Limnol. Oceanogr., 54(5), 2009, 1501–1529

Microbial oceanography of the dark ocean's pelagic realm

Javier Arístegui,[a,*] Josep M. Gasol,[b] Carlos M. Duarte,[c] and Gerhard J. Herndl[d,e]

[a] Facultad de Ciencias del Mar, Universidad de Las Palmas de Gran Canaria, Las Palmas de Gran Canaria, Spain
[b] Institut de Ciències del Mar-CMIMA (CSIC), Barcelona, Spain
[c] IMEDEA (CSIC-UiB), Esporles, Spain
[d] Department of Biological Oceanography, Royal Netherlands Institute for Sea Research (Royal NIOZ), Texel, The Netherlands
[e] University of Vienna, Ecology Center, Department of Marine Biology, Vienna, Austria

Abstract

The pelagic realm of the dark ocean represents a key site for remineralization of organic matter and long-term carbon storage and burial in the biosphere. It contains the largest pool of microbes in aquatic systems, harboring nearly 75% and 50% of the prokaryotic biomass and production, respectively, of the global ocean. Genomic approaches continue to uncover the enormous and dynamic genetic variability at phylogenetic and functional levels. Deep-sea prokaryotes have comparable or even higher cell-specific extracellular enzymatic activity than do microbes in surface waters, with a high fraction of freely released exoenzymes, probably indicative of a life mode reliant on surface attachment to particles or colloids. Additionally, evidence increases that chemoautotrophy might represent a significant CO_2 sink and source of primary production in the dark ocean. Recent advances challenge the paradigm of stable microbial food web structure and function and slow organic-matter cycling. However, knowledge of deep-ocean food webs is still rudimentary. Dynamics of particle transformation and fate of the exported material in deep waters are still largely unknown. Discrepancies exist between estimates of carbon fluxes and remineralization rates. Recent assessments, however, suggest that integrated respiration in the dark ocean's water column is comparable to that in the epipelagic zone, and that the dark ocean is a site of paramount importance for material cycling in the biosphere. The advent of new molecular tools and in situ sampling methodologies will improve knowledge of the dark ocean's microbial ecosystem and resolve current discrepancies between carbon sources and metabolic requirements of deep-sea microbes.

The dark ocean is the largest habitat in the biosphere, comprising 1.3×10^{18} m^3. It is characterized by the absence of sufficient light to support photosynthesis, although the dim light below the epipelagic zone reaching to about 1000-m depth (the so-called "twilight zone") is sufficient to guide organism locomotion and orientation. This realm distinctly differs from the epipelagic ocean by its high pressure, low temperature, and high inorganic nutrient concentrations. It is subdivided into the mesopelagic zone (in this review considered as the depth range from 200- to 1000-m depth), with a water-mass residence time of decades and confined between the seasonal and the permanent thermocline, and the bathypelagic (1000–4000-m depth) and abyssal zones (>4000 m) with water-mass residence times of centuries. Most of the organic carbon resulting from photosynthesis in the epipelagic zone and transported into the dark ocean is respired in the mesopelagic zone by prokaryotes (Arístegui et al. 2005*b*) and hence may be returned back to the atmosphere as carbon dioxide in months to years. However, some of the organic carbon escapes microbial degradation in the mesopelagic and reaches the bathypelagic zone, where it may be respired and sequestered as CO_2 for centuries until the overturning circulation returns this carbon to the upper ocean, allowing exchange with the atmosphere.

The water column >200 m deep contains the largest pool of microbes in aquatic systems (Whitman et al. 1998). More than 95% of the dark ocean's prokaryotes have been reported to thrive as free-living organisms, largely contributing to suspended particulate organic carbon (POC) (Cho and Azam 1988; Turley and Mackie 1994). The remaining fraction of prokaryotes is associated with suspended and sinking particles, considered as "hotspots" for microbial growth and organic matter remineralization (Turley and Mackie 1994; Turley and Stutt 2000). Recent (meta)genomic studies, however, challenge this view that the vast majority of deep-ocean prokaryotes are truly free-living. Also, little is known about how prokaryotes respond to changes in the distribution, composition, and flux of organic carbon (Azam 1998), or which groups of microbes are responsible for most of the carbon recycling in deep waters.

The dark ocean is the largest reservoir of "active" organic carbon in the biosphere, mainly in the form of dissolved organic carbon (DOC) (Libes 1992; Hansell and Carlson 1998; Benner 2002). Most of the deep-ocean DOC pool is derived from biological processes that take place in the upper ocean. The organic carbon may be transported downwards via a suite of processes, including vertical flux from settling particles, active transport by migrating plankton, and DOC transport derived from water-mass sinking with overturning circulation and gradient-driven diffusive fluxes. Yet the realization of the potential importance of the dark ocean as a site of active food webs and carbon cycling is recent.

For a long time, the dark ocean was considered to be a site of negligible biological activity. Holger W. Jannasch, one of the pioneers of deep-sea microbiology, found in the fall of 1969 the contents of a lunch box from the Deep Submergence Vehicle *Alvin* to be well preserved, 10 months after the submersible accidentally sank to the deep sea. This evidence, together with nutrient enrichment experiments

* Corresponding author: jaristegui@dbio.ulpgc.es

carried out in the deep sea, led Jannasch and Wirsen (1973) to suggest that the activity of microorganisms in the deep sea is extremely low and largely confined to intestinal tracts of animals. Reports of relatively intense microbial activity in the dark ocean and the identification of mesopelagic prokaryotes as a major metabolic component of the ocean have emerged only over the last two decades (Cho and Azam 1988; Karl et al. 1988; Nagata et al. 2000). Growing evidence suggests that the mesopelagic realm of the world's ocean harbors active and diverse Bacteria and Archaea (Karner et al. 2001; Lopez-Garcia et al. 2001*b*; Kirchman et al. 2007) and highly diverse viral, protist, zooplankton, and nekton assemblages (Koppelmann and Weikert 1992; Countway et al. 2007; Fukuda et al. 2007).

Recent attempts to derive estimates of carbon cycling in the global ocean conclude that about one-third of biological CO_2 production in the ocean occurs in the dark pelagic layers (del Giorgio and Duarte 2002; Arístegui et al. 2005*b*). Nevertheless, carbon vertical inputs to the dark ocean at regional scales do not commonly match the estimated respiration rates in the water column (Carlson et al. 1994; Reinthaler et al. 2006; Baltar et al. 2009). As we detail, the imbalance can be attributable to uncertainties derived from both unaccounted carbon sources and inaccurate methodological approaches to estimating bulk respiration rates or prokaryotic carbon demand in the dark ocean.

Despite emerging evidence pointing at the dark ocean as a site with a major role in ocean biogeochemistry and as an untapped reservoir of high genetic and metabolic microbial diversity, this realm has been much less studied than surface waters because of difficulty in sampling and the time and expenses involved. Many microplankton (20–200 μm) are too delicate to be sampled with nets or large-volume filtration systems (Bishop et al. 1986), and pressure effects on microorganisms are still uncertain, because of difficulties in measuring microbial activities under in situ pressure conditions. Recent estimates of prokaryotic metabolic rates obtained under in situ pressure (Tamburini 2006; Tamburini et al. in press) contradict early studies (Jannasch and Wirsen 1973), which showed that elevated pressure decreases rates of growth and metabolism of natural microbial populations collected from the deep sea. Unfortunately, studies under in situ conditions are still too scarce to derive any universal conclusion on how pressure affects, directly or indirectly, the metabolism and trophic dynamics of autochthonous and allochthonous (transported via sinking aggregates) microbial communities in the deep ocean.

Here we review the current status of knowledge on sources and fates of organic matter in the dark ocean, phylogenetic and functional diversity and metabolism of dark-ocean microbes, and the contribution of dark-ocean microbes to carbon flux in the meso- and bathypelagic ocean. Where appropriate, comparison is made to the epipelagic (0–200 m) microbial community. We provide evidence for a key contribution of the microbial communities in the pelagic realm of the dark ocean to global carbon cycling, and we identify crucial gaps in knowledge, uncertainties, and future challenges that need to be addressed to provide a comprehensive account of their functioning in the largest habitat of the biosphere.

Organic matter supply to the dark ocean

Microbial communities in the dark ocean are supplied with dissolved organic matter (DOM) and particulate organic matter (POM) largely formed in the epipelagic zone and transported into the deep ocean (Fig. 1). The distinction between DOM and POM is functional because they represent interconnected pools (Simon et al. 2002; Engel et al. 2004; Verdugo et al. 2004). DOM is operationally defined as organic material passing through glass-fiber filters with an effective pore size of about 0.2–0.7 μm. However, colloidal particles >1 nm, which may constitute up to 50% of the DOM pool (Koike et al. 1990; Wells and Goldberg 1994; Kepkay 2000), some picoplankton cells (Li and Platt 1987; Gasol et al. 1997), and essentially all viruses pass through these filters. Additionally, aggregates and living cells may break down during the filtration step. DOM in surface waters consists of a mixture of very old, refractory DOM and a smaller fraction of young, bioavailable DOM (Benner 2002) produced in situ by plankton communities (Druffel et al. 1992). The latter fraction comprises mainly free amino acids, peptides, proteins, and mono- and polysaccharides, which are biodegradable in hours to days. Most of these biochemicals occur in combined form as oligomers and polymers, because concentrations of free sugars and amino acids represent $<10\%$ of total organic carbon in the surface ocean (Benner 2002). Bacteria can directly assimilate only low-molecular-weight compounds such as mono- and oligomers. Thus, extracellular enzymatic hydrolysis is required to cleave these macromolecules prior to uptake. Most of the bioavailable DOM is mineralized in the surface ocean, and a variable fraction is exported to the dark ocean.

Export DOM flux by vertical diffusive mixing through the thermocline is a significant source of organic matter to the upper mesopelagic zone in tropical and subtropical waters (Hansell 2002). However, most of the exported carbon is respired in the upper mesopelagic waters, contributing little to deep-water prokaryotic metabolism or carbon storage in the dark ocean. In contrast, convective mixing and subduction in temperate and cold seas (such as cold-water formation in the North Atlantic) effectively transport DOM down to the dark ocean. The Bermuda Atlantic Time-Series Study (BATS) station provides a well-documented example of the seasonal dynamics of DOM in temperate regions (Carlson et al. 1994). Surface cooling during winter leads to shallow (<300 m) convective mixing, allowing nutrients to be entrained into the upper ocean, supporting phytoplankton blooms (Michaels et al. 1996; Steinberg et al. 2001). Bioavailable DOC, produced during and after the bloom, accumulates within the surface mixed layer once the upper thermocline is established after warming of the surface ocean. The accumulated DOC resists microbial remineralization until the following winter, when it is rapidly metabolized by microorganisms once it is mixed into the mesopelagic zone (Carlson et al. 1994; Hansell and Carlson 2001). Carlson et al. (2004)

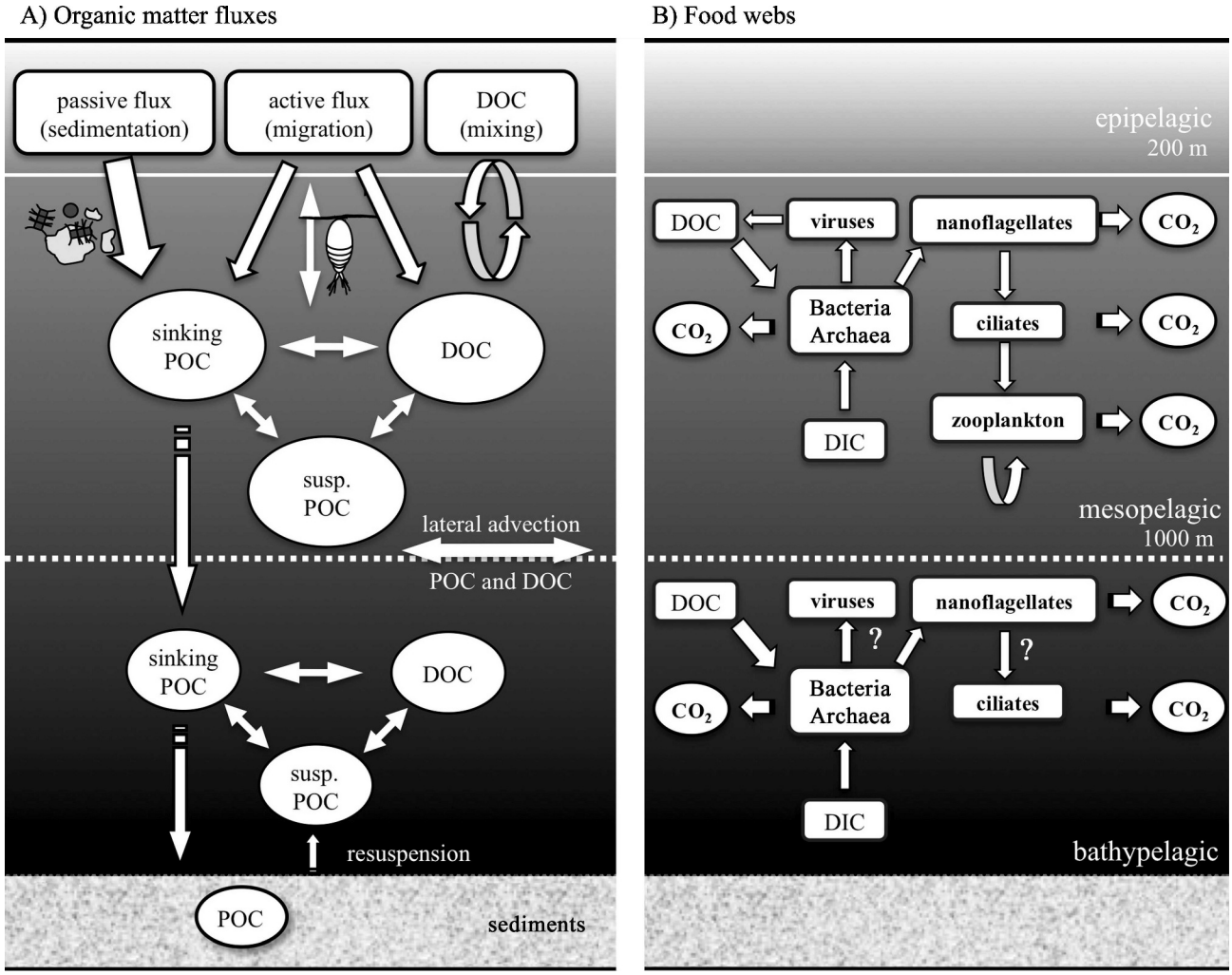

Fig. 1. Schematic simplification of (A) organic matter fluxes and (B) food webs in the mesopelagic and bathypelagic zones of the dark ocean. (A) Three interconnected pools of organic carbon are indicated: dissolved organic carbon (DOC), sinking particulate organic carbon (sinking POC) and suspended particulate organic carbon (susp. POC). DOC and susp. POC are susceptible to lateral transport in both the mesopelagic and the bathypelagic. The flux of carbon from the epipelagic to the dark ocean is because of passive sedimentation of POC, vertical mixing of DOC, or active transport of DOC and POC mediated by migrant zooplankton (*see* text for details). (B) Prokaryotes (Bacteria and Archaea) in the dark ocean may take up both DOC (heterotrophy) and dissolved inorganic carbon (DIC; chemosynthesis). Autotrophic production represents about one-third to one-half of the heterotrophic production in the mesopelagic. The control of prokaryotic assemblages in the mesopelagic zone is channeled through grazing by protozoans and viral lysis, whereas in the bathypelagic zone prokaryotic control by viruses or flagellates and the role of ciliates remain enigmatic (question marks). In the mesopelagic, carnivory by migrant and nonmigrant zooplankton (internal loop) contributes to the carbon fluxes (*see* text for details).

suggest that changes in microbial community structure between surface and deep waters may account, together with other factors, for the utilization of this DOC introduced into the mesopelagic layer. DOC transport by shallow convective mixing (Copin-Montegut and Avril 1993; Carlson et al. 1994; Hansell and Carlson 2001) provides, nevertheless, a relatively short pathway to transport DOC to the dark ocean. A more efficient mechanism of carbon sequestration is the overturning circulation at key sites where intermediate- and deep-water formation takes place (Hansell 2002). Bendtsen et al. (2002) estimated that the biodegradable part of the DOC pool in the dark ocean accounts for 2–15% of the total DOC in the water column, with the highest fraction in the subpolar regions and in areas with a high export production. At these sites, deep convective mixing, and later subduction, removes DOC from surface waters transporting it to the dark ocean (Hansell et al. 2004). The Mediterranean Sea might be an exception to this, because several studies have identified labile DOC transported to the deep ocean as the main source for carbon respiration (Christensen et al. 1989; Lefèvre et al. 1996; Zaccone et al. 2003). Overall, however, DOC exported with the overturning circulation accounts for only 10–20% of the global apparent oxygen utilization in the dark ocean (Arístegui et al. 2002). This supports the general view that the bulk of dark-ocean respiration is driven by the flux of particles (Honjo 1996; Bendtsen et al. 2002; Arístegui et al. 2005*a*).

Two general classes of particles can be distinguished based on their size and sinking properties: "suspended" particles ($\leq 10^2$ μm diameter) and "sinking" particles (10^2–10^4 μm; Verdugo et al. 2004). Particles, however, move up and down the particle size range through aggregation and disaggregation processes. Aggregation can increase the removal rate of suspended material by repackaging small particles into large, rapidly settling particles, whereas disaggregation operates in the opposite way (*see* review by Burd and Jackson 2009).

The majority of POM in the water column is formed by fine, suspended, neutrally buoyant particles (Kepkay 2000; Verdugo et al. 2004), although, paradoxically, the mechanisms of advective transport and remineralization of suspended POM are barely known (Bauer and Druffel 1998), in contrast to sinking POM (*see* review in Boyd and Trull 2007). Suspended POM may, however, represent an important carbon sink in boundary currents and continental margins not accounted for in global-scale carbon budgets. In a recent study, Alonso-González et al. (2009) estimated via a box-model approach the horizontal transport and consumption of suspended POC from surface waters to 3000-m depth in the eastern boundary Canary Current region (subtropical northeast Atlantic). Their results showed that suspended POC could support up to 60% of the carbon respiration in the Central Waters (100–700 m).

Compared to sinking particles collected at comparable depths, suspended particles in the mesopelagic zone of the open ocean have a distinct, more labile chemical composition (Repeta and Gagosian 1984; Wakeham and Canuel 1988; Druffel et al. 1998). Sheridan et al. (2002) found that organic matter alteration in the mesopelagic zone, and not cycling within the epipelagic zone, had the larger effect on the organic composition of deep-water suspended particles in the equatorial Pacific Ocean. They observed that this alteration was caused both by direct degradation of phytodetritus and by the dilution of the latter with material derived from microbial and zooplankton activities. Thus, microbes can directly degrade or alter suspended particles cleaved from the sinking POM pool.

Particles sinking out of the surface ocean originate mainly from phytoplankton and grazing activities (Thornton 2002; Burd and Jackson 2009), although they may be also derived from the aggregation of organic macromolecules (Billett et al. 1983; Small et al. 1983). Phytoplankton and prokaryotes may release gel-forming polysaccharides and macro-gels, which are critical in the process of formation of sinking aggregates as well as in the aggregation of diatom blooms (Alldredge et al. 1993; Passow and Alldredge 1994; Passow 2002). Large gels, several hundred micrometers or larger, may form in the surface ocean from the assemblage of macromolecules or colloidal material, resulting in a significant vector to move organic molecules up the particle size spectrum to become sinking particles (Verdugo et al. 2004). Engel et al. (2004) have recently described polysaccharide aggregation as an important pathway to convert DOC into POC during phytoplankton blooms, contributing effectively to the vertical carbon flux. These authors have suggested that aggregation processes in the ocean cascade from the molecular scale up to the size of fast-settling particles.

Macro-gels, such as transparent exopolymer particles (TEP; Alldredge et al. 1993; Passow 2002) are largely responsible for the aggregation of diatom blooms and the formation of large sinking aggregates (>500 μm), known as "marine snow" (Suzuki and Kato 1953; Alldredge and Silver 1988; Thornton 2002). These comprise complex microenvironments of dead and living plankton cells (Alldredge and Silver 1988), embedded into a sticky matrix of mucopolysaccharides supporting an active microbial community (Alldredge et al. 1986; Kaltenböck and Herndl 1992; Müller-Niklas et al. 1994). The prokaryotes provide an enriched food source for protists, which, in turn, attract larger metazoans (Azam and Long 2001).

Sinking rates of aggregates span three orders of magnitude, varying from <1 m d^{-1} for small particles to >1000 m d^{-1} for large aggregates (Smayda 1970; Alldredge and Gotschalk 1988; Trull et al. 2008). Alldredge and Gotschalk (1988) measured in situ sinking rates in aggregates collected by diving in surface oceanic waters. They observed that the rates varied as a power function of aggregate size and dry weight content, being consistently up to four times slower than rates predicted from laboratory experiments considering sinking velocities derived from the particles' volume and density alone. Nevertheless, there seems not to be a universal and simple relationship between sinking rates and particle dimension, although such relationships may exist locally (Burd and Jackson 2009). The recent use of novel techniques for determining the settling rates of particles in the mesopelagic ocean provides invaluable information to elucidate the mechanisms of biogenic carbon transfer to the deep ocean, although we are still far from knowing the temporal and spatial variability of these processes. Trull et al. (2008) used indented rotating sphere (IRS) sediment traps (Peterson et al. 2005) to estimate settling rates at the stations A Long-Term Oligotrophic Habitat Assessment (ALOHA; 22°45′N, 158°W), in Hawaii, and K2 (47°N, 160°E), in the subarctic north Pacific Ocean. They observed that approximately 50% of the POC flux was sinking at rates higher than 100 m d^{-1} at both stations. However, at ALOHA, $>15\%$ of particles sank at rates >820 m d^{-1}, whereas $<1\%$ of particles did so at K2. Particles with very low sedimentation rates (e.g., <5 m d^{-1}) could have been under-sampled because of the short deployment times (6.5 d) and the brine solution used to fill the collection cups (Trull et al. 2008). The average settling velocity at these two stations was about 200 m d^{-1}, similar to that recorded at the DYFAMED (DYnamique des Flux de mAtière en MEDiterranée; 43°25′N, 7°52′E) station in the Mediterranean Sea (Armstrong et al. in press). These results contrast with those obtained using the same IRS traps, deployed at similar depths, south of the Canary Islands, a region characterized by high remineralization rates in the mesopelagic zone (Arístegui et al. 2005*b*). Particles with settling velocities <5 m d^{-1} contributed $>50\%$ to the POC flux during June to December, but $<5\%$ during the other half of the year, along a 2-yr cycle (I. Alonso-González unpubl.).

