A PRIMER ON DISSOLVED ORGANIC MATERIAL AND HETEROTROPHIC PROKARYOTES IN THE OCEANS

DAVID L. KIRCHMAN
College of Marine Studies, University of Delaware, Lewes, DE 19958, USA
kirchman@udel.edu

INTRODUCTION

Dissolved organic material (DOM) and microbes are well-recognized to be important components of carbon cycles and other biogeochemical cycles in the oceans. The main component of DOM, dissolved organic carbon (DOC), is one of the largest pools of carbon in the biosphere, equaling atmospheric CO_2 in size (ca. 700 X 10^{15} tons) (Hedges and Oades 1997). The flux of carbon through the DOC pool is also substantial. Various approaches indicate that about 50% of primary production is some how routed through bacteria and single-cell eukaryotes (protists) that graze on bacteria, i.e. the microbial loop. Mineralization of DOM to CO_2 and other inorganic nutrients by microbial loop microbes is one of three fates for primary production, the other two being grazing by protists and macrozooplankton (Fig. 1). In the open oceans, most of the carbon produced by phytoplankton (primary production) is mineralized by protist grazers or the microbial loop, leaving <10% for export to the deep ocean via sinking of large phytoplankton, the waste products (fecal pellets) of large zooplankton, or amorphorous aggregates of unclear origin. Carbon can also be exported as DOC in some oceanic regimes, as discussed below.

This chapter attempts to review the basics of DOM and the microbial processes impacting DOM in the oceans. Both topics are thoroughly reviewed by Hansell and Carlson (2002) and Kirchman (2000) who discuss

M. Follows and T. Oguz (eds.), The Ocean Carbon Cycle and Climate, 31–63.

DOM and microbes, respectively, in much more detail than possible here. The goal of this chapter is to introduce some aspects of DOM and microbes that may be useful for those interested in examining the role of DOC-related processes in global carbon cycles.

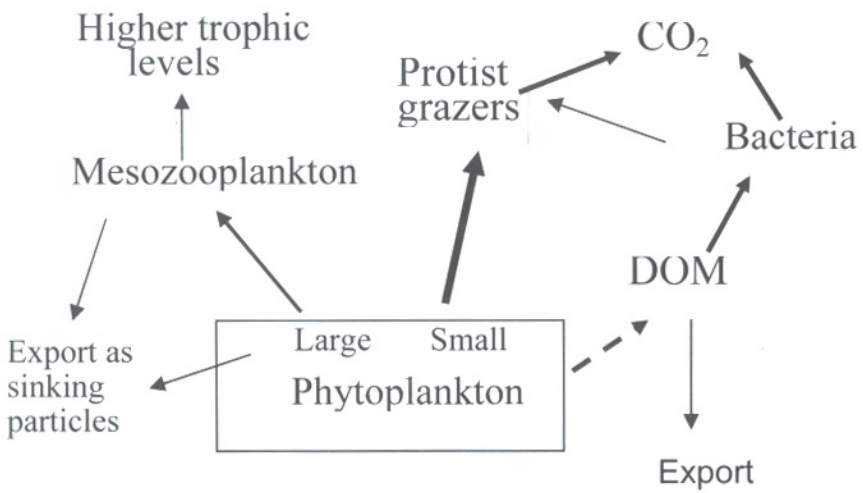

Figure 1. The role of DOM and the microbial loop (DOM–>bacteria and archaea–> grazers) in biogeochemical cycles. The relative size of the flux is indicated by the thickness of the arrow. The arrow for DOM production is dashed because many organisms in addition to phytoplankton produce DOM.

1. WHAT IS DOM?

The DOM pool is operationally defined as any organic material that passes through a GF/F filter which has a nominal pore size of about 0.7 μm. Consequently, "DOM" actually includes small bacteria and archaea, viruses, and detrital particles that are far from being dissolved. Fortunately, particulate carbon in the DOM fraction is usually not large compared to truly dissolved material even if the border between dissolved and particulate can be murky. In fact, DOC concentrations are usually much higher than particulate organic carbon (POC) concentrations, so that sometimes "total organic carbon" (TOC) is measured to avoid contamination caused by filtration. Even ignoring things such as colloids (Wells 2002), the DOM pool is complex and consists of many compounds that differ greatly in structure, molecular size, C:N ratio, and susceptibility to degradation by

microbes, i.e. just about every parameter of importance to understanding its role in biogeochemical processes. To make matters worse, DOM is found in a complex medium, seawater. Because of many analytical difficulties, we know little about the chemical identity of DOM at the molecular level. About 10% of DOM can be identified as amino acids (proteins) and neutral sugars (e.g. glucose) in polysaccharides (reviewed by Benner 2002).

There is a quasi-inverse relationship between our understanding of a DOM component's chemistry and its lability and concentration (Fig. 2). Free amino acids and sugars, whose chemistry can be precisely defined, are used by microbes very rapidly and consequently their concentrations are very low. Biopolymers such as dissolved protein and polysaccharides must be hydrolyzed to smaller free amino acids and sugars before uptake by microbes, so their turnover is a bit slower and concentrations are higher. Likewise, the chemistry of these biopolymers in seawater is not as well understood as that for free amino acids and sugars; the monomers of biopolymers can be examined after acid hydrolysis and HPLC analysis, but the linkages and matrices surrounding targeted biopolymers in seawater are largely unknown.

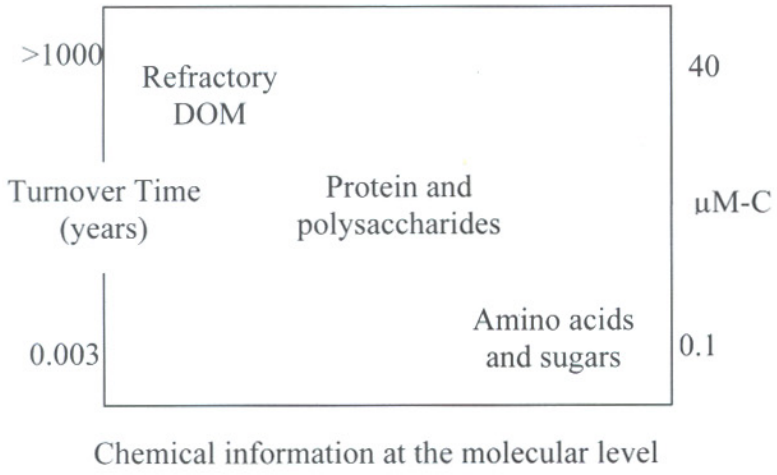

Figure 2. A schematic diagram to illustrate the relationship between turnover time, concentration and our understanding of DOM components at the molecular level. We know the most about the chemistry of the least abundant, fastest turning-over component consisting of compounds like free amino acids and sugars. At the other extreme, we know the least about the component with the highest concentration and slowest turnover time, refractory DOM.

At the other extreme, the largest component of DOM is refractory and a chemical mystery (Benner 2002).

Our fragmentary understanding of DOM chemistry greatly hinders our efforts to examine the role of DOM in biogeochemistry. Most importantly, we could obtain more precise estimates of turnover times and fluxes if armed with more insights into DOM chemistry. For example, because of our ability to measure dissolved free amino acid and neutral sugar concentrations, we can precisely estimate the flux of these compounds; they can account for large fractions of bacterial growth and, ignoring photochemistry, of the total DOM flux (Kirchman 2003).

We have some ideas about concentrations and chemical identify of DOM components such as dissolved protein and polysaccharides, but not enough to devise effective tracers and to estimate fluxes.

