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

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Abstract

Some benthic foraminifera have the ability to incorporate functional chloroplasts from diatoms (kleptoplasty). Our objective was to investigate chloroplast functionality of two benthic foraminifera (*Haynesina germanica* and *Ammonia tepida*) exposed to different irradiance levels (0, 25, 70 µmol photon m^{-2} s^{-1}) using spectral reflectance, epifluorescence observations, oxygen evolution and pulse amplitude modulated (PAM) fluorometry (maximum photosystem II quantum efficiency (*Fv/Fm*) and rapid light curves (RLC)). Our results clearly showed that *H. germanica* was capable of using its kleptoplasts for more than one week while *A. tepida* showed very limited kleptoplastic ability with maximum photosystem II quantum efficiency (*Fv/Fm* = 0.4), much lower than *H. germanica* and decreasing to zero in only one day. Only *H. germanica* showed net oxygen production with a compensation point at 24 µmol photon m^{-2} s^{-1} and a production up to 1000 pmol O_2 $cell^{-1}$ day^{-1} at 300 µmol photon m^{-2} s^{-1}. *Haynesina germanica Fv/Fm* slowly decreased from 0.65 to 0.55

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

1   Introduction

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

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

1   retention may play a major role in foraminiferal survival when facing starvation periods or in

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

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

2   Materials and methods

2.1   Sampling

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

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

2.2 Size and biovolume determination

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

2.3 Spectral reflectance

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

$$\rho = \frac{(Lu - Dn)}{(Ld - Dn)} \qquad (eq.1)$$

2.4 Image analysis

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

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

2.5   Oxygen measurements

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

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

Where D is the oxygen diffusion coefficient (cm² s^{-1}) at experimental temperature (18°C) and salinity (32) (Li and Gregory, 1974), and dC/dx is the oxygen concentration gradient (pmol O_2 cm^{-1}). The O_2 concentration gradients were calculated with the oxygen profiles and using the R² of the regression line to determine the best gradient. Total O_2 consumption and production rates were calculated as the product of O_2 fluxes by the surface area of the micro-

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tube and subsequently divided by the foraminifera number to finally obtain the cell specific rate (pmol O_2 cell^{-1} d^{-1}) (Geslin et al. 2011).

2.6 Fluorescence

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

2.7 Experimental design

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

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

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

Haynesina germanica and *A. tepida* oxygen production and consumption were measured at the beginning of the experiment on three independent replicates with 7 specimens in each replicate. Six different light steps were used to measure O_2 production (0, 25, 50, 100, 200 and 300 µmol photons m^{-2} s^{-1}) for *H. germanica* and only two light steps (0 and 300 µmol photons m^{-2} s^{-1}) for *A. tepida*. Photosynthetic activity (P) data of *H. germanica* were fitted with a Haldane model, as modified by Papacek et al. (2010) and Marchetti et al. (2013) but without photoinhibition (eq. 3).

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

Where Pm is the maximum photosynthetic capacity (pmol O_2 cell^{-1} d^{-1}), I the photon flux density (µmol photons m^{-2} s^{-1}), Ek the half-saturation constant (µmol photons m^{-2} s^{-1}) and Rd the dark respiration, expressed as an oxygen consumption (pmol O_2 cell^{-1} d^{-1}). The initial slope of the P–I (Photosynthesis –Irradiance) curve at limiting irradiance α (pmol O_2 cell^{-1} day^{-1} (µmol photons m^{-2} s^{-1})$^{-1}$)) and the compensation irradiance Ic were calculated according to equations 4 and 5.

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

$$\alpha = \frac{Rd}{Ic} \qquad \text{(eq. 5)}$$

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

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

Rapid light curves were also carried out in all light treatments at the beginning and end of the experiment, after one-hour dark adaptation for the 2 tested species. Additionally, RLC were

carried out daily in an extra triplicate kept in the dark (Dark-RLC) throughout the duration of the experiment.

2.8 Statistical analysis

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

3 Results

3.1 Size and biovolume

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

3.2 Chloroplast functionality

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

Epifluorescence images showed a clear effect of the different light treatments (Dark, Low Light, Hight Light) on *H. germanica* chlorophyll fluorescence (Figure 2). Visual observations showed a clear decrease in chlorophyll fluorescence for the LL and HL treatments from the

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1 beginning of the experiment (Figure 2A) to the end of a 7 day period of light exposure (Figure

2 2C and 2D, respectively). Samples kept in the dark did not show an obvious decrease but

3 showed a more patchy distribution compared to the beginning of the experiment (Figure 2B).

4 This was confirmed by a non-parametric test (Kruskal Wallis) showing that the differences in

5 chlorophyll *a* fluorescence were significant ($p < 0.01$, $Df = 3$, Figure 3). It is also noteworthy

6 to mention that there was a large individual variability within each treatment leading to large

7 standard errors in spite of the number of replicates ($n = 30$).

8 Oxygen measurements carried out at the beginning of the experiment (T0) differed

9 considerably between the two species. *Ammonia tepida* did not show any net oxygen

10 production although respiration rates measured at 300 µmol photons m^{-2} s^{-1} were lower (2485

11 $\pm$ 245 pmol O_2 $cell^{-1}$ d^{-1}) than the ones measured in the dark (3531 $\pm$ 128 pmol O_2 $cell^{-1}$ d^{-1})

12 ($F_{2,2} = 3.7$, $p = 0.02$). *Haynesina germanica* showed lower dark respiration rates (1654 $\pm$ 785

13 pmol O_2 $cell^{-1}$ d^{-1}) and oxygen production quickly increased with irradiance, showing no

14 evidence of photoinhibition within the light range used (Figure 4). Compensation irradiance

15 (Ic) was reached very quickly, as low as 24 µmol photons m^{-2} s^{-1} (95% coefficient bound: 17-

16 30 µmol photons m^{-2} s^{-1}, values calculated from the fitted model eq.4) and the half-saturation

17 constant (Ek) was also reached at very low light levels, i.e. at 17 µmol photons m^{-2} s^{-1}. No

18 photoinhibition was observed under the experimental light conditions (0 to 300 µmol photons

19 m^{-2} s^{-1}), which resulted in an estimation of ~2800 pmol O_2 $cell^{-1}$ d^{-1} for maximum

20 photosynthetic capacity. The P-I curve initial slope at limiting irradiance (α) was estimated at

21 70 pmol O_2 $cell^{-1}$ d^{-1} (µmol photons m^{-2} s^{-1})$^{-1}$ (95% coefficient bound: 58-88).

22 Oxygen measurements carried out at the end of the experiment (T7) showed significant

23 different dark and light respiration rates, with light respiration being lower than dark

24 respiration but not reaching net oxygen production rates (D, LL, HL) (Table 1). Moreover,

25 respiration rates were different between conditions ($p < 0.001$), with significantly lower

26 respiration rates of specimens incubated under High Light conditions than those under Dark

27 and Low Light conditions ($p < 0.05$, Fisher's LSD test).

