

Dear Dr. Middelburg,

Please find enclosed our revised manuscript entitled “**Effect of light on photosynthetic efficiency of intertidal benthic foraminifera**” by Thierry Jauffrais, Bruno Jesus, Edouard Metzger, Jean-Luc Mouget, Frans Jorissen, Emmanuelle Geslin, for submission to Biogeosciences as an original research paper.

We agree with the different comments you have done and modified the manuscript as requested.

We hope that these revisions will fulfill all the requests for publication.

Thank you for your work,
Best regards,



Thierry Jauffrais

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Comment:

- do not use forward referencing: i.e. delete Cesbron et al. (submitted) in the text, p.2, p.3.

Reply:

- Agreed and modified the forward referencing as Cesbron pers. Comm.; the reference of the submitted manuscript was also removed from the reference section.

Comment:

- p. 8, line 25: For all conditions..... F_v/F_m was measured.... period and measurements were done...

Reply:

- Agreed and modified as suggested

Comment:

- p12: line 28: local biogeochemical processes...

Reply:

- Agreed and added as suggested

Comment:

- p14, line 27: replace HL with high light

Reply:

- Agreed and modified as suggested

Comment:

- p16, line 6: why is nitrogen suddenly entering the story here?

Reply:

- Agreed the sentence about nitrogen was removed from the conclusion. It was here to highlight a section on nitrogen assimilation that has been suppressed from the manuscript during the reviewing process.

1 **Effect of light on photosynthetic efficiency of sequestered**
2 **chloroplasts in intertidal benthic foraminifera (*Haynesina***
3 ***germanica* and *Ammonia tepida*)**

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16
17 **Abstract**

18 Some benthic foraminifera have the ability to incorporate functional chloroplasts from
19 diatoms (kleptoplasty). Our objective was to investigate chloroplast functionality of two
20 benthic foraminifera (*Haynesina germanica* and *Ammonia tepida*) exposed to different
21 irradiance levels (0, 25, 70 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$) using spectral reflectance, epifluorescence
22 observations, oxygen evolution and pulse amplitude modulated (PAM) fluorometry
23 (maximum photosystem II quantum efficiency (F_v/F_m) and rapid light curves (RLC)). Our
24 results clearly showed that *H. germanica* was capable of using its kleptoplasts for more than
25 one week while *A. tepida* showed very limited kleptoplastic ability with maximum
26 photosystem II quantum efficiency ($F_v/F_m = 0.4$), much lower than *H. germanica* and
27 decreasing to zero in only one day. Only *H. germanica* showed net oxygen production with a
28 compensation point at 24 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ and a production up to 1000 $\text{pmol O}_2 \text{ cell}^{-1} \text{ day}^{-1}$
29 at 300 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$. *Haynesina germanica* F_v/F_m slowly decreased from 0.65 to 0.55

1 in 7 days when kept in darkness; however, it quickly decreased to 0.2 under high light.
2 Kleptoplast functional time was thus estimated between 11 and 21 days in darkness and
3 between 7 and 8 days at high light. These results emphasize that studies about foraminifera
4 kleptoplasty must take into account light history. Additionally, this study showed that the
5 kleptoplasts are unlikely to be completely functional, thus requiring continuous chloroplast
6 resupply from foraminifera food source. The advantages of keeping functional chloroplasts
7 are discussed but more information is needed to better understand foraminifera feeding
8 strategies.

9 1 Introduction

10 Benthic foraminifera colonize a wide variety of sediments from brackish waters to deep-sea
11 environments and can be the dominant meiofauna in these ecosystems (Gooday 1986; Pascal
12 et al. 2009). They may play a relevant role in the carbon cycle in sediments from deep sea
13 (Moodley et al. 2002) to brackish environments (Thibault de Chanvalon et al. 2015). Their
14 minor role in organic carbon cycling in aerobic sediments, compared to bacteria, contrasts
15 with their strong contribution to anaerobic organic matter mineralisation (Geslin et al. 2011)
16 and they can be responsible for up to 80% of benthic denitrification (Pina-Ochoa et al. 2010;
17 Risgaard-Petersen et al. 2006).

18 Some benthic foraminiferal species are known to sequester chloroplasts from their food
19 source and store them in their cytoplasm (Lopez 1979; Bernhard and Bowser, 1999) in a
20 process known as kleptoplasty (Clark et al. 1990). A kleptoplast is thus a chloroplast,
21 functional or not, that was "stolen" and integrated by an organism. Kleptoplastic foraminifera
22 are found in intertidal sediments (e.g. *Haynesina*, *Elphidium* and *Xiphophaga*) (Lopez 1979;
23 Correia and Lee 2000, 2002a, b; Goldstein et al. 2010; Pillet et al. 2011), low oxygenated
24 aphotic environments (*Nonionella*, *Nonionellina*, *Stainforthia*) (Bernhard and Bowser 1999;
25 Grzyski et al. 2002) and shallow-water sediments (*Bulimina elegantissima*) (Bernhard and
26 Bowser, 1999). The role of chloroplasts sequestered by benthic foraminifera is poorly known
27 and photosynthetic functions have only been studied in a few mudflat species (*Elphidium*
28 *williamsoni*, *Elphidium excavatum* and *Haynesina germanica*) (Lopez 1979; Correia and Lee
29 2000, 2002a, b; Cesbron [pers. comm.](#)). Amongst the deep-sea benthic foraminifer living in the
30 aphotic zone, only *Nonionella stella* has been studied (Grzyski et al. 2002). The authors
31 suggest that the sequestered chloroplasts in this species may play a role in the assimilation of
32 inorganic nitrogen, even when light is absent. It has also been hypothesised that chloroplast

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1 retention may play a major role in foraminiferal survival when facing starvation periods or in
2 anoxic environments (Cesbron [pers. comm.](#)). Under these conditions, kleptoplasts could
3 potentially be used as a carbohydrate source, and participate in inorganic nitrogen
4 assimilation (Falkowski and Raven 2007) or, when exposed to light, to produce oxygen
5 needed in foraminiferal aerobic respiration (Lopez 1979).

6 Foraminifera pigment and plastid ultrastructure studies have shown that the chloroplasts are
7 sequestered from their food source, i.e. mainly from diatoms (Lopez 1979; Knight and
8 Mantoura 1985; Grzymiski et al. 2002; Goldstein 2004). This was confirmed by experimental
9 feeding studies (Correia and Lee 2002a; Austin et al. 2005) and by molecular analysis of
10 kleptoplastic foraminifera from different environments (Pillet et al. 2011, Tsuchiya et al.
11 2015). Foraminifera from intertidal mudflat environments (e.g. *H. germanica*, *A. tepida*) feed
12 mostly on pennate diatoms (Pillet et al. 2011) which are the dominant microalgae in intertidal
13 mudflat sediments (MacIntyre et al. 1996; Jesus et al. 2009). Furthermore, in this transitional
14 coastal environments (e.g. estuaries, bays, lagoons) *A. tepida* and *H. germanica* are usually
15 the dominant meiofauna species in West Atlantic French coast mudflats (Debenay et al. 2000,
16 2006; Morvan et al. 2006; Bouchet et al. 2009; Pascal et al. 2009; Thibault de Chanvalon et
17 al. 2015). Their vertical distribution in the sediment is characterised by a clear maximum
18 density at the surface (Alve and Murray 2001; Bouchet et al. 2009; Thibault de Chanvalon et
19 al. 2015) with access to light, followed by a sharp decrease in the next two centimetres
20 (Thibault de Chanvalon et al., 2015).

