

Potential of Microbial Oceanography to shed light on the Dark Ocean's enigmas

Gerhard J. Herndl*, Thomas Reinthaler and the TRANSAT & ARCHIMEDES shipboard parties

The dark realm of the ocean, below the sunlit surface layer, represents more than 80% of the ocean's volume and harbors about 10 times more carbon than the soil and atmosphere together. Thus, it is evident that the dark ocean plays a crucial role in the global carbon cycling. Although about 75% of the main drivers of the biogeochemical cycles, the marine microbes, reside in the dark ocean, our knowledge on the deep ocean's microbial community and activity is still in its infancy. The emerging field of Microbial Oceanography integrates knowledge on water mass transport, (bio)geochemistry, genomics and microbial ecology to resolve some of the major current uncertainties in the deep ocean carbon flux.

Most of the research on microbial food webs in the open ocean has focused on the sunlit surface waters (euphotic layer) reaching to about 120 m depth into the water column. However, about 75% of the oceanic prokaryotic (bacterial and archaeal) abundance reside in the dark ocean below the euphotic layer sustaining about half of the oceanic heterotrophic prokaryotic activity. This heterotrophic prokaryotic activity in the deep layers is thought to be supported by the organic matter produced by phytoplankton in the euphotic layer. Compilations of sediment trap data from the open ocean reveal a variability of three orders of magnitude in the organic carbon flux in the mesopelagic layer (150-1000 m depth), a much higher variability than the phytoplankton primary

production in the surface layer. The attenuation of the organic carbon flux with depth is generally described by the 'Martin curve', coined after the oceanographer John Martin, who also formulated the 'iron hypothesis'. If we accept the general assumption that deep-water heterotrophs depend on sedimenting organic carbon, then the metabolic activity of the dominant organismal group, the prokaryotes, should decline with depth following the 'Martin curve'. However, this is not the case. One of the enigmas of the dark ocean's heterotrophic prokaryotic activity is that the organic carbon demand of the heterotrophic prokaryotes in the deep ocean is higher than the organic carbon supplied by sedimenting particles collected in sediment traps. This mismatch between prokaryotic carbon demand and supply has been recently reported for the North Atlantic (Fig. 1) and the Pacific. Hence, it is not a regional but rather a global phenomenon that needs further research to arrive at more realistic global carbon budgets and mechanistic understanding of the ocean carbon cycle.

Potential sources of error in our estimates of the carbon flux to the deep ocean and in measuring microbial activity are currently critically reviewed. Here, we rather focus on the microbial side of the problem. One of the major problems in determining microbial activity of deep-sea communities is that the samples collected at depths of several thousand meters (2000 m depth corresponds to a hydrostatic pressure of around 200 bar) are brought on board of the research

vessel and hence, are decompressed prior to performing the actual measurements. This decompression of the samples might lead to alterations in the measured microbial activity. Since there are essentially no microbial activity measurements under *in situ* pressure conditions available for the global ocean at present, the NIOZ Department of Marine Technology has designed and fabricated a high-pressure sampling and incubation device (Fig. 2). The samplers have been extensively

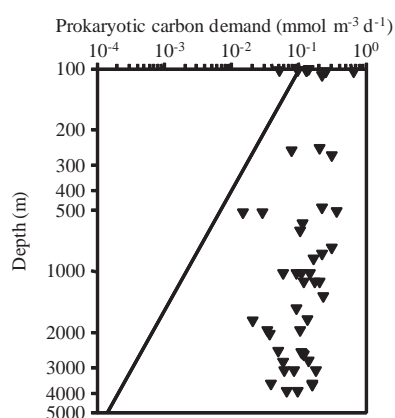


Fig. 1. Heterotrophic prokaryotic carbon demand versus depth in the North Atlantic. Prokaryotic production was measured by ³H-leucine incorporation and the prokaryotic carbon demand was calculated as the sum of production and the respiration measured by oxygen consumption. The line represents the concentration of available carbon as derived from the surface primary production based on the POC-flux model of Antia (2001).

