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Emerging concepts on microbial processes in the bathypelagic ocean – ecology, biogeochemistry, and genomics

Toshi Nagata^{a,*}, Christian Tamburini^b, Javier Arístegui^c, Federico Baltar^c, Alexander B. Bochdansky^d, Serena Fonda-Umani^e, Hideki Fukuda^f, Alexandra Gogou^g, Dennis A. Hansell^h, Roberta L. Hansmanⁱ, Gerhard J. Herndl^j, Christos Panagiotopoulos^b, Thomas Reinthaler^j, Rumi Sohrin^k, Pedro Verdugo^l, Namiha Yamada^m, Youhei Yamashita^{n,1}, Taichi Yokokawa^o, Douglas H. Bartlett^p

^a Marine Biogeochemistry Group, Ocean Research Institute, The University of Tokyo, 1-15-1 Minami-dai, Nakano, Tokyo 164-8639, Japan

^b Université de la Méditerranée, Centre d'Océanologie de Marseille, LMCEM UMR 6117 CNRS-INSU, 163 Avenue de Luminy, 13288 Marseille Cedex 09, France

^c Facultad de Ciencias del Mar, Universidad de Las Palmas de Gran Canaria, 35017 Las Palmas de Gran Canaria, Islas Canarias, Spain

^d Department of Ocean, Earth and Atmospheric Sciences, Old Dominion University, 4600 Elkhorn Ave, Norfolk 23529, VA, USA

^e Department of Life Sciences, University of Trieste, v. Valerio 28/1, Trieste 34127, TS, Italy

^f International Coastal Research Center, Ocean Research Institute, The University of Tokyo, 2-106-1, Akahama, Otsuchi town, Kamihei county, Iwate 028-1102, Japan

^g Hellenic Centre for Marine Research, Institute of Oceanography, 46.7 km Athens-Sounion Av., 19013 Anavyssos, Greece

^h Division of Marine and Atmospheric Chemistry, Rosenstiel School of Marine and Atmospheric Science, University of Miami 4600 Rickenbacker Causeway, Miami, FL 33149, USA

ⁱ Division of Geological and Planetary Sciences, California Institute of Technology, MC 100-23, 1200 E. California Blvd, Pasadena, CA 91125 USA

^j Department of Biological Oceanography, Royal Netherlands Institute for Sea Research (NIOZ), PO box 59, 1790AB Den Burg, the Netherlands & University of Vienna, Department of Marine Biology, Althanstrasse 14, 1090 Vienna, Austria

^k Institute of Geosciences, Shizuoka University, 836 Oya, Suruga-ku, Shizuoka 422-8529, Japan

^l Department of Bioengineering, University of Washington, Friday Harbor Laboratories, 620 University Rd, Friday Harbor 98250, WA, USA

^m National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba AIST West, 16-1 Onogawa, Tsukuba, 305-8569, Japan

ⁿ Southeast Environmental Research Center, Florida International University, OE-148, University Park, Miami, FL 33199, USA

^o Biological Oceanography, Royal Netherlands Institute for Sea Research (NIOZ), PO box 59, 1790AB Den Burg, the Netherlands

^p Marine Biology Research Division, Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California, San Diego, La Jolla, CA 92093-0202, USA

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ABSTRACT

This paper synthesizes recent findings regarding microbial distributions and processes in the bathypelagic ocean (depth > 1000 m). Abundance, production and respiration of prokaryotes reflect supplies of particulate and dissolved organic matter to the bathypelagic zone. Better resolution of carbon fluxes mediated by deep microbes requires further testing on the validity of conversion factors. *Archaea*, especially marine *Crenarchaeota* Group I, are abundant in deep waters where they can fix dissolved inorganic carbon. Viruses appear to be important in the microbial loop in deep waters, displaying remarkably high virus to prokaryote abundance ratios in some oceanic regions. Sequencing of 18S rRNA genes revealed a tremendous diversity of small-sized protists in bathypelagic waters. Abundances of heterotrophic nanoflagellates (HNF) and ciliates decrease with depth more steeply than prokaryotes; nonetheless, data indicated that HNF consumed half of prokaryote production in the bathypelagic zone. Aggregates are important habitats for deep-water microbes, which produce more extracellular enzymes (on a per-cell basis) than surface communities. The theory of marine gel formation provides a framework to unravel complex interactions between microbes and organic polymers. Recent data on the effects of hydrostatic pressure on microbial activities indicate that bathypelagic microbial activity is generally higher under *in situ* pressure conditions than at atmospheric pressures. High-throughput sequencing of 16S rRNA genes revealed a remarkable diversity of *Bacteria* in the bathypelagic ocean. Metagenomics and comparative genomics of piezophiles reveal not only the

* Corresponding author. Present address: Marine Biogeochemistry Group, Atmosphere and Ocean Research Institute, The University of Tokyo, 5-1-5 Kashiwanoha, Kashiwa, Chiba 277-8564, Japan. Tel./fax: +81 4 7136 6090.

E-mail addresses: nagata@ori.u-tokyo.ac.jp (T. Nagata), christian.tamburini@univmed.fr (C. Tamburini), jAristegui@dbio.ulpgc.es (J. Arístegui), federico.baltar102@doctorandos.ulpgc.es (F. Baltar), abochdan@odu.edu (A. Bochdansky), s.fonda@units.it (S. Fonda-Umani), hfukuda@ori.u-tokyo.ac.jp (H. Fukuda), agogou@ath.hcmr.gr (A. Gogou), dhansell@rsmas.miami.edu (D.A. Hansell), rhansman@caltech.edu (R.L. Hansman), gerhard.herndl@univie.ac.at (G.J. Herndl), christos.panagiotopoulos@univmed.fr (C. Panagiotopoulos), thomas.reinthal@univie.ac.at (T. Reinthaler), srshori@ipc.shizuoka.ac.jp (R. Sohrin), verdugo@u.washington.edu (P. Verdugo), namiha-yamada@aist.go.jp (N. Yamada), yamashiy@fu.edu, yamashi@ees.hokudai.ac.jp (Y. Yamashita), taichi.yokokawa@nioz.nl (T. Yokokawa), dbartlett@ucsd.edu (D.H. Bartlett).

¹ Present address: Faculty of Environmental Earth Science, Hokkaido University N10, W5, Kita-ku, Sapporo, Hokkaido 060-0810, Japan.

high diversity of deep sea microbes but also specific functional attributes of these piezophilic microbes, interpreted as an adaptation to the deep water environment. Taken together, the data compiled on bathypelagic microbes indicate that, despite high-pressure and low-temperature conditions, microbes in the bathypelagic ocean dynamically interact with complex mixtures of organic matter, responding to changes in the ocean's biogeochemical state.

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1. Introduction

The bathypelagic zone, at ocean depths > 1000 m, is the largest but least understood aquatic habitat on our planet (accounting for 70% of total seawater volume). This zone receives organic inputs from overlying layers in the form of particulate (POM) and dissolved organic matter (DOM) after substantial remineralization and transformation via complex actions of epi- and mesopelagic biota (Steinberg et al., 2008; Robinson et al., 2010). This “residual” flux of organic matter (deep export), on the order of 1 – 3.6 Pg C year⁻¹ (Martin et al., 1987; Hansell, 2002; Arístegui et al., 2005a), is an important, albeit only weakly constrained, component of global carbon cycles, contributing to carbon sequestration in the time scale of the ocean circulation, i.e. 10³ years (Sarmiento and Gruber, 2006). In order to fully understand oceanic biogeochemical cycles, it is essential to clarify the mechanistic basis, variations in time and space, and controls of the transformation and remineralization of POM and DOM during their transit through, and transformations in, the thick bathypelagic layer.

The importance of water column microbes in planktonic food webs and biogeochemistry has become increasingly apparent during the past three decades. The concept of the microbial loop has been developed (Azam, 1998) and refined (Fuhrman, 1999), indicating that bacteria consume half or more of primary production (Ducklow, 2000; Robinson, 2008) and are in turn consumed by protists (Jürgens and Massana, 2008) and lysed by viruses (Fuhrman, 2000; Breitbart et al., 2008). We now know that the microbial loop exerts the major influence on patterns in fluxes of carbon and nutrients in the ocean (Nagata, 2008). However, our current understanding is mostly based on research conducted in the sunlit layer, with much less information available in the deeper, especially bathypelagic, layers (Herndl et al., 2008; Arístegui et al., 2009). Only recently, studies have begun to reveal remarkable features in distribution patterns of microbes (Nagata et al., 2000; Hansell and Ducklow, 2003; Reinthaler et al., 2006), their community structures (Karner et al., 2001; Sogin et al., 2006), food web interactions (Fukuda et al., 2007; Parada et al., 2007), and metabolic activities (Herndl et al., 2005; Teira et al., 2006a,b) in bathypelagic waters. These studies indicate striking differences between, as well as similarities of, microbial loops in surface and deep oceans.

Importantly, recent evidence challenges the view that activities of microbes in bathypelagic waters are extremely low and largely suppressed under cold and high pressure conditions (Jannasch and Wirsén, 1973), thus displaying minimum responses to the inputs of organic matter and playing only a minor role in biogeochemical cycles. An emerging paradigm suggests that the bathypelagic ocean is a heterogeneous habitat (Hewson et al., 2006) of distinctive and previously unknown communities of remarkably diverse microbes (Sogin et al., 2006; DeLong et al., 2006), interacting dynamically with POM and DOM (Nagata et al., 2000; Baltar et al., 2009a; Teira et al., 2006a). These organisms display unique metabolic capabilities (Vezzi et al., 2005; DeLong et al., 2006) and molecular architectures (Lauro et al., 2007) that allow them to thrive under cold and high-pressure conditions (Lauro and Bartlett, 2008).

The goal of this paper is to synthesize current knowledge and evolving concepts regarding microbial processes in the bathypelagic

oceans, covering several disciplines such as ecology, biogeochemistry, and genomics. We hope that the compilation of the available data and interpretations will guide interdisciplinary interactions, leading to the identification of major areas for future research.

2. Characteristic features of the bathypelagic environment

Table 1 summarizes some physical and chemical features of the bathypelagic ocean. Bathypelagic environments (here, taken to be the depth layer approximately between 1000 and 5000 m) are characterized by high pressure (10 – 50 MPa) and low temperature (-1 to 3 °C, see Table 1 legend for exceptions). In general, the bathypelagic ocean is oxygenated and rich in oxidized forms of inorganic nutrients (NO₃, PO₄), but is depleted of reduced compounds including ammonium. DOM in the bathypelagic layer is largely refractory, as indicated by radiocarbon dating (Druffel et al., 1992). Generally, bathypelagic organic matter (POM and DOM) is more depleted in N relative to C (Hebel and Karl, 2001; Baltar et al., 2009a). The range of salinity in the bathypelagic zone is narrow (34.3 – 35.1), while pH varies in the range of 7.5 – 8.0, being slightly lower than that in the upper ocean (range 7.8 – 8.4). Collectively, the data compiled in Table 1 indicate that physical conditions of the bathypelagic environment are more stable than those in surface oceans, while concentration and composition (as indicated by C:N ratio) of organic constituents display relatively high variability, with a notable example being POM. High variability of some chemical constituents could be due to differences in analytical methods and errors associated with low concentrations (for POC measurements, see Turnwitsch et al., 2007). However, a recent study revealed a close positive correlation between POC concentrations and bacterial respiration in Atlantic deep waters (Baltar et al., 2009a), indicating that the variability was real. Temperature and chemical conditions can deviate substantially from those tabulated in Table 1 in the vicinity of deep-sea hydrothermal systems. Hydrothermal gases can influence large regions in the bathypelagic oceans through the diffusion of vent plumes (Moyer et al., 1998).

3. Prokaryotes

Prokaryotes (organisms without a nucleus) are divided into two domains: *Bacteria* and *Archaea* (Madigan et al., 2006). Although these two domains have been conventionally referred to as “bacteria” in the literature of biological oceanography, *Archaea* are highly abundant in deep waters and differ from *Bacteria* in some fundamental aspects of their metabolic features (see below). In this paper, we use “prokaryote” instead of “bacteria” as a collective term referring to *Archaea* and *Bacteria*.

3.1. Abundance and heterotrophic production

Prokaryote abundance in bathypelagic water columns varies in the range of 0.03 – 2.3 × 10⁵ cells ml⁻¹ (Table 2), typically decreasing with depth (Nagata et al., 2000; Reinthaler et al., 2006; Arístegui et al., 2009). In general, depth-integrated

Table 1

Comparison of environmental characteristics between surface and bathypelagic oceans. The values derived from Carbon Dioxide Information Analysis Center (CDIAC)/World Ocean Circulation Experiment (WOCE) Carbon Data (<http://cdiac.ornl.gov/oceans/datmet.html>) include those collected in the Atlantic, Pacific, and Indian Oceans (except that pH data were not available for the Indian Ocean) including their Antarctic sectors (maximum water depths, 5990 – 6750 m). To avoid the inclusion of anomalous data and to represent the major trend, “outliers” (5% of the data that were furthest away from the mean regardless of if they were too high or low) were excluded by inspecting the histogram of data distribution. When applicable, the ranges of physical and chemical data were compared with maps presented in Millero (2006, 2007) and slight adjustments were made if necessary. For organics, exceptionally high values in surface oceans are not included. The data do not include those from basins with anoxic deep water (e.g. Cariaco Basin, Taylor et al., 2003) or with relatively high temperatures (10–13 °C) throughout the water column (Mediterranean and Sulu Seas, Bethoux et al., 1990; Gamo et al., 2007). DL means that the concentration was below the detection limit. “ND” means that no published data are available.

	Surface (< 100 m)	Bathypelagic (> 1000 m)	Reference ^a
Hydrostatic pressure (MPa)	0.1 – 1	10 – 50 (1000 – 5000 m)	
Temperature (°C)	-1.8 – 30.3	-0.4 – 5.4	CDIAC/WOCE Carbon Data
Salinity	32.6 – 37.6	34.3 – 35.1	CDIAC/WOCE Carbon Data
pH	7.8 – 8.4	7.5 – 8.0	CDIAC/WOCE Carbon Data
Dissolved oxygen (μmol/kg)	3 – 359	29 – 281	CDIAC/WOCE Carbon Data
(Inorganic nutrients)			
Nitrate (μmol/kg)	DL – 30	16 – 43	CDIAC/WOCE Carbon Data
Phosphate (μmol/kg)	DL – 2.1	1.0 – 3.2	CDIAC/WOCE Carbon Data
Silicate (μmol/kg)	DL – 65	10 – 177	CDIAC/WOCE Carbon Data
Ammonium (μmol/kg)	DL – 1	DL	1 – 5
(Organics)			
Particulate organic carbon (μM C)	~2 – 10	0.05 – 3.0	6 – 20
Particulate organic nitrogen (μM N)	0.1 – 1.3	0.006 – 0.3	15 – 24
C:N ratio of particulate organic matter	5 – 8	5 – 20 Higher values (up to 50) reported in ref. 20.	15 – 20, 22, 24
Dissolved organic carbon (μM C)	41 – 84	36 – 46	CDIAC/WOCE Carbon Data
Dissolved organic nitrogen (μM N)	3.5 – 7.5	0.7 – 3.0	25 – 27
Dissolved organic phosphorus (μM P)	0.1 – 0.4	0.02 – 0.15	25, 28
C:N ratio of dissolved organic matter	9 – 18	9 – 34	25, 29
Dissolved combined neutral sugars (nM)	200 – 800	20 – 280	25, 26, 30 – 33
Dissolved combined amino acids (nM)	200 – 500	50 – 300	25, 26, 33 – 36
Dissolved combined amino sugars (nM)	15 – 94	5 – 15	25, 26, 33
Lipids (nM)	0.2 – 0.7	ND	25
Urea (nM)	1.7 – 350	ND	1 – 4, 37
Dissolved free amino acids (nM)	DL – 100	DL	3, 33, 36, 38 – 41
Dissolved free neutral sugars (nM)	DL – 100	DL	30, 32, 33, 38, 41 – 43

^a 1. Varela and Harrison (1999), 2. Kudela and Cochlan (2000), 3. Sambrotto and Mace (2000), 4. Sambrotto (2001), 5. Clark et al. (2008) and references therein, 6. Druffel et al. (1992), 7. Tanoue (1992), 8. Druffel et al. (1996), 9. Druffel et al. (1998), 10. Druffel and Bauer (2000), 11. Gundersen et al. (2001), 12. Hernes and Benner (2002), 13. Gardner et al. (2006), 14. Benner et al. (1997), 15. Tanoue and Handa (1979), 16. Tanoue et al. (1982), 17. Hebel and Karl (2001), 18. Hernes and Benner (2006), 19. Loh et al. (2008), 20. Baltar et al. (2009a), 21. Libby and Wheeler (1997), 22. Hill and Wheeler (2002), 23. Quéguiner and Brzezinski (2002), 24. Romankevich (1984), 25. Benner (2002) and references therein, 26. Davis and Benner (2005), 27. Bronk (2002) and references therein, 28. Karl and Bjorkman (2002) and references therein, 29. Hopkinson and Vallino (2005), 30. Kirchman et al. (2001), 31. Panagiotopoulos and Sempéré (2005) and references therein 32. Sempéré et al. (2008), 33. Kaiser and Benner (2009), 34. Hubberten et al. (1995), 35. Dittmar et al. (2001), 36. Reinthaler et al. (2008), 37. Painter et al. (2008), 38. Rich et al. (1997), 39. Kirchman and Wheeler (1998), 40. Simon and Rosenstock (2007), 41. Keil and Kirchman (1999), 42. Skoog et al. (1999), 43. Rich et al. (1996).

prokaryote abundance in the bathypelagic increases with increasing surface productivity and the sinking flux of POC. Nagata et al. (2000) reported that prokaryote abundance was two- to four-fold higher in subarctic (more productive) than subtropical (less productive) regions of the North Pacific (depth, 1000 – 4000 m). In the Arabian Sea, Hansell and Ducklow (2003) found that the mean annual prokaryote abundance at 2000 m depth was positively correlated with the annual POC flux at that depth. In addition, data collected in the Central Pacific indicates that the extent of the depth-dependent decrease in prokaryote abundance in the subtropical region tended to be more pronounced than that in the subarctic (Sohrin et al., 2010), being consistent with latitudinal variations in the depth-dependent POC flux attenuation across these regions (Steinberg et al., 2008). These findings are consistent with the notion that prokaryote biomass is regulated by the availability of organic carbon in the bathypelagic oceans (bottom up control).