21 Foraminiferal kleptoplast retention times can vary from days to months (Lopez 1979; Lee et
22 al. 1988; Correia and Lee 2002b; Grzymiski et al. 2002). The source of this variation is poorly
23 known but longer kleptoplast retention times were found in dark treatments (Lopez 1979;
24 Correia and Lee 2002b), thus suggesting an effect of light exposure, similar to what is
25 observed in kleptoplastic sacoglossans (Trench et al. 1972; Clark et al. 1990; Evertsen et al.
26 2007; Vieira et al. 2009), possibly related to the absence of some components of the
27 kleptoplast photosynthetic protein complexes in the host (Eberhard et al. 2008).

28 Most recent studies on kleptoplastic foraminifera focused on feeding, genetics and
29 microscopic observation related to chloroplast acquisition (e.g., Austin et al. 2005, Pillet et al.
30 2011, Pillet and Pawlowski 2013). To our knowledge little is known about the effects of
31 abiotic factors on photosynthetic efficiency of sequestered chloroplasts in benthic
32 foraminifera, particularly on the effect of light intensity on kleptoplast functionality. Non-

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1 invasive techniques are ideal to follow photosynthesis and some have already been used to
2 study foraminifera respiration and photosynthesis, e.g. oxygen evolution by microelectrodes
3 (Rink et al. 1998; Geslin et al. 2011) or ^{14}C radiotracer (Lopez, 1979). Recently, pulse
4 amplitude modulated (PAM) fluorometry has been used extensively in the study of
5 kleptoplastic sacoglossans (Vieira et al. 2009; Costa et al. 2012; Jesus et al. 2010; Serodio et
6 al. 2010; Curtis et al. 2013; Ventura et al. 2013). This non-invasive technique has the
7 advantage of estimating relative electron transport rates (rETR) using rapid light curves
8 (RLC) and photosystem II (PSII) maximum quantum efficiencies (F_v/F_m) very quickly and
9 without incubation periods. The latter parameter has been shown to be a good parameter to
10 estimate PSII functionality (e.g. Vieira et al. 2009; Jesus et al. 2010; Serodio et al. 2010;
11 Costa et al. 2012; Curtis et al. 2013; Ventura et al. 2013).

12 The objective of the current work was to investigate the effect of irradiance levels on
13 photosynthetic efficiency and chloroplast functional times of two benthic foraminifera feeding
14 in the same brackish areas, *H. germanica*, which is known to sequester chloroplasts and *A.*
15 *tepida*, not known to sequester chloroplasts. These two species were exposed to different
16 irradiance levels during one week and chloroplast efficiency was measured using
17 epifluorescence, oxygen microsensors and PAM fluorometry.

18

19 **2 Materials and methods**

20 **2.1 Sampling**

21 *Haynesina germanica* and *A. tepida* were sampled in January 2015 in Bourgneuf Bay
22 (47.013°N, 2.019°W), a coastal bay with a large mudflat situated south of the Loire estuary on
23 the French west coast. In this area, all specimens of *A. tepida* belong to genotype T6 of
24 Hayward et al. (2004) (Schweizer pers. comm.). In the field, a large amount (~20 kg) of the
25 upper sediment layer (roughly first 5 mm) was sampled and sieved over 300 and 150 μm
26 meshes using *in situ* sea-water. The 150 μm fraction was collected in dark flasks and
27 maintained overnight in the dark at 18°C in the laboratory. No additional food was added. In
28 the following day, sediment with foraminifera was diluted with filtered (GF/C, 1.2 μm ,
29 Whatman) autoclaved sea-water (temperature: 18°C and salinity: 32) and *H. germanica* and
30 *A. tepida* in healthy conditions (i.e. with cytoplasm inside the test) were collected with a brush
31 using a stereomicroscope (Leica MZ 12.5). The selected specimens were rinsed several times

1 using Bourgneuf bay filtered-autoclaved seawater to minimize bacterial and microalgal
2 contamination.

3 **2.2 Size and biovolume determination**

4 Foraminifera test mean maximal elongation (μm , the length of the axes going from the last
5 chamber to the other side of the test and passing by the umbilicus) was measured using a
6 micrometer mounted on a Leica stereomicroscope (MZ 12.5). Mean foraminiferal volume
7 was approximated with the equation of a half sphere, which is the best resembling geometric
8 shape for *H. germanica* and *A. tepida* (Geslin et al. 2011). The cytoplasmic volume (or
9 biovolume) was then estimated by assuming that the internal test volume corresponds to 75%
10 of the total foraminiferal test volume (Hannah et al. 1994).

11 **2.3 Spectral reflectance**

12 Pigment spectral reflectance was measured non-invasively to determine and compare the
13 relative pigment composition on 50 fresh specimens of *H. germanica*, on 50 fresh specimens
14 of *A. tepida* and on a benthic diatom as explained in Jesus et al. (2008). Concisely, a
15 USB2000 (Ocean Optics, Dunedin, FL, USA) spectroradiometer with a VIS-NIR optical
16 configuration controlled by OObase32 software (Ocean Optics B.V., Duiven, the
17 Netherlands) was used. The spectroradiometer sensor was positioned so that the surface was
18 always viewed from the nadir position. Foraminiferal reflectance spectra were calculated by
19 dividing the upwelling spectral radiance from the foraminifera (L_u) by the reflectance of a
20 clean polystyrene plate (L_d) for both of which the machine dark noise (D_n) was subtracted
21 (eq. 1).

$$22 \quad \rho = \frac{(L_u - D_n)}{(L_d - D_n)} \quad (\text{eq.1})$$

23 **2.4 Image analysis**

24 Foraminifera kleptoplast fluorescence was measured using epifluorescence microscopy
25 ($\times 200$, Olympus Ax70 with Olympus U-RFL-T, excitation wave length 485 nm). Two Tif
26 images (1232×964 px) of each foraminifer were taken (one bright field photography and one
27 epifluorescence photography) using LUCIA GTM software. The bright field photography was
28 used to trace the contours of the foraminifer and an ImageJ macro was used to extract the

1 mean pixel values of the corresponding epifluorescence photography. Higher mean pixel
2 values corresponded to foraminifera emitting more fluorescence and thus, as a proxy, contain
3 more chlorophyll. In a RGB image each channel contains pixels between 0 and 255 values.
4 The majority of the information regarding chlorophyll fluorescence is encoded in the red
5 channel, therefore the green and blue channel were discarded and only the red channel was
6 kept. The images from the different treatments were directly comparable as all images were
7 taken using the same acquisition settings. Thus, the mean red pixel values were used as a
8 proxy for chlorophyll fluorescence.

9 **2.5 Oxygen measurements**

10 Oxygen was measured using advanced Clark type oxygen microelectrodes of 50 μm in
11 diameter (Revsbech, 1989) (OXI50 - Unisense, Denmark). Electrodes were calibrated with a
12 solution of sodium ascorbate at 0.1 M (0%) and with seawater saturated with oxygen by
13 bubbling air (100%). Foraminiferal photosynthesis and oxygen respiration rates were
14 measured following Høglund et al. (2008) and Geslin et al. (2011). Measurements were
15 carried out in a micro-tube made from glass Pasteur pipette tips with an inner diameter of 1
16 mm. The micro-tube was fixed to a small vial, filled with filtered autoclaved seawater from
17 Bourgneuf Bay. The vial was placed in an aquarium with water kept at room temperature
18 (18°C). A small brush was used to position a pool of 7 to 10 foraminifera in the glass micro-
19 tube after removing air bubbles. Oxygen micro-profiles started at a distance of 200 μm above
20 the foraminifers to avoid oxygen turbulences often observed around the foraminifers.
21 Measurements were registered when the oxygen micro-profiles were stable; they were then
22 repeated five time in the centre of the micro-tube, using 50 μm steps until 1000 μm away from
23 the foraminifers (Geslin et al. 2011). The oxygen flux (J) was calculated using the first law of
24 Fick:

$$25 \quad J = -D \times \frac{dC}{dx} \quad (\text{eq. 2})$$

26 Where D is the oxygen diffusion coefficient ($\text{cm}^2 \text{s}^{-1}$) at experimental temperature (18°C) and
27 salinity (32) (Li and Gregory, 1974), and dC/dx is the oxygen concentration gradient (pmol
28 $\text{O}_2 \text{ cm}^{-1}$). The O_2 concentration gradients were calculated with the oxygen profiles and using
29 the R^2 of the regression line to determine the best gradient. Total O_2 consumption and
30 production rates were calculated as the product of O_2 fluxes by the surface area of the micro-

1 tube and subsequently divided by the foraminifera number to finally obtain the cell specific
2 rate ($\text{pmol O}_2 \text{ cell}^{-1} \text{ d}^{-1}$) (Geslin et al. 2011).

