

## ***Interactive comment on “Consequences of respiration in the light on the determination of production in pelagic systems” by O. Pringault et al.***

**O. Pringault et al.**

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### **Replies to Referee 2**

In order to improve the reading and avoid the puzzling description pointed out by the referee, the results and discussion have been modified to present the main results of the study in a clearer manner.

### **Comment 1: Coupling between autotrophic and heterotrophic compartments.**

The coupling between autotrophs and heterotrophs is generally a function of the DOM excreted by the autotrophs which in turn can fuel the respiration activity of the heterotrophs. The composition of exudates can vary as a function of the phytoplankton

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composition which is itself dependent on nutrient supply. In our opinion, it is therefore essential to take into account this coupling for the determination of metabolic processes in natural communities and as stated in the manuscript the traditional Winkler technique used to estimate GPP from NP and R measurements clearly considers this coupling as negligible.

**Comment 2: NP in absolute values greater than  $R_{dark}$  resulting in physiologically impossible negative values of GPP.**

As explained in the manuscript, NP represents the difference between GPP and R. Therefore, if NP in absolute values is greater than  $R_{dark}$  in absolute values, the traditional calculation (Winkler technique) that assumes R in the light equivalent to that in the dark will result in a negative value for GPP. This is of course impossible although as stated in the manuscript, reports of negative values of GPP have been already published. In our opinion, NP values greater (in absolute values) than  $R_{dark}$  provides clear evidence that respiration in the light can not be considered as equivalent to that in the dark.

However, as both reviewers found this part of the manuscript confusing we have improved our explanation in the results and the discussion section to better describe the methodology employed to estimate  $R_{light}$  when NP and  $R_{dark}$  rates resulted in negative values for GPP. We fully agree with the referee regarding the fact that respiration in the light is a function of community composition. In order to improve clarity in the revised version, we have improved the description of Grande et al (1989), already cited in the manuscript (see page 8 lines 19-22). In this study, respiration in the light was estimated for several naturally abundant marine phytoplankton species. Most of the species, including diatoms and cyanobacteria (*Synechococcus* sp) exhibited  $R_{light}$  greater than  $R_{dark}$  with values up to 10-fold those measured in the dark (*Synechococcus* sp). The study of Grande et al (1989) clearly shows that respiration in the light is at least equal to the value measured in the dark and that for most phytoplankton species,  $R_{light}$  is largely greater than  $R_{dark}$ . Moreover, this study was performed using

axenic phytoplankton cultures, which therefore excludes the respiration activity from the heterotrophic community that occurs in natural environments. Heterotrophic respiration can significantly contribute to the respiration in the light in natural systems by degrading organic compounds synthesized by phytoplankton.

**Comment 3: Determination of  $R_{light}$  from the dark period.**

We fully agreed with the referee. As previously indicated in the methods section, the methodology employed does not directly measured respiration in the light, however as described by Falkowski et al (1985), cited in the manuscript, consequences of light exposure on respiration can still be measured few minutes after the onset of darkness. To improve clarity and understanding, we have included, at the beginning of the discussion, a comment on the methodology employed. Moreover, we also refer to the previous work of Weger et al (1989) that confirmed the conclusion of Falkowski et al (1985) by using the stable  $^{18}\text{O}$  isotope technique to estimate  $R_{light}$ .

**Comment 4: Comparison with the stable  $^{18}\text{O}$  isotope technique.**

Comparison with this technique was already made in the previous version (discussion section p1375 lines: 16-20), however, to improve readability the paragraph has been rewritten by firstly comparing our  $R_{light}$  measured in natural field samples with other natural estimates and then by comparing our  $R_{light}$  estimates with values measured in phytoplankton cultures (see page 8 lines 5-22).

