



RESEARCH ARTICLE

# Photosynthetic capacity of the endosymbiotic dinoflagellate *Cladocopium* sp. is preserved during digestion of its jellyfish host *Mastigias papua* by the anemone *Entacmaea medusivora*

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**One sentence summary:** The symbiotic *Cladocopium* cells (Dinoflagellate, Symbiodiniaceae) are photosynthetically active during the whole digestion process of its host, the golden jellyfish *Mastigias papua*, by the asymbiotic sea anemone *Entacmaea medusivora*.

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## ABSTRACT

The sea anemone *Entacmaea medusivora* (Actiniaria, Anthozoa) commonly feeds on the golden jellyfish *Mastigias papua* (Rhizostomeae, Scyphozoa) which harbours an endosymbiotic dinoflagellate of the genus *Cladocopium* (Symbiodiniaceae). In this study, we monitored the photosynthetic activity of the endosymbiotic microalgae while their host jellyfish were ingested and digested by starved medusivorous anemones. By analyzing the photosynthetic yield of photosystem II, we observed that *Cladocopium* cells remain photosynthetically competent during the whole digestion process, thus confirming the exceptional resistance of Symbiodiniaceae to digestive enzymes. In the gastric cavity of *E. medusivora*, *Cladocopium* cells release oxygen, which could broadly stimulate the gastric microbiotic flora of the sea anemone. Ultimately, *E. medusivora* is not able to retain *Cladocopium* cells more than few days and physiologically-unaltered cells are therefore expelled in faecal pellets. The potential contribution of *E. medusivora* to maintain a reservoir of *Cladocopium* symbionts and its role in the life cycle of *M. papua* is discussed.

**Keywords:** endosymbiotic dinoflagellate *Cladocopium*; medusivorous sea anemone; golden jellyfish; photosynthesis; Palau meromictic lake

## INTRODUCTION

Dinoflagellates from the Symbiodiniaceae family have the capacity to establish an endosymbiotic relationship with many species of cnidarians (*e.g.* corals, sea anemones and jellyfish)

(LaJeunesse *et al.* 2018). The establishment of symbiosis implies a multi-step process involving the host and the endosymbiont, the very first step being the physical interaction between both partners. The recognition mechanisms are similar to those acting in

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the recognition of pathogenic organisms and imply the production of pattern recognition receptors (PRR) able to bind to specific microbial compounds (named microbe-associated molecular patterns or MAMPs). In the case of cnidarian–dinoflagellate symbiosis, the PRR are carbohydrate-binding proteins (e.g. lectins) that are either secreted or present at the surface of the host, while the MAMPs are glycans harbored on the cell surface of the symbionts (Davy, Allemand and Weis 2012; Fransolet, Roberty and Plumier 2012). Initial contacts between algae and animal host cells, and the subsequent phagocytosis of algal cells lead to the sorting of the symbionts engulfed in functional structures known as symbiosomes (Fransolet, Roberty and Plumier 2012). On the other hand, many cnidarians such as the actinarian *Nematostella vectensis* (Hand and Uhlinger 1992), the jellyfishes *Clytia hemisphaerica* (hydrozoan) (Houliston, Momose and Manuel 2010) and *Aurelia aurita* (scyphozoan) (Hernroth and Gröndahl 1983), some strains of the freshwater-living polyp *Hydra vulgaris* (Ishikawa et al. 2016), and even some scleractinian corals (Daly et al. 2007; Barbeitos, Romano and Lasker 2010) are asymbiotic.

In the Palau archipelago, the meromictic saline lake on the island of Mecherchar (commonly known as Jellyfish Lake) comprises an upper layer (up to 15 m deep) of oxygenated, nutrient-poor and brackish water which the asymbiotic sea anemone *Entacmaea medusivora* (Fautin and Fitt 1991) and the golden jellyfish *Mastigias papua* (De Souza and Dawson 2018) inhabit (Hamner, Gilmer and Hamner 1982). In Palau, *M. papua* usually lives in symbiosis with dinoflagellates of the genus *Cladocopium* (formerly Clade C) (Krueger et al. 2015; LaJeunesse et al. 2018). About 10% of the jellyfish protein biomass corresponds to endosymbiotic *Cladocopium* cells which reside within the mesoglea of the coelenterate (Muscatine, Wilkerson and McCloskey 1986). Because of this symbiotic relationship, *M. papua* is responsible for about 20% of the carbon primary production of the lake (Hamner, Gilmer and Hamner 1982). These jellyfish swim horizontally close to the surface following the sun position to the shadow line (Dawson and Hamner 2003) and avoid reaching the shoreline where the sea anemone *E. medusivora*, its natural predator, lives (Fautin and Fitt 1991).