3 **2.6 Fluorescence**

4 All pulse amplitude modulated fluorescence measurements were carried out with a Water
5 PAM fluorometer (Walz, Germany) using a blue measuring light. Chloroplast functionality
6 was estimated by monitoring PSII maximum quantum efficiency (F_v/F_m) and by using P-I
7 rapid light curves (RLC, e.g., Perkins et al. (2006)) parameters (α , initial slope of the RLC at
8 limiting irradiance; $rETR_{\text{max}}$, maximum relative electron transport rate; E_k , light saturation
9 coefficient; and E_{opt} , optimum light) (Platt et al. 1980). Rapid light curves were constructed
10 using eight incremental light steps (0, 4, 15, 20, 36, 48, 64, 90 and $128 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$),
11 each lasting 30 seconds. The PAM probe was set up on a stand holder at a 2 mm distance
12 from a group of 10 foraminifera.

13 **2.7 Experimental design**

14 *Haynesina germanica*, a species known to sequester chloroplasts, were placed in plastic Petri
15 dishes and starved during 7 days under three different light conditions: dark (D and Dark-
16 RLC), low light (LL, $25 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$) and high light (HL, $70 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$);
17 whereas for comparison, *A. tepida*, a foraminifer not known to sequester chloroplasts was
18 starved but only exposed to the dark condition. A short term experiment was thus carried out
19 (7 days) to study the effect of light on healthy specimens rather than the effect of
20 starvation. For each condition, ten specimens were used per replicate and three replicates per
21 light treatment; furthermore all plastic Petri dishes were filled with Bourgneuf bay filtered-
22 autoclaved seawater. This experiment was carried out in a thermo-regulated culture room at
23 18°C , equipped with cool light fluorescent lamp (Lumix day light, L30W/865, Osram) and
24 using a 14:10 h (Light:Dark) photoperiod. The distances between the light and the
25 experimental conditions were assessed using a light-meter and a quantum sensor (ULM-500
26 and MQS-B of Walz) to obtain the desirable light intensities. Concerning the dark condition,
27 the Petri dishes were placed in a box covered with aluminium foil.

28 *Haynesina germanica* kleptoplast fluorescence was measured using epifluorescence
29 microscopy, as explain above, before and after the different light treatments. At the beginning
30 of the experiment it was done on 30 independent specimens to assess the natural and initial

1 variation of *Haynesina germanica* kleptoplast fluorescence. At the end of the experiment, the
2 measurement were done on all foraminifera exposed to the different light condition (a total of
3 30 specimens per condition). This was also measured on *A. tepida*, but results are not
4 presented because no chlorophyll fluorescence was observed at the end of the experiment.

5 *Haynesina germanica* and *A. tepida* oxygen production and consumption were measured at
6 the beginning of the experiment on three independent replicates with 7 specimens in each
7 replicate. Six different light steps were used to measure O₂ production (0, 25, 50, 100, 200
8 and 300 μmol photons m⁻² s⁻¹) for *H. germanica* and only two light steps (0 and 300 μmol
9 photons m⁻² s⁻¹) for *A. tepida*. Photosynthetic activity (P) data of *H. germanica* were fitted
10 with a Haldane model, as modified by Papacek et al. (2010) and Marchetti et al. (2013) but
11 without photoinhibition (eq. 3).

$$P(I) = \frac{Pm \times I}{I + Ek} - Rd \quad (\text{eq. 3})$$

13 Where Pm is the maximum photosynthetic capacity (pmol O₂ cell⁻¹ d⁻¹), I the photon flux
14 density (μmol photons m⁻² s⁻¹), Ek the half-saturation constant (μmol photons m⁻² s⁻¹) and Rd
15 the dark respiration, expressed as an oxygen consumption (pmol O₂ cell⁻¹ d⁻¹). The initial
16 slope of the P–I (Photosynthesis –Irradiance) curve at limiting irradiance α (pmol O₂ cell⁻¹
17 day⁻¹ (μmol photons m⁻² s⁻¹)⁻¹) and the compensation irradiance I_c were calculated according
18 to equations 4 and 5.

$$I_c = \frac{Ek \times Rd}{Pm - Rd} \quad (\text{eq. 4})$$

$$\alpha = \frac{Rd}{I_c} \quad (\text{eq. 5})$$

21 Oxygen measurements were repeated at 300 μmol photons m⁻² s⁻¹ and in the dark at the end of
22 the experiment (7 days of incubation) for all different light treatments (D, LL, HL) using 10
23 specimens, to assess their production or consumption of oxygen at these two light levels (300
24 μmol photons m⁻² s⁻¹ and in the dark) in all treatments.

25 For All conditions (D, LL, HL and Dark-RLC) *F_v/F_m* was measured daily at early afternoon,
26 after a one-hour dark adaptation period and measurements were done in triplicate for each
27 Petri Dish.

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1 Rapid light curves were also carried out in all light treatments at the beginning and end of the
2 experiment, after one-hour dark adaptation for the 2 tested species. Additionally, RLC were
3 carried out daily in an extra triplicate kept in the dark (Dark-RLC) throughout the duration of
4 the experiment.

5 **2.8 Statistical analysis**

6 Data are expressed as mean \pm standard deviation (SD) when $n = 3$ or standard error (SE)
7 when $n = 30$. Statistical analyses consisted of a t-test to compare the foraminifera test mean
8 maximal elongation, a non parametric test (Kruskal Wallis) to compare the mean chlorophyll
9 fluorescence of the foraminifera exposed to the different experimental conditions and a
10 multifactor (experimental conditions (D, LL, HL), irradiance ($0-300 \mu\text{mol photons m}^{-2} \text{s}^{-1}$))
11 analysis of variance (ANOVA) with a Fisher's LSD test to compare the respiration rates at the
12 end of the experiment. Differences were considered significant at $p < 0.05$. Statistical analyses
13 were carried out using the Statgraphics Centurion XV.I (StatPoint Technologies, Inc.)
14 software.

15 **3 Results**

16 **3.1 Size and biovolume**

17 *Ammonia tepida* specimens were larger than *H. germanica* with a mean maximal elongation
18 of $390 \pm 42 \mu\text{m}$ (SD, $n = 34$) and $366 \pm 45 \mu\text{m}$ (SD, $n = 122$), respectively ($p < 0.01$, $F_{121,33} =$
19 1.15). This resulted in cytoplasmic biovolumes equal to $1.20 \times 10^7 \pm 3.9 \times 10^6 \mu\text{m}^3$ (SD) and
20 $1.01 \times 10^7 \pm 3.65 \times 10^6 \mu\text{m}^3$ (SD), respectively.