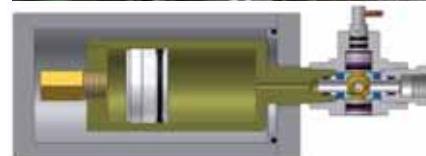


Fig. 2. The titanium high-pressure samplers and incubation devices (HPS) designed and build by the NIOZ department Marine Technology. Top: HPS mounted in the racks in a CTD rosette frame next to NOEX bottles. Bottom: cross-section through a single HPS showing the 50 ml incubation chamber surrounded by the water jacket and the piston at the bottom.

*Corresponding author: gerhard.herndl@nioz.nl

tested over the past two years and are operational now. First data on deep-sea microbial activities measured under *in situ* pressure conditions will become available in the summer of 2009.

Genomic approaches revealed distinct geographic distribution patterns of specific Bacteria and Archaea in the deep ocean over the last five years corresponding to the major deep water masses. A conspicuous feature has been recently reported for the nitrifying Crenarchaeota. Only two marine Crenarchaeota have been cultured thus far, one isolated as a symbiont of a marine sponge and the other, *Nitrosopumilus maritimus*, scratched off from a stone in a tropical aquarium. Both of these isolates are oxidizing ammonia as energy source and use carbon dioxide as carbon source. Hence, it has been believed that all Crenarchaeota found abundantly in the deep ocean are oxidizing ammonia as well. We have shown recently that only in the northern part of the North Atlantic, Crenarchaeota are ammonia oxidizers throughout the water column. A distinct gradient of ammonia-oxidizing Crenarchaeota has been detected in the deep waters from the northern to the equatorial regions of the Atlantic (Fig. 3) coinciding with the decrease in ammonia concentrations in the deep waters from north to the south as the water masses age in the thermohaline ocean circulation. Metagenomic studies revealed that the majority of the deep-sea prokaryotes are adapted to fluctuating environments indicated by a larger genome than surface water prokaryotes. Also, there is evi-

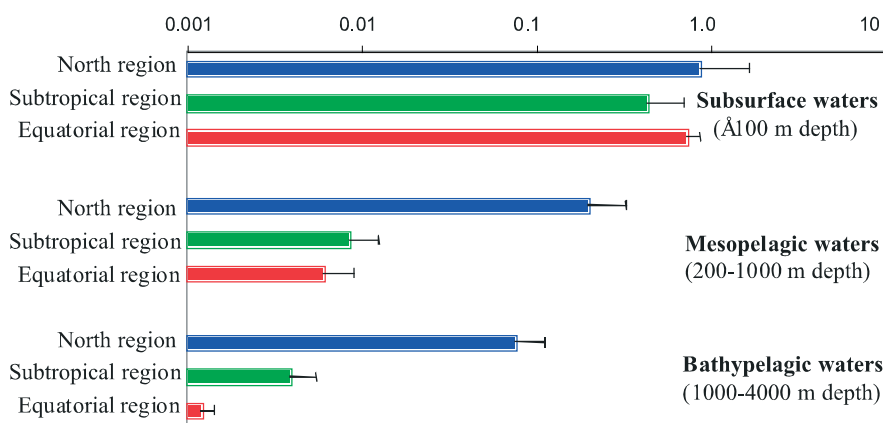


Fig. 3. Latitudinal and depth distribution of the ratio of archaeal *amoA* copies to crenarchaeal 16S rRNA copies per ml of marine water. Total number of samples was 55, bars indicate standard error. Adapted from Agogue et al. 2008, Nature 456: 788-791.

dence for a prevailing particle-attached life mode as indicated by the presence of specific genes. This is somewhat counter-intuitive to the common view that the deep ocean is a rather homogenous and steady environment. Evidence is now accumulating from several fields of research that specific prokaryotes, commonly considered as a single species, can be differentiated in surface and deep-water ecotypes. The deep-water ecotype has specific genes indicative for a preferential particle-attached life mode.

The nature of these particles serving as potential substrate for deep-ocean microbes and leading to a non-random distribution of microbes in the deep ocean needs to be resolved. One hitherto largely ignored source of these deep-water particles might be self-assembling gels. These gels have been reported to be ubiquitously present in the sea including the dark ocean. It is estimated that about 10% of the oceanic dissolved organic carbon has the tendency to form gels poten-

tially further coagulating into microscopically small aggregates (Fig. 4) and providing a surface for attachment of prokaryotes as well as substrate for microbial metabolism. It is expected that these self-assembling gels are of a much higher nutritional value for heterotrophic prokaryotes than truly dissolved organic matter in the deep ocean.