Chemical and biological processes alter the relative composition of aggregates as they sink through the water column, thereby increasing or reducing their settling velocities (Ploug et al. 1999; Grossart and Ploug 2001; Berelson 2002). Minerals (like carbonates and lithogenic and biogenic silicates) influence particle sedimentation rates by adding ballast and accelerating transport to the deep sea (Armstrong et al. 2002; Francois et al. 2002; Klaas and Archer 2002). They also may protect organic matter from remineralization, through association with mineral grains (Hedges et al. 2000), although minerals' dissolution in the deep ocean may affect the preservation of organic matter during its descent (Bidle et al. 2002).

Among the largest sinking materials in the ocean are the pteropod webs, some gelatinous phytoplankton, and the discarded giant mucus houses of larvaceans (Alldredge and Madin 1982; Alldredge and Silver 1988; Silver et al. 1998*b*). These mucus houses (>1 m in diameter) contribute significantly to the vertical carbon flux in some regions, like the deep waters off the Californian coast (Robison et al. 2005). They are under-sampled with classical sediment traps, are very fragile, and can be observed only using undersea vehicles (Davoll and Youngbluth 1990; Silver et al. 1998*a*). When the house is clogged with particles, the animal discards it and generates a new one. The old house collapses and sinks to the ocean floor at a rate about 800 m d^{-1} (Hamner and Robinson 1992), giving little time for microbial remineralization in the mesopelagic zone. Although giant larvacean houses are numerically sparse (~1 per 100 m^3 off California; Silver et al. 1998*b*), their high replacement (once per day) and settling rates makes them an important source of carbon for the dark ocean (Robison et al. 2005).

Finally, migrant mesozooplankton, which engage in diel vertical migration across hundreds of meters, may contribute actively to the vertical flux of carbon into the dark ocean, by feeding on surface waters and releasing part of the ingested carbon to deep-water prokaryotic communities by direct DOC excretion or fecal pellet dissolution (Steinberg et al. 2000). Although some studies point to diel migrants as the main drivers of the vertical carbon flux in the water column (Denis et al. 2003), most of the published data on active transport of carbon by vertical migration of zooplankton fall within the range of 4–34% of the surface-ocean sedimentation carbon flux (Ducklow et al. 2001; Hernández-León and Ikeda 2005). The actual contribution of zooplankton migration to the downward transport of carbon is difficult to constrain, because of the problem of distinguishing fecal pellet production by migrant individuals from that by nonmigrant individuals. Nevertheless, excretion by migrant zooplankton has recently been suggested as one of the main sources of organic carbon supply to mesopelagic prokaryotes to account for their carbon demand (Steinberg et al. 2008). Migrant zooplankton form a layer known as the ''deep scattering layer'' at about 500–700-m depth across the oceans. The presence of local maximums in prokaryotic abundance and production at comparable depths observed in our meta-analysis (*see* following sections) suggests that the carbon flux from migrant zooplankton, which is likely to be highly labile, may indeed be responsible for the presence of a mid-depth maximum in prokaryotic activity. Evidence for this link between migrant zooplankton and enhanced prokaryotic activity at 500–700-m depth is, however, circumstantial and requires further investigation.

Particle transformation in the dark ocean

Overall, about 90% of the POC that sinks below the surface layer does not reach below 1000-m depth, being respired in the mesopelagic zone (Arístegui et al. 2005*a*). Karl et al. (1988) and Smith et al. (1992) argued that this loss of organic carbon could not be attributed only to remineralization processes on sinking particles, because the carbon demand of bacteria colonizing marine aggregates would be so low that months to years would be necessary to consume the particle's carbon. Their conclusion was based on estimations of carbon demand of attached bacteria, derived from bacterial production rates measured in pooled aggregates sedimented in incubation vials. Subsequent experimental studies carried out by Ploug and Grossart (1999) demonstrated that bacterial production is significantly higher when the aggregates are incubated individually and kept in suspension. Using this experimental approach, Ploug and coworkers (Ploug and Grossart 2000; Grossart and Ploug 2001; Ploug 2001) measured respiratory carbon turnover times of aggregates on the order of 1–2 weeks, pointing to a tighter coupling between POC and microbial respiration than previously assumed.

Karl et al. (1988) suggested that particle-associated prokaryotes solubilize large amounts of POC, which is released as DOC. Cho and Azam (1988) and Smith et al. (1992) found support for that theory, showing that bacteria inhabiting marine snow produce large amounts of ectohydrolases transforming POM into DOM at a rate faster than they can take up, leaving a plume of DOM in their wake. Recently, F. Baltar (unpubl.) found that the proportion of dissolved to total extracellular enzymatic activity increased with depth for a suite of enzymes (alpha- and beta-glucosidase, leucine aminopeptidase, and alkaline phosphatase). Based on the notion that particle-attached prokaryotes are releasing ectoenzymes into the environment, one may hypothesize that deep-water prokaryotes are more dependent on aggregated substrates than are surface-water prokaryotic communities. Protease and phosphatase activities are much higher than glucosidase activity, pointing to a preferential nitrogen and phosphorus cleavage compared to carbon, and hence a more efficient carbon export. A preferential decomposition of organic nitrogen as compared to organic carbon was also observed in experimentally derived diatom aggregates by Grossart and Ploug (2001). This ''uncoupled solubilization'' (Smith et al. 1992) could be a biochemical mechanism for a large-scale transfer of organic matter from sinking particles to the dissolved phase. Free-living prokaryotes, which make up the bulk of the microbial populations in deep waters, would benefit from the DOM plume, increasing their growth efficiency and allowing elevated growth rates (Azam and Long 2001; Kiørboe and Jackson 2001).

Free-living prokaryotes probably grow faster when embedded within nutrient patches around detrital particles (Blackburn et al. 1997, 1998). Grossart and Ploug (2000) report a growth efficiency of bacteria associated with fresh aggregates of 0.4–0.5, close to the values of bacteria growing on labile substrates (del Giorgio and Cole 1998). A large fraction (up to 70%) of free-living bacteria are motile (Mitchell et al. 1995; Fenchel 2001; Grossart et al. 2001). Many of these are able to sense nutrient gradients (Jackson 1989; Blackburn and Fenchel 1999; Kiørboe and Jackson 2001) and actively swim towards the aggregates assimilating organic compounds, which are solubilized by the colonizers (Fenchel 2001, 2002; Kiørboe et al. 2002). Once on the aggregate, bacteria may remain attached or detach within a few hours. Whether bacteria remain attached to an aggregate will depend on the inter- and intraspecific interactions among bacteria and between bacteria and their grazers (Grossart et al. 2003; Kiørboe et al. 2003). For instance, many bacterial isolates from marine aggregates display antagonistic activities towards other bacteria, which may inhibit their development and hence influence the community structure associated with particles (Martínez et al. 1996; Grossart et al. 2003).

Azam and Long (2001) suggested that colonizing bacteria may release their progeny into the plume, taking advantage of the DOM emanating from the particle, together with the free-living cells. This hypothesis is, however, questionable, in the light of recent findings that free-living and particle-attached prokaryotic communities are phylogenetically distinct entities sharing only a rather small number of phylotypes (Delong et al. 1993; Rath et al. 1998; Moeseneder et al. 2001*b*), although there are some contradictory results (Hollibaugh et al. 2000). Based on available evidence, Riemann and Winding (2001) postulated that free-living and particle-associated bacterial communities should be considered as interacting, rather than independent, assemblages. Kiørboe et al. (2002) provided further evidence showing that bacteria may attach and detach from aggregates at high rates (presumably to avoid the high grazing pressure by protozoa on particles). The extent of phylogenetic overlap would be dependent on the particle origin and the selective forces driving community succession towards a phylogenetic composition differing from that of the surrounding water.

In addition to prokaryotic activity on sinking aggregates, other physical and biological processes can aggregate and disaggregate particles, transforming small, suspended, and slow-sinking particles into large, fast-sinking ones, and vice versa. For example, during physical coagulation, particles of different sizes collide to form larger aggregates with higher sedimentation rates (McCave 1975; Jackson 1990; Burd and Jackson 2009). Sinking aggregates may scavenge small particles, enhancing their sinking rates (Kepkay 1994). The ''new'' particles would be exposed to intense extracellular enzyme activity in the aggregate, resulting in a gradual accumulation of refractory material and thus prolonging the persistence of the aggregate (Smith et al. 1992). This preferential remineralization of particles may increase particles' sinking rates if it removes relatively buoyant organic material, relative to denser organic material or mineral phases (Boyd and Stevens 2002). Mesopelagic zooplankton also fragment particles by the turbulence induced by their swimming activity or by grazing on them (Dilling et al. 1998; Goldthwait et al. 2004). Alternatively, they can repackage suspended material into denser, rapidly settling fecal pellets (Lampitt et al. 1990; Bochdansky and Herndl 1992; Kiørboe 2001). Conte et al. (2001) observed a marked decrease in the heterogeneity of the bulk composition of the sinking flux with increasing depth in the mesopelagic zone at the BATS station. They suggested that particle aggregation and fragmentation processes, like nonselective grazing by gelatinous zooplankton, homogenize the sinking and suspended particle pools. The latter acts as a reservoir that constantly exchanges with the sinking pool. During periods of low flux rates, net removal of mesopelagic suspended material might be greater than aggregation rates. This may partly explain the observed decoupling between upper-ocean and deep-ocean fluxes, as well as the systematic increase in particle size with depth, which is correlated with the progressive loss of organic carbon with depth, but not with the lithogenic content of the particles (Berelson 2002).

In summary, bioavailable DOM, the primary substrate supporting prokaryotic metabolism, becomes available to dark-ocean microbial communities through transformation and solubilization of particles, rather than via direct export of surface DOM into the dark ocean (Fig. 1). Both microbes and zooplankton are known to contribute to particle transformation (production and destruction) in the surface and subsurface ocean, and thereby to determine the magnitude of POC export, independently in many cases of the amount of net primary production. The relative contribution of these two groups to particle transformation has been, however, rarely quantified (Boyd et al. 1999). Neutrally buoyant suspended particles, which are much more abundant than sinking particles, may result from degradation of sinking material already altered by the activity of microbes and zooplankton, although suspended particles may at the same time aggregate and sink (Fig. 1). This suspended material seems to be more labile than the sinking POM collected at the same depths, and hence might constitute a valuable substrate for prokaryotic growth. Additionally, more refractory suspended POM, partly remineralized in shelf regions, may also be transported into the deep sea (de Haas et al. 2002). The global magnitude of the suspended POM pool still needs to be quantified. Almost all carbon-flux models predicting water column respiration are derived from the attenuation of the sinking POM flux with depth. Only a few incorporate DOM consumption. However, the consumption of the bioavailable fraction of the suspended POM has been almost ignored in carbon flux studies, despite the recognized importance of this pool (Bauer and Druffel 1998). There is evidence of preferential nitrogen and phosphorus cleavage compared to carbon cleavage in deep-water prokaryotes, thus contributing to a more efficient carbon export. However, there is still a major gap in our knowledge of the processes of organic matter decomposition caused by particle-attached and free-living microorganisms. Recent findings have shown that these two

communities can be phylogenetically distinct, and hence one would expect that they are also metabolically different.

Microbial diversity

Phylogenetic diversity of prokaryotes—Over the last decade, the advent of molecular tools in microbial ecology has stirred a revolution in our knowledge of prokaryotic diversity in the dark ocean. Our current perception is that there is a remarkably high diversity, considering the low abundances and production of organic matter in the dark ocean. Assessments of bacterial richness using terminal-restriction length polymorphism fingerprinting at the 16S rRNA level revealed that bacterial richness declines by only about 25% from the epipelagic layer to the bathypelagic waters (Moeseneder et al. 2001*b*; Hewson et al. 2006). Despite this low decline, distinct phylotypes are present in different depth layers, as indicated by fingerprinting techniques (Acinas et al. 1999; Moeseneder et al. 2001*b*; Hewson et al. 2006). Bacterial communities from the same depth layer of different ocean basins are less similar than are those within a specific basin (Hewson et al. 2006). Considerable variability in bacterial composition, however, is occasionally detectable even among adjacent deep-water stations, and is interpreted to result from episodic inputs of organic matter from surface waters (Hewson et al. 2006). This reported variability in deep-ocean prokaryotic communities is in contrast to our perception that the geographic dispersal of deep-water microbes is almost unlimited, resulting in a rather homogenous composition of assemblages derived from that in the deep ocean. However, this reported heterogeneity of prokaryotic communities agrees with the fact that the major deep-water masses maintain their physical and chemical characteristics over thousands of kilometers. Because free-living prokaryotes are non-sinking particles because of their small size, it is reasonable to assume long-term continuity in the prokaryotic communities of distinct water masses, although successional changes in community composition occur over time (Varela et al. 2008*b*). We suggest that the investigation of biogeographic patterns in deep-water prokaryotic communities should take into account the specific water masses, an aspect not adequately considered in studies of deep-water microbial communities thus far. Also, contextual data on water mass characteristics are commonly missing in the databases used to assemble phylogenetic trees for prokaryotic populations and communities, severely limiting the ecological interpretation of the molecular data obtained from microbial communities.

Generally, between 30% and 50% of the prokaryotic phylotypes detectable by fingerprinting techniques in meso- and bathypelagic waters are ubiquitously present, with the remaining phylotypes being present only in specific water masses (Moeseneder et al. 2001*b*; Hewson et al. 2006; Zaballos et al. 2006). Typical deep-water Bacteria are barotolerant or barophilic (also termed piezophilic, i.e., with an affinity to high pressure). Recent evidence suggests that piezophilic Bacteria originate from psychrophiles, because 16S sequences of all the known piezophiles share high similarity with those of non-piezophilic isolates from polar regions (Lauro et al. 2007). Some genera containing piezophiles (*Colwellia*, *Psychromonas*, *Moritella*) appear to be largely restricted to cold waters at various depths, being underrepresented in rRNA surveys (Acinas et al. 2004) and absent from environmental shotgun sequences obtained from temperate to subtropical surface waters (Venter et al. 2004). Low temperature and high pressure are known to affect specific biological processes, such as membrane fluidity and permeability, in a similar way (Delong et al. 1997; Lauro et al. 2007). Both psychrophiles and piezophiles contain lipids with highly unsaturated fatty acids critical to growth at high pressure (Lauro et al. 2007). Thus, it has been hypothesized that evolutionary, initial acclimation to high pressure is facilitated by preexisting adaptation to low temperature (Lauro et al. 2007). In support of this hypothesis, the genome sequence of the piezophile *Photobacterium profundum* (Vezzi et al. 2005) is similar in its gene content to that of the cold-adapted Antarctic bacterium *Pseudoalteromonas haloplanktis* TAC125 (Médigue et al. 2005). A metagenomic comparison of fosmid libraries between a prokaryotic community collected at 3000-m depth in the warm Mediterranean Sea deep waters ($\approx$14°C) and the Pacific station ALOHA indicated that the bathypelagic Mediterranean Sea community more closely resembled the mesopelagic rather than the bathypelagic community at station ALOHA (Martin-Cuadrado et al. 2007). Thus, temperature appears to be a major stratifying factor overriding hydrostatic pressure (Martin-Cuadrado et al. 2007). From the genome analysis of the piezophilic *P. profundum* strain SS9 it appears that adaptation to the deep sea involves a combination of modifications of gene structure and regulation (Vezzi et al. 2005) although piezo-specific genes have not yet been detected. In a recent survey, it has been found that the vast majority of piezophilic bacteria exhibit longer stems in the 16S rRNA gene at helices 10 (*Escherichia coli* positions 184–193) and 11 (*E. coli* positions 198–219) (Lauro et al. 2007). Although these two helices are hypervariable regions of the 16S rRNA, a thorough analysis revealed that the longer stems are an almost exclusive feature of piezophiles (Lauro et al. 2007). The proportion of ribotypes with longer stems is directly related to the optimal growth pressure in the piezophilic strains of *P. profundum* and *Shewanella* (Lauro et al. 2007). However, not all piezophilic gammaproteobacterial 16S rRNA gene sequences available in Genbank exhibit these long loops.

The distinct clusters of bacterial communities found in specific deep-water masses and depth layers as revealed by fingerprinting techniques (*see* above) are caused by the presence of unique phylotypes in distinct water masses or depth layers. Generally, the contribution of gammaproteobacteria to the total bacterial community increases with depth (Lopez-Garcia et al. 2001*a*). Among the bacterial phylotypes typical for meso- and bathypelagic waters, the *Chloroflexi*-type SAR202 cluster (belonging to the gammaproteobacteria group) is a prominent member (Morris et al. 2004; Varela et al. 2008*a*). SAR202 alone has been shown to comprise about 10% of all picoplankton cells in the mesopelagic Pacific and Atlantic (Morris et al. 2004), contributing up to 40% to the bacterial community in the

waters below 2000-m depth in the subtropical North Atlantic (Varela et al. 2008*a*). Remarkably, SAR202 not only increases in its contribution to bacterial abundance with depth but also increases in absolute abundance (Varela et al. 2008*a*). Other gammaproteobacteria typically found in clone libraries of deep waters are related to the genera *Colwellia*, *Shewanella*, *Alteromonas*, *Pseudoalteromonas*, and *Photobacterium* (Lopez-Lopez et al. 2005; Zaballos et al. 2006). Deltaproteobacteria, particularly the SAR324 cluster, are generally more commonly found in meso- and bathypelagic waters than in surface waters, as are the groups *Fibrobacter–Acidobacteria* and Planctomycetes, *Actinobacteria* (Zaballos et al. 2006; Martin-Cuadrado et al. 2007; Quaiser et al. 2008). Members of the Bacteroidetes group, common in near-surface waters, appear to be largely absent in deep waters. Fine-scale analysis of the SAR324 clade has revealed three distinct clusters, two occurring only in tropical and subtropical surface waters <250-m depth, whereas the previously described ubiquitous MGB/SAR324 clade group II occurs across latitudinal gradients but is generally restricted to deep, colder waters in lower latitudes (Brown and Donachie 2007).

In general, most of the sequences retrieved by the different clone libraries show only distant relations to known cultured strains, limiting therefore our ability to make predictions on the function of these Bacteria in the deep ocean. Clone libraries have well-known biases, precluding firm statements concerning the overall abundance of specific groups or phylotypes (Worden et al. 2006). Using pyro-sequencing on meso- and bathypelagic bacterial communities of the North Atlantic, a limited number of abundant groups has been identified belonging to the alpha- and gammaproteobacteria group (Sogin et al. 2006). However, an enormous richness of low-abundant phylotypes was found, indicating that the overall number of deep-water phylotypes is extremely high (Sogin et al. 2006). The evolutionary and ecological implication of this finding awaits further investigation.

Thus far, only the diversity of Bacteria in the deep waters has been discussed. Evidence accumulated over the past decade, however, that non-thermophilic Archaea may be, in terms of abundance, as important as Bacteria in the deep ocean (Karner et al. 2001; Herndl et al. 2005; Teira et al. 2006*a*). Archaeal diversity in the dark ocean appears to be lower than that of Bacteria (Massana et al. 2000). Using fingerprinting techniques for both Bacteria and Archaea on deep-water samples of the North Atlantic, the number of archaeal phylotypes was about half of that for Bacteria (H. Agogué unpubl.). As is true for Bacteria, also in Archaea the number of phylotypes does not decrease significantly with depth (De Corte et al. 2009). Similarly to the Bacteria, clusters of archaeal assemblages can be identified for different depth layers, with a wide mesopelagic cluster resembling the oxygen minimum layer. However, this clustering according to depth layers is not as distinct as it is for Bacteria (H. Agogué unpubl.).

Marine Archaea group I (Crenarchaeota) are particularly ubiquitous in the deep ocean, because (nearly) identical 16 rRNA gene sequences have been detected in different oceanic provinces (Fuhrman and Davis 1997; Massana et al. 2000). Marine Archaea group II (Euryarchaeota) and marine Archaea group IV (Korarchaeota) have been found in clone libraries from the deep waters of several oceanic provinces as well (Moeseneder et al. 2001*a*; Delong et al. 2006). In the upper mesopelagic realm Euryarchaeota exhibit a higher richness than Crenarchaeota, whereas Korarchaeota are generally far less common; however, the latter comprised 12% of the archaeal clones retrieved from the water column of the eastern Mediterranean Sea (Moeseneder et al. 2001*a*).

The distinct clustering according to depth layers and water masses indicates that the vertical and lateral exchange of members of the prokaryotic community is limited, reflecting the persistence of water masses as well-established boundaries in the ocean (Delong et al. 2006). From this distinct clustering, it also follows that the exchange between free-living and particle-attached prokaryotes has to be limited and that niche partitioning is common. Marine snow-type particles sinking through the mesopelagic water column harbor a distinctly different prokaryotic community compared to the free-living assemblages (Moeseneder et al. 2001*b*).

Overall, the diversity of Bacteria and Archaea in the dark ocean is almost as high as in the epipelagic layer, although the concentration of DOC is only about half of that in the sunlit surface ocean and the availability of organic carbon generally limits heterotrophic prokaryotic activity because inorganic nutrients are in excess of their demands.

