other bags, including about 20% diatoms. The distribution of inorganic and organic species for C, N and P is summarised in Figures 3, 4 and 5, respectively.

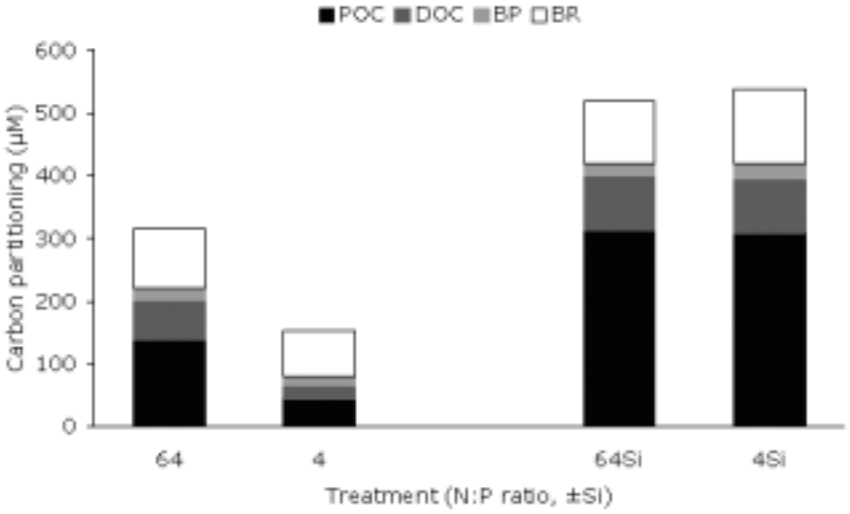

Fig. 3. Partitioning of the carbon production over seven days in four mesocosms treated with different N:P ratios and ±Si dosing.

First thing to notice with respect to the distribution of carbon production is that N and P deficiencies somewhat surprisingly did not affect the amount of new DOC or bacterial activity in the diatom dominated communities (Figure 3). The distribution was 20% to new DOC and 80% to POC. In the bags where Si was not added, the P deficient bag (64) had a higher total production than with N deficiency; however, the carbon partitioning was similar with about 30% to new DOC and 70% to POC. It is evident that the DOC pathway was important for system functioning and explained from 30 to 60% of the total production. All other mesocosms (six more, but not shown) did not deviate from this pattern.

The accumulation of combined neutral sugars explained between 10 to 25% of the new DOC and the higher values were found to be in accordance with the nutrient regime. Depletion of P and N had the higher accumulation values and more so for P than for N depletion (data not shown).

The C:N:P stoichiometry of DOM in ocean surface waters is variable (Hopkinson *et al.,* 2002), but can be approximated to 300:23:1 (Benner, 2002; Karl and Björkman, 2002), thus depleted in N and more so in P. The depletion is even more pronounced in the deep ocean (Benner, 2002). The oceanic C:N end-member ratio about 12 is surprisingly close to what has been found experimentally to be produced during phytoplankton blooms (Norrman *et al.,* 1995; Søndergaard *et al.,* 2000a). However, knowledge on how different nutrient regimes could affect the stoichiometry of newly produced DOM and specifically for DOP is to our knowledge lacking. Intuitively one would expect that N or P deficient situations would result in an efficient recycling of DON and DOP, respectively, unless they are produced as refractory compounds.

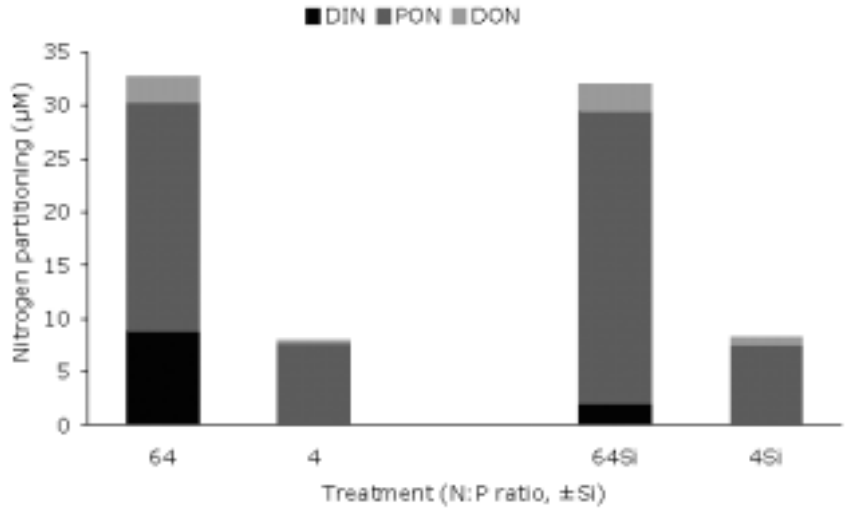

Fig. 4. Partitioning of the net nitrogen production over seven days in four mesocosms treated with different N:P ratios and ±Si dosing. DIN = dissolved inorganic N, PON = particulate organic N and DON = dissolved organic N. The DIN production is added nitrate in surplus.

With exhaustion of inorganic nitrogen and with soluble reactive phosphorus (SRP) in excess, the production of new DON was barely measurable and very uncertain, while the N replete mesocosms showed accumulation of new DON (Figure 4). With inorganic nitrogen present, the partitioning of the organic N production was about 10% to DON and 90% to particulate organic nitrogen (PON).

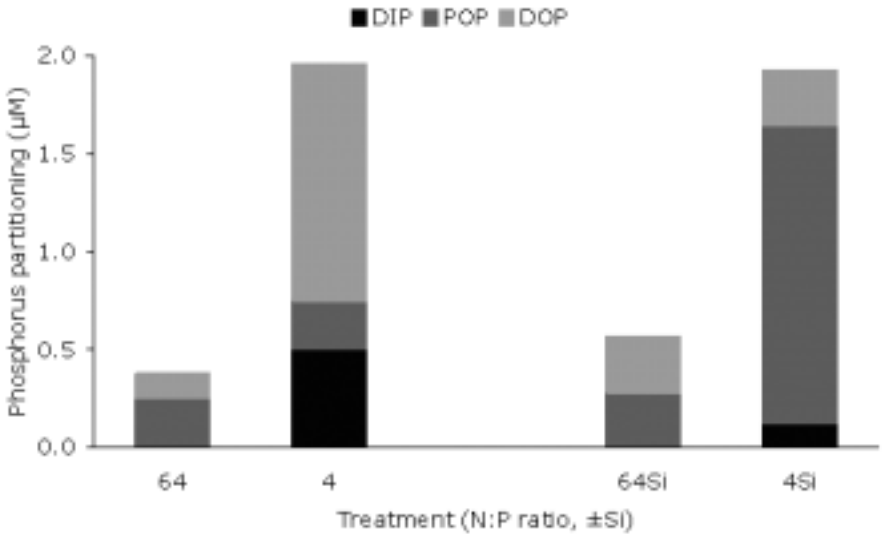

Fig. 5. Partitioning of the net phosphorus production over seven days in four mesocosms treated with different inorganic N:P ratios and ±Si dosing. DIP = dissolved inorganic P, POP = particulate organic P, DOP = dissolved organic P. The DIP production is added phosphate in surplus.

At the most severe P limited treatments (bags 64 and 64Si) new DOP was produced in measurable quantities and accumulating with a partitioning at about 25% to DOP and 75% to particulate phosphorus (POP, Figure 5). At N limitation the diatom dominated community had an ability to store most of the added P in particles and keep the DOP production at the same level as during P limitation. In contrast, DOP accumulated with a partitioning of 4 to 1 in favour of DOP over POP in mesocosm # 4 with an even distribution among the algal groups (Figure 5).

New DOM was accumulating in accordance with the intuitive expectations based on the nutrient regimes and more so for N than for P. A major influence from the biota was also observed. P limitation produced P depleted new DOM and DOC:DOP ratios from about 500:1 to 300:1 (Table 1) and DON:DOP was kept close to 20. Dominance of diatoms kept DOC:DON at 15, a well known value, but without diatom dominance the ratio almost doubled to 26.

Table 1. C:N:P stoichiometry of new DOM and for the total DOM pool in the mesocosms.

	New DOM	DOM in the mesocosms
Before treatment		245: 18:1
64	488: 19:1	257: 12:1
4	17: 2:1	92: 5:1
64Si	297: 20:1	277: 14:1
4Si	297: 2:1	203: 10:1

Nitrogen limitation showed a somewhat extreme pattern with respect to DOM stoichiometry due to high production of DOP (Table 1). Diatoms apparently had the ability to store excess P in the cells (Figure 5) and produced DOM very depleted in nitrogen. Contrary, the very high DOP production in enclosure # 4 without diatom dominance produced DOM with the extreme ratio of 17:2:1 and with a major effect on the whole DOM pool in the mesocosms (Table 1). This result was not erratic or random. The stoichiometric ratios of new DOM in mesocosms dosed with the N:P ratio 8 and 8Si were 29:3:1 and 297:5:1, respectively (data now shown).

The interpretation of the above data needs a precautionary note: the very low values of new DON at nitrogen limited conditions are uncertain and the calculated DOC:DON and DON:DOP ratios of new DOM should be interpreted within at least a factor of 2.

THE FATE OF AUTOCHTHONOUS DOM

The concentrations of DOM in the deep ocean are about 32-40 µM for DOC, DON at 3-5 µM, and DOP at 0.05–0.1 µM. In oceanic surface waters and more coastal stations the concentrations are generally much higher. Two processes – photochemical transformation and microbial degradation – are considered key processes for the removal of DOM and ultimately producing the recalcitrant background values. Adsorptions of DOM to colloids, condensation reactions and flocculation have all been suggested to be active in DOM removal, but quantitative knowledge on such processes is not strong (Carlson, 2002).

Photochemical reactions and specifically those induced by UV-radiation can work in two directions, either producing new molecules that can be utilised by bacteria, thus making the DOM pool more biodegradable or reduce the degradability. Several investigations indicate that new DOC produced by plankton communities becomes more resistant to microbial attack after exposure to UV-radiation (Benner and Biddanda, 1998) while humic material of terrestrial origin becomes more available (Tranvik and Bertilsson, 2001). Interestingly, DOC in seawater sampled out of the euphotic zone became more bioavailable after UV radiation, while DOC in surface water from the same area became more refractory. There seems to be an intricate interaction between photochemical reactions and microbes depending on the origin and perhaps the history of the DOC. The composition of bacterial communities with different physiological capacities

may be part of the explanation. Photochemical reactions typically make DON more available for bacteria and can directly produce ammonium (Bronk, 2002). UV-radiation can also react with the marine DOP pool and produce soluble reactive phosphorus, SRP (Karl and Björkman, 2002), however, once more we can say that knowledge on photochemistry and interactive microbial effects are in the gradient DOC>DON>DOP.

Microbes can partly degrade DOM without interaction from UV-radiation. A literature survey showed that an average of about 14% of DOC in marine surface waters is biodegradable (Søndergaard and Middelboe, 1995). In the Atlantic Bight Hopkinson *et al*. (2002) measured the biodegradability over 180 days and found the DOM pool to be degradable in the order DOP>DON>DOC with average values of the bioavailable fractions at 83, 40 and 30%, respectively. This result provides some insight to understand how the DOM pool becomes carbon rich and impoverished in nutrients but not how and when the recalcitrant background is produced.

Some insight in the production of refractory DOC can be gained from experiments where the biodegradation of new DOC produced by plankton has been investigated. At least the results can provide evidence for the timing in the development of refractory DOC. Apart from a few investigations (Fry *et al*., 1996) the duration for experiments is counted in months and not years and can therefore not be compared with the age of DOC in deep oceans, which is several hundred to thousands of years. No degradation experiment has to our knowledge ever reached the low deep sea concentrations.

Experimental biodegradation studies lasting from 30 days to more than a year have shown that new DOC can become refractory in relatively short time, although at variable relative proportions (Norrman *et al*., 1995; Søndergaard *et al*., 2000b; Meon and Kirchman, 2001; Kragh and Søndergaard, 2004). And, as some of the experiments have been carried out with artificial light, we can also conclude that UV-radiation is apparently not a prerequisite for the production of refractory DOC (Meon and Kirchman, 2001; Kragh and Søndergaard, 2004).

Meon and Kirchman (2001) made interesting observations with respect to the production of refractory DOC. They found that refractory DOC was produced by a nutrient enriched plankton community but not in an un-enriched «control». Furthermore, they identified combined dissolved neutral sugars and combined amino acids to explain some of the difference in end-point DOC values and thus to be members of the refractory pool. The actual nutrient status of their two plankton communities is not known, but as the conditions for decomposition were similar in the two experimental mesocosms it seems fair to conclude that the refractory nature of the produced DOC (and DON) must have developed during the initial 12 days with a net DOC production in the light and not during the following 35 days of microbial degradation in the dark. A similar conclusion may be reached from the degradation experiments carried out with sampled from the mesocosms experiment presented in Figure 3.

The new DOC produced among the different nutrient conditions behaved differently during biodegradation (Figure 6). P-limitation (mesocosms 64 and 64Si) clearly resulted in a larger pool of refractory DOC and more so for the diatom dominated community. At N-limitation, the DOC concentration after 150 days of degradation was not different from the refractory fjord water or only marginally so with diatom dominance. With some caution we conclude that both the nutrient regime and the composition of the community can influence the degradability of new DOC. Unfortunately, we do not know the fate of DON and DOP during degradation. The conditions during degradation were similar for all samples; consequently, the observed differences must have developed in the mesocosms and most probably related to some specific algal products produced during P-limitation or due to microbial processing. It is known that bacteria produce refractory DOM from labile compounds (Tranvik, 1993; Ogawa *et al*., 2001). Effects of UV-radiation cannot be excluded, but apparently need not to be a partner in the production of refractory DOM. Furthermore, optical analyses of DOM in the mesocosms showed that humic-like material was produced and more so at P limiting conditions (Stedmon and Markager, 2005), but their changes over short time interval (days) preclude a refractory nature. The observations

by Stedmon and Markager (2005) partly fit the results from the degradation experiments and also agree with results obtained by Obernosterer and Herndl (1995) on how P-limitation changed the DOM production in an algal culture and the ability of bacteria to utilise the DOM.

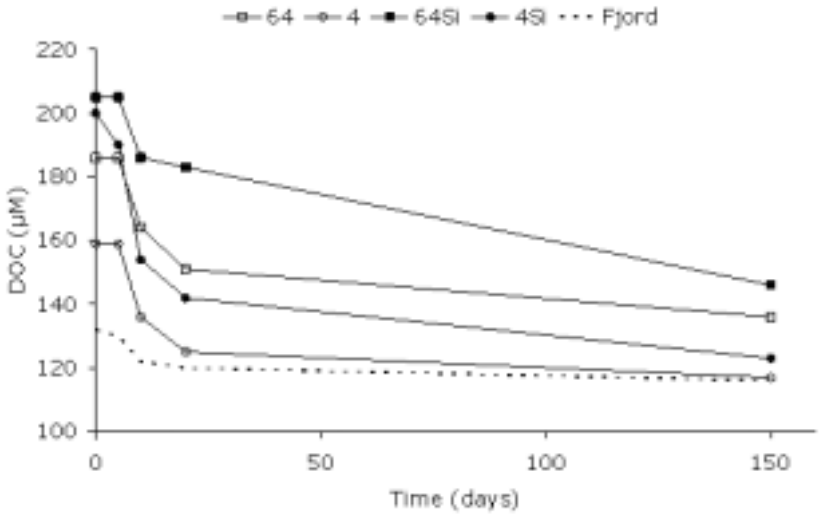

Fig. 6. Biodegradation of DOC produced by plankton communities in four mesocosms treated with different inorganic N:P ratios and ±Si dosing and in a sample of fjord water entering the mesocosms with 10% per day. The samples were collected seven days after the nutrient regime was established, filtered, and added an inoculum of natural bacteria and inorganic nutrients to make carbon the limiting factor.

Having come so far, we can also observe that the DOC «end-point» in the fjord water was 116µM after 150 days of microbial degradation (Figure 6). We still have some way to go to reach the oceans background of about 35 µM DOC.

In conclusion: the nutrient regime and apparently also the community structure can control the partitioning of new DOM in coastal ecosystems. We have no reasons to believe that the observations do not apply to more oceanic waters, however much more difficult to measure. Concerning the Mediterranean Sea with its large P-limited areas it is of special interest to notice the relatively high production of DOP even at P-limitation and the more refractory nature of the newly produced DOC. Future research on autochthonous DOM in the Mediterranean Sea may focus on how different nutrient regimes and community composition in different areas may control DOM production.

Acknowledgements

The experiment in Bergen included the committed participation of Peter J. LeB. Williams, Pascal Conan, Gus Cauwet, Stiig Markager, Colin Stedmon, Mireille Pujo-Pay, Dylan Evans, Bo Riemann, Frede Thingstad, Niels Henrik Borch, Anne J. Jacobsen and more; thanks. The final paper was influenced by the discussions during the workshop.

