Biogeosciences Discussions, 1, 413–428, 2004
www.biogeosciences.net/bgd/1/413/
SRef-ID: 1810-6285/bgd/2004-1-413

Biogeosciences Discussions

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Quantifying the structure of the mesopelagic microbial loop from observed depth profiles of bacteria and protozoa

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Received: 4 August 2004 – Accepted: 16 August 2004 – Published: 19 August 2004

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Abstract

It is widely recognized that organic carbon exported to the ocean aphotic layer is significantly consumed by heterotrophic organisms such as bacteria and zooplankton in the mesopelagic layer. However, very little is known for the trophic link between bacteria and zooplankton or the structure of the microbial loop in this layer. In the northwestern Mediterranean, recent studies have shown that viruses, bacteria, heterotrophic nanoflagellates, and ciliates distribute down to 2000 m with group-specific depth-dependent decreases, and that bacterial production decreases with depth down to 1000 m. Here we show that such data can be analyzed using a simple steady-state food chain model to quantify the carbon flow from bacteria to zooplankton over the mesopelagic layer. The model indicates that a similar amount of bacterial production is allocated to viruses and heterotrophic nanoflagellates, and that heterotrophic nanoflagellates are the important remineralizers.

1 Introduction

The current view of ocean biogeochemistry is that organic carbon (OC) exported from the euphotic layer is mostly remineralized in the mesopelagic layer, otherwise considered buried in the ocean interior (e.g. Fowler and Knauer, 1986). While sinking particulate organic carbon (POC) is consumed by particle-attached bacteria and detritivorous zooplankton during the sinking process (Martin et al., 1987; Cho and Azam, 1988; Smith et al., 1992), dissolved organic carbon (DOC), which is exported from the euphotic layer or released from sinking POC, is accessible only for free-living bacteria. However the trophic link between bacteria and zooplankton, i.e. the structure of the microbial loop, is unknown in the mesopelagic layer, by which our understanding of biological process in global material cycling may be limited.

Recent studies in the northwestern Mediterranean reported vertical and seasonal variations in abundance of viruses, bacteria, heterotrophic nanoflagellates (HNF), and

ciliates, and of bacterial production, in the aphotic layers down to 2000 m (Harris et al., 2001; Tamburini et al., 2002; Tanaka and Rassoulzadegan, 2002; Weinbauer et al., 2003). In addition, Tanaka and Rassoulzadegan (2004) showed that bacteria at 500 m were controlled by both bottom-up (substrate) and top-down (predation) controls. In this paper, we analyzed carbon flow in the mesopelagic microbial loop-zooplankton, using the published data combined with a simple steady-state food chain model. Results indicated that (1) a similar amount of bacterial production is allocated to viruses and HNF, and (2) HNF are the important remineralizers.

2 Study site

The data used in this study were obtained at the French-JGOFS time-series station DYFAMED (43°25.2' N, 07°51.8' E; 2350 m max. depth) in the northwestern Mediterranean, and have been published in Tanaka and Rassoulzadegan (2002, 2004, see also for detailed description of materials and methods). This site is likely independent of anthropogenic and natural dust inputs (Marty et al., 1994; Ridame and Guieu, 2002) and receives very weak lateral flows (Béthoux et al., 1988; Andersen and Prieur, 2000). Water temperature is always ca. 13°C below seasonal thermocline down to 2000 m during the stratified period and in whole water column during the mixing period, and no permanent pycnocline exists (Marty, 2003). This site shows contrasted seasonal patterns of water column structure and biological production in the upper layer (Marty and Chiavérini, 2002), with the consequence that sinking POC fluxes are higher from January to June and smaller from July to December (Miquel et al., 1993, 1994) and that DOC is accumulated in the surface mixed layer during the stratified period and exported during the winter mixing period (Copin-Montégut and Avril, 1993; Avril, 2002). Annual fluxes of sinking POC and exported DOC in the mesopelagic layer (100–1000 m) are estimated to be 0.4 mol C m^{-2} yr^{-1} and 1–1.5 mol C m^{-2} yr^{-1}, respectively (Copin-Montégut and Avril, 1993; Miquel et al., 1994; Avril, 2002). These fluxes correspond to 75% and ~100% of those from the euphotic layer. It is reported

that sinking POC was consumed by detritivorous zooplankton (Carroll et al., 1998) and particle-attached bacteria (Turley and Stutt, 2000) during the sinking process at the same site.