Heterotrophic prokaryote production (HPP) in the bathypelagic varies in the range of 0.0013 – 280 μmol C m⁻³ d⁻¹ (Table 2). This remarkably large variability in HPP, over 6 orders of magnitude, appears to indicate large variation in resource supplies to prokaryotic communities. Errors associated with measurement of low HPP in deep waters may be another source of variability (Section 3.3), although they cannot fully account for large

variability within individual studies, i.e., typical standard deviations for replicate measurements of each sample are 10 – 20% (T. Yokokawa, unpublished). Nagata et al. (2000) found that depth-integrated HPP (1000–4000 m) generally follows the regional trend of prokaryote abundance in the Pacific, suggesting that carbon consumption by prokaryotes was generally coupled with sinking POC. In contrast, Hansell and Ducklow (2003) reported that the meridional variation in HPP in the Arabian Sea was complex and was not correlated with POC flux. They suggested that bathypelagic HPP reflects episodic inputs of organic carbon, whereas prokaryote abundance reflects long term mean inputs of organic matter. Alternatively, relatively constant prokaryote abundance despite large variation in HPP and sinking POC fluxes might be an indication of top-down controls of prokaryote communities in the bathypelagic zone (Sections 4 and 5).

Turnover times of bulk prokaryotic abundance in bathypelagic waters are estimated to vary in the range of 0.1 – 30 years (Nagata et al., 2000; Reinthaler et al., 2006), consistent with the above notion that prokaryote abundance reflects long-term mean input of organic matter. Slow turnover might be in part due to the inclusion of dormant or dead cells in bulk prokaryote counts. However, recent studies using micro-autoradiography combined with fluorescent *in situ* hybridization (FISH) revealed that a significant fraction of *Bacteria* and *Archaea* assimilate substrates

Table 2
 Prokaryotic abundance, prokaryotic production, and respiration in bathypelagic environments. Methods for determination are indicated as follows: [prokaryote abundance] FC, flow cytometry; EM, epifluorescence microscopy; [heterotrophic prokaryote production] Leu, ³H-leucine incorporation; TdR, ³H-thymidine incorporation. The method followed by a notation “HP” indicates that the incorporation rates were determined for samples kept under *in situ*, high-pressure conditions. The method without this notation indicates that the incorporation rates were determined at atmospheric pressures for decompressed samples; [respiration] O₂, consumption of dissolved oxygen; ETS, electron transport system activity. Conversion factor and/or other related assumptions for derivation of the rate variables are indicated by superscript letters on each method (see Table 3 for explanation). Values with an asterisk are those calculated from the source data.

Region	Depth (m)	Abundance (x10 ⁵ cells ml ⁻¹)	Production (μmol C m ⁻³ d ⁻¹)	Respiration (μmol C m ⁻³ d ⁻¹)	Reference
Subtropical Northeast Atlantic	1000	0.6 – 1 (FC)	2 – 20 Leu ^A & TdR ^{E,F}	52 – 146 (O ₂) ^J	Aristegui et al. (2005b)
Eastern and Western North Atlantic	1000 – 3870	0.3 – 1.0 (FC)	0.9 – 2.1 Leu ^B	64 – 224 (O ₂) ^J	Reinthal et al. (2006)
Eastern and Western North Atlantic	1000 – 3870			0.3 – 7 (ETS) ^{I,J}	Reinthal et al. (2006)
North Atlantic (NW African upwelling to offshore of the Canary coastal Transition Zone)	2000	0.1 – 0.4 (FC)	0.5 – 3.7* Leu ^B		Baltar et al. (2007)
Northeast Atlantic (Canary Islands)	1000			1 – 11 (ETS) ^{I,J}	Aristegui et al. (2003)
Northeast Atlantic (Canary Islands)	1000 – 2000			0.2 – 11 (ETS) ^J	J. Aristegui (unpublished)
Northeast Atlantic (Seine SM; Madeira)	1000 – 3000			3 – 10 (ETS) ^{I,J}	J. Aristegui (unpublished)
North Atlantic (Sedlo SM; Azores)	1000 – 3000			2 – 9 (ETS) ^{I,J}	J. Aristegui (unpublished)
Eastern North Atlantic	900 – 5,000	0.2 – 0.7 (FC)	0.1 – 0.4* Leu ^B		Varela et al. (2008a)
Eastern North Atlantic	900 – 5,000			2 – 7 (ETS) ^{I,J}	Baltar et al. (2009a)
Eastern Atlantic	1,000 – 4,000	0.03 – 0.5 (EM)			Patching and Eardly (1997)
Eastern North Atlantic (Iberia & NW Africa)	1,000 – 2,000			7 – 28* (ETS) ^{I,J}	Savenkoff et al. (1993a)
North Atlantic (North of Cape Verde, West Africa)	1,000 – 4,000	0.1 – 1.0 (EM)	0.04 – 0.6 TdR ^{E,H}		Dufour and Torretton (1996)
North Western Atlantic	1,000 – 3,100		0.08* TdR ^D		Turley and Mackie (1994)
North Atlantic (Sargasso Sea)	1,000 – 3,000			0.5 – 2* (ETS) ^{I,J}	Packard et al. (1988)
Northeast Atlantic (Meddies)	1000 – 2000			2 – 38* (ETS) ^{I,J}	Savenkoff et al. (1993a)
Caribbean (Cariaco Basin)	1,000 – 1,300	0.8 – 1.2 (EM)			Taylor et al. (2001)
Mediterranean (Ligurian Sea)	1,000 – 2000	0.4 – 0.8 (EM)	0.3 – 1.0 Leu ^B		Tamburini et al. (2002)
Mediterranean (Tyrrhenian Sea)	1000 – 3500	0.2 – 2.0 (EM)	0.9 – 4.1 Leu HP ^B		
Mediterranean (Ligurian Sea)	800 – 2000	1.1 – 2.3 (EM)	0.4 – 1.7 Leu ^B		Tamburini et al. (2009a)
Mediterranean (Ligurian Sea)	800 – 2000		2.6 – 18.0 Leu HP ^B		
Mediterranean (Ligurian Sea)	800 – 2000		0.03 – 4.2 TdR ^{D,G}		Tholosan et al. (1999)
Mediterranean (Ligurian Sea)	800 – 2000		0.08 – 11.5 TdR HP ^{D,G}		
Mediterranean (Ligurian Sea)	850 – 2000		0.3 – 3.9 TdR ^{D, G}		Tholosan (1999)
Mediterranean (Ligurian Sea)	850 – 2000		1.1 – 4.7 TdR HP ^{D,G}		
Mediterranean (Ligurian Sea)	1000	0.4 – 0.6 (EM)	1.0 – 4.0 Leu ^B		Bianchi et al. (1999b)
Mediterranean (Ligurian Sea)	1000		2.1 – 4.7 Leu HP ^B		
Mediterranean (Ligurian Sea)	1000		0.6 – 1.8 Leu ^A		Tanaka and Rassoulzadegan (2004)
Mediterranean (Ligurian Sea)	1000 – 1200			1 – 48* (ETS) ^{I,J}	Lefevre et al. (1996)
Mediterranean (Ligurian Sea)	1000 – 2000	0.3 ± 0.02 (EM)			Tanaka et al. (2007)
Mediterranean (Aegean and Ionian Sea)	900 – 4350	0.2 – 0.9 (FC)	2.5 – 19.8 Leu ^B		Yokokawa et al. (in press)
Mediterranean (Ionian Sea)	600 – 3000			2 – 17* (ETS) ^{I,J}	La Ferla and Azzaro (2001)
Western Mediterranean	1000 – 2500			0.6 – 4* (ETS) ^{I,J}	Christensen et al. (1989)
Western Mediterranean	1000 – 1870			15 – 32* (ETS) ^{I,J}	Savenkoff et al. (1993b)
Western and Eastern Arabian Arabian	700 – 3600	0.4 – 1.0* (EM)			Koppelman et al. (2005)
Arabian	1000 – 4500	0.1 – 1.5 (EM)	0.8 – 280 TdR ^{E,G}		Hansell and Ducklow (2003)
Indian Ocean (Arabian Sea)	1,000 – 1900			2 – 8 (ETS) ^{I,J}	Naqvi et al. (1996)
Indian Ocean (Bay of Bengal)	1000 – 1900			5 – 6 (ETS) ^{I,J}	Naqvi et al. (1996)
Indian/Southern	1,000 – 4,500		0.0067 – 0.2 TdR ^{C,G}		Moriarty et al. (1997)
Southern Ocean (62°E line)	1,000			3 – 14 (ETS) ^{I,J}	Aristegui et al. (2002)
Southern (the west of the Antarctic peninsula)	1,000 – 5,000	0.2 – 1.5 (EM)			Church et al. (2003)
Southern (150°E - 170°E)	1,000 – 3,800	0.2 – 0.8 (FC)	0.017 – 0.184 Leu ^B		T. Yokokawa et al. (unpublished)
North Pacific (ALOHA)	1,000 – 5,000	0.1 – 0.2 (EM)			Karner et al. (2001)
North Pacific (K2)	750 – 1,100	0.2 – 2.0 (EM)			Steinberg et al. (2008)
North Pacific	1,000 – 5,000	0.1 – 1.6 (EM)	0.0013 – 1.0 Leu ^A		Nagata et al. (2000)
North Pacific (Central North Pacific gyre)	1,000		0.8 – 5.8 TdR ^{D,G}		Cho and Azam (1988)
North Pacific	1000 – 5000			1 – 5* (ETS) ^{I,J}	Packard et al. (1988)
North Pacific	1000 – 6000	0.1 – 0.6 (FC)	0.015 – 0.28 Leu ^B		T. Yokokawa et al. (unpublished)
South Pacific	1000 – 5594	0.1 – 0.8 (FC)	0.005 – 0.70 Leu ^B		T. Yokokawa et al. (unpublished)
Arctic Ocean	1010 – 2025			0.5 – 8* (ETS) ^{I,J}	Packard and Codispoti (2007)

such as amino acids or bicarbonate (Herndl et al., 2005; Teira et al., 2006a).

3.2. Respiration

Respiration in bathypelagic waters varies in the range of $0.2 - 224 \mu\text{mol C m}^{-3} \text{d}^{-1}$ (Table 2). Most previous studies have used the electron transport system (ETS) activity assay. Only two studies (Aristegui et al., 2005b; Reinthaler et al., 2006) have directly examined respiration in bathypelagic waters from the decrease in dissolved oxygen concentration in bottle incubations, a technique widely used for the measurement of respiration in surface waters (Robinson, 2008). In the North Atlantic, Reinthaler et al. (2006) reported an average growth efficiency of 2% due to high prokaryote respiration and low HPP. Such low efficiency, relative to the corresponding value estimated for surface oceans (10%, del Giorgio and Cole, 2000), could be accounted for by low concentrations and low quality (as indicated by high C:N ratio) of substrates in deep waters (Table 1). In a study in the subtropical Northeast Atlantic, Aristegui et al. (2005b) obtained relatively high values of prokaryotic respiration at 1000 m depth ($0.08 \pm 0.03 \mu\text{mol O}_2 \text{ l}^{-1} \text{d}^{-1}$), comparable to those reported by Reinthaler et al. (2006) for the whole bathypelagic North Atlantic Ocean. HPP, however, was higher in the subtropical Atlantic, thus yielding growth efficiencies of $13 \pm 2\%$ at 1000 m. The high growth efficiencies, together with other metabolic proxies, indicated that prokaryotes were actively growing, apparently due to high loadings of organic matter advected from the continental shelf. More recently, Baltar et al. (2009a) found a significant positive correlation between concentrations of POC and respiratory activity in the subtropical Northeast Atlantic, suggesting that suspended POC, presumably transported by lateral advection, represents an important substrate for prokaryotes. Reinthaler et al. (2006) and Baltar et al. (2009a) suggested that prokaryotic carbon consumption far exceeded (up to 2 orders of magnitude) the supply of organic carbon by sinking POC in the investigated regions of the North Atlantic, pointing out an apparent imbalance between estimated carbon supply and demand. This imbalance issue has been extensively reviewed by Burd et al. (2010).

3.3. Uncertainties in rate measurements

There are uncertainties in conversion factors for the measurements of microbial activities (Table 3). The validity of the $^3\text{H-TdR}$ and $^3\text{H-Leu}$ methods as means of estimating HPP has been extensively examined and debated (Ducklow, 2000). Studies have generally concluded, on both empirical and theoretical grounds, that $^3\text{H-TdR}$ and $^3\text{H-Leu}$ methods provide powerful constraints on carbon fluxes mediated by prokaryotes in the upper ocean (Ducklow, 2000). Other investigators point out, though, that the conversion factors are variable for reasons that are still not entirely clear (Gasol et al., 2008). No prior study has determined the conversion factors for prokaryote communities in the bathypelagic ocean. Systematic differences in conversion factors between surface and deep environments would result in systematic biases in HPP determined in the bathypelagic layer. For example, slow growth of prokaryote assemblages in deep waters might imply a higher extent of protein turnover (D.L. Kirchman, personal communication). If true, bathypelagic HPP based on the $^3\text{H-Leu}$ method using a conversion factor derived for surface communities might be too high (i.e. high protein turnover implies that $^3\text{H-Leu}$ incorporated into protein is eventually mineralized with little contribution to the biomass production, Kirchman et al., 1985). Interpretations of the results

obtained by the $^3\text{H-TdR}$ method are complicated due to the unknown extent of thymidine catabolism (incorporation of $^3\text{H-TdR}$ into RNA and protein; Brittain and Karl, 1990) in deep-water communities.

Limited sensitivity of the oxygen method restricts its use as a direct means of determining low microbial respiration rates in deep waters. The ETS assay (Packard, 1971) has been used as an alternative approach that relies on a conversion factor relating the ETS activity to respiration (R) (R:ETS ratio, Table 4). Global estimates of respiration in the dark ocean have been obtained by Aristegui et al. (2003) who used a compiled dataset of ETS activities and a constant R:ETS ratio of 0.086. This conversion factor was derived from empirical R:ETS relationships determined using senescent cultures of bacteria (Christensen et al., 1980). The authors' estimates of the respiration in the mesopelagic zone ranged from 8 to $20 \text{ mmol O}_2 \text{ m}^{-2} \text{d}^{-1}$, which were consistent with the oxygen utilization rates (OUR) inferred from geochemical tracer balances for some oceanic regions (the Atlantic– Jenkins, 1982; Jenkins and Wallace, 1992; the Pacific– Feely et al., 2004). However, a subsequent study of Aristegui et al. (2005b) obtained a R:ETS ratio of 0.68 ± 0.11 on the basis of the direct comparison of oxygen consumption rate and ETS activities using samples collected at 600 and 1000 m depths in the subtropical northeast Atlantic. This high ratio is comparable to that determined for bacterial cultures during an exponential growth phase (average R:ETS = 1.1, range = 0.6 – 1.7). These results suggest that prokaryotes were actively growing (rather than senescent) in deep Atlantic waters and that the respiration (Table 2) derived using low R:ETS ratio (0.086) might be too low. Using a higher conversion factor, the ETS-based respiration rates are much higher than the OUR obtained by geochemical approaches. This discrepancy highlights that the ETS-based estimates of respiration are sensitive to conversion factors that are highly variable depending on the physiological state of microbes. In addition, differences in estimates of oxygen consumption may arise in part from the different time- and spatial scales over which different methods are applied (Burd et al., 2010). The OUR estimated from geochemical mass balances integrate the respiration over large scales of time (> years) and space (regional – basin), whereas microbial estimates are based on the incubation (< days) of bottle contained samples (< 100 ml).

As we will discuss in Section 7, decompression of deep water samples may significantly affect rate processes of deep prokaryotic communities, suggesting that pressures could potentially affect conversion factors involved in $^3\text{H-TdR}$, $^3\text{H-Leu}$ and ETS approaches.

3.4. Community composition and group-specific features

Recent studies have revealed that there are indigenous communities of bathypelagic prokaryotes with distinctive genetic and functional characteristics. One outstanding feature of the bathypelagic prokaryote community is the high relative abundance of *Archaea* (10 – 50% of total prokaryote cell abundance, Karner et al., 2001; Herndl et al., 2005), especially marine *Crenarchaeota* Group I and *Euryarchaeota* Group II (Church et al., 2003; Varela et al., 2008a). Variations in relative abundances of *Bacteria* and *Archaea*, as well as those of *Crenarchaeota* and *Euryarchaeota*, among different water masses and over seasons (Varela et al., 2008a) indicate that these groups have different requirements for growth. In fact, Teira et al. (2006a) found that *Bacteria* and *Archaea* differ in the uptake of enantiomeric amino acids, i.e. *Archaea* were more active than *Bacteria* in assimilating D-aspartic acid, while these two groups were almost equally active in the assimilation of L-aspartic acid in deep Atlantic

Table 3

Conversion factors involved in the calculation of prokaryotic production and respiration reported in Table 2.

Method	Conversion factor or equation	Unit	Superscript in Table 2
Leu (Leu to carbon)	1.6	kg C mol ⁻¹ Leu	A
	3.1	kg C mol ⁻¹ Leu	B
TdR (TdR to cell)	0.5 × 10 ¹⁸	cells mol ⁻¹ TdR	C
	(1.1 – 1.2) × 10 ¹⁸	cells mol ⁻¹ TdR	D
	2 × 10 ¹⁸	cells mol ⁻¹ TdR	E
Cell to carbon	15	fg C cell ⁻¹	F
	20	fg C cell ⁻¹	G
	Norland equation ^a		H
ETS & (R/ETS ratio)	0.086	dimensionless	I
ETS & O ₂ consumption (Respiratory quotient = oxygen to carbon molar ratio)	1	dimensionless	J

^a Norland (1993).**Table 4**

Viral abundance and viral to prokaryote abundance ratio (VPR) in bathypelagic environments. Methods for determination of viral abundance are indicated as follows: EM, epifluorescence microscopy; FC, flow cytometry.