Dissolved primary production and response of prokaryotic heterotrophs

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ABSTRACT

The photosynthetic production of DOC or dissolved primary production (DPP) is now recognized as a significant DOM input in marine systems. Its magnitude relative to total rates (PER, percent extracellular release) can be reasonably well predicted from environmental drivers and is a useful parameter for cross-system comparisons. Among other factors, nutrient conditions, species composition and irradiance may also affect the quality of the released compounds and hence the response of prokaryotic heterotrophs, especially under substrate limitation. Whenever prokaryotic heterotrophs are strongly dependent on phytoplankton-derived DOC and their total carbon requirements are met by DPP we may conclude that both planktonic compartments are tightly coupled. In parallel to an overall decrease in total primary productivity and a relative increase in PER, this phytoplankton-bacterioplankton coupling is hypothesized to strengthen along coastal-offshore gradients. However, the regulation of bacterial growth efficiency arises as a critical factor to further our understanding of the role of prokaryotic heterotrophs on DOM fluxes.

In pelagic aquatic ecosystems, recently fixed photosynthate is made available to other trophic levels of plankton in a *continuum* from particulate to dissolved (Verdugo *et al.,* 2004), since release of dissolved organic carbon (DOC) takes place at the same time as production of particulate organic carbon (POC) by phytoplankton (Fogg, 1983). I hereafter will use the terms dissolved primary production (DPP) and particulate primary production (PPP) to respectively refer to these two processes that make up total primary production (TPP). DPP is still frequently neglected as a carbon flux routine measurement. Although DPP may contribute a low fraction to the overall DOC input to the ocean, it represents an important source of carbon for prokaryotic heterotrophs (Cole *et al.,* 1982; Norrman *et al.,* 1995), commonly called heterotrophic bacteria.

Upon the acceptance of its natural widespread occurrence (Mague *et al.,* 1980), many papers have dealt with methodological aspects of DPP measurements. The separation of organic matter into a dissolved and a particulate fraction is necessarily operational, and in most instances DPP is everything that passes through a filter of ~0.2 µm. However, as important as the choice of an adequate filter (Maske and Garcia-Mendoza, 1994) is the time-scale for the incubations aimed at measuring the rate of production of a rapidly cycled pool of organic matter. Therefore, short (<2 h), and even better time-course resolved incubations (Wiebe and Smith, 1977) are strongly recommended (Lancelot, 1979; Smith and Platt, 1984).

Researchers have proposed different mechanisms to explain the release of dissolved photosynthate, which can be roughly grouped into trophic (i.e. a consequence of protistan or

metazoan grazing on algal cells) and physiological (i.e. direct exudation of healthy phytoplankton). Protistan grazers have been hypothesized to contribute 53% to total recent DOC production, with phytoplankton, zooplankton and viruses each contributing 25%, 12% and 10% (Nagata, 2000). However, DPP estimated by compartmental analyses in kinetic experiments of organic 14C release (Morán and Estrada, 2002) would only include direct release by phytoplankton, with a negligible contribution of micrograzers' sloppy feeding or cell lysis, since this experimental design is specifically aimed at estimating instantaneous DPP and PPP rates. A recent seasonal study suggests that DPP measured in short-term experiments is the result of a purely physiological mechanism of passive DOC release (Marañón *et al.,* 2004). Whichever the exact mechanism, passive diffussion (Bjørnsen, 1988) or an overflow mechanism (Wood and Van Valen, 1990; Baines and Pace, 1991), DPP has been shown to vary, both in absolute terms and relative to total primary production, in response to several biotic and abiotic factors. The percentage of extracellular release (PER), defined as DPP divided by TPP (x100), is a convenient way of comparing the effect of environmental drivers on primary production partitioning across systems of differing trophic status. PER is also usually well-correlated with biomass-specific DPP.

Among the most frequently invoked DPP controlling factors are inorganic nutrient status, community composition and irradiance. Nutrient stress has been shown to increase PER, by as much as 100%, in phosphorus-limited conditions (Obernosterer and Herndl, 1995). The composition of the phytoplankton assemblages also exerts an influence on PER values. For instance, at the same nitrogen concentration in the North Sea, diatoms tend to release relatively less DOC than dinoflagellates, while most TPP appeared extracellularly (70% PER) in waters dominated by *Phaeocystis* colonies (Lancelot, 1983). A rough, alternative approach to species composition is to assess the contribution of different size-fractions. In this regard, high picophytoplanktonic contribution to TPP has been associated with PER enhancement by some authors (Teira *et al.,* 2001a,b; Morán *et al.,* 2002a; but see Marañón *et al.,* 2004 for an exception). Extreme irradiances, both high and low, were found to increase PER in phytoplankton cultures (Zlotnik and Dubinsky, 1989), and recent evidence demonstrates a stronger response to irradiance of PPP than DPP, hence inducing vertical changes in PER (Marañón *et al.,* 2004). Figure 1 shows a consistent increase in PER as the ratio of *in situ* to surface irradiance decreases across different marine ecosystems, suggesting the possible applicability of empirical models for estimating *in situ* DPP. Given the high range of PER variation reported in response to these or other factors such as temperature (Zlotnik and Dubinsky, 1989), it is not difficult to understand why claims of a cross-systems constant value of ~15% PER (Baines and Pace, 1991) have not yet become fully accepted (Teira *et al.,* 2001a; Morán *et al.,* 2002b). The few measurements available for the Mediterranean range from 2 to 44% (Hagström *et al.,* 1988; Fernández *et al.,* 1994; Morán and Estrada, 2001; Morán *et al.,* 2002b). It seems that integrated or volumetric rates behave rather differently, with a trend of integrated PER values to show less variability across the productivity range (Marañón *et al.,* 2004). In any case, integrated values of DPP are of limited relevance when addressing the immediate trophic linkage between primary producers and prokaryotic heterotrophs, since vertical differences in the respective activities of producers and consumers are better represented in volumetric units.

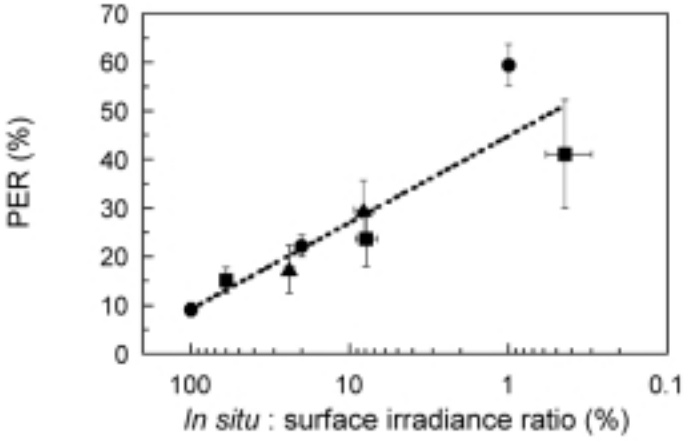

Fig. 1. Overall relation between mean PER values and the mean ratio of *in situ* irradiance at the surface in : ■ SW Mediterranean (Morán and Estrada, 2001), ▲ Southern Ocean (Morán and Estrada, 2002) and ● NE Atlantic coastal waters (Marañón *et al.,* 2004). Bars represent ± standard error.

The relative importance of the above-mentioned factors may influence the quality of released DOC from the *point of view* of prokaryotic heterotrophs, and therefore its fate in the ecosystem. Obernosterer and Herndl (1995) have shown that prokaryotes were less efficient when grown with DOC from P-limited phytoplankton, which can help explain periodic accumulation of DOC in areas such as the northern Adriatic (Fajon *et al.,* 1999). In a study in the Baltic Sea, seasonal changes in DPP were not followed by significant changes in bacterial uptake (Lignell, 1990), suggesting lower quality DOC in summer and/or shifts in bacterial growth efficiency (BGE). It is easy to foresee that overall poor quality DPP would tend to accumulate in the ecosystem as recalcitrant or semi-labile DOC, eventually available for export or subjected to further abiotic transformations. Dissolved photosynthate may also accumulate temporarily in specific instances such as phytoplankton blooms, provided DPP exceeds total carbon requirements by prokaryotic heterotrophs.

However, DPP usually proves a source of substrate rapidly processed by prokaryotic heterotrophs. Indirect evidence of this trophic link includes the finding of diel cycles of prokaryotic heterotrophic activity with maxima around midday, coincident with phytoplanktonic production peaks (Gasol *et al.,* 1998), the rapid decay of algal-derived DOC in the presence of prokaryotic heterotrophs (Chen and Wangersky, 1996) or the positive response of prokaryotic activity to irradiance (Morán *et al.,* 2001), altogether suggesting a strong need of freshly produced photosynthate by prokayotic heterotrophs. Jensen (1983) showed this fine tuning between DPP production and heterotrophic uptake by comparing samples incubated in the presence and absence of antibiotic. Micro-autoradiography has also demonstrated rapid incorporation of DPP by heterotrophs (Iturriaga and Hoppe, 1977).

The recurrent observation of good correlations between bacterial and phytoplankton biomass and production, provided their variation encompasses orders of magnitude (e.g., Gasol and Duarte, 2000), has been frequently associated with a direct dependence of prokaryotic heterotrophs on DPP. Fewer authors, however, have measured the relevant fluxes that underlie this trophic pathway. First, given the moderate to low (<0.30) BGE commonly observed in marine waters (del Giorgio and Cole, 1998), DPP must not only meet bacterial production (BP) but also bacterial respiration (BR), that is to say, bacterial carbon demand (BCD = BP + BR). These variables are in turn related since BGE is simply BP divided by BCD. With these considerations in mind we have recently proposed a *bacteriocentric* definition of "phytoplankton-bacterioplankton coupling" that includes the existence of a significant correlation between DPP and BCD plus a DPP:BCD ratio ≥ 1 (Morán *et al.,* 2002b). Table 1 summarizes the available dataset of simultaneous measurements of these variables in the W Mediterranean.

Table 1. Simultaneous measurements of dissolved primary production (DPP) and estimates of bacterial carbon demand (BCD) at several western Mediterranean sites. Also shown the bacterial growth efficiency (BGE) used and the DPP:BCD ratio.

Region	DPP (mg C m^{-3} h^{-1})	BGE (%)	BCD (mg C m^{-3} h^{-1})	DPP:BCD	Reference
Villefranche-sur-Mer	0.10	60	0.35	0.30	Hagström *et al.,* 1988
Almería-Oran front	0.03 – 0.19	50	0.01 – 0.27	0.17 – 1.00[a]	Fernández *et al.,* 1994
Catalano-Balearic Sea	0.06 – 0.47	3	0.14 – 0.88	0.07 – 1.37	Morán *et al.,* 2002b

[a] DPP assimilated by prokaryotic heterotrophs.

We have found a strong coupling according to the above definition in offshore waters, while in coastal ecosystems DPP was generally far below BCD (Morán *et al.,* 2002b). This is in agreement with other studies performed in coastal areas, which report DPP:BCD ratios <0.3 (Hagström *et al.,* 1988; Lignell, 1990; Baines and Pace, 1991; Nagata, 2000). The immediate conclusion is that additional carbon inputs are needed to fuel the growth and metabolism of prokaryotic heterotrophs in coastal waters. Yet, a recent paper suggests that the response of bacteria in net heterotrophic lakes is still primarily determined by autochthonous rather than terrestrial sources (Kritzberg *et al.,* 2005). The coastal-offshore gradient in the contribution of DPP to BCD was

especially remarkable in the NW Mediterranean, where it followed the same pattern as PER (see Figure 4 in Morán *et al.,* 2002b). Coastal ecosystems are characterized by relatively high volumetric primary production rates (>10 mg C m^{-3} h^{-1}) and low PER values (<5%), while the converse is true for offshore waters (<1 C mg m^{-3} h^{-1}, >10% PER, Figure 2). Our hypothesis that phytoplankton-bacterioplankton coupling becomes tighter as we move from coastal to open ocean ecosystems clearly needs more supporting data than those existing to date. Indirect evidence based on standing stocks rather than production has been recently reported for the western Pacific (Ning *et al.,* 2005).

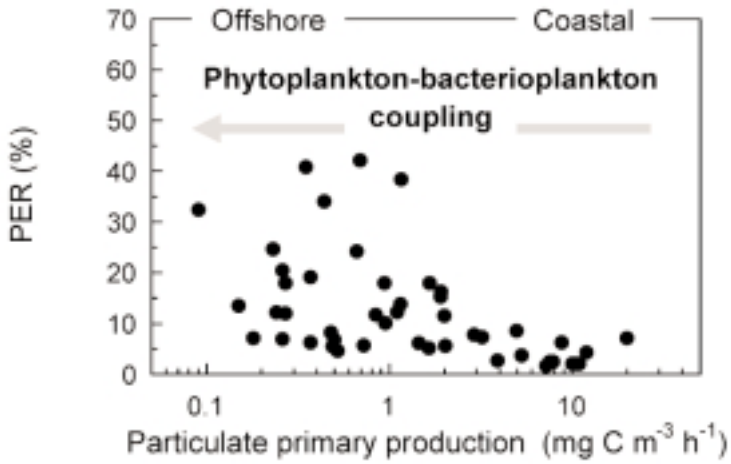

Fig. 2. Relation between PPP and PER for pooled data of the Atlantic and Southern Oceans obtained in time-course experiments of ^{14}C uptake with 0.22 µm membrane filters (Morán *et al.,* 2002b plus unpublished data). The strength of coupling between phytoplankton and prokaryotic heterotrophs by means of DPP across the coastal-offshore gradient is indicated by the arrow.

The use of different methods and conversion factors may strongly affect the conclusions of reports ascertaining the relationship between DPP and BCD (e.g. Morán *et al.,* 2002b; Teira *et al.,* 2003; see also Table 1). However, the critical point with which I would like to end this short review is the poor empirical knowledge of BGE in many systems. In our work (Morán *et al.,* 2002b) we have relied on published models that encompass a great uncertainty [e.g., for the Mediterranean data in Table 1 BGE would be 3% according to del Giorgio and Cole (1998) but 19% according to Rivkin and Legendre (2001)], hoping to constrain the minimum value of BGE, and hence the maximum possible estimate of BCD. If the above-mentioned difference in BGE results in a 6-fold increase in BCD, let us imagine the implications for the carbon budget of even lower or higher BGEs. For a step forward in our predictability of the final fate of the DOC input represented by DPP (i.e. when is it lower than, suffices or exceeds BCD), a better knowledge of the variability of not only BP but also BR is strongly needed before safe conclusions on the outcome of this trophic linkage can be achieved.

Prokaryotic ectoenzyme activity in surface waters of the Mediterranean

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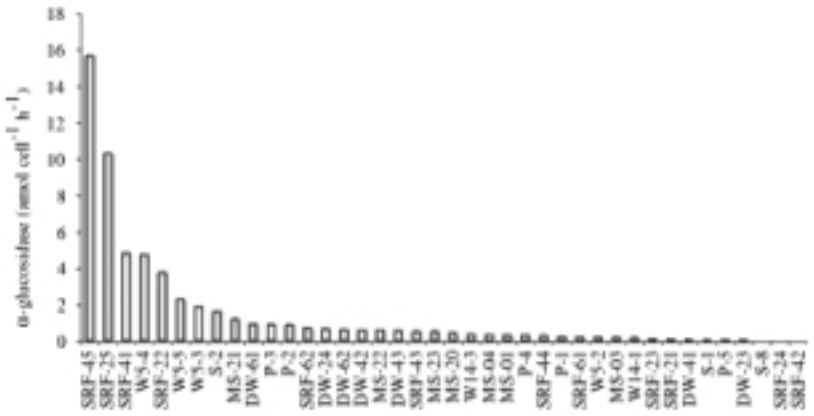
Prokaryotic uptake of organic molecules is mediated by transport systems which can only take up small molecules up to about 600 Da (Payne, 1980). However, most of the biologically available carbon present in the oceans is comprised of molecules larger than 1 kDa (Amon and Benner, 1996). Therefore, these large molecules need to be hydrolyzed prior to prokaryotic uptake, and it has been hypothesized that ectoenzymatic hydrolyisis is the rate-limiting step in the prokaryotic utilization of organic matter in marine environments (Hoppe, 1991). The patterns of hydrolytic enzyme activity expressed by prokaryotic communities can provide great insight on the dynamics of organic matter processing in aquatic systems. In practice, the concentration of the naturally occurring substrates is not measurable with state-of-the art techniques. Therefore, measured rates of enzyme activity are not a direct measurement of the utilization of a given substrate, but rather estimates of the "processing effort" being made by microorganisms. Thus, hydrolytic activity measurements indicate the way prokaryotes "experience" the organic matter field surrounding them. Dissolved organic phosphorus esthers must be hydrolyzed prior to prokaryotic uptake and a dual role of dissolved organic phosphorus as source of either P or C has been hypothesized (Hoppe, 2003). Therefore, phosphatase activity is a particularly interesting parameter in Mediterranean waters since (a) one of the distinct features of the Mediterranean is the apparent phosphorus limitation of primary production as compared to the nitrogen limitation reported for most of the world's oceans (Krom *et al*., 1991) (b) organic P can be the prevalent form of P in surface waters (Karl *et al*., 2001).