3 Model

Our knowledge of the structure and function of the microbial loop is quite limited for the aphotic layer due to the scarcity of direct measurements of biomass and rate process. If one assumes a food-web structure for carbon flow, and combines this with the assumption of an approximate steady-state over the depths (Thingstad, 2000), the data on biomass of microbial heterotrophs and bacterial production can be used to estimate carbon flows between microbial components and zooplankton over the mesopelagic layer (hereafter 110-1000 m). Under the assumption that the mesopelagic food web consists of viruses, bacteria, HNF, ciliates and zooplankton and that only bacteria have two loss processes (viruses and HNF) (Fig. 1), the trophic interaction can be described as:

$$BP = \alpha_H B^* H^* + \delta_{BV} B^* \quad (1)$$

$$Y_H \alpha_H B^* H^* = \alpha_C H^* C^* \quad (2)$$

$$Y_C \alpha_C H^* C^* = \delta_{CZ} C^*, \quad (3)$$

where the asterisk denotes steady state biomass (nmol C L^{-1}) of predator and prey, V, B, H, C and Z are viruses, bacteria, HNF, ciliates and zooplankton, respectively, BP is bacterial production (nmol C L^{-1} d^{-1}), α is predator's clearance rate for prey (L nmol C^{-1} d^{-1}), Y is predator's yield on prey (no dimension), and δ is prey's mortality rate (d^{-1}) by viruses or predators. Arranging the Eqs. (1) and (2) gives:

$$BP/B^* = \alpha_H H^* + \delta_{BV} \quad (4)$$

$$BP/C^* = (\alpha_C / Y_H)H^* + (\alpha_C \delta_{BV})/(Y_H \alpha_H). \quad (5)$$

Because the biomass of HNF and ciliates was not measured simultaneously with bacterial production (see Tanaka and Rassoulzadegan, 2002, 2004), we used annual mean values in biomass of bacteria, HNF and ciliates obtained in 1999–2000, but the depths correspond to those of bacterial production. Two parameters (α_H, δ_{BV}) are directly estimated by linear regression analysis with the data of H^* and BP/B^*. The other parameters (α_C, Y_H and Y_C) can be derived by different ways, depending on the assumptions made. Growth yield is considered variable with environmental conditions, and no data are available for the mesopelagic layer. Clearance rate is assumed to be a function of prey density. It is reported that clearance rate of bacterivorous HNF was ~10 times greater than that of ciliates preying on nanoplankton (Fenchel, 1987). Under the assumption of $\alpha_C = 0.1\ \alpha_H$, Y_H is then calculated by α_C/Y_H or $(\alpha_C \delta_{BV})/(Y_H \alpha_H)$, which were 0.012 and 0.009, respectively. Since the estimated Y_H values were quite similar, the former, which was derived from α_C/Y_H, was used for estimating carbon flow in the microbial loop. Y_C was arbitrarily assumed equal to Y_H.