*E. medusivora* is considered as asymbiotic (Fautin and Fitt 1991). However, given that this sea anemone feeds on symbiotic jellyfish, its gastric cavity is exposed to large amounts of *Cladocopium* cells. The fate of these algae upon digestion is not yet known. However, it has been documented in some symbiotic cnidarian-eating parrotfishes or sea stars that Symbiodiniaceae algae survive digestion and are present in faeces of these animals (Castro-Sanguino and Sánchez 2012; Bachman and Muller-Parker 2007). In this report, we studied the photosynthetic capacity and distribution of *Cladocopium* cells during digestion of golden jellyfish by the sea anemone *E. medusivora*.

## METHODS

### Biological materials

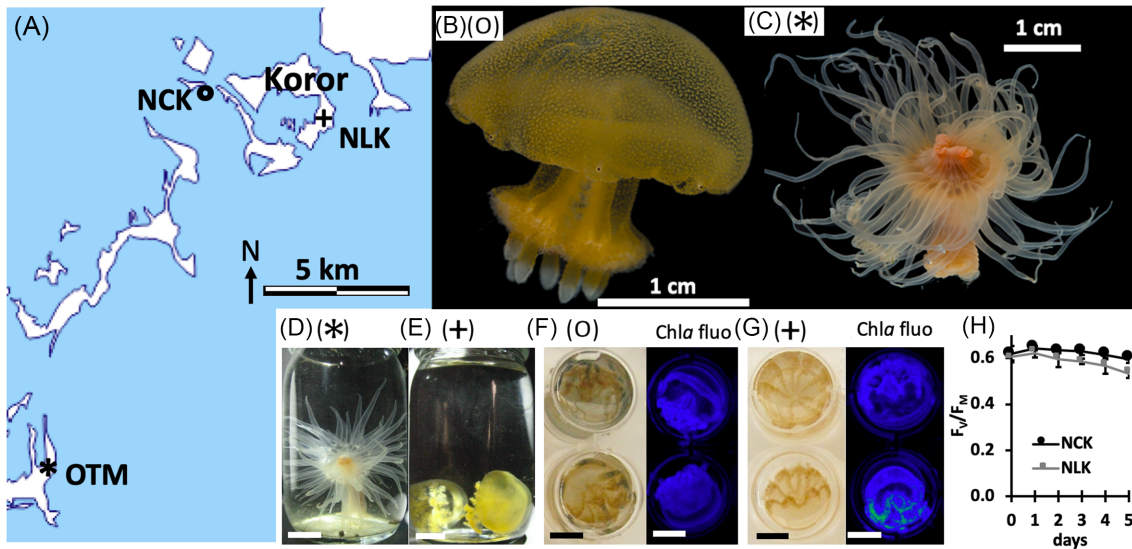
During a sampling campaign conducted in January 2018, six specimens of *E. medusivora* were collected from Jellyfish Lake (Ongeim'l Tketau, OTM) on the island of Mecherchar in the Republic of Palau. Because the population of golden jellyfish *M. papua etpisoni* in OTM dropped to zero in 2016 (the causes are most likely similar to causes of a previous event of disappearance in 1998; see Dawson, Martin and Penland 2001 for details on this matter), *M. papua remeliiki* was sampled in Ngermeuan-gel Lake (NLK) on 17 January 2018, and the ancestral *M. papua*

from Ngerchaol Cove in the Palau lagoon (NCK) on 19 January 2018 (Fig. 1A–E), under Koror State permit n° 027. Four jellyfish specimens were obtained from each site, 1.5–2 cm diameter and 1.5–2 g fresh weight. Symbiodiniaceae associated with NCK and NLK jellyfish were genotyped by T. LaJeunesse at Pennsylvania State University (according to LaJeunesse et al. 2018) which in both populations correspond to *Cladocopium* sp. (T. LaJeunesse, unpublished data). Sea anemones and jellyfish were maintained at the Palau International Coral Research Center (PICRC) for 10–14 days in diluted seawater (salinity of 25 ppt) for specimens from NLK and OTM or natural seawater (salinity of 34 ppt) for jellyfish from NCK. Sea anemones *E. medusivora* were maintained individually in 100 mL cylindrical glass jars covered with a metal mesh. They were starved for at least 7 days before being fed with one or two jellyfish (see results section). Jellyfish were maintained in 2 L open plastic containers, allowing them to swim. Light intensity was low, with a maximum of 100  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$  reached at noon. Water was changed twice a day and water temperature was maintained at  $30 \pm 1^\circ\text{C}$  (day–night variations). This temperature corresponds to the average temperature of different lakes in Palau (Dawson, Martin and Penland 2001) and to the sea surface temperature of the Western Pacific Ocean (Locarnini et al. 2010) in January. The physiological state of sampled golden jellyfish was monitored in the laboratory every day by transferring the jellyfish into standard 12-well plates and subsequently evaluating the frequency of bell contraction and the maximum quantum yield of photosystem II ( $F_V/F_M$ ) by fluorescence imaging (Fig. 1F–H).

