

Interactive comment on “Effect of light on photosynthetic efficiency of sequestered chloroplasts in intertidal benthic foraminifera (*Haynesina germanica* and *Ammonia tepida*)” by Thierry Jauffrais et al.

Anonymous Referee #2

Received and published: 17 March 2016

Author analysed the functionality of chloroplast retained by some species of benthic foraminifera. Study conducted is very interesting, and the techniques used are new and applicable to other organisms, which makes the manuscript relevant to a broad readership. However, methods section needs to be carefully revised as it does not follow a logical sequence, and experimental design needs to be explained in more detail. Moreover, manuscript needs to be proofread and revised by a native English speaker. Many problems with punctuation throughout the text.

Introduction

C1

Page 2

Ln. 19-24: Kleptoplasty is also very common in carbonate reef environments when conditions are favourable (i.e, oligotrophy; e.g., Ziegler and Uthicke 2011).

Ln. 25-28: Studies by Correia and Lee need to be acknowledged and cited here as they represent a good contribution to this research field.

Methods

Page 4

Ln. 11-16: Please provide a rationale for only exposing the specimens to different light levels for one week only.

Page 5

Experimental design: Please, clarify the total number of individual used per replicate and number of replicates per treatment.

Ln. 27-28: Clarify why *A. tepida* specimens were not starved under light conditions, and if *A. tepida* was exposed to different light conditions at all.

Also, please clarify the experimental design. Was *A. tepida* exposed to different light treatments? There is no information in the methods (where it should be). It is surprising that the authors only used one paragraph to explain their experimental design, which is the most important part of the study. There is no way for the reader to know number of replicates, total number of specimens, why conditions were chosen, how light levels were reached, temperature, static or flow-through system? Detail explanation of the experimental design is necessary.

Methods section does not follow a logic sequence when explaining each parameter analysed. This section needs to be carefully revised.

Were all specimens used in the experiment tested for all parameters analysed? Please,

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clarify.

Page 6

Ln. 10-11: Was one individual used at a time or all at once? Please, clarify.

Ln. 20-22: What about inter specific differences? Did the authors use a pool of 7-10 individuals for O₂ consumption measurements? Or the measurements were done individually?

Ln. 24: Authors stated that seven specimens were used, but previously (Ln. 10) mentioned "7 to 10 foraminifera". Please, be consistent.

Ln. 26: Please clarify why only two steps were used for *A. tepida*.

Page 7

Fluoresce measurements: What light was used to measure Fo? Please, clarify

Page 8

Ln. 15-16: It seems that the authors have a blocked design, but it hard to tell based on the current description of the experimental design. For example, if both species were put in the same experimental petri dish or not. That requires a more detailed description of the methods. Therefore, it is impossible to judge if authors conducted the appropriate statistical analyses.

Throughout the methods section author put in brackets "3x10 foraminifera". Please, clarify if this means replicates or trials per parameter analysed.

Ln. 24-25 Please add ", respectively", after "This resulted in cytoplasmic biovolumes equal to $1.20 \times 10^7 \mu\text{m}^3$ (SD = $25.39 \times 10^6 \mu\text{m}^3$) and $1.01 \times 10^7 \mu\text{m}^3$ (SD = $3.65 \times 10^6 \mu\text{m}^3$)"

Page 9

Ln. 5-6: Figure 2 only shows data on *H. germanica* fluorescence. Please, amend the
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sentence accordingly.

Ln. 15-19: The manuscript would improve if all these numbers were put in a table or graph.

Page 10

Ln. 20-22: Please, clarify why data is not shown. Maybe authors could add these results to supplementary material, if possible.

The manuscript would benefit from a figure plotting the relative difference of Fv/Fm between light treatments, specially low and high light levels.

Page 11

Ln. 7-12: Figure 4 does not show this result.

Page 12

Ln. 21-23: This is expected, given that exposure to high light levels generates a lot of reactive oxygen species inside the chloroplasts. This should be mentioned and discussed.

Ln. 21-23: *A. tepida* has no capacity to retain chloroplast according to the results, as fluoresce only persists for a couple of days, and even though some fluorescence is detected, the functionality was not analysed. Therefore, chloroplasts might be present for a couple of days, but not functional. The O₂ consumption is not a proxy of functionally of kleptoplasts, and just because respiration rates were lower at 300 μE does not mean that chloroplasts were functioning. Be careful not to mix up correlation with causation.

Ln. 28-32: This is very interesting. I wonder what caused this significant reduction in tolerance in these chloroplasts. Maybe the lack of a cellular protection? Would be great to see a sentence or two with thoughts from the authors of why such dramatic decrease. It would be possible that in situ the chloroplast are not functional at all.

Page 13

Ln. 7-9: Chloroplasts are naturally hyperoxic and, as mentioned previously, produce reactive oxygen species, which make this organelles susceptible to oxidative stress. Reactive oxygen species in the chloroplast can cause damage to PS II, primarily through oxidative degradation of essential proteins. This is important to be added to the discussion. It would explain why Fv/Fm of *H. germanica* decreases with increases in light level.

Page 15

Ln. 29-30: Please add "within the light range tested in this study": "Comparing *H. germanica* with *A. tepida* showed that the former species potentially has the 30 capacity of retaining functional kleptoplasts up to 21 days, within the light range tested in this study"

Figure 1: Please, mention the species of diatom and reference

Figure 4: Which treatment is plotted in the graph? As stated in the text, P-I curves were measured for all treatments (page 7, ln. 9-11). Please, clarify. It would be interesting to see the P-I curves of specimens exposed to all treatments.

Figure 5: Please add the letters (A, B, C and D) to the legend.

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