Region	Depth (m)	Viral abundance (10 ⁸ L ⁻¹)	VPR	Ref.
Coral Sea	1000 – 4000	2 – 10 (EM)	12 – 20	Fuhrman (2000)
North Atlantic	700 – 5000	13 – 16 (FC)	27 – 110	Parada et al. (2007)
Carioca Basin (anoxic)	1000 – 5000	1 – 60 (EM)	1 – 75	Taylor et al. (2003)
Mediterranean	800 – 2000	1 – 20 (EM)	1 – 6	Weinbauer et al. (2003)
North Pacific	1000 – 5000	0.6 – 5 (EM)	2 – 9	Hara et al. (1996)
N&S Pacific/Southern	1000 – 5000	1 – 12 (FC)	9 – 223	T. Nagata et al. (unpublished)

waters. Compound-specific stable isotope analysis on membrane lipids (ether lipid) specific for *Crenarchaeota* indicated that this group might be autotrophs fixing dissolved inorganic carbon (DIC, Pearson et al., 2001; Wuchter et al., 2003). In support of this hypothesis, Herndl et al. (2005) demonstrated that up to 20% of archaeal cells took up ¹⁴C-bicarbonate in bathypelagic waters (1000–3000 m) of the North Atlantic, whereas the corresponding values for *Bacteria* were low (<2%). A subsequent study by Agogué et al. (2008), however, suggested that *Crenarchaeota* in the bathypelagic layer appeared to be heterotrophic in subtropical deep waters. Using natural abundance radiocarbon measurements of archaeal-specific lipids from 670 m depth in the subtropical North Pacific, Ingalls et al. (2006) determined 83% of archaeal community carbon was derived from autotrophy. Hansman et al. (2009) determined the radiocarbon signature ($\Delta^{14}\text{C}$) of microbial DNA collected from deep Pacific waters (depth, 670 – 915 m) in order to differentiate between the three major carbon pools that are potentially available to prokaryotes: fresh DOC released from POC ($\Delta^{14}\text{C} > +50\%$), ambient DIC ($\Delta^{14}\text{C} \sim -200$ to -100%), and aged bulk DOC ($\Delta^{14}\text{C} = -525\%$). Their results indicated that 1) both DIC and fresh DOC (presumably released from sinking POC) were utilized substantially, 2) ambient DOC did not appear to be a major source of carbon and 3) the extent of total community DIC utilization was correlated with *Crenarchaeota* 16S rRNA and archaeal amoA gene abundances. DIC fixation by *Crenarchaeota* might be fueled by the oxidation of ammonium (i.e., nitrification; Könneke et al., 2005; Wuchter et al., 2006; Beman et al., 2008), although it has yet to be demonstrated directly that such coupling exists in bathypelagic waters (Reinthal et al., 2010).

Hewson et al. (2006) found that bacterial community composition was remarkably heterogeneous at a depth of 3000 m in the oligotrophic North Pacific and Atlantic Oceans. Similarity in community composition decreased with increasing distance

between sampling sites. This finding is consistent with the hypothesis that resources are highly heterogeneous in the bathypelagic environment, although mortality (grazing and viral lysis) may also shape community structure (Thingstad, 2000; Pernthaler, 2005). According to one theory, spatial heterogeneity in community composition and the dispersal of prokaryote species can affect patterns in carbon mineralization in oceanic environments (Miki et al. 2008), providing an impetus for further studies on spatial heterogeneity of prokaryotic communities.

4. Viruses

Viruses are ubiquitous and the most abundant biological entities in marine environments, exerting a major influence on food webs and biogeochemical cycles (Weinbauer, 2004; Breitbart et al., 2008; Motegi et al., 2009). However, our knowledge of the ecology of viruses in the bathypelagic zone is very limited. Viral abundances vary in the range of 0.6 – 60 × 10⁸ viruses l⁻¹ in deep waters (Table 4), about 1/2 to 2 orders of magnitude lower than those in the upper oceans. High viral abundance (up to 1 × 10¹¹ l⁻¹) was found in a deep-sea hydrothermal vent system at a depth of ca. 2000 m (Ortmann and Suttle, 2005). The viral-to-prokaryote abundance ratio (VPR) varies in the range of 1 – 223 (Table 4). This variation may reflect regional variability in VPR as well as methodological artifacts, especially those associated with sample fixation and preservation (Turley and Hughes, 1992; Wen et al., 2004). VPR does not necessarily represent the numerical relationship between individual host and specific viruses, as some members of a prokaryote community may be more susceptible to viral infection than others. There are diverse ranges of viruses of *Bacteria* and those of *Archaea* (Prangishvili et al. 2006). It is unclear if *Bacteria* and *Archaea* (and their subgroups of *Crenarchaeota* and *Euryarchaeota*) differ in susceptibility to viral infection in the deep ocean.

Parada et al. (2007) reported that VPR's were systematically higher in deep than shallow water layers of the North Atlantic, reaching the highest value (110) in the Lower Deep Water (depth, 3500–5000 m). T. Nagata et al. (unpublished) found similar trends in the subtropical Pacific. This increase of VPR over depth was due to the fact that viruses decreased to a lesser degree with depth than prokaryotes. High VPR's in deep waters, about 10 times higher than typical VPR's in surface oceans (i.e., 10–20, Parada et al. 2006), suggest that viral-prokaryote systems in bathypelagic waters differ from those in surface waters. Parada et al. (2007) examined decay rates of viruses based on the decrease in viral abundance over time in 0.2- μm filtered deep water. The first order decay constants (k) were $1.1 - 3.7 \times 10^{-3} \text{ h}^{-1}$, indicating that the turnover times ($1/k$) of viral communities were on the order of 10–40 days. Alternatively, viral turnover time can be calculated from host productivity with steady state assumptions. These assumptions include: 1) production of prokaryotes is balanced by viral-induced mortality and 2) the burst size is constant, i.e., turnover time of viruses = viral abundance/(prokaryote production \times burst size). Using the values reported in Table 5 of Parada et al. (2007), with an assumption that burst size is 20, the estimated turnover times of viruses in bathypelagic waters are on the order of 4000–6000 days. These values are two orders of magnitude higher than those obtained from the decay experiments.

The large discrepancies of viral turnover times obtained using two different approaches highlight major gaps in our understanding of virus-prokaryote interactions in deep waters. Decay experiments would underestimate viral turnover time because of enhanced loss of viral particles [e.g. rupture of viral capsid (Paepe and Taddei, 2006) or adsorption to walls] during the incubation of samples. On the other hand, estimates of viral turnover time, on the basis of steady state assumptions, might be too long because of the ignorance of spatial and temporal heterogeneity in the viral-prokaryote relationship. Viral turnover could be more rapid than predicted by the model during periods of episodic inputs of organic matter as well as on (or inside) aggregates where abundance and activity of prokaryotes can be high (Section 6.1).

We know little about the role of lysogeny in the bathypelagic ocean. Weinbauer et al. (2003) found that lysogenized bacteria were abundant in the deep (at 2000 m) Mediterranean Sea. This

finding is consistent with the notion that lysogeny is common in oligotrophic environments where host activities are low (Williamson and Paul, 2004). Switching from lysogenic to lytic modes of reproduction is triggered by UV irradiation, chemical inducers, and changes in nutrient conditions (Williamson and Paul, 2004; Motegi and Nagata, 2007). However, factors that control lysogenic to lytic conversion of viral populations in deep waters remain unclear.

Interpretations of spatial and temporal variations in viral abundance in deep waters are complicated by allochthonous inputs of viruses. In the Cariaco Basin, Taylor et al. (2003) reported that the vertical fluxes of viruses at a depth of 1200 m varied seasonally, ranging from $10^9 - 10^{10}$ viruses $\text{m}^{-2} \text{d}^{-1}$. Advective transport associated with overturning circulation is an alternative mechanism by which viruses are transported into deep waters. Parada et al. (2007) suggested that allochthonous inputs of viruses account for high VPR in deep waters of the North Atlantic.

5. Protists

Heterotrophic nanoflagellates (HNF) are a phylogenetically highly diverse functional group of protists and dominate grazing of prokaryotes in marine surface waters (Jürgens and Massana, 2008). HNF are also the dominant morphotype in the deep sea with ciliates and amoebae occurring at more than an order of magnitude lower abundances (Table 5, Arndt et al., 2003). Early studies reported the occurrence of HNF throughout the water column of the Atlantic Ocean, providing taxonomic descriptions based on light and electron microscopy (Patterson et al., 1993). Studies also examined growth and morphology of some isolates of piezophilic and piezotolerant HNF (Turley et al., 1988; Turley and Carstens, 1991). Recently, tremendous diversity of small-sized protists (size range 0.2–5 μm) was discovered in bathypelagic waters (Lopez-Garcia et al., 2001; Arndt et al., 2003; Not et al., 2007; Countway et al., 2007). These studies either used live observations (Arndt et al., 2003) or culture independent techniques to analyze polymerase chain reaction (PCR) clone libraries targeted to 18S rRNA genes directly extracted from seawater (Worden and Not, 2008). In deep Antarctic waters (200–3500 m), Lopez-Garcia et al. (2001) discovered abundant and diverse

Table 5

Abundance and biomass of protists in bathypelagic environments. Methods indicate the type of microscopy (Epi, epifluorescence microscopy, LM, transmission light microscopy). In parentheses after microscopy method- dyes (or technique) used for staining are presented for the count of flagellates and small eukaryotes, whereas fixatives used are presented for the count of ciliates.

Protist group	Region	Depth (m)	Cell abundance (cell ml^{-1})	Biomass ($\mu\text{g C L}^{-1}$)	Method	Reference
Flagellates	Mid North Atlantic	1000 – 2700	10 – 20		Epi (AO)	Patterson et al. (1993)
Eukaryotic microbes	Subtropical and tropical Atlantic (eastern basin)	900 – 5000	0.03 – 3.88	-	Epi (TSA-FISH & DAPI)	Morgan-Smith (unpublished)
Flagellates	Eastern Mediterranean	1000 – 4000	0 – 0.067		LM(Live count)	Arndt et al. (2003)
Flagellates (< 20 μm)	Eastern Mediterranean (Levantine Basin)	1000 – 1750	3.4 – 7.0		Epi (DAPI)	Tanaka et al. (2007)
Flagellates (< 20 μm)	Northwest Mediterranean	1000 – 2000	1.2 – 15	0.002 – 0.05	Epi (Proflavine & DAPI)	Tanaka and Rassoulzadegan (2002)
Dinoflagellates	Mediterranean	1000 – 4000	0 – 0.015	0 – 1.4	LM (formaldehyde)	Fonda-Umani (unpublished)
Flagellates	Western North Pacific	1000 – 5800		0.03 – 0.3	Epi (FITC&DAPI)	Yamaguchi et al. (2004)
Dinoflagellates	Western North Pacific	1000 – 5800		0.007 – 0.2	Epi (FITC&DAPI)	Yamaguchi et al. (2004)
Flagellates (< 20 μm)	Subarctic Pacific	1000 – 3500	1.4 – 12	0.0020 – 0.017	Epi (FITC&DAPI)	Fukuda et al. (2007)
Flagellates (< 20 μm)	Central Pacific	1000 – 5000	5.5 – 13	0.0077 – 0.070	Epi (FITC&DAPI)	Sohrin et al. (2010)
Ciliates	Northwest Mediterranean	1000 – 2000	0.0008 – 0.029	0.001 – 0.1	LM (acid Lugol)	Tanaka and Rassoulzadegan (2002)
Ciliates	Mediterranean	1000 – 4000	0.001 – 0.052	0.0002 – 0.33	LM (formaldehyde)	Fonda-Umani (unpublished)
Ciliates	Western North Pacific	1000 – 5800		0 – 0.01	LM (formaldehyde)	Yamaguchi et al. (2004)
Ciliates	Central Pacific	1000 – 5000	< 0.0001 – 0.010	0 – 0.018	LM (acid Lugol)	Sohrin et al. (2010)

sequences of 18S rRNA genes belonging to two novel clades of *Alveolata*. Alveolates include well known groups of protists such as ciliates and dinoflagellates, but the novel sequences were grouped into previously unknown major clades. Subsequent studies revealed that these novel alveolates are widespread throughout the water column of the oceans (Not et al., 2007; Countway et al., 2007). However, the physiology and trophic modes of the affiliated organisms from which the sequences are derived are not entirely clear (Worden and Not, 2008). Countway et al. (2007) characterized protist diversity in samples from euphotic (< 125 m) and bathypelagic (2500 m) zones of the western North Atlantic. Analyses of clone libraries showed that protist assemblages in the euphotic and bathypelagic zones were very different: only 28 out of the 324 operational taxonomic units were detected in both habitats. In short, largely distinctive protistan assemblages inhabit bathypelagic water, with only minor contributions from surface-dwelling taxa.

Abundances of HNF in deep water vary in the range of $< 0.02 - 20 \text{ cells ml}^{-1}$ (Table 5). While some of the differences in flagellate abundance can be attributed to the different methods used for enumeration, some variability may be due to differences in productivity of the overlying water masses among these diverse geographic regions (Table 5). The depth dependent pattern in full depth distributions of HNF can be described by a power function, with exponents being greater than those for prokaryotes (Tanaka and Rassoulzadegan, 2002; Arístegui et al., 2009). The vertical pattern of HNF abundance below 1000 m depth is less evident, being either highly variable (Morgan-Smith et al., unpublished) or relatively constant (Sohrin et al., 2010). The abundance ratio of HNF to prokaryote tends to decrease from a typical value of 10^{-3} in surface oceans to around $1 - 2 \times 10^{-4}$ in bathypelagic layers (Tanaka and Rassoulzadegan, 2002; Fukuda et al., 2007), although exceptions dependant upon oceanic region have also been noted (Sohrin et al., 2010).

Five- to 10-fold increases in the abundance ratio of prokaryote to HNF might imply that HNF play only a minor role in control of prokaryotes in deep waters. However, data obtained by Fukuda et al. (2007) did not support this hypothesis. They found that HNF consumed half of the prokaryote production in the bathypelagic zone (1000–3500 m) of the subarctic Pacific; this study estimated HNF grazing from total cell volume of HNF and a volume-specific clearance rate of HNF determined in the mesopelagic zone (Cho et al., 2000). In addition, they found that turnover time of prokaryotes in the bathypelagic was significantly and negatively correlated with the abundance of HNF. A tight predator-prey interaction between prokaryote and HNF in bathypelagic waters seems to occur despite the low average abundance of prokaryotes (on the order of $10^4 \text{ cells ml}^{-1}$, Table 2). If prokaryotes were evenly distributed, these densities may be below the threshold level at which growth of flagellates is no longer sustainable (ca. $10^5 \text{ cells ml}^{-1}$; Andersen and Fenchel, 1985; Wikner and Hagström, 1991). One hypothesis to explain this apparent paradox is that most of the trophic interactions between HNF and prokaryotes take place inside and on the surface of aggregates (Section 6) where prokaryote abundance is high (Aldredge and Youngbluth, 1985; Simon et al., 2002). In support of this notion, video profiles have shown that particles are highly abundant in bathypelagic layers down to a depth of 6000 m (Bochdansky et al., in press). Association with particles, however, would make current estimates of HNF feeding difficult to interpret, i.e. clearance rate estimates by Fukuda et al. (2007) assume homogeneous distribution of prey. An alternative explanation of the observed high grazing impact on prokaryote communities is that HNF in the deep sea are adapted to much lower prey concentrations, and that their threshold prey density is much lower than that of surface-dwelling flagellates.

Abundances of ciliates vary in the range of $< 0.0001 - 0.052 \text{ cells ml}^{-1}$ in deep waters (Table 5). Decrease with depth in the abundance of ciliates is even steeper than that of HNF (Tanaka and Rassoulzadegan, 2002; Sohrin et al., 2010); Sohrin et al. (2010) found that ciliate biomass was significantly and positively correlated with prokaryote biomass but not with HNF biomass in the bathypelagic zone of the central Pacific, reflecting the difference in microbial food-web structures between the bathypelagic and upper oceans. However, data on geographic distributions and activity of ciliates are scarce and their contributions to the microbial food webs in bathypelagic environments remain unclear.

6. POM - microbe interactions

6.1. Aggregates

There are several classes of marine detrital particles with different sizes and properties (Nagata, 2008). Large marine particles are called organic aggregates since they are composed of smaller particles (Simon et al., 2002). Macroscopic forms of the aggregates ($> 500 \mu\text{m}$), known as marine snow, play an important role as vehicles for the vertical transport of carbon and other elements (Aldredge and Youngbluth, 1985; Simon et al., 2002). These larger particles occur at high and variable abundances throughout the bathypelagic environment with abundances and sizes characteristic for individual water masses (Bochdansky et al., in press). The abundance of these large particles frequently peaks at the interface between water masses (Bochdansky et al., in press).

Various types of organic and inorganic components, with variable chemical properties and origins, contribute to the formation of aggregates. Investigators have suggested that “gels” are abundant and important components of aggregates throughout the water column of the oceans (Verdugo et al., 2004; Verdugo and Santschi, 2010). Gels are a unique form of molecular organization in which the polymer chains are interconnected by entanglements and cation or hydrophobic crosslinks (Chin et al., 1998; Ding et al., 2008). The gel phase spans a large size spectrum, from colloids to marine snow, mediating conversion of DOM to POM via self-assembly of polymers. Therefore, aggregates may not only be delivered to the bathypelagic zone via sinking, but also via self assembly of micro-gels (Fig. 1). It is likely that high hydrostatic pressures in deep water environments may affect organization of polymer assemblies, although how changes in the

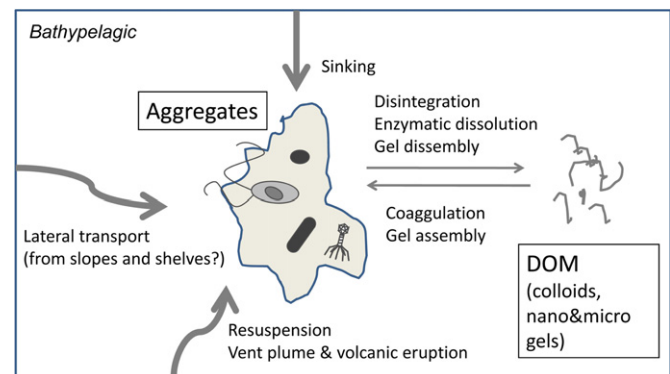


Fig. 1. Aggregates in the bathypelagic ocean. The major routes of the delivery of aggregates to bathypelagic water include sinking from the upper layer, lateral transport from slopes and shelves, resuspension of bottom sediments, and volcanic eruptions and hydrothermal vents. Coagulation and self assembly of gels account for *in situ* formation of aggregates in deep waters. Illustration indicates that aggregates provide a habitat for various microbes including prokaryotes, protists, and viruses.

structure of organic matter affect assimilation patterns by microbes have yet to be investigated.