Much has been learned about the role of DOM in the oceanic carbon cycle from data on DOC concentrations over time and space. For example, DOC concentrations usually are higher in the euphotic zone and then decrease with depth (Fig. 3A), indicating the impact of biology on DOM processes. Depth profiles of concentrations can be used to divide the DOC pool into three components (Kirchman et al. 1993), based on lability (Table 1). The smallest pool, the labile pool, consists of those compounds that can be chemically identified at the molecular level and are used rapidly by microbes. Although concentrations of the labile pool are quite low (< 1 μM-C), fluxes can be high due to high turnover. The other extreme is the refractory pool whose concentration is taken to be that of DOC in the deep ocean (>1000 m). Williams and Druffel (1987) used ^{14}C dating to estimate that deep DOC turns over on the order of thousands of years. Since this turnover is longer than the turnover of the oceans, Williams and Druffel (1987) reasoned that the most refractory component of DOC must be uniform with depth (Fig. 3B). Consequently, concentrations of DOC found in the deep ocean provide an estimate of refractory DOC in the surface layer. Then, the semi-labile pool of the surface layer is the difference between total DOC concentrations in the surface layer and the deep DOC with the labile pool contributing only trivial amounts. There are few good estimates of the turnover time for the semi-labile pool, but weeks-to-months is probably the right time scale (Table 1).

I would like to emphasize two caveats to keep in mind when using the three component model. As with any simplification, it is difficult to fit all DOM into just three components, and the boundaries can be blurry. For example, dissolved polysaccharides are generally considered to be labile, yet their concentrations can be quite high (ca. 20% of surface layer DOC) (Pakulski and Benner 1994) and there is some evidence that they build up over the summer (Williams 1995), two characteristics of the semi-labile

pool. The other caveat is that lability is currently defined strictly by bioassays or changes in concentrations in the water column; no chemical property is a good predictor of how fast a compound will be used by microbes. For these reasons, it is difficult and probably futile to try to assign particular compounds into one of the three compartments.

Table 1. The three compartment model of DOC in the upper layer of the oceans. The boundaries separating these three compartments are not strict, and there is currently no chemical parameter that can be used consistently and accurately to define these pools. The size of semi-labile pool versus that of the refractory pool also varies among the oceans.

Name	% of total DOC	Turnover Time
Labile	<1	minutes to days
Semi-labile	50	weeks to months
Refractory	50	years to millenia

It is not clear why microbes do not degrade semi-labile DOC, allowing it to increase in concentration during the growth season. One obvious answer is that the structure of semi-labile compounds simply makes them less available to microbes. This explanation seems more likely than another one often invoked: that microbes are limited by the low supply or concentration of some other element (e.g. phosphorus). Because our understanding of DOC chemistry is so limited, we do not have much evidence in support of the first hypothesis. But a few studies offer evidence against the second. Addition of phosphate and other inorganic nutrients does not appear to stimulate the use of the semilable DOC, even in environments such as the Sargasso Sea where bacterial growth may be limited by phosphate (Carlson et al. 2002).

2. FLUXES OF DOM THROUGH THE MICROBIAL LOOP

Heterotrophic prokaryotes are responsible for most of DOM mineralization in the oceans. Prokaryotes consist of Archaea and Bacteria, with the latter dominating the surface layer of the ocean (see below). Cyanobacteria are phylogenetically "bacteria" but functionally they are the same as eukaryotic algae or phytoplankton. Like eukaryotic algae, cyanobacteria are primary producers (CO_2 fixers) that account for >80% of primary production in the open oceans.

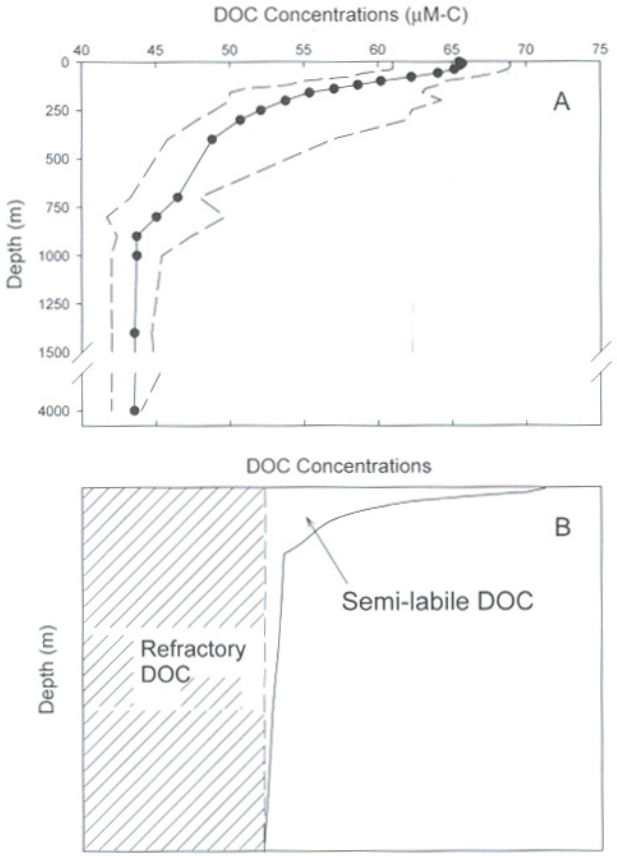

Figure 3. Dissolved organic carbon along a depth profile of the North Atlantic.

A) Concentrations of dissolved organic carbon (DOC) at the Bermuda Times Series Station in 1994 – 1998. The two dashed lines without data points follow the minimum and maximum concentrations. Data from Hansell and Carlson (2001).

B) Schematic diagram of the depth profile data, illustrating how deep DOC concentrations are used to estimate refractory DOC concentrations. The difference between deep and surface concentration is an estimate of the semi-labile pool. Concentrations of the labile pool are <1 μM-C and trivial compared to the other two DOC pools.

There is some evidence of DOM use by heterotrophic protists (single cell eukaryotes) (Sherr 1988), phytoplankton (Palenik and Morel 1990), and even invertebrate larvae. However, DOM uptake by these eukaryotes is not substantial even when it is essential for the organism. One reason for the predominance of prokaryotes in DOM uptake is that they are the most numerous organism in the biosphere (Whitman et al. 1998), and their

biomass can rival that of even phytoplankton in the euphotic zone of open oceans (Ducklow 2000). Although uptake by eukaryotes can be ignored, photochemical oxidation of DOM may be an important alternative pathway to consider (Moran and Zepp 2000). In tributaries of the Amazon River, for example, photochemical oxidation of DOC to CO_2 can exceed microbial respiration (Amon and Benner 1996). Photochemistry is probably less important in the open ocean where concentrations of DOM capable of absorbing light, i.e. colored DOM, are low. For this reason, we can focus on microbially-mediated oxidation of DOM in thinking about oceanic carbon cycles.

What is the evidence that about half of primary production is somehow routed through the DOM pool and the microbial loop? Three interrelated approaches support the 50% estimate. The first two examine the decrease (uptake) over time of DOC or of oxygen (respiration) in surface water incubations of the small size fraction that includes mostly heterotrophic bacteria. These data are reviewed by Williams (2000) and Carlson (2002). Independently, these studies show the importance of DOM uptake. The data on DOC depletion and respiration are often used to estimate growth efficiency (BGE), which is an essential parameter in the third approach for estimating uptake of DOM by bacteria. Growth efficiency is defined as bacterial biomass production (BP) divided by total organic carbon uptake (U), i.e. BGE = BP/U.

The third approach estimates DOM uptake by combining estimates of growth efficiency and biomass production of heterotrophic bacteria: U = BP/BGE. Analogous to primary production, rates of heterotrophic bacterial production reflect the synthesis of new biomass before mortality (grazing and viral lysis) has taken its toll. Respiration is not included in bacterial production estimates. In the absence of mortality, bacterial biomass would increase over time initially at the rate of bacterial production. A hidden assumption of this approach is that heterotrophic bacteria rely mostly on DOM rather than particulate organic carbon. Except in rare cases, this assumption is a safe one because of the large number and high concentration of free-living bacteria and DOC relative to attached bacteria and particulate organic carbon.

