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Structure and function of the mesopelagic microbial loop in the NW Mediterranean Sea

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ABSTRACT: The mesopelagic layer is where the majority of the particulate organic carbon exported from the epipelagic layer is remineralized to CO_2. Recent studies at the DYFAMED time-series station in the NW Mediterranean Sea have shown that dominance of prokaryotes (*Bacteria* and *Archaea*) within the microbial community represented by biomass data increases with depth. The studies have also shown that the depth-integrated biomass of protists (heterotrophic flagellates and ciliates) in the mesopelagic layer is as great as that in the epipelagic layer, and that biomass of mesopelagic prokaryotes is controlled by both substrate availability (bottom-up control) and predation and viral infection (top-down control). Data on prokaryotic growth efficiency (derived from the prokaryotic heterotrophic production and the loss of organic carbon flux in the mesopelagic layer on an annual scale) suggest that mesopelagic prokaryotes are not simply remineralizers of organic carbon, but also play an important role in supporting the production of organisms belonging to higher trophic levels. Analysis using a simple food chain model suggests that heterotrophic nanoflagellates may be important remineralizers of organic carbon produced by prokaryotes, and that the viral loop may enhance remineralization of organic carbon in the mesopelagic layer. There are, however, uncertain elements in measuring organic carbon flux and biomass and activity of the microbial components in the mesopelagic layer. By comparing the results obtained at the DYFAMED station with those obtained from other environments, this study summarizes our current knowledge on the structure and function of the mesopelagic microbial loop in the NW Mediterranean Sea as well as in other oceanic regions.

KEY WORDS: Mesopelagic microbial loop · Protists · Carbon flux · NW Mediterranean Sea

INTRODUCTION

The biological carbon pump, consisting of a suite of biologically mediated processes, plays an important role in the global carbon cycle (Volk & Hoffert 1985). The processes include conversion of inorganic carbon to organic carbon by phytoplankton, transport of organic carbon from the surface to deep waters via sinking of particles, water mixing, and active transport by animals. One can assume that an efficient biological carbon pump requires a condition in which the extent of mineralization of organic carbon (i.e. conversion of organic carbon back to inorganic CO_2) is reduced before the organic carbon is sequestered into ocean interiors. The general view in the field of marine biogeochemistry is that a large portion of organic carbon exported from the epipelagic layer is remineralized to CO_2 in the mesopelagic layer (e.g. Fowler & Knauer 1986). It has been demonstrated that a large portion of sinking particulate organic carbon (POC) is consumed by both zooplankton (e.g. Banse 1990, Steinberg et al. 1994) and prokaryotes during the sinking process (e.g. Cho & Azam 1988, Smith et al. 1992, Ducklow 1993, Turley & Mackie 1994).

The decrease in sinking POC flux in the mesopelagic layer, however, appears to be insufficient to support the carbon demand of prokaryotes in the open Arabian Sea (Ducklow 1993), the NE Atlantic (Turley & Mackie 1994), and the subarctic Pacific (Nagata et al. 2001), as well as the carbon demand of both prokaryotes and zooplankton in the oligotrophic subtropical Pacific gyre and the northwest subarctic

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Pacific (Steinberg et al. 2008). These studies collectively suggest the presence of additional mechanisms for supplying organic carbon to the mesopelagic layer, such as the lateral and vertical transport of dissolved organic carbon (DOC) and diel vertical migration of zooplankton feeding in the surface waters. Such organic carbon deficits are also suggested from the comparison between prokaryotic carbon demand and the amount of potentially available POC at given depths in the bathypelagic layer: Prokaryotic carbon demand is up to an order of magnitude higher in the subtropical North Atlantic (Baltar et al. 2009) and 2 to 3 orders of magnitude higher in the North Atlantic basin (Reinthaler et al. 2006) than POC. It should be noted that DOC, produced in or supplied to the mesopelagic layer, is accessible only to prokaryotes. Regardless of the spatial and/or temporal variations in the contribution of sinking POC and exported DOC in supplying organic carbon to the mesopelagic layer, previous studies strongly suggest the necessity to better understand the role of prokaryotes in the loss of organic carbon in the mesopelagic layer.

In the epipelagic layer, a significant portion of carbon fixed by photosynthesis is lost to the DOC pool through a multitude of processes, which can be reincorporated into the food web via prokaryotes (Cole et al. 1988). Reincorporation of DOC via microbial organisms is referred to as the microbial loop (Azam et al. 1983). Growth of prokaryotes through the consumption of degradable DOC can be controlled by the availability of inorganic nutrients (bottom-up control) (e.g. Havskum et al. 2003, Thingstad et al. 2008), while the biomass of prokaryotes is controlled by predation and viral infection (top-down control). The function of the food web in terms of material cycling is therefore intimately linked to the food web structure, that is, the (functional) types of organisms that are present, the extent of their interactions, and the external (physical and chemical) driving forces that potentially affect the structure (Thingstad & Rassoulzadegan 1999). It is, therefore, important to elucidate the factors controlling the food web structure and their effects on prokaryotes (e.g. substrate availability and trophic interactions) for better understanding of the biogeochemical role of prokaryotes, especially those that are present in the mesopelagic layers.

In this review, previous studies performed in the mesopelagic layer of the NW Mediterranean Sea will be compared with other published works conducted in other regions. Moreover, our current knowledge on the structure and function of the mesopelagic microbial loop in the NW Mediterranean Sea as well as in other oceanic regions will be summarized in order to identify which areas of study require future attention.