4 Results and discussion

Tanaka and Rassoulzadegan (2002) demonstrated that bacteria, HNF and ciliates were always detected throughout the water column during an annual study, with one, two and three orders of magnitude of depth-dependent decrease (5–2000 m), respectively, at the DYFAMED site (Fig. 2). Under the assumption that the food web was close to steady state, this suggests that rate processes (i.e. growth and loss rates) are less variable for bacteria than for protozoa over the depth, and that the density-dependent predator-prey relationship becomes less coupled between three microbial heterotrophs with increasing depth down to 2000 m. While bacterial biomass and production showed depth-dependent decreases over the 110–1000 m layer, both parameters were seasonally variable down to 300 m and 500 m, respectively, likely due to seasonal variations in

OC supply to these depths (Tanaka and Rassoulzadegan, 2004). In the 1000–2000 m, bacterial production showed seasonal variations but did not decrease with depth (Tamburini et al., 2002).

Controlling factors on bacteria at 500 m were assessed by the comparison in changing rate of bacterial abundance in different treatments, which was designed to identify effects of predation and substrate availability on bacteria at 500 m, and to evaluate relative difference in substrate availability between 500 m and 110 m (Fig. 3). Results suggest that bacteria at 500 m were controlled by both bottom-up and top-down controls and that the availability of dissolved organic matter was seasonally variable (Tanaka and Rassoulzadegan, 2004).

Significant linear relationships in both Eqs. (4) and (5) suggest that the assumed food-web structure approximates the microbial loop in the mesopelagic layer of the study site (Table 1; Fig. 4). HNF growth efficiency on bacteria was estimated to be 1%. Since the study site is likely semi-enclosed, annual bacterial growth efficiency has been estimated to be 19–39% for the same layer by using the data of OC flux and annual bacterial production (Tanaka and Rassoulzadegan, 2004). This suggests that the bacterial ingestion by HNF functions as remineralization rather than energy transfer to higher trophic levels.

A back-calculation, using the estimated parameters and the annual mean of integrated biomass of bacteria, HNF and ciliates, suggests a similar role of viruses and HNF in bacterial mortality in the mesopelagic layer (Table 2). Note that effects of viruses and HNF on bacteria are functionally to produce dissolved and particulate organic matter, respectively, in the food web. Of total bacterial production that is equivalent to bacterial mortality, 0.4% and 0.004% are transferred to ciliates and zooplankton, respectively, which suggests a distance of the trophic link between “viruses, bacteria and HNF” and “ciliates and zooplankton”. This however may be contrary to a recent suggestion that virus-induced mortality of bacteria is low (3–6%) in the mesopelagic and bathypelagic layers at the same site (Weinbauer et al., 2003). Because the empirical model to estimate virus-induced mortality of bacteria is not derived from

mesopelagic and bathypelagic layers and data on virus-induced mortality of bacteria in the ocean aphotic layer have been limited to this study site (Weinbauer et al., 2003), it may be difficult to validate these estimates at present. Our estimate of clearance rate of HNF for bacteria is within the range reported for the upper 500 m of the East Sea (Cho et al., 2000), by using the mean cell volume of HNF 20 μm^3 $cell^{-1}$ (Tanaka and Rassoulzadegan, 2002) and a carbon to volume conversion factor 183 fg C μm^{-3} (Caron et al., 1995). Conceptually, specialized zooplankton (e.g. appendicularians and salps) that can consume particles as small as bacteria may reconcile this discrepancy by making a shortcut between the microbial loop and zooplankton. The observation of pellet fluxes at 500 m at the study site suggested the presence of mesopelagic appendicularians (Carroll et al., 1998), which were dominant in the macrozooplankton community around 400 m near the study site (Laval et al., 1989). Due to the paucity of data on zooplankton distribution and feeding, effect of such specialized zooplankton on our model remains to be open. Precision in our estimates of carbon flow may have suffered from the relatively low precision in microscope-based biomass estimates.