Prokaryotic metabolic diversity—Comparative environmental genomics is emerging as a tool to predict metabolic modes of non-cultivable microorganisms (Delong et al. 2006). The only available integrated genomic survey of microbial communities along well-defined open-ocean environmental gradients (Delong et al. 2006) reports a high abundance of genes responsible for glyoxylate and dicarboxylate metabolism, corresponding to the relatively more oxidized and degraded DOM present in the deep ocean as compared to surface-water DOM (Benner 2002). Furthermore, deep-water prokaryotes are more enriched in genes for transposases, polysaccharide and antibiotic synthesis, protein export, and urea metabolism (Delong et al. 2006). The observed enrichment in polysaccharide and antibiotic synthesis genes in deeper-water samples has been interpreted as an indication of a potentially greater role for a particle-attached life style in deeper-water microbial communities. Transposases originate from diverse microbial taxa and might represent a mechanism to maintain genetic flexibility under slow growth conditions (Delong et al. 2006). Metagenomic analysis of a prokaryotic community collected at 3000-m depth in the Mediterranean Sea also revealed a high abundance of genes encoding transporters for dipeptides and oligopeptides and branched-chain amino acids (Martin-Cuadrado et al. 2007). The relatively high number of peptide and branched-chain amino acid transporters might indicate that proteins, potentially associated with marine snow or colloidal particles, are an important carbon source for

bathypelagic prokaryotes (Martin-Cuadrado et al. 2007). Evidence of the potential importance of a particle-attached life mode for deep-sea prokaryotes has been presented not only on the community or metagenomic level but also on the ecotype level (Ivars-Martinez et al. 2008). The deep-water ecotype of the common heterotrophic gammaproteobacterium *Alteromonas macleodii* exhibits a DNA sequence similarity to its counterpart, the surface ecotype, which is close to the border delineating the same species. The deep-water ecotype harbors genes indicative for life under microaerophilic conditions, for the degradation of recalcitrant compounds, and for particle attachment, whereas the surface ecotype has a greater potential to utilize sugars and amino acids.

High levels of chaperone-encoding genes were found in deep-sea prokaryotes as well (Delong et al. 2006). Chaperones maintain the tertiary structure of specific molecules such as ectoenzymes, essential under high-pressure conditions to ensure their functioning under changing pressure conditions (Boonyaratanakornit et al. 2002). This may be particularly relevant for particle-attached prokaryotes sedimenting through the meso- and bathypelagic water column but should be less relevant for non-sinking free-living cells. Taken together, the high levels of polysaccharide, antibiotic, and chaperone synthesis genes may indicate that microbial life in the meso- and bathypelagic realm is more concentrated on particles than assumed hitherto. Clearly, sampling strategies are needed to selectively collect deep-ocean particles to decipher the relative role of particle-attached vs. free-living microbes.

Remarkably high ectoenzymatic activities (alpha- and beta-glucosidase, aminopeptidase, alkaline phosphatase) have been detected in the bathypelagic deep ocean with cell-specific ectoenzymatic activity higher than in surface waters (F. Baltar unpubl.). These deep-water microbial ectoenzymes are probably well adapted to operate under high-pressure conditions in the cold, as shown for Arctic bacteria with temperature optima of below 20°C (Huston et al. 2000).

Generally, the cycling of organic carbon in the deep ocean proceeds at orders-of-magnitude lower rates than that in the surface ocean (Nagata et al. 2000; Reinthaler et al. 2006), as discussed in more detail below. Biogeochemically, the main role of the dark ocean's prokaryotes is the remineralization of organic compounds into carbon dioxide and inorganic nutrients, and hence the heterotrophic processing of organic matter using oxygen as terminal electron acceptor. In addition, a number of electron donors other than organic matter and electron acceptors other than oxygen are used in the oxygenated meso- and bathypelagic water column.

Novel metabolic pathways in deep-sea prokaryotes—Novel metabolic pathways in the dark ocean include genes encoding for dehydrogenases, which are more abundant than other enzyme-coding genes such as oxidases, reductases, and oxidoreductases in the bathypelagic Mediterranean Sea (Martín-Cuadrado et al. 2007). Among these dehydrogenase genes, those encoding for the different subunits of carbon monoxide dehydrogenase (*cox*L, *cox*M, *cox*S) were most represented (Martín-Cuadrado et al. 2007). In the metagenomic analysis of the surface waters of the Sargasso Sea (Venter et al. 2004), only *cox*L was detected in rather low abundance, whereas *cox*L gene abundance at Station ALOHA was much higher in the meso- and bathypelagic waters than in surface waters (Delong et al. 2006). The capacity to oxidize CO aerobically without a direct link to autotrophy, because these bacteria lack an autotrophic carbon fixation pathway, has been shown for several bacteria (King and Weber 2007). Hence, these bacteria are supplementing heterotrophy with the use of CO. In the deep waters of the Mediterranean Sea, *cox*L was related to Alphaproteobacteria, Actinobacteria, and Chloroflexi homologues (Martín-Cuadrado et al. 2007).

Another novel metabolic pathway represents the anaerobic methane oxidizers, forming consortia with sulfate reducers detected in cold seep sediments (Boetius et al. 2000). Although the occurrence of these associations in the deep-ocean water column is still uncertain, anaerobic ammonia oxidizers (anammox) of the *Planctomycetales* have been found recently in suboxic waters underlying upwelling regimes, such as the Benguela and the Peruvian systems, and the mesopelagic, oxygen-depleted waters of the Black Sea (Kuypers et al. 2003, 2005; Hamersley et al. 2007). At both upwelling sites, phylogenetic analyses of the *Planctomycetes*-specific 16S rDNA clone libraries indicated sequence similarities of >98% to the anammox bacteria candidate *Scalindua sorokinii* found in the Black Sea (Kuypers et al. 2003). It remains to be shown whether these anammox *Planctomycetales* occur in all the oxygen minimum zones of the global ocean. Apparently, anammox *Planctomycetales* are not restricted to the typical oxygen minimum zones, because they have also been found in the deep Mediterranean, leading the authors to speculate that they might originate from sinking particles with anoxic niches (Martín-Cuadrado et al. 2007). Present knowledge on the distribution of anammox *Planctomycetales* is that they thrive in very-low-oxygen environments (<10 μmol L^{-1} O_2), such as in the mesopelagic layers of upwelling areas or the oxygen minimum zone of the Arabian Sea (Jaeschke et al. 2007; Woebken et al. 2007).

The oxygen minimum zone of the Arabian Sea is the largest hypoxic region in the global open ocean, and plays an important role in the marine nitrogen cycle, because N loss has been estimated to amount to between 10 and 44 Tg N yr^{-1} in its oxygen minimum zone (Naqvi et al. 1998), representing between 10% and 40% of the global ocean's N loss (Devol et al. 2006). Measurements of nitrogen gas excess, however, predict a nitrogen anomaly larger than estimated by classical stoichiometric methods (Devol et al. 2006). This mismatch is assumed to result from incorrect assumptions of Redfield stoichiometry inherent to the nitrate deficit calculation, input of new nitrogen through N fixation, N_2 contributions from sedimentary nitrification along the continental margins, and the anammox reaction.

Non-thermophilic pelagic Crenarchaea have also been shown to be involved in the nitrogen cycle. The only Crenarchaea in culture thus far oxidizes ammonia as energy source (Könneke et al. 2005), and hence harbors the *amo*A gene, encoding one of the key enzymes, ammonia monooxygenase, common to all nitrifiers. A quantitative

survey of bacterial vs. crenarchaeal ammonia oxidizers in the mesopelagic waters of the North Atlantic and Pacific revealed that crenarchaeal *amo*A abundance outnumbers that of beta-proteobacterial *amo*A by at least 10-fold, whereas no gamma-proteobacterial ammonia oxidizers were detected (Wuchter et al. 2006; Mincer et al. 2007). Interestingly, also in soils crenarchaeal *amo*A genes are far more abundant than bacterial *amo*A genes (Leininger et al. 2006). Taken together, these studies from different environments indicate that Crenarchaea might be prominent ammonia oxidizers, particularly in the mesopelagic layer, where the main nitrification activity takes place. It has been hypothesized that ammonia-oxidizing Crenarchaea might synergistically interact with anammox *Planctomycetales* by providing nitrite as electron acceptor for anammox in suboxic pelagic waters to generate N_2. This hypothesis needs be tested thoroughly; however, recent evidence from the Black Sea provides some evidence for this mutual interaction (Lam et al. 2007).

Marine planktonic Crenarchaea show considerable sympatric sequence variation (Garcia-Martinez and Rodriguez-Valera 2000). Whether this sympatric sequence variation of some clusters translates into coexisting, ecologically different genomes is unclear at the moment. There is evidence that planktonic Archaea, both Euryarchaea and Crenarchaea, are taking up amino acids, thus exhibiting heterotrophy (Ouverney and Fuhrman 2000; Kirchman et al. 2007). Crenarchaea in bathypelagic waters take up D-amino acids (commonly known to be taken up at rather low rates) at least as efficiently as L-amino acids (Teira et al. 2006*a*,*b*). This peculiar feature of bathypelagic Crenarchaea may represent an adaptation to utilize an organic substrate barely used by other groups. It is likely that L-amino acids with their high turnover rates are depleted in the bathypelagic ocean. The heterotrophic life mode of bathypelagic Crenarchaea is further supported by the fact that archaeal *amo*A copy numbers, here indicative for an autotrophic life mode, decrease in the bathypelagic North Atlantic, particularly in the North Atlantic Deep Water (NADW), from north to south following the decline of ammonia as the NADW ages (Agogué et al. 2008). Similarly, archaeal *amo*A copy numbers decreased by one to two orders of magnitude from the mesopelagic to the bathypelagic waters of the eastern Mediterranean Sea, whereas crenarchaeal abundance did not exhibit a clear depth-related trend (De Corte et al. 2009). Genomic analysis of *Cenarchaeum symbiosum* revealed genes encoding for a modified 3-hydroxypropionate cycle of autotrophic carbon assimilation but also for the oxidative tricarboxylic acid cycle, indicating consumption of organic carbon (Hallam et al. 2006). This novel 3-hydroxypropionate-co-4-hydroxybutyrate CO_2 assimilation pathway has been shown to be of global significance (Berg et al. 2007).

C. symbiosum has the potential to function either as a strict autotroph, or as a mixotroph utilizing both carbon dioxide and organic matter as carbon sources (Hallam et al. 2006). The mixotrophic potential of marine Crenarchaeota group I has been confirmed by analyzing metagenomic libraries from different bathypelagic regions (Martín-Cuadrado et al. 2008). Indicative for the energy metabolism in marine Crenarchaeota group I, genes predicted to encode ammonia monooxygenase subunits, ammonia permease, urease, and urea transporters have been identified (Hallam et al. 2006). Evidence for a partly heterotrophic life mode of Crenarchaea has also been found by ^{14}C analysis of Crenarchaea-specific lipids at about 700-m depth in the Pacific Ocean off Hawaii, suggesting that about 70% of the crenarchaeal carbon requirements are covered by inorganic carbon fixation whereas 30% of the carbon demand is covered by organic matter utilization (Ingalls et al. 2006).

Planktonic group II Euryarchaeota are much less studied than group I Crenarchaeota. Consequently, much less is known about their metabolic requirements. Frigaard et al. (2006) found that surface-water Euryarchaea possess, like many other surface-water prokaryotes, proteorhodopsin, whereas deep-water Euryarchaea lack this pigment that acts as a light-driven proton pump. A comparative metagenomic analysis of group II Euryarchaeota from different bathypelagic regions including the South Atlantic and the Mediterranean Sea revealed that this group is enriched in putative anaerobic respiration components, suggesting that they gain energy by anaerobically respiring various compounds (Martín-Cuadrado et al. 2008).

Taken together, these recent findings shed new light on the magnitude of dark-ocean autotrophy. Together with Euryarchaeota and possibly also anammox *Planctomycetales*, Crenarchaeota seem to thrive in a predominately autotrophic life mode in the mesopelagic realm. The amount of inorganic carbon fixed in the meso- and bathypelagic North Atlantic has been estimated to amount to about 1 mmol C m^{-2} d^{-1} (Herndl et al. 2005). As the most important energy source driving this inorganic carbon fixation, the oxidation of ammonia has been generally assumed to be particularly mediated by nitrifying Crenarchaeota and Bacteria (Francis et al. 2007). Based on the integrated heterotrophic prokaryotic carbon production in the North Atlantic of 3.7 mmol C m^{-2} d^{-1} and a prokaryotic growth efficiency (PGE) of 4% for the mesopelagic realm, the heterotrophic carbon demand amounts to 92.5 mmol C m^{-2} d^{-1} (Reinthaler et al. 2006). Of this carbon demand, about 89 mmol C m^{-2} d^{-1} is respired and 13.5 mmol N m^{-2} d^{-1} released, assuming Redfield stoichiometry. Assuming that one carbon atom is fixed for ≈10 nitrogen molecules oxidized according to the nitrification stoichiometry, our measured inorganic carbon fixation of 1 mmol C m^{-2} d^{-1} would result in the oxidation of about 10 mmol NH_3 m^{-2} d^{-1}, which is close to the estimated release of 13.5 mmol N m^{-2} d^{-1} based on the heterotrophic prokaryotic carbon utilization in the mesopelagic realm of the North Atlantic. Thus, the measured prokaryotic organic matter utilization in the mesopelagic North Atlantic is potentially capable of sustaining the ammonia requirements as an energy source to maintain the measured inorganic carbon fixation rates. The other approach to constraining the predominately mesopelagic inorganic carbon fixation is via the sedimenting organic N flux. A compilation of sediment trap data from the North Atlantic (Antia et al. 2001) revealed a grand average of 5.2 mmol C m^{-2} d^{-1} or about 0.5 mmol N m^{-2} d^{-1} assuming a sinking POC : PN ratio of around 10 (Trull et al. 2008). Thus, the sinking particulate nitrogen flux as

determined by sediment traps is about 20 times lower than the estimated requirement to sustain the measured inorganic carbon fixation if ammonia oxidation is assumed as the sole energy source driving chemolithotrophy in the mesopelagic North Atlantic. Apparently, we are facing a similar problem with deep-ocean chemolithotrophy as with heterotrophic prokaryotic carbon demand. Although the nitrogen requirements to support chemolithotrophy might be met by heterotrophic organic matter remineralization, the sedimenting organic matter flux as revealed is at least one order of magnitude too low. Clearly, this discrepancy needs to be resolved. In any case, however, the measured inorganic carbon fixation of the deep ocean, presumably mainly by Crenarchaea, presents a newly synthesized organic matter source for the deep ocean's biota. If this measured chemolithotrophy is mediated mainly by free-living Crenarchaea, it will contribute to the non-sinking organic matter pool. Although the estimated 1 mmol C m^{-2} d^{-1} of deep-ocean inorganic carbon fixation represents only a fraction of the inorganic carbon fixed by phytoplankton production in the sunlit surface waters, it influences the dark ocean's carbon budget, because these prokaryotes represent a thus far largely unrecognized sink of carbon dioxide in the mesopelagic realm and are a source of newly fixed carbon in the deep ocean.

Protistan diversity—Compared to prokaryotic community composition, protistan diversity of the ocean has generally received much less attention. Although a number of protistan species can be determined microscopically because of specific morphological features such as ciliates (Foissner 1998; Katz et al. 2005), picoeukaryotes mostly lack diagnostic features to distinguish species under the microscope. Similarly to prokaryotic communities, molecular tools are required to shed light onto picoeukaryotic community composition. Most of the studies on picoeukaryotic community composition have focused on surface waters (Diez et al. 2001, 2004) or on specific deep-ocean sites, such as hydrothermal vents (Edgcomb et al. 2002; López-Garcia et al. 2003) or anoxic basins (Stoeck et al. 2003). Only a few studies report on the diversity and community composition of the picoeukaryotes in the oxygenated deep ocean (López-Garcia et al. 2001*b*; Lovejoy et al. 2006; Not et al. 2007).

The few studies focusing on meso- and bathypelagic picoeukaryotic diversity generally report a high diversity of thus far uncultured organisms, similarly to the situation in the meso- and bathypelagic prokaryotic communities (Countway et al. 2007; Not et al. 2007). Also similarly to deep-water prokaryotes, the picoeukaryotic communities inhabiting the dark ocean are distinctly different from the epipelagic communities, sharing only about 10% of picoeukaryotic taxa in the western North Atlantic (Not et al. 2007). Thus, there is an apparent stratification of protistan communities in the oceanic water column, as indicated by the higher similarity of protistan communities of the same depth strata of widely separated stations than within-station similarity across depth (Lovejoy et al. 2006; Countway et al. 2007). Generally, there is only a rather limited number of abundant picoeukaryotic taxa and a large number of rare picoeukaryotic taxa (Countway et al. 2007), again corresponding to recent findings of prokaryotic diversity in meso- and bathypelagic waters (Sogin et al. 2006). Based on cloning and sequencing efforts, it has been estimated that there are about 700–800 protistan taxa in the global ocean, with a diversity in the deep ocean about 30–60% (depending on the diversity indices used) lower than in the epipelagic layer (Countway et al. 2007). Thus, it appears that protistan diversity declines with depth slightly more than prokaryotic diversity does. This pronounced decline of protistan diversity with depth has been interpreted as an indication of a finite number of ecological niches present in the deep ocean for protists (Countway et al. 2007).

Apparently, many of the protistan taxa are globally distributed, because they have been reported from diverse sites in the global ocean (Lovejoy et al. 2006; Massana et al. 2006; Not et al. 2007). Among them are protistan taxa that appear more frequently in deep-water clone libraries than in libraries from the epipelagic waters. Based on the classification scheme for eukaryotes of Adl et al. (2005) dividing the eukaryotes into six super-groups, deep-water protists are distributed mainly over three super-groups: Rhizaria, Chromalveolata, and Excavata are commonly reported in the deep ocean, as well as fungi belonging to the Opisthokonta (Countway et al. 2007; Not et al. 2007). Stramenopiles occur also in deep waters (Countway et al. 2007); however, they are more common in the epipelagic realm (Lovejoy et al. 2006; Massana et al. 2006). The most abundant protistan taxa in the deep waters are Radiolaria, with the Acantharia and Polycystinea and Alveolata of the Chromalveolata super-group (Countway et al. 2007; Not et al. 2007). Alveolates comprised the major fraction of clone libraries from the deep western North Atlantic, with a high proportion of known ciliate and dinoflagellate lineages as well as the novel alveolate Groups I and II (Countway et al. 2007). Euglenozoa are more abundant in deep-sea than in epipelagic libraries (Countway et al. 2007). They have been reported from a wide range of deep-sea habitats, including the Polar Frontal Zone of the Southern Ocean (López-Garcia et al. 2001*b*), the anoxic Cariaco Basin (Stoeck et al. 2003), and hydrothermal vents (Edgcomb et al. 2002).

In general, the few studies available on protistan diversity of the oxygenated meso- and bathypelagic ocean indicate that the dark ocean harbors largely unique protistan communities with only minor contributions from epipelagic taxa. Only a limited number of protistan taxa occurs at high abundances, whereas a large number of rare protistan appears to thrive in the dark ocean, corresponding to our view on deep-water prokaryotic communities. As indicated by rarefaction analysis of sequence data from protistan clone libraries, larger clone libraries need to be sequenced to reveal the apparently large number of rare protistan taxa, again corroborating the notion emerging from studies on deep-water prokaryotic assemblages.

The dark-ocean microbial food web: Abundances and rates

For this review, we have compiled all data on microbial abundance and activity in the dark ocean published up to

mid-2007 to describe patterns of variability in the meso- and bathypelagic realms (database available from corresponding author). For all the vertical profiles where meso- or bathypelagic data were collected, we also registered the epipelagic values to compare the decrease with depth of the different variables, as well as the epi-, meso-, and bathypelagic integrated values (Table 1). From the prokaryotic abundance profiles, ca. 200 mesopelagic and ca. 100 bathypelagic profiles were sufficiently complete to allow integration over the specific depth layers and obtain estimates of integrated values. Fewer profiles were available for integrating production. Many fewer estimates were found for flagellate and viral abundances (Table 1). The rates of change with depth are expressed as slopes of the log (variable)–log (depth) relationship, although an alternative exponential model, wherein the exponential slopes represent the fractional change per meter, is also presented in Table 2.

Prokaryotic abundance—Prokaryotic abundance in the dark ocean declines logarithmically with depth (Fig. 2A). As an average, at mesopelagic depths prokaryotic abundance is 25% of the mean prokaryotic abundance in the epipelagic and 7% in the bathypelagic layer (Table 1); the exponential rate of decrease is −0.9, or a decrease by about 90% per 1000 m. A log–log representation of abundance vs. depth can be used to show the main trends in the change with depth. This rate of decrease is constant throughout all depths (Fig. 2A), although there are well-described ocean basin differences in the rates of decrease (Nagata et al. 2000; Yamaguchi et al. 2002). The overall log–log slope of decrease with depth (−0.553; Table 2) is similar to that determined by Tanaka and Rassoulzadegan (2002, 2004). Yamaguchi et al. (2002) report a range of −0.37 to −1.05 and Nagata et al. (2000) reported a range from −0.47 to −1.19. These latter slopes are nevertheless calculated in a slightly different way, and from 100-m depth downwards only.

The specific vertical profiles indicate that the decrease in abundance with depth is not monotonic, and that there are mid-depth anomalies corresponding to abundance maximums, particularly between 400- and 800-m depth in the mesopelagic (Sorokin 1971; Karl 1980; Carlucci et al. 1986), associated with water mass discontinuities. Sorokin et al. (1985), for example, found consistent abundance peaks at the upper boundary of the Antarctic Intermediate Water, and indicated that they seemed to be a general feature in the dark ocean. In some cases, these complex vertical profiles have been reported to be caused by lateral organic carbon supply (Nagata et al. 2000).

On average, integrated prokaryotic biomass in the epipelagic (133.8 ± 7.4 mmol C m^{-2}) is lower than the mesopelagic (average 204.5 ± 13 mmol C m^{-2}) and bathypelagic (209.3 mmol C m^{-2}) integrated biomass (Table 1; Fig. 3A). The integrated epipelagic prokaryotic biomass is closely related to the meso- and bathypelagic integrated biomasses (Fig. 4A). These relationships are best described by log–log least squares linear regression equations with slopes of 0.83 ± 0.05 and 0.74 ± 0.13 for the meso- and bathypelagic realms, and intercepts of 1.08 ± 0.25 and 1.44 ± 0.61, respectively ($n = 188$, $r^2 = 0.57$ for epipelagic–mesopelagic relationships; $n = 53$, $r^2 = 0.39$ for epipelagic–bathypelagic relationships). Overall, the compilation of values indicates that the epipelagic ocean comprises 24%, the mesopelagic ocean 37%, and the bathypelagic 38% of the total water column prokaryotic biomass (Fig. 3A). Thus, the dark ocean contains three-quarters of the pelagic marine prokaryotic biomass.