Another major, thus far largely ignored, aspect of organic matter synthesis in the mesopelagic ocean is the autotrophic activity of prokaryotes, synthesizing organic carbon via the uptake of carbon dioxide. It has been estimated that this mesopelagic dark ocean's primary production mediated by autotrophic prokaryotes equals about 15% of the phytoplankton production exported from the sunlit surface layer. This newly synthesized organic matter in the mesopelagic has not been previously exposed to degradation unlike the sedimenting particles collected with sediment traps. Hence, the newly produced deep-water organic matter, i.e., autotrophic prokaryotes, is of high nutritional value and will remain present in the water column as individual microbial cells are too small to sink. Thus, this newly produced material likely represents an essential food source for the deep-sea biota albeit it is not captured by sediment traps and is consequently missing in the current carbon budgets. Overall, the dark ocean's microbial food web seems to be fundamentally different from its well-studied surface counterpart (Fig. 5). The Microbial Oceanography approach has revealed a wealth of information on the dark

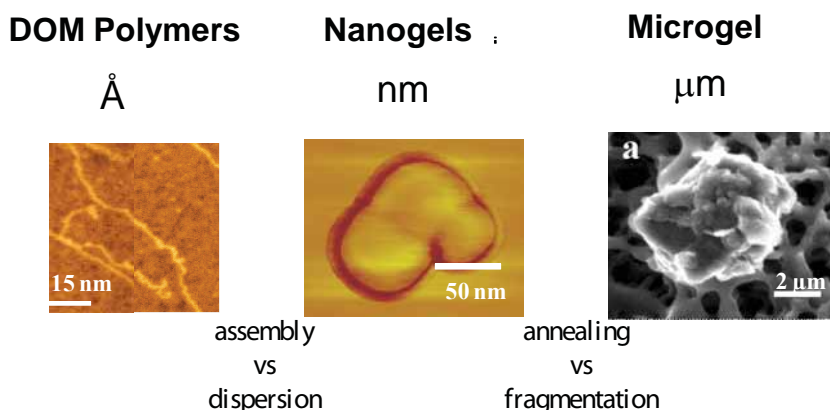


Fig. 4. Scheme of formation and degradation of self-assembling gels from nm-sized DOM polymers to μmeter-sized microgels in the ocean

ocean's biogeochemistry and biota over the past few years and likely will continue to revolutionize our view on the dark ocean's microbial food web in the coming years.

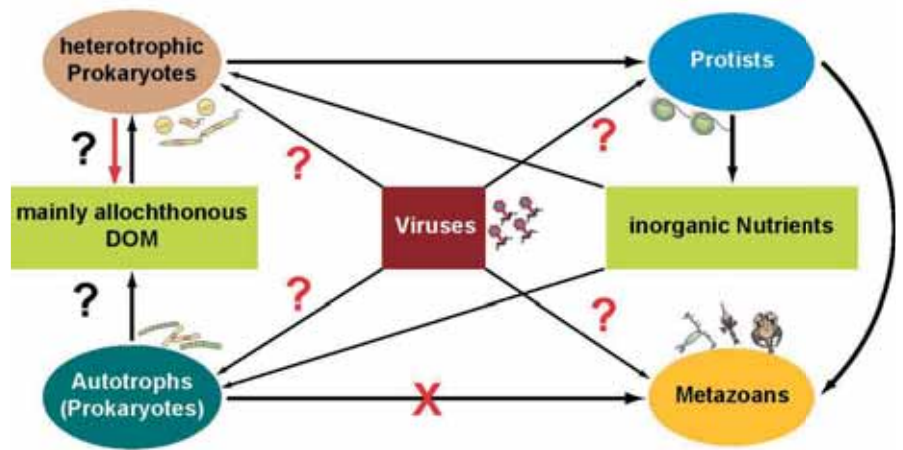


Fig. 5. Scheme of the microbial food web structure and cycling of organic and inorganic matter pool in the meso- and bathypelagic realm. The black arrows between the different biotic and abiotic compartments indicate the interactions as known from the euphotic layer. Red question marks indicate major uncertainties on the importance of these interactions in the dark ocean. Red cross denotes the lack of evidence that this flux is significant in the deep.