21 **3.2 Chloroplast functionality**

22 Fresh *Haynesina germanica* and *A. tepida* showed very different spectral reflectance
23 signatures (Figure 1). *Haynesina germanica* showed a typical diatom spectral signature with
24 high reflectance in the infrared region ($>740 \text{ nm}$) and clear absorption features around 585,
25 630 and 675 nm; the absorption feature around 675 nm correspond to the presence of
26 chlorophyll *a*; the 585 nm feature is the result of fucoxanthin and the 630 nm absorption
27 feature is the result of chlorophyll *c* (arrows, Figure 1). *Ammonia tepida* showed no obvious
28 pigment absorption features apart from 430 nm (Figure 1).

1 Epifluorescence images showed a clear effect of the different light treatments (Dark, Low
2 Light, High Light) on *H. germanica* chlorophyll fluorescence (Figure 2). Visual observations
3 showed a clear decrease in chlorophyll fluorescence for the LL and HL treatments from the
4 beginning of the experiment (Figure 2A) to the end of a 7 day period of light exposure (Figure
5 2C and 2D, respectively). Samples kept in the dark did not show an obvious decrease but
6 showed a more patchy distribution compared to the beginning of the experiment (Figure 2B).
7 This was confirmed by a non-parametric test (Kruskal Wallis) showing that the differences in
8 chlorophyll *a* fluorescence were significant ($p < 0.01$, $Df = 3$, Figure 3). It is also noteworthy
9 to mention that there was a large individual variability within each treatment leading to large
10 standard errors in spite of the number of replicates ($n = 30$).

11 Oxygen measurements carried out at the beginning of the experiment (T0) differed
12 considerably between the two species. *Ammonia tepida* did not show any net oxygen
13 production although respiration rates measured at $300 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ were lower (2485
14 $\pm 245 \text{ pmol O}_2 \text{ cell}^{-1} \text{ d}^{-1}$) than the ones measured in the dark ($3531 \pm 128 \text{ pmol O}_2 \text{ cell}^{-1} \text{ d}^{-1}$)
15 ($F_{2,2} = 3.7$, $p = 0.02$). *Haynesina germanica* showed lower dark respiration rates (1654 ± 785
16 $\text{pmol O}_2 \text{ cell}^{-1} \text{ d}^{-1}$) and oxygen production quickly increased with irradiance, showing no
17 evidence of photoinhibition within the light range used (Figure 4). Compensation irradiance
18 (I_c) was reached very quickly, as low as $24 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (95% coefficient bound: 17-
19 $30 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, values calculated from the fitted model eq.4) and the half-saturation
20 constant (E_k) was also reached at very low light levels, i.e. at $17 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. No
21 photoinhibition was observed under the experimental light conditions (0 to $300 \mu\text{mol photons}$
22 $\text{m}^{-2} \text{s}^{-1}$), which resulted in an estimation of $\sim 2800 \text{ pmol O}_2 \text{ cell}^{-1} \text{ d}^{-1}$ for maximum
23 photosynthetic capacity. The P-I curve initial slope at limiting irradiance (α) was estimated at
24 $70 \text{ pmol O}_2 \text{ cell}^{-1} \text{ d}^{-1} (\mu\text{mol photons m}^{-2} \text{s}^{-1})^{-1}$ (95% coefficient bound: 58-88).

25 Oxygen measurements carried out at the end of the experiment (T7) showed significant
26 different dark and light respiration rates, with light respiration being lower than dark
27 respiration but not reaching net oxygen production rates (D, LL, HL) (Table 1). Moreover,
28 respiration rates were different between conditions ($p < 0.001$), with significantly lower
29 respiration rates of specimens incubated under High Light conditions than those under Dark
30 and Low Light conditions ($p < 0.05$, Fisher's LSD test).

31 PAM fluorescence rapid light curve (RLC) parameters (α , $rETR_{\text{max}}$, E_k and E_{opt}) showed
32 significant differences between foraminiferal species and over the duration of the experiment

1 (Figures 5 and 6). Highest rETR_{max}, α and E_{opt} were always observed in *H. germanica*.
2 After only one starvation day *A. tepida* RLC parameters dropped to zero or close to zero.
3 Contrastively, *H. germanica* RLC parameters showed a slow decrease throughout the
4 experiment (Figures 5 and 6) with rETR_{max} and α decreasing from 6 to 4 and 0.22 to 0.15,
5 respectively (Figures 6A and B). The parameters E_k and E_{opt} stayed constant over the 7 days
6 of the experiment, with values oscillating around 30 and 90, respectively (Figures 6C and D).
7 PSII maximum quantum yields (*F_v/F_m*) were clearly affected by light and time (Figure 7).
8 Both species showed high initial *F_v/F_m* values, i.e. > 0.6 and 0.4 for *H. germanica* and *A.*
9 *tepida*, respectively (Figure 7). However, while *A. tepida* *F_v/F_m* values quickly decreased to
10 zero after only one starvation day, *H. germanica* exhibited a large variability between light
11 conditions (D, LL, HL) throughout the duration of the experiment (Figure 7); decreasing from
12 0.65 to 0.55 in darkness (D), from 0.65 to 0.35 under low light (LL) conditions and from 0.65
13 to 0.20 under high light (HL). Using these *F_v/F_m* decreases, *H. germanica* kleptoplast
14 functional times were estimated between 11-21 days in the dark (D), 9-12 days in low light
15 (LL) and 7-8 days in high light (HL); depending if an exponential or linear model was
16 applied. *Ammonia tepida* chloroplast functional times were estimated between 1-2 days
17 (exponential and linear model, respectively) and light exposure reduced the functional time to
18 less than one day (data not shown).

19

20 **4 Discussion**

21 **4.1 Chloroplast functionality**

22 Our results clearly show that only *H. germanica* was capable of carrying out net
23 photosynthesis. *Haynesina germanica* had typical diatom reflectance spectra (Figure 1),
24 showing the three major diatom pigment absorption features: chlorophyll *a*, chlorophyll *c*, and
25 fucoxanthin (Meleder et al. 2003; Jesus et al. 2008; Kazemipour et al. 2012; Meleder et al.
26 2013). Conversely, in *A. tepida* these absorption features were not detected, suggesting that
27 diatom pigments ingested by this species were quickly digested and degraded to a degree
28 where they were no longer detected by spectral reflectance measurements. These non-
29 destructive reflectance measurements are thus in accordance with other studies on benthic
30 foraminifera pigments by HPLC showing that *H. germanica* feed on benthic diatoms (Knight

1 and Mantoura, 1985). Similarly, Knight and Mantoura (1985) also detected higher
2 concentrations and less degraded diatom pigments in *H. germanica* than in *A. tepida*.

3 Furthermore, *H. germanica* has the ability to produce oxygen from low to relatively high
4 irradiance, as shown by the low compensation point (I_c) of $24 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ and the
5 high onset of light saturation ($>300 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$) (Figure 4). Thus, *H. germanica*
6 seems to be well adapted to cope with the high light variability observed in intertidal
7 sediments that can range from very high irradiance levels ($>1000 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$) at the
8 surface of the sediment during low tide to very low levels within the sediment matrix or
9 during high tide in turbid mudflat waters. *Ammonia tepida* was found to carry out aerobic
10 respiration, but respiration rates measured at $300 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ were lower than those
11 measured in the dark. We thus suppose that in *A. tepida* oxygen production by ingested
12 diatom or chloroplasts might be possible, provided that this species is constantly supplied
13 with fresh diatoms. However, another possibility to explain this reduction in oxygen
14 consumption could be a decrease of its metabolism or activity under light exposure. The light
15 and dark oxygen production or consumption values measured for both species are in
16 accordance with previous studies (Geslin et al. 2011).

