

## **Anonymous Referee #2**

We thank this reviewer for his/her comments, which have helped us in improving our manuscript.

**1. I believe that the authors have ignored some important factors in their assessment of their results. The most significant, perhaps, is the lack of acknowledgement, up to the front, that DOCp, as measured by  $^{14}\text{C}$ -DOC released from phytoplankton, will not only represent exudation of photosynthate from healthy cells, but will include DOC released as a result of cell death and disintegration through grazing and viral lysis for example. How is that distinguished from exudates using this protocol.**

As properly stated, what we measure with the  $^{14}\text{C}$  protocol of DOCp is the appearance in dissolved form of the labeled C that has recently been fixed intracellularly, which besides being exudates from phytoplankton cells, could also be the result of cell breakage or grazing processes such as egestion or sloppy feeding (Nagata, 2000). It is not possible with our protocol to distinguish the various mechanisms that originate  $\text{DO}^{14}\text{C}$ . However, previous studies with kinetic experiments had shown that production and accumulation of this dissolved organic carbon starts immediately after the experiment begins, increases linearly during light hours, and stops once the dark period begins. We believe that if the main mechanisms involved in DOC release were related to grazing processes,  $\text{DO}^{14}\text{C}$  accumulation should continue to accumulate during the whole incubation period.

**2. The other main point is the statement that bacterial carbon demand seems to exceed the DOCp. However, DOCp here is a net measurement, after 24 hours incubation. This would affect the estimated rate of DOCp due to consumption of DOCp by, presumably heterotrophic bacteria. Have you ever looked at the DOCp just after the end of the light period or in time course incubations to assess the potential loss of DOCp by heterotrophic consumption?**

We don't have data from kinetic experiments for BOUM cruise. However, the BCD estimates were on average 6- to 14-fold higher than DOC<sub>p</sub> rates, therefore we believe that even though we report net rates the main conclusion remains the same.

**3. Also, there is no mentioning of the size of the ambient DOC pool as reference to BCD. What turnover times would that pool have (I presume long)?**

We have to keep in mind that most of the DOC pool is refractory, and what we measure (DOC<sub>p</sub>) is a small fraction of the labile (or semilabile if we consider that it tends to accumulate in surface waters during the summer period) part, which in turn represents a small percentage of the whole DOC pool. Due to the lack of information on the rates of production of these other longer-residence time, DOC pools, we are not able to provide the turnover time of DOC.

**4. If the DOC exudate, as suggested, is of low quality in P-limited environments, would that not increase BCD? i.e. increase respirations, and decrease BGE, and hence draw down the more of the DOC pool?**

There is experimental evidence that suggest that not only the availability of dissolved organic matter but its nature might be a key issue in regulating bacterial metabolism. Puddu et al. (2003) showed that all metabolic rates (i.e. growth, production and respiration) were significantly lower on bacterioplankton cells fed on P-depleted exudates of the diatom *Cylindrotheca closterium* than on cells fed on P-balanced DOC. In Mediterranean waters, especially under summer conditions, it has been suggested that not only phytoplankton cells but also bacteria are P-limited, and due to a low assimilation of organic material when P is not available for bacterial metabolism, an accumulation of the dissolved organic substrates might occur in surface waters of the Mediterranean Sea (Thingstad and Rassoulzadegan, 1995) and some other oligotrophic areas (Karl et al., 1998).

**5. On the algorithms to compute BGE (equations 1 and 2), add a brief explanation to what the different factors are in these. For example what does the 1.8 represent in eq.1? Also, what limitations are associated with these two approaches.**

With the aim to explore the importance of DOCp as source of organic material to sustain bacterial consumption, we compare our DOCp data with bacterial carbon demand rates (BCD). In order to obtain this parameter, two different models were used to get the bacterial growth efficiency (BGE). The first model used  $[BGE = (0.037 + 0.65 BP) / (1.8 + BP)]$ , was obtained by fitting bacterial production rates (BP) and BGE, from a data set of 237 paired observations of bacterial respiration (BR) and BP data taken from the literature (del Giorgio and Cole, 1998). The equation represents a rectilinear hyperbole with a fixed lower limit.

The second equation ( $BGE = 1 - [1 / (0.727 \times [Chl-a / (Chl-a + 4.08)] + 1.02)]$ ) describes the dependence of BGE on resource availability by using the chlorophyll concentration (Chl-a) as a proxy (López-Urrutia and Morán, 2007). The model was obtained from a large data set of BP measured by the incorporation of <sup>3</sup>H-Leucine. The equation was derived from a general model proposed for bacterial metabolism, where the total carbon assimilated by a bacteria cell (BCD) depends of both temperature and resource availability according to the equation:

$$BCD = b_0 e^{-E/kT} [Chl / Chl + K_m]$$

Where  $b_0$  is a normalization constant independent of temperature and resource availability;  $e^{-E/kT}$  is Boltzmann's factor where E is the average activation energy for bacterial metabolism and k is Boltzmann's constant;  $Chl / Chl + K_m$  is the Michaelis-Menten functional response of bacterial metabolism to resource availability, where Chl serve as proxy for resource concentration. Part of the assimilated carbon is devoted to cell maintenance and respired as CO<sub>2</sub>. However, both approximations had intrinsic limitations, which directly affect the magnitude of BGE, and that are inherent to the method used to measure bacterial production (<sup>3</sup>H-leucine incorporation) in the data sets used in both studies.