THE DYFAMED TIME-SERIES STATION

The Mediterranean Sea is a unique oceanic regime in terms of its hydrography and biogeochemistry (reviewed by Krom et al. 2003). Nutrient concentrations, the integrated chlorophyll and primary production in the epipelagic layer, and the POC export from the epipelagic layer all decrease in the Mediterranean Sea from west to east (Moutin & Raimbault 2002). In the Levantine Basin of the eastern Mediterranean Sea, the surface waters above the chlorophyll maximum contain very low nutrient and low chlorophyll concentrations (30 to 80 nM ammonia, <1 to 10 nM nitrate, <2 to 4 nM phosphate, 20 to 50 ng l^{-1} chlorophyll) during the summer (Krom et al. 2005). The low nutrient status is caused by anti-estuarine circulation in which nutrient-poor surface water flows eastward through the straits of Gibraltar and Sicily becoming progressively more saline to the east, while a counter-current of (relatively) nutrient-rich water flows out of the basin. The deep waters have a nitrate:phosphate ratio of 22:1 in the western Mediterranean Sea (Béthoux et al. 1992) and 25:1 to 28:1 in the Eastern Mediterranean Sea (Krom et al. 1991, Kress & Herut 2001). In other words, these waters are P-starved compared to other oceanic regions whose ratios of nitrate to phosphate conform to what is known as the Redfield ratio of 16. Many studies report that Mediterranean surface waters become P-limited for prokaryotes and phytoplankton during the stratified period (e.g. Zweifel et al. 1993, Vaulot et al. 1996, Thingstad et al. 1998, Zohary et al. 2005). One theoretical consideration suggests that the carbon accumulated in deep waters due to the sinking of particles (i.e. one of the processes constituting the biological carbon pump) is stored more stably in a P-limited system than in an N-limited system (Thingstad & Rassoulzadegan 1995). The Mediterranean Sea thus offers a unique set of conditions as a natural laboratory to study the interplay among biological, biogeochemical, and hydrographical processes (Thingstad & Rassoulzadegan 1999).

In the NW Mediterranean Sea, a multidisciplinary study has been performed since 1987 at the DYFAMED time-series station (43° 25.2′ N, 07° 51.8′ E; 2350 m max depth) as a part of the French Joint Global Ocean Flux Study (JGOFS) and in the framework of many French national programs (Marty 2002). The DYFAMED site is characterized by different water types (water masses), i.e. surface water with seasonally variable temperature, intermediate water of Levantine origin (LIW) which is present between 300 and 500 m depths and exhibits the maxima of temperature and salinity, and deep water which is colder and less saline than LIW. It should be noted that water temperature is always ca. 13°C below the seasonal thermocline down

to the bottom and throughout the water column during the winter mixing period (e.g. Marty 2003). This site does not receive significant anthropogenic or natural dust inputs (Marty et al. 1994, Ridame & Guieu 2002) and receives very weak lateral flows (Béthoux et al. 1988, Andersen & Prieur 2000). Seasonal patterns in the water column structure and biological production in the epipelagic layer (Marty & Chiavérini 2002) result in sinking POC fluxes that are higher from January to June and lower from July to December (Miquel et al. 1993, 1994). DOC accumulates in the surface mixed layer during the stratified period and is exported to the deeper layer during the winter mixing period (Copin-Montégut & Avril 1993, Avril 2002). The dissolved oxygen minimum is 170 µmol kg^{-1} around 200 to 300 m depth (Copin-Montégut & Bégovic 2002), which is far too low to allow nitrate reduction to occur. The mesopelagic layer is usually referred to as the layer below the epipelagic layer down to either ca. 1000 m, where a permanent pycnocline usually exists as a persistent barrier to deep vertical mixing, or the maximum depth of winter mixing where the permanent pycnocline is absent (Legendre & Rivkin 2002). At the DYFAMED site, the pycnocline in deep water does not exist and the depth of water column homogenization during the winter is variable between years but has always been <1000 m (Copin-Montégut & Bégovic 2002). Hereafter, the 5 to 110, 110 to 1000, and 1000 to 2000 m layers of this site will be referred to as the epipelagic, mesopelagic, and bathypelagic layers, respectively.

DISTRIBUTION OF MICROBIAL PROKARYOTES AND EUKARYOTES IN DEEP WATERS

Abundance and biomass of 9 microbial groups collected from the DYFAMED site at 13 depths, ranging from 5 to 2000 m, were measured 10 times between May 1999 and March 2000 (Tanaka & Rassoulzadegan 2002 unpubl. data). It is known that Archaea's contribution to the prokaryotic community in terms of abundance and biomass increases in the dark ocean (Fuhrman & Davis 1997, Karner et al. 2001). Both counting of prokaryotes based on DNA-staining dyes such as DAPI and SYBR Green (Porter & Feig 1980, Jacquet et al. 1998) and measurement of radioisotope-labeled leucine incorporation by prokaryotes (Kirchman 1992) in the aphotic layer result in the measurement of the total abundance and heterotrophic activity of both *Bacteria* and *Archaea* combined. It should also be noted here that the abundance of cyanobacteria containing phycoerythrin pigments has generally been measured separately from their heterotrophic counterparts, and thus the term 'prokaryotes' used herein does not include the cyanobacterial cells containing phycoerythrin. Nine microbial groups consisted of 2 prokaryotic groups, i.e. cyanobacterial cells containing phycoerythrin pigments (hereafter referred to as cyanobacteria) and prokaryotes including both *Bacteria* and *Archaea* but excluding cyanobacteria (hereafter referred to as prokaryotes), and 7 eukaryotic groups, i.e. heterotrophic pico- and nanoflagellates (HPF, HNF), ciliates, tintinnids, cyanobacteria, autotrophic pico- and nanoflagellates (APF, ANF), and *Mesodinium rubrum* Lohmann 1908 (=*Myrionecta rubra* Jankowski 1976). Tanaka & Rassoulzadegan (2002) reported that prokaryotes, HNF, and ciliates were always detected throughout the water column. Abundances of prokaryotes, HNF, and ciliates all decreased with depth, from 2.1×10^9 to 2.2×10^7, 1.2×10^6 to 1.2×10^3, and 1.5×10^4 to 1 cells l^{-1} for prokaryotes, HNF, and ciliates, representing 1, 2, and 3 orders of magnitude of decrease (5 to 2000 m), respectively (Fig. 1). The log-log linear regression analysis for abundance versus depth showed that the regression slope values indicating the magnitude of depth-dependent decrease in microbial abundance were relatively constant (mean ± SD = −0.557 ± 0.125 for prokaryotes, −0.886 ± 0.125 for HNF, and −1.102 ± 0.246 for ciliates). The analysis also showed that the magnitude of depth-dependent decreases in abundance was significantly smaller for prokaryotes than for protists. Under the assumption that the food web is close to steady-state, the results suggest that the balance between process rates (i.e. growth versus mortality rates) is less variable with depth for prokaryotes than for protists, and that the density-dependent predator–prey relationship between the 3 microbial components becomes less coupled with depth down to 2000 m.