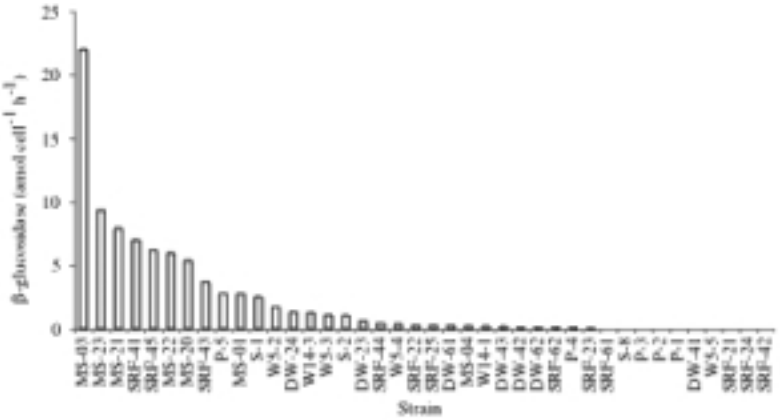


Fig. 1.

Bulk hydrolytic enzyme activities of whole prokaryotic communities have been measured extensively all over the world. However, not all the prokaryotes share the same hydrolytic abilities. For example, one of the differences between the two recently described *Prochlorococcus* ecotypes is the acquisition of an alkaline phosphatase like gene which enables growth on organic phosphate sources (Rocap *et al.,* 2003). Besides, even when the genetic information is present, different prokaryotes express completely different levels of activity under a given set of conditions. The results in Figure 1 show the per-cell levels of α- and β-glucosidase of 40 different bacterial strains isolated from surface waters, marine snow particles and the sediment sources in the Northern Adriatic Sea (Herndl *et al.,* 1999a). The per cell activity for this admittedly small sample of 40 different strains is not distributed as a Gaussian curve with most species having intermediate levels of activity, but rather follows a power-law in which a few strains have very high activity and the majority of the strains has very little or none at all. Although these are bacterial isolates cultured in rich medium and represent a tiny fraction of their original prokaryotic communities, activities in the original communities are likely to follow the same distribution. The consequences of such a distribution pattern are quite striking. If we made a theoretical assemblage mixing equal numbers of these isolates, we would have an artificial community in which about 20% of the bacteria would be responsible for about 80% of the total α- or β-glucosidase activity 1 (Figure 2). Moreover, if we removed the one species with the highest per cell activity (only 2.5% of the cells), the total activity would decrease by about 25%. This example illustrates the importance of species composition in determining the activity of natural prokaryotic communities.

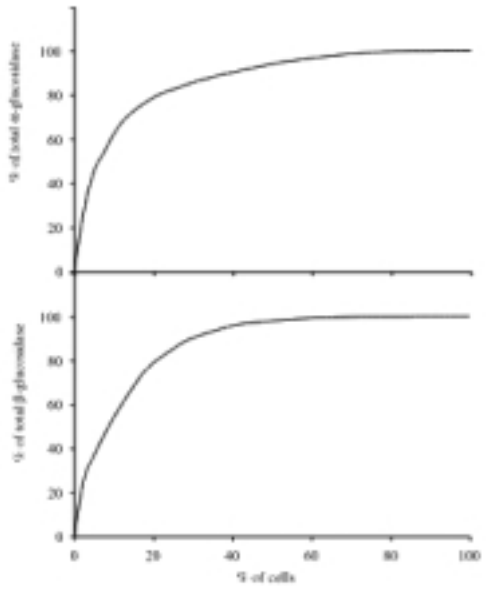


Fig. 2.

Thus, in order to elucidate the dynamics of ectoenzyme producing cells, single-cell approaches would be desirable. Direct labeling of ectoenzyme producing cells by means of fluorogenic substrates which precipitate upon hydrolysis have been used to detect phosphatase-producing cells in freshwaters (Nedoma *et al.,* 2003). However, this approach is limited by the availability of substrates for different enzyme activities. As most of the phosphatase activity in marine systems is often found as dissolved extracellular enzymes, this further limits the usability of these substrates for single-cell analysis.

The bulk enzyme activity commonly measured comprises the dynamics of different enzymes, probably produced by different organisms which are able to process a given substrate. Although we cannot identify the different organisms responsible for the bulk activity, we can at least estimate how many different enzymes are being produced. We developed an assay based on capillary electrophoresis, which allowed us to detect the different β-glucosidases expressed by a natural prokaryotic community. We observed the appearance and disappearance of eleven different prokaryotic β-glucosidases during the wax and vane of phytoplankton in the coastal North Sea (Arrieta and Herndl, 2002). Up to eight different β-glucosidases could be detected concurrently in the same water sample. The patterns of appearance of new enzymes coincided with a succession of bacterial species, proving the importance of species composition in determining the bulk activities of prokaryotic communities. However, there are factors other than species composition, which also play a role in shaping the ectoenzyme activity of a microbial assemblage. Conversely, we observed enhanced synthesis of existing β-glucosidases and induction of new enzymes in the absence of significant community changes during the development of an iron-induced phytoplankton bloom in the Southern Ocean (Arrieta *et al.,* 2004). This contrasting pattern can be explained by the fact that the synthesis of enzymes is an energy requiring process and therefore it is tightly regulated. Thus, most hydrolases are not constitutively expressed and will not be synthesized if the cost-benefit relationship of enzyme synthesis does not pay off (ie. when target substrate is not present in utilizable amounts or when the metabolic state of the cell cannot support the subsequent utilization of the products of enzyme activity).

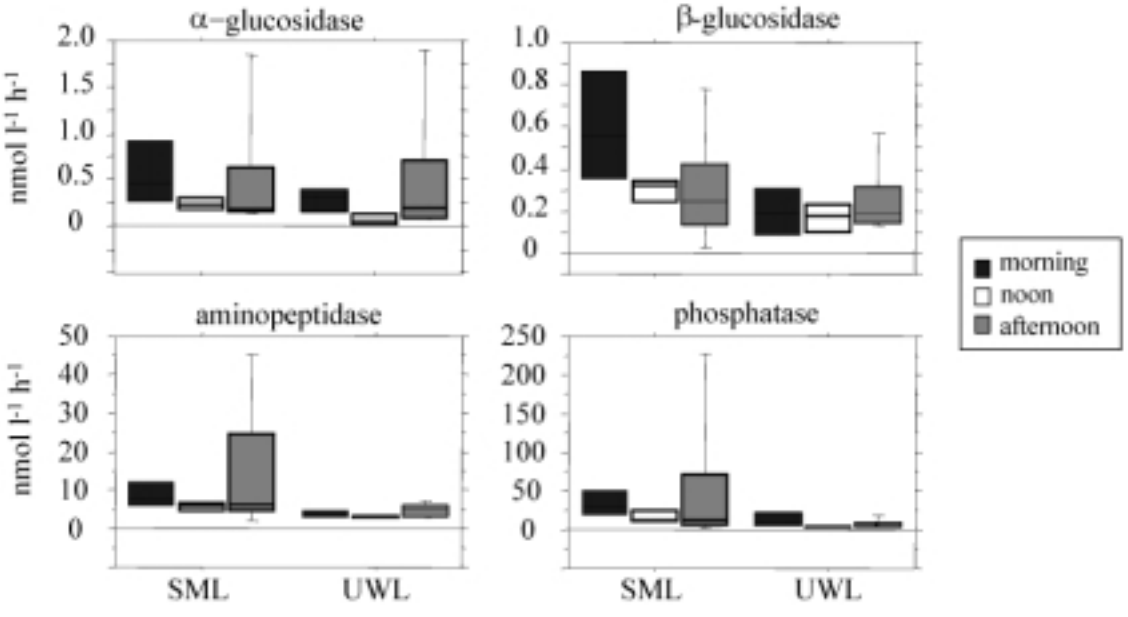


Fig. 3.

In addition to biological factors, abiotic factors such as ultraviolet radiation (UV) play a key role in the transformation of the dissolved organic matter (DOM) field in surface waters. UV radiation can increase the degradability of refractory compounds, decrease the availability of labile compounds (Obernosterer *et al.,* 1999) and impair prokaryotic activity, including ecto- and

extracellular enzyme activity (Herndl *et al*., 1999b). The diel dynamics of ectoenzyme activity in surface waters of the Mediterranean show clear evidence of this. Figure 3 shows the average bulk activities of four different hydrolases in a mesoscale eddy in the western Mediterranean. Samples were collected from the surface microlayer (SML) or the underlying surface waters (UWL), in the morning, noon or early evening on four different dates. Despite the high variability of the morning and evening samples, there is clear evidence of inhibition of enzyme activity at noon, most probably due to UV radiation. Thus, measurements taken at noon would tend to underestimate the actual rates of enzyme activity while measurements taken in the early morning or evening would lead to an overestimation of the actual rates of enzyme activity. Aminopeptidase and phosphatase activity were mainly free extracellular enzymes while almost all the α- and β-glucosidase was found in the fraction bigger than 0.2 µm. Yet another physical factor, windspeed seems to explain most of the variability of these free dissolved enzymes in the SML in the morning and evening samples (Figure 4). These data agree with the increase in SML thickness in response to increasing wind speeds up to about 10m s^{-1} reported previously (Falkowska, 1999). These findings suggest that at moderate wind speed, surface-active compounds, such as proteins would be more efficiently transported to the SML, where they would become effectively trapped and subsequently inactivated by the high UV intensities reaching the SML. This would, in turn, increase the turnover of dissolved enzymes from deeper layers, a major factor to be taken into account when interpreting bulk activity data.

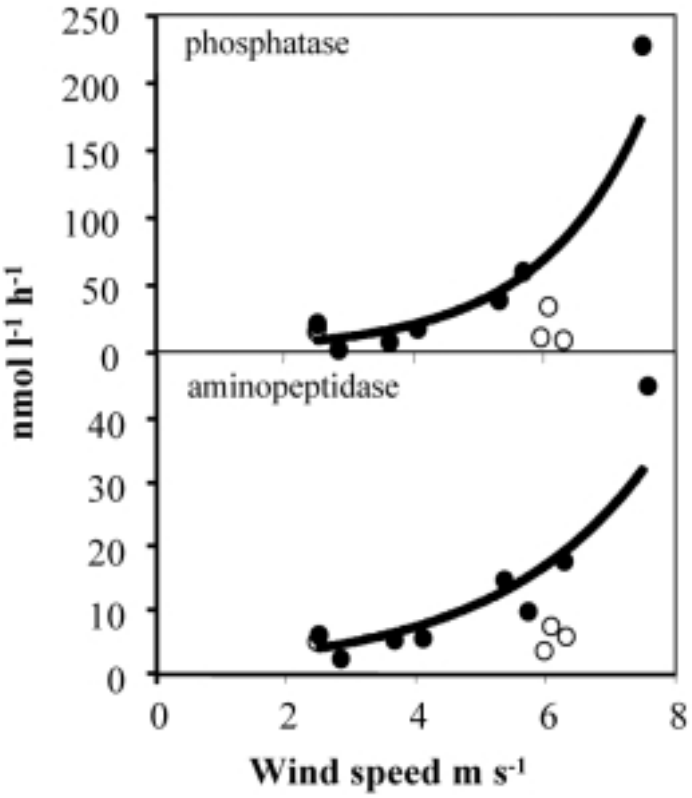


Fig. 4.

Characterisation and cycling of Dissolved Organic Matter in the north-western Mediterranean Sea

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ABSTRACT

Dissolved organic matter (DOM) represents one of the largest reservoirs of carbon on Earth, however its chemical composition remains largely unknown. This study consists in a detailed examination of the chemical composition of DOM at station DYFAMED, NW Mediterranean Sea. Samples were collected between April and December 2004 and high molecular weight DOM was isolated by ultrafiltration. Dissolved organic carbon (DOC) concentrations were highest at surface (73.30±3.80µM, n=4), decreasing with depth to 52.90±10.07µM (n=4) and 61.16±15.14µM (n=4) at 500m and 1500m, respectively. All samples had a significant monosaccharide component and the relative distribution of individual monosaccharides was consistent between samples and similar to that previously reported for DOM from other oceanic locations. Amino acid and amino acid enantiomer analysis suggested that the bacterial contribution to the DOM pool was minor (in the order of <1%). Protein analysis by gel electrophoresis (SDS-PAGE) revealed several distinct protein bands in most samples, while the majority of the dominant proteins appeared to be >50kDa. Nuclear magnetic resonance (NMR) analysis revealed a consistent ratio of three major biochemicals (carbohydrate/lipid/CHx) in all samples, similar to that reported previously for DOM, with the exception of the July surface sample which showed the highest levels of carbohydrate. This coincided with the lowest bacterial contribution to DOM and the previously-reported accumulation of transparent exopolymer particles at DYFAMED (Mari *et al.*, 2001). These results therefore suggest the presence of an annual cycle in the quality of DOM, potentially leading to the accumulation of a large, non-Redfield (carbon-rich) standing stock of dissolved and particulate organic matter in the euphotic zone during the stratification period.

1. INTRODUCTION

Dissolved organic matter (DOM) is one of the largest and least understood reservoirs of carbon (700 GT), nitrogen (35 GT), and phosphorus (2 GT) on the planet today. DOM supports most

bacterial respiration in seawater, limits the depth of the euphotic zone, binds micronutrient trace metals (Fe, Co, Zn), enhances the solubility of anthropogenic pollutants and affects their fate and transport. Previous analyses of the composition of oceanic dissolved organic matter (DOM) indicate that bulk C:N ratios range from 16-38 (Hansell *et al.,* 1993; Karl *et al.,* 1993), deviating significantly from Redfield stoichiometry. Thus, the production of high C:N DOM might be one explanation for observations of non-Redfield utilization of inorganic carbon and nitrogen in both coastal and oceanic regions (Karl *et al.,* 1991; Sambrotto *et al.,* 1993).

Our ability to study DOM composition and to use DOM in laboratory experiments is limited by our ability to recover DOM from fresh and marine waters. Investigations of DOM in seawater over the last two decades have followed mainly a holistic approach, focusing primarily on the total concentration, bulk properties and collective behavior of the mixture of molecules that make up the DOM pool. This approach comprises measurements of the total dissolved organic carbon (DOC), dissolved organic nitrogen (DON) and less often dissolved organic phosphorous (DOP) concentrations, and determinations of bulk spectral or isotopic compositions. Although this strategy presents the advantage of yielding characteristics that are representative of the entire DOM pool, the information obtained for the ecological and biogeochemical cycling of DOM is limited and highly biased toward the less reactive components of the DOM mixture (Hedges, 2002).

Our understanding of the composition and cycling of DOM in the oceans has changed tremendously since the 1990s. The application of large volume ultrafiltration technology resulted in a rapid advance in our understanding of dissolved organic matter composition over the last decade. Through the use of new analytical tools, we have a greater appreciation of the complexity of DOM and we can, at the same time, view its biomolecular building blocks with greater clarity. We now know that 22-33% of the total DOC has a molecular size>1kDa and consists largely in carbohydrate material, with C: N ratios of 15-22 (Benner *et al.,* 1992; Aluwihare *et al.,* 1997).

Despite these developments, the chemical composition of oceanic DOM remains largely elusive. This study aims to combine the results of a broad set of chemical characterisation techniques, in order to obtain a comprehensive view of the molecular composition of DOM in the NW Mediterranean, an area where such a study has not been conducted to date to our knowledge. The final goal is to consider the relative importance of different processes in the production of DOM (e.g. algal versus microbial processes) and reflect on potential links between the composition of DOM and the annual trophic cycle at the study site. Our approach was to study DOM composition firstly at the *monomeric* level, by amino acid enantiomer and monosaccharide analysis, then at the *macromolecular* level, by considering intact proteins using gel electrophoresis, and finally at the *structural* level by 1H NMR.

2. Methods and materials

2.1. Sampling site and sample collection

Sampling was carried out at station DYFAMED (Dynamics of Atmospheric Fluxes in the Mediterranean Sea; primary station: 43º 25´ N, 7º 52´E) in the NW Mediterranean Sea. The site is subject to monthly monitoring of several parameters, such as nutrient and pigment concentrations, as part of the French JGOFS programme. DYFAMED exhibits deep-water formation events and strong stratification in the summer and is presumed to be relatively isolated from terrestrial inputs due to the presence of the Ligurian current in the area. As a result DYFAMED is considered to be a good proxy for open ocean environments (Marty *et al.,* 2005). Concentrations of DOC (Table 1) are highest at the surface (73±3.7µM, n=9), decreasing to 52±10.87µM (n=9) at 500m and 61.16±15.13µM at 1500m (n=9).

Samples were collected three times during the year, in April, July, and October 2005, in order to cover the spring bloom, stratification and winter mixing periods. 200 liters of water were collected at the surface, 500m and 1500m, using Teflon tubing only, and filtered on-board through 0.2µm polysulphone cartridges. In order to concentrate the high molecular weight DOM (HMW DOM) samples were then processed through an ultrafiltration system (Separation Engineering, CA, USA) with a molecular weight cut-off of 1kDa, until the volume was reduced to

Table 1. DOC concentrations (µM) for initial seawater sample, after ultrafiltration, after desalting and respective DOC recoveries (%) for each sample.
[2] From published DOC data for DYFAMED (Avril, 2002).
* no data available.
† all sample desalted.