17 According to Lopez (1979), measured oxygen data can be used to estimate *H. germanica*
18 carbon fixation rates. Thus, using $1000 \text{ pmol O}_2 \text{ cell}^{-1} \text{ d}^{-1}$ at $300 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$, ~ 200 to
19 $4000 \text{ cells per } 50 \text{ cm}^3$ in the top 0.5 cm (Morvan et al. 2006; Bouchet et al. 2007) and
20 assuming that photosynthesis produced one mol O_2 per mol of C fixed, *H. germanica* primary
21 production would be between 1.8×10^{-5} and $4.0 \times 10^{-4} \text{ mol C m}^{-2} \text{ d}^{-1}$. This is a very low value
22 compared to microphytobenthos primary production in Atlantic mudflat ecosystems, which
23 usually range from 1.5 to $5.9 \text{ mol C m}^{-2} \text{ d}^{-1}$ (e.g. Brotas and Catarino 1995, reviewed in
24 MacIntyre et al. 1996). The estimated values represent thus less than 0.1% of
25 microphytobenthos fixated carbon and are in the same range of values than what has been
26 described by Lopez (1979) using ^{14}C radioactive tracers. These results should be interpreted
27 with caution because a wide variety of factors probably affect *H. germanica in situ* primary
28 production, e.g. diatom availability, kleptoplast densities, nutrient supply, light exposure, sea
29 water turbidity, [local biogeochemical processes](#) and migration capability are all factors that
30 can potentially affect *H. germanica* kleptoplast functionality. Nevertheless, although carbon
31 fixation seems not to be relevant at a global scale, the oxygen production could be important

1 at a microscale and relevant in local mineralization processes in/on mudflat sediments (e.g.
2 iron, ammonium, manganese).

3 At sampling time (T0) *H. germanica* rETR and *Fv/Fm* values were similar to
4 microphytobenthic species (i.e. *Fv/Fm* > 0.65) (Perkins et al. 2001), suggesting that the
5 kleptoplast PSII and electron transport chain were not much affected after incorporation in the
6 foraminifers' cytoplasm. In contrast, *A. tepida* *Fv/Fm* and RLC parameters were already
7 much lower on the sampling day and quickly decreased to almost zero within 24 hours,
8 suggesting that plastids were not stable inside the *A. tepida* cytoplasm. Complete diatoms
9 inside *A. tepida* were already observed in feeding studies (Le Kieffre, pers. com), this low
10 *Fv/Fm* value might thus come from recently ingested diatoms by *A. tepida*. *Fv/Fm* has
11 previously been used to determine kleptoplast functional times and to follow decrease in
12 kleptoplast efficiency in other kleptoplastic organisms, e.g. the sea slug *Elysia viridis* (Vieira
13 et al. 2009). *Fv/Fm* measurements carried out on *H. germanica* at different light conditions
14 showed that light had a significant effect on the estimation of kleptoplast functional time, with
15 the longest functional time estimated at 21 days for dark condition. This time frame would
16 qualify *H. germanica* as a long term kleptoplast retention species (Clark et al. 1990);
17 however, our seven days estimation for the high light treatment would place *H. germanica* in
18 the medium-term retention group. This clearly shows that light exposure has an important
19 effect on this species kleptoplast functionality. Concerning *A. tepida*, the short dark diatom or
20 chloroplast functional time (<2 days) places this species directly in the short or medium-term
21 retention group.

22 Additionally, *H. germanica* kept in darkness showed a slow decrease of the RLC parameters,
23 α and rETR_{max}, throughout the seven experimental days; this decrease is likely related to
24 overall degradation of the light-harvesting complexes and of other components of the
25 photosynthetic apparatus, which gradually induced a reduction of light harvesting efficiency
26 and of carbon metabolism. This decrease was much amplified in low and high irradiance and
27 it should be pointed out that the actual light level of the HL treatment (i.e. 70 $\mu\text{mol photons}$
28 $\text{m}^{-2} \text{s}^{-1}$) is very low as compared to irradiances in their natural environment, which are easily
29 going above 1000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, showing that the foraminifera kleptoplasts lack the
30 high photoregulation capacity exhibited by the benthic diatoms that they feed upon
31 (Cartaxana et al. 2013). This is consistent with the observation at the end of the experiment
32 that no net oxygen production was occurring under the different light conditions.

1 Nevertheless, a small difference was still found between dark and light respiration (Table 1),
2 suggesting that some oxygen production was still occurring but it was not sufficient to
3 compensate for the respiration oxygen consumption. We also noticed that the respiration was
4 higher in the foraminifera maintained in low light and dark conditions in comparison to the
5 high light foraminifera. In the line of the lower F_v/F_m values observed, this suggests that
6 kleptoplasts and possibly other metabolic pathways might have been damaged by the excess
7 of light. Clearly, in *H. germanica* light exposure had a strong effect on PSII maximum
8 quantum efficiency and on the retention of functional kleptoplasts (Figure 7), which can
9 explain the absence of net oxygen production after the 7 days of the experiments. Comparable
10 results for *H. germanica* were also obtained by counting the number of chloroplasts over time
11 with cells exposed or not to light (Lopez 1979). One of the most probable explanations for the
12 observed F_v/F_m decrease is the gradual inactivation of the protein D1 in PSII reaction
13 centres. This protein is an essential component in the electron transport chain and its turnover
14 rate is frequently the limiting factor in PSII repair rates (reviewed in Campbell and Tyystjärvi
15 2012). Normally, protein D1 is encoded in the chloroplast and is rapidly degraded and
16 resynthesized under light exposure with a turnover correlated to irradiance (Tyystjärvi and
17 Aro 1996). However, although D1 is encoded by the chloroplast genome, its synthesis and
18 concomitant PSII recovery require further proteins that are encoded by the algal nuclear
19 genome (Yamaguchi et al. 2005). Thus, when D1 turnover is impaired it will induce an F_v/F_m
20 decrease correlated to irradiance (Tyystjärvi and Aro 1996) consistent to what was observed
21 in the present study. In another deep sea benthic species (*Nonionella stella*) the D1 and other
22 plastid proteins (RuBisCO and FCP complex) were still present in the foraminifer one year
23 after sampling (Grzyski et al. 2002). This shows that some foraminifera can retain both
24 nuclear (FCP) and chloroplast (D1 and RuBisCO) encoded proteins. However, contrary to *H.*
25 *germanica*, *N. stella* lives in deeper environments never exposed to light and thus is unlikely
26 to carry out oxygenic photosynthesis (Grzyski et al. 2002). This fundamental difference
27 could explain why kleptoplast functional times are much longer in *N. stella*, reaching up to
28 one year in specimens kept in darkness (Grzyski et al. 2002). On the other hand, it has been
29 shown that isolated chloroplasts are able to function for several months in Sacoglossan sea
30 slugs provided with air and light in aquaria (Green et al. 2001; Rumpho et al. 2001), which
31 demonstrates the existence of interactions between the kleptoplast and the host genomes,
32 and/or of mechanisms facilitating and supporting such long-lasting associations. In *H.*
33 *germanica* exposed to high light it is also possible that reactive oxygen species (ROS)

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1 production rates of the sequestered chloroplasts might exceed the foraminifera capacity to
2 eliminate those ROS, thus inducing permanent damage to the foraminifera. This ROS
3 production could also eventually damage the kleptoplasts resulting in higher kleptoplast
4 degradation rates.