### In vivo chlorophyll fluorescence imaging

True-colour pictures along with chlorophyll *a* fluorescence imaging were used to monitor the distribution of symbiotic microalgae and overall shape during the feeding and digestion events by anemones. Distribution of chlorophyll *a* fluorescence yield in jellyfish and sea anemones was determined at room temperature with a fluorescence imaging system (SpeedZen, BeamBio/API, France). Chlorophyll *a* fluorescence was excited by an array of blue LEDs (450–470 nm), filtered with a long-pass red filter and recorded by a CCD camera (UI-3240CP-NIR-GL Rev.2, IDS, Obersulm, Germany). The anemones change their overall shape during digestion and move inside the glass containers, so the geometry of the fluorescence imaging system is different from one image to another. Accordingly, it was not possible to quantitatively compare the overall amount of fluorescence at the different time points.

To determine if algal cells were photosynthetically active, we measured the relative electron transfer rate of photosystem II (rETR-PSII) at different continuous actinic light intensities (up to 1000  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ) using an LED array emitting at 660 nm, and also delivering 200 ms 6000  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$  saturating pulses. The maximum quantum yield of photosystem II was measured as  $F_V/F_M$ , where  $F_V = F_M - F_0$ ,  $F_0$  is the initial fluorescence level in dark-adapted sample and  $F_M$  is the maximum fluorescence level after a saturating pulse of light (Kitajima and Butler 1975). After acclimation to different light intensities,  $F_S$  and  $F_M$  (stationary fluorescence level in continuous light and maximum fluorescence emission induced by saturating pulse of light, respectively) were measured and the effective quantum yield of photosystem II at steady state ( $\phi\text{PSII} = (F_M - F_S)/F_M$ , Genty, Briantais and Baker 1989) was calculated. The rETR-PSII was calculated as the product of  $\phi\text{PSII}$  and the actinic light intensity (Ralph and Gademann 2005). Because rETR-PSII value is proportional to a ratio of fluorescence measurements, its value is



**Figure 1.** Geographic origin, aspect and size of the golden jellyfish *M. papua* and the medusivorous anemone *E. medusivora* from Palau used in this study. (A) Partial map of Palau Archipelago indicating the sampling sites: (\*) Ongeim'l Tketau on Mecherchar (OTM, Jellyfish Lake), 7°09'40.2"N 134°22'35.0"E; (+) Ngermeuangel Lake in Koror (NLK), 7°19'28.5"N 134°30'31.6"E; (o) Ngerchaol Cove in the Koror lagoon (NCK), 7°20'17.6"N 134°27'04.6"E. The map was obtained at <https://d-maps.com/carte.php?num.car=3326>. (B,F) *M. papua* from NCK. (C,D) *E. medusivora* from OTM. (E,G) *M. papua remeliiki* from NLK. (H) Maximal PSII quantum yield ( $F_v/F_m$ ) at day 0 (after sample harvesting) and during 5 days in aquariums. Measurements were performed by chlorophyll fluorescence imaging of jellyfish in standard 12-well plates (mean of four jellyfish with standard errors). Scale bars on (B–G) correspond to 1 cm.

much less affected by the geometry of our experimental setup and the data can be quantitatively compared.

### Polarographic measurements of oxygen

Measurements of light-dependent oxygen release of *M. papua* and *E. medusivora* (24 h after having being fed with one jellyfish) were carried out by using a Clark-type electrode in a 2.5 mL chamber (Oxygraph+, Hansatech, UK). The translucent acrylic chamber (DW1, Hansatech, UK) was set next to the SpeedZen camera, which was placed in a horizontal position. The same red actinic light intensity used to perform fluorescence measurements was used to monitor oxygen exchange. Measurement of light intensity was performed using a submersible spherical micro quantum sensor (US-SQS, Walz, Germany) connected to a LI-250A light meter (Li-Cor, USA).