Collection date	Collection depth (m)	initial c. DOC (µM)	c. DOC after ultra-filtration (µM)	c. DOC after desalting (µM)	DOC recovery after ultra-filtration (%)	DOC recovery after desalting (%)
April	0	82.0[2]	321.2	139.5	4.0	1.8
April	500	50.0[2]	212.5	116.2	4.5	3.4
April	1500	46.4	318.4	96.3	8.4	3.0
July	0	76.8	707.7	389.2	10.1	6.5
July	500	58.0	354.6	222.1	6.4	4.3
July	1500	73.4	*	*	*	*
October	0	69.2	1874.2	334.5	18.6	4.1
October	500	41.3	1362.6	312.7	23.1	9.2
October	1500	44.2	†	539.3	n/a	16.6

approximately 2l. The 'DOM concentrate' was then desalted and lyophilised. Dissolved organic carbon (DOC) recoveries using this procedure varied from 3.9% to 26.7% before desalting and 1.8% to 16.6% after desalting, hence resulting in DOC concentrations in the final sample of 212.5µM-1456.5µM and 96.31µM-539µM for non-desalted and desalted samples respectively (Table 1).

2.2. Analytical procedures

Amino acid enantiomer analysis was carried out by high performance liquid chromatography (HPLC), as in Jones *et al*. (2005), based on the method of Kaufman and Manley (1998). Polysaccharide analysis was carried out by gas chromatography-flame ionization detection (GC-FID), as in York *et al*. (1985). Samples were prepared and analysed for intact protein, as in Jones *et al*. (2004), by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Finally, ^{1}H nuclear magnetic resonance analysis was carried out as in Aluwihare *et al*. (1997).

3. Results and Discussion

3.1. Monomeric level

3.1.1. Amino acid enantiomers

Proteins contain exclusively L-amino acids, while peptidoglycan, a component of the bacterial cell wall, contains certain of the more unusual D-amino acids. The 'percent peptidoglycan contribution to amino acid nitrogen' (PG-AA-N%; calculated as in McCarthy *et al*. (1998), based on D-alanine concentrations and the average peptidoglycan structure) was employed here as a measure of the bacterial contribution to DOM. PG-AA-N % was 14.61±3.2% (n=8), thus indicating that peptidoglycan accounts for a minor portion of the HMW DOC pool at DYFAMED (estimated <1%).

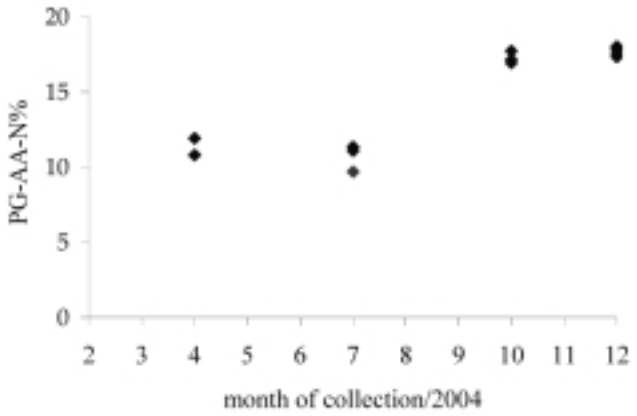


Fig. 1. Peptidoglycan contribution to amino acid nitrogen (PG-AA-N%; calculated as in McCarthy *et al.,* 1998) in the HMW fraction of DOM, throughout the year. The lowest PG-AA-N % value was observed for the July surface sample.

Furthermore, the distribution of D-amino acids in the sample was not characteristic of peptidoglycan (e.g. high levels of D-serine) and suggests the presence of additional sources to the D-amino acid pool (Jones *et al.,* 2005), such as archaea, diatoms or siderophores. As a result, calculations which assume that all D-amino acid originate from peptidoglycan could overestimate the bacterial input, which may hence be even smaller than the one postulated above. This is in agreement with a number of recent studies (Aluwihare *et al.,* 2005; Kaiser and Benner, personal comm.), which have demonstrated that peptidoglycan does not account for a significant portion of DOM, contrary to models proposed in the late'90s (e.g. McCarthy *et al.,* 1998.

3.1.2. Neutral sugar analysis

Data for neutral sugars are plotted in Figure 2, in order to illustrate the mole percent monosaccharide distribution of each sample. Despite differences in the monosaccharide yield between surface and deep waters and between different seasons, the distribution of neutral sugars in the DYFAMED HMW DOM was consistent between samples and in agreement with the distribution that had been observed previously for oceanic DOM samples (e.g. Aluwihare *et al.,* 1997). On average, mannose was the most abundant sugar, followed by fucose, rhamnose, xylose, arabinose, glucose and mannose at similar relative levels. Interestingly, the July surface sample, which, as mentioned in Section 3.1.1 showed the lowest PG-AA-N% value, also had the highest carbohydrate content (21.4% of HMW DOC).

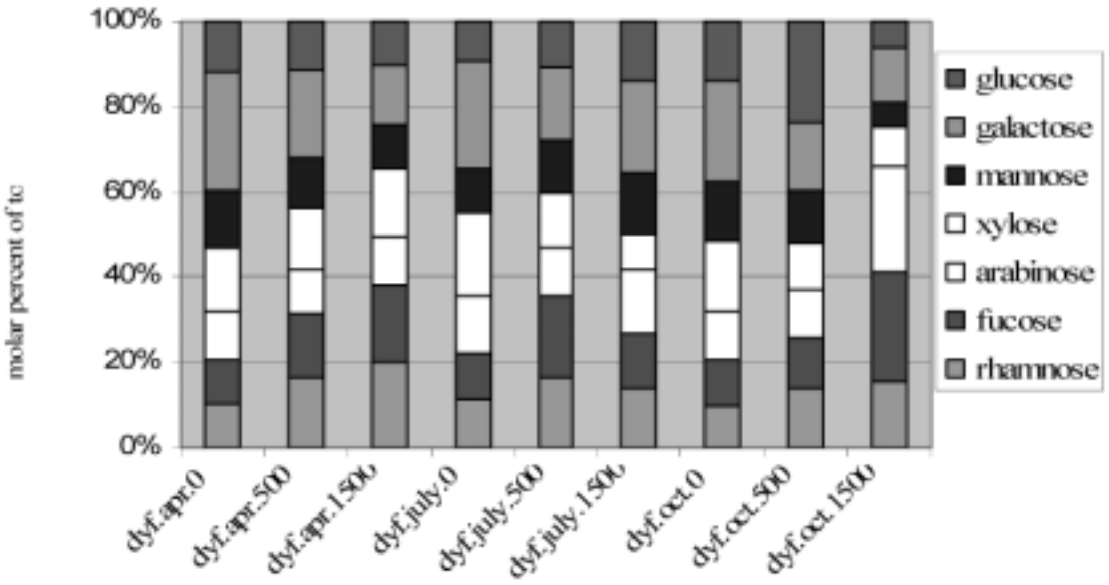


Fig. 2. Relative amounts of neutral sugars present in the sample collected at DYFAMED (as molar percentage of the total). Note that results for the October 1500m samples are suspected to be biased by contamination from the ultrafiltration membrane.

3.2. Macromolecular level: intact protein analysis

The amino acid composition of DOM is relatively invariable between different environments, hence providing little information on the biogeochemistry of the parent protein molecules. SDS-PAGE has recently been applied to aquatic samples (e.g. Tanoue *et al.,* 1995; Jones *et al.,* 2004) and permits the analysis of intact proteins, hence potentially providing a considerably higher level of information on the sources and cycling of DOM.

Sodium dodecyl sulphate polyacrylamide gel electroporesis (SDS-PAGE) analysis produced gels with intense background staining, which is characteristic of DOM samples (e.g. Tanoue *et al.,* 1995; Jones *et al.,* 2004), as well as contamination by skin keratin in the 50-70 kDa area (Jones *et al.,* 2004). Distinct bands were, however, detected in all samples collected in April, in the July 500m and 1500m samples and the October surface sample (Figure 3).

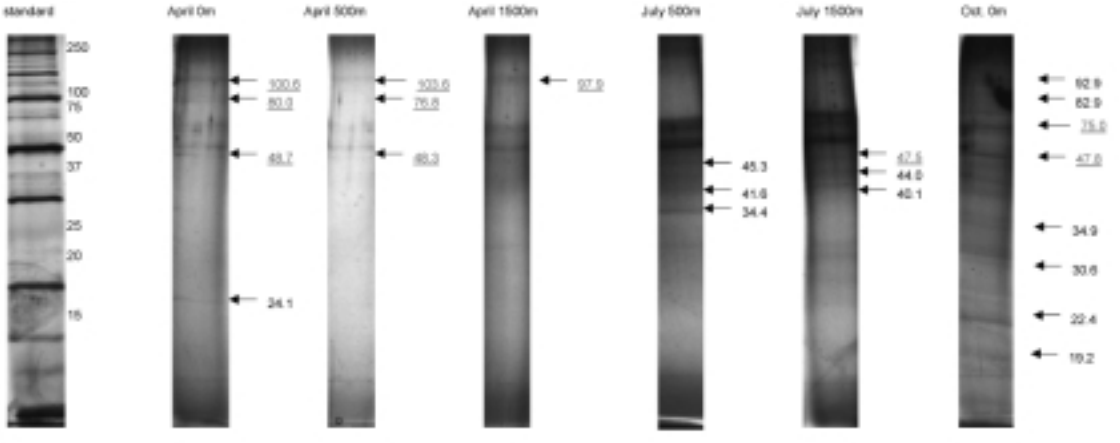


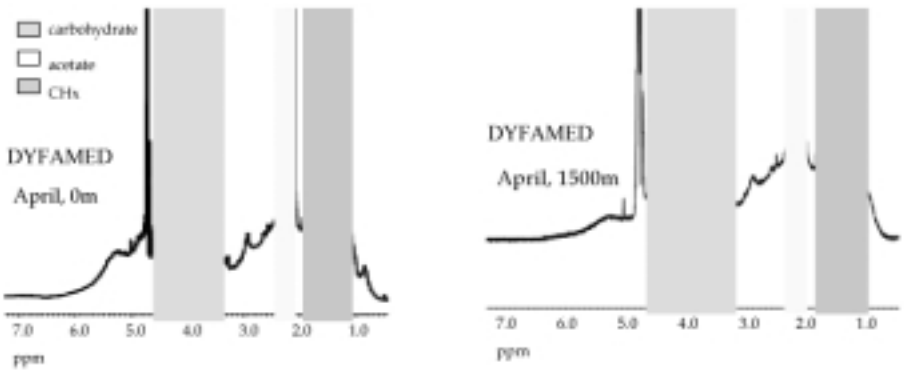
Fig. 3. SDS-PAGE of DOM samples collected at DYFAMED. Bands were detected and molecular size attributed using the software PD-Quest (Bio-Rad). Major bands and their approximate molecular size (kDa) are noted (underlined bands observed in more than one samples). When interpreting the gels, a precision of ±3kDa was assumed for bands representing molecular sizes > 50kDa and ±1kDa for bands representing molecular sizes ≤ 50kDa. Due to keratin contamination the 50-70kDa region has been excluded from interpretation.

The majority of the bands were present in the >50kDa area. None of the bands were ubiquitous, but several bands were present in more than one sample (e.g. 100kDa, 80kDa). However, due to the low resolution of SDS-PAGE, one cannot assume that these represent the same protein molecules. The 48kDa band, which corresponds to the molecular size of the bacterial protein reported as ubiquitous in a wide range of oceanic locations (e.g. Tanoue *et al.,* 1995), was only detected in some samples (April 0m, April 500m, July 1500m, October 0m).

3.3. Structural level: 1H Nuclear Magnetic Resonance Spectroscopy

H^1 NMR has been used to study the structural characteristics of aquatic samples to reveal a constant ratio of three major biochemicals, carbohydrate/acetate/lipid (Aluwihare *et al.,* 1997; Repeta *et al.,* 2002).

The ^{1}H NMR spectra of all the DYFAMED samples showed major resonances corresponding to carbohydrate (5-5.5ppm), acetate (2ppm) and CHx (1.3ppm). The ratio of carbohydrate/acetate/CHx was 8.7/1.0/1.3 (n=2[1]) at surface, while the carbohydrate component decreased in deeper water samples, resulting in ratios of 3.8/1.0/1.0 (n=2) and 3.9/1.0/1.2 (n=2) at 500m and 1500m, respectively (Figure 4). The July surface sample was particularly carbohydrate-rich, with a ratio of carbohydrate/acetate/CHx of 10.3/1/1.5 (Figure 4).



1 only April and July samples included, heavy contamination by the ultrafiltration membrane hindered 1H NMR analysis for October sample

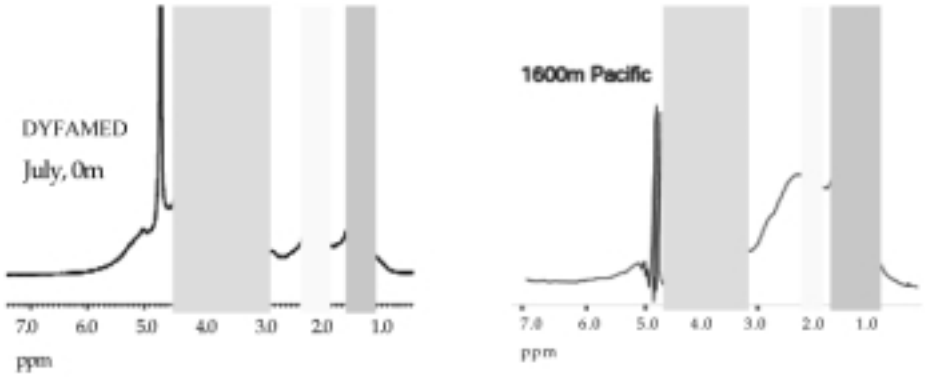


Fig. 4. 1H NMR spectra of: April surface, April 1500m and July surface samples collected at DYFAMED and sample collected at 1600m in the Pacific Ocean (Aluwihare, 1999).

The NMR spectra obtained for the DYFAMED samples are remarkably similar to those reported for samples collected from a wide variety of aquatic environments, ranging from open ocean locations to coastal waters (Figure 4; Aluwihare *et al.,* 1997; Aluwihare, 1999; Repeta *et al.,* 2002; Figure 4). Therefore, our results support the hypothesis that HMW DOM consists in a structurally consistent biopolymer, with a significant carbohydrate component throughout the world's oceans (Repeta, unpubl.).

Moreover, the NMR patterns obtained suggest the presence of particular conditions at surface in July at DYFAMED, as DOM appears to have a higher than usual amount of carbohydrate. This is in agreement with the monosaccharide analysis, which showed the highest carbohydrate content for the July surface sample and also coincides with the lowest PG-AA-N% value. These results, therefore, provide some evidence of a cycle in the quality of DOM at DYFAMED. This may be linked to the annual trophic cycle, with the presence of particularly 'fresh' DOM in the surface waters at the beginning of the stratification period. The pigment data collected at DYFAMED suggest that this carbohydrate maximum July does not coincide with the chlorophyll *a* bloom (April), but rather with a secondary bloom of cyanobacteria (J. Ras and J.-C. Marty, personal comm.).

4. SYNOPSIS AND CONCLUSIONS

a) There was a striking similarity between the NMR spectra obtained for the DYFAMED samples and samples collected from other oceanic environments. The samples appear to have a significant carbohydrate component and the distribution of individual neutral sugars is consistent between samples, but also similar to that reported previously for DOM samples.

b) The bacterially-derived component appears to be minor (in the order of <1% of DOC). This is supported by the amino acid enantiomer and gel electrophoresis analyses.

c) The results presented here may provide evidence of an annual cycle in the quality of DOM linked to the trophic cycle at DYFAMED. At the beginning of the stratification period (July) the carbohydrate content of DOM appears to increase, while the relative importance of the bacterial input decreases. This accumulation of carbohydrate-rich DOM during the summer coincides with the accumulation of TEP, polysaccharide-rich particles, at DYFAMED (Mari *et al.,* 2001).

d) The above support the hypothesis proposed previously (Karl *et al.,* 1991; Sambrotto *et al.,* 1993) that a large, non-Redfield (carbon-rich) standing stock of dissolved and particulate organic matter could accumulate in the euphotic zone during the stratification period. This pool may represent an important intermediate in the ocean carbon cycle in oligotrophic regions.

Despite recent advances, many important questions remain unanswered regarding the chemical composition of DOM and provide impetus for continued research. With the advent and dissemination of new tools in molecular biology, we expect that coupling of the two approaches

in the future will yield new insights into the cycling of organic nutrients by microorganisms. Knowing the DOM structure will allow us to address the environmental impact of this pool, regarding its production (algae/bacteria), uptake by microorganisms, transport depth, chelation of metals and dissolved organic nutrient uptake by phytoplankton.

Acknowledgments

The authors would like to acknowledge M.-D. Pizay for the carrying out the DOC analysis and the Marie Curie post doctoral fellowship programme as well as the National Science Foundation for financial support.