28 PAM fluorescence rapid light curve (RLC) parameters (α, rETRmax, Ek and Eopt) showed

29 significant differences between foraminiferal species and over the duration of the experiment

30 (Figures 5 and 6). Highest rETRmax, α and Eopt were always observed in *H. germanica.*

31 After only one starvation day *A. tepida* RLC parameters dropped to zero or close to zero.

32 Contrastively, *H. germanica* RLC parameters showed a slow decrease throughout the

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1 experiment (Figures 5 and 6) with rETRmax and α decreasing from 6 to 4 and 0.22 to 0.15,

2 respectively (Figures 6A and B). The parameters Ek and Eopt stayed constant over the 7 days

3 of the experiment, with values oscillating around 30 and 90, respectively (Figures 6C and D).

4 PSII maximum quantum yields (*Fv/Fm*) were clearly affected by light and time (Figure 7).

5 Both species showed high initial *Fv/Fm* values, i.e. > 0.6 and 0.4 for *H. germanica* and *A.*

6 *tepida,* respectively (Figure 7)*.* However*, w*hile *A. tepida Fv/Fm* values quickly decreased to

7 zero after only one starvation day, *H. germanica* exhibited a large variability between light

8 conditions (D, LL, HL) throughout the duration of the experiment (Figure 7); decreasing from

9 0.65 to 0.55 in darkness (D), from 0.65 to 0.35 under low light (LL) conditions and from 0.65

10 to 0.20 under high light (HL). Using these *Fv/Fm* decreases, *H. germanica* kleptoplast

11 functional times were estimated between 11-21 days in the dark (D), 9-12 days in low light

12 (LL) and 7-8 days in high light (HL); depending if an exponential or linear model was

13 applied. *Ammonia tepida* chloroplast functional times were estimated between 1-2 days

14 (exponential and linear model, respectively) and light exposure reduced the functional time to

15 less than one day (data not shown).

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4 Discussion

4.1 Chloroplast functionality

19 Our results clearly show that only *H. germanica* was capable of carrying out net

20 photosynthesis. *Haynesina germanica* had typical diatom reflectance spectra (Figure 1),

21 showing the three major diatom pigment absorption features: chlorophyll *a*, chlorophyll *c*, and

22 fucoxanthin (Meleder et al. 2003; Jesus et al. 2008; Kazemipour et al. 2012; Meleder et al.

23 2013). Conversely, in *A. tepida* these absorption features were not detected, suggesting that

24 diatom pigments ingested by this species were quickly digested and degraded to a degree

25 where they were no longer detected by spectral reflectance measurements. These non-

26 destructive reflectance measurements are thus in accordance with other studies on benthic

27 foraminifera pigments by HPLC showing that *H. germanica* feed on benthic diatoms (Knight

28 and Mantoura, 1985). Similarly, Knight and Mantoura (1985) also detected higher

29 concentrations and less degraded diatom pigments in *H. germanica* than in *A. tepida.*

30 Furthermore, *H. germanica* has the ability to produce oxygen from low to relatively high

31 irradiance, as shown by the low compensation point (Ic) of 24 μmol photons m^{-2} s^{-1} and the

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

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

At sampling time (T0) *H. germanica* rETR and *Fv/Fm* values were similar to microphytobenthic species (i.e. *Fv/Fm* > 0.65) (Perkins et al. 2001), suggesting that the kleptoplast PSII and electron transport chain were not much affected after incorporation in the

12

foraminifers' cytoplasm. In contrast, *A. tepida* *Fv/Fm* and RLC parameters were already much lower on the sampling day and quickly decreased to almost zero within 24 hours, suggesting that plastids were not stable inside the *A. tepida* cytoplasm. Complete diatoms inside *A. tepida* were already observed in feeding studies (Le Kieffre, pers. com), this low *Fv/Fm* value might thus come from recently ingested diatoms by *A. tepida*. *Fv/Fm* has previously been used to determine kleptoplast functional times and to follow decrease in kleptoplast efficiency in other kleptoplastic organisms, e.g. the sea slug *Elysia viridi*s (Vieira et al. 2009). *Fv/Fm* measurements carried out on *H. germanica* at different light conditions showed that light had a significant effect on the estimation of kleptoplast functional time, with the longest functional time estimated at 21 days for dark condition. This time frame would qualify *H. germanica* as a long term kleptoplast retention species (Clark et al. 1990); however, our seven days estimation for the high light treatment would place *H. germanica* in the medium-term retention group. This clearly shows that light exposure has an important effect on this species kleptoplast functionality. Concerning *A. tepida,* the short dark diatom or chloroplast functional time (<2 days) places this species directly in the short or medium-term retention group.

Additionally, *H. germanica* kept in darkness showed a slow decrease of the RLC parameters, α and rETRmax, throughout the seven experimental days; this decrease is likely related to overall degradation of the light-harvesting complexes and of other components of the photosynthetic apparatus, which gradually induced a reduction of light harvesting efficiency and of carbon metabolism. This decrease was much amplified in low and high irradiance and it should be pointed out that the actual light level of the HL treatment (i.e. 70 μmol photons m^{-2} s^{-1}) is very low as compared to irradiances in their natural environment, which are easily going above 1000 μmol photons m^{-2} s^{-1}, showing that the foraminifera kleptoplasts lack the high photoregulation capacity exhibited by the benthic diatoms that they feed upon (Cartaxana et al. 2013). This is consistent with the observation at the end of the experiment that no net oxygen production was occurring under the different light conditions. Nevertheless, a small difference was still found between dark and light respiration (Table 1), suggesting that some oxygen production was still occurring but it was not sufficient to compensate for the respiration oxygen consumption. We also noticed that the respiration was higher in the foraminifera maintained in low light and dark conditions in comparison to the high light foraminifera. In the line of the lower *Fv/Fm* values observed, this suggests that kleptoplasts and possibly other metabolic pathways might have been damaged by the excess