Aggregates are active sites of transformations of materials and are characterized by high local concentrations of labile organic matter that nourishes dense microbial communities, although some aggregates are scarcely populated by microbes (Karl et al., 1988; Alldredge et al., 1993). Studies have shown that prokaryotes associated with aggregates produce large amounts of extracellular enzymes to hydrolyze polymers, resulting in high release of DOM, subsequently utilized by free-living prokaryotes to support their production in deep waters (Cho and Azam, 1988; Nagata et al., 2000). Consistent with this notion, compound-specific radiocarbon analyses of high-molecular-weight DOM revealed that a fraction of neutral sugars at 600 m in the North Pacific are introduced from large, rapidly sinking particles (Repeta and Aluwihare, 2006).

Kjørboe and Jackson (2001) developed a model to explain the formation of a plume of DOM streaming behind sinking particles. They predicted that free-living prokaryotes can take advantage of the plume to fuel their growth, creating a highly patchy environment in terms of biological activity.

6.2. Extracellular hydrolytic enzyme activities

A few studies examined extracellular enzyme activities in bathypelagic waters (Table 6). Extracellular enzyme activities normalized to prokaryote cell abundance or production in the bathypelagic zone are much higher than those in surface waters, a fact that stresses the importance of polymeric substrates for prokaryotes at depth (Hoppe et al., 2002; Tamburini et al.,

Table 6

Extracellular enzyme activities in bathypelagic environments. Activities were determined at atmospheric pressure (i.e., after decompression) except for studies conducted by Tamburini et al. (2002), Tamburini (2002), and Tamburini et al., 2009a, 2009b in which activities were determined at both *in situ* pressure conditions and at atmospheric pressure. Presented in Remarks is the range of ratios of the activities determined at *in situ* pressure to those determined after decompression (IN-SITU/ATM: a ratio > 1 means that the activities determined at *in situ* pressure conditions were higher than those determined after decompression). Standard deviations associated with measurements of enzyme activities for individual samples are generally ~15% (F. Baltar, unpublished), indicating that much of the variability for each study cannot be simply accounted for by analytical errors; rather it may reflect spatio-temporal variability in the oceans. However, data should be carefully compared among studies, because variations due to differences in assay protocols among different laboratories are unresolved.

Region	Depth (m)	Enzyme	Activity (nmole L ⁻¹ h ⁻¹)	Remarks	Reference
Eastern and Western subtropical Atlantic	1000 – 4500	Alpha-glucosidase	< 0.02 – 0.16	Maximum activity estimated from kinetic analysis (added substrate concentration, 0.6 – 1,200 μM)	Baltar et al. (2009b)
		Beta-glucosidase	< 0.02 – 0.07		
		Leu aminopeptidase Alkaline phosphatase	0.6 – 9.2 0.04 – 3.7		
Central Pacific	1000 – 4000	Alpha-glucosidase	0.0001 – 0.0037	Particulate fraction (> 0.2 μm). Added substrate concentration, 100 – 150 μM	Koike and Nagata (1997)
		Beta-glucosidase Alkaline phosphatase	0.0002 – 0.0046 0.031 – 0.35		
Indian Ocean	1000 – 3000	Beta-glucosidase	1.4 – 2.5	Added substrate concentration, 250 μM	Hoppe and Ullrich (1999)
		Leu aminopeptidase Alkaline phosphatase	6 – 10 2.2 – 4.0		
Antarctic (Ross Sea)	1750 – 1815	Beta-glucosidase	2.2 – 2.4	Added substrate concentrate, 50 – 100 μM	Celussi et al. (2009)
		Leu aminopeptidase	6.9 – 9.3		
		Alkaline phosphatase	11 – 12		
		Chitinase Lipase	1.7 – 2.2 5 – 15		
NW Mediterranean (DYFAMED site)	1000 – 2000	Leu aminopeptidase	0.1 – 1.3	Maximum activity estimated from kinetic analysis (added substrate concentration, 0.05 – 5 μM). IN-SITU/ATM = 1.5 to 2.8.	Tamburini et al. (2002)
		Alkaline phosphatase	0.01 – 1.8		
Eastern Mediterranean (Ionian Sea)	1000 – 3000	Leu aminopeptidase	0.01 – 0.27	Maximum activity estimated from kinetic analysis (added substrate concentration, 0.05 – 5 μM) over 10 hours time-course experiment IN-SITU/ATM = 2.1 to 3.5	Tamburini (2002)
Tyrrhenian Sea (Mediterranean Sea)	1000 – 3500	Leu aminopeptidase	0.51 – 8.60	Maximum activity estimated from kinetic analysis (added substrate concentration, 0.05 – 5 μM) over 10 hours time-course experiment IN-SITU/ATM = 1.4 to 5.8	Tamburini et al. (2009a)
		Alkaline phosphatase	0.21 – 9.90		
Mediterranean (Ionian Sea)	700 – 3500 (including bottom boundary layer)	Beta glucosidase	0.02 – 0.07	Maximum activity estimated from kinetic analysis (added substrate concentration, 0.1 – 20 μM)	Zaccone et al. (2003)
		Leu aminopeptidase	0.03 – 0.8		
		Alkaline phosphatase	0.08 – 0.4		

2003; Baltar et al., 2009b). In deep Mediterranean waters, Tamburini et al. (2003) found a tight coupling between POC fluxes, HPP and extracellular enzyme activities. In the central Atlantic deep waters, Baltar et al. (2010) found high extracellular enzymatic activities in the dissolved fraction, leading to the hypothesis that microbes associated with aggregates released large amounts of free enzymes. As we discuss in Section 7, decompression can affect ectoenzymatic hydrolytic activities.

Koike and Nagata (1997) found that alkaline phosphatase (APase) activities associated with particles were high in the deep (2000–4000 m) central Pacific. Their results are inconsistent with the notion that extracellular enzyme synthesis is suppressed under the presence of sufficient amounts of monomeric substrate (Hoppe et al., 2002). Other studies have confirmed this observation in other oceanic regions (Hoppe and Ullrich, 1999; Tamburini et al., 2002; Tamburini et al., 2009a; Baltar et al., 2009b). Hypotheses to explain this paradox include the delivery of APase to deep water with sinking aggregates (Koike and Nagata, 1997) and prokaryotic expression of APase activity to utilize carbon rather than the phosphorus moiety of organic phosphorus (Hoppe and Ullrich, 1999). Regardless of the mechanisms, the widespread occurrence of high APase activity in the bathypelagic ocean has potentially important implications for global cycling of phosphorus (Colman et al., 2005).

6.3. Microbial communities associated with aggregates

In the upper ocean, several studies have documented differences in the composition of *Bacteria* between particle-associated and free-living communities (DeLong et al., 1993; Simon et al., 2002). However, the data are scarce on compositions of particle-associated microbes in the bathypelagic ocean. Using a metagenomic approach (Section 8), DeLong et al. (2006) suggested that a surface-attached lifestyle is important in bathypelagic microbial communities. They found that genes encoding pilus, polysaccharide, and antibiotic synthesis, which are generally involved in bacterial attachment on surfaces and microbial interactions in biofilms, were more enriched in deeper (down to 5,000 m) than shallower water samples collected in the subtropical Pacific. In deep waters of the eastern Mediterranean Sea, Moeseneder et al. (2001) found that community composition and stratification patterns of free-living prokaryotes differed from those of particle-associated prokaryotes; the two communities shared only a limited number of phylotypes. Turley and Carstens (1991) found that pressure tolerance differed greatly among species of HNF associated with particles, indicating that sinking particles may experience a succession of bacterivorous HNF during their transit through deep water. Tamburini et al. (2006, 2009b) examined effects of pressure on community compositions of prokaryotes developed on aggregates. They found that the increase in hydrostatic pressure induced variable responses of different phylogenetic groups, resulting in the loss of some metabolic capabilities of microbial communities to degrade organic matter (cf. Turley, 1993).

7. Pressure effects on microbial activity

The available data on HPP (Table 2), respiration (Table 2) and extracellular enzyme activities (Table 6) in the bathypelagic ocean were mostly obtained from the incubation of water samples under atmospheric pressure conditions. Questions arise regarding the accuracy and relevance of the values determined on decompressed samples, given that hydrostatic pressure can have substantial impacts on the physiology and activities of microbes. Using a pressurized vessel to obtain re-pressurized deep-sea samples, early studies found that pressure affects bacterial physiology and growth (ZoBell and Oppenheimer, 1950). Our knowledge of pressure effects on marine microbes has been improved by laboratory

experiments using pure cultures of high pressure-adapted microbes or piezophiles (from the Greek verb *piezo*, to press). These studies have elucidated microbial adaptations to high hydrostatic pressure in respect to growth (Abe et al., 1999), membrane lipids (Yayanos, 1995), membrane protein (Bartlett et al., 1989), enzymes (Kato et al., 2008), respiratory chain (Yamada et al., 2000) and DNA replication and translation (Bartlett et al., 1995).

In order to examine microbial activities under *in situ*, high pressure conditions, pressure-retaining samplers have been developed (Jannasch et al., 1973; Tabor et al., 1981; Bianchi et al., 1999b). Using one of these samplers, Jannasch and Wirsén (1973) concluded that elevated pressure substantially decreases rates of growth and metabolism of microbes collected from deep waters. However, subsequent studies have reported conflicting results: the mode (positive or negative) and extent of pressure effects vary depending on several factors including the type of substrates used for the determination of activities (Patching and Eardly, 1997) and mixing condition of the water column (Bianchi and Garcin, 1993). For samples collected at a deep water-sediment interface, Danovaro et al. (2008) found no pressure effect on HPP.

Despite apparent complexity in pressure effects on microbial activities, the data obtained in Mediterranean deep waters during the stratified period revealed a general trend. A compilation of the data obtained by a series of studies conducted by French researchers (Bianchi et al., 1999a, 1999b; Tamburini, 2002; Tamburini et al., 2002, 2009b; Tholosan, 1999; Tholosan et al., 1999) showed that the average value of HPP determined at *in situ* pressure conditions ($2.5 \pm 2.1 \mu\text{mol C m}^{-3} \text{d}^{-1}$, mean \pm SD) was significantly higher (paired *t*-test, $t = 5.538$, $p < 0.001$, $n = 41$) than the HPP determined under atmospheric pressure conditions ($1.3 \pm 1.3 \mu\text{mol C m}^{-3} \text{d}^{-1}$). A pairwise comparison of the HPP determined at *in situ* pressure conditions (IN-SITU) relative to those determined at atmospheric pressure conditions (ATM) yielded an average IN-SITU/ATM ratio of 3.4 ± 4.1 (\pm S.D., $n = 41$, Fig. 2). The large standard deviation indicates that

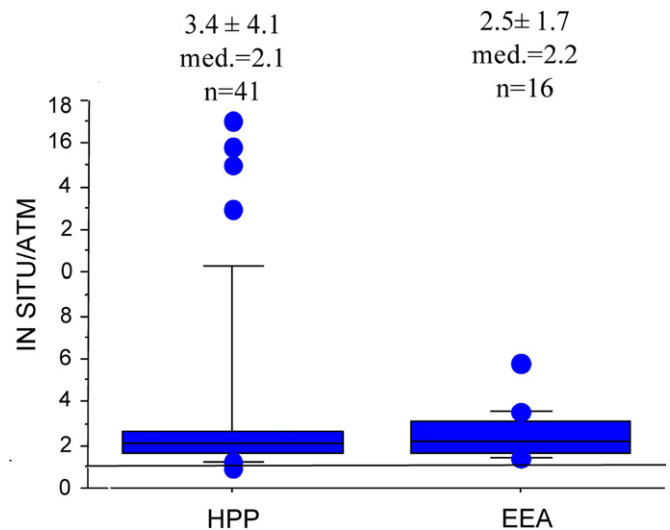


Fig. 2. Box-plot of an index of pressure effect (IN SITU/ATM) for heterotrophic prokaryotic production (HPP) and extracellular hydrolytic enzyme activities (EEA: including the activities of leu-aminopeptidase and alkaline phosphatase). HPP and EEA were determined for samples maintained and incubated under *in situ* high pressure conditions (IN-SITU) and for samples incubated at atmospheric pressure after decompression (ATM). The top and bottom of each box-plot represent 75th and 25th percentiles, respectively. A line within the box marks the median. Whiskers (error bars) above and below the box indicate the 90th and 10th percentiles. The outliers are shown as full circles. Values on each plot (from top to bottom) are as follows: Average \pm standard deviation, med. = median, n = number of samples. Data are from Bianchi et al. (1999b), Tamburini (2002), Tamburini et al. (2002, 2009a), Tholosan (1999) and Tholosan et al. (1999) for HPP and from Tamburini (2002), Tamburini et al. (2002, 2009a) for EEA.

HPP determined at atmospheric pressures is a weak predictor of HPP under *in situ* pressure conditions. However, mechanisms underlying variations in the IN-SITU/ATM ratio remain unclear.

The above studies have also found that hydrostatic pressure affects extracellular hydrolytic enzyme activities. From a comparison of 16 samples, they found that extracellular hydrolytic enzyme activities determined under *in situ* pressure condition were significantly higher than the corresponding activities determined after decompression, deriving an average IN-SITU/ATM ratio of 2.5 ± 1.7 (\pm S.D., $n = 16$, Fig. 2). Similar results have been obtained using a bacterial isolate. Kato et al. (1995) reported that alkaline serine ectoprotease activity of a strain of *Sporosarcina* sp. isolated from the Japan Trench at 6500 m was nearly doubled at 60 MPa compared to the rate measured at atmospheric pressure. They suggested that this protease may be adapted to the high-pressure environment found in the deep sea.

Investigators hypothesized that: 1) allochthonous microbes are inactive under deep- water, high-pressure conditions, but they account for bulk activities determined under decompressed conditions, and 2) autochthonous microbes are less active under atmospheric pressure conditions but they account for the bulk activities determined under *in situ*, high- pressure conditions (Eardly et al., 2001; Tamburini et al., 2006; Deming, 2007). Hypothesis #1 was supported by data demonstrating that activities of microbes attached to particles decreased with increasing pressure (Turley et al., 1995; Tamburini et al., 2006, 2009b).

In short, evidence has begun to indicate that indigenous microbial communities in deep waters express higher activities under pressurized (*in-situ*) conditions than the same communities incubated under atmospheric pressures. However, the extent of the pressure effect appears to be highly variable for reasons that are still not clear.

8. New insights derived from high throughput sequencing, genomic, and metagenomic data

Examination of an organism's complete genetic material (genomics) and that of genomes from natural microbial communities using DNA extracted directly from the environment (metagenomics, also referred to as "environmental genomics" or "community genomics") has become a powerful approach to explore novel ecological processes and genetic bases of biogeochemical processes mediated by microbes in the oceans (Moran, 2008). In addition, studies using high throughput sequencing techniques have begun to reveal remarkable diversity of marine microbes (Sogin et al., 2006). This section discusses some results obtained using these new approaches.

8.1. Diversity and compositional characteristics of deep-sea microbes

Sogin et al. (2006) sequenced $\approx 118,000$ PCR amplicons that span the V6 hypervariable region of ribosomal RNAs from environmental DNA preparations from different sites of the meso- and bathypelagic realm of the North Atlantic down to about 4100 m. One of the striking findings from this work is that while microbial abundances and biomass decrease with depth (Section 3.1), there may still be one hundred times greater diversity in deep ocean samples compared to surface water samples (Quince et al., 2008; Sogin et al., 2006). Another important aspect is the nature of the changes in microbial community structure. When a subset (low temperature component) of data obtained by Sogin et al. (2006) was re-examined, it was found that *Alphaproteobacteria* and *Gammaproteobacteria* dominated most of the samples, but increasing depth was associated with proportional increases in the phyla *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, *Gemmatimonadetes*, *Lentisphaera*, *Nitrospirae*, *Planctomycetes* and *Verrucomicrobia* and the subphylum *Deltaproteobacteria* (Lauro and Bartlett, 2008). Some of the general characteristics of members of these groups are listed in Table 7. Interestingly, many of the microbial groups that are suited to deep oceanic conditions are also dominant members of terrestrial soil environments.

A few additional high throughput-sequencing efforts have been undertaken in other deep-sea settings. DeLong et al. (2006) obtained samples from various depths down to 4000 m at the ALOHA station in the North Pacific subtropical gyre (11 Mbp sequence information obtained) and Martín-Cuadrado et al. (2007) from a depth of 3000 m in the Ionian Sea (7.2 Mbp sequence information obtained). In contrast to the study of Sogin et al. (2006), these studies cloned and sequenced large (40 kbp) environmental DNA fragments from microbes (metagenomics). DeLong et al. (2006) found that a larger proportion of *Bacteria* were related to *Alphaproteobacteria*, *Gammaproteobacteria*, *Deltaproteobacteria*, *Actinobacteria*, *Chloroflexi* and *Planctomycetes* sequences in the deep-water samples compared to their shallow-water counterparts of the subtropical Pacific. Martín-Cuadrado et al. (2007) found similar trends in the Ionean Sea: they noted high levels of *Alphaproteobacteria* (especially *Rhizobiales*) and *Gammaproteobacteria* followed by *Deltaproteobacteria*, *Acidobacteria*, *Chloroflexi* and *Planctomycetes*.

Stratified microbial community composition also extends to the domains *Archaea* and *Eukarya*. The above-mentioned environmental genomic studies noted high abundances of marine *Crenarchaeota* Group I at depth, consistent with the prior studies (Section 3.4). In the case of the *Eukarya*, small subunit rRNA gene

Table 7

Examples of bacterial groups increasing in abundance with ocean depth. General characteristics include functional and physiological features, taxonomic signatures, and typical habitats (in parentheses) that characterize previously known species and genera belonging to the corresponding major phylogenetic group.