Nagata et al. (2000) provided evidence of latitudinal changes in the amount of biomass accumulated in the meso- and bathypelagic. They showed an apparent coupling with POC sinking rates, and suggested that this implied that the POC–DOC–bacteria pathway dominates the carbon flux in the dark ocean. Their data, in fact, suggest that prokaryotic biomass at 1000-m depth is linked to upper water column production and to latitude, but the slope of change with depth below 1000-m depth is fairly constant across their study sites. These latitudinal trends have not been found by Yamaguchi et al. (2004), who only detected latitudinal trends in mesozooplankton biomass. Also, the direct link of prokaryotic biomass to POC flux has not been found in the deep waters of the Arabian Sea using the extensive data set of the US–Joint Global Ocean Flux Study Arabian Sea program (Hansell and Ducklow 2003). This might indicate that bacterial activity is linked to suspended POC or DOC rather than to vertical POC flux.

There are few reports of how average prokaryotic size changes with depth (and thus, the slopes of abundance and biomass are very similar, because one is calculated from the other with a constant factor in Table 2). However, Rosso and Azam (1987) and Patching and Eardly (1997) report that the average cell size in the mesopelagic is higher than in the epipelagic realm. Bacteria of ~0.07 μm^3 biovolume were obtained from a depth of 1000 m, compared to the 0.04 μm^3 typical of epipelagic bacteria. There is evidence that the percentage of cells containing high levels of nucleic acids increases with depth in the North Atlantic and Antarctic oceans (Andrade et al. 2003; Corzo et al. 2005; Reinthaler et al. 2006). High-nucleic-acid–containing cells might reflect an overall larger genome size in deep sea than in epipelagic prokaryotes, possibly reflecting a higher level of cell-specific activity or a higher metabolic versatility at depth or a bigger cell size in the meso- and bathypelagic ocean.

Relative activity of single cells—Commonly, meso- and bathypelagic prokaryotes are thought to be less active than those inhabiting the epipelagic (Morita 1984), assuming that the low temperatures and substrate concentrations would necessarily lead to low activity. However, sufficient evidence has emerged over the last years to challenge this view. Several studies have shown seasonality in prokaryotic abundance in the dark ocean. Nagata et al. (2000) report a twofold change in prokaryotic abundance from a station sampled twice over a period of a few years. In the Mediterranean Sea, relatively large seasonal changes have been observed, particularly in the mesopelagic realm (Tanaka and Rassoulzadegan 2002, 2004). Meso- and bathypelagic prokaryotic communities should be active to exhibit these seasonal changes in abundance. Other

Table 1. Layer-averaged and layer-integrated values of the different variables, as well as the number of data considered (n). Because most of the data distributions are skewed, we also present the median values. The epipelagic data correspond only to stations for which meso- or bathypelagic data were available. Epipelagic from 0- to 200-m depth; mesopelagic from 200- to 1000-m depth; and bathypelagic from 1000- to 4000-m depth. Prokaryotic (Prok) abundance was converted to biomass assuming a carbon content of 15 fg C cell^{-1}, unless the authors provided their own estimates of both variables. Leucine incorporation was converted to carbon production using 1.55 kg C mol^{-1} leucine. HNF, heterotrophic nanoflagellate; PFR, Prok : HNF ratio; VLP, virus-like particles; VPR, VLP : Prok ratio.

	Prok abundance (cells mL^{-1})			Prok biomass (mmol C m^{-3})		Heterotrophic production (μmol C m^{-3} d^{-1})			Cell−specific production (fmol C cell^{-1} d^{-1})			Growth rate (d^{-1})		
	n*	Average±SE	Median	Average±SE	Median	n	Average±SE	Median	n	Average±SE	Median	n	Average±SE	Median
Epipelagic	1626	$7.85\times10^5(\pm0.24)$	5.27×10^5	0.96(±0.03)	0.66	874	136.5(±8.8)	35.8	701	0.199(±0.018)	0.063	676	0.157(±0.014)	0.050
Mesopelagic	933	$1.94\times10^5(\pm0.06)$	1.51×10^5	0.24(±0.01)	0.19	569	24.4(±5.0)	2.8	439	0.112(±0.017)	0.022	426	0.090(±0.014)	0.018
Bathypelagic	724	$0.59\times10^5(\pm0.02)$	0.43×10^5	0.07(±0.01)	0.05	425	4.0(±0.6)	1.1	385	0.076(±0.010)	0.023	375	0.061(±0.008)	0.019

	HNF abundance (cells mL^{-1})			PFR		VLP (particles mL^{-1})			VPR	
	n†	Average±SE	Median	Average±SE	Median	n‡	Average±SE	Median	Average±SE	Median
Epipelagic	324	$1.68\times10^3(\pm0.18)$	0.83×10^3	1239(±96)	771	126	$6.07\times10^6(\pm0.69)$	3.30×10^6	13.83(±0.91)	12.64
Mesopelagic	133	$0.14\times10^3(\pm0.01)$	0.09×10^3	1924(±276)	1707	74	$1.08\times10^6(\pm0.15)$	0.60×10^6	10.58(±1.06)	8.10
Bathypelagic	75	$0.13\times10^3(\pm0.06)$	0.01×10^3	4987(±715)	3331	64	$0.53\times10^6(\pm0.10)$	0.19×10^6	10.15(±0.91)	9.13

	Prok abundance (cells m^{-2})		Prok biomass (mmol C m^{-2})		Heterotrophic production (mmol C m^{-2} d^{-1})		HNF abundance (cells m^{-2})		VLP abundance (particles m^{-2})	
	n	Average±SE	n	Average±SE	n	Average±SE	n	Average±SE	n	Average±SE
Epipelagic	162	$1.10\times10^{14}(\pm0.08)$	205	133.8(±7.4)	151	15.26(±2.25)	25	$1.72\times10^{11}(\pm0.48)$	23	$0.95\times10^{15}(\pm0.15)$
Mesopelagic	164	$1.71\times10^{14}(\pm0.10)$	218	204.5(±13.0)	160	8.72(±1.90)	29	$1.01\times10^{11}(\pm0.06)$	21	$0.75\times10^{15}(\pm0.09)$
Bathypelagic	64	$1.94\times10^{14}(\pm0.17)$	96	209.3(±21.8)	37	5.45(±1.49)	8	$2.59\times10^{11}(\pm0.06)$	13	$1.46\times10^{15}(\pm0.13$

* n applies to Prok abundance and Prok biomass.
† n applies to HNF abundance and PFR.
‡ n applies to VLP and VPR.

Table 2. Log–log slopes and fits between depth and the different variables; also, the exponential slope of the decrease with depth of the different variables ("Exponential slope," $\ln X = a + b \times \text{depth}$, depth expressed in km). Either all data or data binned by depth are analyzed. The depth bins used had range limits of 0, 50, 100, 200, 400, 600, 1000, 2000, 3000, and the bottom. The data on prokaryotic respiration have been recalculated from Arístegui et al. (2003).*

Variable	*n*	r^2	Log–log slope±SE	*p* (slope = 0)	Exponential slope (km^{-1})	*p*
All data						
Prokaryotic abundance	3249	0.50	−0.553(±0.009)	<0.0001	−0.901	<0.0001
Prokaryotic biomass	3244	0.51	−0.551(±0.009)	<0.0001	−0.903	<0.0001
Heterotrophic production	1930	0.48	−0.968(±0.023)	<0.0001	−1.381	<0.0001
Prokaryotic growth rate	1546	0.11	−0.354(±0.026)	<0.0001	−0.502	<0.0001
HNF abundance	385	0.38	−0.655(±0.042)	<0.0001	−0.726	<0.0001
Prokaryote : HNF ratio	363	0.05	0.140(±0.034)	0.001	—	—
VLP abundance	264	0.39	−0.542(±0.042)	<0.0001	−0.880	<0.0001
Virus : prokaryote ratio	254	0.02	−0.069(±0.028)	0.017	—	—
Depth-binned data						
Prokaryotic abundance	9	0.99	−0.687(±0.022)	<0.0001	−0.818	0.002
Prokaryotic biomass	9	0.99	−0.683(±0.023)	<0.0001	−0.817	0.002
Heterotrophic production	9	0.95	−0.960(±0.087)	<0.0001	−1.162	0.002
Heterotrophic production†	7	0.87	−0.969(±0.164)	0.002	−0.875	0.002
Prokaryotic respiration†	7	0.78	−0.614(±0.143)	0.008	−0.605	<0.0001
Prokaryotic growth rate	9	0.68	−0.238(±0.062)	0.006	—	—
HNF abundance	9	0.67	−0.556(±0.148)	0.007	—	—
Prokaryote : HNF ratio	9	0.04	—	—	—	—
VLP abundance	9	0.91	−0.620(±0.072)	<0.0001	−0.631	0.003
Virus : prokaryote ratio	9	0.26	—	—	—	—

* HNF, heterotrophic nanoflagellate; VLP, virus-like particles.
† Only data from >100-m depth included.

evidences of relatively active populations in the mesopelagic ocean come from the work by Church et al. (2003), who found a higher proportion of cells at depth than near the surface detected by fluorescence in situ hybridization (FISH), assumed to correspond to high rRNA content. Arístegui et al. (2005*b*) obtained about the same proportion of actively respiring cells in the mesopelagic of the northwest Atlantic and in the epipelagic; however, the per-cell respiration was higher in the meso- than in the epipelagic. J. M. Gasol (unpubl.) observed in the same region that the rate of decrease with depth of cells with intact membrane potential was higher (average slope −0.91) than that of respiring cells (slope −0.51) and the average bulk cell abundance (−0.49). The rate of decrease with depth of high nucleic-acid content cells was also significantly slower (−0.31), suggesting that different processes act on each of these physiological properties, leading to shifts in the physiological performance of the community with increasing depth. Recently, micro-autoradiography in combination with catalyzed reporter deposition FISH (MICRO-CARD-FISH; Teira et al. 2004) indicated that about 16–20% of the heterotrophic prokary-

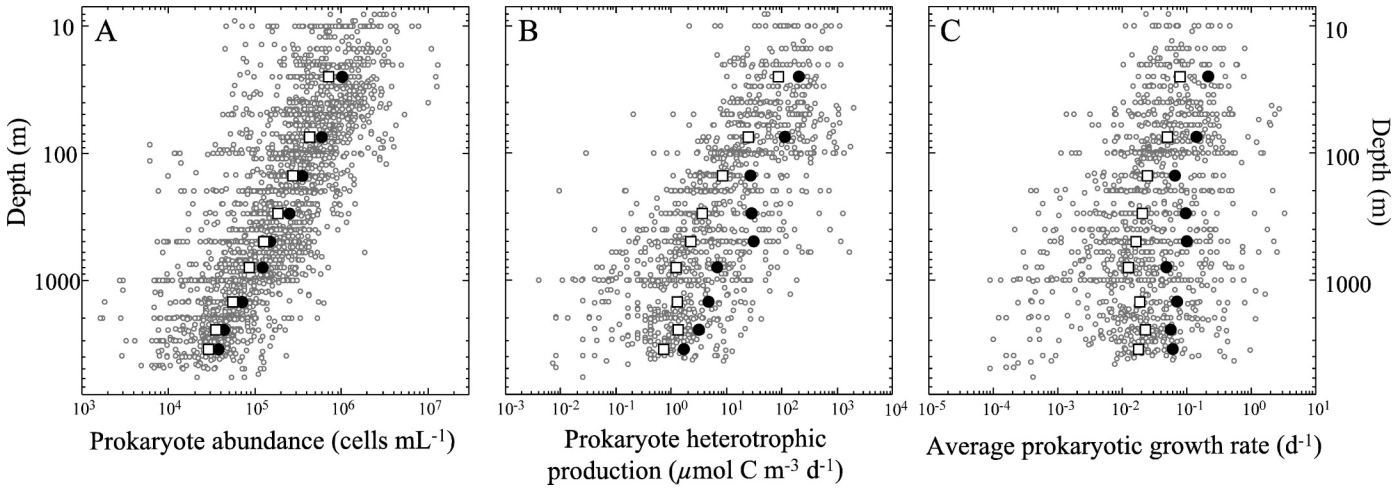

Fig. 2. Depth distribution of (A) prokaryotic abundances, (B) heterotrophic production, and (C) growth rates in the compiled dataset. We have eliminated all data <7-m depth, and computed the average value in variable depth bins (shown in all graphs as large black dots), and the median value per depth bin (grey dots).

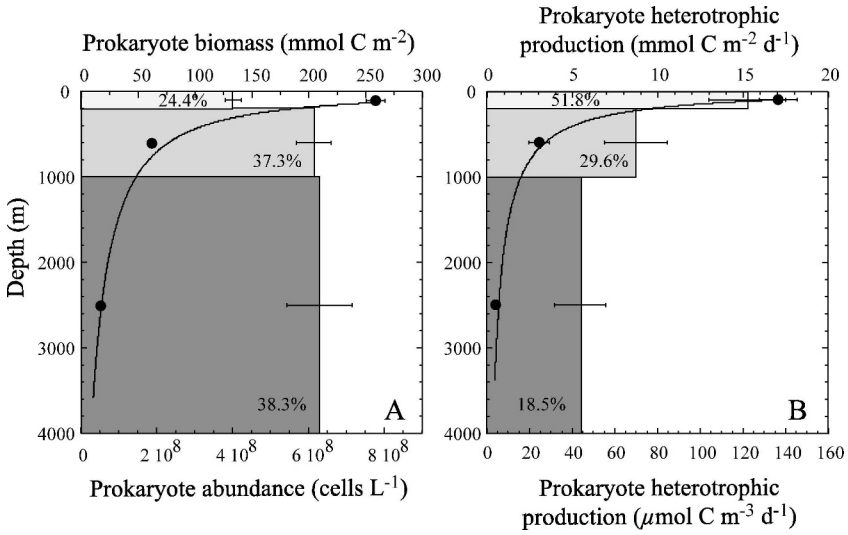

Fig. 3. (A) Average integrated prokaryotic biomass and (B) heterotrophic production in each of the three water layers ± SE (upper scale, bars), and percentage of total water column–integrated biomass that each water layer represents (number in boxes). Average water-layer prokaryotic abundance ± SE (lower scale, points) is given in panel A. Graph inspired by a figure in Turley (2002).

otic community of the meso- and bathypelagic North Atlantic is taking up leucine and can be considered as metabolically active (Herndl et al. 2005; Teira et al. 2006*a*), a percentage similar to that reported for epipelagic waters (del Giorgio and Gasol 2008). In the data set we have compiled, the cell-specific leucine incorporation rate varies less than threefold between the epi- and bathypelagic (Table 1), from 0.2 ± 0.02 fmol C cell^{-1} d^{-1} to 0.08 ± 0.01 fmol C cell^{-1} d^{-1} in deep waters. This 3-fold variation contrasts with the >10-fold decrease in abundance (Table 1).

Although the available information clearly indicates that deep-ocean prokaryotes are often as active as those living in the surface waters, further data are needed to resolve whether this is a general pattern in all oceanic regions or a peculiarity of specific regions.

Prokaryotic activity: Production, respiration, growth and efficiency—Vertical profiles of prokaryotic heterotrophic production exhibit a steep exponential decline in production with depth, spanning two to three orders of magnitude from the epi- to the bathypelagic ocean (Fig. 2B). On average, the volumetric heterotrophic production amounts to ~18% and 2% of the epipelagic production in the meso- and bathypelagic, respectively (Table 1). The decrease of production with depth is much steeper (average log–log slope −0.968) than that of biomass (Table 2), indicating that the cell-specific (or biomass-specific) rates of activity also decline with depth (with a slope of −0.35; Fig. 2C; Table 2). The slope for the depth-dependent decline in production given in Table 2 is similar to the mean log–log slope of −1.15 reported for the Mediterranean Sea (Tanaka and Rassoulzadegan 2004).

Our layer-averaged or median heterotrophic prokaryotic production data are higher than some of the values given in specific studies. Azam et al. (1992) report mesopelagic prokaryotic activity 1000–10,000 times lower than in the epipelagic zone in the Southern California Bight, and Dufour and Torreton (1996) report values of 0.002 μg C l^{-1} d^{-1} for the Atlantic Ocean. However, in a North Atlantic basin study, Reinthaler et al. (2006) report a heterotrophic prokaryotic production of 0.012 μg C l^{-1} d^{-1} (1 μmol m^{-3} d^{-1}) for the deep waters of the Central Atlantic. These values are all included in our data set, which implies that the range of variability of heterotrophic prokaryotic production in the dark ocean is as large as three orders of magnitude at any given depth (Fig. 2B). Furthermore, heterotrophic prokaryotic production may change temporally. Sherry et al. (1999) reported a fivefold seasonal variation for the northeast Pacific. This again indicates that our perception of a homogeneous dark ocean with steady slow processes requires revision.

As with prokaryotic biomass, peaks in biomass production have been detected at density interfaces (Sorokin 1973). A general trend of higher activity at ca. 500–600-m depth is frequently observed (Karl 1980). The presence of metabolically highly active microbial communities at intermediate depth layers might have a profound influence on the distribution of nonconservative chemical properties of seawater, the diagenesis and decomposition of sedimenting organic matter and the trophic organization of mesopelagic communities.

Determinations of prokaryotic heterotrophic production in the dark ocean must be considered with a certain degree of caution, because they depend critically on several assumptions. First, all the measurements reported in this review have been taken from decompressed samples. The effect of in situ pressure on prokaryotic production is still a matter of debate (Tamburini 2006), but several studies show prokaryotic production to be higher in samples incubated at in situ pressure than in depressurized samples (Tamburini et al. 2002). It is uncertain, however, whether the effect is derived from responses of the organisms themselves, the effect of pressure on the structure and

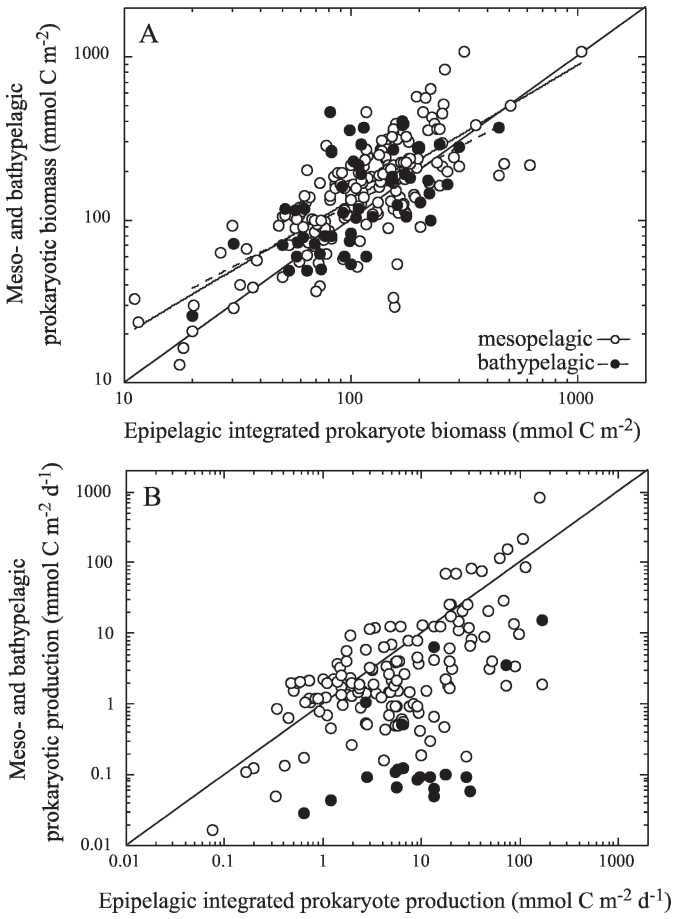

Fig. 4. (A) Relationship between the epipelagic-integrated prokaryotic biomass and the mesopelagic-integrated prokaryotic biomass (open circles) and the bathypelagic-integrated biomass (solid circles). (B) Relationship between the epipelagic-integrated prokaryotic heterotrophic production and the mesopelagic- (open circles) and bathypelagic-integrated heterotrophic production (solid circles). Shown are the regression lines (except in B), and the 1 : 1 relationship.

lability of the organic material, or both. Second, estimates are also affected by a complete lack of knowledge on the variability in the carbon-to-leucine conversion factor in the deep ocean, which in surface waters communities has been reported to vary >60 times (Alonso-Sáez et al. 2007). With all these caveats in mind, our data compilation shows that the decrease in production with depth is much higher than that in abundance (Table 2). Likewise, the decrease in production is higher than that of prokaryotic respiration, estimated also from decompressed samples, either from enzymatic electron transport system (ETS) activity (Arístegui et al. 2003) or actual oxygen consumption rates (Reinthaler et al. 2006). Provided the log–log slopes of −0.9 for the decline in production and −0.6 for the decline in respiration with depth (Table 2), PGE can be predicted to decrease as the −0.3 power of depth. Only a few data are available in the literature to test this hypothesis. If we assume a mean PGE in the epipelagic zone of ≈0.15 (del Giorgio and Cole 2000), then the predicted PGE in the bathypelagic realm, according to the above calculation, should be ≈0.04. Comparable PGEs have been reported for the deep waters of the North Atlantic (Reinthaler et al. 2006), whereas other studies report higher PGEs, either because of the methodologies used (Zaccone et al. 2003) or because the studies were carried out in productive boundary areas such as the mesopelagic zone of the Canary Current in the North Atlantic (Arístegui et al. 2005*b*). Needless to say, many more estimates of PGE must be obtained to validate our predictions and resolve patterns in the variability of PGE in the deep ocean.

Particularly, the meager database on prokaryotic respiration must be extended, and both prokaryotic heterotrophic production and respiration estimates from incubations should be performed under in situ conditions to account for pressure effects and avoid depressurization artifacts. By incubating under in situ conditions (i.e., without water manipulation and at in situ pressure), bottle effects would be minimized. However, respiration rates are so low in the deep ocean that very long (days) incubations are necessary to resolve changes in oxygen concentrations in the incubation bottles, increasing the risk of artifacts derived from changes in community structure. A promising future solution would be the use of high-precision oxygen electrodes placed inside large in situ incubation chambers (Drazen et al. 2005; Lefèvre and Tamburini pers. comm.). Although currently available oxygen sensors are still not sensitive enough as to sort out microbial respiration rates in the deep ocean, new generations of optical oxygen sensors (micro-optodes) may yield the necessary precision. Nevertheless, the derivation of reliable respiration rates in the deep ocean is a time-consuming exercise, which would constrain the data eventually available to map respiration in the global ocean. It would, however, contribute critical knowledge on the variability of the ratio between actual and potential respiration (R : ETS) in the deep ocean, allowing the use of enzymatic ETS activities as proxies to respiration rates (Arístegui et al. 2005*b*). This approach will permit the database to grow sufficiently as to be comparable with large-scale biogeochemical estimates of oxygen utilization rates (OUR) in the dark ocean (*see* final section).