5 **4.2 Possible advantages of kleptoplasty for intertidal benthic foraminifera**

6 Much is still unknown about the relationship between kleptoplastic benthic foraminifera and
7 their sequestered chloroplasts. The relevance of the photosynthetic metabolism compared to
8 predation or organic matter assimilation is unknown; however, it would be of great interest to
9 understand the kleptoplast role in the foraminiferal total energy budget. Oxygenic
10 photosynthesis comprises multiple reactions leading to the transformation of inorganic carbon
11 to carbohydrates. However, to produce these carbohydrates all the light driven reactions have
12 to be carried out, as well as the Calvin cycle reactions. With fresh kleptoplasts this hypothesis
13 seems possible (e.g. Lopez 1979), especially if the plastid proteins are still present and
14 functional. However, we showed that the maximum quantum efficiency of the PSII decreased
15 quickly under light exposure, suggesting that substantial direct carbohydrate production is
16 unlikely without constant chloroplast replacement. Conversely, the production of intermediate
17 photosynthetic products such as adenosine triphosphate (ATP) and nicotinamide adenine
18 dinucleotide phosphate (NADPH) could be possible and would be of metabolic value for the
19 foraminifera. It is also possible that *in situ* the foraminifera have better photoregulation
20 capacities. Not only they will have easy access to fresh diatom chloroplasts, as *H. germanica*
21 is mainly living in the first few mm of the superficial sediment (Alve and Murray 2001,
22 Thibault de Chanvalon et al. 2015), but they will also have the possibility of migrating within
23 the sediment (Gross 2000) using this behavioural feature to enhance their photoregulation
24 capacity, similarly to what is observed in benthic diatoms from microphytobenthic biofilms
25 (e.g. Jesus et al. 2006; Mouget et al. 2008; Perkins et al. 2010). However, below the photic
26 limit (max 2 to 3 mm in estuarine sediments (reviewed in MacIntyre et al. 1996, Cartaxana et
27 al. 2011)) it is unlikely that oxygenic photosynthesis will occur, even if live *H. germanica* are
28 also found below this limit (Thibault de Chanvalon et al. 2015, Cesbron et al. in press).

29 **5 Conclusion**

30 Comparing *H. germanica* with *A. tepida* showed that the former species potentially has the
31 capacity of retaining functional kleptoplasts up to 21 days, much longer than *A. tepida* that

1 showed almost no PSII activity after 24 hours. Nevertheless, the capacity of *H. germanica* to
2 keep functional kleptoplasts was significantly decreased by exposing it even to low irradiance
3 levels, which resulted in low F_v/F_m values and decreased oxygen production. This shows
4 clearly that in our experimental conditions, *H. germanica* had reduced photoregulation
5 capacities. These results emphasize that studies on kleptoplast photophysiology of benthic
6 foraminifera must be interpreted with care, as results are strongly influenced by the
7 foraminiferal light history before incubation. Additionally, this study shows that the cellular
8 machinery necessary for chloroplast maintenance is unlikely to be completely functional,
9 suggesting that *H. germanica* has to continuously renew its chloroplasts to keep them
10 functional. We hypothesize that kleptoplasts might have an added value by providing extra
11 carbon, mainly under light exposure, but also as energy stock to be digested during food
12 impoverished periods, in dark or light conditions.

Supprimé: and fueling nitrogen metabolic pathways to foraminifera

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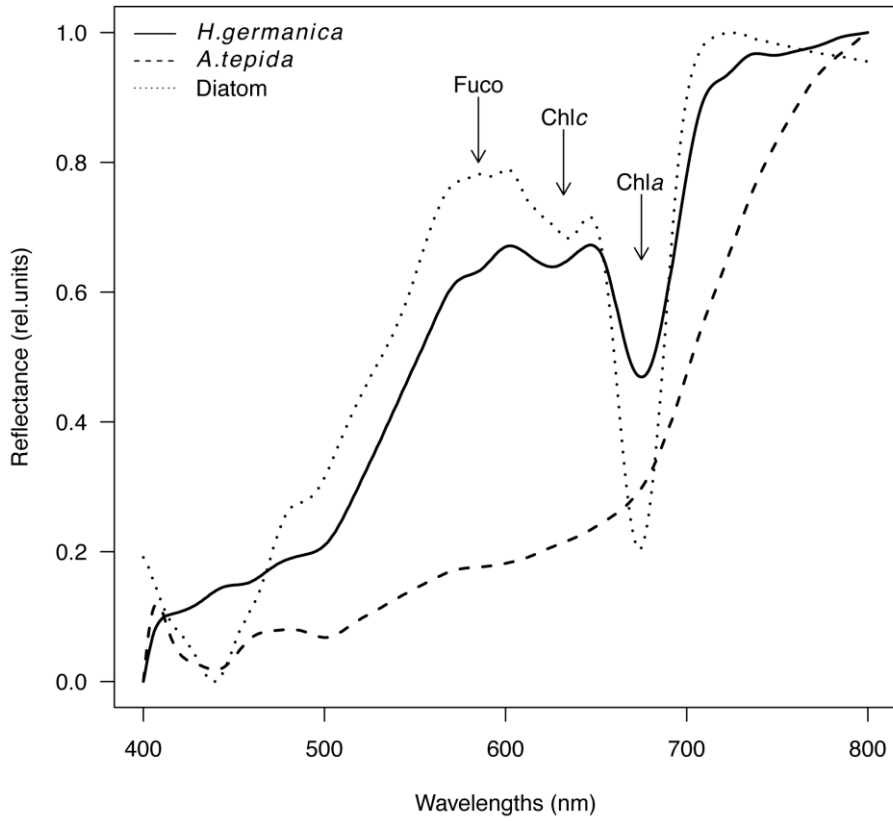
1 Table 1. Light and dark respiration rates ($\mu\text{mol O}_2 \text{ cell}^{-1} \text{ d}^{-1}$) \pm SD of *Haynesina germanica* in
 2 the three experimental conditions (Dark, Low Light and High Light) at the end of the
 3 experiment (Df, degree of freedom, PFD Photon Flux Density).

4

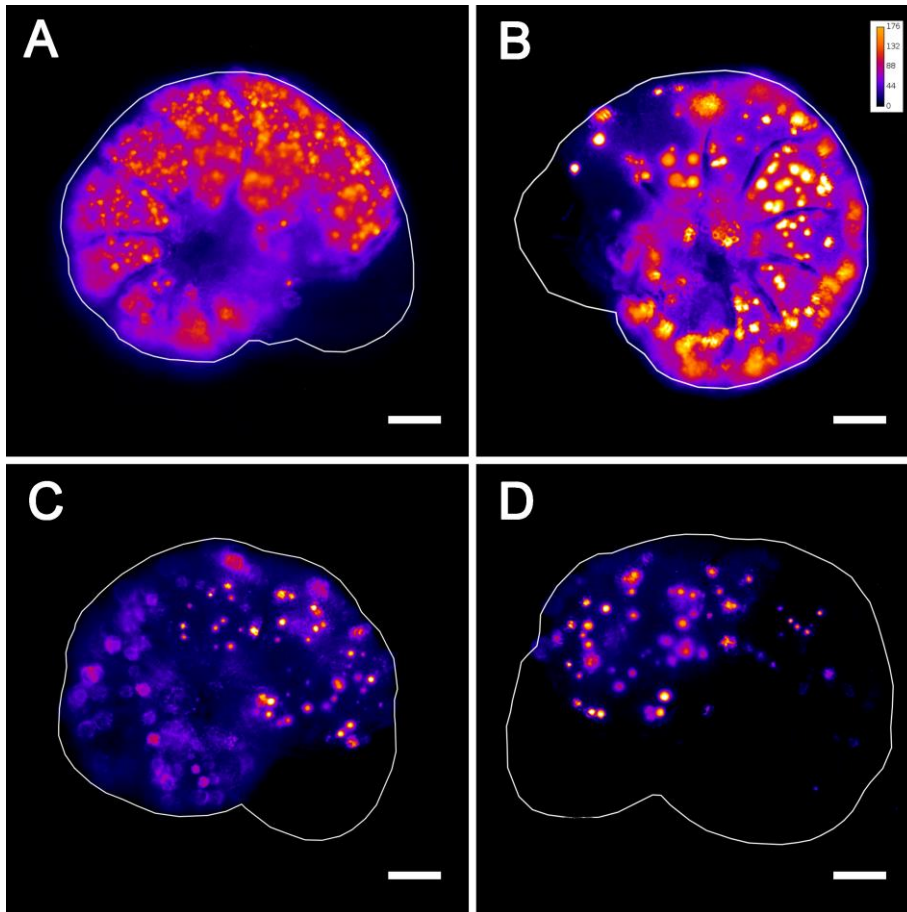
Condition	PFD	Respiration Rate ($\mu\text{mol O}_2 \text{ cell}^{-1} \text{ d}^{-1}$)		
D	300	2452 \pm 537		
	0	3542 \pm 765		
LL	300	3468 \pm 305		
	0	4015 \pm 110		
HL	300	1179 \pm 261		
	0	1905 \pm 235		
Anova		Df	F-test	p
Condition	p ($\alpha=0.05$)	2	13.1	<0.001
PFD	p ($\alpha=0.05$)	1	5.4	0.026
Interaction	p ($\alpha=0.05$)	2	0.3	0.78