A more serious problem with using bacterial production to examine DOM fluxes is that the growth efficiency can vary in ways that are still poorly understood. Growth efficiency depends on many factors that affect bacterial metabolism and varies among aquatic habitats as a function of trophic status. Freshwaters and estuaries have the highest (35%) and the open oceans (15%) the lowest BGE values reported to date (del Giorgio and Cole 2000). There are also problems associated with estimating bacterial production by current methods (incorporation of thymidine and leucine) (see

Ducklow 2000). Even ignoring the methodological problems, some investigators argue that we should concentrate on measuring bacterial respiration rather than production because respiration is the larger of the two processes. Also, because DOM can be re-cycled (in a sense) more than once, bacterial production can theoretically exceed primary production (Anderson and Ducklow 2001; Strayer 1988) and thus respiration is more directly comparable to primary production. These criticisms are quite valid, but the difficulties of directly measuring DOC depletion and respiration prevent these approaches from becoming common practice. Also, rates measured with the bacterial size fraction may not be accurate because the food web producing DOM and inorganic nutrients has been disrupted by filtration. In contrast, the ease of measuring thymidine and leucine incorporation allows much greater spatial and temporal coverage of heterotrophic bacteria-phytoplankton interactions in a nearly unperturbed state. Even given the ease of the methodology, the number of estimates of bacterial production for the open ocean is small compared to the vastness of the oceans and the sample density of primary production.

Generally, heterotrophic bacterial production correlates with primary production in the open oceans over sufficiently large and long space and time scales (Fig 4). The relationship between bacterial (BP) and primary production (PP) is often parameterized by the ratio of the two rates (BP:PP). The data in Figure 4 suggest that BP:PP values for the open oceans are generally lower than that seen in freshwaters, estuaries and the few coastal ocean samples included in the old review of Cole et al. (1988), as Ducklow (2000) first observed. Indeed, the average BP:PP in Figure 2 (the slope of the line) is 0.087 ± 0.046, over 2-fold lower than the BP:PP found by Cole et al. (1988). This issue needs to be examined by a more quantitative analysis, including an update of Cole et al. (1988).

We can use BP:PP and BGE values to estimate the fraction of primary production that is routed through the DOM pool (U:PP).

It can be shown that U:PP = (BP:PP)/BGE. Table 2 illustrates how U:PP varies with BP:PP and BGE. Taking BP:PP= 0.0867 and BGE= 0.15 (or 15%) implies that U:PP = 0.578, that is, somewhat higher than the 50% estimate quoted above. Ducklow et al. (2002) recently discussed balancing BP:PP and BGE in a carbon budget for a spring bloom in the North Atlantic. They conclude that some of the original BP:PP ratios estimated for the North Atlantic are too high to fit in our current understanding of food web dynamics and carbon cycles. Still, even the lower BP:PP ratios and our best estimate of BGE indicate that 50% or more of primary production is processed by the microbial loop via the DOM pool.

In fact, recently Hoppe et al. (2002) reported even higher BP:PP ratios and suggested that areas of the tropical Atlantic Ocean are net heterotrophic, i.e. organic carbon consumption exceeds primary production.

Table 2. Percentage of primary production that is routed through the DOM pool as a function of the ratio of bacterial production to primary production (BP:PP) and growth efficiency, expressed as a fraction. Currently, BP:PP and the growth efficiency are thought to be <0.1 and 0.15 (15%), respectively, which would imply that >50% of primary production passes through the DOM pool.

	Growth efficiency		
BP:PP	0.5	0.20	0.15
0.3	60%	150%	200%
0.2	40%	100 %	133%
0.1	20%	50%	67%

These data add to an ongoing debate about the balance between autotrophy and heterotrophy in the oceans (del Giorgio et al. 1997; Duarte et al. 2001; Williams 1998), although that question is best examined with direct measures of oxygen production and consumption (Serret et al. 2001). I side with Williams and Bowers (1999) and doubt that large oceanic regions can be heterotrophic for long. Regardless, the bacterial production data point to the importance of DOM fluxes.

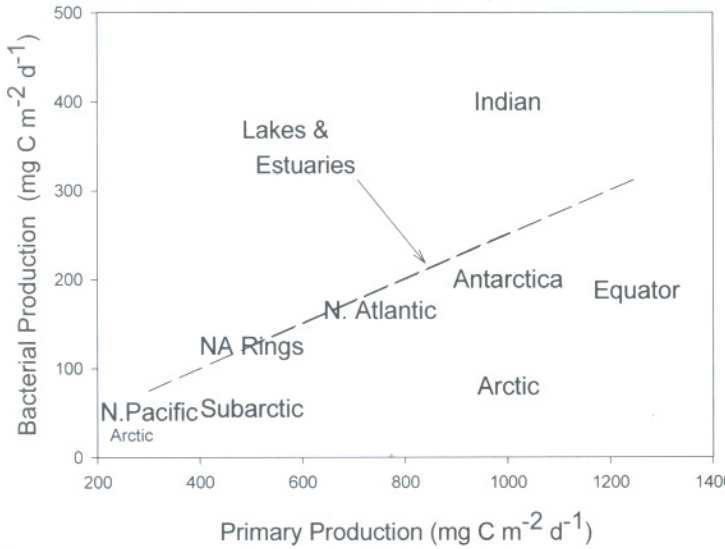

Figure 4. The mean bacterial production and ^{14}C particulate primary production for the indicated oceanic regimes, taken from Ducklow (2000) with two values from the Arctic provided by Rich et al. (1997). The dotted line labeled with "lakes and estuaries" is from Cole et al. (1988)

3. NET PRODUCTION AND EXPORT OF DOC

With few exceptions, net changes in DOC concentrations are not included in estimates of DOM fluxes (production and uptake), at least the fluxes defined above that depend on microbial activity, because net changes are generally small compared to DOM fluxes estimated by other approaches (Table 3). In fact, as a first approximation, the net daily change in total DOC is nil while there is always some bacterial growth and respiration, i.e. a measurable DOM flux. Individual labile compounds, such as free amino acids and sugars (Rich et al. 1996), can vary several fold over a couple days, but the impact of these changes on total DOC is negligible, since concentrations of labile compounds are so small (nanomoles per liter). The implication is that production of DOM is closely balanced by consumption.

Table 3. Net changes in DOC concentrations and DOM fluxes calculated from bacterial production and bacterial growth efficiency assumed to be 0.15. The bottom end of the range for net changes was set at zero because usually the net changes in DOC on a day time scale is below detection. The upper limit was taken as the range of concentration observed over the entire sampling period. The concentration range was then divided by days in the sampling period and multiplied by the euphotic zone to obtain an integrated daily rate comparable to the DOM flux.

Regime	DOM flux (mmol C m^{-2} d^{-1})	Potential Net Changes in DOC (mmol C m^{-2} d^{-1})	Reference
Equatorial. Pacific	58-158	0-10	Ducklow (2000), Carlson and Ducklow (1995)
Sargasso	39	0-12	Carlson et al. (1994)
Ross Sea	31	0-3	Ducklow et al. (2001), Carlson et al. (2000)

However, occasionally DOM production can greatly exceed consumption over short time scales and even relatively small daily changes in DOC can accumulate. These changes can be a substantial part of the overall carbon budget. In the few oceanic habitats with available data, it is evident that DOC can build up in the upper 100 m during the growing season in spite of continuous bacterial activity (Fig. 3A) (Hansell and Carlson 1998b). The cause of this build up is unknown (see above), but its size can be gauged by comparing it to the simultaneous depletion of nitrate, one measure of net primary production. In most of the oceans with available data, it seems that about 20% of net primary production accumulates as DOC (Table 4). The

Sargasso Sea, where DOC has been examined probably more extensively than any other open ocean, is an interesting case. During the spring bloom, 59-70% of net primary production accumulates as DOC but when calculated for the entire year, this percentage drops to <10% (Hansell and Carlson 1998b; Hansell 2002). These percentages indicate that net DOC accumulation can be substantial relative to net primary production.

Table 4. Net DOC production compared with net community (primary) production (PP). Based on the review by Hansell (2002). Net primary production was estimated from nitrate depletion.