Ectoenzymatic hydrolytic activity is considered the rate-limiting step for use of organic matter by prokaryotic plankton. Surprisingly, high phosphatase activities were detected in the mesopelagic waters of the Indian Ocean despite high phosphate concentrations (Hoppe and Ullrich 1999). This paradox has been interpreted as a strategy to cleave organic carbon moieties from macromolecules rather than acquiring phosphate in the generally carbon-limited dark ocean (Hoppe and Ullrich 1999). Higher cell-specific ectoenzymatic activities in the meso- and bathypelagic layers than in epipelagic waters are apparently a common feature of deep-sea prokaryotes (Hoppe et al. 1993; Tamburini et al. 2002), perhaps also a consequence of the larger cell size of dark-ocean prokaryotes. Recently, F. Baltar (unpubl.) found cell-specific alpha- and beta-glucosidase activities increasing from 100-m to about 3000-m depth by a factor of about 6 in the eastern Atlantic, whereas leucine aminopeptidase activity increased over the same depth range by a factor of about 4. Remarkably, alkaline phosphatase activity increased by more than

sixfold with depth, despite the high end product concentrations, i.e., phosphate, commonly found in deep waters. Thus, deep-sea prokaryotes might have to express more enzymes than epipelagic prokaryotes to cleave the same amount of hydrolysis products (Tamburini et al. 2002; F. Baltar unpubl.), which may also be a result of the lower hydrolysis rates associated with the low temperature of the dark ocean. This conclusion agrees with the low PGE commonly reported for the deep ocean (*see* above), and the higher rates of amino acid respiration in deep waters (Carlucci et al. 1986).

Whereas L-amino acids dominate the ambient amino acid pool and are commonly released by phytoplankton and through grazing, D-amino acid production in the marine environment is largely restricted to the peptidoglycan layer of bacteria (Pérez et al. 2003; Kawasaki and Benner 2006). Commonly, prokaryotic uptake of D-amino acids is rather low in surface waters compared to L-amino acid uptake (D : L-amino acid uptake ratio $\approx$0.01; Pérez et al. 2003). Consequently, the D-amino acids have been reported to be part of the recalcitrant DOM pool (Amon et al. 2001). Interestingly, however, the D : L-amino acid uptake ratio increases with depth, reaching D : L-amino acid uptake ratios of >1 below 1000-m depth in the Atlantic (Pérez et al. 2003). Recent work has shown that deep mesopelagic and bathypelagic Crenarchaea are preferentially utilizing D-amino acids (Teira et al. 2006*b*). Using MICRO-CARD-FISH, it has been shown that there are about twice as many crenarchaeal cells taking up D-amino acids than taking up L-amino acids in the NADW (Teira et al. 2006*b*).

Cell-specific production rates in mesopelagic waters may be considerably higher in particle-associated than in free-living bacteria (Karner and Herndl 1992; Turley and Stutt 2000). It has been proposed that large sinking particles are solubilized by their associated bacterial consortia releasing a fraction of the solubilized DOM into the ambient water, supporting free-living bacterial production both in the mesopelagic and bathypelagic zone (Cho and Azam 1988; Karl et al. 1988; Smith et al. 1992). However, apparent contradictory findings on the role of sinking detrital particles on mesopelagic prokaryotic production have been reported (*see* above sections). Sedimenting particles were found to substantially support prokaryotic activity in the mesopelagic waters of the Gulf of Oman, whereas in the Arabian Sea the sedimenting particle flux did not account for the prokaryotic production (Ducklow 1993).

Based on the idea that the substrate pool for prokaryotes is different at different depths, Harris et al. (2001) proposed that the rate of decrease in prokaryotic production with depth should be best described by two consecutive slopes: a steeper slope describing a faster rate of DOM utilization in the epipelagic zone, and a lower slope reflecting the slower utilization rate of the more recalcitrant DOM pool in the mesopelagic waters. The resulting contrasting rates varied fivefold among the two layers in the NW Mediterranean (Harris et al. 2001).

Nagata et al. (2000) studied the relationship between mesopelagic heterotrophic prokaryotic production and some potentially regulating factors, including temperature, to show that production was related to the semi-labile DOC concentration in the northern Pacific Ocean. They suggested that this deep-water DOC was introduced into the system by lateral isopycnal transport from adjacent highly productive oceanic areas. Nagata et al. (2000) also claimed that in the subarctic Pacific deep waters, prokaryotic activity is related to sinking POC flux, and thus, to primary production. However, they did not directly test this. In contrast, a thorough analysis of data available from the Arabian Sea led Hansell and Ducklow (2003) to conclude that only prokaryotic abundance at 200-m depth, and not prokaryotic activity, was related to the POC flux. Their results suggested that mesopelagic prokaryotic abundance and production might depend more on the long-term average POC input than those measured by short-term trap deployments. Simon et al. (2004) contributed to this debate by measuring mesopelagic prokaryotic heterotrophic production in the Polar Frontal Zone in the Atlantic Sector of the Southern Ocean and found that prokaryotic production in the mesopelagic waters is well correlated to surface chlorophyll and primary production. All these apparently contradictory results suggest that it is likely that the hydrographic regimens of specific areas and ocean basins determine whether meso- and bathypelagic prokaryotic abundance and activity are linked to the POC flux.

About 30% of the water column–integrated prokaryotic heterotrophic production occurs in the mesopelagic and $\approx$50% of the total water column heterotrophic prokaryotic production takes place below the epipelagic layer (Fig. 3B). Thus, the assumption of negligible prokaryotic activity in the dark ocean is unsubstantiated. Deep-water heterotrophic prokaryotes exhibit relatively lower growth efficiencies than their epipelagic counterparts, probably because of low organic matter supply combined with a more recalcitrant nature of the bulk DOM reaching the dark ocean. The supply rate of organic matter, however, might be sporadically high (Arístegui et al. 2005*b*). Our dataset on the meso- and bathypelagic prokaryotic abundance and activity indicates considerable variability (Fig. 2) suggesting that the dark ocean's realm is more dynamic and less homogenous than commonly assumed. The observation of significant cell-specific production rates together with moderate temporal variability in abundance strongly suggests that the microbial food web of the dark ocean is highly dynamic, and hence, prokaryote predators must play an important role in the dark-ocean ecosystem.

Heterotrophic nanoflagellate abundance—Pomeroy and Johannes (1968) were probably the first to report the presence of 2–10-μm-sized flagellates in the dark ocean. The first detailed flagellate profiles throughout an open ocean water column were presented by Sorokin et al. (1985), showing that flagellates are relatively abundant, particularly at those depths where prokaryotes accumulated (between 500- and 700-m depth). In these early papers, the abundance of flagellates at some depths was even higher than that of prokaryotes, reflecting the inadequate methods used to enumerate prokaryotes.

Mesopelagic heterotrophic nanoflagellate (HNF) abundance averages a few hundred cells mL^{-1} (Table 1;

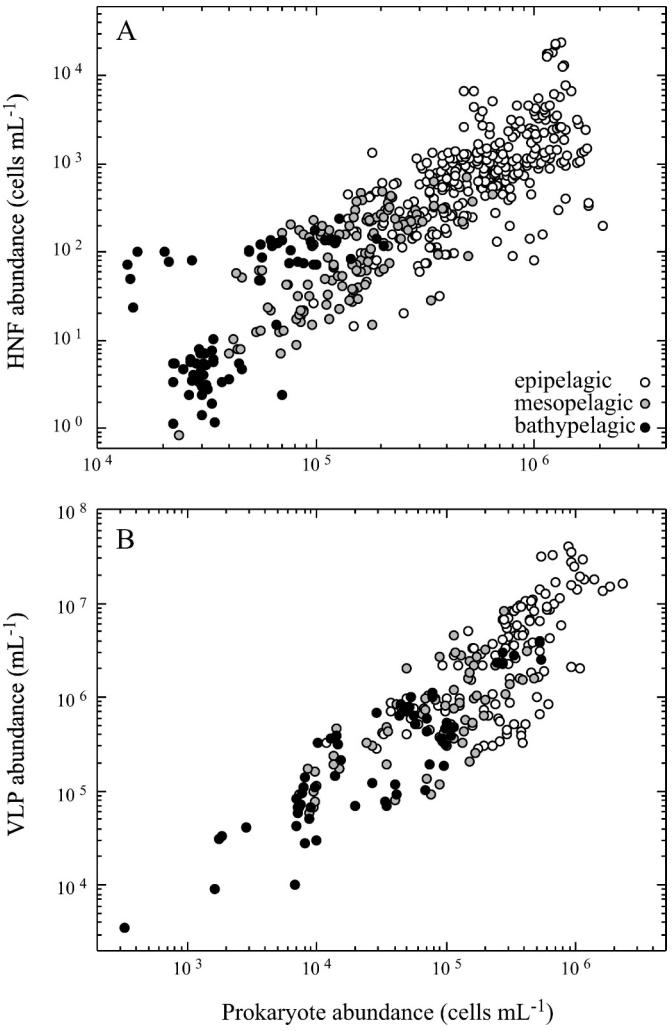

Fig. 5. Relationships between prokaryotic abundance and (A) heterotrophic nanoflagellate abundance and (B) viral abundance based on the dataset analyzed. Epipelagic (open circles), mesopelagic (grey circles) and bathypelagic (black circles) data.

Fig. 5A), whereas in the bathypelagic realm, HNF abundance ranges between a few organisms per mL to ≈100 cells mL^{-1} (Fig. 5A). Indeed, our analysis of available data shows HNF abundance to decrease with depth with log–log regression slopes rather similar to those for prokaryotic abundance (Table 1). A direct examination of the relationship between HNF and prokaryotic abundance shows that HNF abundances are significantly related to prokaryotic abundances (Fig. 5A), increasing more than prokaryotes, as reflected in a log–log slope significantly higher than 1 ($n = 545$, $r^2 = 0.68$, slope = 1.360 ± 0.040). This observation suggests that the prokaryote : flagellate ratio (PFR) should be lower in surface waters than at depth (Table 1). An analysis of the full dataset suggests, however, that there are less HNF than expected (or higher PFR) in the deep ocean. A closer look at the data indicates that this is because of the Mediterranean Sea data, with a much lower HNF abundance at similar depths than in the Atlantic or Pacific waters (Fig. 6A). This implies that the PFR is rather constant with depth, with a ratio of around 1000 prokaryotes per HNF (Fig. 6B), except in the Mediterranean Sea. The lower HNF abundance at a given prokaryotic abundance in the Mediterranean could be related to the relatively high abundance of ciliates in Mediterranean waters or to the higher temperature of meso- and bathypelagic Mediterranean waters. Some basic energetic considerations (Gasol 1994) suggest that the abundance of HNF in the dark ocean is likely constrained by the available food in the form of prokaryotic biomass, and that there are no additional sources of carbon for flagellates, nor any significant top-down control of HNF abundance in the dark ocean, except, perhaps, in the Mediterranean.

Deep-ocean HNFs are smaller than surface ones in the subarctic Pacific, with a mean HNF biovolume of 10.5 μm^3 in the epipelagic realm, compared to 7.11 and 6.57 μm^3 in the meso- and bathypelagic layers, respectively (Fukuda et al. 2007). In the Mediterranean Sea, however, the

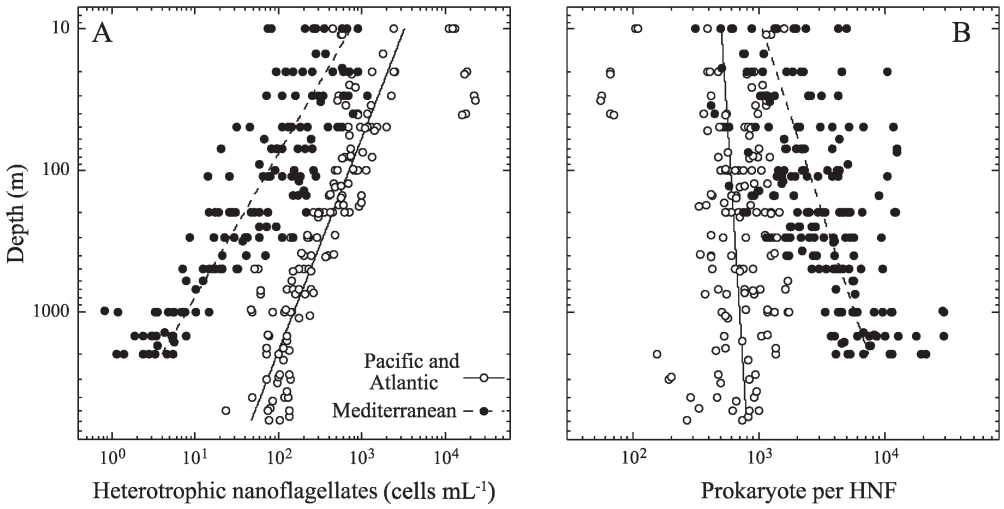

Fig. 6. (A) Depth profiles of heterotrophic nanoflagellate (HNF) abundance in the Pacific and Atlantic Oceans (open circles) vs. the Mediterranean (solid circles). The slope of the Mediterranean data is −0.99, whereas that of the other data is −0.66. (B) Number of prokaryotes per HNF in the Pacific and Atlantic Oceans (open circles) and the Mediterranean (solid circles).

biovolume of epipelagic HNF does not change significantly with depth (Tanaka and Rassoulzadegan 2002). The decrease in HNF biovolume with depth would contrast with the tendency of deep-ocean prokaryotes to increase in volume as compared to epipelagic prokaryotes (Patching and Eardly 1997).

Our global database on dark-ocean prokaryotes and HNFs suggests that HNFs should be as important as predators of prokaryotes at greater depth as in the epipelagic. In fact, the only study measuring mesopelagic flagellate grazing rates reported HNF clearance rates and volume-specific HNF clearance rates in the same range as obtained for epipelagic layers in the East Sea (1–11 nL HNF^{-1} h^{-1}) with peaks in the mesopelagic of 16 nL HNF^{-1} h^{-1} (Cho et al. 2000). These authors measured total HNF grazing rates of 0.1–4 $\times$ 10^3 cells mL^{-1} h^{-1} in the mesopelagic (Cho et al. 2000). With a typical mesopelagic prokaryotic abundance of 1.6 $\times$ 10^5 mL^{-1} (Table 1), these grazing rates imply specific prokaryotic loss rates of 0.02–0.6 d^{-1} (compare to average growth rates in Table 1). Grazing rates in the meso- and bathypelagic layers, however, are lower than they are in the epipelagic because of the temperature effect, well-known to affect HNF grazing rates (Peters 1994; Vaqué et al. 1994) and consistent with the metabolic effects of temperature. Average flagellate-specific grazing rates for epipelagic waters were 3.4 prokaryotes HNF^{-1} h^{-1} and 2 prokaryotes HNF^{-1} h^{-1} in the mesopelagic (Vaqué et al. 1994). Applying the equation given in Vaqué et al. (1994) and average HNF abundance and temperature, we arrive at an average flagellate grazing rate of $\approx$5 $\times$ 10^3 cells mL^{-1} d^{-1} and at a corresponding loss rate of ca. 0.03 d^{-1}. These values are lower than those measured by Cho et al. (2000), yet they represent the most likely rates one would expect. Although clearance rates are small relative to prokaryotic abundance, their slow growth rates suggest that grazing should remove most of the prokaryotic production, thereby exploiting their prey at the maximum sustainable rates. For the meso- and bathypelagic waters of the subarctic Pacific and Bering Strait, Fukuda et al. (2007) used typical clearance rates to calculate that HNF have the potential to consume most (48–70%) of the prokaryotic production. In conclusion, the few estimates available all point towards a high potential of HNFs to efficiently control the prokaryotic community of the dark ocean, at least in the mesopelagic zone (*see* below).

HNF predators are probably not very relevant in the deep ocean. Even though it is well known that ciliates inhabit sedimenting particles (Silver et al. 1984), few studies have measured the abundance of ciliates or other potential HNF predators. An abundance of 1–2 ciliates L^{-1} has been reported for the mesopelagic waters of the Arabian Sea (Gowing et al. 2003), whereas the mesopelagic waters of the western Mediterranean Sea harbor a ciliate abundance of 1–100 cells L^{-1} (Tanaka and Rassoulzadegan 2002). The slope of change of ciliate abundance with depth (average -1.1) obtained by Tanaka and Rassoulzadegan (2002) is much steeper than those for prokaryotes (-0.56) and HNF (-0.89), indicating that ciliate abundance is depleted in the bathypelagic waters relative to abundances of prokaryotes and HNFs. However, Yamaguchi et al. (2004) report twice as high dinoflagellate abundances in the deep-water layers of the north Pacific Ocean than HNF abundance at specific stations. These authors also stress that radiolarians may be an important component of the protist community at some stations (Yamaguchi et al. 2004). They also found zooplankton biomass exceeding microbial biomass in the mesopelagic layers. However, the rate of decrease of zooplankton biomass with depth is twice as steep as that of prokaryotes and protists, again indicating that the relative contribution of mesozooplankton to the total plankton biomass decreases with depth (Koppelmann et al. 2005). Protists, including HNFs, have been rarely studied in the dark ocean. Consequently, from a functional and diversity point of view, we know little about organisms other than prokaryotes in the deep ocean.

Based on the decrease in abundance of HNFs and prokaryotes with depth, the question emerges of how HNFs survive in an environment where the potential prey is rather diluted (prokaryotic abundance $<1 \times 10^5$ mL^{-1}). A threshold abundance of $\approx$2–3 $\times$ 10^5 prokaryotes mL^{-1} has been reported to provide sufficient encounter probability for HNFs to survive (Fenchel 1986). This threshold abundance of prokaryotes is widely accepted even though firm experimental tests about its validity are mostly lacking. Our compiled dataset on deep-ocean microbial abundance suggests that HNFs in the deep ocean are actively feeding on prokaryotes at concentrations below this threshold. It is possible, however, that HNFs are feeding on hotspots of prokaryotes associated with suspended POM or sinking marine snow–type particles. This possibility needs to be experimentally tested and requires the sampling of the fragile particles in the dark ocean to ensure the integrity of these hotspots.

Viral abundance and activity—Viruses are present in the surface waters of all aquatic environments at concentrations ranging from 10^6 to 10^9 mL^{-1} (Weinbauer 2004). They are also present in the meso- and bathypelagic ocean at concentrations ranging from 4 $\times$ 10^4 to 10^7 mL^{-1} according to the rather limited number of studies reporting viral abundance for the deep ocean (Hara et al. 1996; Wommack et al. 2004; Ortmann and Suttle 2005). Viral abundance is well correlated to that of prokaryotes (Fig. 5B; $n = 254$, $r^2 = 0.72$, slope 0.969 $\pm$ 0.04), with some scatter probably because of the different methods used to enumerate the viruses. Interestingly, some bathypelagic viral abundances are rather high ($\approx$3 $\times$ 10^6 mL^{-1}), particularly at sites where prokaryotic abundance is also relatively high (4–5 $\times$ 10^5 mL^{-1}) such as near hot vents (Ortmann and Suttle 2005). For the central Atlantic Ocean, high viral abundances have been found, with up to 1.6 $\times$ 10^6 viruses at 4000-m depth in the Antarctic Bottom Water (Parada et al. 2007). Some of the earlier reports on deep-water viral abundance might underestimate the actual viral abundance because viral decay in preserved samples can be rather high. Thus, prolonged storage of samples, as done in most of the earlier studies, might have led to substantial underestimates of the actual number of viruses present in situ (Wen et al. 2004).

In general, the relatively high viral abundance in the bathypelagic ocean is surprising, as is the fact that they are

related in the same way to prokaryotic abundance as in the epi- and mesopelagic realm (Fig. 5B). Overall, viral abundance decreases from the surface to deeper water layers with a rate (slope −0.54; Table 2) very similar to that of prokaryotic abundance. Although the decrease is often monotonic (Noble and Fuhrman 1998), in some cases, local depth-specific maximums do occur (Weinbauer et al. 2003). The virus : prokaryote ratio (VPR) decreases little with depth, from ≈14 in the epipelagic to 10 in the meso- and bathypelagic layer (Table 1). The rate of decrease with depth of the VPR is not significant (Table 2). In contrast, the Central Atlantic data of Parada et al. (2007), which are not included in our data analysis, indicate an increase in the VPR with depth, from 9 in the epipelagic to 110 in the Lower Deep Water (≈3500-m depth). For the Gulf of Mexico and for the Pacific, however, there was no systematic change in the VPR detectable from the epipelagic to the deeper water layers (2.8 ± 0.2 viruses prokaryote^{-1}), whereas in the Arctic, the Western Pacific, and the Mediterranean, the VPR in the epipelagic was higher (13 ± 1 viruses prokaryote^{-1}) than in the mesopelagic (5.6 ± 0.6 viruses prokaryote^{-1}) (Boehme et al. 1993; Hara et al. 1996).

Generally, viruses and flagellates are the main sources of mortality for prokaryotes. These two factors were compared in a single station in the Arctic, where viral lysis in the epipelagic was found to be slightly more important than flagellate grazing (2–16% of prokaryotic production and 1–8% in the mesopelagic; Steward et al. 1996). However, on average, only 13% of total prokaryotic production was explained by these two loss terms. Because other mortality sources could not be identified, this raises doubts about the methods used. Our average rates of change of viral and HNF abundance with depth (Table 2) indicate that HNF should be a more important source of mortality than viruses in the deep ocean, because, with the exception of the Mediterranean data discussed above, the PFR decreased with depth whereas the VPR did not.