13

of light. Clearly, in *H. germanica* light exposure had a strong effect on PSII maximum quantum efficiency and on the retention of functional kleptoplasts (Figure 7), which can explain the absence of net oxygen production after the 7 days of the experiments. Comparable results for *H. germanica* were also obtained by counting the number of chloroplasts over time with cells exposed or not to light (Lopez 1979). One of the most probable explanations for the observed *Fv/Fm* decrease is the gradual inactivation of the protein D1 in PSII reaction centres. This protein is an essential component in the electron transport chain and its turnover rate is frequently the limiting factor in PSII repair rates (reviewed in Campbell and Tyystjärvi 2012). Normally, protein D1 is encoded in the chloroplast and is rapidly degraded and resynthesized under light exposure with a turnover correlated to irradiance (Tyystjärvi and Aro 1996). However, although D1 is encoded by the chloroplast genome, its synthesis and concomitant PSII recovery require further proteins that are encoded by the algal nuclear genome (Yamaguchi et al. 2005). Thus, when D1 turnover is impaired it will induce an *Fv/Fm* decrease correlated to irradiance (Tyystjärvi and Aro 1996) consistent to what was observed in the present study. In another deep sea benthic species (*Nonionella stella*) the D1 and other plastid proteins (RuBisCO and FCP complex) were still present in the foraminifer one year after sampling (Grzymski et al. 2002). This shows that some foraminifera can retain both nuclear (FCP) and chloroplast (D1 and RuBisCO) encoded proteins. However, contrary to *H. germanica*, *N. stella* lives in deeper environments never exposed to light and thus is unlikely to carry out oxygenic photosynthesis (Grzymski et al. 2002). This fundamental difference could explain why kleptoplast functional times are much longer in *N. stella*, reaching up to one year in specimens kept in darkness (Grzymski et al. 2002). On the other hand, it has been shown that isolated chloroplasts are able to function for several months in Sacoglossan sea slugs provided with air and light in aquaria (Green et al. 2001; Rumpho et al. 2001), which demonstrates the existence of interactions between the kleptoplast and the host genomes, and/or of mechanisms facilitating and supporting such long-lasting associations. In *H. germanica* exposed to HL it is also possible that reactive oxygen species (ROS) production rates of the sequestered chloroplasts might exceed the foraminifera capacity to eliminate those ROS, thus inducing permanent damage to the foraminifera. This ROS production could also eventually damage the kleptoplasts resulting in higher kleptoplast degradation rates.

4.2 Possible advantages of kleptoplasty for intertidal benthic foraminifera

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

5 Conclusion

Comparing *H. germanica* with *A. tepida* showed that the former species potentially has the capacity of retaining functional kleptoplasts up to 21 days, much longer than *A. tepida* that showed almost no PSII activity after 24 hours. Nevertheless, the capacity of *H. germanica* to keep functional kleptoplasts was significantly decreased by exposing it even to low irradiance levels, which resulted in low *Fv/Fm* values and decreased oxygen production. This shows clearly that in our experimental conditions, *H. germanica* had reduced photoregulation capacities. These results emphasize that studies on kleptoplast photophysiology of benthic

15

foraminifera must be interpreted with care, as results are strongly influenced by the foraminiferal light history before incubation. Additionally, this study shows that the cellular machinery necessary for chloroplast maintenance is unlikely to be completely functional, suggesting that *H. germanica* has to continuously renew its chloroplasts to keep them functional. We hypothesize that kleptoplasts might have an added value by providing extra carbon and fueling nitrogen metabolic pathways to foraminifera, mainly under light exposure, but also as energy stock to be digested during food impoverished periods, in dark or light conditions.

Acknowledgements

References

Alve, E. and Murray, J.W.: Temporal variability in vertical distributions of live (stained) intertidal foraminifera, southern England. J. Foraminifer. Res. 31: 12-24, 2001.

Austin, H.A., Austin, W.E. and Paterson, D.M.: Extracellular cracking and content removal of the benthic diatom *Pleurosigma angulatum* (Quekett) by the benthic foraminifera *Haynesina germanica* (Ehrenberg). Mar. Micropaleontol. 57: 68-73, 2005.

Bernhard, J.M. and Bowser, S.S.: Benthic foraminifera of dysoxic sediments: chloroplast sequestration and functional morphology. Earth Sci. Rev. 46: 149-165, 1999.

Bouchet, V.M.P., Debenay, J.-P., Sauriau, P.-G., Radford-Knoery, J. and Soletchnik, P.: Effects of short-term environmental disturbances on living benthic foraminifera during the Pacific oyster summer mortality in the Marennes-Oleron Bay (France). Mar. Environ. Res. 64: 358-383, 2007.

Bouchet, V.M.P., Sauriau, P.-G., Debenay, J.-P., Mermillod-Blondin, F., Schmidt, S., Amiard, J.-C. and Dupas, B.: Influence of the mode of macrofauna-mediated bioturbation on the vertical distribution of living benthic foraminifera: First insight from axial tomodensitometry. J. Exp. Mar. Biol. Ecol. 371, 20-33, 2009.

Brotas, V. and Catarino, F.: Microphytobenthos primary production of Tagus estuary intertidal flats (Portugal). Neth. J. Aquat. Ecol. 29: 333-339, 1995.

16

Cartaxana, P., Domingues, N., Cruz, S., Jesus, B., Laviale, M., Serôdio, J. and Marques da Silva, J.: Photoinhibition in benthic diatom assemblages under light stress. Aqua. Microb. Ecol. 70: 87-92, 2013.

Cartaxana, P., Ruivo, M., Hubas, C., Davidson, I., Serôdio, J. and Jesus, B.: Physiological versus behavioral photoprotection in intertidal epipelic and epipsammic benthic diatom communities. J. Exp. Mar. Biol. Ecol. 405: 120-127, 2011.

Campbell, D. A., and Tyystjarvi, E.: Parameterization of photosystem II photoinactivation and repair, Biochim. Biophys. Acta-Bioenerg., 1817, 258-265, 2012.

Cesbron F., Geslin E., Le Kieffre C., Jauffrais T., Nardelli M.P., Langlet D., Mabilleau G., Jorissen, F., Jézéquel, D., Metzger E., 201X. Sequestered chloroplasts in the benthic foraminifer *Haynesina germanica*: cellular organization, oxygen fluxes and potential ecological implications. J. Foram. Res. Submitted

Cesbron F., Geslin E., Jorissen F.J., Delgard M.L., Charrieau L., Deflandre B., Jézéquel D., Anschutz P. and Metzger E.: Vertical distribution and respiration rates of benthic foraminifera: Contribution to aerobic remineralization in intertidal mudflats covered by *Zostera noltei* meadows. Estuar. Coast. Shelf Sci. In press

Clark, K.B., Jensen, K.R. and Stirts, H.M.: Survey for functional kleptoplasty among West Atlantic Ascoglossa (=Sacoglossa) (Mollusca: Opisthobranchia). Veliger. 33: 339-345, 1990.

Correia, M.J. and Lee, J.J.: Chloroplast retention by *Elphidium excavatum* (Terquem). Is it a selective process? Symbiosis. 29: 343-355, 2000.

Correia, M.J. and Lee, J.J.: Fine structure of the plastids retained by the foraminifer *Elphidium excavatum* (Terquem). Symbiosis. 32: 15-26, 2002a.

Correia, M.J. and Lee, J.J.: How long do the plastids retained by *Elphidium excavatum* (Terquem) last in their host? Symbiosis. 32: 27-37, 2002b.

Costa, J., Gimenez-Casalduero, F., Melo, R., Jesus, B.: Colour morphotypes of *Elysia timida* (Sacoglossa, Gastropoda) are determined by light acclimation in food algae. Aqua. Biol. 17: 81-89, 2012.