	% of net PP	Reference
Ross Sea	8-20	Carlson et al. (2000)
Equatorial Pacific	20	Archer et al. (1997), Hansell et al. (1997)
Equatorial Atlantic	20	Thomas et al. (1995)
Iberian margin	20	Alvarez-Salgado et al. (2001)
Greenland Sea	50	Noji et al. (1999)
Sargasso Sea		Carlson et al. (1994), Hansell and Carlson (2001)
Spring bloom	59-70	
Entire year	8	

This accumulated DOM is a potential source of carbon and other essential elements (e.g. nitrogen and phosphorus) for bacterial growth in the surface layer. The oft-observed lag between bacterial and primary production can be explained by use of DOM produced and accumulated during previous periods of high primary production (Ducklow et al. 2002). This uncoupling is taken to its extreme by those arguing for large net heterotrophic regions in the oceans. Accumulated DOM, produced in a highly productive location, has been called upon to support excess heterotrophic bacterial activity in other, more oligotrophic locations (del Giorgio et al. 1997). There are two problems with this mechanism. First, to make up the oxygen and carbon deficit of some presumed heterotrophic oceanic regions, accumulated DOC produced in productive areas would have to be carried away by surface currents larger than the Gulf Stream (Williams and Bowers 1999), currents that have escaped the attention of physical oceanographers to date. Another problem is that the DOM has to be refractory enough to accumulate and survive during transport but then it is has to be labile enough to support substantial heterotrophic bacterial activity in the oligotrophic regions. Some DOM has the right degree of lability to be

exported both laterally and vertically (see below), but probably not to the extent argued by some investigators.

Another fate of accumulated DOM is export from the surface layer to the mesopelagic zone and deep ocean (Hansell and Carlson 2001). As Hansell (2002) points out, DOM export requires biology to produce DOM and physics to move water with its dissolved constituents to deeper layers of the oceans. One could add that in addition to biology and physics, chemistry is needed to make DOC refractory enough to survive immediate degradation by heterotrophic bacteria and to accumulate in the surface layer. Substantial export of DOC is known to occur with the formation of North Atlantic Deep Water (NADW) (Hansell and Carlson 1998a) and North Pacific Intermediate Water (Hansell et al. 2002).

One way to estimate DOC export is to examine the relationship between apparent oxygen utilization (AOU) and DOC concentrations in various density strata (e.g. Fig. 5). The extent to which the variation in AOU is explained by DOC concentrations is a measure of how much oxygen use (total respiration) is fueled by DOC. Since little DOC is produced below the euphotic zone, DOC used by respiration must have been exported from the surface layer. The approach cannot be used to examine DOC-supported respiration in the upper 100 m where DOC-AOU correlations are not statistically significant because atmospheric exchange prevents any deficit in oxygen concentrations. At the other extreme, DOC-AOU correlations are again insignificant in the deepest ocean where DOC concentrations are relatively constant. Between those two extremes in depth, however, there are several depth strata with significant DOC-AOU correlations. At these locations, DOC appears to explain 15-47% of AOU, with each oceanic regime varying about two-fold with depth (Table 5). Hansell and Carlson (pers. comm.) estimated that global DOC export to intermediate, deep and bottom water is about 9.8 $x10^{12}$ mol C y^{-1} or about 10% of total export (oxygen utilization) to depths >500 m. Export to shallower waters is higher, as summarized in Table 5.

Table 5. DOC export, estimated from the correlation between DOC concentrations and apparent oxygen utilization (AOU). Based on the review by Hansell (2002).

Region	% AOU explained By DOC	Depth (m)	Reference
North Pacific Gyre	40	75-125	Emerson et al. (1997)
South Pacific	21-47	<500	Doval and Hansell (2000)
Indian Ocean	18-43	<500	Doval and Hansell (2000)
Gulf of Mexico	20-30*	0-250	Guo et al. (1994)
Western North Pacific	33	400-1000	Hansell et al. (2003)

*Upper limit, as the covariance between AOU and DOC was not corrected for mixing effects.

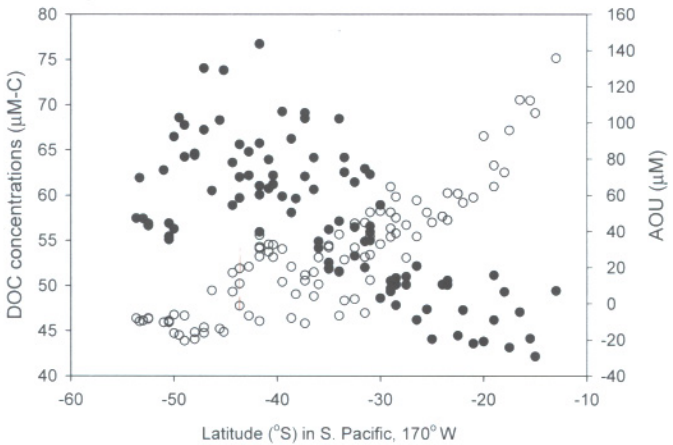

Figure 5. Example of data on DOC and apparent oxygen demand (AOU) at a density of σ_θ =26-26.5. Modified from Doval and Hansell (2000). The filled-in circles are DOC concentrations whereas the open circles are AOU.

4. CARBON STORAGE AND DOM UPTAKE IN THE DEEP OCEAN

A major issue in climate change studies is to understand the mechanisms by which carbon can be sequestered out of contact with the atmosphere on various time scales. DOC can be viewed as one such sequestration mechanism. Because of its concentration and the volume of the ocean, oceanic DOC is a major store of carbon in the biosphere. The largest fraction, which is also the most refractory, would seem not to impact atmospheric CO_2 on time scales <1000 years. The model of Anderson and Williams (1999) indicates that changes in refractory DOC can be ignored when examining global change over 200 years. However, any changes in refractory DOC, especially in the deepest depths where most of the oceanic volume lies, could have large impacts on atmospheric CO_2 on longer time scales.

It would be extremely difficult to directly measure changes over time in deep oceanic DOC, given that its turnover exceeds 1000 years. Indeed, Hansell (2002) points out that there is no evidence that deep oceanic DOC changes over time, at least during six years of work in the Sargasso Sea and over a year of study in the Arabian Sea. But DOC concentrations do appear to decrease along the conveyor belt of deep oceanic currents that cover the world's ocean (Hansell and Carlson 1998). DOC is 43-48 µM-C in deep

waters of the North Atlantic (1000-4800 m), near sites of North Atlantic Deep Water (NADW) formation, and it decreases to 34-39 μM-C at 900-5000 m in the North Pacific Ocean. This deep North Pacific Water is NADW with some influence from Antarctic Bottom Water. The drop in DOC parallels the increase in concentrations of inorganic nutrients such as phosphate along the conveyor belt.

Hansell (2002) points out that we do not know if the decrease in deep oceanic DOC is due to microbial activity or to some abiotic mechanism, such as the scavenging of DOC on sinking particles. He reasoned that the amount of useable DOC is too small and the time frame too long to select for much bacterial activity. However, Williams (2000) argued that it is energetically worthwhile for a microbe to attempt to take up even a single glucose molecule dissolved in several oceans. In fact, there is enough microbial activity at depth to explain the gradient in deep oceanic DOC. If we take the microbial activity estimates (8.33×10^{-5} μmol-C liter^{-1} d^{-1}) of Nagata et al. (2000) and assume deep oceanic DOC to be 40 μM-C, the implied deep oceanic DOC turn over is on the order of 1300 years, faster than the turnover estimated from ^{14}C-dating. The model of Bendtsen et al. (2002) indicates that the observed gradient in DOC in the deep North Atlantic can be explained by temperature-dependent bacterial activity.

Bacterial activity is high in the deep oceans relative to the vertical flux of particulate organic carbon, even though deep sea activity is 100-fold (or more) lower than rates in the surface layer. Measured rates of bacterial production (mainly leucine incorporation) and assumed growth efficiencies can be used to estimate the amount of carbon used by bacteria in the mesopelagic zone of the oceans. In the North Pacific gyre (Cho and Azam 1988), other North Pacific regimes (Nagata et al. 2000; Simon et al. 1992) and the Arabian Sea (Ducklow 1993), the estimated carbon use by heterotrophic bacteria is a large fraction (30-100%) of total organic carbon supplied by sinking particles as measured by sediment traps. The numbers and activity of truly attached bacteria are low at all depths in the oceans, so this carbon mineralization in deep waters is by unattached bacteria using labile DOM. The DOC fueling deep bacteria could be from DOM exported directly from the surface layer, a possibility that has not been examined thoroughly. More likely, sinking particles disintegrate to DOM because of abiotic hydrolysis and microbial activity (Smith et al. 1992). We know little about this process in spite of its obvious importance for understanding carbon fluxes.