In the dark and cold deep ocean, viral decay will not be mediated by the same physical factors (such as ultraviolet radiation) as in the ocean's epipelagic layer, and it could be expected that the life span of free viruses is much longer in the dark ocean, so that the high VPR does not reflect higher mortality, but higher accumulation of free viruses. However, the low abundance of prey suggests that the observed free viruses are in a state of maintenance and are unlikely to infect. To estimate the role of viruses on prokaryotic mortality, the frequency of visibly infected cells is commonly determined. In the Arctic, the frequency of visibly infected cells was similar in the meso- and epipelagic waters (Steward et al. 1996), but lower at the Mediterranean DYFAMED station, where 8.5–12% of the prokaryotes were infected in the epipelagic layer and only 1–6% in the mesopelagic (Weinbauer et al. 2003). The calculated virally induced mortality was also higher in the epipelagic (13–15%) than in the mesopelagic (3–5% of prokaryotic production), indicating that viruses are more important agents of prokaryotic mortality in epipelagic waters than at depth. However, estimating the relative importance of lysogeny using mitomycin C induction indicated that the frequency of lysogenic cells is significantly higher in the mesopelagic (16–39%) than in the epipelagic (11–13%) layers (Weinbauer et al. 2003). Analyzing data from other environments, these authors concluded that the contribution of lysogeny to prokaryotic mortality is negatively related to total prokaryotic abundance (Weinbauer et al. 2003). This may represent a survival strategy at low host abundance and activity such as in the dark ocean.

Indeed, a comparison of the predictions of the Gasol (1994) model relating prokaryotic and flagellate abundance and that of Weinbauer and Peduzzi (1995) relating viral and prokaryotic abundance suggests that HNFs should be the major source of prokaryotic mortality at low prokaryotic abundance. Viruses should be the most important source of mortality at high prokaryotic abundance. Thus, in the sparsely populated dark ocean, flagellates should be the main agents of prokaryotic mortality.

The relatively high viral abundance coinciding with the low prokaryotic abundance and production leads to the conclusion that the measured viral abundance cannot be sustained by the prokaryotes, and hence it is likely that the deep ocean viral community might originate, at least partly, from sedimenting particles or other allochthonous sources (Hara et al. 1996; Parada et al. 2007). Indeed, viruses have also been found in material collected in sediment traps and on sinking particles, where they may have infected up to 37% of the prokaryotic cells (Proctor and Fuhrman 1991). Similarly to prokaryotes, viruses might be adsorbed to particles where viral decay may be reduced (Wells and Deming 2006). Viral activity might also contribute to the dissolution of the particle by lysing attached prokaryotes and releasing DOC, thereby enhancing prokaryotic growth.

Parada et al. (2007) measured substantially longer viral turnover times (11–39 d) in the deep North Atlantic than the commonly reported viral turnover times of 1–2 d for epipelagic waters (Noble and Fuhrman 2000; Bongiorni et al. 2005; Cheng et al. 2007). The viral turnover times of 11–39 d reported by Parada et al. (2007) are, furthermore, much shorter than those of the co-occurring prokaryotes. Pulsefield gel electrophoresis fingerprints obtained from the viral community in the deep layers of the North Atlantic revealed a rather simple viral community, with a maximum of only 4 bands at 4000-m depth. Thus, there is an apparent paradox: a relatively high viral abundance in the deep ocean with a relatively long turnover time, coinciding with even longer prokaryotic turnover times, cannot be explained unless additional viral sources other than lytic production are invoked. Parada et al. (2007) suggest that there must be a rather large allochthonous viral input to the deep ocean via sedimenting particles or lateral input and, perhaps simultaneously, large lysogenic or pseudolysogenic viral production mechanisms. Taken together, these results also point towards a lack of viral control on the deep-water prokaryotic community. The apparent paradox, however, needs to be addressed in future studies.

Dark-ocean prokaryotes and the global carbon cycle

The view emerging from this synthesis is that prokaryotes in the dark ocean are as active on a per-cell level as in

the epipelagic layer, but they are much less concentrated. Flagellates, like prokaryotes, are also less concentrated than in the epipelagic zone, and probably exhibit lower activity but might control prokaryotic abundance in the mesopelagic zone in the same way as in epipelagic waters (Fig. 1). The role of viruses is less clear, but they are probably less active in the deep ocean than in the epipelagic layer, and the virus : prokaryote ratio tends to decrease with depth. The relatively high abundance of viruses in the dark-ocean waters cannot be explained at the moment but may be a result of fewer losses of free virus and a corresponding slow turnover of the free virus pool, allowing relatively high abundance despite low production rates. The low abundance of prokaryotes in the dark ocean may affect the probability of viruses encountering their proper host, a view not shared by some model analyses (Tanaka et al. 2005). In a now-vintage paper, Sorokin (1971) measured what he called ''potential bacterial production,'' which was the production developing after 0.45-μm–filtering water. Because the ''potential production'' was much higher than the actual production, he claimed there should be strong limitation by bacterial predators. Three decades later, our analysis supports this claim, but the direct measurements to support it are largely lacking. We have not learned much since 1971, and accurate rate measurements in the dark ocean are clearly on the agenda of needs.

This review also indicates that the dark ocean is a site of significant prokaryotic activity and associated carbon remineralization. Conventional depictions of the global carbon cycle, however, assign a remineralization role to the dark ocean involving the production of 11 Pg CO_2 yr^{-1} (IPCC 2001). This value represents one-third of the heterotrophic remineralization assumed to occur in the epipelagic zone (34 Pg C yr^{-1}; IPCC 2001), in contrast with the results derived from this synthesis indicating that dark-ocean prokaryotes account for nearly 75% and 50% of prokaryotic biomass and production, respectively, in the ocean (Fig. 3). Indeed, the role of the dark ocean in organic carbon respiration as depicted by the IPCC (2001) is not derived from an assessment of remineralization rates, but is calculated from the estimated vertical flux of POC and DOC of 11 Pg C yr^{-1}, accounting for these discrepancies. Recent assessments indicate that respiration rates by dark-ocean prokaryotes are likely to be much higher. Del Giorgio and Duarte (2002) estimated that respiration by dark-ocean prokaryotes is on the order of 22.3–29.6 Pg C yr^{-1}. Their rates are within the 20–33.3 Pg C yr^{-1} independently estimated by Arístegui et al. (2003) on the basis of a compilation of ETS measurements assuming an R : ETS ratio of 0.09, which had been derived experimentally from marine bacterial cultures in senescent phase (Christensen et al. 1980). These rates, expressed in oxygen units and in a volumetric basis, are in the range of 8–20 μmol O_2 m^{-3} d^{-1} for the mesopelagic zone, in close agreement with OUR inferred from large-scale tracer balances in the Atlantic Ocean (Jenkins 1982; Jenkins and Wallace 1992) and the Pacific Ocean (Feely et al. 2004). OUR are in theory a better approach than ETS to estimate respiration, because they integrate long-term and large-scale respiration rates and do not rely on conversion factors. Unfortunately, application of the method is restricted to a few oceanic regions and depth ranges where water mixing is minimal and the age of the water mass can be accurately estimated. Thus, a global-ocean respiration rate cannot be inferred from this method. More recently, Arístegui et al. (2005*a*) concluded, on the basis of a review of respiration rates and carbon inputs to the dark ocean, that dark-ocean respiration is on the order of 14.4–16.8 Pg C yr^{-1}, but indicated that the estimates might reach 26 Pg C yr^{-1}, although these rates would conflict with reported organic carbon supplies that reach a maximum of 19 Pg C yr^{-1} if DOC flux and lateral input of organic carbon are considered (Arístegui et al. 2005*a*). Moreover, Arístegui et al. (2005*b*) report that direct estimates of mesopelagic respiration rates in the subtropical NE Atlantic are much higher than those derived from ETS activity rates using conventional conversion factors, which assumed prokaryotes cells to be senescent, suggesting that microbial communities in the dark ocean may be far more active than hitherto assumed, as supported also by the review of prokaryote activity rates reported in the preceding sections. Indeed, our synthesis portrays a level of prokaryotic activity in the dark ocean comparable to that in the epipelagic that sets upper estimates of global prokaryotic remineralization rates in the dark ocean at about the 25–30 Pg C yr^{-1} of del Giorgio and Duarte (2002) and Arístegui et al. (2005*a*). In particular, our results show that the vertically integrated heterotrophic prokaryotic production in the dark ocean constitutes about 50% of the water-column total. Our results also show that PGE declines with depth and is threefold to fourfold lower, on average, in the dark ocean (0.04) than in the epipelagic (0.15). Because total carbon flux through prokaryotes equals the product of production and PGE, these robust findings suggest, taken in concert, that global prokaryotic remineralization rates should far exceed those in the epipelagic.

Such high remineralization rates seem quite phenomenal and difficult to reconcile with presently assumed estimates of organic carbon supply to the dark ocean, which would account for only slightly more than half of the prokaryotic carbon demand. Constraining this imbalance is a huge challenge for understanding of the global carbon cycle but will give us mechanistic insights into the functioning of deep-ocean microbial food webs and the amount of carbon sequestered in the ocean interior. Most likely, entire revision of the global carbon cycle will be necessary, particularly the export production estimates, to accommodate the emerging major role of the prokaryotic community in the dark ocean. In order to reconcile uncertainties between sources and sinks, accurate estimates of PGE and respiration rates are needed. The meager database of ''reliable'' ecological measurements in the dark ocean needs to be expanded by increasing the sensitivity of methods and by addressing bottle and pressure effects. This enhancement could be used to validate biogeochemical estimates, which are in theory the gold standard for microbial rate measurements, because they integrate large temporal and spatial scales, but which are potentially biased as well because of mixing of water masses. Also needed are more robust estimates of organic carbon input, because there is

evidence of severe underestimation by conventional techniques such as sediment traps. Several hypotheses have been formulated to address the mismatch between carbon inputs and remineralization rates in the dark ocean, such as a major role of migrant metazoans in the vertical transport of organic matter (Dam et al. 1995; Steinberg et al. 2000, 2008), or the unaccounted role of suspended particles as a carbon sink in the ocean (Alonso-González et al. 2009). Most importantly, this major discrepancy should also be considered as a major warning to remain alert to the possibility that autotrophic prokaryotic production in the dark ocean, through the recently discovered anammox and metabolic pathways yet to be discovered, may be far greater than currently assumed (Herndl et al. 2005), potentially helping to resolve the apparent discrepancy between organic carbon supply and microbial carbon demand. The application of functional genomic tools to assess metabolic diversity of dark-ocean microbes has already delivered new insights but is still in its infancy. We might expect major breakthroughs in the near future as understanding of the metabolic diversity of dark-ocean prokaryotes advances. Far from being the azoic waters they were once believed to be, the dark ocean will prove to be—through still largely enigmatic microbes and their metabolic diversity—a site of paramount importance for material cycling in the biosphere.

Acknowledgments

We thank H. Agogué, F. Baltar, R. Danovaro, T. Reinthaler, T. Tanaka, T. Yokokawa, and A. Yamaguchi for providing access to their raw data, and to all our colleagues for sharing their views on the deep ocean with us. Thanks are also due to Ramón Massana for reviewing the eukaryote diversity section. Two anonymous reviewers provided valuable comments that helped to improve the manuscript. This review was stimulated by K. Timmis and benefited from the collaborative projects ''Remolinos Oceánicos y Deposición Atmosférica'' (CTM2004-06842-C03) and MALASPINA 2010, funded by the Spanish National I+D Plan, and ''Transformation of dissolved organic matter (DOM) in the North Atlantic Deep Water and intermediate waters: assessing the functional and phylogenetic variability of marine bacterioplankton communities in relation to the quality of DOM'' (TRANSAT), ''Activity, rates, carbon use and high-pressure microbial ecology'' (ARCHIMEDES), and ''Influence of deep ocean viruses on prokaryotes'' (VIPeR) funded by the Dutch Science Fund, Earth and Life Science Branch (NWO-ALW) to G.J.H. This is a contribution to the European Union Networks of Excellence EurOceans and MarBef.

References

Acinas, S. G., J. Anton, and F. Rodriguez-Valera. 1999. Diversity of free-living and attached bacteria in offshore Western Mediterranean waters as depicted by analysis of genes encoding 16S rRNA. Appl. Environ. Microbiol. **65:** 514–522.

———, and others. 2004. Fine-scale phylogenetic architecture of a complex bacterial community. Nature **430:** 551–554.

Adl, S. M., and others. 2005. The new higher level classification of eukaryotes with emphasis on the taxonomy of protists. J. Eukaryot. Microbiol. **52:** 399–451.

Agogué, H., M. Brink, J. Dinasquet, and G. J. Herndl. 2008. Major gradients in putatively nitrifying and non-nitrifying Archaea in the deep North Atlantic. Nature **456:** 788–791.

Alldredge, A. L., J. J. Cole, and D. A. Caron. 1986. Production of heterotrophic bacteria inhabiting macroscopic organic aggregates (marine snow) from surface waters. Limnol. Oceanogr. **31:** 68–78.

———, and C. Gotschalk. 1998. In situ settling behavior of marine snow. Limnol. Oceanogr. **33:** 339–351.

———, and L. P. Madin. 1982. Pelagic tunicates: Unique herbivores in the marine plankton. Bioscience **32:** 655–661.

———, U. Passow, and B. Logan. 1993. The abundance and significance of a class of large, transparent organic particles in the ocean. Deep-Sea Res. I **40:** 1131–1140.

———, and M. W. Silver. 1988. Characteristics, dynamics and significance of marine snow. Prog. Oceanogr. **20:** 41–82.

Alonso-González, I. J., J. Arístegui, J. C. Vilas, and A. Hernández-Guerra. 2009. Lateral transport and consumption in surface and deep waters of the Canary Current: A box model study. Global Biogeochem. Cycles **23:** GB2007, doi:10.1029/2008GB003185.

Alonso-Sáez, L., J. M. Gasol, J. Arístegui, J. C. Vilas, D. Vaqué, C. M. Duarte, and S. Agustí. 2007. Large-scale variability in surface bacterial carbon demand and growth efficiency in the subtropical northeast Atlantic Ocean. Limnol. Oceanogr. **52:** 533–546.

Amon, R. M. W., H.-P. Fitznar, and R. Benner. 2001. Linkages among the bioreactivity, chemical composition, and diagenetic state of marine dissolved organic matter. Limnol. Oceanogr. **46:** 287–297.

Andrade, L., A. M. Gonzalez, F. V. Araujo, and R. Paranhos. 2003. Flow cytometry assessment of bacterioplankton in tropical marine environments. J. Microbiol. Methods **55:** 841–850.

Antia, A. N., and others. 2001. Basin-wide particulate carbon flux in the Atlantic Ocean: Regional export patterns and potential for atmospheric CO_2 sequestration. Global Biogeochem. Cycles **15:** 845–862.

Arístegui, J., S. Agustí, and C. M. Duarte. 2003. Respiration in the dark ocean. Geophys. Res. Lett. **30:** 1041.

———, ———, J. J. Middelburg, and C. M. Duarte. 2005*a*. Respiration in the mesopelagic and bathypelagic zones of the ocean, p. 181–205. *In* P. A. D. Giorgio and P. J. L. Williams [eds.], Respiration in aquatic ecosystems. Oxford Univ. Press.

———, C. M. Duarte, S. Agustí, M. Doval, X. A. Álvarez-Sagado, and D. A. Hansell. 2002. Dissolved organic carbon support of respiration in the dark ocean. Science **298:** 1967.

———, ———, J. M. Gasol, and L. Alonso-Sáez. 2005*b*. Active mesopelagic prokaryotes support high respiration in the subtropical northeast Atlantic Ocean. Geophys. Res. Lett. **32:** L03608, doi:10.1029/2004GL021863.

Armstrong, R. A., C. Lee, J. I. Hedges, S. Honjo, and S. G. Wakeham. 2002. A new, mechanistic model for organic carbon fluxes in the ocean based on the quantitative association of POC with ballast minerals. Deep-Sea Res. II **49:** 219–236.

———, M. L. Peteerson, C. Lee, and S. G. Wakeham. In press. Settling velocity spectra and the ballast ratio hypothesis. Deep-Sea Res. II.

Azam, F. 1998. Microbial control of oceanic carbon flux: The plot thickens. Science **280:** 694–696.

———, and R. A. Long. 2001. Sea snow microcosms. Nature **414:** 495–498.

———, D. C. Smith, and A. F. Carlucci. 1992. Bacterial transformation and transport of organic matter in the Southern California Bight. Prog. Oceanogr. **30:** 151–166.

Baltar, F., J. Arístegui, J. M. Gasol, E. Sintes, and G. J. Herndl. 2009. Evidence of prokaryotic metabolism on suspended particulate organic matter in the dark waters of the subtropical North Atlantic. Limnol. Oceanogr. **54:** 182–193.

Bauer, J. E., and E. R. M. Druffel. 1998. Ocean margins as a significant source of organic matter to the deep open ocean. Nature **392:** 482–485.

Bendtsen, J., C. Lundsgaard, M. Middelboe, and D. Archer. 2002. Influence of bacterial uptake on deep-ocean dissolved organic carbon. Global Biogeochem. Cycles **16:** 1127.

Benner, R. 2002. Chemical composition and reactivity, p. 59–90. *In* D. A. Hansell and C. A. Carlson [eds.], Biogeochemistry of marine dissolved organic matter. Elsevier Science.

Berelson, W. M. 2002. Particle settling rates increase with depth in the ocean. Deep-Sea Res. II **49:** 237–251.

Berg, I. A., D. Kockelkorn, W. Buckel, and G. Fuchs. 2007. A 3-hydroxypropionate/4-hydroxybutyrate autotrophic carbon dioxide assimilation pathway in Archaea. Science **318:** 1782–1786.

Bidle, K. D., M. Manganelli, and F. Azam. 2002. Regulation of oceanic silicon and carbon preservation by temperature control on bacteria. Science **298:** 1980–1984.

Billett, D. S. M., R. S. Lampitt, A. L. Rice, and R. F. C. Mantoura. 1983. Seasonal sedimentation of phytoplankton to the deep-sea benthos. Nature **302:** 520–522.

Bishop, J. K. B., M. H. Conte, P. H. Wiebe, M. R. Roman, and C. Langdon. 1986. Particulate matter production and consumption in deep mixed layers: Observations in a warm-core ring. Deep-Sea Res. **33:** 1813–1841.

Blackburn, N., F. Azam, and A. Hagstrom. 1997. Spatially explicit simulations of a microbial food web. Limnol. Oceanogr. **38:** 36–51.

———, and T. Fenchel. 1999. Influence of bacteria, diffusion and shear on micro-scale nutrient patches, and implications for bacterial chemotaxis. Mar. Ecol. Prog. Ser. **189:** 1–7.

———, ———, and J. Mitchell. 1998. Microscale nutrient patches in planktonic habitats shown by chemotactic bacteria. Science **282:** 2254–2256.

Bochdansky, A. B., and G. J. Herndl. 1992. Ecology of amorphous aggregations (marine snow) in the Northern Adriatic Sea: III. Zooplankton interactions with marine snow. Mar. Ecol. Prog. Ser. **87:** 135–146.

Boehme, J., and others. 1993. Viruses, bacterioplankton, and phytoplankton in the southeastern Gulf of Mexico: Distribution and contribution to oceanic DNA pools. Mar. Ecol. Prog. Ser. **97:** 1–10.

Boetius, A., and others. 2000. A marine microbial consortium apparently mediating anaerobic oxidation of methane. Nature **407:** 623–626.

Bongiorni, L., M. Magagnini, M. Armeni, R. Noble, and R. Danovaro. 2005. Viral production, decay rates, and life strategies along a trophic gradient in the North Adriatic Sea. Appl. Environ. Microbiol. **71:** 6644–6650.

Boonyaratanakornit, B. B., C. B. Park, and D. S. Clark. 2002. Pressure effects on intra- and intermolecular interactions within proteins. Biochim. Biophys. Acta **1595:** 235–249.

Boyd, P. W., and C. L. Stevens. 2002. Modelling particle transformation and the downward organic carbon flux in the NE Atlantic Ocean. Prog. Oceanogr. **52:** 1–29.

———, and T. W. Trull. 2007. Understanding the export of biogenic particles in oceanic waters: Is there a consensus? Prog. Oceanogr. **72:** 276–312.

———, and others. 1999. Transformation of biogenic particulates from the pelagic to the deep ocean realm. Deep-Sea Res. II **46:** 2761–2792.

Brown, M. V., and S. P. Donachie. 2007. Evidence for tropical endemicity in the Deltaproteobacteria Marine Group B/SAR324 bacterioplankton clade. Aquat. Microb. Ecol. **46:** 107–115.

Burd, A. B., and G. A. Jackson. 2009. Particle aggregation. Annu. Rev. Mar. Sci. **1:** 65–90.

Carlson, C. A., H. W. Ducklow, and A. F. Michaels. 1994. Annual flux of dissolved organic carbon from the euphotic zone in the northwestern Sargasso Sea. Nature **371:** 405–408.

———, S. J. Giovannoni, D. A. Hansell, S. J. Goldberg, R. Parsons, and K. Vergin. 2004. Interactions among dissolved organic carbon, microbial processes, and community structure in the mesopelagic zone of the northwestern Sargasso Sea. Limnol. Oceanogr. **49:** 1073–1083.

Carlucci, A. F., D. B. Craven, and K. J. Robertson. 1986. Microheterotrophic utilization of dissolved free amino acids in depth profiles of Southern California Borderland basin waters. Oceanol. Acta **9:** 89–96.

Cheng, K., Y. Zhao, X. Du, Y. Zhang, S. Lan, and Z. Shi. 2007. Solar radiation-driven decay of cyanophage infectivity, and photoreactivation of the cyanophage by host cyanobacteria. Aquat. Microb. Ecol. **48:** 13–18.

Cho, B. C., and F. Azam. 1988. Major role of bacteria in biogeochemical fluxes in the ocean's interior. Nature **332:** 441–443.

———, S. C. Na, and D. H. Choi. 2000. Active ingestion of fluorescently labeled bacteria by mesopelagic heterotrophic nanoflagellates in the East Sea, Korea. Mar. Ecol. Prog. Ser. **206:** 23–32.

Christensen, J. P., T. G. Owens, A. H. Devol, and T. T. Packard. 1980. Respiration and physiological state in marine bacteria. Mar. Biol. **55:** 267–276.