5

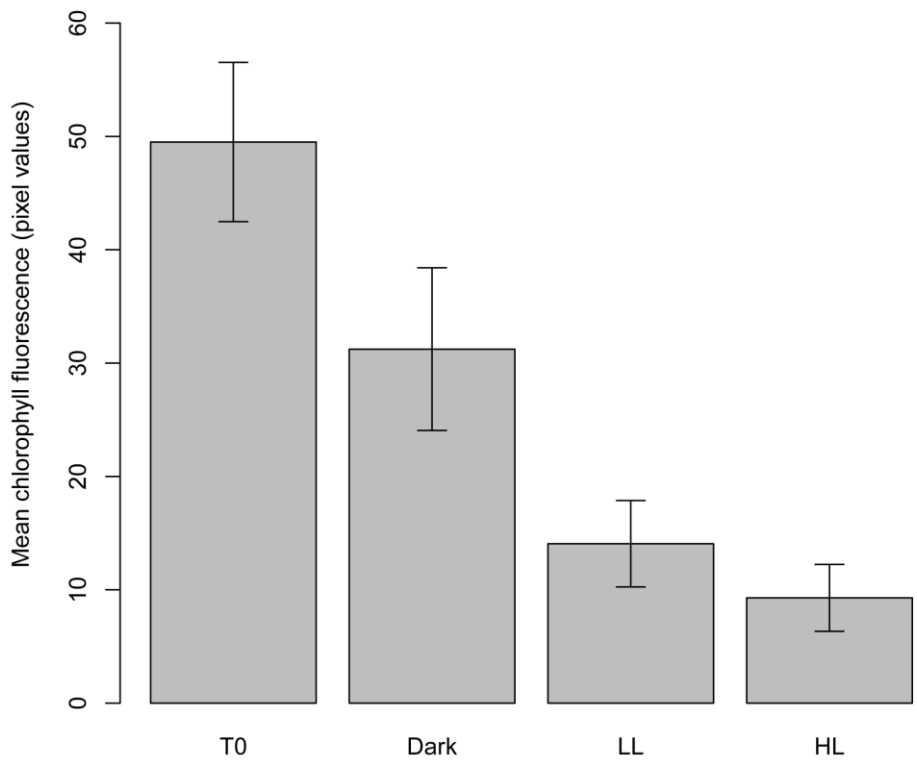
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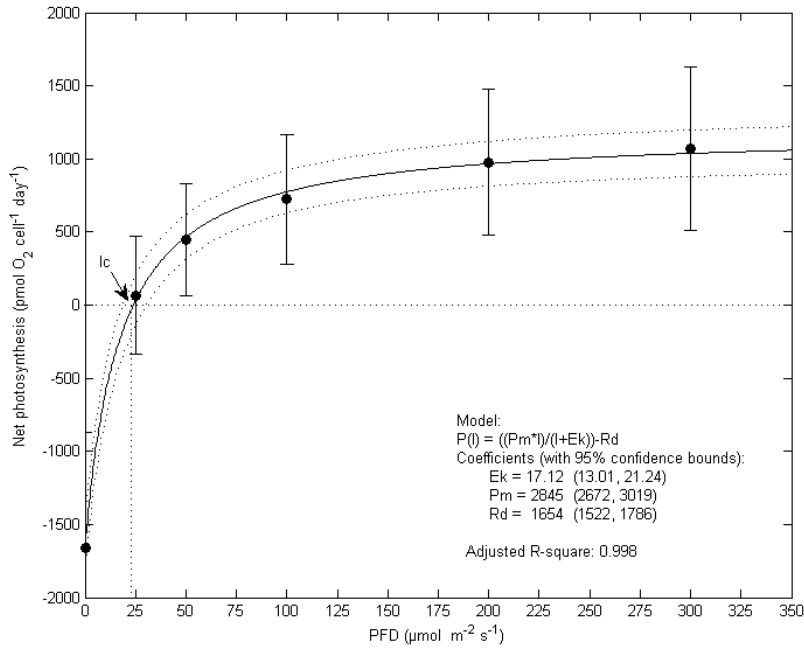
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 2 Figure 1. Spectral reflectance signatures of *Haynesina germanica*, *Ammonia tepida* and of a
 3 benthic diatom in relative units (X-axis legend: Wavelength (nm)).
 4



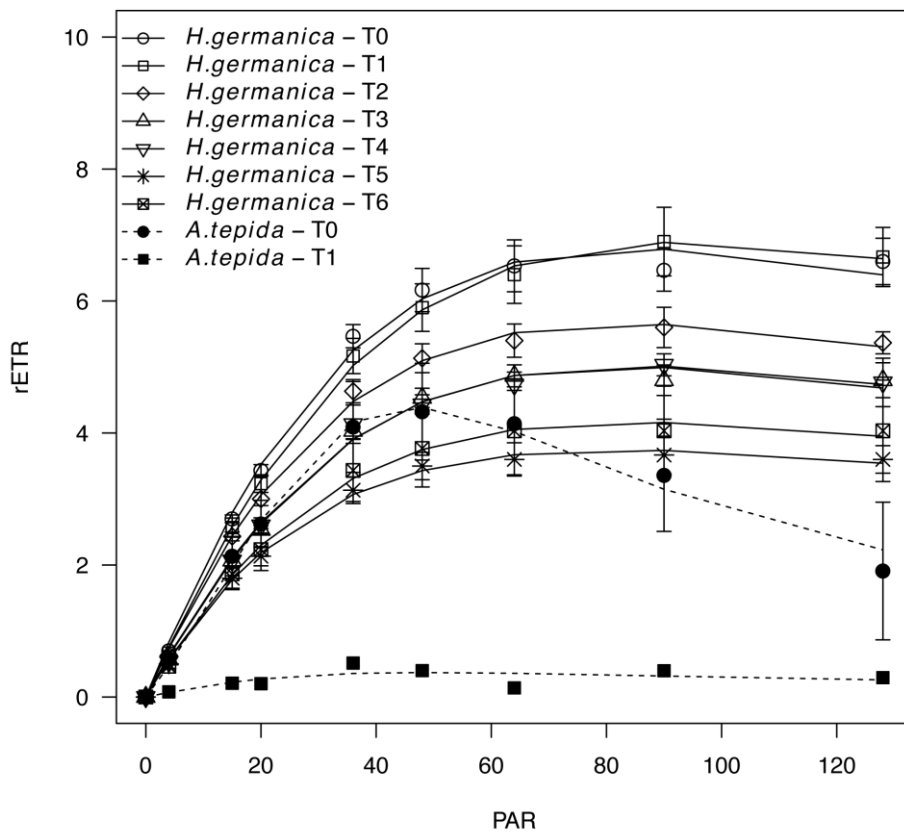
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 2 Figure 2. Illustration of *Haynesina germanica* chloroplast content at the beginning (A) and at
 3 the end of the experiment for the three experimental conditions, Dark (B), Low Light (C) and
 4 High Light (D). Higher colour scale values correspond to foraminifera emitting more
 5 fluorescence and likely containing more chlorophyll *a*; fluorescence in pixel values between 0
 6 and 255, (scale bar = 50 μm).
 7