We need more work on DOC and microbial activity in the deep ocean. Williams (2000) pointed out that most studies of microbial activity are restricted to the upper 100 m, probably because of the emphasis on primary production, leaving the vast interior of the ocean grossly undersampled.

Unlike deep DOC, it is possible to measure changes in surface layer DOC. Church et al. (2002) reported that surface layer DOC concentrations in the North Pacific Gyre increased at a rate of 0.3 mol- C m^{-2} y^{-1} from 1993-1999. While this DOC accumulation is only about 2% of primary production, it is 15% of total organic carbon export in the North Pacific, given that export is about 2 mmol-C m^{-2} y^{-1} in this region (Emerson et al. 1997). Concentrations of dissolved organic nitrogen (DON) also increased, but dissolved organic phosphorus did not (Church et al. 2002). The implications of these changes are still not clear, but the changes seem large enough to be considered in carbon budgets and models.

5. WHAT MECHANISMS PRODUCE LABILE AND REFRACTORY DOM?

The mechanisms producing labile DOM probably differ greatly from those producing refractory DOM, and as a first approximation we can consider the two DOM pathways to be completely separate. We know the most about how labile DOM is produced.

5.1 LABILE DOM

While only one microbial group, heterotrophic bacteria (and perhaps archaea), are the main users of DOM, several organisms and processes potentially produce DOM. Direct release or excretion by phytoplankton has been examined more extensively than any other DOM production mechanism, in part because of the ease of using ^{14}C to trace carbon from the inorganic carbon pool through phytoplankton and into DOM and heterotrophic bacteria. There are many generalizations that are often quoted (e.g. DOC is released by nutrient stress nearing the end of phytoplankton blooms) but with conflicting supporting data at best (Williams 1990). In a review of the topic, Nagata (2000) concludes that phytoplankton release only about 12% of ^{14}C-primary production as labile DOM (Fig. 6). The ^{14}C-based studies used by Nagata (2000) for his 12% estimate may have underestimated release of polysaccharides and other polymers normally associated with cell walls or bound to the outside of phytoplankton (and perhaps bacteria), because these polymers would not be labeled adequately in the typical short incubation with ^{14}C. Polysaccharides are large DOM constituents (Pakulski and Benner 1994), which can build up over the growing season (Kirchman et al. 2001). In any event, the 12% estimate seems a reasonable average for direct release by phytoplankton.

Nagata (2000) then assumes that total DOM uptake and release account for 49% of primary production, implying that direct phytoplankton release is roughly 20% of total DOM production (10%/50%). The contribution by viral lysis is set at 5% of primary production or 20% of total DOM production, the most arbitrary and least-constrained part of Nagata's budget. The remaining 65% of total DOM production has to be due to release of DOM by grazers, an estimate that is supported by direct measurements (Strom et al. 1997). Nagata (2000) estimates that protist grazers account for most of this release (53% of total DOM production) with DOM release by mesozooplankton contributing the last 6%, reflecting the fact that protists graze more of phytoplankton production than do mesozooplankton (by nearly 20-fold in Nagata's budget) in the open ocean, which again is supported by direct measurements of grazing rates (Landry et al. 1995).

That grazers, not phytoplankton, are the main source of DOM has many implications for understanding carbon cycles and trophic dynamics. It could explain why bacterial production often is not correlated with primary production and more specifically why bacterial activity often lags behind phytoplankton. Whether grazers or phytoplankton (or viruses) produce the most DOM is likely to impact virtually all biogeochemical properties of DOM. For example, we may expect DOM from grazers to be partially degraded and thus less labile than DOM directly from phytoplankton.

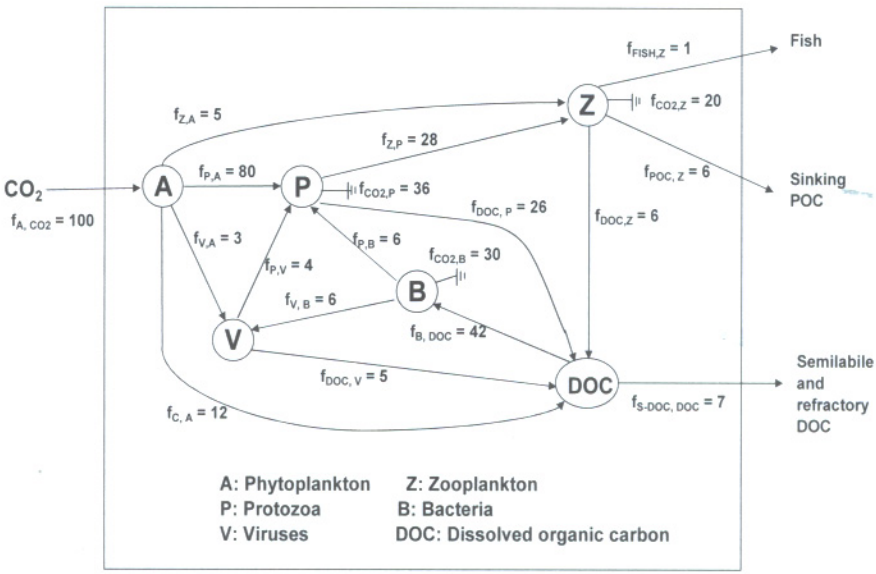

Figure 6. A budget of carbon compartments and flow used to account for DOC production. The symbols in the form of $f_{x,y}$ denote the flux from compartment y to compartment x. All fluxes are normalized to primary production ($f_{A,CO2}$) or the flux from CO_2 to phytoplankton (A). Modified from Nagata (2000).

5.2 REFRACTORY DOM

We know little about how refractory DOM is produced in the oceans. ^{13}C data indicate that it does not come from terrestrial sources, and the concentrations of known terrestrial compounds, the most important one being lignin, are very low in oceanic waters (Benner 2002). The implication is that organic material originally produced by phytoplankton is somehow transformed to refractory DOM. What complicates matters is that only a very small fraction (<1%) of this phytoplankton-produced organic carbon needs to be transformed to refractory DOM; the vast majority is mineralized back to CO_2 and inorganic nutrients mostly in the upper layer where organic material is produced.

Refractory DOM is often said be "humic material", formed by the abiotic condensation of labile, low molecular weight (LMW) DOM. Borrowing from studies on soil formation, humic material is operationally defined as the organic material that can be isolated by sorption onto XAD resins after acidification. While the same term is used for material isolated by this method for soils and seawater, it has been clear since the initial studies that seawater humic substances are not the same as those compounds isolated from soils. Regardless, the classic condensation hypothesis did seem to explain the basic dilemma of turning labile into refractory DOM; compounds synthesized abiotically are generally less available to enzymatic hydrolysis than organic material synthesized by enzymes. The hypothesis, however, always suffered from the problem that known condensation reactions required higher concentrations than observed for LMW DOM. More critically, the classic condensation model leads to predictions that are not supported by the data.

One prediction of the condensation hypothesis is that high molecular weight (HMW) DOM, if resulting from abiotic condensation of labile LMW precursors, should be more refractory than LMW DOM. In fact, the opposite seems to be the case. HMW DOM (>1000 Da) isolated by ultrafiltration is used much more quickly than its LMW DOM counterpart (Amon and Benner 1994). It remains true that biopolymers larger than about 500 Da must be hydrolyzed by ectoenzymes before the LMW byproducts can be transported into the cell and catabolized. However, it is quite misleading to assume that LMW byproducts of biopolymer hydrolysis are representative of LMW DOM, since concentrations of byproducts such as free amino acids and sugars are very low and are a trivial fraction of LMW DOM found in seawater. In contrast, biopolymers such as polysaccharides make up a large fraction of HMW DOM (Benner 2002), which is one big reason why it is more labile than LMW DOM. Benner (2002) summarizes the data indicating the LMW DOM is more refractory than HMW DOM.