In the mesopelagic layer of the NW Mediterranean Sea, abundance and biomass were respectively in the range of 3.1×10^7 to 3.4×10^8 cells l^{-1} and 42 to 4200 nmol C l^{-1} for prokaryotes, 1.2×10^3 to 1.8×10^5 cells l^{-1} and 0.83 to 49 nmol C l^{-1} for HNF, and 1.6 to 376 cells l^{-1} and 0.33 to 100 nmol C l^{-1} for ciliates (Fig. 1; Tanaka & Rassoulzadegan 2002). A similar range of abundance is reported for prokaryotes (2.4×10^7 to 4.1×10^8 cells l^{-1}) and HNF (8.3×10^2 to 2.8×10^5 cells l^{-1}) in the 100 to 1000 m depth layer in the eastern Mediterranean Sea (Tanaka et al. 2007). This was in contrast to the known longitudinal difference of epipelagic productivity across the Mediterranean (Turley et al. 2000, Moutin & Raimbault 2002). A comparison of data on prokaryotic abundance compiled from different oceanic areas showed that the prokaryotic abundance was in the order of 10^7 to 10^8 cells l^{-1} in the 200 to 1000 m depth layer (Koppelmann et al. 2005, Arístegui et al. 2009). Arístegui et al. (2009) also showed that HNF abundance in the 100 to 1000 m

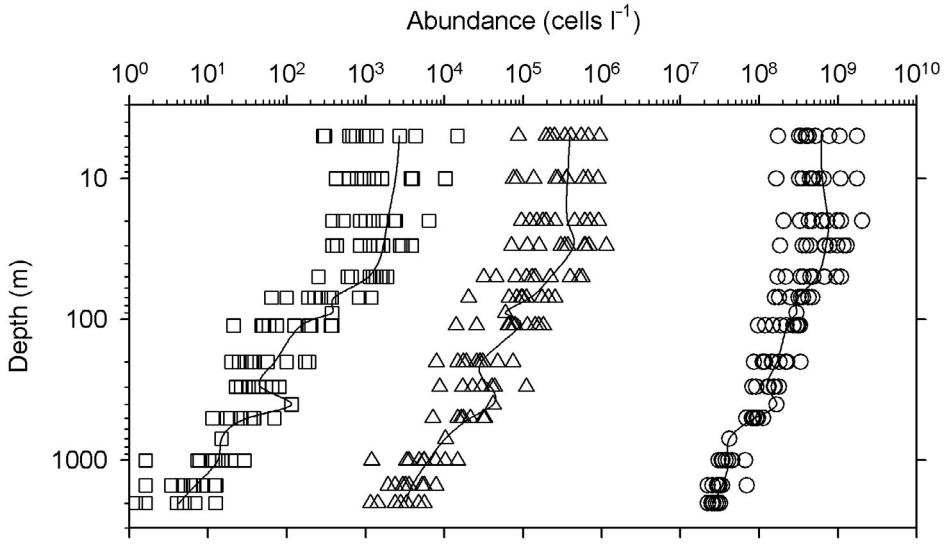

Fig. 1. Abundances (cells l^{-1}) of prokaryotes, heterotrophic nanoflagellates (HNF), and ciliates between 5 and 2000 m depth from May 1999 to March 2000 at the DYFAMED site (redrawn from Tanaka & Rassoulzadegan 2002). Prokaryotes (*Bacteria* + *Archaea*) were counted by epifluorescence microscope after staining with 4′6-diamidino-2-phenylindole (DAPI) (Porter & Feig 1980); all flagellates including HNF were counted and sized by epifluorescence microscope after staining with DAPI and proflavine (Haas 1982); all ciliates were counted and sized with an inverted microscope. Open squares, triangles, and circles denote ciliates (only oligotrich ciliates), HNF, and prokaryotes, respectively; lines denote the annual average at each depth

depth layer was generally in the order of 10^4 to 10^5 cells l^{-1}, and noted that HNF abundances at similar depth are much lower in the NW Mediterranean Sea than in Atlantic or Pacific waters. They suggested that the lower HNF abundance in the NW Mediterranean Sea could be related to the relatively high abundance of ciliates in Mediterranean waters or to the relatively high temperature of meso- and bathypelagic Mediterranean waters (ca. 13°C). To our knowledge, these data on the vertical distribution of the abundance and biomass of ciliates, which are comparable to those reported in Tanaka & Rassoulzadegan (2002), have not been published elsewhere.

Depth-integrated biomass in the epipelagic layer showed the dominance of prokaryotes (mean = 48%, range = 39 to 57%), followed by cyanobacteria (mean = 20%, range = 9 to 32%), ciliates (mean = 13%, range = 5 to 24%), ANF (mean = 11%, range = 4 to 25%), and HNF (mean = 6%, range = 2 to 13%) (Fig. 2A). The contribution of other groups to the depth-integrated biomass was on average 0.2 to 1.5% in the epipelagic layer. The dominance of prokaryotes in microbial biomass further increased with depth, and comprised up to 92% (range = 87 to 96%) of total microbial biomass in the bathypelagic layer. Abundance of autotrophic groups rapidly decreased with depth in the aphotic layers, and their contribution to total microbial biomass was 3.8% in the mesopelagic layer and 0.5% in the bathypelagic layer. Note that autotrophic groups were sometimes detected in deeper layers (down to 500 m for *Mesodinium rubrum*, 1500 m for APF, and 2000 m for cyanobacteria and ANF). The presence of microbial autotrophs down to the bathypelagic layer suggests their rapid transport due to the vertical migration of zooplankton and sinking of particles rather than winter vertical mixing (Gowing et al. 2003, Richardson & Jackson 2007). Depth-integrated biomasses of HPF, HNF, and ciliates in the mesopelagic layer were similar to those in the epipelagic layer (Fig. 2B). In contrast, the prokaryotic biomass distribution in the mesopelagic layer (52%) was twice as high as that in the epipelagic and the bathypelagic layers (27 and 21%, respectively). The greatest decrease in depth-integrated biomass from the epipelagic to the mesopelagic layer was observed for tintinnids among the microbial predators. The integrated biomass of these microbial groups in the bathypelagic layer always showed the lowest values amongst the layers (4.6 to 21%). At the DYFAMED site, although the biomass data suggested the dominance of prokaryotes among the microbial groups in the mesopelagic and bathypelagic layers (Fig. 2A), the data on depth-integrated biomass of HPF, HNF, and ciliates suggested that the importance of these microbial groups in the epipelagic layer remained relatively unchanged in the mesopelagic layer (Fig. 2B).