Microbial Group	Examples	General characteristics	Reference
<i>Acidobacteria</i> phylum	<i>Holophaga</i> , <i>Geothrix</i> , <i>Acidobacterium</i>	(soil, sediment)	Jones et al. (2009)
<i>Bacteroidetes</i> phylum	<i>Bacteroidetes</i> , <i>Flavobacteria</i> , <i>Sphingobacteria</i>	Biopolymer degraders	Kirchman (2002)
<i>Actinobacteria</i> phylum	<i>Frankia</i> , <i>Mycobacterium</i> , <i>Streptomyces</i>	High GC% Gram positives (soil, sediment)	Jensen and Lauro (2008)
<i>Deltaproteobacteria</i> subphylum	<i>Myxococcus</i> , <i>Bdellovibrio</i>	Diverse lifestyles, Sulfate reducers (soil, sediment)	Rodionov et al. (2004)
<i>Planctomycetes</i> phylum	<i>Planctomyces</i> , <i>Pirellula</i> .	Biopolymer degraders, Anaerobic ammonia oxidizers (sewage, sediment)	Woebken et al. (2007)
<i>Firmicutes</i> phylum	<i>Bacillus</i> , <i>Clostridium</i> and <i>Desulfotomaculum</i>	Low GC% Gram positives (soil, sediment)	Onyenwoke et al. (2004)
<i>Nitrospirae</i> phylum	<i>Nitrospira</i> , <i>Leptospirillum</i> , <i>Thermodesulfobacter</i>	Nitrite and iron oxidizers, Sulfate reducers (aquatic, terrestrial, sewage)	Maixner et al. (2008)
<i>Gemmatimonadetes</i> phylum	<i>Gemmatimonas aurantiaca</i>	Polyphosphate accumulation (sludge, soil, sediment)	Zhang et al. (2003)
<i>Lentisphaerae</i> phylum	<i>Lentisphaera araneosa</i> , <i>Victivallis vadensis</i>	Transparent exopolymer paraticles production (marine, anaerobic digester)	Cho et al. (2004)
<i>Verrucomicrobia</i> phylum	<i>Acidimethylsilex</i> , <i>Alterococcus</i> , <i>Chthoniobacter</i>	Degraders of plant saccharides, Low pH methanotrophs (soils, animals, mud volcano)	Wagner and Horn (2006)

sequencing has been performed on DNA obtained from the Marmara Sea, Ionian Sea and the South and North Atlantic. The results of this work indicate that flagellate species affiliated with the two major uncultured diplomemid clades preferentially inhabit the deep ocean (Lara et al., 2009).

8.2. Functional attributes and deep-sea adaptations of microbes: Comparative genomics

8.2.1. Transposable elements and systems related to the utilization of organic matter

The first genome sequence to be obtained from a low temperature-adapted deep-sea microbe (psychropiezophiles) came from the bacterium *Photobacterium profundum* strain SS9 (Vezi et al., 2005; Bartlett et al., 2007, 2008). This moderate piezophile was found to have an exceptionally large number of transposable elements, and duplications of genes encoding components of systems known to be pressure-sensitive in mesophilic bacteria, including the F_1F_0 ATPase and *cbb3* cytochrome oxidase genes. Carbon and energy utilization also appeared to be different. An amino acid fermentation pathway previously known only in strict anaerobes (i.e. the Stickland reaction) was present along with systems related to those for the utilization of various complex polymers including xenobiotics, chitin, pullulan and cellulose. The latter is particularly relevant since more recalcitrant dissolved organic carbon may be utilized in deeper ocean environments (Carlson et al., 2004). Another interesting feature of the SS9 genome is what is not there: a deoxyribodipyrimidine photolyase gene. This absence presumably reflects the lack of need for a light-driven DNA repair system in the dark ocean, an observation that has also been made in subsequent deep-sea genome analyses (DeLong et al., 2006; Wang et al., 2008).

8.2.2. Large number of rRNA genes, larger intergenic regions, and larger genome size

Full or partial genome sequence information is available for other psychropiezophiles including *Shewanella* KT99, *Moritella* sp. PE36, *Psychromonas* sp. CNPT3 and *Carnobacterium* AT7 (Lauro and Bartlett, 2008). Compared to related non-piezophiles, these microbes contain larger numbers of rRNA genes and larger intergenic regions, perhaps reflecting adaptations for more rapid responsiveness to environmental change and more cis-acting regulatory features. More recently the genome of the piezo-tolerant *Shewanella* strain WP3 has been obtained (Wang et al., 2008). As with the piezophiles, it has a larger genome size and contains extra genes for transport, secretion, metabolism (including sulfated glycopolymer utilization), transcriptional regulation, and tRNA and rRNA modification.

8.2.3. Unsaturated membrane fatty acids

All of these deep-sea organisms also have enzymes for maintaining highly unsaturated membrane fatty acids, including β -ketoacyl-ACP synthases, desaturases and polyketide synthases for omega-3 polyunsaturated fatty acid production. The critical need for producing unsaturated membrane fatty acids by deep-sea bacteria has been amply demonstrated. Genetic experiments coupled with fatty acid supplementation experiments show the need for monounsaturated fatty acids in SS9 growth at high pressure (Allen et al., 1999; Allen and Bartlett, 2002). The disruption of the phosphopantetheinyl transferase gene in strain WP3, which is required for omega-3 polyunsaturated fatty acid production, resulted in both cold-sensitive and high-pressure sensitive growth (Wang et al., 2008).

8.2.4. Elongation of rRNA

Lauro et al. (2007) investigated the 16S rRNA sequence of piezophiles and found that there is a strong correlation between piezophily in some genera and elongation of helices in the 16S rRNA. Insertions were found in helices: 10, 11, and 44 (Brimacombe, 1995). *Colwellia* and *Photobacterium* species primarily had insertions in helix 10 whereas *Shewanella* exhibited an insertion in helix 11. Based on the presence or absence of these insertions, five ribotypes were identified in *Photobacterium profundum* SS9 (Lauro et al., 2007). Despite having different ribotypes, it was found that regardless of the pressure the cells were grown at, all of the ribotypes were constitutively expressed (Lauro et al., 2007). Helices 10, 11, and 44 have all been implicated in interactions with ribosomal protein S20 which is necessary for ribosome assembly. Pressure-sensitive transposon mutant derivatives of SS9 have been obtained and their Tn5 or Tn10 insertion positions mapped to the genome sequence. Many of these mutants are defective in aspects of chromosome replication, ribosome structure and function, and metabolism (Lauro et al., 2008).

8.2.5. Sensor protein, transporters, and DNA replication

Comparative genomics between SS9 and two other members of the same species identified six piezo-specific genes of which expression is high-pressure inducible. They encode a sensor protein, three transport proteins and two hypothetical proteins (Campanaro et al., 2005). Transporters are the most represented category of positively selected piezophile genes (Campanaro et al., 2008); they include ABC permeases such as those involved with nucleotide and inorganic ion transport and TonB type proton motive force-dependent transporters. Other functions most selected for change with depth are associated with translation, DNA replication and membrane imbedded porins and multidrug efflux pumps. One of the positively selected SS9 genes is *subB*, whose product is required for protein synthesis. SS9 *subB* transposon mutants are both cold-sensitive and pressure-sensitive (Lauro et al., 2008).

8.2.6. Surface-associated life style and swimming behavior

It has already been mentioned that deep-sea microbes have a strong reliance on surfaces (Section 6.3). This appears to also be the case for SS9. The SS9 genome contains two flagellar gene clusters: a polar flagellum gene cluster (PF) and a putative lateral flagellum gene cluster (LF) which is used for surface movement. The LF cluster is not present in the pressure-sensitive *P. profundum* strain 3TCK, but is present in the other piezophilic *P. profundum* strain, DSJ4. LF systems are also present in the piezophilic or piezotolerant deep-sea bacteria *Moritella* sp. PE36 (unpublished results) and *Shewanella* sp. WP3 (Wang et al., 2008). The LF system of SS9 is induced under conditions of high pressure and high viscosity and requires the presence of a functional polar flagellum. Planktonic swimming motility is also important in the bathypelagic environment. Direct swimming velocity measurements obtained using a high-pressure microscopic chamber indicate that the SS9 polar flagellum system is operational for at least short periods well above the known pressure limit for life: 150 MPa (Eloe et al., 2008).

8.3. Functional attributes and deep-sea adaptations of microbes: Metagenomics

The aforementioned metagenomic studies in the North Pacific subtropical gyre (DeLong et al., 2006) and the Ionean Sea (Martín-Cuadrado et al., 2007) have shed much light on life in the dark ocean. Reduced sugar transporters and more ABC transporters and

TRAP transporters (which bring substrates into cells using electrochemical ion gradients rather than ATP as an energy source) have been observed. DeLong et al. (2006) made the same observation first noted by Vezzi et al. (2005) that life at depth is associated with increased numbers of transposable elements, perhaps reflecting less purifying selection (DeLong et al., 2006). In addition, as already mentioned (Section 6.3) they discovered that there is a preponderance of genes associated with a surface existence including genes for polysaccharide, pilus and secondary metabolite production. This is consistent with other work showing that deep-sea microbial abundance and activity is influenced by fluxes of particulate organic carbon (Nagata et al., 2000; Hansell and Ducklow, 2003; Baltar et al., 2009a). Another intriguing issue associated with deep-sea microbial communities is the extent of autotrophy. The environmental genomics data of Martín-Cuadrado et al. (2007) suggest that carbon monoxide oxidation could be an important source of energy for carbon dioxide fixation in the deep Ionea Sea.

Since the pioneering work of DeLong et al. (2006) and Martín-Cuadrado et al. (2007) additional analyses of deep-ocean, large-insert libraries have been performed, making possible additional comparative metagenomic analyses. One such study reinforced results showing increases in *Acidobacteria* with depth and identified a dioxygenase that is presumably used in polyaromatic hydrocarbon degradation by deep-sea members of this phylum (Quaiser et al., 2008). Fosmid sequence analyses of 4000 m deep-sea crenarchaeal sequences from the North Pacific subtropical gyre indicate that the predominant deep-sea population is genetically distinct and characterized by low sequence divergence (Konstantinidis and DeLong, 2008). Deep-sea Group II euryarchaeotal fosmid sequence analyses suggest that they may gain energy by anaerobically respiring various substrates (Martín-Cuadrado et al., 2007).

9. Summary and future directions

Recent studies have begun to reveal that microbes in the bathypelagic ocean are highly diverse in phylogenetic composition and versatile in metabolic capabilities, despite their low activities and the relatively stable physical conditions of the deep sea. These attributes are largely distinctive from surface dwelling microbial communities. Data on spatial variations in microbial abundance and activity support the notion that deep-water microbes respond dynamically to variations in organic matter delivery to the bathypelagic realm. Although our knowledge is still limited regarding how physical forces (e.g., temperature, hydrostatic pressure, turbulence) and biotic interactions (e.g., protist grazing, viral lysis) affect biogeochemical cycles mediated by microbes in deep oceans, recent data obtained using contemporary genetic approaches (genomics and metagenomics) have provided important insights into biochemical and physiological mechanisms by which carbon and nutrient cycles are driven by microbial communities at depth. We conclude that future studies are needed to explore emergent themes (as listed below) in order to fill the major gaps in our understanding of ecology and biogeochemistry in bathypelagic oceans:

1. Unambiguous resolution of carbon fluxes mediated by microbes (HPP and respiration) in the bathypelagic zone requires further testing and improvement of methodologies. Conversion factors involved in microbial rate measurements should be evaluated with considerations of adaptive features of indigenous microbes thriving under high pressure, low temperature, and energy-limited conditions. In addition to theoretical and empirical approaches to determine “bulk” conversion factors (Ducklow, 2000), single-cell detection techniques (e.g. microautoradiography combined with FISH) are promising tools to provide valuable information regarding taxon-specific features in microbial metabolism (Herndl et al., 2005; Varela et al., 2008b).
2. Aggregates are regarded as “hot spots” of microbial activity in the ocean (Simon et al., 2002). Indirect evidence suggests that a particle-associated life style is important for bathypelagic microbial communities (DeLong et al., 2006, Baltar et al., 2009a, 2010). However, the dichotomy of particle-associated and free-living forms is probably too simplistic for thorough consideration of microbial systems in the deep sea. Marine organic matter spans from “truly dissolved” organic matter to visible marine snow; in between is a diverse range of abundant particles including colloids and submicron particles (Koike et al., 1990; Nagata, 2008). Emerging concepts on marine gels provide one possible framework to unravel complex interactions between microbes and the organic matter continuum (Verdugo and Santchi, 2010). It remains to be seen how high pressure affects self assembly and enzymatic cleavage (prokaryote consumption) of polymer gels.
3. Microbial food webs in the bathypelagic ocean appear to differ from those in the upper ocean, being characterized by higher abundance of viruses (Parada et al., 2007, T. Nagata, unpublished) and lower abundance of HNF (Fukuda et al., 2007) relative to prokaryote abundance, although there are geographic variations (Aristegui et al., 2009). It is important to determine the rates at which prokaryotes are consumed by HNF and lysed by viruses. These data on prokaryote mortality are essential to improve parameterization of prokaryote dynamics and carbon and nutrient cycling in the bathypelagic ocean, and to explain prokaryotic community composition and diversity. We know very little about the role of ciliates, suggesting a need of further studies to identify the closure of the microbial loop at depth.
4. One of the prominent features in biological oceanography is the presence of large-scale spatial patterns in ecological properties (Longhurst, 1998). Together with the data collected by long-term monitoring at fixed stations, these results provide a solid foundation for the refinement of biogeochemical and ecological models. However, such data on microbial parameters in the bathypelagic ocean are scarce. Extensive investigation of the deep sea ecosystem is a challenging task, which needs to overcome technical, budgetary and intellectual barriers. The collected data should provide clues to identify sources of POM and DOM in the bathypelagic ocean. The carbon fluxes derived from microbial rate parameters should supplement the data obtained by sediment traps and geochemical models. Effective samplers that retain high pressure conditions, autonomous vehicles, and automated mooring systems (e.g. for determination of *in situ* activity measurements) are among the tools to be improved for the execution of such a task.
5. The “molecular revolution” has radically revised our view on prokaryote community composition in the oceans. This is particularly true in the bathypelagic realm. The most astonishing results obtained in deep waters during the past decade include the discoveries of previously unknown communities of microbes and novel metabolic capabilities of *Archaea* (i.e. autotrophy). Metagenomics and comparative genomics of piezophiles have begun to provide enormous amounts of genetic information regarding evolution, physiology, biochemistry, and community structure (DeLong et al., 2006). These data help to develop inferences on the life style and adaptations of deep-sea microbes (Lauro and Bartlett, 2008). However, there are major gaps in our understanding of the link

between genetic information and biogeochemical cycles at depth. Clearly, we need more information on the extent of archaeal autotrophy to evaluate the contributions of this process to carbon and nitrogen cycling in the bathypelagic ocean. Although the discovery of remarkably high diversity of deep-sea microbes is important in its own right, characteristic features (e.g., growth, mortality and substrate uptake) of each taxonomic group need to be clarified if we are to embed the community structures of microbes into biogeochemical models (Yokokawa and Nagata, 2010). Probes that detect specific functional genes (e.g. *amoA*) are now used to indicate the presence of the specific function (e.g. nitrification). However, these approaches are probably most effective when the data on the corresponding fluxes are available.