Another prediction of the condensation hypothesis is that the biochemical composition of LMW DOM should be most similar to that of organisms, and HMW DOM should be composed of more unknown compounds. In fact, again the opposite is true. There is a general decrease in the fraction of material that can be identified as typical biochemicals along the size gradient from particulate material (plankton) to LMW DOM. About 85% of the organic carbon in plankton can be chemically-identified at the molecular level whereas this percentage decreases to 15% and 4% for HMW and LMW DOM, respectively (calculated from data presented by Benner 2002). So, the most refractory material is in the smallest molecular size fraction that contains the lowest percentage of known biochemicals. There appears to be a correlation between lability and the fraction of organic material identifiable at the molecular level, and perhaps this is no mere coincidence. As compounds move out of the analytical windows of our chemical methods, they also seem to become less accessible to enzymatic attack. Microbial enzymes and our analytical methods both seem focused on the compounds directly synthesized by organisms.

Another set of studies using NMR techniques have shed some light on the composition of HMW DOM (reviewed by Benner 2002). In brief, these studies indicated that the chemical bonds found in HMW DOM are similar to those of the plankton and are not those expected from condensation reactions. In particular, McCarthy et al. (1997) reported ^{15}N-NMR results indicating that most of the nitrogen in HMW DOM is in amide bonds, which make up common biopolymers, including protein, peptidoglycan (found in bacterial cell wall) and chitin (common in invertebrates and selected algae). The structural compounds, peptidoglycan and chitin, are most likely the major components of HMW DOM, because they are thought to be more resistant to microbial attack than protein (but see below). These results do not rule out all abiotic reactions, but they do indicate that HMW DOM is not completely from the condensation or coagulation of LMW DOM.

It has been implied that phytoplankton are the source for the refractory DOM. Several lines of evidence indicate, however, that refractory compounds in fact come from bacteria. Tanoue et al. (1995) reported one of the first sightings of a bacterial biomarker, a membrane protein (porin) unique to bacteria, in the HMW fraction of both surface and deep DOM. Subsequently, McCarthy et al. (1998) found more evidence, high amounts of D-amino acids, that bacterial cell wall remains make up a large fraction of HMW DOM. Abiotic racimerization or the switching of L-amino acids (the form found in proteins) to the D form is too slow to explain the high D/L ratios measured by McCarthy et al. (1998). The most likely source of the D-amino acids is the peptide bridge found in peptidoglycan, a biopolymer synthesized only by bacteria. These results are consistent with the ^{15}N-NMR

results (McCarthy et al. 1997), suggesting peptidoglycan as one possible source of the amide bonds found in HMW DOM.

More recent data complicate the peptidoglycan story. First, concentrations of the amino sugar, muramic acid, which is unique to peptidoglycan, are not as high as one may expect if peptidoglycan were common in the HMW DOM fraction (Benner and Kaiser 2003). Second, turnover of peptidoglycan does not appear to be any slower than protein turnover, at least in short term incubations (Nagata et al. 2003). However, the experiments of Nagata et al. (2003) revealed one crucial difference between peptidoglycan and protein degradation. Unlike protein degradation, LMW byproducts were released during the degradation of peptidoglycan. What is even more interesting is that the composition of these byproducts could not be identified by the same chemical methods that successfully characterized the starting material. In short, it seems that material reminiscent of refractory DOM was produced during peptidoglycan degradation (Nagata et al 2003).

While the precise precursor may still be in doubt, it is clear that bacteria can be a source, if not the sole mechanism for producing refractory DOM. The role of heterotrophic bacteria was clearly demonstrated by Ogawa et al. (2001) who fed glucose to a bacterial size fraction and measured glucose and DOC concentrations over time. They found that within two days glucose dropped to unmeasurable levels while concentrations of DOC, which initially was nearly all glucose, also decreased, but less than expected, considering how much glucose had disappeared. The difference between the expected and the actual decrease in DOC concentrations indicates the formation of unknown organic material in the DOM pool; after the glucose disappeared, <15% of the DOM could be identified. Ogawa et al. (2001) calculated turnover times exceeding a year for this unknown DOM.

The mechanism by which glucose was somehow transformed into refractory DOM is not clear. Ogawa et al. (2001) recognized that viruses and protists may have been involved, but the authors favor another mechanism: nonspecific activity by bacterial enzymes produce compounds which escape further bacterial attack and detection by standard chemical analysis. Regardless of the precise mechanism, this experiment rules out several mechanisms and indicates that light, phytoplankton, zooplankton and sediments are all unnecessary in the production of refractory DOM.

Perhaps most important, the experiment indicates that refractory DOM can be produced quite quickly; organic compounds with turnover times exceeding a year were produced within a couple days. Similarly, Norrman et al. (1995) observed production of refractory DOC over a few days during a phytoplankton bloom in a mesocosm. In both cases, the production rates are undoubtedly abnormally high because of high DOC concentrations (glucose

in the experiments of Ogawa et al. (2001)) or of excessive phytoplankton activity (in the eutrophic mesocosm of Norrman et al. (1995)). The oceans would quickly fill up with refractory DOM if produced at the rate suggested by these two studies. Even so, these data suggest that refractory DOM can be produced on much shorter time scales than it is degraded. This apparent uncoupling between production and mineralization of refractory DOM seems quite important in thinking about DOM-microbe interactions and in including DOC in carbon budgets.

6. CHARACTERIZING BACTERIA COMMUNITIES IN THE OCEAN

Just as the DOM pool is a complex mixture of several compounds that differ greatly in biogeochemically-relevant properties, so too is the prokaryotic community a complex assemblage of many organisms that potentially differ in their biogeochemical roles. Again analogous to DOM, we still do not know much about the taxonomic composition ("community structure") of prokaryotic communities in the oceans, in spite of extensive work over the last 15 years. The fundamental problem is, few marine microbes can be isolated and cultured in the lab, which is the first step in traditional methods for identifying microbes. Consequently, microbial ecologists have to turn to molecular methods and other techniques for deducing the role of uncultured microbes in biogeochemical processes such as the uptake and production of DOM.

Giovannoni and Rappé (2000) and Kirchman (2002) provide more complete reviews than possible here about microbial community structure in the oceans. This chapter will discuss some observations that I think are most relevant to thinking about microbial processes in carbon cycle models.

Starting with the highest phylogenetic level, prokaryotes consist of two domains, bacteria and archaea, which share some morphological traits (they cannot be distinguished with simple microscopy) but differ in several other ways. The first archaea to be characterized were extremophiles (e.g. hyperthermophiles and halophiles) able to survive and grow under extreme conditions using unique metabolisms, such as the production of methane (methanogens). Recent work, however, indicates that most marine archaea are not extremophiles and are not just in environments with high temperatures, pH or salinity. They can be found virtually in all marine habitats, but archaea appear to be particularly abundant in the deep ocean (Karner et al. 2001). The number of crenarchaeota, one of the three main groups of archaea, is about equal to bacterial abundance in the North Pacific below 1000 m (Karner et al. 2001). Another main archaeal group,

euryarchaeota, is relatively rare throughout the water column (Karner et al. 2001). While archaea are abundant in the deep ocean, bacteria still greatly outnumber archaea in the surface layer.

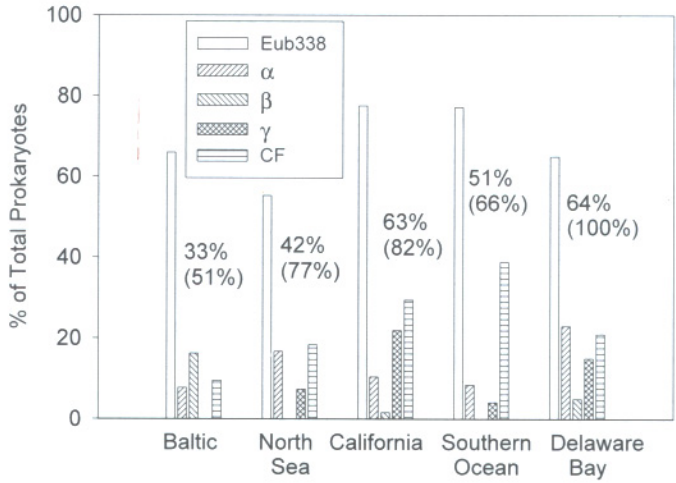

Figure 7. The major bacterial groups in selected oceanic regimes and the Delaware Bay. The oceanic data are summarized by Kirchman (2002) and the Delaware Bay data are from Cottrell and Kirchman (2003). The first percentage is the fraction of total bacterial abundance (DAPI direct counts) accounted for by the four major groups. The percentage in parenthesis is the fraction of Eub338-positive microbes (Eub338 targets all bacteria) accounted for by the four groups.