Using conversion factors, the biomass of microbes was calculated in carbon units from cell numbers or volume, which were measured for laboratory-grown strains or the microbes collected from the epipelagic

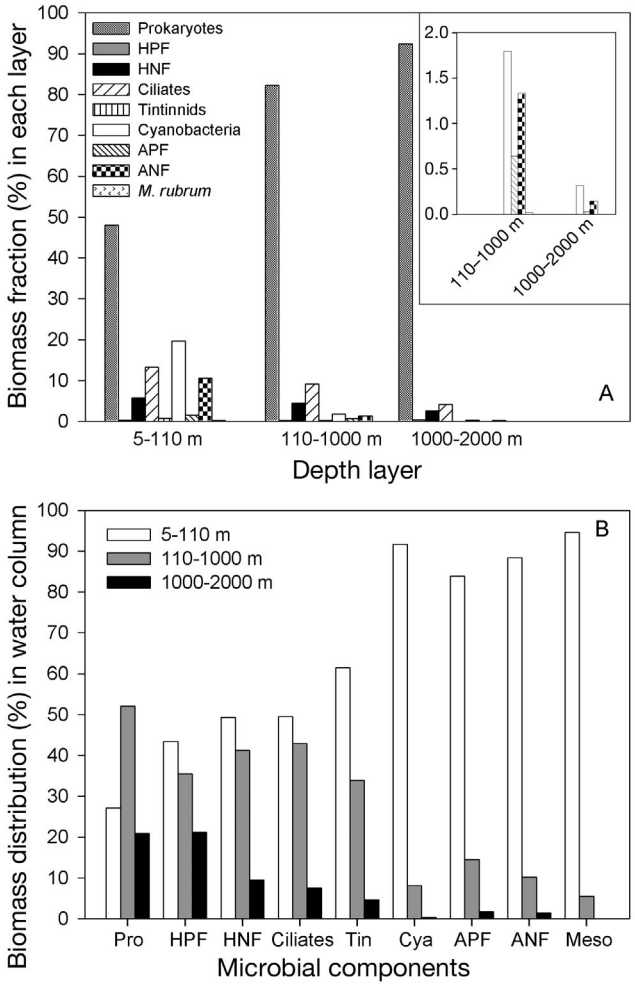

Fig. 2. Distribution (%) of the microbial components (data from Tanaka & Rassoulzadegan unpubl. data). To calculate biomass in carbon, the following conversions factors were used: 15 fg C cell^{-1} for prokaryotes, 200 fg C cell^{-1} for cyanobacteria, 183 fg C µm^{-3} for flagellates (Caron et al. 1995), 0.19 pg C µm^{-3} for ciliates (Putt & Stoecker 1989), and 0.053 pg C µm^{-3} for tintinnids (Verity & Langdon 1984). (A) Percent biomass of the microbial components in each depth layer; inset emphasizes the biomass penetration of phytoplankton in deeper waters (axes units are the same). (B) Percent biomass in 3 depth layers for each microbial component. Pro: prokaryotes; HPF: heterotrophic picoflagellates; HNF: heterotrophic nanoflagellates; Tin: tintinnids; Cya: cyanobacteria; APF: autotrophic picoflagellates; ANF: autotrophic nanoflagellates; Meso: *Mesodinium rubrum*

layers in different marine systems (see Tanaka & Rassoulzadegan 2002). Direct measurement of carbon content showed 4 times as much difference in the cellular carbon content among the prokaryotes collected from the epipelagic layers of various oceanic regions (Fukuda et al. 1998). This suggests that the application of a single conversion factor for calculating the biomass of various types of microbes collected from vertically diverse environments ranging from epipelagic to bathypelagic layers can lead to significant errors in biomass estimates. This may lead to difficulties in understanding the role of the microbial loop below the epipelagic layer in biogeochemical cycling of elements. To our knowledge, no direct determination of cellular elemental composition has been reported for microbes in the mesopelagic or bathypelagic layers.