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References

- Abe, F., Kato, C., Horikoshi, K., 1999. Pressure-regulated metabolism in microorganisms. *Trends in Microbiology* 7, 447–453.
- Agogue, H., Brink, M., Dinasquet, J., Herndl, G.J., 2008. Major gradients in putatively nitrifying and non-nitrifying Archaea in the deep North Atlantic. *Nature* 456 (7223), 788–791.
- Allredge, A.L., Passow, U., Logan, B.E., 1993. The abundance and significance of a class of large, transparent organic particles in the ocean. *Deep-Sea Research I* 40 (6), 1131–1140.
- Allredge, A.L., Youngbluth, M.J., 1985. The significance of macroscopic aggregates (marine snow) as sites for heterotrophic bacterial production in the mesopelagic zone of the subtropical Atlantic. *Deep-Sea Research* 32 (12), 1445–1456.
- Allen, E.E., Bartlett, D.H., 2002. Piezophiles: microbial adaptation to the deep-sea environment. In: Gerday, C. (Ed.), *Extremophiles*. Eolss Publishers Co. Ltd, Oxford, U. K.
- Allen, E.E., Facciotti, D., Bartlett, D.H., 1999. Monounsaturated but not polyunsaturated fatty acids are required for growth at high pressure and low temperature in the deep-sea bacterium *Photobacterium profundum* strain SS9. *Applied and Environmental Microbiology* 65 (4), 1710–1720.
- Andersen, P., Fenchel, T., 1985. Bacterivory by microheterotrophic flagellates in seawater samples. *Limnology and Oceanography* 30 (1), 198–202.
- Aristegui, J., Agusti, J., Middelburg, J.J., Duarte, C.M., 2005a. Respiration in the mesopelagic and bathypelagic zones of the oceans. In: Del Giorgio, P.A., Williams, P.J.L. (Eds.), *Respiration in Aquatic Ecosystems*. Oxford University Press, Oxford, pp. 182–206.
- Aristegui, J., Agusti, S., Duarte, C.M., 2003. Respiration in the dark ocean. *Geophysical Research Letters* 30 (2), doi: 10.1029/2002GL016227.
- Aristegui, J., Denis, M., Almunia, J., Montero, M.F., 2002. Water-column remineralization in the Indian sector of the Southern Ocean during early spring. *Deep Sea Research Part II* 49, 1707–1720.
- Aristegui, J., Duarte, C.M., Gasol, J.M., Alonso-Sáez, L., 2005b. Active mesopelagic prokaryotes support high respiration in the subtropical northeast Atlantic Ocean. *Geophysical Research Letters*, 32, doi:10.1029/2004GL021863.
- Aristegui, J., Gasol, J.M., Duarte, C.M., Herndl, G.J., 2009. Microbial oceanography of the dark ocean's pelagic realm. *Limnology and Oceanography* 54, 1501–1529.
- Arndt, H., Hausmann, K., Wolf, M., 2003. Deep-sea heterotrophic nanoflagellates of the Eastern Mediterranean Sea: qualitative and quantitative aspects of their pelagic and benthic occurrence. *Marine Ecology Progress Series* 256, 45–56.
- Azam, F., 1998. Microbial control of oceanic carbon flux: the plot thickens. *Science* 280 (5364), 694–696.
- Baltar, F., Aristegui, J., Gasol, J.M., Hernández-León, S., Herndl, G.J., 2007. Strong coast–ocean and surface–depth gradients in prokaryotic assemblage structure and activity in a coastal transition zone region. *Aquatic Microbial Ecology* 50, 63–74.
- Baltar, F., Aristegui, J., Gasol, J.M., Sintes, E., Herndl, G.J., 2009a. Evidence of prokaryotic metabolism on suspended particulate organic matter in the dark waters of the subtropical North Atlantic. *Limnology and Oceanography* 54, 182–193.
- Baltar, F., Aristegui, J., Sintes, E., van Aken, H., Gasol, J.M., Herndl, G.J., 2009b. Prokaryotic extracellular enzymatic activity in relation to biomass production and respiration in the meso- and bathypelagic waters of the (sub) tropical Atlantic. *Environmental Microbiology* 11 (8), 1998–2014.
- Baltar, F., Aristegui, J., Gasol, J.M., Sintes, E., van Aken, H.M., Herndl, G.J., 2010. High dissolved extracellular enzymatic activity in the deep Central Atlantic Ocean. *Aquatic Microbial Ecology* 58, 287–302.
- Bartlett, D.H., Ferguson, G., Valle, G., 2008. Adaptation of the psychrotolerant piezophile *Photobacterium profundum* strain SS9. In: Michiels, C., Bartlett, D.H., Aertsen, A. (Eds.), *High-Pressure Microbiology*. ASM Press, Washington, D. C. pp. 319–337.
- Bartlett, D.H., Kato, C., Horikoshi, K., 1995. High pressure influences on gene and protein expression. *Research in Microbiology*. 146 (8), 697–706.
- Bartlett, D.H., Lauro, F.M., Eloe, E.A., 2007. Microbial adaptation to high pressure. In: Gerday, C., Glandsdorf, N. (Eds.), *Physiology and Biochemistry of Extremophiles*. American Society for Microbiology Press, Washington, D.C. pp. 333–348.
- Bartlett, D.H., Wright, M., Yayanos, A.A., Silverman, M., 1989. Isolation of a gene regulated by hydrostatic pressure in a deep-sea bacterium. *Nature* 342 (6249), 572–574.
- Beman, J.M., Popp, B.N., Francis, C.A., 2008. Molecular and biogeochemical evidence for ammonia oxidation by marine Crenarchaeota in the Gulf of California. *The ISME Journal* 2 (4), 429–441.
- Benner, R., 2002. Chemical composition and reactivity. In: Hansell, D.A., Carlson, C.A. (Eds.), *Biogeochemistry of Marine Dissolved Organic Matter*. Academic Press, San Diego, pp. 59–90.
- Benner, R., Biddanda, B., Black, B., McCarthy, M., 1997. Abundance, size distribution, and stable carbon and nitrogen isotopic compositions of marine organic matter isolated by tangential-flow ultrafiltration. *Marine Chemistry* 57 (3–4), 243–263.
- Bethoux, J.P., Gentili, B., Raunet, J., Tailliez, D., 1990. Warming trend in the western Mediterranean deep-water. *Nature* 347 (6294), 660–662.
- Bianchi, A., Garcin, J., 1993. In stratified waters the metabolic rate of deep-sea bacteria decreases with decompression. *Deep-Sea Research I* 40 (8), 1703–1710.
- Bianchi, A., Garcin, J., Gorsky, G., Poulicek, M., Tholosan, O., 1999a. Stimulation du potentiel de dégradation dans les eaux marines profondes par les bactéries barotolérantes colonisant les fèces du plancton migrant. *Comptes Rendus de l'Académie des Sciences de Paris* 322, 1113–1120.
- Bianchi, A., Garcin, J., Tholosan, O., 1999b. A high-pressure serial sampler to measure microbial activity in the deep sea. *Deep-Sea Research I* 46 (12), 2129–2142.
- Bochdansky, A.B., van Aken, H.M., Herndl, G.J., 2010. Role of macroscopic particles in deep-sea oxygen consumption. *Proceedings of the National Academy of Sciences of the United States of America*, in press.
- Breitbar, M., Middelboe, M., Rohwer, F., 2008. Marine viruses: Community dynamics, diversity and impact on microbial processes. In: Kirchman, D.L. (Ed.), *Microbial Ecology of the Oceans* 2nd edition. John Wiley and Sons, Hoboken, NJ, pp. 443–479.
- Brimacombe, R., 1995. The structure of ribosomal RNA: a three-dimensional jigsaw puzzle. *European Journal of Biochemistry* 230 (2), 365–383.
- Brittain, A.M., Karl, D.M., 1990. Catabolism of tritiated thymidine by aquatic microbial communities and incorporation of tritium into RNA and protein. *Applied and Environmental Microbiology* 56 (5), 1245–1254.
- Bronk, D.A., 2002. Dynamics of DON. In: Hansell, D.A., Carlson, C.A. (Eds.), *Biogeochemistry of Marine Dissolved Organic Matter*. Academic Press, Amsterdam, pp. 153–247.
- Burd, A.B., Hansell, D.A., Steinberg, D.K., Anderson, T.R., Aristegui, J., Baltar, F., Beupré, S.R., Buesseler, K.O., DeHairs, F., Jackson, G.A., Kadko, D.C., Koppelman, R., Lampitt, R.S., Nagata, T., Reinthaler, T., Robinson, C., Robison, B.H., Tamburini, C., Tanaka, T., 2010. Assessing the apparent imbalance between geochemical and biochemical indicators of meso- and bathypelagic biological activity: What the @#! is wrong with present calculations of carbon budgets? *Deep-Sea Research II* 57 (16), 1557–1571.

- Campanaro, S., Treu, L., Valle, G., 2008. Protein evolution in deep sea bacteria: an analysis of amino acids substitution rates. *BMC Evolutionary Biology* 8 (1), 311.
- Campanaro, S., Vezzi, A., Vitulo, N., Lauro, F.M., D'Angeo, M., Simonato, F., Cestaro, A., Malacrida, G., Bertoloni, G., Valle, G., Bartlett, D.H., 2005. Laterally transferred elements and high pressure adaptation in *Photobacterium profundum* strains. *BMC Genomics* 6, 122.
- Carlson, C.A., Giovannoni, S.J., Hansell, D.A., Goldberg, S.J., Parsons, R., Vergin, K., 2004. Interactions among dissolved organic carbon, microbial processes, and community structure in the mesopelagic zone of the northwestern Sargasso Sea. *Limnology and Oceanography* 49 (4), 1073–1083.
- Celussi, M., Cataletto, B., Fonda Umani, C., Del Negro, P., 2009. Depth profiles of bacterioplankton assemblages and their activities in two different areas of the Ross Sea (Antarctica). *Deep-Sea Research I* 56 (12), 2193–2205.
- Chin, W.-C., Orellana, M.V., Verdugo, P., 1998. Spontaneous assembly of marine dissolved organic matter into polymer gels. *Nature* 391, 568–572.
- Cho, B.C., Azam, F., 1988. Major role of bacteria in biogeochemical fluxes in the oceans interior. *Nature* 332 (6163), 441–443.
- Cho, B.C., Na, S.C., Choi, D.H., 2000. Active ingestion of fluorescently labeled bacteria by mesopelagic heterotrophic nanoflagellates in the East Sea. *Korea Marine Ecology Progress Series* 206, 23–32.
- Cho, J.C., Vergin, K.L., Morris, R.M., Giovannoni, S.J., 2004. *Lentisphaera araneosa* gen. nov., sp. nov., a transparent exopolymer producing marine bacterium, and the description of a novel bacterial phylum, *Lentisphaerae*. *Environmental Microbiology* 6 (6), 611–621.
- Christensen, J.P., Owens, T.G., Devol, A.H., Packard, T.H., 1980. Respiration and physiological state in marine bacteria. *Marine Biology* 55 (4), 267–276.
- Christensen, J.P., Packard, T.T., Dortch, F.Q., Minas, H.J., Gascard, J.C., Richez, C., Garfield, P.C., 1989. Carbon oxidation in the deep Mediterranean Sea: Evidence for a dissolved organic carbon source. *Global Biogeochemical Cycles* 3 (4), 315–335.
- Church, M.J., DeLong, E.F., Ducklow, H.W., Karner, M.B., Preston, C.M., Karl, D.M., 2003. Abundance and distribution of planktonic Archaea and Bacteria in the waters west of the Antarctic Peninsula. *Limnology and Oceanography* 48 (5), 1893–1902.
- Clark, D.R., Rees, A.P., Joint, I., 2008. Ammonium regeneration and nitrification rates in the oligotrophic Atlantic Ocean: Implications for new production estimates. *Limnology and Oceanography* 53 (1), 52–62.
- Colman, A.S., Blake, R.E., Karl, D.M., Fogel, M.L., Turekian, K.K., 2005. Marine phosphate oxygen isotopes and organic matter remineralization in the oceans. *Proceedings of the National Academy of Sciences of the United States of America* 102 (37), 13023–13028.
- Countway, P.D., Gast, R.J., Dennett, M.R., Savai, P., Rose, J.M., Caron, D.A., 2007. Distinct protistan assemblages characterize the euphotic zone and deep sea (2500 m) of the western North Atlantic (Sargasso Sea and Gulf Stream). *Environmental Microbiology* 9 (6), 1219–1232.
- Danovaro, R., Dell'Anno, A., Corinaldesi, C., Magagnoli, M., Noble, R., Tamburini, C., Weinbauer, M., 2008. Major viral impact on the functioning of benthic deep-sea ecosystems. *Nature* 454 (7208), 1084–1087.
- Davis, J., Benner, R., 2005. Seasonal trends in the abundance, composition and bioavailability of particulate and dissolved organic matter in the Chukchi/Beaufort Seas and western Canada Basin. *Deep-Sea Research II* 52 (24–26), 3396–3410.
- del Giorgio, P.A., Cole, J.J., 2000. Bacterial energetics and growth efficiency. In: Kirchman, D.L. (Ed.), *Microbial Ecology of the Oceans*. Wiley-Liss, New York, pp. 289–325.
- DeLong, E.F., Franks, D.G., Alldredge, A.L., 1993. Phylogenetic diversity of aggregate-attached vs. free-living marine bacterial assemblages. *Limnology and Oceanography* 38 (5), 924–934.
- DeLong, E.F., Preston, C.M., Mincer, T., Rich, V., Hallam, S.J., Frigaard, N.U., Martinez, A., Sullivan, M.B., Edwards, R., Brito, B.R., Chisholm, S.W., Karl, D.M., 2006. Community genomics among stratified microbial assemblages in the ocean's interior. *Science* 311, 496–503.
- Deming, J.W., 2007. Extreme high-pressure marine environments. In: Hurst, C.J., Crawford, R.L., Garland, J.L., Mills, A.L., Stetzenbach, L.D. (Eds.), *ASM Manual of Environmental Microbiology Third Edition*. ASM Press, Washington, D.C, pp. 575–590.
- Ding, Y.-X., Chin, W.-C., Rodriguz, A., Hung, C.-C., Santschi, H.P., Verdugo, P., 2008. Amphiphilic exopolymers from *Sagittula stellata* induce DOM self-assembly and formation of marine microgels. *Marine Chemistry* 112 (1–2), 11–19.
- Dittmar, T., Fitznar, H.P., Kattner, G., 2001. Origin and biogeochemical cycling of organic nitrogen in the eastern Arctic Ocean as evident from D- and L-amino acids. *Geochimica et Cosmochimica Acta* 65 (22), 4103–4114.
- Druffel, E.R.M., Bauer, J.E., 2000. Radiocarbon Distributions in Southern Ocean Dissolved and Particulate Organic Matter. *Geophysical Research Letters* 27 (10), 1495–1498.
- Druffel, E.R.M., Bauer, J.E., Williams, P.M., Griffin, S., Wolgast, D., 1996. Seasonal variability of particulate organic radiocarbon in the northeast Pacific Ocean. *Journal of Geophysical Research* 101 (C9), 20543–20552.
- Druffel, E.R.M., Griffin, S., Bauer, J.E., Wolgast, D.M., Wang, X.C., 1998. Distribution of particulate organic carbon and radiocarbon in the water column from the upper slope to the abyssal NE Pacific Ocean. *Deep-Sea Research II* 45 (4–5), 667–687.
- Druffel, E.R.M., Williams, P.M., Bauer, J.E., Ertel, J.R., 1992. Cycling of dissolved and particulate organic matter in the open ocean. *Journal of Geophysical Research* 97 (C10), 15639–15659.
- Ducklow, H.W., 2000. Bacterial production and biomass in the oceans. In: Kirchman, D.L. (Ed.), *Microbial Ecology of the Oceans*. Wiley-Liss, New York, pp. 85–120.
- Dufour, P.H., Torreton, J.P., 1996. Bottom-up and top-down control of bacterioplankton from eutrophic to oligotrophic sites in the tropical northeastern Atlantic Ocean. *Deep-Sea Research I* 43 (8), 1305–1320.
- Eardly, D.F., Carton, M.W., Gallagher, J.M., Patching, J.W., 2001. Bacterial abundance and activity in deep-sea sediments from the eastern North Atlantic. *Progress in Oceanography* 50 (1–4), 249–259.
- Eloe, E.A., Lauro, F.M., Vogel, R.F., Bartlett, D.H., 2008. The deep-sea bacterium *Photobacterium profundum* SS9 is capable of swimming and swarming at high pressure. *Applied and Environmental Microbiology* 74 (20), 6298–6305.
- Feely, R.A., Sabine, C.L., Schlitzer, R., Bullister, J.L., Mecking, S., Greely, D., 2004. Oxygen utilization and organic carbon remineralization in the upper water column of the Pacific Ocean. *Journal of Oceanography* 60, 45–52.
- Fuhrman, J.A., 1999. Marine viruses and their biogeochemical and ecological effects. *Nature* 399, 541–548.
- Fuhrman, J., 2000. Impact of viruses on bacterial processes. In: Kirchman, D.L. (Ed.), *Microbial Ecology of the Oceans*. Wiley-Liss, New York, pp. 327–350.
- Fukuda, H., Sohrin, R., Nagata, T., Koike, I., 2007. Size distribution and biomass of nanoflagellates in meso- and bathypelagic layers of the subarctic Pacific. *Aquatic Microbial Ecology* 46 (2), 203–207.
- Gamo, T., Kato, Y., Hasumoto, H., Kakiuchi, H., Momoshima, N., Takahata, N., Sano, Y., 2007. Geochemical implications for the mechanism of deep convection in a semi-closed tropical marginal basin: Sulu Sea. *Deep-Sea Research II* 54 (1–2), 4–13.
- Gardner, W.D., Mishonov, A.V., Richardson, M.J., 2006. Global POC concentrations from in-situ and satellite data. *Deep-Sea Research II* 53 (5–7), 718–740.
- Gasol, J.M., Pinhassi, J., Alonso-Saez, L., Ducklow, H., Herndl, G.J., Koblizek, M., Labrenz, M., Luo, Y., Moran, X.A.G., Reinthaler, T., Simon, M., 2008. Towards a better understanding of microbial carbon flux in the sea. *Aquatic Microbial Ecology* 53 (1), 21–38.
- Gundersen, K., Orcutt, K.M., Purdie, D.A., Michaels, A.F., Knap, A.H., 2001. Particulate organic carbon mass distribution at the Bermuda Atlantic Time-series Study (BATS) site. *Deep-Sea Research II* 48 (8–9), 1697–1718.
- Hansell, D.A., 2002. DOC in the global ocean carbon cycle. In: Hansell, D.A., Carlson, C.A. (Eds.), *Biogeochemistry of Marine Dissolved Organic Matter*. Academic Press, pp. 685–715.
- Hansell, D.A., Ducklow, H.W., 2003. Bacterioplankton distribution and production in the bathypelagic ocean: Directly coupled to particulate organic carbon export? *Limnology and Oceanography* 48 (1) 150–156.
- Hansman, R.L., Griffin, S., Watson, J.T., Druffel, E.R.M., Ingalls, A.E., Pearson, A., Aluwihare, L.I., 2009. The radiocarbon signature of microorganisms in the mesopelagic ocean. *Proceedings of the National Academy of Sciences of the United States of America* 106 (16), 6513–6518.
- Hara, S., Koike, I., Terauchi, K., Kamiya, H., Tanoue, E., 1996. Abundance of viruses in deep oceanic waters. *Marine Ecology Progress Series* 145, 269–277.
- Hebel, D.V., Karl, D.M., 2001. Seasonal, interannual and decadal variations in particulate matter concentrations and composition in the subtropical North Pacific Ocean. *Deep-Sea Research II* 48 (8–9), 1669–1695.
- Herndl, G.J., Agogue, H., Balter, F., Reinthaler, T., Sintez, E., Varela, M.M., 2008. Regulation of aquatic microbial processes: the microbial loop of the sunlit surface waters and the dark ocean dissected. *Aquatic Microbial Ecology* 53 (1), 59–68.
- Herndl, G.J., Reinthaler, T., Teira, E., Aken, H., Veth, C., Pernthaler, A., Pernthaler, J., 2005. Contribution of *Archaea* to total prokaryotic production in the deep Atlantic Ocean. *Applied and Environmental Microbiology* 71 (5), 2303–2309.
- Hernes, P.J., Benner, R., 2002. Transport and diagenesis of dissolved and particulate terrigenous organic matter in the North Pacific Ocean. *Deep-Sea Research I* 49 (12), 2119–2132.
- Hernes, P.J., Benner, R., 2006. Terrigenous organic matter sources and reactivity in the North Atlantic Ocean and a comparison to the Arctic and Pacific oceans. *Marine Chemistry* 100 (1–2), 66–79.
- Hewson, I., Steele, J.A., Capone, D.G., Fuhrman, J.A., 2006. Remarkable heterogeneity in meso- and bathypelagic bacterioplankton assemblage composition. *Limnology and Oceanography* 51 (3), 1274–1283.
- Hill, J.K., Wheeler, P.A., 2002. Organic carbon and nitrogen in the northern California current system: comparison of offshore, river plume, and coastally upwelled waters. *Progress in Oceanography* 53 (2–4), 369–387.
- Hopkinson, C.S., Vallino, J.J., 2005. Efficient export of carbon to the deep ocean through dissolved organic matter. *Nature* 433 (7022), 142–145.
- Hoppe, H.-G., Arnosti, C., Herndl, G.J., 2002. Ecological significance of bacterial enzymes in the Marine Environment. In: Burns, R.G., Dick, R.P. (Eds.), *Enzymes in the Environment*. Marcel Dekker, Germany, pp. 73–107.
- Hoppe, H.-G., Ullrich, S., 1999. Profiles of ectoenzymes in the Indian Ocean: phenomena of phosphatase activity in the mesopelagic zone. *Aquatic Microbial Ecology* 19 (2), 139–148.
- Hubberten, U., Lara, R.J., Kattner, G., 1995. Refractory organic compounds in polar waters: Relationship between humic substances and amino acids in the Arctic and Antarctic. *Journal of Marine Research* 53 (1), 137–149.
- Ingalls, A.E., Shah, S.R., Hansman, R.L., Aluwihare, L.I., Santos, G.M., Druffel, E.R.M., Pearson, A., 2006. Quantifying archaeal community autotrophy in the mesopelagic ocean using natural radiocarbon. *Proceedings of the National Academy of Sciences of the United States of America* 103 (17), 6442–6447.