Increase in number of trophic levels generally results in less efficient material transfer from lower to higher trophic levels or more efficient remineralization in the food web, which has been addressed as a function of the microbial loop in the euphotic layer (Azam et al., 1983). This context may be reflected in the mesopelagic layer, where all microbial heterotrophs and zooplankton exist and constitute the mesopelagic food web. Our model analysis suggests that half of the mesopelagic bacterial production goes to "DOC-bacteria-viruses" circuit and the rest is "DOC-microbial loop" circuit with HNF as the important remineralizers. This may challenge to the current view of ocean biogeochemistry with bacteria as the principal remineralizers in the mesopelagic layer (Cho and Azam, 1988; Smith et al., 1992). Our results thus urge to measure not only bacterial but also other biological rate processes in this layer.

Acknowledgements. This work was supported by the EC through contracts EVK3-CT-1999-00009 (Cycling of Phosphorus in the Mediterranean) and EVK3-CT-2001-00049 (Detection and Analysis of Nutrient Limitation) and by the EU through contract HPRI-1999-CT-00056 (Bergen Marine Food Chain Research Infrastructure).

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Table 1. Estimated parameter values in Eqs. (4) and (5) based on a linear regression model I.

Variables (X, Y)	Model	Slope (±SE)	Y-int. (±SE)	n	r^2	F	Significance F-test
H^*, BP/B^*	$Y = \alpha_H X + \delta_{BV}$	0.0007 ±0.0001	0.0069 ±0.002	29	0.469	24.5	<0.0001
H^*, BP/C^*	$Y = (\alpha_C/Y_H)X + (\alpha_C\delta_{BV})/(Y_H\alpha_H)$	0.0061 ±0.0018	0.0733 ±0.0244	29	0.291	11.5	0.0021

Table 2. Carbon flow estimates for the mesopelagic layer (110–1000 m), which are based on the estimated parameters of α_H (0.0007), α_C (0.00007), δ_{BV} (0.0069). Data on annual mean biomass are from Tanaka and Rassoulzadegan (2002).

	Mean biomass (mmol C m^{-2})	Carbon flow (mmol C m^{-2} yr^{-1})
Bacteria	130	327 (to viruses) 249 (to HNF)
HNF	6.7	2.2 (to ciliates)
Ciliates	11.7	0.03 (to zooplankton)

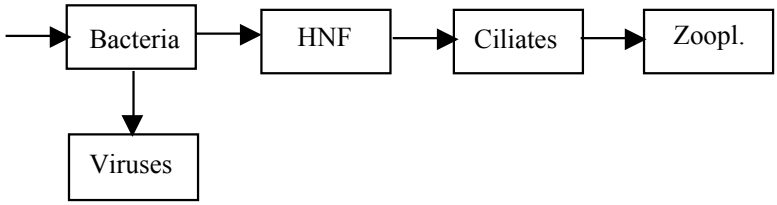

Fig. 1. Flow structure of the model used for analyzing carbon flow in the mesopelagic layer of the northwestern Mediterranean. The model consists of viruses, bacteria, HNF (heterotrophic nanoflagellates), ciliates, and zooplankton.

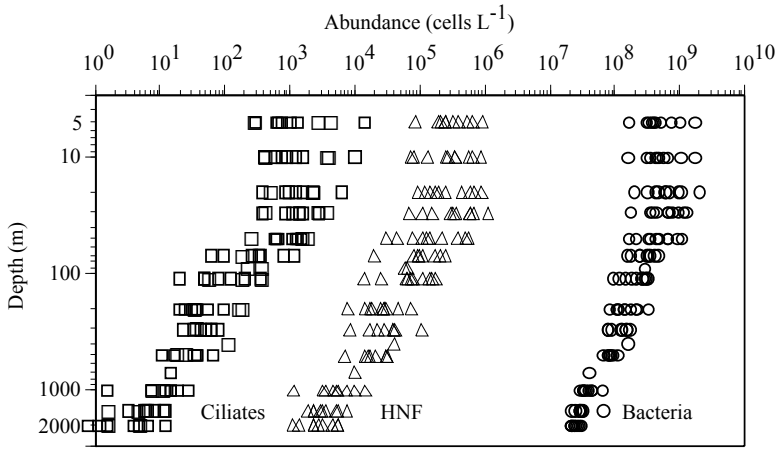

Fig. 2. Distributions of bacteria, heterotrophic nanoflagellates (HNF) and ciliates. Measurements were monthly done at 13 depths between 5 and 2000 m from May 1999 to March 2000 at the DYFAMED site (Redrawn from Tanaka and Rassoulzadegan, 2002). Circles, triangles and squares denote bacteria, HNF and ciliates, respectively.