Composition and formation of macroaggregates in the northern Adriatic Sea

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ABSTRACT

Composition of macroaggregates in the northern Adriatic Sea, presently considered as a product of aggregation of macromolecular dissolved organic matter into macrogels, was studied using spectroscopic methods (NMR, FTIR). The macroaggregates are composed of aliphatic chains and polysaccharides bonded through ester and amide groups, and organosilicon compounds. The formation of macroaggregates includes processes which transform macromolecular dissolved organic matter, mostly the product of phytoplankton extracellular release, into colloidal organic matter and successively into particulate organic matter. The aggregation and stability of macrogels is most probably enhanced by interaction between organic matter and particles (cells, minerals, etc.).

1. INTRODUCTION

Macroaggregates in the northern Adriatic offer a rare opportunity to study the assembling of macromolecular DOM into macrogels. The mucous macroaggregates in the northern Adriatic, recently observed in the summer of 1997 and late spring of 2000, 2002 and 2004, were primarily the product of "new" phytoplankton production during favorable environmental conditions (Faganeli *et al.,* 1995). The episodic mucilage phenomenon has a natural origin and events of massive mucous formation in the northern Adriatic have been recorded for centuries (Fonda-Umani *et al.,* 1989). Mucous macroaggregates are found in a variety of forms, including small flocs, macroflocs, stringers, tapes, creamy surface and gelatinous layers (Stachowitsch *et al.,* 1990), with heterogeneous distribution and accumulation within the stratified water column. Macroaggregates contain various entrapped phytoplankters, microzooplankters, bacteria, and detrital and mineral particles (Stachowitsch *et al.,* 1990; Baldi *et al.,* 1997). The chemical composition of northern Adriatic macroaggregates was studied (Kovac *et al.,* 1998, 2002, 2004) using different spectroscopic techniques (^{1}H NMR, ^{13}C NMR, FTIR). These studies revealed a very complex structure of macroaggregates, in general composed of carbohydrates, aliphatic chains, organosilicon compounds and an inorganic fraction composed of calcite, quartz and clays. In this contribution the results of these spectroscopic studies and a hypothesis about aggregation processes are presented.

2. COMPOSITION

2.1. NMR spectroscopy

The ^{1}H-NMR spectrum of macroaggregates (Figure 1) shows intense peaks in the region δ=0-2.5 ppm, which are due to signals from paraffinic protons. The peaks around δ=0.8-0.9 ppm are assigned to protons of the methyl groups. The region at δ=1.2-1.6 ppm is attributed to methylene and peaks at δ=1.8 to methyne protons. The signals at δ=1.8-2.5 ppm can be assigned to protons (bonded to alpha carbon atoms) adjacent to functional groups, such as carboxyl and amide, however, the carbonyl, ester and methyl groups of aliphatic ketones could also occur in this region. Resonances from proteins also appear between δ=1.5-2.8 ppm. The broad peak in the region of δ=3.3-4.8 ppm and the signals between δ=5.0-5.7 ppm (anomeric protons) are assigned to carbohydrates. Additionally, resonances at δ=8.2 ppm in the aromatic and conjugated olefinic region, normally observed between δ=6-9 ppm, are also present.

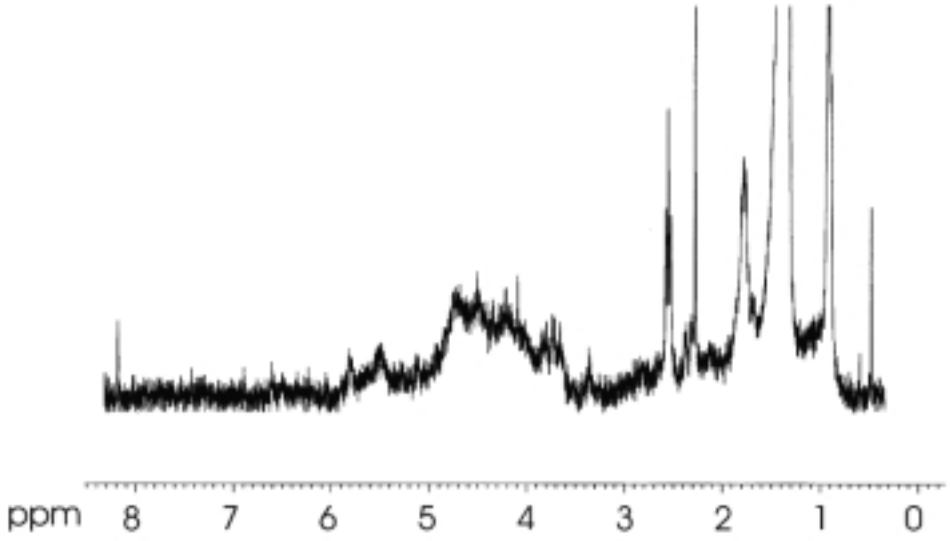

Fig. 1. ^{1}H-NMR spectrum of macroaggregate (Kovac *et al.,* 2002).

In general, from our ^{1}H-NMR spectra four major classes of structural elements can be identified: carbohydrates, an aliphatic component, functional groups such as ester and amide groups and organosilicon compounds (Kovac *et al*., 2002).

Considering H integrals in NMR spectra the temporal variations of the relative composition of the macroaggregate samples were followed. These variations resulted in a relative decrease in the content of carbohydrates and a contemporaneous rise in the content of the aliphatic and organosilicon component. The content of protons bonded to α-carbon atoms in carboxylic and amide groups decreased indicating that some transformations in this part of the molecule occurred.

The partition of macroaggregates into water-soluble and water-insoluble fractions revealed significant differences in their composition. According to the ^{1}H-NMR spectra the water-insoluble fraction of macroaggregate samples consists mainly of aliphatic structures probably bonded to carbohydrates through ester and amide bonds, and organosilicon compounds of diatomaceous frustulae (Kovac *et al*., 2004). On the other hand, the water-soluble fraction is mostly composed of carbohydrates with a minor amount of the aliphatic component. The ^{1}H NMR spectrum of this fraction revealed structural similarities to UDOM isolated from the cultured diatom *C. fusiformis*. This is in accordance with the general view that high concentrations of phytoplankton exudates, mainly composed of polysaccharides (Mingazzini and Thake, 1995), are most probably the precursors of mucilage. The aromatic signal around δ=8.4

ppm present in the ^{1}H-NMR spectrum of the water-soluble fraction and the UDOM of cultured *C. fusiformis,* assigned to amino acids, also confirms the importance of the diatom UDOM for macroaggregate formation.

Solid-state ^{13}C-NMR confirmed the general composition of macroaggregates in the northern Adriatic (Kovac *et al.,* 2004) obtained from the ^{1}H NMR spectra. The ^{13}C-NMR spectrum (Figure 2) shows major spectral bands including alkyl C (δ = 0-45 ppm), O/N-alkyl C (δ = 45-110 ppm), olefinic/aromatic C (δ = 110-160 ppm) and carboxyl/carbonyl/amide C (δ = 160-220 ppm) resonances (Keim *et al.,* 2000; Hedges, *et al.,* 2002). The broad signal between δ = 15-50 ppm indicates heterogeneity of alkyl carbon (Guggenberger *et al.,* 1994). The peak at δ = 30 ppm corresponds to $(CH_2)n$ chains and the signals at δ = 22-23 ppm are due to tertiary or quaternary carbons while the peaks around δ = 15 ppm indicate the contribution of CH_3 groups (Zegouagh *et al.,* 1999). The other main peaks were assigned to C-O or C-N functions (δ = 57 ppm), while the signals around δ = 75 ppm and δ = 103-105 ppm (glycosidic C-O) represent mostly polysaccharides. Olefinic and /or aromatic C=C (δ = 120-130 ppm), and ester and/or amide C (δ = 173 ppm) signals are also present.

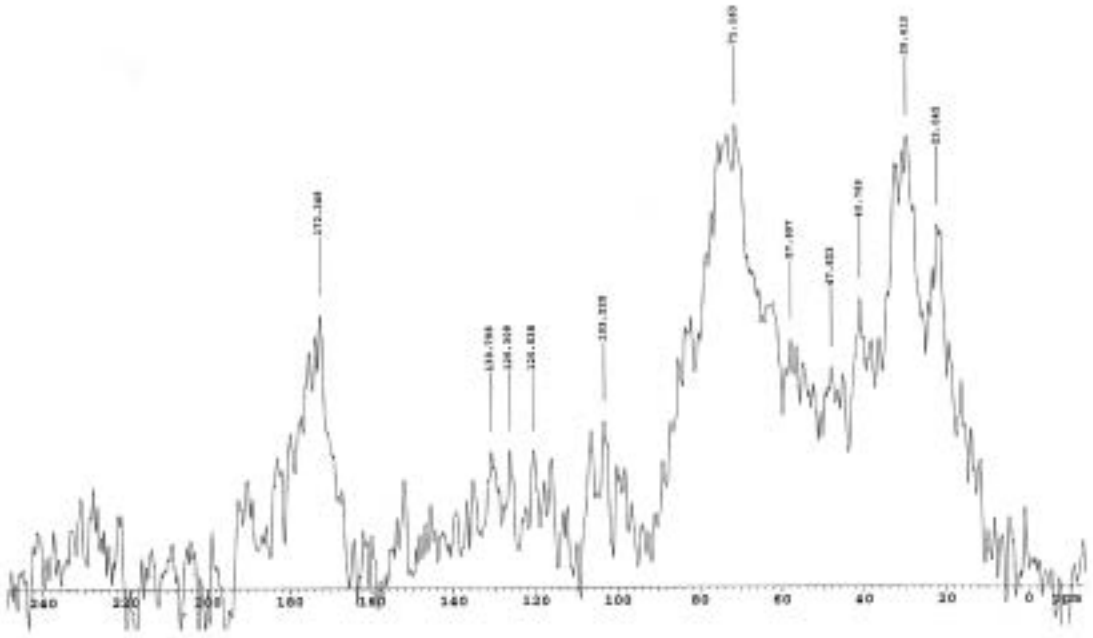

Fig. 2. Solid-state ^{13}C-NMR spectrum of macroaggregate sample collected in the Gulf of Trieste on June 26, 2000. Spectrum shows major resonances of alkyl C (δ = 0-42 ppm), O/N-alkyl C (δ = 45-110 ppm), olefinic and aromatic C (δ = 110-160 ppm) and carboxyl/carbonyl/amide C (δ = 160-220 ppm).

2.2. FTIR spectroscopy

The FT-IR spectrum of the macroaggregate sample (Figure 3) exhibits bands in the range 3600-2800 cm^{-1} corresponding to the presence of the hydroxyl group and N-H stretching (Zegouagh *et al.,* 1999) and a group of much weaker bands in the range 2800-3000 cm^{-1} originating from the aliphatic component (methylene and methyl groups; Arouri *et al.,* 1999). In the range 400-1800 cm^{-1}, numerous bands are noted and attributed to the vibrations of the organic (proteins and polysaccharides) and inorganic components (carbonates and silicates). A clear distinction between them is difficult to make because they overlap and possible frequency shifts due to the particular chemical environment of the gel skeleton could not be observed. The bands at 1737, 1649, 1543 and 1237 cm^{-1} are assigned to the organic fraction corresponding to carbonyl (C=O) (1737 cm^{-1}) and C-O-C bond modes (1237 cm^{-1}) while nearby bands at 1649 cm^{-1} and 1543 cm^{-1} are probably due to the C=O stretching vibrations of amide I and the N-C=O stretching vibrations of amide II. The band centered at 1649 cm^{-1} may also include some contribution of aromatic and olefinic C=C. However, this band cannot be assigned unequivocally because it coincides with the

water deformational mode (1640 cm^{-1}). The strong bands at 1000 - 1100 cm^{-1} originate from polysaccharides (Guggenberger *et al.,* 1994) and other C-O groups. However, the most characteristic bands of silicates appear in the range 1000 – 1100 cm^{-1} coinciding with the most characteristic bands of polysaccharides (~ 1100 cm^{-1}) (Bourdon *et al.,* 2000). The existence of silicates in the samples can also be observed from the 1080 cm^{-1} and around the 1049 cm^{-1} band which are superimposed on the broad band in this region. Nevertheless, these bands alone are not sufficient to distinguish between various silicates. Considering the 1044 – 1034 cm^{-1} band and some other bands of lower intensities (527 – 535 and 468 cm^{-1}), the organosilicon compounds (Si-CH_x groups) and α-SiO_2 as one of the mineral components are the most important (Anderson, 1974; Moenke, 1974).

In addition, the spectrum displays calcite absorption peaks. The bands at 877 cm^{-1}, 712 cm^{-1} and 1380 – 1480 cm^{-1} (centered at 1426 cm^{-1}) can undoubtedly be attributed to $CaCO_3$. The signals around 1430 cm^{-1} could also be due to C-H aliphatic deformation vibrations in the methyl and methylene groups. The signal from CH_3 symmetric bending also appears at 1384 cm^{-1}.

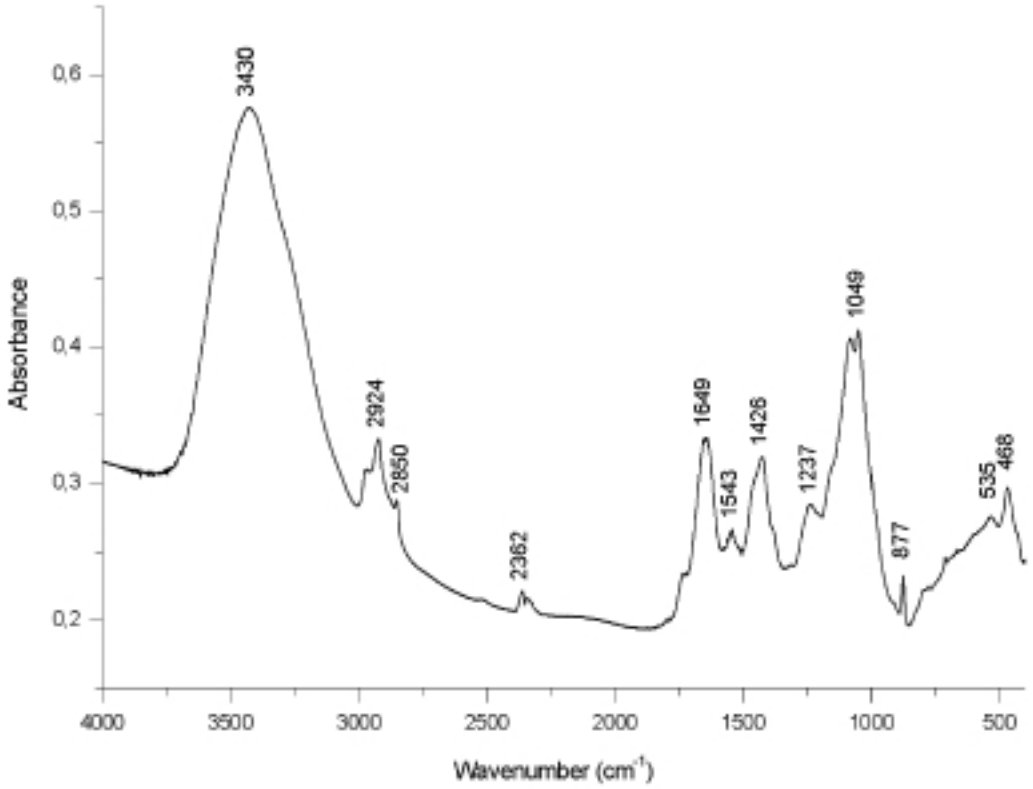

Fig. 3. FT-IR spectrum of macroaggregate sample collected in the Gulf of Trieste on July 31, 1997.

3. FORMATION OF MACROAGGREGATES

Mucous macroaggregates are defined as macrogels produced by phytoplankton. Their formation includes processes transforming macromolecular dissolved organic matter (DOM) into colloidal organic matter (COM) and successively into particulate organic matter (POM). The most important sources of COM are phytoplankton-derived colloids mostly originating in phytoplankton exudation (polysaccharidic) and cell lyses. The aggregation process could be explained via polymer gel theory (Chin *et al.,* 1998) and the formation of nanogels and, further, of microgels (Verdugo *et al.,* 2004) that continue to agglomerate in particulate organic matter (POM). Since dissolved organic matter concentrates at phase boundaries such as the sediment-water, air-sea interfaces, and pycnocline and due to the presence of mineral particles (sediment,

atmospheric dust), the COM and mucous macroaggregates agglomerate and further accumulate in those layers. The transformation of DOM to COM includes the increase in the size and changes in the reactivity of the material (Haygood *et al.,* 1993). Photopolymerization (Kovac *et al.,* 1998) leading to high molecular organic matter, also seems important as a transformation process of COM. The high molecular weight of the water-soluble fraction of mucous macroaggregates was confirmed by size exclusion chromatography (SEC). Macromolecules represent the substrate less available to bacteria, at least for a certain length of time, and so they could concentrate. The isolation of the northern part of the Adriatic Sea with its special physical summer conditions (development of a pronounced pycnocline and, stable summer conditions, etc.) additionally enable the subsequent concentration and agglomeration of macromolecular organic matter (and phytoplankton cells in the presence of mineral particles). The mineral particles and/or ions such as Ca^{2+} and Fe^{3+} seem to be very efficient cross-linkers (Verdugo, 1994; Chin *et al.,* 1998) between marine hydrogels. This was confirmed by laboratory experiments showing that flocculation of gels into macrogels started immediately after the addition of calcite, silicates and $FeCl_3$ from seawater ("supersaturated gel solution") sampled at the beginning of a mucous appearance (Kovac *et al.,* 2002) (Figure 4).