- Jannasch, H.W., Wirsén, C.O., 1973. Deep-sea microorganisms : *in situ* response to nutrient enrichment. *Science* 180, 641–643.
- Jannasch, H.W., Wirsén, C.O., Winget, C.L., 1973. A bacteriological pressure-retaining deep-sea sampler and culture vessel. *Deep-Sea Research* 20 (7), 661–664.
- Jenkins, W.J., 1982. Oxygen utilization rates in the North Atlantic Subtropical Gyre and primary production in oligotrophic systems. *Nature* 300, 246–248.
- Jenkins, W.J., Wallace, D.W.R., 1992. Tracer based inferences of new primary production in the sea. In: Falkowski, P.G., Woodhead, A.D. (Eds.), *Primary productivity and biogeochemical cycles in the sea*. Plenum, pp. 292–316.
- Jensen, P.R., Lauro, F.M., 2008. An assessment of actinobacterial diversity in the marine environment. *Antonie Van Leeuwenhoek* 94, 51–62.
- Jones, R.T., Robeson, M.S., Lauber, C.L., Hamady, M., Knight, R., Fierer, N., 2009. A comprehensive survey of soil acidobacterial diversity using pyrosequencing and clone library analyses. *The ISME Journal* 3, 442–453.
- Jürgens, K., Massana, R., 2008. Protistan grazing on marine bacterioplankton. In: Kirchman, D.L. (Ed.), *Microbial Ecology of the Oceans* 2nd edition John Wiley and Sons, Inc., Hoboken, N.J., pp. 383–441.
- Kaiser, K., Benner, R., 2009. Biochemical composition and size distribution of organic matter at the Pacific and Atlantic time-series stations. *Marine Chemistry* 113, 63–77.
- Karl, D.M., Bjorkman, K.M., 2002. Dynamics of DOP. In: Hansell, D.A., Carlson, C.A. (Eds.), *Biogeochemistry of Marine Dissolved Organic Matter*. Academic Press, Amsterdam, pp. 249–366.
- Karl, D.M., Knauer, G.A., Martin, J.H., 1988. Downward flux of particulate organic matter in the ocean: a particle decomposition paradox. *Nature* 332, 438–441.
- Karner, M.B., DeLong, E.F., Karl, D.M., 2001. Archaeal dominance in the mesopelagic zone of the Pacific Ocean. *Nature* 409 (6819), 507–510.
- Kato, C., Suzuki, S., Hata, S., Ito, T., Horikoshi, K., 1995. The properties of a protease activated by high pressure from *Sporosarcina* sp. Strain DSK25 isolated from deep-sea sediment. *Jamsteer* 32, 7–13.
- Kato, C., Sato, T., Abe, F., Ohmae, E., Tamegai, H., Nakasone, K., Siddiqui, K.S., Thomas, T., 2008. Protein adaptation to high-pressure environments. In: Thomas, T., Siddiqui, K.S. (Eds.), *Protein Adaptation in Extremophiles*. Molecular Anatomy and Physiology of Proteins Series. Nova Science Publisher, pp. 167–192.
- Keil, R.G., Kirchman, D.L., 1999. Utilization of dissolved protein and amino acids in the northern Sargasso Sea. *Aquatic Microbial Ecology* 18 (3), 293–300.
- Kjørboe, T., Jackson, G.A., 2001. Marine snow, organic solute plumes, and optimal chemosensory behavior of bacteria. *Limnology and Oceanography* 46 (6), 1309–1318.
- Kirchman, D., 2002. The ecology of Cytophaga–Flavobacteria in aquatic environments. *FEMS Microbiology Ecology* 39 (2), 91–100.
- Kirchman, D., K'Ness, E., Hodson, R., 1985. Leucine incorporation and its potential as a measure of protein synthesis by bacteria in natural aquatic systems. *Applied and Environmental Microbiology* 49 (3), 599–607.
- Kirchman, D.L., Meon, B., Ducklow, H.W., Carlson, C.A., Hansell, D.A., Steward, G.F., 2001. Glucose fluxes and concentrations of dissolved combined neutral sugars (polysaccharides) in the Ross Sea and Polar Front Zone, Antarctica. *Deep-Sea Research II* 48 (19–20), 4179–4197.
- Kirchman, D.L., Wheeler, P.A., 1998. Uptake of ammonium and nitrate by heterotrophic bacteria and phytoplankton in the sub-Arctic Pacific. *Deep-Sea Research I* 45 (2–3), 347–365.
- Koike, I., Hara, S., Terauchi, K., Kogure, K., 1990. The role of submicrometer particles in the ocean. *Nature* 345, 242–244.
- Koike, I., Nagata, T., 1997. High potential activity of extracellular alkaline phosphatase in deep waters of the central Pacific. *Deep-Sea Research I* 44 (9–10), 2283–2294.
- Könneke, M., Bernhard, A.E., de la Torre, J.R., Walker, C.B., Waterbury, J.B., Stahl, D.A., 2005. Isolation of an autotrophic ammonia-oxidizing marine archaeon. *Nature* 437 (7058), 543–546.
- Konstantinidis, K.T., DeLong, E.F., 2008. Genomic patterns of recombination, clonal divergence and environment in marine microbial populations. *The ISME Journal* 2, 1052–1065.
- Koppelman, R., Zimmermann-Timm, H., Weikert, H., 2005. Bacterial and zooplankton distribution in deep waters of the Arabian Sea. *Deep-Sea Research I* 52 (11), 2184–2192.
- Kudela, R.M., Cochlan, W.P., 2000. Nitrogen and carbon uptake kinetics and the influence of irradiance for a red tide bloom off southern California. *Aquatic Microbial Ecology* 21 (1), 31–47.
- La Ferla, R., Azzaro, M., 2001. Microbial respiration in the Levantine Sea: evolution of the oxidative processes in relation to the main Mediterranean water masses. *Deep-Sea Research I* 48 (10), 2147–2159.
- Lara, E., Moreira, D., Vereshchaka, A., Lopez-Garcia, P., 2009. Pan-oceanic distribution of new highly diverse clades of deep-sea diplomonads. *Environmental Microbiology* 11 (1), 47–55.
- Lauro, F.M., Bartlett, D.H., 2008. Prokaryotic lifestyles in deep-sea habitats. *Extremophiles* 12 (1), 15–25.
- Lauro, F.M., Chastain, R.A., Blankenship, L.E., Yayanos, A.A., Bartlett, D.H., 2007. The unique 16S rRNA genes of piezophiles reflect both phylogeny and adaptation. *Applied Environmental Microbiology* 73, 838–845.
- Lauro, F.M., Tran, K., Vezzi, A., Vitulo, N., Valle, G., Bartlett, D.H., 2008. Large-scale transposon mutagenesis of *Photobacterium profundum* SS9 reveals new genetic loci important for growth at low temperature and high pressure. *Journal of Bacteriology* 190 (5), 1699–1709.
- Lefevre, D., Denis, M., Lambert, C.E., Miquel, J.C., 1996. Is DOC the main source of organic matter remineralization in the ocean water column? *Journal of Marine Systems* 7 (2–4), 281–291.
- Libby, P.S., Wheeler, P.A., 1997. Particulate and dissolved organic nitrogen in the central and eastern equatorial Pacific. *Deep-Sea Research I* 44 (2), 345–361.
- Loh, A.N., Canuel, E.A., Bauer, J.E., 2008. Potential source and diagenetic signatures of oceanic dissolved and particulate organic matter as distinguished by lipid biomarker distributions. *Marine Chemistry* 112 (3–4), 189–202.
- Longhurst, A., 1998. *Ecological Geography of the Sea*. Academic Press, San Diego.
- Lopez-Garcia, P., Rodriguez-Valera, F., Pedros-Alio, C., Moreira, D., 2001. Unexpected diversity of small eukaryotes in deep-sea Antarctic plankton. *Nature* 409, 603–606.
- Madigan, M.T., Martinko, J.M., Brock, T.D., 2006. *Brock biology of microorganisms*. Pearson Prentice Hall, Upper Saddle River, NJ.
- Maixner, F., Wagner, M., Lückner, S., Pelletier, E., Schmitz-Esser, S., Hace, K., Spieck, E., Konrat, R., Le Paslier, D., Daims, H., 2008. Environmental genomics reveals a functional chlorite dismutase in the nitrite-oxidizing bacterium *Candidatus Nitrospira defluvi*. *Environmental Microbiology* 10 (11), 3043–3056.
- Martin, J.H., Knauer, G.A., Karl, D.M., Broenkow, W.W., 1987. VERTEX – Carbon cycling in the Northeast Pacific. *Deep-Sea Research Part A* 34 2, 267–285.
- Martin-Cuadrado, A.-B., López-García, P., Alba, J.-C., Moreira, D., Monticelli, L., Strittmatter, A., Gottschalk, G., Rodríguez-Valera, F., 2007. Metagenomics of the deep Mediterranean, a warm bathypelagic habitat. *PLoS One* 2, e914.
- Miki, T., Yokokawa, T., Nagata, T., Yamamura, N., 2008. Immigration of prokaryotes to local environments enhances remineralization efficiency of sinking particles: a metacommunity model. *Marine Ecology Progress Series* 366, 1–14.
- Millero, F.J., 2006. *Chemical Oceanography*. CRC Press.
- Millero, F.J., 2007. The marine inorganic carbon cycle. *Chemical Review* 2007 (107), 308–341.
- Moeseneder, M.M., Winter, C., Herndl, G.J., 2001. Horizontal and vertical complexity of attached and free-living bacteria of the eastern Mediterranean Sea, determined by 16S rDNA and 16S rRNA fingerprints. *Limnology and Oceanography* 46 (1), 95–107.
- Moran, M.A., 2008. Genomics and metagenomics of marine prokaryotes. In: Kirchman, D.L. (Ed.), *Microbial Ecology of the Ocean* 2nd edition John Wiley and Sons, Inc., Hoboken, NJ, pp. 91–129.
- Moriarty, D.J.W., Bianchi, M., Talbot, V., 1997. Bacterial productivity and organic matter flux in the Southern Ocean and in the Antarctic Intermediate Water and Mode Water of the Indian Ocean. *Deep-Sea Research II* 44 (5), 1005–1015.
- Motegi, C., Nagata, T., 2007. Enhancement of viral production by addition of nitrogen or nitrogen plus carbon in subtropical surface waters of the South Pacific. *Aquatic Microbial Ecology* 48, 27–34.
- Motegi, C., Nagata, T., Miki, T., Weinbauer, M.C., Legendre, L., Rassoulzadegan, F., 2009. Viral control of bacterial growth efficiency in marine pelagic environments. *Limnology and Oceanography* 54 (6), 1901–1910.
- Moyer, C.L., Tiedje, J.M., Dobbs, F.C., Karl, D.M., 1998. Diversity of deep-sea hydrothermal vent Archaea from Loihi seamount, Hawaii. *Deep-Sea Research II* 45 (1–3), 303–317.
- Nagata, T., 2008. Organic matter-bacterial interactions in seawater. In: Kirchman, D.L. (Ed.), *Microbial Ecology of the Oceans* 2nd edition John Wiley and Sons, Inc., Hoboken, NJ, pp. 207–241.
- Nagata, T., Fukuda, H., Fukuda, R., Koike, I., 2000. Bacterioplankton distribution and production in deep Pacific waters: Large-scale geographic variations and possible coupling with sinking particle fluxes. *Limnology and Oceanography* 45 (2), 426–435.
- Naqvi, S.W.A., Shailaja, M.S., Kumar, M.D., SenGupta, R., 1996. Respiration rates in subsurface waters of the northern Indian Ocean: Evidence for low decomposition rates of organic matter within the water column in the Bay of Bengal. *Deep-Sea Research II* 43 (1), 73–81.
- Norland, S., 1993. The relationship between biomass and volume of bacteria. In: Kemp, P., Sherr, B.F., Sherr, E.B., Cole, J. (Eds.), *Handbook of Methods in Aquatic Microbial Ecology*. Lewis Publish, pp. 303–308.
- Not, F., Gausling, R., Azam, F., Heidelberg, J.F., Worden, A.Z., 2007. Vertical distribution of picoeukaryotic diversity in the Sargasso Sea. *Environmental Microbiology* 9 (5), 1233–1252.
- Onyenwoke, R.U., Brill, J.A., Farahi, K., Wiegel, J., 2004. Sporulation genes in members of the low G+C Gram-type-positive phylogenetic branch (Firmicutes). *Archives of Microbiology* 182, 182–192.
- Ortmann, A.C., Suttle, C.A., 2005. High abundances of viruses in a deep-sea hydrothermal vent system indicate viral mediated microbial mortality. *Deep-Sea Research I* 52 (8), 1515–1527.
- Packard, T.T., 1971. The measurement of electron respiratory transport activity in marine phytoplankton. *Journal of Marine Science* 29, 235–244.
- Packard, T.T., Codispoti, L.A., 2007. Respiration, mineralization, and biochemical properties of the particulate matter in the southern Nansen Basin water column in April 1981. *Deep-Sea Research I* 54 (3), 403–414.
- Packard, T.T., Denis, M., Rodier, M., Garfield, P., 1988. Deep-ocean metabolic CO₂ production – Calculations from ETS activity. *Deep-Sea Research Part A* 35 3, 371–382.
- Paepe, M.D., Taddei, F., 2006. Viruses' life history: towards a mechanistic basis of a trade-off between survival and reproduction among phages. *PLoS Biology* 4 (193), 193.