We know little about the metabolic capacities of these marine archaea and whether they differ greatly from bacteria. Marine archaea do appear capable of using DOM (Ouverney and Fuhrman 2000), but much more work on this topic is needed.

Of the over 50 divisions or phyla of bacteria found in the biosphere (Rappé and Giovannoni 2003), only three are usually abundant in surface waters of the oceans (Giovannoni and Rappé 2000). One of these is the cyanobacterial group consisting of three genera: *Synechococcus*, *Prochlorococcus*, and *Trichodesmium*. The other two divisions are the proteobacteria and the Bacteriodetes. The latter division includes *Cytophaga-Flavobacterium*. Proteobacteria include several subdivisions of which two, alpha- and gamma-proteobacteria, are most abundant in the oxic waters of the oceans (Fig. 7). Beta-proteobacteria are common in lakes and freshwater reaches of estuaries, but are usually <10% of abundance in marine waters. Epsilon-proteobacteria, which include sulfate reducers, are

abundant only in anoxic habitats. Usually *Cytophaga-Flavobacterium* and alpha-proteobacteria are the two most abundant bacterial groups in marine surface waters (Kirchman 2002), as determined by one molecular approach, fluorescence in situ hybridization (FISH) with oligonucleotide probes. We are still in the early days of describing the variation in these bacterial groups and understanding what factors determine this variation.

The simplicity implied by stating that the oceans are dominated by a few bacterial groups is misleading. Each of these groups contains many different bacteria with potentially different biogeochemical roles. As measured by 16S rRNA sequences, the phylogenetic marker of choice, the difference within a proteobacterial subdivision or the *Cytophaga-Flavobacterium* cluster is greater than the difference among the common phytoplankton groups, such as diatoms and coccolithophorids or among common zooplankton, such as copepods and euphausids. The number of bacterial genera and species in the ocean is also not clear, nor is it obvious whether bacterial communities are dominated by a few species or are evenly divided among several species. A recent study using fluorescence in situ hybridization (FISH) suggests that one alpha proteobacterial group, SAR11, is abundant and ubiquitous in the oceans (Morris et al. 2002), but even this group is quite diverse (Giovannoni and Rappé 2000). And there is even more diversity at even finer phylogenetic levels than indicated by commonly used 16S rRNA gene sequences. It is well known that 16S rRNA is not a good phylogenetic marker for closely related organisms. For example, different ecotypes of the abundant cyanobacterial genus *Prochlorococcus* are known to inhabit different depths and light regimes in oligotrophic oceans, but these ecotypes have virtually the same 16S rRNA signature (<3% difference) (Moore et al. 1998). In short, while only a few of the many bacterial divisions are found in the oceans, the diversity within bacterial divisions is incredibly high with unclear implications for understanding the role of bacteria in biogeochemical cycles. The different levels of bacterial phylogeny are summarized in Figure 8.

I think the critical question microbial ecologists have to answer is, what phylogenetic level is most appropriate for describing bacterial activity in the oceans and other aquatic environments? The answer probably varies with the particular problem being addressed. Consider two extremes. Microbial ecologists have long discussed DOM uptake by "bacteria", i.e. the action of the entire bacterial domain (not to mention archaea), without apparently needing to know which bacterial group was doing what. At the other extreme, we know that some strains of a bacterial species are pathogenic while others are not; many strains of *E.coli* are common, harmless residents of the human gut microflora whereas others cause dysentery and even death.

It remains to be seen whether we need to consider finer phylogenetic levels (the extreme being strains) in describing DOM uptake.

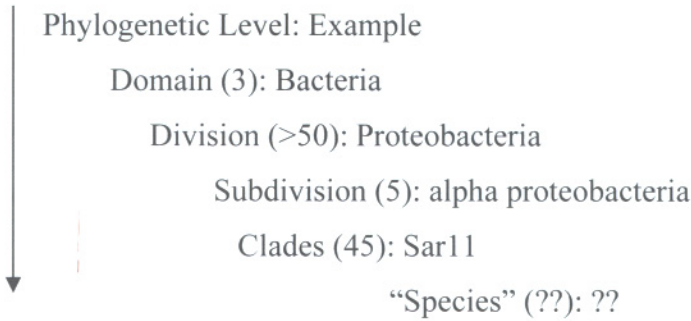

Figure 8. Summary of the major phylogenetic levels used to organize bacterial diversity and an example for each level. The arrow indicates the progression from the broadest level to the finest. "Species" is not a well defined concept in bacterial taxonomy. The numbers in parenthesis refer to subgroups at each level. There are three domains of which Bacteria is one. There are about 52 subdivisions of Bacteria of which Proteobacteria is one, and so on. Based on Giovannoni and Rappé (2000) and Rappé and Giovannoni (2003).

7. UPTAKE OF SELECT DOM COMPONENTS BY AQUATIC BACTERIAL GROUPS

Current models of carbon cycles in the ocean depict bacteria by a single compartment (Fasham et al. 1999)–if bacteria are included at all. The implied assumption of a single compartment is that all bacteria are using all DOM components equally. Unfortunately, there are few data available to test this assumption. Laboratory-bound microbiologists would immediately point to the wealth of data showing how different bacterial species differ in using organic compounds, and some of this information is useful for understanding natural bacterial assemblages. However, it is quite difficult to apply information about cultured bacteria to the oceans because so few marine bacteria can be cultured and because those that can be cultured differ greatly from those that cannot (Giovannoni and Rappé 2000). Furthermore, even if we could culture the major oceanic bacteria, it would be difficult to extrapolate from laboratory experiments to the ocean because of the difficulties in reproducing oceanic conditions in the lab.

Only a few studies provide data that bear on the question of whether all uncultured bacteria take up DOM equally. Cottrell and Kirchman (2000a) combined microautoradiography and FISH (Micro-FISH) to examine uptake

of selected ^{3}H-labeled organic compounds by bacteria identifiable by FISH probes for the proteobacterial subdivisions and the *Cytophaga-Flavobacterium*. Cottrell and Kirchman (2000a) found that not even free amino acids were taken up equally by the bacterial groups they examined. Their data suggest that the alpha-proteobacteria seem to dominate use of LMW DOM (free amino acids and N-acetylglucosamine) whereas *Cytophaga-Flavobacterium* dominated uptake of HMW organic compounds (protein and chitin). This proficiency in using biopolymers is consistent with laboratory experiments with *Cytophaga-Flavobacterium* (Kirchman 2002).

Other approaches can be used to examine DOM uptake by specific bacterial groups or at least to make inferences about specific DOM-bacteria interactions. One approach is to follow the incorporation of radiolabeled or stable isotopically-labeled compounds into taxon-specific RNA or phospholipids (Boschker et al. 1998). Another approach is to follow bacterial community structure following the addition of various DOM components (e.g. Carlson et al. 2002); the increase of a bacterial group in response to the addition implies, although does not necessarily prove, that these bacteria are keying in on the added organic carbon. Covert and Moran (2001) isolated the LMW and HMW fractions of DOM, added back an inoculum of a natural bacterial assemblage and examined with a DNA fingerprint method the bacterial communities that grew up in each incubation. The bacterial groups that grew on these DOM fractions differed from those expected from the results of Cottrell and Kirchman (2000a), for many possible reasons. The two studies used different techniques to examine the microbes in two different locations. Also, the compounds used by Cottrell and Kirchman (2000) may not model the LMW and HMW material examined by Covert and Moran (2001). Perhaps even more problematically, Covert and Moran (2001) found that a second experiment did not yield the same communities as in the first. Differences in starting LMW and HMW DOM could explain this lack of consistency between experiments. More troubling, the results may indicate the need to look at finer phylogenetic levels than examined by Covert and Moran (2000) and certainly by Cottrell and Kirchman (2000a).