The increased assembly rate of marine gels leads to an increase of POM (Verdugo *et al.,* 2004), i.e. larger particles contribute to clarification of the sea water column which was usually observed before the greater macroscopic mucous appearance. In this case, mucous macroaggregates play an important role in scavenging processes and, further, in the transformation of marine organic matter.

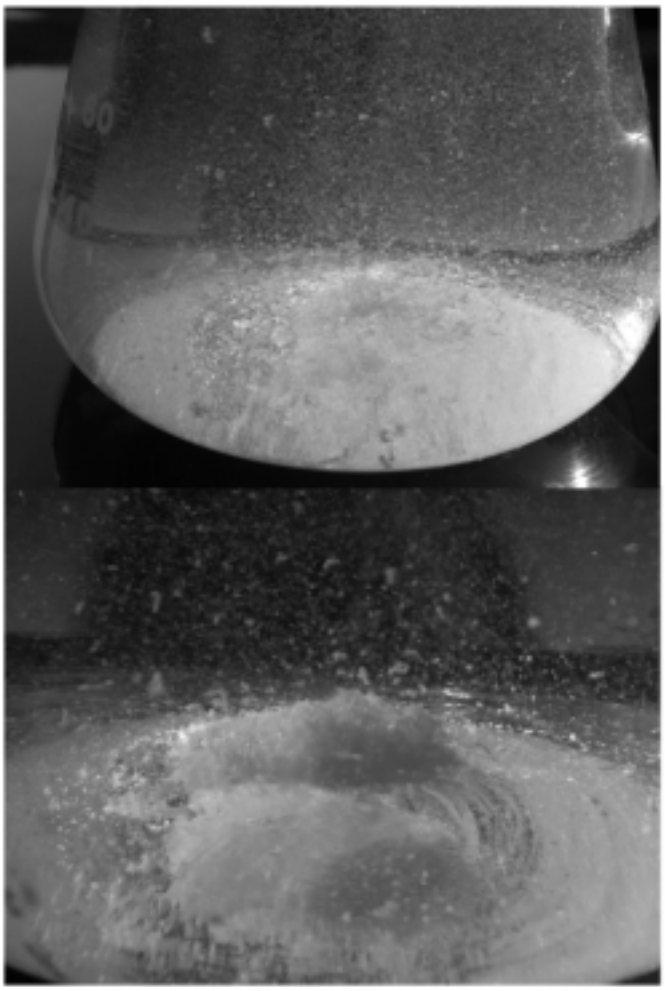

Fig. 4. Flocculation of gels into macrogels after the addition of selected minerals to seawater sample (Kovac *et al.,* 2002).

CONCLUSIONS

According to FT-IR and NMR spectra, macroaggregates in the northern Adriatic, are composed of aliphatic chains and polysaccharides bonded through ester and amide groups and organosilicon compounds. This structure seems similar to that of marine UDOM and UDOM in phytoplankton exudates, suggesting that aggregates are mostly the product of phytoplankton extracellular release (exudation, cell lyses) and the subsequent aggregation of macromolecular DOM and particles (cells, minerals, etc.).

The formation of macroaggregates includes processes which transform macromolecular dissolved organic matter (DOM) into colloidal organic matter (COM) and successively into particulate organic matter (POM). The aggregation and stability of the macrogels of aggregates is most probably enhanced by interaction between organic matter and mineral particles mostly consisting of calcite, quartz and clay minerals.

Bacterial heterotrophic activity in the sea surface microlayer of the coastal Mediterranean Sea

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ABSTRACT

We determined the concentration of dissolved and particulate organic carbon and the abundance of the major members of the microbial community in the sea surface microlayer and the subsurface waters of the coastal Mediterranean Sea. We further compared heterotrophic bacterial activity in the surface microlayer and subsurface waters and we investigated the impact of surface solar radiation on bacterial activity in these layers. Based on these results, we discuss the potential role of bacterioneuston in the transformation of organic matter in the sea surface microlayer.

INTRODUCTION

All exchange of gaseous, liquid and particulate material between the ocean and the atmosphere must pass the sea surface microlayer (SML). It represents therefore an accumulation and weathering point of material from the atmosphere and the water column. Traditionally, the SML has been defined as the uppermost 1-1000 µm (Liss *et al.,* 1997) of aquatic environments, but alternative conceptual models consider the upper 1 m water layer containing a series of sub-layers (Hardy, 1997). The origin of material and its (photo)chemical and biological processing in the SML determine important features such as bioavailability, and thus its impact on marine biogeochemical cycles. Organisms inhabiting the SML (so-called neuston) play an important role, as they are intermediary sources and sinks of organic and inorganic matter in the SML.

Bacterioneuston activity has been a focus of a number of studies; the results, however are largely contradictory. The enrichment of the SML in organic matter could favor heterotrophic bacterial activity while physical properties such as short-term variability in temperature and salinity as well as the exposure to high intensities of solar radiation could retard overall metabolic activity in the SML. *In situ* heterotrophic bacterioneuston activity (measured via radiotracer incorporation) is reported to be enriched (Sieburth *et al.,* 1976; Carlucci *et al.,* 1986), depleted (Bell and Albright, 1982; Bailey *et al.,* 1983) or not substantially different in the SML as compared to the subsurface waters (SSW) (Hermannson and Dahlback, 1983; Garabétian, 1990; Agogué *et al.,* 2004). The balance between growth-promoting and growth-inhibiting processes likely varies among systems and determines whether the SML represents a habitat of enhanced heterotrophic bacterioneuston activity.

We present here several chemical and biological characteristics of the SML in the coastal Mediterranean Sea and we discuss the impact of solar radiation on bacterioneuston activity and the potential role of bacterioneuston in the transformation of organic matter at the air-water interface.

STUDY SITE AND SEA SURFACE MICROLAYER SAMPLING

In July 2003, SML samples were collected at Station SOLA (Station d'Observation Laboratoire Arago), located in the Bay of Banyuls-sur-Mer (NW Mediterranean Sea). Station SOLA is a coastal, shallow (26.5 m) oligotrophic site, with concentrations of ~ 1 µg chlorophyll (chl) *a* l^{-1} in the water column during the sampling period (www.obs-banyuls.fr/sola/sola.html). SML samples were collected between 07:00-09:00 h from a zodiac with a metal screen that collects roughly the upper 250 µm water layer (Garrett, 1965). The subsurface waters (SSW) were sampled by submerging a closed Pyrex flask that was opened at ~ 0.3 m.

Enrichment factors (EF) were determined as the ratio between the concentration or activity in the samples collected by the metal screen (SML) and the subsurface waters (SSW).

CHARACTERISTICS OF THE SML

Overall, the SML revealed consistent, but variable enrichment in organic matter and heterotrophic and autotrophic microorganisms (Table 1). Concentrations of dissolved (DOC) and particulate organic carbon (POC) were, on average 1.3 and 14-fold, respectively, higher in the SML than in the SSW. This resulted in a contribution of POC to total organic carbon of 40% and 6% in the SML and in the SSW, respectively. We observed a small, but consistent enrichment in the SML in heterotrophic bacteria, *Synechococcus* spp., pico- and nanoeukaryotes by factors varying between 1.1-1.7. Heterotrophic nanoflagellates exhibited particularly high EF (on average 2.7). The abundance of particle-attached bacteria was ~ 10-fold higher in the SML (mean 16.8 x 10^5 cells l^{-1}, n = 10) than in the SSW (mean 1.5 x 10^5 cells l^{-1}, n = 10). However, particle-attached bacteria contributed for only 3% and 0.3% of total bacterial abundance in the SML and the SSW, respectively.

Table 1. Concentration of dissolved (DOC) and particulate organic carbon (POC) and abundance of different members of the heterotrophic and autotrophic community in subsurface waters (SSW) with respective enrichment factors (EF) for the surface microlayer (SML). Mean values ± SD are given.

	SSW	Enrichment Factor
DOC (µM)	89±10 (n=6)	1.3±0.2
POC (µM)	5±1 (n=2)	14.0±6
Heterotrophic Bacteria (x 10^9 l^{-1})	0.75±0.2 (n=9)	1.2±0.1
Heterotrophic Nanoflagellates (x 10^6 l^{-1})	0.9±0.6 (n=9)	2.7±1.9
Synechococcus spp. (x 10^7 l^{-1})	3.5±1.7 (n=9)	1.1±0.2
Picoeukaryotes (x 10^6 l^{-1})	1.7±0.5 (n=9)	1.2±0.2
Nanoeukaryotes (x 10^6 l^{-1})	0.6±0.3 (n=9)	1.7±0.5
Bacterial Production (µg C l^{-1} h^{-1})		
Raw seawater	0.23±0.09 (n=4)	0.3±0.1
< 0.8 µm size-fraction	0.10±0.03 (n=4)	0.9±0.6

HETEROTROPHIC BACTERIAL ACTIVITY IN THE SML

Heterotrophic bacterial production (measured as ^{3}H-leucine incorporation) in unfiltered seawater was, on average, 3-fold higher in the SSW (0.076±0.026 µg C $l^{-1}h^{-1}$) than in the SML (0.234±0.088µg C $l^{-1}h^{-1}$) (Table 1). This suggests that bacterioneuston activity was severely inhibited in July in the SML of the Bay of Banyuls. Size-fractionation experiments revealed no differences between bacterial production in the unfiltered and 0.8-µm filtered water from the SML. However, in the SSW, bacterial production in the < 0.8-µm fraction accounted for only 46% of the bacterial production in unfiltered seawater. It was surprising to observe similar bacterial activities in unfiltered seawater from the SML and the < 0.8-µm size fraction. The relatively high concentrations of POC and the high abundances of heterotrophic nanoflagellates in the SML suggest an important contribution of these parameters to the functioning of the microbial food web. One could, for example, expect a higher contribution of particle-attached bacteria in the SML. Bacterial abundance in the < 0.8-µm fraction, however, accounted for 90% of the bacterial abundance in unfiltered seawater in the SML and the SSW. Our results from the size-fractionation experiment thus indicate that interactions among heterotrophic bacteria and other components of the microbial food web are more important in the SSW, resulting in pronounced differences in bacterial activity in unfiltered seawater and the < 0.8 µm size fraction. We conclude that primarily environmental factors, such as high intensities of solar radiation, account for the observed low bacterial activity in the SML.

IMPACT OF SOLAR RADIATION ON BACTERIAL ACTIVITY

In a set of experiments, we investigated the impact of surface solar radiation on heterotrophic bacteria from the SML and the SSW. We therefore performed 24h-surface incubations of unfiltered seawater from the SML and the SSW, starting off with an 8h light period (see Figure 1). Changes in bacterial activity were followed throughout the incubation period.

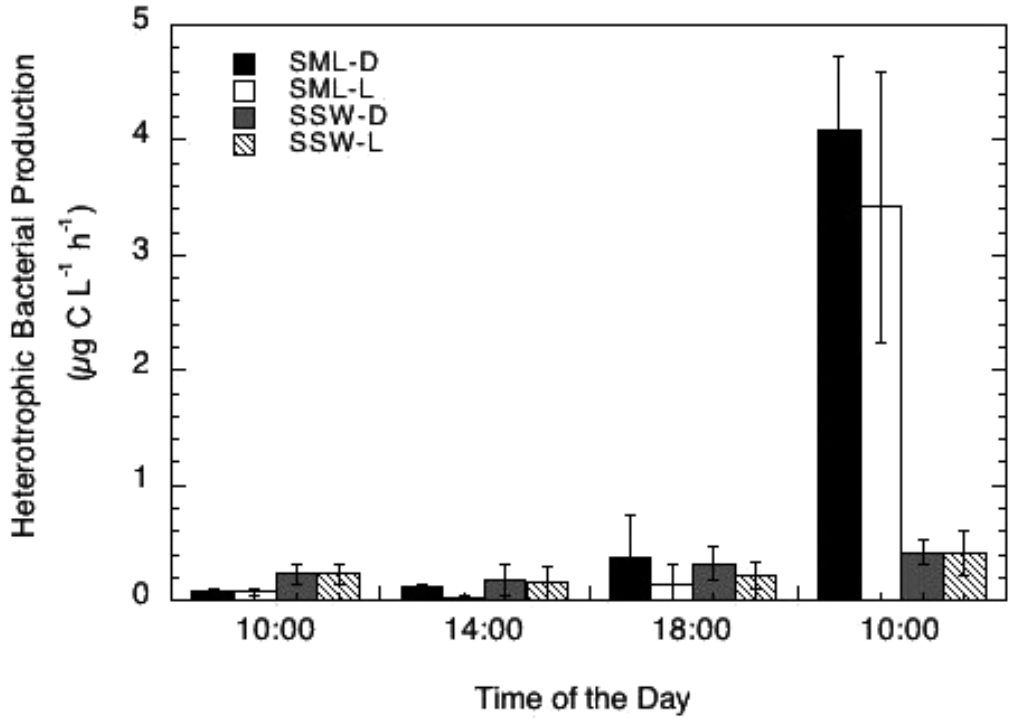

Fig. 1. Changes in heterotrophic bacterial production in unfiltered seawater from the sea-surface microlayer (SML) and subsurface waters (SSW) exposed to solar radiation (L) or kept in the dark (D). Error bars represent the SD of 4 replicate experiments performed on different days in June 2003.

Exposure of seawater resulted in a substantial decrease in heterotrophic bacterial production and this was more pronounced for bacterioneuston (by 73% as compared to the dark control) than for bacterioplankton (by 34% as compared to the dark control) (Figure 1). During the subsequent dark incubation, however, bacterioneuston activity increased by a factor of 65, while the increase in bacterioplankton activity remained relatively low (1.6-fold; Figure 1).

These results suggest that bacterioneuston are more sensitive to solar radiation than bacterioplankton. Furthermore for both bacterioneuston and bacterioplankton we have observed rapid recovery from solar radiation induced stress. In the case of the SML, the pronounced increase in bacterial production during the dark period most likely reflects a response to the substantially higher concentrations of organic carbon. This results from the incubation experiment can only be considered as a potential of organic matter transformation by bacterioneuston as it contradicts our *in situ* measurements of bacterioneuston activity. Combining the results from *in situ* measurements and incubation experiments performed at different times of the year will help elucidate the role of heterotrophic bacteria in organic matter transformation in the SML.

Ultra-violet radiation may affect bacterial cycling of dissolved organic matter in the Mediterranean Sea: a case study, a review.

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Dissolved organic matter in the Ocean

It is currently believed by marine biogeochemists that dissolved organic carbon (DOC) which is the main reservoir of organic carbon in the Ocean is made up of several components including refractory material with turnover times of millennia, semi-labile material with turnover times of months to years and labile material with turnover times of hours to days (Sempéré *et al.,* 2003; Hansell *et al.,* 1995). In oceanic waters, DOC which accounts for most of the total organic carbon (TOC) stock is the result of the imbalance between the inputs and outputs, of *in situ* biological production and degradation processes including bacterial consumption, particle adsorption and photomineralization (Hansell, 2001; Tedetti and Sempéré, unpubl.; and references therein). Previous studies in the Mediterranean basin have shown that the main external source of organic carbon was Atlantic water spreading through the Gibraltar Strait (Copin-Montégut, 1993; Dafner *et al.,* 2001a, c), this is followed by the contribution by rivers (Ludwig and Probst., 1998; Sempéré *et al.,* 2000), atmospheric deposition (Loÿe-Pillot *et al.,* 1992) and finally the Black Sea (Polat and Tugrul, 1996; Sempéré *et al.,* 2002). However, there are no reports dealing on a global scale with the mineralization of DOC by bacteria. Such a study could help estimate the quantity of CO2 produced through bacterial respiration and lead to a better understanding of the carbon cycle in the Mediterranean Sea.

UV radiation on the Earth's surface

Solar radiation comprises a wide wavelength spectrum that includes UV radiation (UVR 200-400 nm), photosynthetically available radiation (PAR 400-700 nm), and infrared (IR 700-1000 nm). UVR that reaches the Earth's surface is arbitrarily divided into UV-B radiation (UVBR 280-315 nm) and UV-A radiation (UVAR 315-400 nm). UVBR is efficiently absorbed by stratospheric ozone and therefore represents a small amount of UVR reaching the Earth's surface (5%) compared to UVAR (95%). Both UVBR and UVAR are affected by the solar zenith angle (SZA) that leads to their strong dependence on latitude, season and local time. They are also affected by cloud cover, surface albedo (snow cover and sea ice), aerosols, altitude and pollution (tropospheric ozone, sulfur and nitrogen dioxides, volatile organic compounds and carbon monoxide).