Curtis, N.E., Middlebrooks, M.L., Schwartz, J.A. and Pierce, S.K.: PAM analysis of 3 sacoglossan species reveals differences in photosynthetic function and chloroplast longevity. Integr. Comp. Biol. 53: 272-272, 2013.

17

1 Debenay, J.P., Bicchi, E., Goubert, E. and du Chatelet, E.A.: Spatio-temporal distribution of
2 benthic foraminifera in relation to estuarine dynamics (Vie estuary, Vendee, W France).
3 Estuar. Coast. Shelf Sci. 67: 181-197, 2006.

4 Debenay, J.-P., Guillou, J.-J., Redois, F. and Geslin, E.: Distribution trends of foraminiferal
5 assemblages in paralic environments. In: Martin, R.E. (Ed.), Environmental
6 Micropaleontology. Springer US, pp. 39-67, 2000.

7 Eberhard, S., Finazzi, G. and Wollman, F.-A.: The dynamics of photosynthesis, Annu. Rev.
8 Genet., pp. 463-515, 2008.

9 Evertsen, J., Burghardt, I., Johnsen, G. and Wagele, H.: Retention of functional chloroplasts
10 in some sacoglossans from the Indo-Pacific and Mediterranean. Mar. Biol. 151: 2159-2166,
11 2007.

12 Falkowski, P.G. and Raven, J.A.: Aquatic photosynthesis, second ed. Princeton Universty
13 Press, 2007.

14 Geslin, E., Risgaard-Petersen, N., Lombard, F., Metzger, E., Langlet, D. and Jorissen, F.:
15 Oxygen respiration rates of benthic foraminifera as measured with oxygen microsensors. J.
16 Exp. Mar. Biol. Ecol. 396: 108-114, 2011.

17 Goldstein, S.T., Bernhard, J.M. and Richardson, E.A. Chloroplast sequestration in the
18 foraminifer *Haynesina germanica*: Application of high pressure freezing and freeze
19 substitution. Microsc. Microanal. 10: 1458-1459, 2004.

20 Goldstein, S.T., Habura, A., Richardson, E.A. and Bowser, S.S.: *Xiphophaga minuta*, and *X.*
21 *allominuta*, nov. gen., nov. spp., new monothalamid Foraminifera from coastal Georgia
22 (USA): cryptic species, gametogenesis, and an unusual form of chloroplast sequestration. J.
23 Foraminifer. Res. 40: 3-15, 2010.

24 Gooday, A.J.: Meiofaunal foraminiferans from the bathyal Porcupine Seabight (northeast
25 Atlantic): size structure, standing stock, taxonomy composition, species diversity and vertical
26 distribution in the sediment. Deep-Sea Res. Part I-Oceanogr. Res. Pap. 33: 1345-1373, 1986.

27 Green B.J., Li W.-Y., Manhart J.R., Fox T.C., Summer E.J., Kennedy R.A., Pierce S.K. and
28 Rumpho M.E.: Mollusc-algal chloroplast endosymbiosis. Photosynthesis, thylakoid protein
29 maintenance, and chloroplast gene expression continue for many months in the absence of the
30 algal nucleus. Plant Physiol. 124: 331-342, 2001.

Gross, O.: Influence of temperature, oxygen and food availability on the migrational activity of bathyal benthic foraminifera: evidence by microcosm experiments. Hydrobiol. 426: 123-137, 2000.

Grzymski, J., Schofield, O.M., Falkowski, P.G. and Bernhard, J.M.: The function of plastids in the deep-sea benthic foraminifer, *Nonionella stella*. Limnol. Oceanogr. 47: 1569-1580, 2002.

Hannah, F., Rogerson, A. and Laybournparry, J.: Respiration rates and biovolumes of common benthic foraminifera (Protozoa). J. Mar. Biol. Assoc. U.K. 74: 301-312, 1994.

Hayward, B.W., Holzmann, M., Grenfell, H.R., Pawlowski, J. and Triggs, C.M.: Morphological distinction of molecular types in Ammonia - towards a taxonomic revision of the world's most commonly misidentified foraminifera. Mar. Micropal. 50: 237-271, 2004.

Hogslund, S., Revsbech, N.P., Cedhagen, T., Nielsen, L.P., and Gallardo, V.A.: Denitrification, nitrate turnover, and aerobic respiration by benthic foraminiferans in the oxygen minimum zone off Chile. J. Exp. Mar. Biol. Ecol. 359: 85-91, 2008.

Jesus, B., Brotas, V., Ribeiro, L., Mendes, C.R., Cartaxana, P. and Paterson, D.M.: Adaptations of microphytobenthos assemblages to sediment type and tidal position. Cont. Shelf Res. 29: 1624-1634, 2009.

Jesus, B., Mouget, J.-L. and Perkins, R.G.: Detection of diatom xanthophyll cycle using spectral reflectance. J. Phycol. 44: 1349-1359, 2008.

Jesus, B., Perkins, R.G., Consalvey, M., Brotas, V. and Paterson, D.M.: Effects of vertical migrations by benthic microalgae on fluorescence measurements of photophysiology. Mar. Ecol. Prog. Ser. 315: 55-66, 2006.

Jesus, B., Ventura, P. and Calado, G.: Behaviour and a functional xanthophyll cycle enhance photo-regulation mechanisms in the solar-powered sea slug Elysia timida (Risso, 1818). J. Exp. Mar. Biol. Ecol. 395: 98-105, 2010.

Kazemipour, F., Launeau, P. and Méléder, V.: Microphytobenthos biomass mapping using the optical model of diatom biofilms: Application to hyperspectral images of Bourgneuf Bay. Remote Sens. Environ. 127: 1-13, 2012.

Supprimé: Grossman, A. and Takahashi, H.: Macronutrient utilization by photosynthetic eukaryotes and the fabric of interactions. Annu. Rev. Plant Physiol. Plant Molec. Biol. 52: 163-210, 2001.¶

Knight, R. and Mantoura, R.F.C.: Chlorophyll and carotenoid pigments in foraminifera and their symbiotic algae: analysis by high performance liquid chromatography Mar. Ecol. Prog. Ser. 23: 241-249, 1985.

Lee, J.J., Lanners, E. and Ter Kuile, B.: The retention of chloroplasts by the foraminifera *Elphidium crispum*. Symbiosis. 5: 45-60, 1988.

Li, Y.H. and Gregory, S.: Diffusion of ions in sea-water and deep-sea sediments. Geochim. Cosmochim. Acta. 38: 703-714, 1974.

Lopez, E.: Algal chloroplasts in the protoplasm of three species of benthic foraminifera: taxonomic affinity, viability and persistence. Mar. Biol. 53: 201-211, 1979.

MacIntyre, H.L., Geider, R.J. and Miller, D.C.: Microphytobenthos: The ecological role of the "secret garden" of unvegetated, shallow-water marine habitats .1. Distribution, abundance and primary production. Estuaries. 19: 186-201, 1996.