POTENTIAL ROLE OF MESOPELAGIC PROKARYOTES IN THE DECREASE OF ORGANIC CARBON FLUX

Another study at the DYFAMED site reported prokaryotic abundance and prokaryotic heterotrophic production (PHP) in the mesopelagic layer between July 2001 and October 2002 (Tanaka & Rassoulzadegan 2004). Prokaryotic abundance ranged from 4.3 × 10^7 to 4.4 × 10^8 cells l^{-1}, and PHP ranged from 0.3 to 15.3 nmol C l^{-1} d^{-1} (mean = 2.7 nmol C l^{-1} d^{-1}, median = 1.5 nmol C l^{-1} d^{-1}). These ranges are comparable to those reported in other studies conducted in the mesopelagic layers of the NW Mediterranean Sea (e.g. Turley & Stutt 2000, Harris et al. 2001, Tamburini et al. 2002, Tanaka & Rassoulzadegan 2002, Mével et al. 2008). Compared to a data set recently compiled for mesopelagic PHP (mean ± SE = 24.4 ± 2.8 nmol C l^{-1} d^{-1}, median = 2.8 nmol C l^{-1} d^{-1}, Arístegui et al. 2009), the PHP values in the mesopelagic layer of the NW Mediterranean Sea fall within the lower range of values. The magnitude of depth-dependent decrease was greater for PHP than for prokaryotic abundance, and was relatively constant despite seasonal variations in prokaryotic abundance and PHP down to 300 m and 500 m, respectively (mean ± SD of the log-log linear regression slopes = −0.662 ± 0.133 for prokaryotic abundance and −1.154 ± 0.297 for PHP). Sharper depth-dependent decrease in PHP compared to prokaryotic abundance suggests that the cell-specific rates of activity also decline with depth (Arístegui et al. 2009). These slope values were similar to those obtained from the compiled data set (−0.553 for abundance and −0.968 for production) (Arístegui et al. 2009). This indicates that, while PHP in the mesopelagic layer tends to be lower in the NW Mediterranean Sea than in other oceanic regions, the extent of the depth-dependent decrease in prokaryotic abundance and PHP in the NW Mediterranean Sea is similar to that in other regions. At the DYFAMED site, seasonal variations in PHP were more evident in the upper 500 m, and a vertical decrease in PHP was not always evident between 200 and 300 m or 300 and 500 m (Tanaka & Rassoulzadegan 2004). This suggests that seasonal rather than vertical variations in the size of

the organic carbon pool available for prokaryotic production were more profound down to 500 m at the DYFAMED site.

Prokaryotic turnover time derived from prokaryotic biomass and PHP is an index of prokaryotic activity indicating how fast (slow) prokaryotic biomass is renewed by PHP. Prokaryotic turnover time at the DYFAMED site varied from 31 to 283 d (mean = 94 d, n = 37) in the 110 to 1000 m depth layer. This variation in prokaryotic turnover time was similar to that in the tropical northeastern Atlantic Ocean (ca. 10 to 400 d; Dufour & Torréton 1996). The mean prokaryotic turnover times reported from other regions were shorter: 7.7 d (n = 29) in the NW African Shelf and the open Atlantic Ocean (Aristegui et al. 2005), and 39 and 68 d in the western and the eastern North Atlantic basin, respectively (Reinthaler et al. 2006). The mean turnover time of 11 d from the data set compiled by Arístegui et al. (2009) also indicated that the prokaryotic turnover times in the NW Mediterranean Sea were longer than those found in other regions. Turnover times, calculated as the depth-integrated values of prokaryotic turnover time, varied from 55 to 141 d (mean = 81 d, n = 7) at the DYFAMED site. In the subarctic Pacific, turnover times integrated for the 100 to 1000 m depth layer varied from 170 to 648 d (mean = 317 d, n = 18; Nagata et al. 2001) and 20 to 160 d (mean = 97 d, n = 6; Fukuda et al. 2007).

Tanaka & Rassoulzadegan (2004) compared their data with the data of Nagata et al. (2001) and noted that: (1) the range of integrated PHP at the DYFAMED site was in the lower part of the range reported from other oceanic regions (1.8 to 10 mmol C m^{-2} d^{-1}) (see Nagata et al. 2001 and references in their Table 6); (2) there was no significant difference in the integrated chlorophyll *a* values between the subarctic Pacific (21 to 218 mg m^{-2} in the 0 to 100 m depth layer, Nagata et al. 2001) and the DYFAMED site (12 to 230 mg m^{-2} in the 0 to 200 m depth layer for 1991 to 1999, Marty et al. 2002); and (3) the water temperature in the mesopelagic layer was lower in the subarctic Pacific (3 to 7°C) than in the NW Mediterranean Sea (ca. 13°C). Tanaka & Rassoulzadegan (2004) suggested that this temperature difference (6 to 10°C) may explain the 4-fold difference in prokaryotic growth rates between the 2 studies by assuming that Q_{10} values were 2 to 4 for prokaryotic growth (e.g. White et al. 1991, Nagata et al. 2001). However, the accumulated data suggest that regional differences in turnover time of prokaryotic biomass cannot be explained by water temperature alone. Such differences are likely attributed to the size, form, and composition of organic carbon available for mesopelagic prokaryotes, the phylogenetic composition of prokaryotes, and predation on and viral infection of prokaryotes. Episodic increases of PHP were observed in the mesopelagic layer of the NW Mediterranean Sea (Misic & Fabiano 2006, Mével et al. 2008), suggesting the necessity for a sampling with shorter spatial and temporal intervals to better understand *in situ* PHP variation.

Prokaryotic growth efficiency (PGE, Reinthaler et al. 2006), defined as the ratio of PHP to prokaryotic carbon demand (PCD), is a useful indicator in understanding the role of prokaryotes in organic carbon flux. PCD is the sum of PHP and prokaryotic respiration. Although mesopelagic prokaryotic respiration was not measured in Tanaka & Rassoulzadegan (2004), PGE was approximated based on the assumptions that (1) lateral transport of organic carbon is negligible and (2) decrease of organic carbon flux in the mesopelagic layer is mostly due to the consumption of organic carbon by prokaryotes. PGE values were then calculated by replacing PCD with the decrease of organic carbon flux in the mesopelagic layer (Tanaka & Rassoulzadegan 2004). An annual study using sediment traps at the DYFAMED site reports that the decrease in sinking POC between 100 and 1000 m depths was 0.4 mol C m^{-2} yr^{-1}, equivalent to 75% of the sinking POC flux at 100 m depth (Miquel et al. 1994). DOC, which accumulates in the epipelagic layer during the stratified period, is exported to deeper layers through winter vertical mixing at the same site (Copin-Montégut & Avril 1993, Avril 2002). Since this mixing depth does not exceed 1000 m and the DOC concentration below 1000 m depth is very stable, most of the exported DOC (1 to 1.5 mol C m^{-2} yr^{-1}) is thought to be consumed within the mesopelagic layer (Avril 2002). This suggests that the decrease of organic carbon flux in the mesopelagic layer is dominated by the exported DOC, and is approximately 1.4 to 1.9 mol C m^{-2} yr^{-1} in total. Another approach to estimate the decrease of organic carbon flux in the mesopelagic layer is based on potential export production including both POC and DOC exports (cf. Dugdale & Goering 1967). Potential export production was estimated from the primary production data and F_p ratio (as a proxy of the *f*-ratio) based on a phytoplankton pigment analysis (see Claustre 1994 for details). F_p ratio is an estimator of the biomass fraction of phytoplankton groups that potentially contribute to new production in a phytoplankton community, and *f*-ratio is the ratio of new production to total (new + regenerated) production. Mean potential export production at the study site was calculated to be 3.5 mol C m^{-2} yr^{-1} for the 1993–1999 period (Marty & Chiavérini 2002). By fitting the sediment trap data (Miquel et al. 1993, J. C. Miquel unpubl. data) to the Martin curve (Martin et al. 1987), the extent of decrease in export production (i.e. exponent term in the Martin curve) was calculated to be –0.789. The decrease of organic carbon flux between 110 and 1000 m depth was calcu-