The decline of the stratospheric ozone layer (due to the release of CFCs into the atmosphere; Molina and Rowland, 1974) observed over the Antarctic continent (Hofmann *et al.,* 1994) and more recently over the Arctic and mid-latitudes of both hemispheres during various periods of the

year (McKenzie *et al.,* 1999), has enabled the UVBR at the Earth's surface to increase (Herman *et al.,* 1996). Additionally, there is evidence that UVR may be affected, both positively and negatively, by the effects of climate change (Moran and Zepp, 2000; Kerr *et al.,* 2000). These effects are twofold: the first "indirect" effect results from global warming that influences the total ozone and consequently affects UVBR. It includes increase in greenhouse gases, cooling of stratosphere, decrease in atmospheric chlorine and bromide compounds, increase in atmospheric circulation and increase in aircraft activity. The second "direct" effect is the result of climate changes that affect UVBR and UVAR. It includes variation in cloudiness, increase in tropospheric extinctions, decrease in surface albedo and increase in aviation activity. In turn, increasing UVR can also influence global warming by variations in the photochemical production of ozone and in the sea-air exchange of gases. For example, during summer in the urban region of the Mediterranean Basin, the intensity of UVBR could influence the appearance of ozone photochemical pollution peaks. These variations of UVR levels at the Earth's surface induced by global warming/ozone depletion have been shown to affect aquatic ecosystems (Häder *et al.,* 1998).

UV IMPACT ON MARINE BACTERIA

In the marine environment, UVR can have important effects on bacterial activities, phytoplankton photosynthesis and photochemical transformations of dissolved organic matter (DOM). In the oceanic surface layer, the DNA is one of the most prominent targets for solar UVR-induced damage in a variety of organisms including bacteria, cyanobacteria and phytoplankton (Häder and Sinha, 2005). UVR exposure of DNA causes lesions that alter its configuration. The two major UVBR-induced DNA lesions are cyclobutane pyrimidine dimers (CPDs), mainly thymine dimers (TT) and pyrimidine pyrimidone photoproducts (6-4PPs) and their Dewar valence isomers (Sinha and Häder, 2002). Many studies have reported the inhibition of bacterial activities in different marine ecosystems after exposure to UVBR through the production of CPDs (Jeffrey *et al.,* 1996; Kaiser and Herndl, 1997; Buma *et al.,* 2003). Bacteria are usually too small to effectively protect themselves against solar UVR by absorbing substances. As a result, they are more susceptible to UVBR damage and may serve as a more sensitive indicator of UV stress than other organisms.

UV IMPACT ON DISSOLVED ORGANIC MATTER (DOM) CYCLING BY BACTERIA

The attenuation of light into the water column is controlled by two physical processes: scattering and absorption, which are dependent on the optical properties of seawater. Chromophoric (or colored) dissolved organic matter (CDOM) is the major contributor to the attenuation of UVR in oceanic water (Moran *et al.,* 2000; Mopper and Kieber, 2002; Obernosterer and Benner, 2004). The absorption coefficient for CDOM increases exponentially with decreasing wavelength (Blough *et al.,* 1993). The absorption of UVR by CDOM affects its structure through photolysis and photobleaching. Recent studies have shown that UVR exposure of DOM originating from systems dominated by terrestrial-derived inputs leads to an increase of its bacterial utilization (Moran *et al.,* 2000; Obernosterer and Benner, 2004). This positive effect on DOM bioreactivity has been largely attributed to the photoproduction of biologically labile low molecular weight (LMW) organic compounds including carboxylic acids, aldehydes and ketones (Mopper *et al.,* 1991; Moran and Zepp, 1997; Pullin *et al.,* 2004). In contrast, UVR exposure of DOM originating from systems dominated by phytoplankton-derived inputs leads to a decrease of its bacterial utilization (Obernosterer *et al.,* 1999; Tranvik and Bertilsson, 2001). This negative effect on DOM bioreactivity can be explained by the phototransformation of bioreactive to biorefractory substrates. Photochemical degradation of DOM can also lead to the production of CO and CO_2 (Miller and Moran, 1997; Goldstone *et al.,* 2002) removing organic carbon from the pool of potentially bioavailable carbon compounds.

The concentration of CDOM and the mechanisms that influence its abundance (e.g. seasonal changes in the transparency; Kuwahara *et al.,* 2000; Dring *et al.,* 2001) are likely to change in the next few decades due to global warming. Consequently, these modifications will have an important impact on the penetration of UVR and its subsequent related effects (Hessen *et al.,* 2001). According to Häder *et al.* (2003), global warming may initiate the increased penetration of UVBR and UVAR into aquatic environments, predominantly through a decrease in the

attenuation of radiation by DOM. In addition, the impact of UVR could be modified in the next years by variations in the depth and the rate of mixing layer (Diaz *et al*., 2000; Huot *et al*., 2000).

Here we report preliminary results related to the UVECO programme (http://www.com.univ-mrs.fr/LMGEM/uveco/; Pis F. Joux and R. Sempéré) which evaluates the effect of UVR on microbial communities, on OH. radical production, and on bacterial cycling of specific marine dissolved organic matter (DOM) including dicarboxylic acids and polysaccharides in Mediterranean Sea which is referenced as a low nutrient and a low chlorophyll (LNLC) system. Our first results indicate that, in the summer season, the sunlight exposure of 0.2 µm filtrated surface coastal seawater with addition of CDOM and/or NO_3^- increases the production of OH. radicals and monosaccharides including sucrose and glucose (Figure 1).

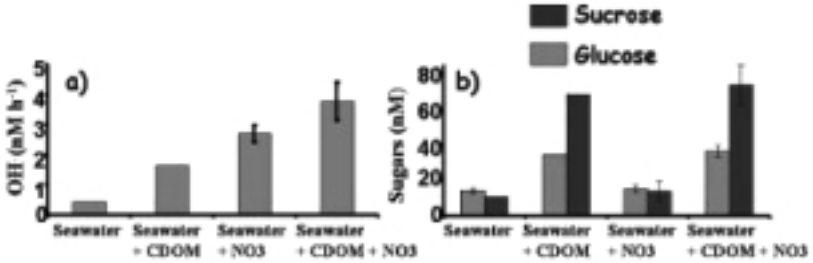

Fig. 1. (**a**) Production of OH. (nM h^{-1}) and (**b**) concentration of dissolved sugars (glucose and sucrose) (nM) after 12 h exposition of 0.2 µm filtered seawater from the SOLA station in Banyuls-sur-mer under solar simulator (ORIEL 1000 W; 60 % FS) in June 2004.

The mixing of this solution with an unexposed-bacterial assemblage (0.8 µm filtrated surface coastal seawater) induced an increase of bacterial production in the final solution in early spring. This phenomenon is significantly favoured with full sun radiation (including visible, UV-A and UV-B radiations) exposure (compared to visible, and visible + UV-A radiations exposures) and when NO_3^- is added to the solution before exposure to the sunlight. On the other hand, and slightly after Spring bloom, though full sun exposure induced still an increase of OH. production, the results showed rather a production saccharose and a decrease of the bacterial production compared to visible and visible + UV-A radiations exposure (Figure 2). Therefore, the results indicate possible major UV-B effects on the molecular distribution of surface DOM and subsequently on its cycling by marine bacteria. This effect seasonally varies in relation with the OH. photoproduction from NO_3^- and also very likely with the degree of freshness of dissolved organic matter.

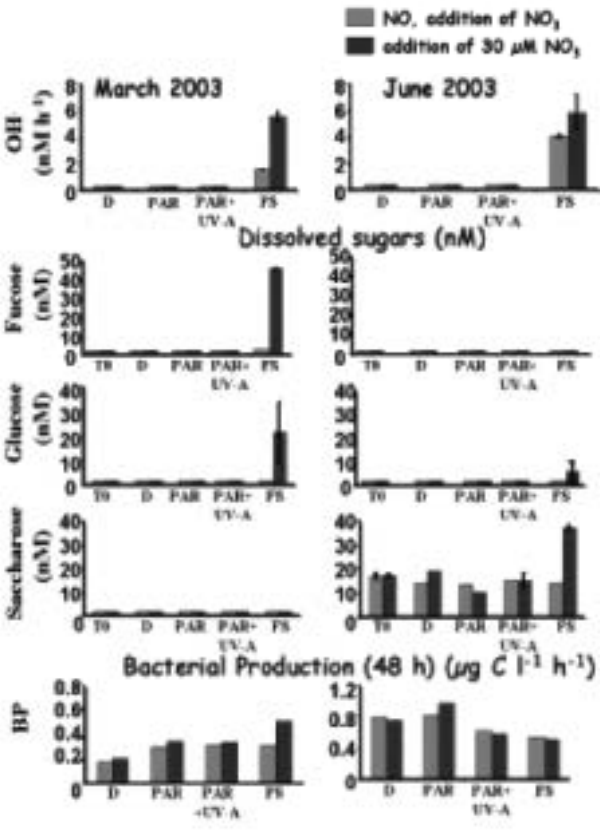

Fig. 2. Production of OH (nM h^{-1}), concentration of fucose, glucose and sucrose (nM) after natural sunlight exposure of 0.2 µm- filtered seawater. Bacterial Production [BP (µg C l^{-1} h^{-1})] after mixing of 0.2 µm- filtered seawater exposed to natural sunlight with bacterial innoculum (0.8 µm- filtered seawater). Experiments were undertaken with and without addition of NO_3^- in March and June 2003. Doses were measured with the ELDONET radiometer on 25 March and 27 June 2003 and were 7377, 1067, 17.5 and 8322, 1366, 30.1 kJ m^{-2} for PAR, UV-A, and UV-B, respectively.

Struggling with the ill-defined notions of stability, resistance and lability

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ABSTRACT

The paper examines the questions of the persistence of dissolved organic material in the oceans. It lays down a basic separation of the reason for persistence into two categories. 1) *Stability* – i.e. will not break down, which in principle is an absolute property and an innate property of the molecule. 2) *Resistance* – not inclined to breakdown. Both may be further divided into two subcategories, respectively stability into *thermodynamic and molecular* stability; resistance into *inbuilt resistance* and *circumstantial resistance*.

Stability: we presently have no molecular basis nor broad theory to understand why organic material persists in the oceans, although it is suggested that it may be profitable to explore the possibility of well-established xenophoric substitutions.

Resistance: physicochemical calculations suggest that there is no evidence for either external thermodynamic or kinetic blocks. Their dilution would not stop the assimilation of dissolved organic material either due to the energy required for uptake organic compounds or their rate of arrival at the cell surface. There are stoichiometric constraints to assimilation – organic substrates with no nitrogen will call for the organism to assimilate inorganic nitrogen, putting them in competition with the photosynthetic algae. Stoichiometric constraints give rise to a trophic basis to persistence.

The deep water profiles DOC and properties (concentration and age) cannot be described by single exponential models. They can be satisfactorily reproduced by double exponential models; however the rate constants to account for the observed deep water DOC age call for turnover times so long (decades) that the models are thought not to be realistic. The conclusion is that there must be some form of discontinuous decomposition.

INTRODUCTION

It is conventional to pay greatest attention to the rates of removal of organic material as they set the rate at which biogeochemical cycles in fact spin round – thus the widespread interest in measuring lability and the rates of decomposition. There is a strong argument that runs: what persists has greater impact on the ecology, as it sets the size of pools, the export from one pool to the other, the timescale of cycling within the ecosystem and the broad structure of the biochemical cycles themselves. It is also the property we can easily measure. Figure 1, taken

from Lenton and Watson (2000), shows the flows and feedbacks; negative feedbacks, which are essential to the stabilisation of an ecosystem, are shown as dotted lines; characteristically are associated with the accumulation of material.

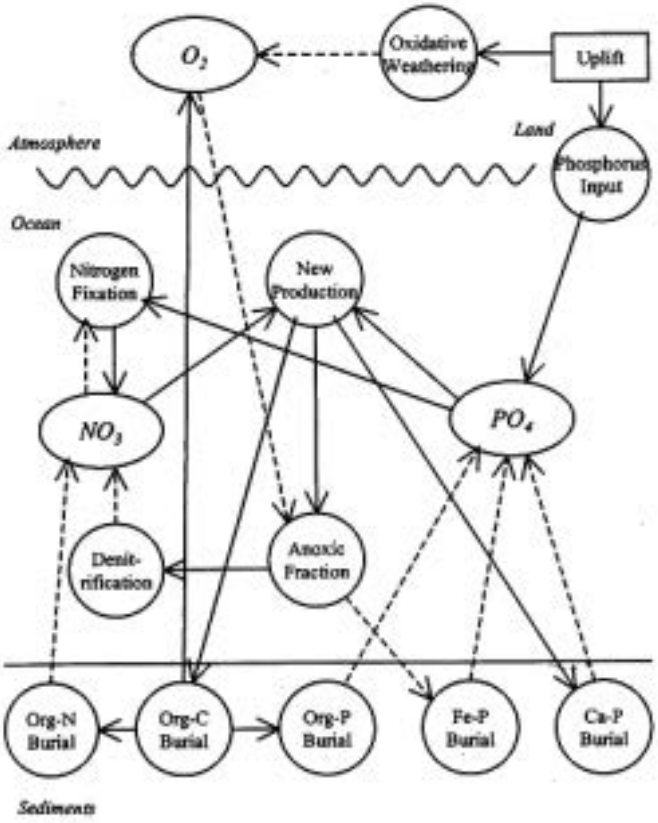

Fig. 1. Flows and controls within the oceanic biogeochemical cycle (Lenton and Watson, 2000).

The terms "labile", "semi-labile" and "resistant" are purely empirical and provide no insight into the mechanism giving rise to these properties. It is far easier to address stability than lability. In the first part of this paper, I will explore the proposal that we learn more by asking why an organic compound **is not** broken down than why **it is**.

A CONCEPTUAL BASIS FOR PERSISTENCE

The following is an extension of the discussion in Williams (2000) with additions and clarifications.

It may be useful to recognise two bases for persistence:

Stability – i.e. will not break down. In principle this is an absolute property and an innate property of the molecule. This may be further divided into two subcategories:

- thermodynamic;
- molecular.

Resistance – not inclined to breakdown. This also may be divided into two subcategories:

- inbuilt resistance, a property of the molecule;
- circumstantial resistance, an ephemeral property set by the prevailing circumstances.

Stability

Thermodynamic stability: this is associated with the energy released by the decomposition of the molecule – the Gibbs free energy. As the Gibbs free energy is determined by the reaction, the reactants, other than the molecule, play a role. Thus a grey area exists between this and

circumstantial resistance. It is axiomatic that whereas thermodynamics can predict whether or not a molecule will persist in a particular circumstance it can give no insight into the rate of decomposition: thermodynamics cannot predict kinetics.

In a way this is illustrated in Figure 2a, where a set of molecules have been placed in rank order of their reactivity, and then matched against the free energy of their oxidation. If anything, there is an anti-correlation, certainly no correlation.

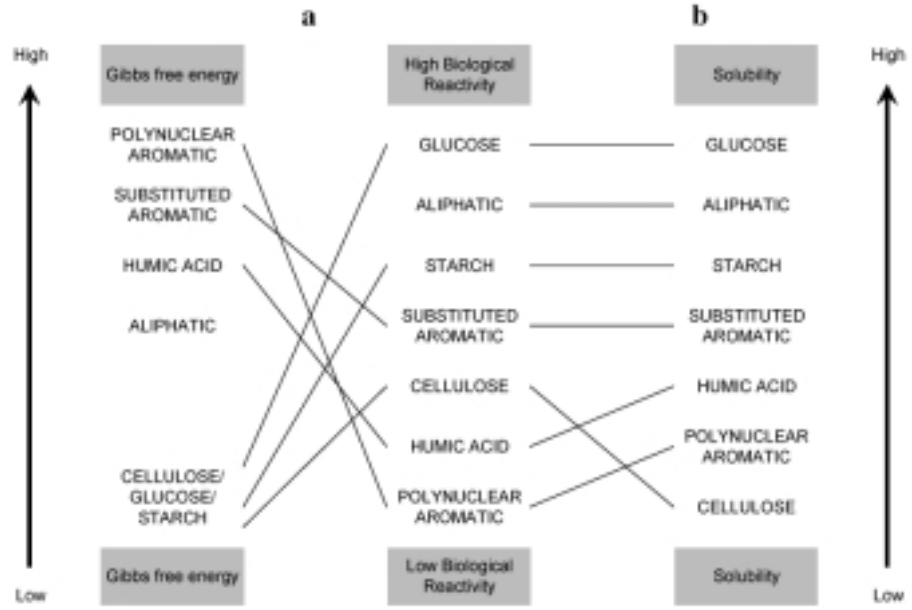

Fig. 2. **a**) Microbiological reactivity and Gibbs free energy, ranked for various organic molecules; **b**) with the addition of solubility.

Molecular stability: the history of foaming problems in water treatment works associated with the early synthetic detergents made from branched hydrocarbons led to an understanding that, for steric reasons, certain molecular structures were resistant to microbial decomposition and the concept of xenophoric structures evolved. In simple terms, there are a variety of substitutions into a linear carbon chain that enhance resistance to microbial decomposition – notably the tertiary carbon structure and the elements Cl, Br, nitrate and sulphate, interestingly all molecules present in seawater (see Figure 3). It is not clear whether this confers stability or simplicity enhances resistance. In principle it ought to be an all or nothing effect unless there is some degree of tautomerism which might release the steric hindrance. There is an interesting asymmetry here between chemical and microbial reactivity: whereas the substitution of these groups confers resistance to microbial decomposition, they increase the chemical reactivity. This phenomenon may have important consequences on the decomposition of the more resistant components of marine DOC.