Marchetti, J., Bougaran, G., Jauffrais, T., Lefebvre, S., Rouxel, C., Saint-Jean, B., Lukomska, E., Robert, R. and Cadoret, J.P.: Effects of blue light on the biochemical composition and photosynthetic activity of *Isochrysis* sp. (T-iso). J. Appl. Phycol. 25: 109-119, 2013.

Meleder, V., Barille, L., Launeau, P., Carrere, V. and Rince, Y.: Spectrometric constraint in analysis of benthic diatom biomass using monospecific cultures. Remote Sens. Environ. 88: 386-400, 2003.

Meleder, V., Laviale, M., Jesus, B., Mouget, J.L., Lavaud, J., Kazemipour, F., Launeau, P., and Barille, L.: In vivo estimation of pigment composition and optical absorption cross-section by spectroradiometry in four aquatic photosynthetic micro-organisms. J. Photochem. Photobiol. B-Biol. 129: 115-124, 2013.

Moodley, L., Middelburg, J.J., Boschker, H.T.S., Duineveld, G.C.A., Pel, R., Herman, P.M.J. and Heip, C.H.R.: Bacteria and Foraminifera: key players in a short-term deep-sea benthic response to phytodetritus. Mar. Ecol. Prog. Ser. 236: 23-29, 2002.

Morvan, J., Debenay, J.-P., Jorissen, F., Redois, F., Beneteau, E., Delplancke, M. and Amato, A.-S. Patchiness and life cycle of intertidal foraminifera: Implication for environmental and paleoenvironmental interpretation. Mar. Micropaleontol. 61: 131-154, 2006.

Mouget J.-L., Perkins R.G., Consalvey M. and Lefebvre S.: Migration or photoacclimation to prevent photoinhibition and UV-B damage in marine microphytobenthic communities. Aquat. Microb. Ecol. 52: 223-232, 2008.

Papacek, S., Celikovsky, S., Rehak, B. and Stys, D.: Experimental design for parameter estimation of two time-scale model of photosynthesis and photoinhibition in microalgae. Math. Comput. Simul. 80: 1302-1309, 2010.

Pascal, P.-Y., Dupuy, C., Richard, P., Mallet, C., du Chatelet, E.A. and Niquil, N.: Seasonal variation in consumption of benthic bacteria by meio- and macrofauna in an intertidal mudflat. Limnol. Oceanogr. 54: 1048-1059, 2009.

Perkins, R.G., Lavaud, J., Serodio, J., Mouget, J.L., Cartaxana, P., Rosa, P., Barille, L., Brotas, V. and Jesus, B.M.: Vertical cell movement is a primary response of intertidal benthic biofilms to increasing light dose. Mar. Ecol. Prog. Ser. 416: 93-103, 2010.

Perkins, R.G., Mouget, J.-L., Lefebvre, S. and Lavaud, J.: Light response curve methodology and possible implications in the application of chlorophyll fluorescence to benthic diatoms. Mar. Biol. 149: 703-712, 2006.

Perkins, R.G., Underwood, G.J.C., Brotas, V., Snow, G.C., Jesus, B. and Ribeiro, L.: Responses of microphytobenthos to light: primary production and carbohydrate allocation over an emersion period. Mar. Ecol. Prog. Ser. 223: 101-112, 2001.

Pillet, L., de Vargas, C. and Pawlowski, J.: Molecular identification of sequestered diatom chloroplasts and kleptoplastidy in foraminifera. Protist. 162: 394-404, 2011.

Pillet, L. and Pawlowski, J.: Transcriptome analysis of foraminiferan *Elphidium margaritaceum* questions the role of gene transfer in kleptoplastidy. Mol. Biol. Evol. 30: 66-69, 2013.

Pina-Ochoa, E., Hogslund, S., Geslin, E. and others: Widespread occurrence of nitrate storage and denitrification among foraminifera and gromiida. Proc. Natl. Acad. Sci. U.S.A. 107: 1148-1153, 2010.

Platt, T., Gallegos, C.L. and Harrison, W.G.: Photoinhibition of photosynthesis in natural assemblages of marine phytoplankton. J. Mar. Res. 38: 687-701, 1980.

Revsbech, N.P.: An oxygen microsensor with a guard cathode. Limnol. Oceanogr. 34: 474-478, 1989.

Rink, S., Kuhl, M., Bijma, J. and Spero, H.J.: Microsensor studies of photosynthesis and respiration in the symbiotic foraminifer *Orbulina universa*. Mar. Biol. 131: 583-595, 1998.

Risgaard-Petersen, N., Langezaal, A.M., Ingvardsen, S. and others: Evidence for complete denitrification in a benthic foraminifer. Nature. 443: 93-96, 2006.

Rumpho, M.E., Summer, E.J., Green, B.J., Fox, T.C. and Manhart, J.R.: Mollusc/algal chloroplast symbiosis: how can isolated chloroplasts continue to function for months in the cytosol of a sea slug in the absence of an algal nucleus? Zoology. 104: 303-312, 2001.

Serodio, J., Pereira, S., Furtado, J., Silva, R., Coelho, H. and Calado, R.: In vivo quantification of kleptoplastic chlorophyll a content in the "solar-powered" sea slug *Elysia viridis* using optical methods: spectral reflectance analysis and PAM fluorometry. Photochem. Photobiol. Sci. 9: 68-77, 2010.

Thibault de Chanvalon, A., Metzger, E., Mouret, A., Cesbron, F., Knoery, J., Rozuel, E., Launeau, P., Nardelli, M. P., Jorissen, F. J., and Geslin, E.: Two-dimensional distribution of living benthic foraminifera in anoxic sediment layers of an estuarine mudflat (Loire estuary, France), Biogeosciences, 12:6219-6234, 2015..

Trench, R.K., Trench, M.E. and Muscatin. L.: Symbiotic chloroplasts; their photosynthetic products and contribution to mucus synthesis in two marine slugs. Biol. Bull. 142: 335-349, 1972.

Tsuchiya, M., Toyofuku, T., Uematsu, K., Brüchert, V., Collen, J., Yamamoto, H., Kitazato, H.: Cytologic and genetic characteristics of endobiotic bacteria and kleptoplasts of *Virgulinella fragilis* (Foraminifera). J. Euk. Microbiol. 62, 454-469, 2015.

Tyystjärvi, E. and Aro, E.M.: The rate constant of photoinhibition, measured in lincomycin-treated leaves, is directly proportional to light intensity. Proc. Natl. Acad. Sci. U.S.A. 93: 2213-2218, 1996.

Ventura, P., Calado, G. and Jesus, B.: Photosynthetic efficiency and kleptoplast pigment diversity in the sea slug *Thuridilla hopei* (Verany, 1853). J. Exp. Mar. Biol. Ecol. 441: 105-109, 2013.

Vieira, S., Calado, R., Coelho, H. and Serodio, J.: Effects of light exposure on the retention of kleptoplastic photosynthetic activity in the sacoglossan mollusc *Elysia viridis*. Mar. Biol. 156: 1007-1020, 2009.