- Painter, S.C., Sanders, R., Waldron, H.N., Lucas, M.I., Torres-Valdes, S., 2008. Urea distribution and uptake in the Atlantic Ocean between 50°N and 50°S. *Marine Ecology Progress Series* 368, 53–63.
- Panagiotopoulos, C., Sempéré, R., 2005. Analytical methods for the determination of sugars in marine samples: A historical perspective and future directions. *Limnology and Oceanography: Methods* 3, 419–454.
- Parada, V., Herndl, G.J., Weinbauer, M.G., 2006. Viral burst size of heterotrophic prokaryotes in aquatic systems. *Journal of Marine Biological Association, U.K.* 86, 613–621.
- Parada, V., Sintés, E., Aken, H.M.v., Weinbauer, M.G., Herndl, G.J., 2007. Viral abundance, decay, and diversity in the meso- and bathypelagic waters of the North Atlantic. *Applied and Environmental Microbiology* 73 (14), 4429–4438.
- Patching, J.W., Eardly, D., 1997. Bacterial biomass and activity in the deep waters of the eastern Atlantic - evidence of a barophilic community. *Deep-Sea Research I* 44 (9–10), 1655–1670.
- Patterson, D.J., Nygaard, K., Steinberg, G., Turley, C.M., 1993. Heterotrophic flagellates and other protists associated with oceanic detritus throughout the water column in the mid North Atlantic. *Journal of the Marine Biological Association of the United Kingdom* 73, 67–95.
- Pearson, A., McNichol, A.P., Benitez-Nelson, B.C., Hayes, J.M., Eglinton, T.I., 2001. Origins of lipid biomarkers in Santa Monica Basin surface sediment: A case study using compound-specific $D^{13}C$ analysis. *Geochimica et Cosmochimica Acta* 65 (18), 3123–3137.
- Pernthaler, J., 2005. Predation on prokaryotes in the water column and its ecological implications. *Nature Reviews Microbiology* 3 (7), 537–546.
- Prangishvili, D., Forterre, P., Garrett, R.A., 2006. Viruses of the *Archaea*: a unifying view. *Nature Reviews Microbiology* 4 (11), 837–848.
- Quaiser, A., Lopez-Garcia, P., Zivanovic, Y., Henn, M.R., Rodriguez-Valera, F., Moreira, D., 2008. Comparative analysis of genome fragments of *Acidobacteria* from deep Mediterranean plankton. *Environmental Microbiology*. 10 (10), 2704–2717.
- Quéguiner, B., Brzezinski, M.A., 2002. Biogenic silica production rates and particulate organic matter distribution in the Atlantic sector of the Southern Ocean during austral spring 1992. *Deep-Sea Research II* 49 (9–10), 1765–1786.
- Quince, C., Curtis, T.P., Sloan, W.T., 2008. The rational exploration of microbial diversity. *The ISME Journal* 2, 997–1006.
- Reinthal, T., van Aken, H., Veth, C., Aristegui, J., Robinson, C., Williams, P.J.L.R., Lebaron, P., Herndl, G.J., 2006. Prokaryotic respiration and production in the meso- and bathypelagic realm of the eastern and western North Atlantic basin. *Limnology and Oceanography* 51 (3), 1262–1273.
- Reinthal, T., Sintés, E., Herndl, G.J., 2008. Dissolved organic matter and bacterial production and respiration in the sea-surface microlayer of the open Atlantic and the western Mediterranean Sea. *Limnology and Oceanography* 53 (1), 122–136.
- Reinthal, T., van Aken, H.M., Herndl, G.J., 2010. Major contribution of autotrophy to microbial carbon cycling in the deep North Atlantic's interior. *Deep-Sea Research II* 57 (16), 1572–1580.
- Repeta, D.J., Aluwihare, L.I., 2006. Radiocarbon analysis of neutral sugars in high-molecular-weight dissolved organic carbon: Implications for organic carbon cycling. *Limnology and Oceanography* 51 (2), 1045–1053.
- Rich, J.H., Ducklow, H.W., Kirchman, D.L., 1996. Concentrations and uptake of neutral monosaccharides along 140°W in the equatorial Pacific: Contribution of glucose to heterotrophic bacterial activity and the DOM flux. *Limnology and Oceanography* 41 (4), 595–604.
- Rich, J.H., Gosselin, M., Sherr, E., Sherr, B., Kirchman, D.L., 1997. High bacterial production, uptake and concentrations of dissolved organic matter in the Central Arctic Ocean. *Deep-Sea Research II* 44 (8), 1645–1663.
- Robinson, C., 2008. Heterotrophic bacterial respiration. In: Kirchman, D.L. (Ed.), *Microbial Ecology of the Oceans*, 2nd edition, Hoboken N.J., pp. 299–334.
- Robinson, C., Steinberg, D.K., Koppelman, R., Robinson, B., Andersen, T.R., Aristegui, J., Carlson, C.A., Frost, J.R., Ghiglione, J.-F., Hernández-León, S., Jackson, G., Queguiner, B., Ragueneau, O., Rassoulzadegan, F., Tamburini, C., Tanaka T., Wishner, K.F., Zhang, J., 2010. Mesopelagic zone ecology and biogeochemistry a synthesis. *Deep-Sea Research II* 57 (16), 1504–1518.
- Rodionov, D.A., Dubchak, I., Arkin, A., Alm, E., Gelfand, M.S., 2004. Reconstruction of regulatory and metabolic pathways in metal-reducing delta-proteobacteria. *Genome Biology* 5, R90.
- Romankevich, E.A., 1984. *Geochemistry of Organic Matter in the Ocean*. Springer, Berlin.
- Sambrotto, R.N., 2001. Nitrogen production in the northern Arabian Sea during the Spring Intermonsoon and Southwest Monsoon seasons. *Deep-Sea Research II* 48 (6–7), 1173–1198.
- Sambrotto, R.N., Mace, B.J., 2000. Coupling of biological and physical regimes across the Antarctic Polar Front as reflected by nitrogen production and recycling. *Deep-Sea Research II* 47 (15–16), 3339–3367.
- Sarmiento, J.L., Gruber, N., 2006. *Ocean Biogeochemical Dynamics*. Princeton University Press, Princeton, N.J.
- Savenkoff, C., Lefevre, D., Denis, M., Lambert, C.E., 1993a. How do microbial communities keep living in the Mediterranean outflow within Northeast Atlantic Intermediate Waters? *Deep-Sea Research II* 40 (1–2) 627–641.
- Savenkoff, C., Prieur, L., Reys, J.-P., Lefevre, D., Dallot, S., Denis, M., 1993b. Deep microbial communities evidenced in the Liguro-Provençal front by their ETS activity. *Deep-Sea Research II* 40 (4), 709–725.
- Sempéré, R., Teddetti, M., Panagiotopoulos, C., Charrière, B., Van Wambeke, F., 2008. Distribution and bacterial availability of dissolved neutral sugars in the South East Pacific. *Biogeosciences* 5 (4), 1165–1173.
- Simon, M., Grossart, H.-P., Schweitzer, B., Ploug, H., 2002. Microbial ecology of organic aggregates in aquatic ecosystems. *Aquatic Microbial Ecology* 28, 175–211.
- Simon, M., Rosenstock, B., 2007. Different coupling of dissolved amino acid, protein, and carbohydrate turnover to heterotrophic picoplankton production in the Southern Ocean in austral summer and fall. *Limnology and Oceanography* 52 (1), 85–95.
- Skoog, A., Biddanda, B., Benner, R., 1999. Bacterial utilization of dissolved glucose in the upper water column of the Gulf of Mexico. *Limnology and Oceanography* 44 (7), 1625–1633.
- Sogin, M.L., Morrison, H.G., Huber, J.A., Welch, D.M., Neal, P.R., Arrieta, J.M., Herndl, G.J., 2006. Microbial diversity in the deep sea and the underexplored "rare biosphere". *Proceedings of the National Academy of Sciences of the United States of America* 103 (32), 12115–12120.
- Sohrin, R., Imazawa, M., Fukuda, H., Suzuki, Y., 2010. Full-depth profiles of prokaryotes, heterotrophic nanoflagellates, and ciliates along a transect from the equatorial to the subarctic central Pacific Ocean. *Deep-Sea Research II* 57 (16), 1537–1550.
- Steinberg, D.K., Van Mooy, B.A.S., Buesseler, K.O., Boyd, P.W., Kobari, T., Karl, D.M., 2008. Bacterial vs. zooplankton control of sinking particle flux in the ocean's twilight zone. *Limnology and Oceanography* 53 (4), 1327–1338.
- Tabor, P.S., Deming, J.W., Ohwada, K., Davis, H., Waxman, M., Colwell, R.R., 1981. A pressure-retaining deep ocean sampler and transfer system for measurement of microbial activity in the deep sea. *Microbial Ecology* 7, 51–65.
- Tamburini, C., 2002. La dégradation du matériel organique profond par les microflores profondes : de la mesure des vitesses potentielles au flux de CO_2 généré *in situ*. Ph.D. Thesis. Université de la Méditerranée, Marseille.
- Tamburini, C., Garcin, J., Bianchi, A., 2003. Role of deep-sea bacteria in organic matter mineralization and adaptation to hydrostatic pressure conditions in the NW Mediterranean Sea. *Aquatic Microbial Ecology* 32, 209–218.
- Tamburini, C., Garcin, J., Grégori, G., Leblanc, K., Rimmelin, P., Kirchman, D.L., 2006. Pressure effects on surface Mediterranean prokaryotes and biogenic silica dissolution during a diatom sinking experiment. *Aquatic Microbial Ecology* 43 (3), 267–276.
- Tamburini, C., Garcin, J., Ragot, M., Bianchi, A., 2002. Biopolymer hydrolysis and bacterial production under ambient hydrostatic pressure through a 2000 m water column in the NW Mediterranean. *Deep-Sea Research II* 49 (11), 2109–2123.
- Tamburini, C., Garel, M., Al Ali, B., Mérigot, B., Kriwy, P., Charrière, B., Budillon, G., 2009a. Distribution and activity of *Bacteria* and *Archaea* in the different water masses of the Tyrrhenian Sea. *Deep-Sea Research II* 56 (11–12), 700–712.
- Tamburini, C., Goux, M., Guigue, C., Garel, M., Lefèvre, D., Charrière, B., Sempéré, R., Pepa, S., Peterson, M.L., Wakeham, S., Lee, C., 2009b. Effects of hydrostatic pressure on microbial alteration of sinking fecal pellets. *Deep-Sea Research II* 56 (18), 1533–1546.
- Tanaka, T., Rassoulzadegan, F., 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 Research II* 49 (11), 2093–2107.
- Tanaka, T., Rassoulzadegan, F., 2004. Full-depth profile (0–2000 m) of bacteria, heterotrophic nanoflagellates 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 Research I* 51 (4), 531–544.
- Tanaka, T., Zohary, T., Krom, M.D., Law, C.S., Pitta, P., Psarra, S., Rassoulzadegan, F., Thingstad, T.F., Tselepidis, A., Woodward, E.M.S., Flaten, G.A.F., Skjoldal, E.F., Zodiatis, G., 2007. Microbial community structure and function in the Levantine Basin of the eastern Mediterranean. *Deep-Sea Research I* 54 (10), 1721–1743.
- Tanoue, E., 1992. Occurrence and characterization of particulate proteins in the Pacific Ocean. *Deep-Sea Research Part A* 39 (5), 743–761.
- Tanoue, E., Handa, N., 1979. Distribution of particulate organic carbon and nitrogen in the Bering Sea and Northern North Pacific Ocean. *Journal of the Oceanography* 35, 47–62.
- Tanoue, E., Handa, N., Kato, M., 1982. Horizontal and vertical distributions of particulate organic matter in the Pacific sector of the Antarctic Ocean. *Transactions of the Tokyo University of Fisheries* 5, 65–83.
- Taylor, G.T., Hein, C., Iabichella, M., 2003. Temporal variations in viral distributions in the anoxic Cariaco Basin. *Aquatic Microbial Ecology* 30 (2), 103–116.
- Taylor, G.T., Iabichella, M., Ho, T.Y., Scranton, M.I., Thunell, R.C., Muller-Karger, F., Varela, R., 2001. Chemoautotrophy in the redox transition zone of the Cariaco Basin: A significant midwater source of organic carbon production. *Limnology and Oceanography* 46 (1), 148–163.
- Teira, E., Aken, H.v., Veth, C., Herndl, G.J., 2006a. Archaeal uptake of enantiomeric amino acids in the meso- and bathypelagic waters of the North Atlantic. *Limnology and Oceanography* 51 (1), 60–69.
- Teira, E., Lebaron, P., Aken, H.v., Herndl, G.J., 2006b. Distribution and activity of *Bacteria* and *Archaea* in the deep water masses of the North Atlantic. *Limnology and Oceanography* 51 (5), 2131–2144.
- Thingstad, T.F., 2000. Elements of a theory for the mechanisms controlling abundance, diversity, and biogeochemical role of lytic viruses in aquatic systems. *Limnology and Oceanography* 45, 1320–1328.
- Tholosan, O., 1999. Activités microbiennes dans les eaux et les sédiments profonds. Rôle de la pression hydrostatique., Ph.D. Thesis. Université de la Méditerranée, Marseille.

- Tholosan, O., Garcin, J., Bianchi, A., 1999. Effects of hydrostatic pressure on microbial activity through a 200 m deep water column in the NW Mediterranean Sea. *Marine Ecology Progress Series* 183, 49–57.
- Turley, C.M., 1993. The effect of pressure on leucine and thymidine incorporation by free-living bacteria and by bacteria attached to sinking oceanic particles. *Deep-Sea Research I* 40 (11–12), 2193–2206.
- Turley, C.M., Carstens, M., 1991. Pressure tolerance of oceanic flagellates: implications for remineralization of organic matter. *Deep-Sea Research* 38 (4), 403–413.
- Turley, C.M., Hughes, D.J., 1992. Effects of storage on direct estimates of bacterial numbers of preserved seawater samples. *Deep-Sea Research* 39 (3–4), 375–394.
- Turley, C.M., Lochte, K., Lampitt, R.S., 1995. Transformation of biogenic particles during sedimentation in the northeastern Atlantic. *Philosophical Transactions of the Royal Society, Biological Science* N 348, 179–189.
- Turley, C.M., Lochte, K., Patterson, D.J., 1988. A barophilic flagellate isolated from 4500m in the mid-North Atlantic. *Deep-Sea Research* 35 (7), 1079–1092.
- Turley, C.M., Mackie, P.J., 1994. Biogeochemical Significance of Attached and Free-Living Bacteria and the Flux of Particles in the NE Atlantic-Ocean. *Marine Ecology Progress Series* 115 (1–2), 191–203.
- Turnwitsch, R., Springer, B.M., Kiriakoulakis, K., Vilas, J.C., Aristegui, J., Wolff, G., Peine, F., Werk, S., Graf, G., Waniek, J.J., 2007. Determination of particulate organic carbon (POC) in seawater: The relative methodological importance of artificial gains and losses in two glass-fiber-filter-based techniques. *Marine Chemistry* 105, 208–228.
- Varela, D.E., Harrison, P.J., 1999. Seasonal variability in nitrogenous nutrition of phytoplankton assemblages in the northeastern subarctic Pacific Ocean. *Deep-Sea Research II* 46 (11–12), 2505–2538.
- Varela, M.M., van Aken, H.M., Sintès, E., Herndl, G.J., 2008a. Latitudinal trends of Crenarchaeota and Bacteria in the meso- and bathypelagic water masses of the Eastern North Atlantic. *Environmental Microbiology* 10 (1), 110–124.
- Varela, M.M., van Aken, H.M., Herndl, G.J., 2008b. Abundance and activity of *Chloroflexi*-type SAR202 bacterioplankton in the meso- and bathypelagic waters of the (sub)tropical Atlantic. *Environmental Microbiology* 10, 1903–1911.
- Verdugo, P., Alldredge, A.L., Azam, F., Kirchman, D.L., Passow, U., Santschi, P.H., 2004. The oceanic gel phase: a bridge in the DOM-POM continuum. *Marine Chemistry* 92 (1–4), 67–85.
- Verdugo, P., Santschi, P.H., 2010. Polymer dynamics of DOC networks and gel formation in seawater. *Deep-Sea Research II* 57 (16), 1486–1493.
- Vezzi, A., Campanaro, S., D'Angelo, M., Simonato, F., Vitulo, N., Lauro, F.M., Cestaro, A., Malacrada, G., Simionati, B., Cannata, N., Romualdi, C., Bartlett, D.H., Valle, G., 2005. Life at depth: *Photobacterium profundum* genome sequence and expression analysis. *Science* 307, 1459–1461.
- Wagner, M., Horn, M., 2006. The Planctomycetes, Verrucomicrobia, Chlamydiae and sister phyla comprise a superphylum with biotechnological and medical relevance. *Current Opinion in Biotechnology* 17, 241–249.
- Wang, F., Wang, J., Jian, H., Zhang, B., Li, S., Wang, F., Zeng, X., Gao, L., Bartlett, D.H., Yu, J., Hu, S., Xiao, X., 2008. Environmental adaptation: genomic analysis of the piezotolerant and psychrotolerant deep-sea iron reducing bacterium *Shewanella piezotolerans* WP 3 (4), e1937PLoS One 3 (4), e1937 Erratum in: PLoS ONE. 2008;3(5) doi: 10.1371/annotation/744d7c8d-db8a-4ad4-.
- Weinbauer, M.G., 2004. Ecology of prokaryotic viruses. *FEMS Microbiology Review* 28, 1127–1181.
- Weinbauer, M.G., Brettar, I., Höfle, M.G., 2003. Lysogeny and virus-induced mortality of bacterioplankton in surface, deep, and anoxic marine waters. *Limnology and Oceanography* 48 (4), 1457–1465.
- Wen, K., Ortmann, A.C., Suttle, C.A., 2004. Accurate estimation of viral abundance by epifluorescence microscopy. *Applied and Environmental Microbiology* 70 (7), 3862–3867.
- Wikner, J., Hagström, Å., 1991. Annual study of bacterioplankton community dynamics. *Limnology and Oceanography* 36, 1313–1324.
- Williamson, S.J., Paul, J.H., 2004. Nutrient stimulation of lytic phage production in bacterial populations of the Gulf of Mexico. *Aquatic Microbial Ecology* 36, 9–17.
- Woebken, D., Teeling, H., Wecker, P., Dumitriu, A., Kostadinov, I., Delong, E.F., Amann, R., Glöckner, F.O., 2007. Fosmids of novel marine *Planctomycetes* from the Namibian and Oregon coast upwelling systems and their cross-comparison with planctomycete genomes. *The ISME Journal* 1, 419–435.
- Worden, A.Z., Not, F., 2008. Ecology and diversity of picoeukaryotes. In: Kirchman, D.L. (Ed.), *Microbial Ecology of the Ocean* 2nd edition John Wiley and Sons, Inc., Hoboken, N.J., pp. 159–205.
- Wuchter, C., Abbas, B., Coolen, M.J.L., Herfort, L., van Bleijswijk, J., Timmers, P., et al., 2006. Archaeal nitrification in the ocean. *Proceedings of the National Academy of Sciences of the United States of America*. 103 (33), 12317–12322.
- Wuchter, C., Schouten, S., Boschker, H.T.S., Damsté, J.S.S., 2003. Bicarbonate uptake by marine Crenarchaeota. *FEMS Microbiology Letter* 219, 203–208.
- Yamada, M., Nakasone, K., Tamegai, H., Kato, C., Usami, R., Horikoshi, K., 2000. Pressure regulation of soluble cytochromes *c* in a deep-sea piezophilic bacterium, *Shewanella violacea*. *Journal of Bacteriology* 182 (10), 2945–2952.
- Yamaguchi, A., Watanabe, Y., Ishida, H., Harimoto, T., Furusawa, K., Suzuki, S., Ishizaka, J., Ikeda, T., Takahashi, M.M., 2004. Latitudinal differences in the planktonic biomass and community structure down to the greater depths in the western North Pacific. *Journal of Oceanography* 60, 773–787.
- Yayanos, A.A., 1995. Microbiology to 10,500 meters in the deep sea. *Annual Reviews in Microbiology* 49, 777–805.
- Yokokawa, T., De Corte, D., Sintès, E., Herndl, G.J., in press. Spatial patterns of bacterial abundance, activity and community composition in relation to water masses in the Eastern Mediterranean Sea. *Aquatic Microbial Ecology*.
- Yokokawa, T., Nagata, T., 2010. Linking bacterial community structure to carbon fluxes in marine environments. *Journal of Oceanography* 66, 1–12.
- Zaccaro, R., Monticelli, L.S., Seritti, A., Santinelli, C., Azzaro, M., Boldrin, A., LaFerla, R., D'Alcala, M.R., 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. *Journal of Geophysical Research* 108 (C9), 8117, doi:10.1029/2002JC001625.
- Zhang, H., Sekiguchi, Y., Hanada, S., Hugenholtz, P., Kim, H., Kamagata, Y., Nakamura, K., 2003. *Gemmatimonas aurantiaca* gen. nov., sp. nov., a Gram-negative, aerobic, polyphosphate-accumulating micro-organism, the first cultured representative of the new bacterial phylum *Gemmatimonadetes* phyl. nov. *International Journal of Systematic and Evolutionary Microbiology* 53, 1155–1163.
- ZoBell, C.E., Oppenheimer, C.H., 1950. Some effects of hydrostatic pressure on the multiplication and morphology of marine bacteria. *Journal of Bacteriology* 60, 771–781.