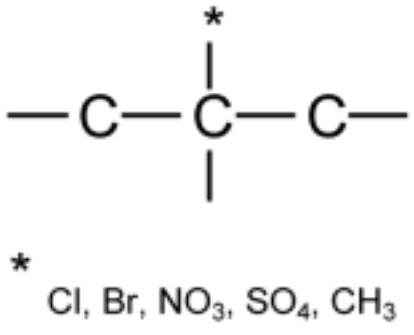

Fig. 3. Molecular substitutions conferring recalcitrance.

It is axiomatic that all biologically produced molecules are susceptible to biological decomposition. If such compounds occur naturally in the environment (and natural chlorocarbons are produced in the sea) then either the axiom cannot be sustained or there must be chemical production. The matter could be very subtle, in that biologically produced molecules could subsequently undergo chemical reactions giving rise to xenophoric structures. There is no reason why such reactions could not occur very rapidly, indeed could occur within the cell.

Resistance

Inbuilt Resistance: we are aware that certain molecules tend to persist, where others are prone to rapid decomposition. Glucose would be an example of the latter, whilst its polymer – cellulose – would be an example of the former. This example in isolation could lead to the conclusion that molecular weight could be a main determinant of resistance. But as soon as we introduce the second polymer of glucose – starch – this explanation collapses. Over everything but very short timescales, starch is every bit as readily decomposable as glucose. Figure 2b, shows the solubility generally correlated with reactivity and in the case of glucose and its polymers it may be a major determinant – but it cannot be a universal explanation. Indeed one would be unwise to search for single universal controls on reactivity.

Circumstantial Resistance: It is becoming realised that the reactivity of molecules may vary with the environmental circumstances. Figure 4 illustrates a simple case. Replicate mesocosms were dosed with glucose, in one case (the upper graph) the dosing started on day 5 and continued to the end of the study, in the second case (the lower graph) dosing was deferred until day 15. In the first case (Figure 4a) the glucose (strictly hexoses) accumulated, essentially at the rate of addition, for 6 days, after which it started to be removed. In the second instance (Figure 4b), when the glucose addition was deferred until day 15, glucose never accumulated; it appeared to be assimilated immediately. The study clearly shows that there are features other than the properties of the molecule itself that determine it persistence, and in a given environment they may change with time.

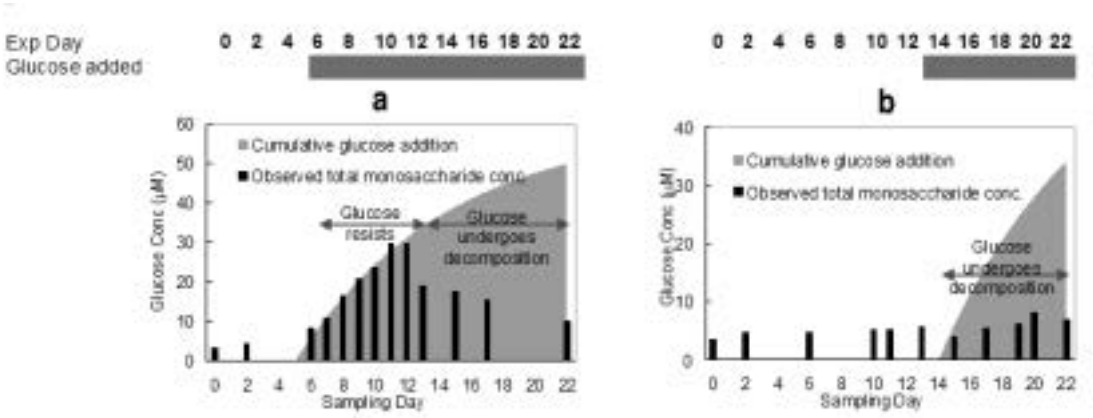

Fig. 4. Mesocosm study in which glucose addition was initiated in replicate enclosures after 5 days (**a**) and 15 days (**b**). The vertical bars represent the observed hexose concentrations, the shaded areas the calculated cumulative addition.

There are four circumstantial mechanisms that may effect control on reactivity:

- thermodynamic mechanisms;
- kinetic mechanisms;
- stoichiometric mechanisms;
- trophic mechanisms.

Two purely physicochemical causes for persistence have been raised on occasions: that taking up the substrate is not economic as it requires more energy to take it up than is gained from its subsequent respiration *(thermodynamic mechanisms)* or that the concentrations in seawater

become so low that their rate of arrival at the cell surface is too slow to sustain some critical rate of growth *(kinetic mechanisms)*. These propositions can be analysed and neither turn out to have any likely basis. They are introduced here mainly to eliminate them.

Thermodynamic constraint to acquisition: work has to be done to take up a molecule against a diffusion gradient and the energy for this work has to be acquired from the respiration of the molecule. This is a different issue to the work for the transport of the molecule across the membrane. The calculation (Box 1) has as much entertainment as scientific value – the outcome of the calculation is that the work required to concentrate material against a concentration gradient is disappearing small.

Box 1. Thermodynamic constraints to acquisition

Problem: at what concentration does it become uneconomical to take up organic compounds; i.e. when does the energy required to concentrate the material exceed that obtained from its respiration?

This can be calculated by reversing the Heat of Dilution calculation

Heat of Dilution:- $\Delta F = RT \ln (C_1/C_2)$; when $-\Delta F = -\Delta G$, then there is no surplus energy
$-\Delta G$ for the oxidation of glucose is 2870 kJ/mole

Then: $2870 \times 10^3 = 8.3 \times 290 \times 2.303 \times \log(C_1/C_2) = 5.5 \times 10^3 \times \log(C_1/C_2)$
$\log(C_1/C_2) = 2870 \times 10^3 / 5.5 \times 10^3 = 517$

Thus : $(C_1/C_2) = 10^{517}$; if C_1 = 10-4 M, then $C_2 = 10^{-521}$ M

Take Avogadro's number as 6×10^{23} and the oceans as 10^{21} litres, then one molecule in the whole of the oceans would have a concentration of $\sim 10^{-45}$ M

Thus it would be economical to acquire one molecule of glucose from 10^{476} oceans

Kinetic constraint to acquisition: the calculation (given in Box 2) poses the question "at what concentration does the arrival of molecules limit growth"? The calculation, other than the required arrival rate at the surface, is fairly routine; the other major uncertainty is the fraction of successful collisions. I have taken a cautious value of 1 in 1000; given this, concentrations would need to fall to 10^{-11} molar before they would limit growth. Presently our analytical methods for hexoses and free amino acids are probably limited to concentrations no lower that 10^{-8} molar. Thus in principle if we can detect a molecule to be present it will be arriving at a sufficient frequency to sustain microbial growth.

Box 2. Kinetic constraint to acquisition

Problem: do molecules arrive at a sufficient rate to sustain metabolism?

Consider bacteria at a concentration of 12 µgC (i.e. 1 µM) /dm3, growing at 1 div/day with a growth efficiency of 25%

This would require $1 \times 4 \times 10^{-6} \times 6 \times 10^{23}$ molecules /day = 24×10^{15} molecules/day = 2.78×10^{13} per second

The bacterial surface area in the sea is approximately $1 dm^2/dm^3$

The calculated **minimum** arrival must be **3×10^{13} molecules/sec.cm^2**

Such a collision rate would be sustained by 10^{10} molecules/dm^3; i.e. **$\sim 10^{-14}$M**

This step in the calculation incurs the greatest uncertainty in the overall calculation one that physical chemists are very reluctant to make it

Assume the successful collision was 1 in 10^3 then the **minimum** concentration of the food substrate would need to be **10^{-11}M,** i.e. 10510 times greater than the calculated thermodynamic constraint (see Box 3) but not a long way from the **10^{-8} M observed**

Stoichiometric mechanisms: if bacteria are using exclusively organic material for growth then they must obtain their cell nitrogen from their organic substrate. The maximum C/N ratio of an organic substrate that can by itself support growth is calculated in Box 3. The outcome of the calculation is interesting when the organic nitrogen containing compound has a C/N ratio near 18. Assimilation of organic compounds with C/N ratios above 18 will leave the cell N-deficient, ratios below 18 will result in inorganic nitrogen excretion. C/N ratios of nitrogen-containing biochemicals vary from 2-9 (e.g. glycine and tyrosine) then infinity (e.g. glucose). Thus, no single biochemical has a C/N ratio in the range >9 to infinity! If there are nitrogen containing organic compounds available to the organism, then it may assimilate some non-nitrogen organics (that is why the exact value for Y_C is not critical in the argument in Box 1). If no nitrogen containing compounds are available then the micro-organism needs to resort to assimilating inorganic nitrogen, typically ammonia as a nitrogen source. (Some micro-organisms can use nitrate also.) If there is no inorganic nitrogen available then non-nitrogenous organics will accumulate. This, however, is not thought to be the basis for the accumulation of glucose seen in Figure 4.

Box 3. Stoichiometric argument

The bacterial C/N quota is low (~ 4.5) and has to be met.
The quotient of the Cell Quota ($Q_{C/N}$) and the Carbon Growth Yield (Y_C)

i.e. $(Q_{C/N})/(Y_C)$
gives the maximum C/N of a satisfying substrate

Assume $Q_{C/N} = 4.5$
and $Y_C = 0.25$ (below $Y_C = 0.5$, (the exact value used is not critical)

Then maximum C/N substrate = 4.5/0.25 = 18

Substrates with higher C/N ratios can only be assimilated, with the associated assimilation of inorganic nitrogen

In the euphotic zone, assimilation of inorganic nitrogen brings the bacteria into competition with the photosynthetic organisms. Thus, stoichiometric control can lead to one form of trophic control.

Trophic mechanisms: this area has been explored by Thingstadt and the following (Figures 5a and 5b) are pictorial account of his proposals. A simple plankton food web, comprising two size groups of algae (the autotrophic nanoflagellates and the diatoms), two of protozoa: the herbivorous microzooplankton (the ciliates) and the heterotrophic nanoflagellates; and at the top of the food web the mesozooplankton (eg the copepods) and the bacteria at the bottom. All but the mesozooplankton organisms have similar growth rates. The bacteria are predated upon by the heterotrophic nanoflagellates and when using non-nitrogenous organic substrates they compete with the diatoms but mainly the autotrophic nanoflagellates for inorganic nitrogen – typically ammonia.

Top down control, by the mesozooplankton can be seen to determine the success of their attempt to assimilate non-nitrogenous compounds. The dynamics are shown pictorially in Figures 5a and b.

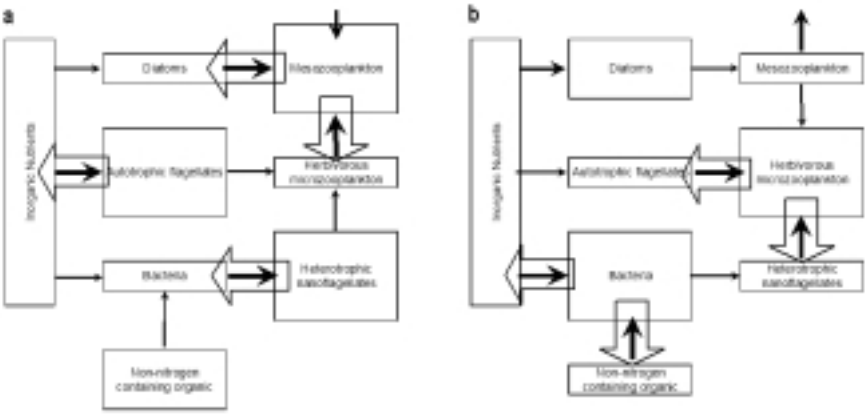

Fig. 5. The flow during the second two phases of the mesocosm study shown in Fig. 4. The solid arrows indicate the scale of flow, the open arrows indicate major sites of top down control.

The sequence 5a to 5b is hypothesised to be the basis of the phases of glucose non-lability and lability shown respectively in Figures 4a and 4b.

CONCEPTUAL MODELS OF OCEANIC DOC PROFILES

In this second section, also largely drawn from Williams (2000), I look at the fundamental structure of models used to describe DOC persistence through the water column. The main additions to the earlier account is the discussion of the combined modelling of age and concentration and more detail on the modelling of the participation of photochemistry in decomposition.

Various modes of decomposition were considered by Williams (20000):

- continuous decomposition;
- discontinuous decomposition, this may be further divided into:
  - periodic decomposition;
  - intermittent decomposition.

Continuous decomposition

Early water quality work used first order models of decomposition. It is easy, using "depth for time" models, to write equations that give very adequate representations of DOC profiles. (see Figure 6). However, there is a second constraint that any deep water DOC model must comply with – the observed age of the DOC.

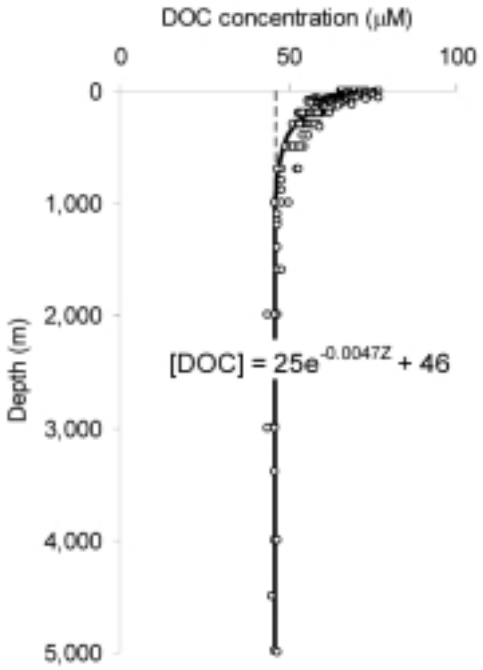

Fig. 6. A simple one exponent model for the vertical distribution of DOC with depth.

When exploring this problem in models, one finds that a doubling or halving of the rate constant gives a roughly comparable change in the modelled age. This means that, unless the rate constant of decomposition is essentially a fixed property, then the age cannot also be a constant property. The uncomfortable aspect of these types of continuous models is that they inevitably require exceedingly low decomposition rates – the half-life of the organic material must be comparable to its age (6,000 years). It is difficult to envisage bacterial metabolic or growth rates this slow: turnover times of a decade or so, but as it is a volumetric rate, not a per cell rate this could be produced by low bacterial biomasses. However, they would need to be some 10^4 less than numbers at the surface and this is simply not observed.

Periodic decomposition: this dilemma is circumvented if decomposition is periodically switched on and off. Among various switches discussed by Williams (2000), the only one that stood up to close analysis was the switching on and off of photochemical reactions. This type of model of decomposition has built into it conceptually the notion of recalcitrant molecules (i.e. xenophoric structures) that are susceptible only to photochemical attack. Anderson and Williams (1999) worked with a model of this type (Figure 7) and were able to produce profiles of DOC concentration that matched the classical profiles. Furthermore, with no fitting of constants, they obtained an age of 2300 years for the deepwater DOC.

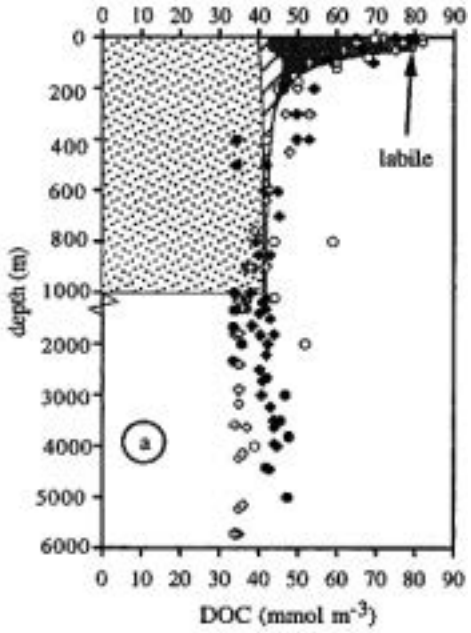

Fig. 7. The structure of a model that incorporates both bacterial and photochemical decomposition.

Intermittent decomposition: the above model would fit the old Ryther and Menzel (1968) model of refractory deepwater DOC, but would not account for the more recent observations of Hansen and Carlson (1998). Hansen and Carlson observed a fall in DOC concentration from 48 mmol/m^3 to 34 mmol/m^3 over the time (the order 1000 years) that deep water took to travel the N. Atlantic to the N. Pacific. This takes us back to the problem raised earlier – the ultra slow decomposition rate: in this case a turnover time of bacteria of seven years is required. Thus, again some form of intermittency in decomposition needs to be occurring. Whether it takes the form of decomposition associated with descending particles (see Williams, 2000) or some other mechanism is not known.

CONCLUSIONS

1. We presently have no molecular basis to understand why organic material persists in the oceans;
2. It may be profitable to explore the possibility of well-established xenophoric substitutions;
3. Stoichiometric constraints give rise to a trophic basis to persistence;
4. The concentration of individual organics may affect the rate of uptake but not give rise to persistence;
5. Single exponential models cannot describe deep water DOC properties;
6. Dual exponent models can, but require very slow (?unacceptable) bacterial turnovers;
7. Periodic decomposition on ocean circulation timescales can explain profiles;
8. Intermittent decomposition on particles, in principle, can explain much of what we know.