lated to be 2.9 mol C m^{-2} yr^{-1}. Using a trapezoidal equation, the annual PHP integrated for the mesopelagic layer was estimated to be 0.55 mol C m^{-2} yr^{-1}. PGE was then estimated to be 19 to 39% in the mesopelagic layer on an annual scale (Tanaka & Rassoulzadegan 2004).

In the ocean epipelagic layer, the compiled data on PHP and prokaryotic respiration show that the median PGE is 9% (mean ± SD = 15 ± 12%) (del Giorgio & Cole 2000). The concentration of organic matter available for prokaryotes is generally lower in deeper waters (Williams 2000), and thus one may expect that the PGE value is smaller in the mesopelagic layer than in the epipelagic layer. In this context, our PGE values in the mesopelagic layer seem unrealistically high. However, since sinking POC is consumed not only by prokaryotes (Turley & Stutt 2000) but also by detritivorous zooplankton (Carroll et al. 1998) at the study site, the extrapolated PCD values (Tanaka & Rassoulzadegan 2004) are, to an extent, overestimated and therefore should be regarded as conservative. It should be noted that the time scale is different between the PGE values based on the data compiled by del Giorgio & Cole (2000) and the PGE values from Tanaka & Rassoulzadegan (2004). The former are mostly based on the volumetric values of PHP and prokaryotic respiration measured for relatively short periods (hours and days, respectively) in the epipelagic layer. The reliability of our PGE estimates depends on the extent of uncertainty associated with the measurement of PHP and sinking POC flux by a sediment trap, the extrapolation of PHP to an annual scale, and an estimate of DOC exported to the mesopelagic layer. The coefficient of variation in PHP measurement by ^{3}H-leucine incorporation was approximately 20% (T. Tanaka & F. Rassoulzadegan unpubl. data). The use of a sediment trap may underestimate the downward flux of POC by a factor of 2 (Buesseler et al. 2007). It is difficult to evaluate the extent of uncertainty associated with the measurement of exported DOC flux or the determination of depth ranges where the exported DOC is mostly consumed by prokaryotes. At the Bermuda Atlantic Time-series study site in the NW Sargasso Sea, DOC, accumulated during the stratified period and exported during winter vertical mixing, is consumed only in the upper part of the mesopelagic layer after winter vertical mixing (Carlson et al. 1994). Even if the decrease of sinking POC flux were underestimated by a factor of 2 (i.e. 0.8 mol C m^{-2} yr^{-1}) and PHP was 20% overestimated (i.e. 0.44 mol C m^{-2} yr^{-1}), PGE would have been 19%. Further studies are needed to reduce the degree of uncertainty regarding the PGE estimate.

The PGE values calculated from the data obtained by the ^{3}H-thymidine incorporation method and the electron transport system assay ranged from 1 to 36% in the 100 to 700 m depth layer at the DYFAMED site (Martin et al. 1994). The yield of ^{14}C-glutamic acid assimilation measured under *in situ* hydrostatic pressure was 36% in the spring and 13% in the fall for the 200 to 1000 m depth layer at the same site (Tamburini et al. 2002). Reinthaler et al. (2006) measured ^{3}H-leucine incorporation and oxygen consumption, and reported on average a PGE value of 2% in the 180 to 1030 m depth layer (oxygen minimum zone) in the North Atlantic Basin. In the NW African shelf and the open Atlantic Ocean, PGE, which was estimated from PHP using the ^{3}H-thymidine/^{3}H-leucine incorporation method and the back-scaled prokaryotic respiration derived from the prokaryotic abundance, was 18 ± 3% at 600 m and 13 ± 2% at 1000 m (Arístegui et al. 2005). These results suggest that the PGE estimates from Tanaka & Rassoulzadegan (2004) are not complete outliers; otherwise, significant errors have been involved in the studies in the NW Mediterranean Sea. Uncertainties regarding the PGE estimate aside, not very small PGE values in Tanaka & Rassoulzadegan (2004) suggest that mesopelagic prokaryotes are not merely remineralizers of organic carbon but also support higher trophic levels of the mesopelagic plankton food web in the NW Mediterranean Sea. In other words, the PGE estimates suggest that the prokaryotic biomass in the mesopelagic layer is not simply controlled by substrate availability (bottom-up control) but also by predation and virus infection (top-down control).