———, T. T. Packard, F. Q. Dortch, H. J. Minas, J. C. Gascard, C. Richez, and P. C. Garfield. 1989. Carbon oxidation in the deep Mediterranean Sea: Evidence for dissolved organic carbon source. Global Biogeochem. Cycles **3:** 315–335.

Church, M. J., E. F. Delong, H. W. Ducklow, M. B. Karner, C. M. Preston, and D. M. Karl. 2003. Abundance and distribution of planktonic *Archaea* and *Bacteria* in the waters west of the Antarctic Peninsula. Limnol. Oceanogr. **48:** 1893–1902.

Conte, M. H., N. Ralph, and E. H. Ross. 2001. Seasonal and interannual variability in deep ocean particle flux at the Oceanic Flux Program (OFP)/Bermuda Atlantic Time Series (BATS) site in the western Sargasso Sea near Bermuda. Deep-Sea Res. II **48:** 1471–1505.

Copin-Montegut, G., and B. Avril. 1993. Vertical distribution and temporal variation of dissolved organic carbon in the north-western Mediterranean Sea. Deep-Sea Res. I **40:** 1963–1972.

Corzo, A., S. Rodriguez-Gálvez, L. Lubian, C. Sobrino, P. Sangra, and A. Martinez. 2005. Antarctic marine bacterioplankton subpopulations discriminated by their apparent content of nucleic acids differ in their response to ecological factors. Polar Biol. **29:** 27–39.

Countway, P. D., R. J. Gast, M. R. Dennett, P. Savai, J. M. Rose, and D. A. Caron. 2007. Distinct protistan assemblages characterize the euphotic zone and deep sea (2500 m) of the western North Atlantic (Sargasso Sea and Gulf Stream). Environ. Microbiol. **9:** 1219–1232.

Dam, H. G., X. Zhang, M. Butler, and M. R. Roman. 1995. Mesozooplankton grazing and metabolism at the equator in the central Pacific: Implications for carbon and nitrogen fluxes. Deep-Sea Res. II **42:** 735–756.

Davoll, P. J., and M. J. Youngbluth. 1990. Heterotrophic activity on appendicularian (Tunicata: Appendicularia) houses in mesopelagic regions and their potential contribution to particle flux. Deep-Sea Res. I **37:** 285–294.

De Corte, D., T. Yokokawa, M. M. Varela, H. Agogué, and G. J. Herndl. 2009. Spatial distribution of *Bacteria* and *Archaea* and *amo*A gene copy numbers throughout the water column of the Eastern Mediterranean Sea. ISME J. **3:** 147–158.

de Haas, H., T. C. E. van Weering, and H. de Stigter. 2002. Organic carbon in shelf seas: Sinks or sources, processes and products. Cont. Shelf Res. **22:** 691–717.

del Giorgio, P. A., and J. J. Cole. 1998. Bacterial growth yield efficiency in natural aquatic systems. Annu. Rev. Ecol. Syst. **29:** 503–541.

———, and ———. 2000. Bacterial energetics and growth efficiency, p. 289–325. *In* D. L. Kirchman [ed.], Microbial ecology of the oceans. Wiley-Liss.

———, and C. M. Duarte. 2002. Respiration in the open ocean. Nature **420:** 379–384.

———, and J. M. Gasol. 2008. Physiological structure and single-cell activity in marine bacterioplankton, p. 243–298. *In* D. L. Kirchman [ed.], Microbial ecology of the ocean. Wiley.

Delong, E. F., D. G. Franks, and A. L. Alldredge. 1993. Phylogenetic diversity of aggregate-attached vs. free-living marine bacterial assemblages. Limnol. Oceanogr. **38:** 924–934.

———, ———, and A. A. Yananos. 1997. Evolutionary relationships of cultivated psychrophilic and barophilic deep-sea bacteria. Appl. Environ. Microbiol. **63:** 2105–2108.

———, and others. 2006. Community genomics among stratified microbial assemblages in the ocean's interior. Science **311:** 496–503.

Denis, M., and others. 2003. Pulsed remineralization in the northwestern Mediterranean Sea: A hypothesis. J. Mar. Syst. **39:** 19–41.

Devol, A. H., and others. 2006. Denitrification rates and excess nitrogen gas concentrations in the Arabian Sea oxygen deficient zone. Deep-Sea Res. I **53:** 1533–1547.

Díez, B., R. Massana, M. Estrada, and C. Pedrós-Alió. 2004. Distribution of eukaryotic picoplankton assemblages across hydrographic fronts in the Southern Ocean, studied by denaturing gradient gel electrophoresis. Limnol. Oceanogr. **49:** 1022–1034.

———, C. Pedrós-Alió, and R. Massana. 2001. Study of genetic diversity of eukaryotic picoplankton in different oceanic regions by small-subunit rRNA gene cloning and sequencing. Appl. Environ. Microbiol. **67:** 2932–2941.

Dilling, L., J. Wilson, D. Steinberg, and A. Alldredge. 1998. Feeding by the euphausiid *Euphausia pacifica* and the copepod *Calanus pacificus* on marine snow. Mar. Ecol. Prog. Ser. **170:** 189–201.

Drazen, J. C., L. E. Bird, and J. P. Barry. 2005. Development of a hyperbaric trap-respirometer for the capture and maintenance of live deep-sea organisms. Limnol. Oceanogr. Methods **3:** 488–498.

Druffel, E. R. M., S. Griffin, J. E. Bauer, D. M. Wolgast, and X.-C. Wang. 1998. Distribution of particulate organic carbon and radiocarbon in the water column for the upper slope to the abyssal northeastern Pacific Ocean. Deep-Sea Res. II **45:** 667–687.

———, P. M. Williams, J. E. Bauer, and J. Ertel. 1992. Cycling of dissolved and particulate organic matter in the open ocean. J. Geophys. Res. **97:** 15539–15659.

Ducklow, H. W. 1993. Bacterioplankton distribution and production in the northwestern Indian Ocean and Gulf of Oman, September 1986. Deep-Sea Res. **11:** 753–771.

———, D. K. Steinberg, and K. O. Buesseler. 2001. Upper ocean carbon export and the biological pump. Oceanography **14:** 50–58.

Dufour, P., and J.-P. Torreton. 1996. Bottom-up and top-down control of bacterioplankton from eutrophic to oligotrophic sites in the tropical northeastern Atlantic Ocean. Deep-Sea Res. I **43:** 1305–1320.

Edgcomb, V. P., D. T. Kysela, A. Teske, A. de Vera Gomez, and M. L. Sogin. 2002. Benthic eukaryotic diversity in the Guaymas Basin hydrothermal vent environment. Proc. Natl. Acad. Sci. USA **99:** 7658–7662.

Engel, A., S. Thoms, U. Riebesell, E. Rochelle-Newall, and I. Zondervan. 2004. Polysaccharide aggregation as a potential sink of marine dissolved organic carbon. Nature **428:** 929–932.

Feely, R. A., C. L. Sabine, R. Schlitzer, J. L. Bullister, S. Mecking, and D. Greely. 2004. Oxygen utilization and organic carbon remineralization in the upper water column of the Pacific Ocean. J. Oceanogr. **60:** 45–52.

Fenchel, T. 1986. The ecology of heterotrophic microflagellates. Adv. Microb. Ecol. **9:** 57–97.

———. 2001. Eppur si muove: Many water column bacteria are motile. Aquat. Microb. Ecol. **24:** 197–201.

———. 2002. Microbial behavior in an heterogeneous world. Science **296:** 1068–1071.

Foissner, W. 1998. An updated compilation of world soil ciliates (Protozoa, Ciliophora), with ecological notes, new records, and descriptions of new species. Eur. J. Protistol. **34:** 195–235.

Francis, C. A., J. M. Beman, and M. M. M. Kuypers. 2007. New processes and players in the nitrogen cycle: The microbial ecology of anaerobic and archaeal ammonia oxidation. ISME J. **1:** 19–27.

Francois, R., S. Honjo, R. Krishfield, and S. Manganini. 2002. Factors controlling the flux of organic carbon to the bathypelagic zone of the ocean. Global Biogeochem. Cycles **16:** 1087, doi:10.1029/2001GB001722.

Frigaard, N.-U., A. Martinez, T. J. Mincer, and E. F. Delong. 2006. Proteorhodopsin lateral gene transfer between marine planktonic Bacteria and Archaea. Nature **439:** 847–850.

Fuhrman, J. A., and A. A. Davis. 1997. Widespread Archaea and novel bacteria from the deep sea as shown by 16S rRNA gene sequences. Mar. Ecol. Prog. Ser. **150:** 275–285.

Fukuda, H., R. Sohrin, T. Nagata, and I. Koike. 2007. Size distribution and biomass of nanoflagellates in meso- and bathypelagic layers of the subarctic Pacific. Aquat. Microb. Ecol. **46:** 203–207.

Garcia-Martinez, J., and F. Rodriguez-Valera. 2000. Microdiversity of uncultured marine prokaryotes: The SAR11 cluster and the marine Archaea of group I. Mol. Ecol. **9:** 935–948.

Gasol, J. M. 1994. A framework for the assessment of top-down vs bottom-up control of heterotrophic nanoflagellate abundance. Mar. Ecol. Prog. Ser. **113:** 291–300.

———, P. A. del Giorgio, and C. M. Duarte. 1997. Biomass distribution in marine planktonic communities. Limnol. Oceanogr. **42:** 1353–1363.

Goldthwait, S., J. Yen, J. Brown, and A. Alldredge. 2004. Quantification of marine snow fragmentation by swimming euphausiids. Limnol. Oceanogr. **49:** 940–952.

Gowing, M. M., D. L. Garrison, K. F. Wishner, and C. Gelfman. 2003. Mesopelagic microplankton of the Arabian Sea. Deep-Sea Res. I **50:** 1205–1234.

Grossart, H.-P., T. Kiørboe, K. Tang, and H. Ploug. 2003. Bacterial colonization of particles: Growth and interactions. Appl. Environ. Microbiol. **69:** 3500–3509.

———, and H. Ploug. 2000. Bacterial production and growth efficiencies: Direct measurements on riverine aggregates. Limnol. Oceanogr. **45:** 436–445.

———, AND ———. 2001. Microbial degradation of organic carbon and nitrogen on diatom aggregates. Limnol. Oceanogr. **46:** 267–277.

———, L. RIEMANN, AND F. AZAM. 2001. Bacterial motility in the sea and its ecological implications. Aquat. Microb. Ecol. **25:** 247–258.

HALLAM, S. J., T. J. MINCER, C. SCHLEPER, C. M. PRESTON, K. ROBERTS, P. M. RICHARDSON, AND E. F. DELONG. 2006. Pathways of carbon assimilation and ammonia oxidation suggested by environmental genomic analyses of marine *Crenarchaeota*. PLoS Biol. **4:** e95, doi:10.1371/journal.pbio.0040095.

HAMERSLEY, M. R., AND OTHERS. 2007. Anaerobic ammonium oxidation in the Peruvian oxygen minimum zone. Limnol. Oceanogr. **52:** 923–933.

HAMNER, W. M., AND B. H. ROBINSON. 1992. In situ observations of giant appendicularians in Monterey Bay. Deep-Sea Res. **39:** 1299–1313.

HANSELL, D. A. 2002. DOC in the global ocean cycle, p. 685–715. *In* D. A. Hansell and C. A. Carlson [eds.], Biogeochemistry of marine dissolved organic matter. Elsevier.

———, AND C. A. CARLSON. 1998. Deep-ocean gradients of dissolved organic carbon. Nature **395:** 263–266.

———, AND ———. 2001. Biogeochemistry of total organic carbon and nitrogen in the Sargasso Sea: Control by convective overturn. Deep-Sea Res. II **48:** 1649–1667.

———, AND H. W. DUCKLOW. 2003. Bacterioplankton distribution and production in the bathypelagic ocean: Directly coupled to particulate organic carbon export? Limnol. Oceanogr. **48:** 150–156.

———, ———, A. M. MACDONALD, AND M. O. N. BARINGER. 2004. Metabolic poise in the North Atlantic Ocean diagnosed from dissolved organic matter transports. Limnol. Oceanogr. **49:** 1084–1094.

HARA, S., I. KOIKE, K. TERAUCHI, H. KAMIYA, AND E. TANOUE. 1996. Abundance of viruses in deep oceanic waters. Mar. Ecol. Prog. Ser. **145:** 269–277.

HARRIS, J. R. W., E. D. STUTT, AND C. M. TURLEY. 2001. Carbon flux in the northwest Mediterranean estimated from microbial production. Deep-Sea Res. I **48:** 2631–2644.

HEDGES, J. I., AND OTHERS. 2000. The molecularly uncharacterized component of nonliving organic matter in natural environments. Org. Geochem. **31:** 945–958.

HERNÁNDEZ-LEÓN, S., AND T. IKEDA. 2005. Zooplankton respiration, p. 57–82. *In* P. A. Del Giorgio and P. J. L. Williams [eds.], Respiration in aquatic ecosystems. Oxford Univ. Press.

HERNDL, G. J., T. REINTHALER, E. TEIRA, H. VAN AKEN, C. VETH, A. PERNTHALER, AND J. PERNTHALER. 2005. Contribution of *Archaea* to total prokaryotic production in the deep Atlantic Ocean. Appl. Environ. Microbiol. **71:** 2303–2309.

HEWSON, I., J. A. STEELE, D. G. CAPONE, AND J. A. FUHRMAN. 2006. Remarkable heterogeneity in meso- and bathypelagic bacterioplankton assemblage composition. Limnol. Oceanogr. **51:** 1274–1283.

HOLLIBAUGH, J. T., P. S. WONG, AND M. C. MURRELL. 2000. Similarity of particle-associated and free-living bacterial communities in northern San Francisco Bay, California. Aquat. Microb. Ecol. **21:** 103–114.

HONJO, S. 1996. Fluxes of particles to the interior of the open oceans, p. 91–154. *In* V. Ittekot, P. Schäfer, S. Honjo and P. J. Depetris [eds.], Particle flux in the ocean. Wiley.

HOPPE, H.-G., H. DUCKLOW, AND B. KARRASCH. 1993. Evidence for dependency of bacterial growth on enzymatic hydrolysis of particulate organic matter in the mesopelagic ocean. Mar. Ecol. Prog. Ser. **93:** 277–283.

———, AND S. ULLRICH. 1999. Profiles of ectoenzymes in the Indian Ocean: Phenomena of phosphatase activity in the mesopelagic zone. Aquat. Microb. Ecol. **19:** 139–148.

HUSTON, A. L., B. B. KRIEGER-BROCKETT, AND J. W. DEMING. 2000. Remarkably low temperature optima for extracellular enzyme activity from Arctic bacteria and sea ice. Environ. Microbiol. **2:** 383–388.

INGALLS, A. E., S. R. SHAH, R. L. HANSMAN, L. I. ALUWIHARE, G. M. SANTOS, E. R. M. DRUFFEL, AND A. PEARSON. 2006. Quantifying archaeal community autotrophy in the mesopelagic ocean using natural radiocarbon. Proc. Natl. Acad. Sci. USA **103:** 6442–6447.

IPCC. 2001. Climate change 2001: The scientific basis, p. 267–269. *In* J. T. Houghton, Y. Ding, D. J. Griggs, M. Noguer, P. J. van der Linden, X. Dai, K. Maskell and C. A. Johnson [EDS.], Contribution of working group I to the third assessment report of the intergovernmental panel on climate change. Cambridge Univ. Press.

IVARS-MARTINEZ, E., AND OTHERS. 2008. Comparative genomics of two ecotypes of the marine planktonic copiotroph *Alteromonas macleodii* suggests alternative lifestyles associated with different kinds of particulate organic matter. ISME J. **2:** 1194–1212.

JACKSON, G. A. 1989. Simulation of bacterial attraction and adhesion to falling particles in an aquatic environment. Limnol. Oceanogr. **34:** 514–530.

———. 1990. A model of the formation of marine algal flocs by physical coagulation processes. Deep-Sea Res. **37:** 1197–1211.

JAESCHKE, A., E. C. HOPMANS, S. G. WAKEHAM, S. SCHOUTEN, AND J. S. S. DAMSTÉ. 2007. The presence of ladderane lipids in the oxygen minimum zone of the Arabian Sea indicates nitrogen loss through anammox. Limnol. Oceanogr. **52:** 780–786.

JANNASCH, H. W., AND C. O. WIRSEN. 1973. Deep-sea microorganisms: In situ response to nutrient enrichment. Science **180:** 641–643.

JENKINS, W. J. 1982. Oxygen utilization rates in the North Atlantic Subtropical Gyre and primary production in oligotrophic systems. Nature **300:** 246–248.

———, AND D. W. R. WALLACE. 1992. Tracer based inferences of new primary production in the sea, p. 292–316. *In* P. G. Falkowski and A. D. Woodhead [eds.], Primary productivity and biogeochemical cycles in the sea. Plenum.

KALTENBÖCK, E., AND G. J. HERNDL. 1992. Ecology of amorphous aggregations (marine snow) in the Northern Adriatic Sea: IV. Dissolved nutrients and the autotrophic community associated with marine snow. Mar. Ecol. Prog. Ser. **87:** 147–159.

KARL, D. M. 1980. Cellular nucleotide measurements and applications in microbial ecology. Microbiol. Rev. **44:** 739–796.

———, G. A. KNAUER, AND J. H. MARTIN. 1988. Downward flux of particulate organic matter in the ocean: A particle decomposition paradox. Nature **332:** 438–441.

KARNER, M. B., E. F. DELONG, AND D. M. KARL. 2001. Archaeal dominance in the mesopelagic zone of the Pacific Ocean. Nature **409:** 507–510.

———, AND G. J. HERNDL. 1992. Extracellular enzymatic activity and secondary production in free-living and marine snow associated bacteria. Mar. Biol. **113:** 341–347.

KATZ, L. A., G. B. MCMANUS, O. L. O. SNOEYENBOS-WEST, A. GRIFFIN, K. PIROG, B. COSTAS, AND W. FOISSNER. 2005. Reframing the 'Everything is everywhere' debate: Evidence for high gene flow and diversity in ciliate morphospecies. Aquat. Microb. Ecol. **41:** 55–65.

KAWASAKI, N., AND R. BENNER. 2006. Bacterial release of dissolved organic matter during cell growth and decline: Molecular origin and composition. Limnol. Oceanogr. **51:** 2170–2180.

Kepkay, P. E. 1994. Particle aggregation and the biological reactivity of colloids. Mar. Ecol. Prog. Ser. **109:** 293–304.

———. 2000. Colloids and the ocean carbon cycle, p. 35–56. *In* P. Wangersky [ed.], Handbook of environmental chemistry. Springer.

King, G. M., and C. F. Weber. 2007. Distribution, diversity and ecology of CO-oxidizing bacteria. Nat. Rev. Microbiol. **5:** 107–118.

Kiørboe, T. 2001. Formation and fate of marine snow: Small-scale processes with large-scale implications. Sci. Mar. **65:** 55–71.

———, H-P. Grossart, H. Ploug, and K. Tang. 2002. Mechanisms and rates of bacterial colonization of sinking aggregates. Appl. Environ. Microbiol. **68:** 3996–4006.

———, and G. A. Jackson. 2001. Marine snow, organic solute plumes, and optimal chemosensory behavior of bacteria. Limnol. Oceanogr. **46:** 1309–1318.

———, K. Tang, H-P. Grossart, and H. Ploug. 2003. Dynamics of microbial communities on marine snow aggregates: Colonization, growth, detachment, and grazing mortality of attached bacteria. Appl. Environ. Microbiol. **69:** 3036–3047.

Kirchman, D. L., H. Elifantz, A. I. Dittel, R. R. Malmstrom, and M. T. Cottrell. 2007. Standing stock and activity of Archaea and Bacteria in the western Arctic Ocean. Limnol. Oceanogr. **52:** 495–507.

Klaas, C., and D. E. Archer. 2002. Association of sinking organic matter with various types of mineral ballast in the deep sea: Implications for the rain ratio. Global Biogeochem. Cycles **16:** 1116, doi:10.1029/2001GB001765.

Koike, I., S. Hara, K. Terauchi, and K. Kogure. 1990. Role of sub-micrometre particles in the ocean. Nature **345:** 242–244.

Könneke, M., A. E. Bernhard, J. R. de la Torre, C. B. Walker, J. B. Waterbury, and D. A. Stahl. 2005. Isolation of an autotrophic ammonia-oxidizing marine archaeon. Nature **437:** 543–546.

Koppelmann, R., and H. Weikert. 1992. Full-depth zooplankton profiles over the deep bathyal of the NE Atlantic. Mar. Ecol. Prog. Ser. **86:** 263–272.

———, H. Zimmermann-Timm, and H. Wikert. 2005. Bacterial and zooplankton distribution in deep waters of the Arabian Sea. Deep-Sea Res. I **52:** 2184–2192.

Kuypers, M. M., and others. 2003. Anaerobic ammonium oxidation by anammox bacteria in the Black Sea. Nature **422:** 608–611.

———, and ———. 2005. Massive nitrogen loss from the Benguela upwelling system through anaerobic ammonium oxidation. Proc. Natl. Acad. Sci. USA **102:** 6478–6483.

Lam, P., and others. 2007. Linking crenarchaeal and bacterial nitrification to anammox in the Black Sea. Proc. Natl. Acad. Sci. USA **104:** 7104–7109.

Lampitt, R. S., T. Noji, and B. von Bodungen. 1990. What happens to zooplankton faecal pellets? Implications for material flux. Mar. Biol. **104:** 15–23.

Lauro, F. M., R. A. Chastain, L. E. Blankenship, A. A. Yayanos, and D. H. Bartlett. 2007. The unique 16S rRNA genes of piezophiles reflect both phylogeny and adaptation. Appl. Environ. Microbiol. **73:** 838–845.

Lefevre, D., M. Denis, C. E. Lambert, and J.-C. Miquel. 1996. Is DOC the main source of organic matter remineralization in the ocean water column? J. Mar. Syst. **7:** 281–291.

Leininger, S., and others. 2006. Archaea predominate among ammonia-oxidizing prokaryotes in soils. Nature **442:** 806–809.

Li, W. K. W., and T. Platt. 1987. Photosynthetic picoplankton in the ocean. Sci. Prog. **71:** 117–132.

Libes, S. M. 1992. An introduction to marine biogeochemistry. Wiley.