### Electrochromic shift spectra

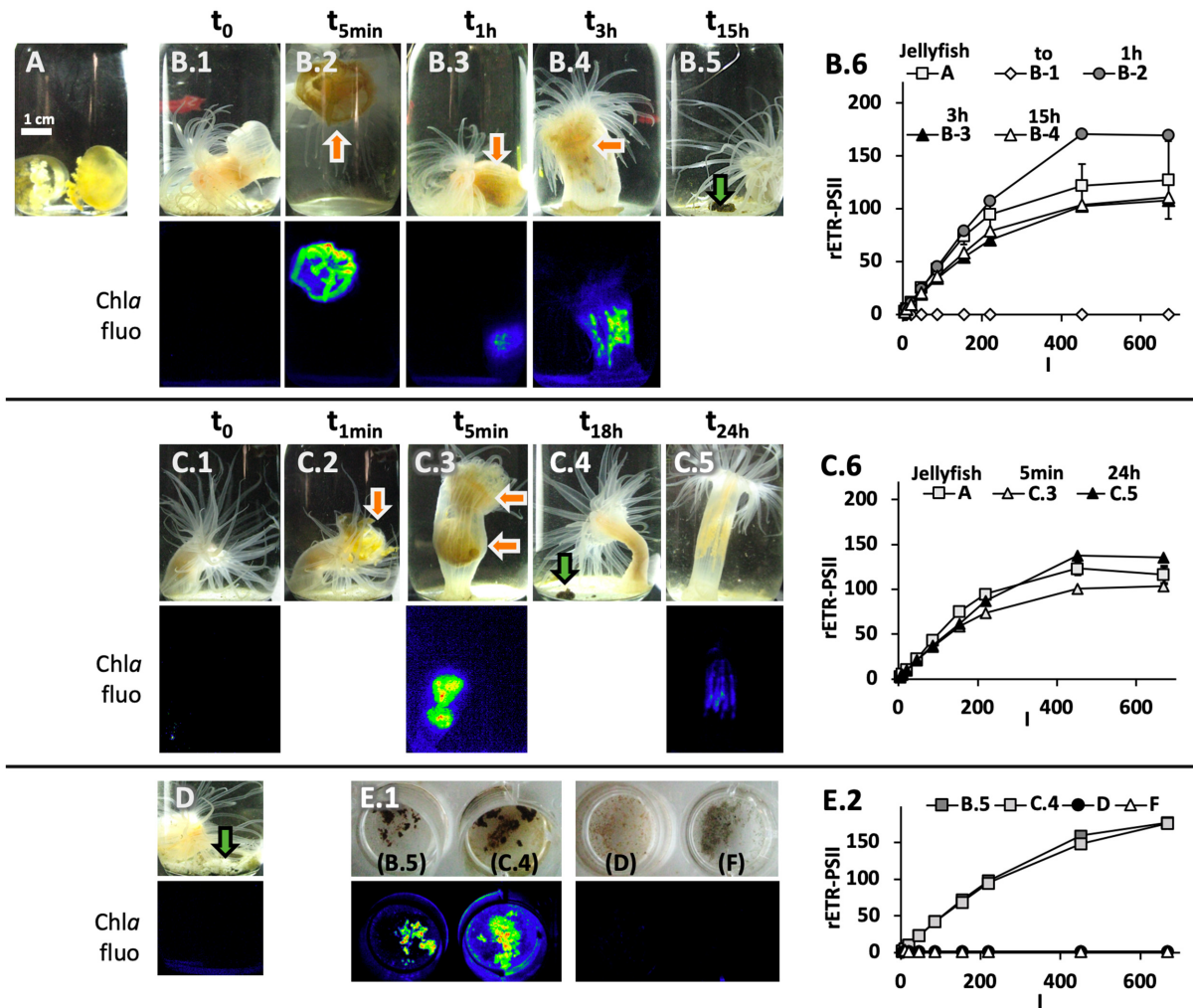
The response of photosynthetically competent microalgae to actinic radiation was also assessed by electrochromic shift signal (ECS) detection using a JTS-10 LED pump-probe spectrophotometer (Biologic, France). ECS occurs only in response to an electric potential across the thylakoid membrane (Bailleul et al. 2010). The shape of the ECS spectrum also reflects the membrane pigment composition and is therefore different between various photosynthetic eukaryote lineages (Bailleul et al. 2010). Spectral characteristics of ECS were determined from *M. papua* and *E. medusivora* 24 h after being fed with one NLK jellyfish. Absorbance changes were measured in a glass visible-spectrometer cuvette cell (path length of 1 cm), from 480 to 540 nm every 10 nm, using BrightLine (Semrock) single-band band-pass filters (full width at half maximum bandwidth of ~15 nm). They were determined during 4 ms after a short saturating pulse of red light (9000  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ , 660 nm), as previously described for cultured *Symbiodinium* (formerly temperate Clade A) cells (Roberty et al. 2014).