Supprimé: Teugels, B., Bouillon, S., Veuger, B., Middelburg, J.J. and Koedam, N.: Kleptoplasts mediate nitrogen acquisition in the sea slug Elysia viridis. Aquat. Biol. 4: 15-21, 2008.¶

Yamaguchi, K., Mayfield, S., and Sugita, M.: Transcriptional and Translational Regulation of Photosystem II Gene Expression. In: Wydrzynski, T., Satoh, K., Freeman, J. (Eds.), Photosystem II. Springer Netherlands, pp. 649-668, 2005.

Supprimé: Zehr, J.P., and Falkowski, P.G.: Pathway of ammonium assimilation in a marine diatom determined with radiotracer N-13. J. Phycol. 24: 588-591, 1988.¶

Table 1. Light and dark respiration rates (pmol O_2 cell^{-1} d^{-1}) ± SD of *Haynesina germanica* in the three experimental conditions (Dark, Low Light and High Light) at the end of the experiment (Df, degree of freedom, PFD Photon Flux Density).

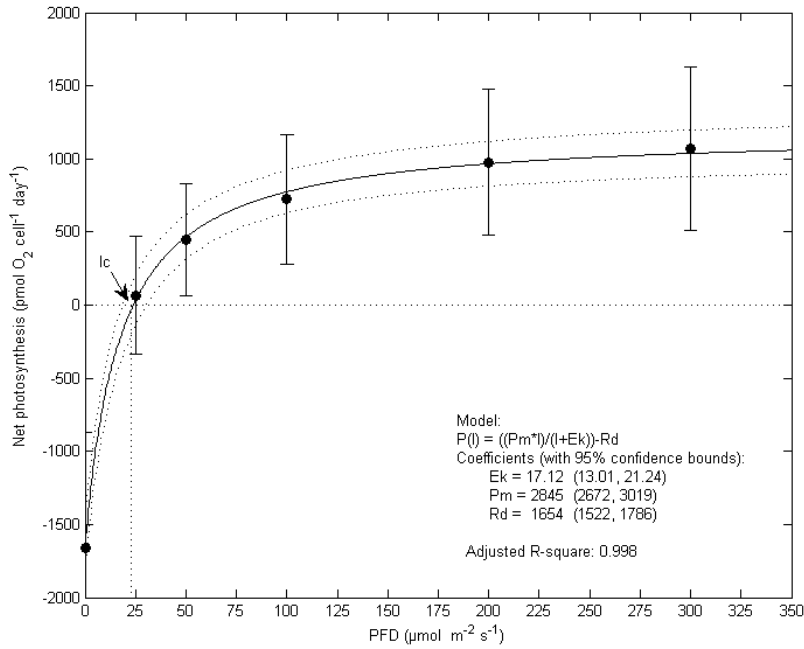| Condition	PFD	Respiration Rate (pmol O_2 cell^{-1} d^{-1})		
D	300	2452 ± 537		
	0	3542 ± 765		
LL	300	3468 ± 305		
	0	4015 ± 110		
HL	300	1179 ± 261		
	0	1905 ± 235		
Anova		Df	F-test	p
Condition	p (α=0.05)	2	13.1	<0.001
PFD	p (α=0.05)	1	5.4	0.026
Interaction	p (α=0.05)	2	0.3	0.78
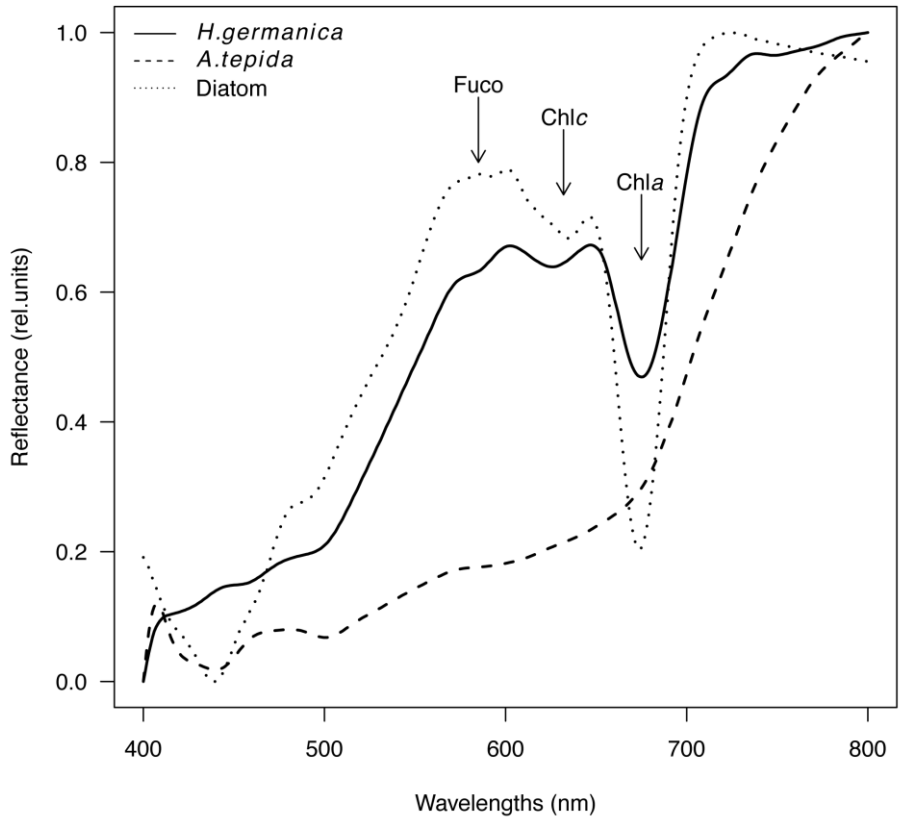
1