López-Garcia, P., A. López-López, D. Moreira, and F. Rodríguez-Valera. 2001*a*. Diversity of free-living prokaryotes from a deep-sea site at the Antarctic Polar Front. FEMS Microbiol. Ecol. **36:** 193–202.

———, F. Rodríguez-Valera, C. Pedrós-Alió, and D. Moreira. 2001*b*. Unexpected diversity of small eukaryotes in deep-sea Antarctic plankton. Nature **409:** 603–606.

———, H. Philippe, F. Gail, and D. Moreira. 2003. Autochthonous eukaryotic diversity in hydrothermal sediment and experimental microcolonizers at the Mid-Atlantic Ridge. Proc. Natl. Acad. Sci. USA **100:** 697–702.

López-López, A., S. G. Bartual, L. Stal, O. Onyshenko, and F. Rodríguez-Valera. 2005. Genetic analysis of housekeeping genes reveals a deep-sea ecotype of *Alteromonas macleodii* in the Mediterranean Sea. Environ. Microbiol. **7:** 649–659.

Lovejoy, C., R. Massana, and C. Pedrós-Alió. 2006. Diversity and distribution of marine microbial eukaryotes in the Arctic Ocean and adjacent seas. Appl. Environ. Microbiol. **72:** 3085–3095.

Martín-Cuadrado, A.-B., and others. 2007. Metagenomics of deep Mediterranean, a warm bathypelagic habitat. PLoS ONE **2:** e914, doi:10.1371/journal.pone.0000914.

———, and ———. 2008. Hindsight in the relative abundance, metabolic potential and genome dynamics of uncultivated marine archaea from comparative metagenomic analyses of bathypelagic plankton of different oceanic regions. ISME J. **2:** 865–886.

Martínez, J., D. C. Smith, G. F. Steward, and F. Azam. 1996. Variability in ectohydrolytic enzyme activities of pelagic marine bacteria and its significance for substrate processing in the sea. Aquat. Microb. Ecol. **10:** 223–230.

Massana, R., E. F. Delong, and C. Pedrós-Alió. 2000. A few cosmopolitan phylotypes dominate planktonic archaeal assemblages in widely different oceanic provinces. Appl. Environ. Microbiol. **66:** 1777–1787.

———, R. Terrado, I. Forn, C. Lovejoy, and C. Pedrós-Alió. 2006. Distribution and abundance of uncultured heterotrophic flagellates in the world oceans. Environ. Microbiol. **8:** 1515–1522.

McCave, I. N. 1975. Vertical flux of particles in the ocean. Deep-Sea Res. **22:** 491–502.

Médigue, C., and others. 2005. Coping with cold: The genome of the versatile marine Antarctica bacterium *Pseudoalteromonas haloplanktis* TAC125. Genome Res. **15:** 1325–1335.

Michaels, A. F., and others. 1996. Inputs, losses and transformations of nitrogen and phosphorus in the pelagic North Atlantic Ocean. Biogeochemistry **35:** 181–226.

Mincer, T. J., M. J. Church, L. T. Taylor, C. Preston, D. M. Karl, and E. D. Delong. 2007. Quantitative distribution of presumptive archaeal and bacterial nitrifiers in Monterey Bay and the North Pacific Subtropical Gyre. Environ. Microbiol. **9:** 1162–1175.

Mitchell, J. G., L. Pearson, and S. Dillon. 1995. Clustering of marine bacteria in seawater enrichments. Appl. Environ. Microbiol. **62:** 3716–3721.

Moeseneder, M. M., C. Winter, J. M. Arrieta, and G. J. Herndl. 2001a. Terminal restriction fragment length polymorphism (T-RFLP) screening of a marine archaeal library to determine the different phylotypes. J. Microbiol. Methods **44:** 159–172.

———, ———, and G. J. Herndl. 2001b. Horizontal and vertical complexity of attached and free-living bacteria of the eastern Mediterranean Sea, determined by 16S rDNA and 16S rRNA fingerprints. Limnol. Oceanogr. **46:** 95–107.

Morita, R. Y. 1984. Feast or famine in the deep sea. Developments in industrial microbiology. Soc. Int. Microbiol. **25:** 5–16.

Morris, R. M., M. S. Rappé, E. Urbach, S. A. Connon, and S. J. Giovannoni. 2004. Prevalence of the *Chloroflexi*-related SAR202 bacterioplankton cluster throughout the mesopelagic zone and deep ocean. Appl. Environ. Microbiol. **70:** 2836–2842.

Müller-Niklas, G., S. Schuster, E. Kaltenböck, and G. J. Herndl. 1994. Organic content and bacterial metabolism in amorphous aggregations of the northern Adriatic Sea. Limnol. Oceanogr. **39:** 58–68.

Nagata, T., H. Fukuda, R. Fukuda, and I. Koike. 2000. Bacterioplankton distribution and production in the deep Pacific waters: Large-scale geographic variations and possible coupling with sinking particle fluxes. Limnol. Oceanogr. **45:** 426–435.

Naqvi, S. W. A., and others. 1998. Budgetary and biogeochemical implications of N_2O isotope signatures in the Arabian Sea. Nature **394:** 422–424.

Noble, R. T., and J. A. Fuhrman. 1998. Use of Sybr Green I for rapid epifluorescence counts of marine viruses and bacteria. Aquat. Microb. Ecol. **14:** 113–118.

———, and ———. 2000. Rapid virus production and removal as measured with fluorescently labeled viruses as tracers. Appl. Environ. Microbiol. **66:** 3790–3797.

Not, F., R. Gausling, F. Azam, J. F. Heidelberg, and A. Z. Worden. 2007. Vertical distribution of picoeukaryotic diversity in the Sargasso Sea. Environ. Microbiol. **9:** 1233–1252.

Ortmann, A. C., and C. A. Suttle. 2005. High abundances of viruses in a deep-sea hydrothermal vent system indicate viral mediated microbial mortality. Deep-Sea Res. I **52:** 1515–1527.

Ouverney, C. C., and J. A. Fuhrman. 2000. Marine planktonic archaea take up amino acids. Appl. Environ. Microbiol. **66:** 4829–4833.

Parada, V., E. Sintes, H. M. van Aken, M. G. Weinbauer, and G. J. Herndl. 2007. Viral abundance, decay, and diversity in the meso- and bathypelagic waters of the North Atlantic. Appl. Environ. Microbiol. **73:** 4429–4438.

Passow, U. 2002. Transparent exopolymer particles (TEP) in aquatic environments. Prog. Oceanogr. **55:** 287–333.

———, and A. L. Alldredge. 1994. Distribution, size and bacterial colonization of transparent exopolymer particles (TEP) in the ocean. Mar. Ecol. Prog. Ser. **113:** 185–198.

Patching, J. W., and D. Eardly. 1997. Bacterial biomass and activity in the deep waters of the eastern Atlantic—evidence of a barophilic community. Deep-Sea Res. I **44:** 1655–1670.

Pérez, M. T., C. Pausz, and G. J. Herndl. 2003. Major shift in bacterioplankton utilization of enantiomeric amino acids between surface waters and the ocean's interior. Limnol. Oceanogr. **48:** 755–763.

Peters, F. 1994. Prediction of planktonic protistan grazing rates. Limnol. Oceanogr. **39:** 195–206.

Peterson, M. L., S. G. Wakeham, C. Lee, M. A. Askea, and J. C. Miquel. 2005. Novel techniques for collecting of sinking particles in the ocean and determining their settling rates. Limnol. Oceanogr. Methods **3:** 520–532.

Ploug, H. 2001. Small-scale oxygen fluxes and remineralization in sinking aggregates. Limnol. Oceanogr. **46:** 1624–1631.

———, and H.-P. Grossart. 1999. Bacterial production and respiration in suspended aggregates—a matter of the incubation method. Aquat. Microb. Ecol. **20:** 21–29.

———, and ———. 2000. Bacterial growth and grazing on diatom aggregates: Respiratory carbon turnover as a function of aggregate size and sinking velocity. Limnol. Oceanogr. **45:** 1467–1475.

———, ———, F. Azam, and B. B. Jørgensen. 1999. Photosynthesis, respiration, and carbon turnover in sinking marine snow from surface waters of Southern California Bight: Implications for the carbon cycle in the ocean. Mar. Ecol. Prog. Ser. **179:** 1–11.

Pomeroy, L. R., and R. E. Johannes. 1968. Occurrence and respiration of ultraplankton in the upper 500 m of the ocean. Limnol. Oceanogr. **15:** 381–391.

Proctor, L. M., and J. A. Fuhrman. 1991. Roles of viral infection in organic particle flux. Mar. Ecol. Prog. Ser. **69:** 133–142.

Quaiser, A., P. López-Garcia, Y. Zivanovic, M. R. Henn, F. Rodriguez-Valera, and D. Moreira. 2008. Comparative analysis of genome fragments of *Acidobacteria* from deep Mediterranean plankton. Environ. Microbiol. **10:** 2704–2717.

Rath, J., K. Y. Wu, G. J. Herndl, and E. F. Delong. 1998. High phylogenetic diversity in a marine snow-associated bacterial assemblage. Aquat. Microb. Ecol. **14:** 261–269.

Reinthaler, T., and others. 2006. Prokaryotic respiration and production in the meso- and bathypelagic realm of the eastern and western North Atlantic basin. Limnol. Oceanogr. **51:** 1262–1273.

Repeta, D. J., and R. B. Gagosian. 1984. Transformation reactions and recycling of carotenoids and chlorins in the Peru upwelling region (15°S, 75°W). Geochim. Cosmochim. Acta **48:** 1265–1277.

Riemann, L., and A. Winding. 2001. Community dynamics of free-living and particle-associated bacterial assemblages during a freshwater phytoplankton bloom. Microb. Ecol. **42:** 274–285.

Robison, B. H., K. R. Reisenbichler, and R. E. Sherlock. 2005. Giant larvacean houses: Rapid carbon transport to the deep sea floor. Science **308:** 1609–1611.

Rosso, A. L., and F. Azam. 1987. Proteolytic activity in coastal oceanic waters: Depth distribution and relationship to bacterial populations. Mar. Ecol. Prog. Ser. **41:** 231–240.

Sheridan, C. C., C. Lee, S. G. Wakeham, and J. K. B. Bishop. 2002. Suspended particle organic composition and cycling in surface and midwaters of the equatorial Pacific Ocean. Deep-Sea Res. I **49:** 1983–2008.

Sherry, N. D., P. W. Boyd, K. Sugimoto, and P. J. Harrison. 1999. Seasonal and spatial patterns of heterotrophic bacterial production, respiration, and biomass in the subarctic NE Pacific. Deep-Sea Res. II **46:** 2557–2578.

Silver, M. W., S. L. Coale, C. H. Pilskaln, and F. P. Chavez. 1998*a*. Exploratory observations of marine aggregates at subeuphotic depths. Deep-Sea Res. II **45:** 1839–1861.

———, ———, ———, and D. R. Steinberg. 1998*b*. Giant aggregates: Importance as microbial centers and agents of material flux in the mesopelagic zone. Limnol. Oceanogr. **43:** 498–507.

———, M. M. Gowing, D. C. Brownlee, and J. O. Corliss. 1984. Ciliated protozoa associated with oceanic sinking detritus. Nature **309:** 246–248.

Simon, M., H. P. Grossart, B. Schweitzer, and H. Ploug. 2002. Microbial ecology of organic aggregates in aquatic systems. Aquat. Microb. Ecol. **28:** 175–211.

———, B. Rosenstock, and W. Zwisler. 2004. Coupling of epipelagic and mesopelagic heterotrophic picoplankton production to phytoplankton biomass in the Antarctic polar frontal region. Limnol. Oceanogr. **49:** 1035–1043.

Small, L. F., S. W. Fowler, S. A. Moore, and J. Larosa. 1983. Dissolved and fecal pellet carbon and nitrogen release by zooplankton in tropical waters. Deep-Sea Res. **30:** 1199–1220.

Smayda, T. J. 1970. The suspension and sinking of phytoplankton in the Sea. Oceanogr. Mar. Biol. Ann. Rev. **8:** 353–414.

Smith, D. C., M. Simon, A. L. Alldredge, and F. Azam. 1992. Intense hydrolytic enzyme activity on marine aggregates and implications for rapid particle dissolution. Nature **359:** 139–142.

Sogin, M. L., and others. 2006. Microbial diversity in the deep sea and the under-explored "rare biosphere." Proc. Natl. Acad. Sci. USA **103:** 12115–12120.

Sorokin, Y. I. 1971. Bacterial populations as components of oceanic ecosystems. Mar. Biol. **11:** 101–105.

———. 1973. Data on biological productivity of the western tropical Pacific Ocean. Mar. Biol. **20:** 177–196.

———, A. I. Kopylov, and N. V. Mamaeva. 1985. Abundance and dynamics of microplankton in the central tropical Indian Ocean. Mar. Ecol. Prog. Ser. **24:** 27–41.

Steinberg, D. K., C. A. Carlson, N. R. Bates, S. A. Goldthwait, L. P. Madin, and A. F. Michaels. 2000. Zooplankton vertical migration and the active transport of dissolved organic and inorganic carbon in the Sargasso Sea. Deep-Sea Res. I **47:** 137–158.

———, ———, ———, R. J. Johnson, A. F. Michaels, and A. H. Knap. 2001. Overview of the US JGOFS Bermuda Atlanric Time-series Study (BATS): A decade-scale look at ocean biology and biogeochemistry. Deep-Sea Res. II **48:** 1405–1447.

———, B. A. S. van Mooy, K. O. Buesseler, P. W. Boyd, T. Kobari, and D. M. Karl. 2008. Bacterial vs. zooplankton control of sinking particle flux in the ocean's twilight zone. Limnol. Oceanogr. **53:** 1327–1338.

Steward, G. F., D. C. Smith, and F. Azam. 1996. Abundance and production of bacteria and viruses in the Bering and Chukchi Sea. Mar. Ecol. Prog. Ser. **131:** 287–300.

Stoeck, T., G. T. Taylor, and S. S. Epstein. 2003. Novel eukaryotes from the permanently anoxic Cariaco Basin (Caribbean Sea). Appl. Environ. Microbiol. **69:** 5656–5663.

Suzuki, N., and K. Kato. 1953. Studies on suspended materials marine snow in the sea. Part I. Sources of marine snow. Hakkaido Univ. Fac. Fish. Bull. **4:** 132–135.

Tamburini, C. 2006. Life under pressure. Deep-sea microbial ecology, p. 1–17. *In* J. Seckbach [ed.], Life as we know it. Series: Cellular origin and life in extreme habitats and astrobiology. Springer.

———, J. Garcin, M. Ragot, and A. Bianchi. 2002. Biopolymer hydrolysis and bacterial production under ambient hydrostatic pressure through a 2000 m water column in the NW Mediterranean. Deep-Sea Res. II **49:** 2109–2123.

———, M. Garel, A. Al Ali, B. Mérigot, P. Kriwy, B. Charrière, and G. Budillon. In press. Distribution and activity of bacteria ad archaea in the different water masses of the Tyrrhenian Sea. Deep-Sea Res. II.

Tanaka, T., and F. Rassoulzadegan. 2002. Full-depth profile (0–2000 m) of bacteria, heterotrophic nanoflagellates and ciliates in the NW Mediterranean Sea: Vertical partitioning of microbial trophic structures. Deep-Sea Res. II **49:** 2093–2107.

———, and ———. 2004. Vertical and seasonal variations of bacterial abundance and production in the mesopelagic layer of the NW Mediterranean Sea: Bottom-up and top-down controls. Deep-Sea Res. I **51:** 531–544.

———, ———, and T. F. Thingstad. 2005. Analyzing the trophic link between the mesopelagic microbial loop and zooplankton from observed depth profiles of bacteria and protozoa. Biogeosciences **2:** 9–13.

Teira, E., P. Lebaron, H. van Aken, and G. J. Herndl. 2006b. Distribution and activity of Bacteria and Archaea in the deep water masses of the North Atlantic. Limnol. Oceanogr. **51:** 2131–2144.

———, T. Reinthaler, A. Pernthaler, J. Pernthaler, and G. J. Herndl. 2004. Combining catalyzed reporter deposition-fluorescence in situ hybridization and microautoradiography to detect substrate utilization by Bacteria and Archaea in the deep ocean. Appl. Environ. Microbiol. **70:** 44411–44414.

———, H. van Aken, C. Veth, and G. J. Herndl. 2006*a*. Archaeal uptake of enantiomeric amino acids in the meso- and bathypelagic waters of the North Atlantic. Limnol. Oceanogr. **51:** 60–69.

Thornton, D. C. O. 2002. Diatom aggregation in the sea: Mechanisms and ecological implications. Eur. J. Phycol. **37:** 149–161.

Trull, T. W., S. G. Bray, K. O. Buesseler, C. H. Lamborg, S. Manganini, C. Moy, and J. Valdes. 2008. *In situ* measurements of mesopelagic particle sinking rates and the control of carbon transfer to the ocean interior during the Vertical Flux in the Global Ocean (VERTIGO) voyages in the North Pacific. Deep-Sea Res. II **55:** 1684–1695.

Turley, C. M. 2002. The importance of marine snow. Microbiol. Today **29:** 177–179.

———, and P. J. Mackie. 1994. Biogeochemical significance of attached and free-living bacteria and the flux of particles in the NE Atlantic Ocean. Mar. Ecol. Prog. Ser. **115:** 191–203.

———, and E. D. Stutt. 2000. Depth-related cell-specific bacterial leucine incorporation rates on particles and its biogeochemical significance in the northwest Mediterranean. Limnol. Oceanogr. **45:** 419–425.

Vaqué, D., J. M. Gasol, and C. Marrasé. 1994. Grazing rates on bacteria: The significance of methodology and ecological factors. Mar. Ecol. Prog. Ser. **109:** 263–274.

Varela, M. M., H. M. van Aken, and G. J. Herndl. 2008a. Abundance and activity of *Chloroflexi*-type SAR202 bacterioplankton in the meso- and bathypelagic waters of the (sub)tropical Atlantic. Environ. Microbiol. **10:** 1903–1911.

———, ———, E. Sintes, and G. J. Herndl. 2008b. Latitudinal trends of *Crenarchaeota* and *Bacteria* in the meso- and bathypelagic waters of the North Atlantic. Environ. Microbiol. **10:** 110–124.

Venter, J. C., and others. 2004. Environmental genome shotgun sequencing of the Sargasso Sea. Science **304:** 66–74.

Verdugo, P., A. L. Alldredge, F. Azam, D. L. Kirchman, U. Passow, and P. H. Santschi. 2004. The oceanic gel phase: A bridge in the DOM-POM continuum. Mar. Chem. **92:** 67–85.

Vezzi, A., and others. 2005. Life at depth: *Photobacterium profundum* genome sequence and expression analysis. Science **307:** 1459–1461.

Wakeham, S., and E. A. Canuel. 1988. Organic geochemistry of particulate matter in the eastern tropical North Pacific Ocean: Implications for particle dynamics. J. Mar. Res. **46:** 183–213.

Weinbauer, M., I. Brettar, and M. G. Höfle. 2003. Lysogeny and virus-induced mortality of bacterioplankton in surface, deep, and anoxic marine waters. Limnol. Oceanogr. **48:** 1457–1465.

Weinbauer, M. G. 2004. Ecology of prokaryotic viruses. FEMS Microbiol. Rev. **28:** 1127–1181.

———, and P. Peduzzi. 1995. Significance of viruses versus heterotrophic nanoflagellates for controlling bacterial abundance in the northern Adriatic Sea. J. Plankton Res. **17:** 1851–1856.

Wells, L. E., and J. W. Deming. 2006. Effects of temperature, salinity and clay particles on inactivation and decay of cold-active marine bacteriophage 9A. Aquat. Microb. Ecol. **45:** 31–39.

Wells, M. L., and E. D. Goldberg. 1994. The distribution of colloids in the North Atlantic and Southern Oceans. Limnol. Oceanogr. **39:** 286–302.

WEN, K., A. C. ORTMANN, AND C. A. SUTTLE. 2004. Accurate estimation of viral abundance by epifluorescence microscopy. Appl. Environ. Microbiol. **70:** 3862–3867.

WHITMAN, W. B., D. C. COLEMAN, AND W. J. WIEBE. 1998. Prokaryotes: The unseen majority. Proc. Natl. Acad. Sci. USA **95:** 6578–6583.

WOEBKEN, D., B. M. FUCHS, M. M. M. KUYPERS, AND R. AMANN. 2007. Potential interactions of particle-associated anammox bacterial with bacterial and archaeal partners in the Nambian Upwelling System. Appl. Environ. Microbiol. **73:** 4648–4657.

WOMMACK, K. E., S. J. WILLIAMSON, A. SUNDBERGH, R. E. HELTON, B. T. GLAZER, K. PORTUNE, AND S. C. CARY. 2004. An instrument for collecting discrete large-volume water samples suitable for ecological studies of microorganisms. Deep-Sea Res. I **51:** 1781–1792.

WORDEN, A. Z., M. L. CUVELIER, AND D. H. BARTLETT. 2006. In-depth analyses of marine microbial community genomics. Trends Microbiol. **14:** 331–336.

WUCHTER, C., AND OTHERS. 2006. Archaeal nitrification in the ocean. Proc. Natl. Acad. Sci. USA **103:** 12317–12322.

YAMAGUCHI, A., AND OTHERS. 2002. Structure and size distribution of plankton communities down to the greater depths in the western North Pacific Ocean. Deep-Sea Res. II **49:** 5513–5529.

———, AND ———. 2004. Latitudinal differences in the planktonic biomass and community structure down to greater depths in the Western North Pacific. J. Oceanogr. **60:** 773–787.

ZABALLOS, M., AND OTHERS. 2006. Comparison of prokaryotic diversity at offshore oceanic locations reveals a different microbiota in the Mediterranean Sea. FEMS Microbiol. Ecol. **56:** 389–405.

ZACCONE, R., AND OTHERS. 2003. Bacterial processes in the intermediate and deep layers of the Ionian Sea in winter 1999: Vertical profiles and their relationship to the different water masses. J. Geophys. Res. **108:** 8117, doi:10.1029/2002JC001625.

Associate editor: Peter A. Jumars

Received: 23 July 2008
Accepted: 16 March 2009
Amended: 16 April 2009