## RESULTS

Specimens of the sea anemone *E. medusivora* collected from Jellyfish Lake (OTM) in the Republic of Palau were on average the same size, about 3 cm high when individuals were fully extended (Fig. 1D). Before being fed, the anemones did not show any chlorophyll a fluorescence signal (Fig. 2B.1, C.1 and D). We first fed an anemone with small *M. papua* jellyfish collected at NLK. From the moment when a jellyfish was caught by anemone tentacles, it took approximately 5 min for *E. medusivora* to fully ingest their prey (Fig. 2B.2, C.2 and C.3). The shape of the jellyfish could be distinguished up to 1 h after ingestion inside the sea anemone column (Fig. 2B.3). From 3 h after ingestion, the shape of the jellyfish was no longer distinguishable and chlorophyll a fluorescence was distributed in mesenteries of the anemone (Fig. 2B.4 and C.5), indicating that digestion had occurred. The overall trend was a decrease in the chlorophyll fluorescence (Fig. 2A and 2B) that fully disappeared after 3–4 days (data not shown).

To determine if these fluorescence signals corresponded to photosynthetically active microalgae upon digestion, light saturation curves of the rETR-PSII were established. When comparing the light curve obtained with free jellyfish, ingested jellyfish (5 min–1 h) and mesenteries of anemones (from 3 h to 24 h after digestion), no significant differences were found both in the maximum rate of rETR-PSII and in the initial slope of the light curve (Fig. 2B.6 and C.6). These results strongly suggest that an important fraction of *Cladocodium* cells remain photosynthetically competent during the digestion process of symbiotic jellyfish by *E. medusivora*.

We also collected faecal pellets expelled by these anemones 12–18 h after feeding with jellyfish (Fig. 2B.5 and C.4) and these excretions were fluorescent (see B.5 and C.4 in Fig. 2E.1). In contrast, faeces from another anemone fed with mussel flesh or waste matter from a starved specimen were not fluorescent (see D and F in Fig. 2E.1). rETR-PSII of the fluorescent faecal matter (Fig. 2E.2) were similar to the one of intact or digested jellyfish.



**Figure 2.** Photosynthetic activity of *Cladocopium* upon digestion of its host golden jellyfish *M. papua remeliiki* NLK by the anemone *E. medusivora*. (A) *M. papua* from NLK (see Fig. 1 for details). A starved *E. medusivora* was fed with one (B.1–5) or two (C.1–5) NLK jellyfish. Orange arrows point to jellyfish whose shape is still visible inside the anemone. (D) *E. medusivora* was fed with (non-photosynthetic) mussel flesh collected on the shore of OTM, Jellyfish Lake. (E.1) Faeces from anemones (B.5, C.4 and D; green arrows) and waste matter at the bottom of the glass pot of a starved anemone (F) were transferred in standard 12-well plates. Imaging of the maximal yield of chlorophyll fluorescence ( $F_M$ , Chl-*a* fluo) is shown for selected pictures: before ingestion of jellyfish (B.1, C.1); at various moments during digestion of jellyfish (B.2–4, C.3, C.5); after feeding with mussels (D) and for faeces (F). (B.6, C.6, E.2) rETR-PSII as a function of the actinic light intensity ( $I$ ,  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) was calculated from chlorophyll fluorescence signals of anemones from B and C series ( $n = 1$ ) and intact jellyfish (A,  $n = 3$ ; the series shown in B.6 and C.6 correspond to independent triplicates). Scale bar in (A) corresponds to 1 cm.