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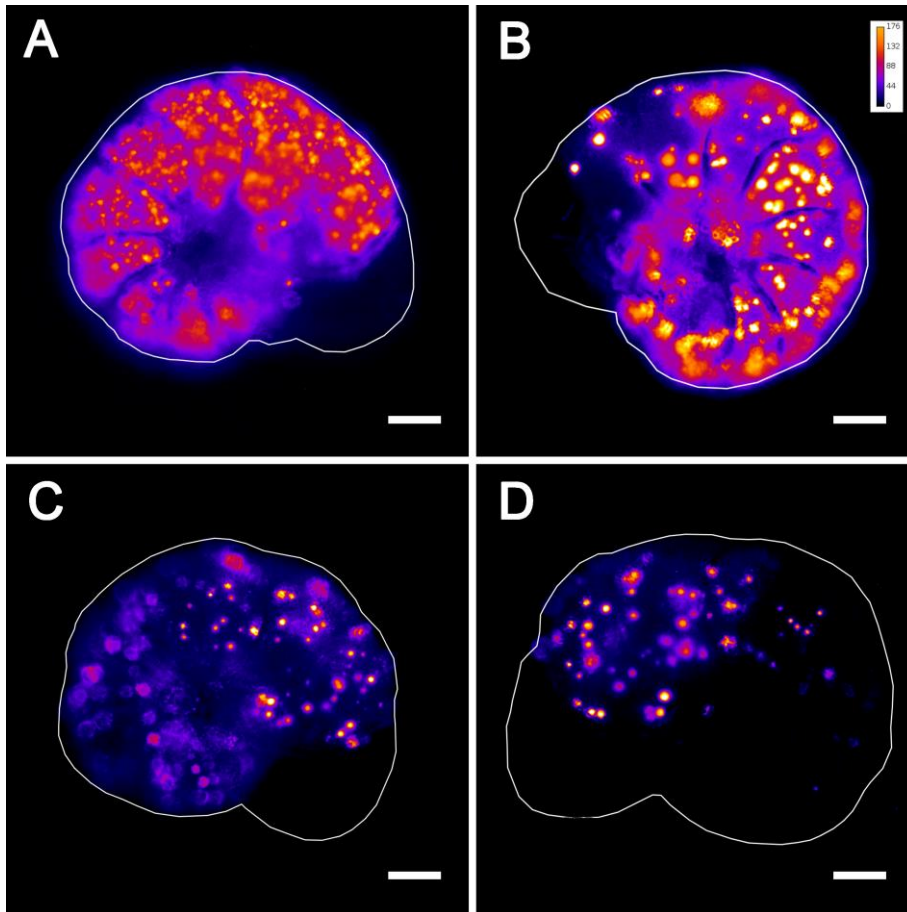
Figure 2. Illustration of *Haynesina germanica* chloroplast content at the beginning (A) and at the end of the experiment for the three experimental conditions, Dark (B), Low Light (C) and High Light (D). Higher colour scale values correspond to foraminifera emitting more fluorescence and likely containing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

26
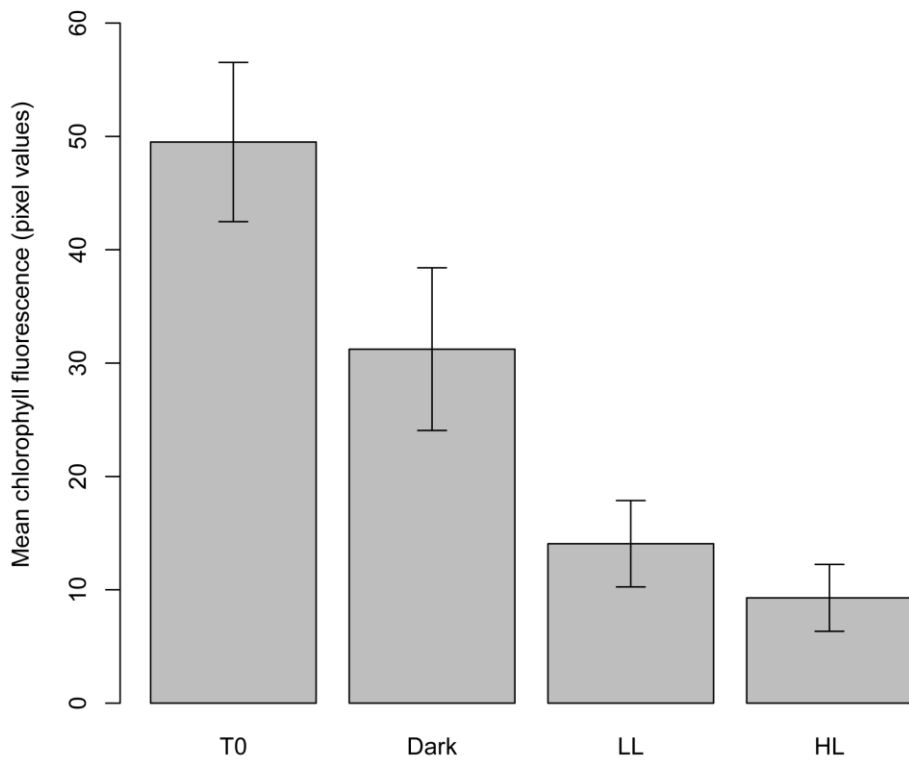
Figure 3. Mean chlorophyll *a* fluorescence (± SE, n = 30) at the end for the three experimental conditions (Dark, Low Light and High Light) and the beginning (T0) of the experiment using *Haynesina germanica*. Higher mean values likely corresponded to foraminifera containing more chlorophyll.

Model:
P(I) = ((Pm*I)/(I+Ek))-Rd
Coefficients (with 95% confidence bounds):
Ek = 17.12 (13.01, 21.24)
Pm = 2845 (2672, 3019)
Rd = 1654 (1522, 1786)

Adjusted R-square: 0.998

Net photosynthesis (pmol O_2 cell^{-1} day^{-1})

PFD (μmol m^{-2} s^{-1})