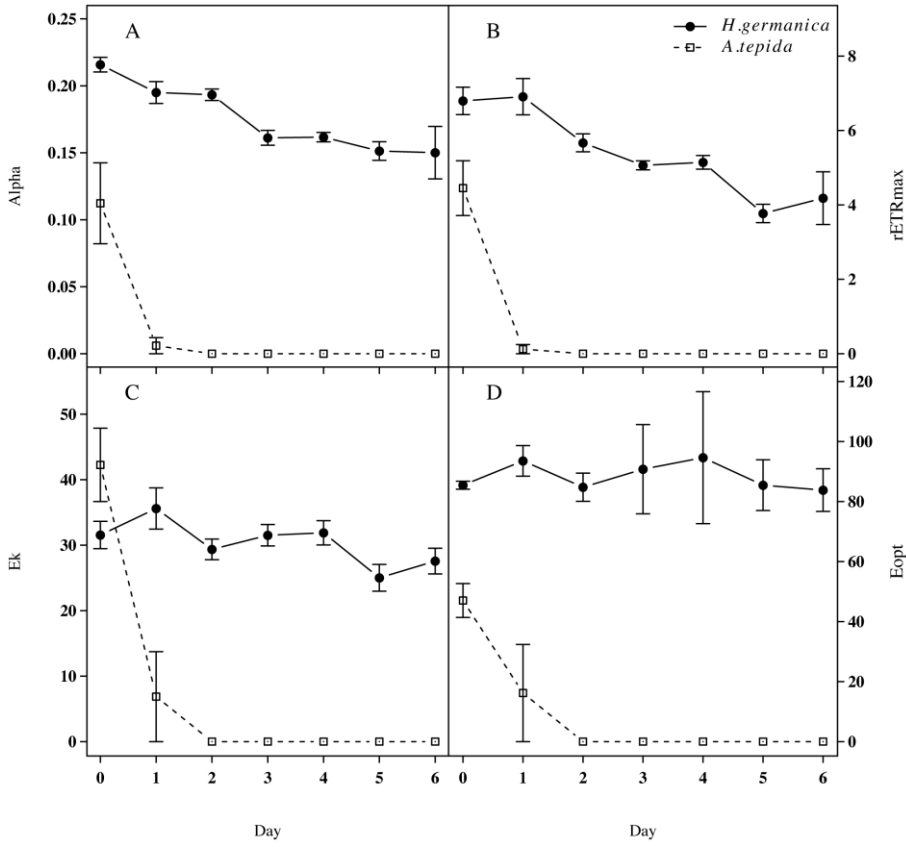
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 2 Figure 3. Mean chlorophyll *a* fluorescence (\pm SE, n = 30) at the end for the three experimental
 3 conditions (Dark, Low Light and High Light) and the beginning (T0) of the experiment using
 4 *Haynesina germanica*. Higher mean values likely corresponded to foraminifera containing
 5 more chlorophyll.
 6



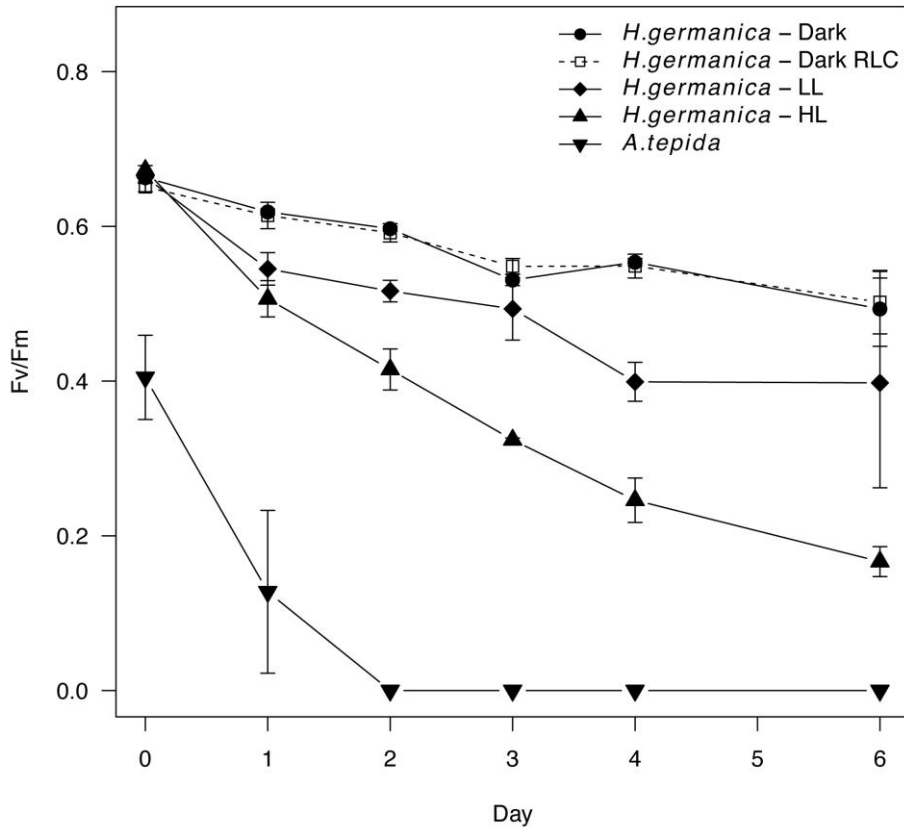
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 2 Figure 4. Net photosynthesis of *Haynesina germanica* ($\text{pmol O}_2 \text{ cell}^{-1} \text{ d}^{-1}$) as a function of the
 3 photon flux density (PFD, $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$). The half-saturation constant, E_k , was found
 4 at 17 (13-21), the dark respiration, R_d , at 1654 (1522-1786) $\text{pmol O}_2 \text{ cell}^{-1} \text{ d}^{-1}$ and the
 5 maximum photosynthetic capacity, P_m , at 2845 (2672-3019) $\text{pmol O}_2 \text{ cell}^{-1} \text{ d}^{-1}$. The I_c ,
 6 calculated compensation irradiance (24 (17-30) $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$). The adjusted R^2 of the
 7 model was equal to 0.998, $n = 3$.
 8



1
 2 Figure 5. Rapid light curves (RLC, n = 3) expressed as the relative electron transport rate
 3 (rETR) as a function of the photosynthetic active radiation (PAR in $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) of
 4 *Haynesina germanica* (black lines) and *Ammonia tepida* (black dashed lines) during the seven
 5 days of the experiment.
 6



1
 2 Figure 6. Rapid light curve (RLC, n = 3) parameters for *Haynesina germanica* (Dark-RLC)
 3 and *Ammonia tepida* maintained in the dark during the experiment, Alpha is the initial slope
 4 of the RLC at limiting irradiance, rETRmax is the maximum relative electron transport rate,
 5 Ek is the light saturation coefficient and Eopt is the optimum light, all of them were estimated
 6 by adjusting the experimental data to fit the model of Platt et al. (1980).
 7



1
 2 Figure 7. Maximum quantum efficiency of the photosystem II (F_v/F_m , $n = 3$) during the
 3 experiment for the different applied conditions (Dark, Low Light and High Light) and species
 4 (*Haynesina germanica* and *Ammonia tepida*).