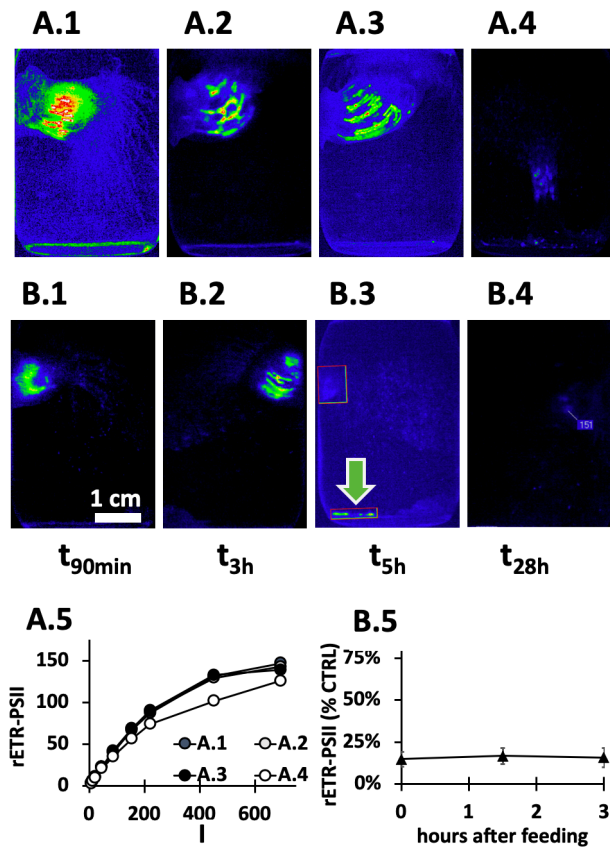
These results indicate that *Cladocopium* cells can resist digestive enzymes of *E. medusivora*.

When *M. papua* (lagoon form) collected in NCK (Fig. 1B and F) was used to feed another starved anemone, the same set of events was observed, meaning that the jellyfish is digested and *Cladocopium* cells stay photosynthetically active during digestion (Fig. 3A). Because chlorophyll fluorescence is emitted in all directions and may therefore diffuse into the animal tissues, we could not fully exclude that some *Cladocopium* cells are not located outside the mesenteries. For instance, tentacles were faintly fluorescent in some pictures (see for instance Fig. 3A.1). No chlorophyll *a* fluorescence was however detected in separated tentacles. All anemones fed only once with jellyfish (NLK or NCK) were also starved thereafter. No remaining fluorescence was detected in these animals after 3 days (data not shown).

In parallel, another *E. medusivora* was fed with a lagoon NCK jellyfish poisoned with 3-(3,4-dichlorophenyl)-1,1-dimethylurea

(DCMU), a potent inhibitor of photosystem II. Although rETR-PSII of the ingested jellyfish was initially <10% of the control (Fig. 3B.5), the same temporal set of events occurred. The overall amount of fluorescence however decreased rapidly in the DCMU-poisoned experiment, being very low 5 h after feeding (compare Fig. 3B.3 and A.3). This suggests that DCMU-poisoned *Cladocopium* cells lost their fluorescence due to damage to the photosynthetic apparatus and might have been digested or died and been expelled with excrement (green arrow in Fig. 3B.3).

To confirm that *Cladocopium* cells were still photosynthetically active inside the anemone 1 day after being fed with unpoisoned jellyfish, we examined if an ECS could be measured. A similar ECS spectrum could be determined for both types of golden jellyfish as well as for an anemone fed with a jellyfish from NLK (Fig. 4A). These ECS spectra of *Cladocopium* are similar to those of cultured *Symbiodinium microadriaticum* (formerly Clade A) cells, which are characterized by a maximum at 510 nm and a minimum at 550 nm (Roberty et al. 2014). These



**Figure 3.** Comparison of photosynthetic activities in DCMU-poisoned and unpoisoned *Cladocodium* during digestion of *M. papua* NCK by *E. medusivora*. (A.1–4) A starved *E. medusivora* was fed with *M. papua* jellyfish from NCK (see Fig. 1 for details). Pictures were obtained by *in vivo* fluorescence imaging of the maximal yield of chlorophyll fluorescence at various moments (from 90 min to 28 h) during digestion of the jellyfish. (A.5) rETR-PSII as a function of the actinic light intensity ( $I$ ,  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) was calculated from chlorophyll fluorescence signals of A series ( $n = 1$ ). (B.1–4) A starved *E. medusivora* was fed with NCK *M. papua* jellyfish poisoned by 20  $\mu\text{M}$  DCMU during 5 min of prior ingestion. (B.5) Ratio between rETR-PSII from DCMU-treated (B series) and control experiment (A series) ( $n = 1$ ). A green arrow indicates fecal pellets. Averaged values and standard deviations were calculated from values at eight different light intensities. Scale bar in (B.1) corresponds to 1 cm.

results confirm that *Cladocodium* cells were still photosynthetically active in the mesenteries and that membrane pigments responsible for ECS were not impaired.