Ic

Figure 4. Net photosynthesis of *Haynesina germanica* (pmol O_2 cell^{-1} d^{-1}) as a function of the photon flux density (PFD, μmol photons m^{-2} s^{-1}). The half-saturation constant, Ek, was found at 17 (13-21), the dark respiration, Rd, at 1654 (1522-1786) pmol O_2 cell^{-1} d^{-1} and the maximum photosynthetic capacity, Pm, at 2845 (2672-3019) pmol O_2 cell^{-1} d^{-1}. The Ic, calculated compensation irradiance (24 (17-30) μmol photons m^{-2} s^{-1}). The adjusted R² of the model was equal to 0.998, n = 3.
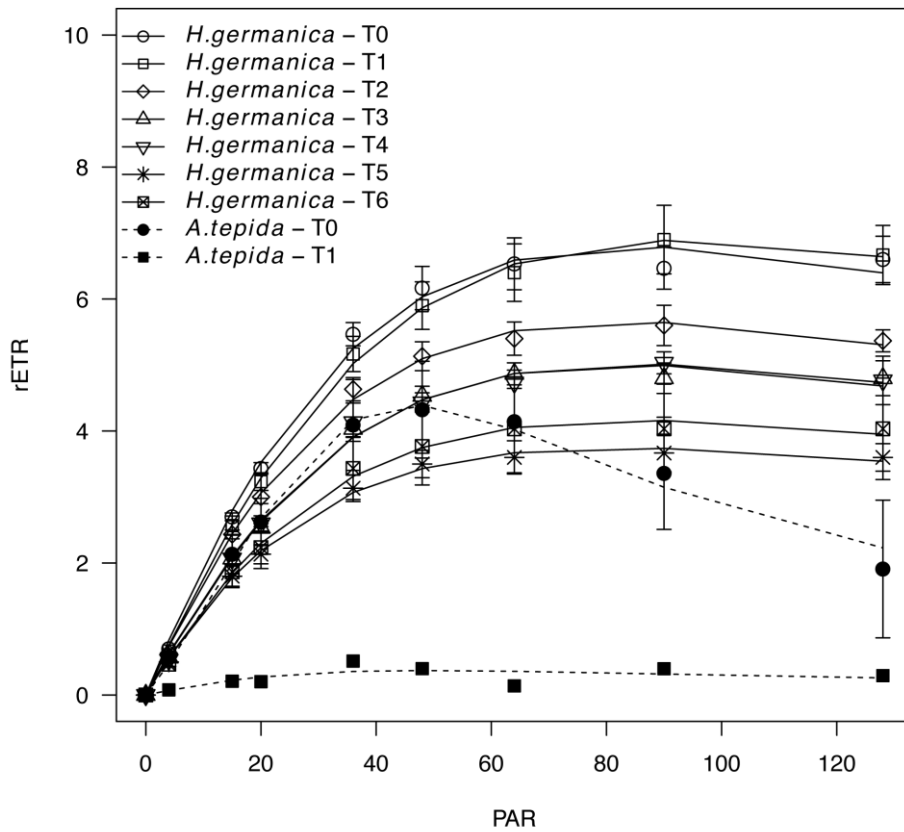
Figure 5. Rapid light curves (RLC, n = 3) expressed as the relative electron transport rate (rETR) as a function of the photosynthetic active radiation (PAR in μmol photons m^{-2} s^{-1}) of *Haynesina germanica* (black lines) and *Ammonia tepida* (black dashed lines) during the seven days of the experiment.
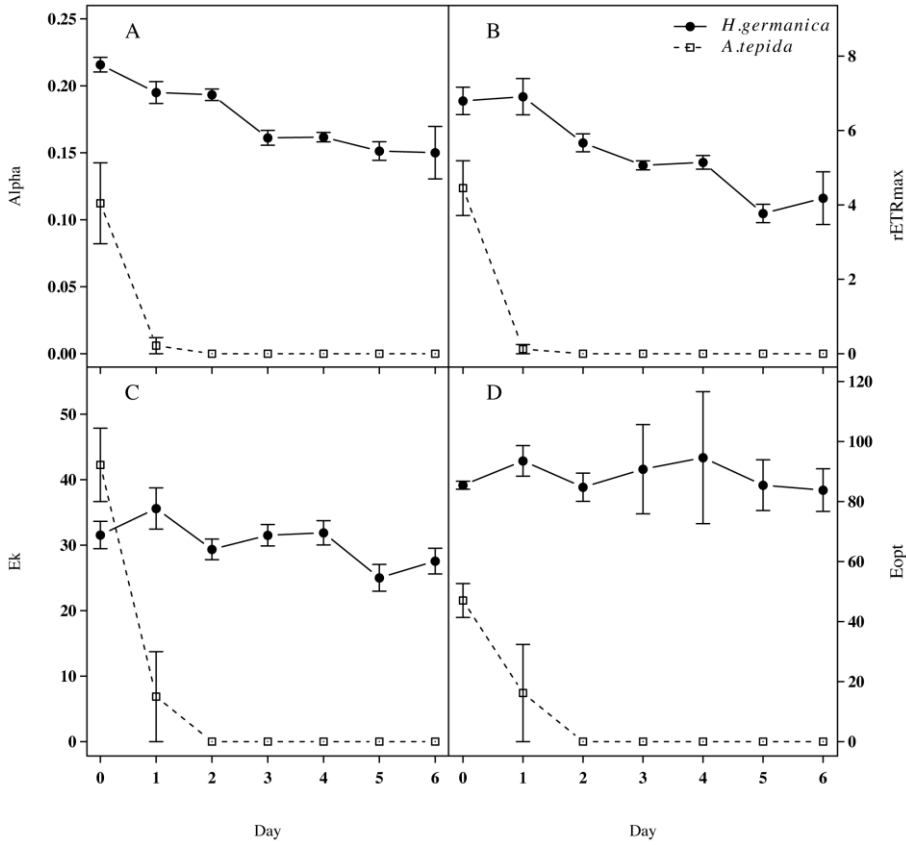
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2 Figure 6. Rapid light curve (RLC, n = 3) parameters for *Haynesina germanica* (Dark-RLC)

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).

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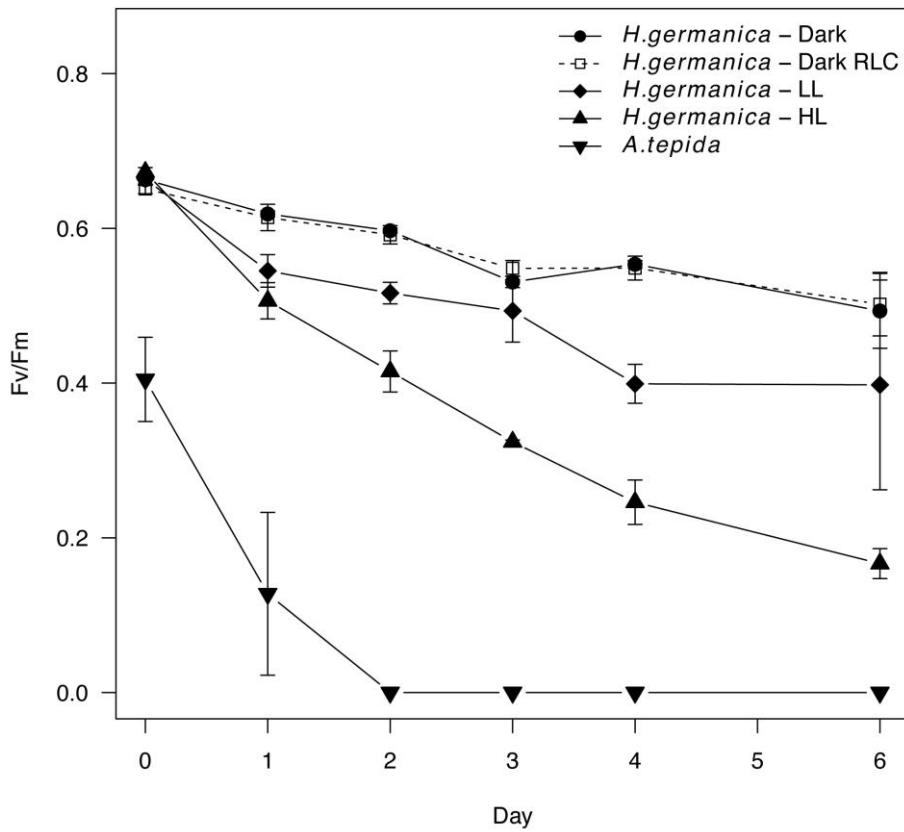
Figure 7. Maximum quantum efficiency of the photosystem II (*Fv/Fm*, n = 3) during the
experiment for the different applied conditions (Dark, Low Light and High Light) and species
(*Haynesina germanica* and *Ammonia tepida*).