**Comment 5: Presentation of highly unexpected results which is not discussed.**

The data presented in figure 2B is actually a clear demonstration of the strong coupling between production and respiration resulting in a physiologically negative value of  $\text{GPP}$  if  $R$  in the light is assumed to be equivalent to that in the dark. The results presented in figure 2B are discussed in the previous version (discussion page 1376, lines 22-29). We fully agreed with the referee that this result can be considered as unexpected since monitoring of  $\text{O}_2$  in natural field water samples to estimate metabolic processes

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are relatively rare in literature. However, under net heterotrophic conditions ( $NP < 0$ ), a stimulation of  $R$  in the light can lead to a stronger decrease in oxygen during the light incubation relative to the dark incubation. Therefore, since the phenomenon described in figure 2B and observed in some occasions under net heterotrophic conditions (see table 1) is evidence of a light stimulation of  $R$ , we have modified the results and the discussion sections to improve clarity and understanding in order to better describe this "unexpected" result. See reply to comment 2.

#### **Comment 6: respiration rates oxygen dependent.**

Oxygen consumption can of course be limited by oxygen supply, however,  $K_m$  values for  $O_2$  estimated in natural field samples are around 1-10  $\mu M$  (see Epping et al L&O 1999, 44:1936-1948), values that are far lower than those observed in our study which are close to saturation level.

#### **Comment 7: Is a fast responding microsensor really needed?**

Since the methodology employed focused on the few minutes consecutive to darkness, a fast-responding microsensor provides a more accurate estimation of  $R_{light}$  by providing a larger number of data which allows a better understanding of the oxygen changes during the transient light to dark state. A compromise between stability of the signal and fast response can be obtained by designing an oxygen sensor with a high signal but that are still fast responding (less than 1 sec). Furthermore, the most important thing is to have an oxygen sensor that does not consume oxygen. For this purpose, an oxygen sensor should be designed as described by Revsbech (1989 L&O 34:474-476) with a guard cathode which greatly reduces  $O_2$  consumption by the measuring electrode. This paper is cited in the manuscript.

#### **Replies to Referee 1**

As suggested by referee 1 the methods and the results sections have been rewritten to improve clarity and avoid repetition.

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## Material and methods.

The incubation procedure has been better explained by giving the water temperature conditions (see table 1) as suggested by Referee 1. We also included a paragraph about the reproducibility of the method (see page 3 lines 22-27)

## Result section.

The results section has been modified to avoid repetitions pointed out by the referee. Furthermore, to improve clarity, we have added titles to the different results sections.

### Page 1372 lines 16-21.

The paragraph has been rewritten.

### Page 1372 line 21. NP greater in absolute values than $R_{dark}$ .

See reply to comment 2 of Referee 2.

### Page 1372 line 25. What does in situ hourly rates mean?

We agree with the referee, the term "in situ" was confusing since incubations were performed under laboratory conditions. We have deleted the term in the revised version.

### Page 1373 variable PFD.

The effects of a variable PFD on  $R_{light}$  has been estimated from measurements performed with a phytoplankton culture (natural mixed assemblage). In the revised version we now explain how this calculation was made (see page 6 lines 6-13). We fully agree with the referee that the assumption of a fixed PFD over 12 hours should be subject to caution. However, the light incubations were performed under saturating light conditions, conditions that are prevalent during the majority of the day. Measurements conducted at the sampling sites have shown that phytoplankton photosynthesis is subject to saturating irradiances during on 80-90% of the day. Furthermore, previous studies performed with phytoplankton cultures cited in the manuscript have shown that light

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respiration was strongly coupled with photosynthesis, exhibiting maximum rates under saturating light conditions. Similarly, assuming a constant  $R_{dark}$  value over 12 hours is also subject to caution, since we show that exposure during 12 hours of light might severely affect respiration rate in the dark during the period immediately following this 12-hour light exposure. Indeed, the respiration values remain higher than those estimated prior to illumination (see figure 3). Therefore, it is unlikely that under natural conditions respiration rates remain constant in both the dark and in the light. However, obtaining a better estimation of the daily rates would require incubating for at least 24 hours. Such incubations can be problematic since it is known that, changes in both biomass and community structure are likely to occur even over the period of 24 hours, thus rendering the interpretation of such results difficult.

### Discussion section.

#### Page 1376 line 6-8.

In the revised version, we have modified the text accordingly.

#### Page 1376 line 22-24.

This part of the discussion has been rewritten. (See reply to comment 2 of Referee 2).

#### Table 1 and 2.

As now indicated in the revised version, experiments were not performed in replicates however reproducibility between two similar samples was regularly checked.

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