Finally, net oxygen exchange rate ( $V_{\text{O}_2}$ ) of the anemone analyzed in Fig. 4A was compared with  $V_{\text{O}_2}$  values of an intact golden jellyfish (Fig. 4B). The dark respiratory rate per animal of both cnidarians was similar. For both organisms, a light-dependent oxygen evolution was observed. Because the experiment was performed only once we cannot comment on the absolute values. Still, this result is another confirmation that *Cladocodium* cells are able to perform photosynthesis inside the gastric cavity of the anemone.

## DISCUSSION

Overall, our observations indicate that a significant fraction of *Cladocodium* cells are not altered and remain photosynthetically active from ingestion by symbiotic jellyfish *M. papua* to expulsion of excrements by the sea anemone *E. medusivora*. The sea anemone *E. medusivora* has been found so far only in Jellyfish

Lake located on Mecherchar Island in Palau (Fautin and Fitt 1991) and in Kakaban Lake in Indonesia (Hoeksema, Tuti and Becking 2015). These anemones notably feed on various jellyfish species such as *Aurelia aurita* (the moon jellyfish), *M. papua* (golden jellyfish) and *Cassiopea* sp. (upside-down jellyfish) (Fautin and Fitt 1991; Hoeksema, Tuti and Becking 2015). These two last species live in symbiosis with photosynthetic dinoflagellates of the Symbiodiniaceae family (LaJeunesse et al. 2018). In Palau, the population of *M. papua etpisoni* disappeared from Jellyfish Lake (OTM) in 2016 following a strong El Niño—southern oscillation event—and began recovering towards the end of 2018 (Coral Reef Research Foundation, pers. comm.). Therefore, *E. medusivora* sampled in January 2018 had been deprived of photosynthetic jellyfish for several months. Upon digestion of *M. papua* by starved *E. medusivora*, the vast majority of *Cladocodium* chlorophyll fluorescence is distributed in areas that most likely correspond to mesenteries, where digestive enzymes are present. Despite the high amount of algae that remain photosynthetically active from ingestion by their host jellyfish to expulsion with undigested matter, *E. medusivora* does not establish stable symbiosis with photosynthetic *Cladocodium* cells. The lack of chlorophyll fluorescence measured *in vivo* in individuals just after sampling, or several days after feeding with *M. papua*, confirms that *E. medusivora* is asymbiotic. This is in good agreement with a previous study that failed to identify dinoflagellate cells using electron microscopy (Fautin and Fitt 1991). But this contrasts with the fact that this sea anemone belongs to a genus containing symbiotic species, such as the bubble-tip anemone *Entacmaea quadricolor* (Scott and Harrison 2007). Several assumptions can explain why ingested *Cladocodium* do not establish endosymbiosis: (1) the absence of a recognition mechanism between the two species; (2) the sea anemone recognizes *Cladocodium* as a prey; (3) *Cladocodium* is recognized and then phagocytized but the phagosome does not mature into a symbiosome and fuses with lysosomes. In this respect Fautin and Fitt (1991) suggested that some Symbiodiniaceae are phagocytized into host digestive cells when introduced with *Artemia nauplii*, but do not remain in the *E. medusivora* digestive cavity for more than 1 week. A few cases of non-photosynthetic anemones capable of establishing a symbiotic relationship with algae have however been documented, e.g. asymbiotic strains of *Hydra vulgaris* (Hydrozoa) may establish endosymbiosis with *Chlorococcum* green algae under laboratory conditions (Ishikawa et al. 2016).

Although *E. medusivora* is unable to establish a lasting relationship with *Cladocodium*, it is tempting to suggest that the transient presence of a large amount of photosynthetically active Symbiodiniaceae inside its digestive cavity may have a positive impact on the physiology of the anemone. As the body of *E. medusivora* is translucent, *Cladocodium* cells located inside the gastric cavity perform photosynthesis and release  $\text{O}_2$ . Carbon fixed by photosynthesis may also be exported by the microalgae. Indeed, it has been previously reported that isolated Symbiodiniaceae from the sea anemone *Exaiptasia* released ~5% of their photosynthates. This proportion even increased to 14–25% in response to extracts of host tissue (host-release factor; Davy and Cook 2001). Therefore, we suggest that the release of photosynthetic products may directly contribute to the metabolism of *E. medusivora*. This needs to be further investigated by the use of stable isotope tracers of carbon or nitrogen, with labelling of the microalgae before ingestion by anemones (Cleveland, Verde and Lee 2011). The beneficial effects may also be indirect through the stimulation of the bacterial community inhabiting the gastric cavity (Agostini et al. 2012; Cleary et al. 2016).