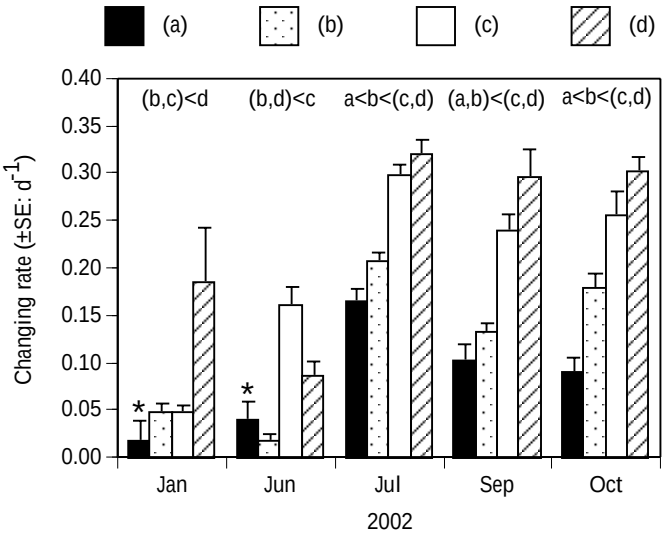

Fig. 3. Effects of substrate availability and predation on mesopelagic bacteria. Measurements were done from January to October 2002 at the DYFAMED site (Data from Tanaka and Rassoulzadegan, 2004). Four different treatments were prepared, and bacterial abundance was measured at the start and the end of the incubation from separate triplicate bottles. Shown are mean changing rates ± standard errors (day^{-1}) of bacterial abundance. Bars denote whole water from 500 m **(a)**, filtrate of 0.8 μm from 500 m **(b)**, filtrate of 0.8 μm from 500 m diluted by filtrate of 0.2 μm from 500 m **(c)**, and filtrate of 0.8 μm from 500 m diluted by filtrate of 0.2 μm from 110 m **(d)**. Changing rates were calculated by semi-log linear regression (n=6 except Treatment b in January, n=5). Comparison between treatments were shown (p <0.05, t-test). Asterisks denote insignificant changes of bacterial abundance during the incubation (p >0.05, t-test).

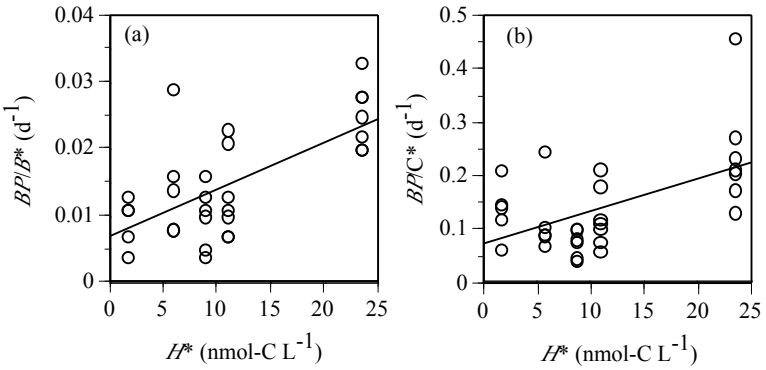

Fig. 4. Rate estimations by fitting the model-derived equations. **(a)**: Relationship between BP/B^* and H^*, **(b)**: Relationship between BP/C^* and H^* in the 110–1000 m. The lines were calculated with a linear regression model I.