To investigate the factors potentially controlling the abundance of mesopelagic prokaryotes, bioassay experiments were performed in different seasons using the seawater collected from the DYFAMED site (Tanaka & Rassoulzadegan 2004). Four different experimental conditions were set up: (1) control (unfiltered seawater from 500 m), (2) bacterivore-free (<0.8 µm filtered seawater from 500 m), (3) bacterivore-free (<0.8 µm filtered seawater from 500 m) diluted by <0.2 µm filtered seawater from 500 m, and (4) bacterivore-free (<0.8 µm filtered seawater from 500 m) diluted by <0.2 µm filtered seawater from 110 m. Seawater was incubated for 4 d at *in situ* temperatures (±1°C) in the dark under atmospheric pressure, and the instantaneous growth rate of prokaryotes was determined by linear regression of the natural log of prokaryotic abundance during the incubation (Fig. 3). Significant differences in prokaryotic growth rate among different experimental conditions (*t*-test, $p < 0.05$), i.e. 1 < 2, 2 < 3, and 3 < 4, indicate the effects of predation on prokaryotes at 500 m, substrate availability (or bottom-up control) at 500 m, and the difference in DOM availability for mesopelagic prokaryotes between 500 and 110 m depth. Disruption of living cells and detritus could occur during filtration even at a low pressure differential, enhancing

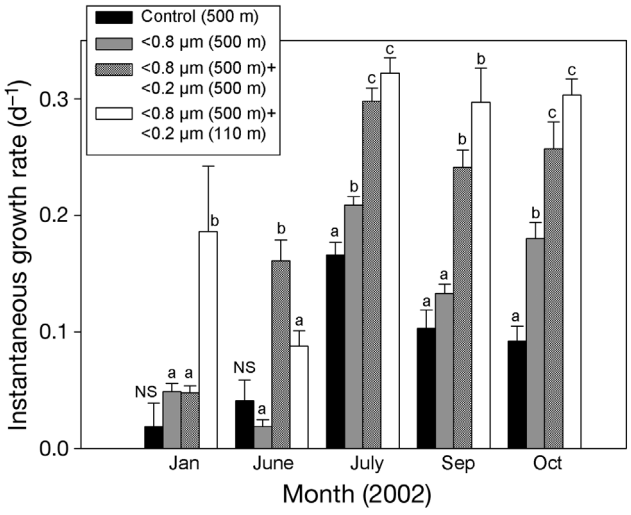

Fig. 3. Instantaneous growth rates (±SE; d^{-1}) of prokaryotes in 4 different treatments during 4 d incubation at *in situ* temperature in the dark (data from Tanaka & Rassoulzadegan 2004). Values are means of 3 replicate experimental bottles except for '<0.8 µm (500 m) + <0.2 µm (110 m)' in January (n = 5). Mean values were compared using a *t*-test. Different letters indicate significant difference ($p < 0.05$). NS: not significant

the concentration of DOM in filtrates. If the extent of this disruption was significant, there should have been a systematic difference in instantaneous growth rate of prokaryotes among different experimental conditions (i.e. 1 < 2 < 3 < 4). However, such a systematic difference was not evident (Fig. 3), indicating that the enhancement of DOM concentration in the filtrates was not a significant artifact in this bioassay. The results suggest that both predation and substrate availability were generally affecting the prokaryotic abundance significantly at 500 m. A significant predation on mesopelagic prokaryotes indicated by our study at the DYFAMED site supports the previous measurement of the HNF predation rate on prokaryotes down to 500 m in the Sea of Japan (Cho et al. 2000). Also, Tanaka & Rassoulzadegan (2002) suggested that the concentration of DOM available for mesopelagic prokaryotes at 110 m was, compared to that at 500 m, at a high level in January, at similar levels in July, September, and October, and at a low level in June. The water column structure at the DYFAMED site was characterized by the mixing period from December to March, the stratified period from June to September, and the semi-stratified period in May and October (see Tanaka & Rassoulzadegan 2002). Seasonal variations in sinking POC fluxes and DOC export are related to the water column structure (Copin-Montégut & Avril 1993, Miquel et al. 1994). Seasonal variations in the availability of semi-labile DOC, which is resistant to rapid microbial degradation in the epipelagic layer but available for the mesopelagic prokaryotes (Carlson et al. 2004), may explain the differences in the prokaryotic growth rates obtained from experimental conditions 3 and 4. Overall, the bioassay experiments suggest that the abundance of mesopelagic prokaryotes is governed by both bottom-up and top-down controls.

POTENTIAL ROLE OF THE MESOPELAGIC MICROBIAL LOOP IN CARBON FLOW

If one assumes a food chain structure in an approximate steady state over different depths and time scales for carbon flow (e.g. Thingstad 2000), the data on microbial biomass and PHP can be used to estimate the carbon flow between microbial components and zoo-

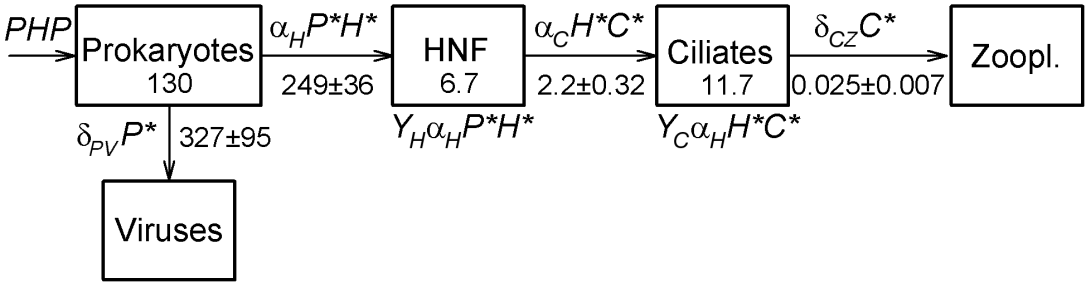

Fig. 4. Flow structure of the model used for analyzing carbon flow over the mesopelagic layer (110–1000 m depth layer) at the DYFAMED site (Tanaka et al. 2005). The model consists of viruses, prokaryotes, heterotrophic nanoflagellates (HNF), ciliates, and zooplankton. Because of the limited knowledge on trophic interactions in the mesopelagic plankton community, a simple food chain, in which only prokaryotes have 2 loss processes, was assumed. *: steady state concentrations of predator and prey; *P*, *H*, *C*: biomass of prokaryotes, HNF, and ciliates, respectively; *PHP*: prokaryotic heterotrophic production; α: predator's clearance rate for prey; *Y*: predator's yield on prey; δ: prey's specific mortality rate. $\alpha_C = 0.1\ \alpha_H$, $Y_C = Y_H$ was assumed. Values in each box denote annual mean biomass (mol C m^{-2} yr^{-1} from Tanaka & Rassoulzadegan 2002) and those between boxes denote estimate carbon flow (mmol C m^{-2} yr^{-1} from Tanaka et al. 2005). *PV*: specific mortality of prokaryotes by viral infection; *CZ*: specific mortality rate of ciliates by zooplankton

