

## **Interactive comment on “Ecology of aerobic anoxygenic phototrophic bacteria along an oligotrophic gradient in the Mediterranean Sea” by D. Lamy**

We would like to greatly thank the reviewers for their thoughtful comments on our manuscript. The comments and suggestions were much appreciated.

Both reviewers had minor comments.

Below a general reply stating about the general changes made on the ms, we answer point-by-point in the issues raised by the reviewer #1 and clearly state how we addressed the comments in the revised ms.

### **General changes**

Co-authors were added in the paper due to their relevant contribution to this work: M. Pujo-Pay, L. Oriol and V. Cornet-Barthaux for providing the nutrients and organic matter concentrations and P. Catala and L. Bariat for flow cytometry analyses.

Claude Courties is acknowledged in the revised version of the paper for his contribution to the flow cytometry analyses. Michal Koblížek is acknowledged for his help with detection of AAP bacterial colonies using the infra-red system available in his laboratory.

According to the comments of the reviewer #2, the map and its figure caption were changed to make a difference between the stations not sampled and those sampled for the assessment of the different AAP bacterial-related parameters and the experiments conducted on the effects of nutrients amendments on the AAP bacterial growth.

The order of the columns in Table 1 was changed to organize it from western (left column) to eastern (right column) Mediterranean as it was also done in the figures.

A companion paper, published in the same special issue in BGD and unveiling the diversity of cultivated and metabolically active AAP during the same Mediterranean survey has been cited in the revised version of the ms (page 4 line 63-65).

“In a companion paper, Jeanthon et al. (2011) reported the isolation of AAP bacteria and unveiled the diversity of the active AAP populations retrieved during the BOUM cruise.”

A short paragraph on the relative abundance of AAP bacteria (percent to total prokaryotes) was added in the Results section of the revised version of the ms (page 12 line 247-251).

“Relative abundance of AAP bacteria in subsurface waters did not vary substantially along the longitudinal transect, ranging from 0.4 to 0.9 % of total prokaryote abundance. The relative abundance maxima were at or just above the DCM at all the stations, reaching up to 4% in the western part (station 21 at 50 m) and less than 1% in the eastern part.”

### **Specific comments of reviewer #1**

*Page 329, Line 18: The absolute detection limit of 2 ng BChl-a L<sup>-1</sup> for the IR kinetic fluorometer was inconsistent with the BChl-a concentrations determined by HPLC. Fig. 3 showed that almost all BChl-a concentrations were < 2 ng L<sup>-1</sup> in this study area, while the authors still gave BChl-a fluorescence signals.*

This comes from a typing error. The actual detection limit of the IR kinetic fluorometer is indeed 0.2 ng BChl-a L<sup>-1</sup>, as it is indicated in Koblížek et al. (2007). It is now corrected in the revised ms (page 7 line 128).

*Page 337, Line 26: “strongly followed the depth distribution of cyanobacteria” is not supported by Fig. 4A.*

We agree and the sentence was removed from the discussion section.

*Page 339, Line 1-3: Why the BChl-a quotas in the Mediterranean Sea are much higher than the values observed in the previous studies? The authors suggested that reliance on phototrophy varied along the oligotrophic gradient and that nutrient and/or carbon limitation favors BChl-a synthesis in natural communities. So the authors should explain whether the Mediterranean Sea is more oligotrophic than the South Pacific Ocean, the Sargasso Sea, and the Mid-Atlantic Bight in the previous studies?*

The comparison of our environmental data with those shown in the previous studies highlights that the Mediterranean Sea is more oligotrophic than the South Pacific Ocean, the Sargasso Sea, and the Mid-Atlantic Bight.

Indeed, higher concentrations of phosphate (0.13 - 0.34  $\mu\text{M}$ ), nitrate (below the detection limit - 3.05  $\mu\text{M}$ ) and chlorophyll *a* (0 - 0.27  $\mu\text{g L}^{-1}$ ) were found in the South Pacific Ocean (Lami et al., 2007) compared to those observed in the Mediterranean Sea during our study.

The chlorophyll *a* concentrations in the Sargasso Sea (Sieracki et al., 2006) ranged from 0.09 to 2.83  $\mu\text{g L}^{-1}$  and were higher than the concentrations we detected.

Cottrell et al. (2006) reported also higher concentrations of nitrate + nitrite (0.18 - 2.7  $\mu\text{M}$ ), phosphate (0.07 - 0.39  $\mu\text{M}$ ) and chlorophyll *a* (0.04 - 0.25  $\mu\text{g L}^{-1}$ ) in the Mid-Atlantic Bight (Gulf Stream) than in the Mediterranean Sea.

According to the comment of the reviewer, we added a short comment in the discussion section of the revised paper (page 18 line 396-399).

“Nutrients and Chl-*a* concentrations measured during the BOUM cruise suggest that the oligotrophic conditions experienced by AAP bacteria during the stratification period of the Mediterranean Sea were more severe than that observed in the three latter oligotrophic regions.”

*Page 339, Line 21-25: This part is not easy to be understood. The authors should give a clearer discussion on the link between the hypothesis and the observation by this study.*

We agree and have changed this discussion part to be clearer (page 18-19 line 414-427).

”On the basis of our nutrient-addition experiments, we cannot speculate about P-limitation of AAP bacteria since growth of AAP and total prokaryotes were not stimulated by P addition, although higher heterotrophic bacterial activity was observed. Consistent with our results, Tanaka et al. (2011) did not find any indication of P-alone limitation although a clear P-starved status in the Mediterranean basins was observed. Moreover, we observed that net growth of AAP bacteria was enhanced by glucose and nitrogen additions in the eastern basin whereas net growth of total prokaryotes was not stimulated by these additions. This result does not favor the hypothesis that light-derived energy could serve for supporting the nutrient acquisition in a nutrient-depleted environment. The problem may be in trying to deduce the impact of the additions by measuring net growth, which is a function of the bacterial response and mortality factors. However, AAP bacteria and total prokaryotes responded differently in terms of net variation of their abundances, which suggest different growth and mortality controls between these two populations.”

*Technical corrections: Page 326, Line 24: Should be “A few”. Page 334, Line 24: Should be “6.90 x 10<sup>5</sup> cells ml<sup>-1</sup>”.*

These editing corrections were made in the revised ms.