8. NEW TYPES OF BACTERIAL METABOLISMS–EVEN MORE BACTERIAL COMPONENTS FOR MODELS?

Many other members of the bacterial community are well known to participate in several biogeochemical processes important in the oceans

(Capone 2002). One example worth mentioning is the group of chemoautotrophic microbes that oxidize ammonium eventually to nitrate (nitrification). Much less is known about two new types of microbes that are potentially involved in uptake, mineralization, and perhaps production of DOM. Both types harvest light energy while using DOM to supplement their energy and carbon needs, as illustrated in Figure 9.

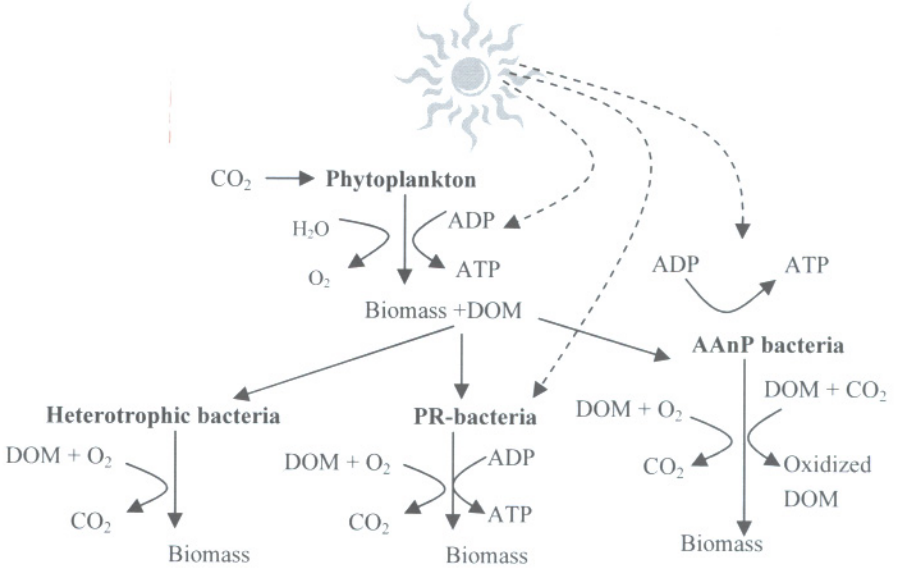

Figure 9. A schematic diagram illustrating the use of light by microbes, including two newly discovered bacterial groups, aerobic anoxygenic photosynthesizing (AAnP) bacteria and bacteria containing proteorhodopson (PR). Heterotrophic bacteria also synthesize ATP during the oxidation of DOM, which is not depicted in this diagram. Based on a more detailed diagram in Karl (2002).

One new microbial group, aerobic anoxygenic photosynthesizing (AAP) bacteria, is really not new in some sense, since culturable representatives had been isolated from sediments and the water column from a couple marine habitiats (Yurkov and Beatty 1998). In culture, these microbes use bacteriochlorophyll a (bchl a) to harvest light energy to carry out CO_2 fixation, analogous to oxygenic photosynthesizing cyanobacteria and eukaryotic algae and plants. Unlike oxygenic photosynthetic organisms, however, AAP bacteria do not use H_2O as the reductant for the synthesis of NADH needed for reducing CO_2; hence AAP bacteria do not evolve O_2 (they are anoxygenic) even though they tolerate aerobic conditions and even require O_2 (they are aerobic). Bacteria belonging to the alpha-proteobacterial subdivision with cultured AAP representatives had been

detected in the oceans (Giovannoni et al. 1990), but initial attempts to find bchl were unsuccessful, suggesting that oceanic AAP was insignificant.

More recently, Kolber et al. (2001) and Kolber et al. (2000) used biophysical techniques to detect AAP activity in the surface ocean and isolated AAP bacteria from a variety of oceanic habitats. The isolates were all closely related to *Erythrobacter*, a subgroup of alpha-proteobacteria. Using a molecular approach Beja et al. (2002) found *puf* genes, which direct the synthesis of the AAP light-harvesting complex, in uncultured marine bacteria more closely related to beta-proteobacteria than to alpha-proteobacteria. The reductant for these AAP bacteria remains unknown, but DOM is one possibility (Yurkov and Beatty 1998).

The other new microbial group, proteorhodopson-containing bacteria, is also not so new. Beja et al. (2000) completely sequenced a 130,000 base clone from a library constructed with DNA isolated from uncultured bacteria taken from the Pacific Ocean; they concentrated on this clone because screening for 16S rRNA genes indicated that it contained DNA from the SAR86 subgroup of gamma-proteobacteria. SAR86 had been found previously (Giovannoni et al. 1990), but more sequence data than just from the 16S rRNA gene were needed to indicate the presence of proteorhodopson. Rhodopson is a well characterized membrane protein found in the eyes of animals and in extreme halophilic archaea. In archaea, rhodopson can function as chloride ion pumps, photo-receptors, or light-driven proton pumps, the latter being capable of driving ATP synthesis. Likewise, proteorhodopson is thought to enable SAR86 bacteria to use light energy to synthesize ATP. Prior to the work of Beja et al. (2000), rhodopson had not been found in bacteria. In addition to the gene, Beja et al. (2001) found evidence of active proteorhodopson in surface seawater samples and suggested that the light absorption properties of proteorhodopson vary among oceanic habitats in response to the light environment.

The biogeochemical significance of these two new phototrophic microbes is still unclear. CO_2 fixation by AAP is probably much less than 10% of primary production (Kolber et al. 2001) and bchl a is <2% of chlorophyll a present in oxygenic primary producers (Goericke 2002). Kolber et al. (2001) estimated the abundance of AAP bacteria to be about 10% of total prokaryotic abundance, based on biophysical data, although molecular analysis indicates that the number could be much higher. Using FISH, Beja et al. (2001) found SAR86 to also comprise about 10% of total prokaryotic abundance in the Pacific Ocean, although Cottrell and Kirchman (2000b) were not able to detect it in coastal waters off California.

We need more information about both microbial groups because they have radically new, to the euphotic zone of the ocean, types of metabolisms. CO_2 fixation by AAP is at most a small fraction of total primary production,

and light harvesting by both groups is probably trivial compared to that of oxygenic phototrophic cyanobacteria and eukaryotic phytoplankton. What is potentially much more significant is their use of DOM. For starters, it is possible that our current methods for measuring heterotrophic bacterial production (thymidine and leucine incorporation) do not include these two new metabolic types, if for no other reason than bacterial production is measured in dark incubations. All other measures of DOM use are also done in dark incubations. DOM uptake cannot be greatly underestimated by our current methods, because that possibility has already been discounted by analyses of bacterial production and growth efficiencies (Ducklow et al. 2002). Still, at least one form of DOM use by AAP bacteria may not be captured by current measurements and budgets. To obtain reducing power for CO_2 reduction, these bacteria may partially oxidize DOM, without carbon assimilation, oxygen consumption or CO_2 production, and release a more oxidized, perhaps more refractory organic compound into the DOM pool. In short, our concepts about many aspects of DOM processes may be affected if these light-dependent microbes are abundant and active in the oceans.

9. SUMMARY

The community structure of marine bacterial and archaeal assemblages remains poorly understood in spite of much work over the past 15 years. In addition to more widespread use of current molecular methods, the application of novel techniques in genomics should greatly help us understand these uncultured microbes and reveal new types of microbes, as already demonstrated by the work on AAP and proteorhodopsin. It is now technically possible (although expensive) to completely sequence the microbial assemblage from a water sample and to reconstruct the genome of each microbe. Whole genome sequences from an increasing number of microbes in pure cultures will also help field-orientated microbial ecologists as will other genomic-based technology.

Once armed with this wealth of molecular data, microbial ecologists interested in DOM face two challenges. The first challenge is to use the molecular data to understand DOM uptake and in particular the detailed interactions between microbes and DOM. While molecular microbial ecologists have been describing the composition of microbial communities, molecular organic geochemists have been elucidating the composition of DOM and the mechanisms by which it is produced. Combining data from these two fields will not be easy but it will undoubtedly yield many insights into microbe-DOM interactions. As these interactions become clear, the second challenge will become more important to face: how do we use this

information to better understand carbon cycles and other biogeochemical processes at basin and global scales?

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