plankton in the mesopelagic layer (Tanaka et al. 2005). In the idealized food chain (Fig. 4), the number of equations required for describing the steady state can be narrowed down to 2, in which the data on microbial biomass and PHP were used to estimate 3 process parameters by linear regression analysis. Estimated specific clearance rate (±SE) of HNF for prokaryotes in the mesopelagic depth layer (0.0007 ± 0.0001 l nmol C^{-1} d^{-1}) was equivalent to 8.9 ± 1.3 nl HNF^{-1} h^{-1}, using the mean cell volume at the DYFAMED site (Tanaka & Rassoulzadegan 2002) and the carbon to volume conversion factor of 183 fg C μm^{-3} (Caron et al. 1995). The result falls within the upper part of the range (1 to 11 nl HNF^{-1} h^{-1}) reported from the Sea of Japan based on the measurement of uptake rates of fluorescently labeled prokaryotes by HNF down to 500 m (Cho et al. 2000). HNF growth efficiency on prokaryotes was estimated to be 1.12 ± 0.15% (Tanaka et al. 2005). This estimate is below the reported range (4 to 49%) for flagellates under variable experimental conditions (e.g. temperature and prey concentration) (reviewed by Caron & Goldman 1990) and much lower than the estimated PGE values (19 to 39%) in the mesopelagic layer. This suggests that the prokaryotic ingestion by HNF functions as a remineralization rather than an energy transferring process to higher trophic levels in the mesopelagic layer at the DYFAMED site.

A back-calculation, using the estimated parameters and the annual mean of integrated biomass, suggests that 40 to 48% of prokaryotic mortality is due to HNF ingestion and the rest is due to viral lyses in the mesopelagic layer (Fig. 4). This, however, may be contrary to a suggestion that virus-induced mortality of prokaryotes is low (3 to 6%) in the mesopelagic and bathypelagic layers at the same site (Weinbauer et al. 2003). A similar magnitude of vertical decrease between HNF and viruses (Tanaka & Rassoulzadegan 2002, Weinbauer et al. 2003) may suggest that the prokaryotic mortality by HNF and viruses decreases similarly over the mesopelagic layer at the DYFAMED site. PHP transferred to ciliates and zooplankton was estimated to be low (0.36 to 0.43% and 0.039 to 0.0046%, respectively). This suggests a distance within the trophic link between the group of microbial components including viruses, prokaryotes, and HNF and that including ciliates and zooplankton over the mesopelagic layer at the DYFAMED site.

Increase in the number of trophic levels generally results in less efficient material transfer from a lower trophic level to a higher trophic level or more efficient remineralization of organic carbon in the food web, the outcome of which has been addressed as a function of the microbial loop in the epipelagic layer (Azam et al. 1983, but see also Thingstad et al. 2005). A similar generalization may be applied to the case found in the mesopelagic layer, where viruses, prokaryotes, HNF, ciliates, and zooplankton are all present to constitute the mesopelagic plankton food web. The model analysis by Tanaka et al. (2005) suggests that mesopelagic PHP is allocated either almost equally to a DOC–prokaryote–virus circuit (cf. viral loop: Bratbak et al. 1990) and a DOC–microbial loop circuit, or 1.5 times greater to the former than the latter, and that HNF are potentially important remineralizers of the organic carbon produced by mesopelagic prokaryotes.

CONCLUSIONS

In the NW Mediterranean Sea, it has been shown that: (1) the depth-dependent decrease in abundance and biomass is greater for protists than for prokaryotes; (2) the dominance of prokaryotes in terms of microbial biomass increases with depth; (3) the integrated biomass of protists in the mesopelagic layer is as great as that in the epipelagic layer; (4) turnover times of prokaryotic biomass at the DYFAMED site are longer than those in most other oceanic regions; (5) production of mesopelagic prokaryotes is governed by both bottom-up and top-down controls; (6) the currently approximated PGE values suggest that mesopelagic prokaryotes are not simply remineralizers of organic carbon, and that PHP supports the production of organisms in higher trophic levels; and (7) the analysis using a simple food chain model suggests that HNF may be important remineralizers consuming the organic carbon produced by prokaryotes, and that the viral loop may enhance remineralization of organic carbon by reducing the transfer of POC from prokaryotes to higher trophic levels in the mesopelagic layer.

Future studies, however, need to clarify a multitude of uncertainties associated with the parameters used for estimating organic carbon flux and biomass and activity of the microbial components in the mesopelagic layer. Compared to the data on distribution, diversity, and activity of prokaryotes in the mesopelagic layer, data on viruses and protists in the mesopelagic layer are currently very limited. Moreover, very little is known about predator–prey and virus–host relationships among the microbial components constituting the mesopelagic plankton food web. Although the importance of sinking particles and the activity of particle-attached microbial community in the organic carbon flux is known (e.g. Alldredge & Silver 1988, Cho & Azam 1988, Turley & Mackie 1994), the structure and function of the microbial food web in and around such hot spots remains unclear (cf. Azam 1998). The simple food chain model used by Tanaka et al. (2005) (see Fig. 4) does not take particle-attached microbial communities into account because of the

lack of such data. Zooplankton (e.g. appendicularians and salps) that can specifically consume particles as small as prokaryotes may reduce the distance between the microbial loop and zooplankton within the conceptual food web by making a shortcut between the two. The observation of pellet fluxes at 500 m at the DYFAMED site suggests the presence of mesopelagic appendicularians (Carroll et al. 1998), which were the dominant macrozooplankton around 400 m near the DYFAMED site (Laval et al. 1989). The biogeochemical cycling of elements is the result of element transfers between biological and non-biological compartments. To better to understand the structure and function of the mesopelagic plankton food web and its contribution to the biogeochemical cycling of elements in the mesopelagic layer, one of the great challenges is to quantify the interactions (e.g. predator– prey and virus–host relationships) among the microbial components constituting the mesopelagic plankton food web.

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