**6. P 8601, ln. 10: What was the relative contribution of the larger phytoplankton components? There is no data presented here on the size, or taxon, distribution of the community along the transect. Was there any data collected on size fractionated primary production?**

We found that the relative contribution to total phytoplankton biomass, as estimated from euphotic layer-integrated Chl-a in two different size class ( $0.2-2.0 \mu\text{m}^3$  and  $>2.0 \mu\text{m}^3$ ) was 56% and 44%, respectively, and the contribution from each class remained quite constant along the longitudinal transect within the three sampled sites. We don't have data from size fractionated primary production, but it can be expected that they will follow the same pattern found for size-fractionated Chl-a data.

**7. Throughout the manuscript the wording “importance” of DOCp is used, without a qualifier as to what is important. I suggest changing this to “contribution” or “relative fraction” as “importance” seems to be used in reference to the relative amount of DOCp to POCp.**

Changed as suggested.

**8. I would have welcomed a more thorough material and method sections (despite the reference to Moutin et al.). This is especially so far for data is used in the manuscript, such as chlorophyll.**

The suggestion was followed and a more detailed description of the methods was included.

**9. P 8595, ln 10: suggest adding –For a full description... see Moutin et al (Moutin et al.).**

Changed as suggested.

**10. P 8595, ln 13: Were the six depths determined by the light level, or were they fixed depths?**

They were fixed depths.

**11. P 8595, ln 20: Did you observed temperature effects when using on-deck incubations vs. in situ, given that the on deck incubators were cooled with surface water only?**

We did not have simultaneous measurements of both incubations, therefore it is difficult to establish if there are significant differences between both kinds of incubations. However, we have alternate experiments, and we didn't find any systematic difference between one day *in situ* incubation and the on deck incubation performed at the following day.

**12. P 8596 ln 5: What brand name was the cocktail?**

Ultima Gold XR (Perkin Elmer)

**13. P 8596 Ln15: suggest stressing here that DOCp is a net measurement and that a portion of it most likely is consumed, especially during the dark period when new production should be limited.**

Done as suggested.

**P 85497, ln 6: ...“close to detection limit”... by what method?**

Samples for nitrate (NO<sub>3</sub>), nitrite (NO<sub>2</sub>) and phosphate (PO<sub>4</sub>) were analyzed using the automated colorimetric technique (Tréguer and Le Corre, 1975; Wood et al., 1967), on a segmented flow Bran Luebbe autoanalyser II. Detection limits were 0.02 μM for NO<sub>3</sub>, 0.01 μM for NO<sub>2</sub> and 0.01 for PO<sub>4</sub>. Fluorometric determination of NH<sub>4</sub> (Holmes et al., 1999) was performed on a fluorometer Jasco FP-2020. The detection limit for the procedure was 3 nM. For a full description see Pujo-Pay et al. (2010)

**P 8597, ln 17: ... “dominated mostly by”... suggest omitting “mostly” here.**

Changes were done in the text.

**P 8598, ln 1-4: Are there any error estimates in the integrated production values?**

**Your samples were in triplicates for each depth, so there should be data).**

The standard deviation is indicated as suggested.

**P8598, ln 10: Change “tree” to “ three”.**

Correction done.

**P 8598, ln 10-12: Also, why is an approximate value used sometimes, where precise values are used elsewhere when describing ranges?**

Changed accordingly.

**P 8598, ln 13: Change “tend” to “tended”**

Changed as suggested.

**P 8598, ln 17-18: Suggest rewording to “the fraction of DOCp to total primary production was relatively constant throughout the study area”**

Changed as suggested.

**P 8600, ln 1: Suggest rewording to “the consistency in the relative contribution of DOCp”**

Changed as suggested.

**P 8600, ln 3: suggest changing “taking into account all the sampled stations” to “across all sections”**

Changed as suggested.

**P 8600, ln 15-20: How do you distinguish the DOCp derived from exudates or through cell death via grazing, viral lysis etc?**

As we already mentioned elsewhere, what we measure with the  $^{14}\text{C}$  protocol of DOCp is the appearance in dissolved form of the labeled C that has recently been fixed intracellular, which could come from any grazing process or due to cell breakage. Therefore, it is not possible to distinguish between different sources.

**P 8602, ln 3: Suggest rewording: “...has resulted in that during most field studies DOCp measurements are not routinely...” to “has resulted in that routine DOCp measurements are not carried out”**

Changed as suggested.

**P8602, ln 19: Change analized to analyzed.**

Correction done.

**P 8604, ln 18: Change Hangström et al. to Hagström et al.**

Correction done.