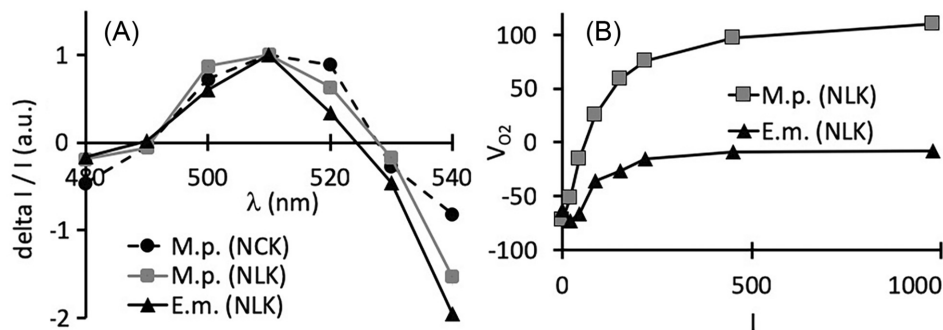


Figure 4. Comparison of photosynthetic features from golden jellyfish *M. papua remeliiki* and *E. medusivora* 24 h after feeding with NLK golden jellyfish. (A) ECS spectra upon 4 ms of illumination. (B) Net oxygen exchange rate ( $V_{O_2}$ ;  $10^{-1}$  nmol  $O_2$   $s^{-1}$  per animal,  $n = 1$ ) as a function of light intensity ( $I$ ,  $\mu$ mol photons  $m^{-2}$   $s^{-1}$ , 3 min steps). M.p. (NLK), M.p. (NCK), *M. papua* from NLK and NCK respectively; E.m. (NLK), *E. medusivora* fed with NLK golden jellyfish.

Our study also demonstrates that expelled *Cladocopium* from *E. medusivora* after digestion of *M. papua* are still photosynthetically competent. Similar results have been previously reported in other corallivorous organisms. For instance, some parrot fishes can carry Symbiodiniaceae algae in their digestive system and expel them alive within their faeces (Castro-Sanguino and Sánchez 2012). The faeces of the sea star *Dermasterias imbricata* fed with the symbiotic anemone *Anthopleura elegantissima* also contains almost physiologically-unaltered symbiotic microalgae (Bachman and Muller-Parker 2007). These observations in three different species indicate a general resistance capacity of Symbiodiniaceae to the digestion process of various animals. This is not really surprising since a method aiming to isolate intact and viable Symbiodiniaceae from host tissues requires the use of 1 M NaOH (Zamoum and Furla 2012). Such a resistance to host lysosomal enzymes could be considered as a fundamental prerequisite to the establishment of a symbiotic relationship (*e.g.* Kodama and Fujishima 2014).

Similarly to *Cassiopea* (Lampert 2016), a Rhizostomeae jellyfish closely related to the golden jellyfish, the transmission of endosymbiotic dinoflagellates in *M. papua* occurs horizontally. Indeed, eggs, testicular vesicles and planula larvae of both species are free of endosymbionts, and only scyphistomae (polyps) acquire them during growth (Sugiura 1964; Hofmann, Fitt and Fleck 1996). The colonization of the polyps is even required to initiate the strobilation and the production of symbiont-containing ephyra larvae and adults of *M. papua* (Sugiura 1964). Consequently, the Symbiodiniaceae community hosted by the jellyfish necessarily originates from an environmental reservoir of free-living symbionts present in sediments and in the water column. This reservoir can be constituted by the release of Symbiodiniaceae (1) from *Mastigias* polyps, ephyra and adults into the surrounding water (Sugiura 1964), release that could be constant as in symbiotic corals (Hoegh-Guldberg, McCloskey and Muscatine 1987); (2) from the degradation of dead adult jellyfish; (3) from predators feeding on cnidarians (Muller-Parker 1984; Castro-Sanguino and Sánchez 2012; Bachman and Muller-Parker 2007).

Based on the results presented in this report, we propose that, by feeding on *M. papua*, the medusivorous anemone could significantly contribute to increase the speed of algal release. Because *Mastigias scyphistomae* and *E. medusivora* are living in close vicinity to each other in Jellyfish Lake (Dawson 2005), this proximity could contribute to the maintenance of an environmental reservoir of free-living *Cladocopium*. Hence, by eating some jellyfish, and participating in microalgal cycling, *E. medusivora* could play a key role in the life cycle of *M. papua*.

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