



# Biophysical analysis of bioenergetics on coral slices

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## Introduction

Corals possess endosymbiotic dinoflagellate microalgae from the *Symbiodiniaceae* family (Lajeunesse et al. 2018). Algal symbionts provide energy to the animal cells by exporting photosynthetic products.

Many biophysical studies on photosynthesis have been conducted on cultured symbiotic dinoflagellate (e.g. Hennige et al. 2009), but few less on symbiotic corals due to technical constraints to have optically optimal biological samples (e.g. Szabó et al. 2017).

Here we describe the use of small fragments of the model coral *Stylophora pistillata* to study bioenergetic processes. Our aim is to understand the role of respiration and photosynthesis and their regulatory mechanisms in the healthiness of coral reefs.

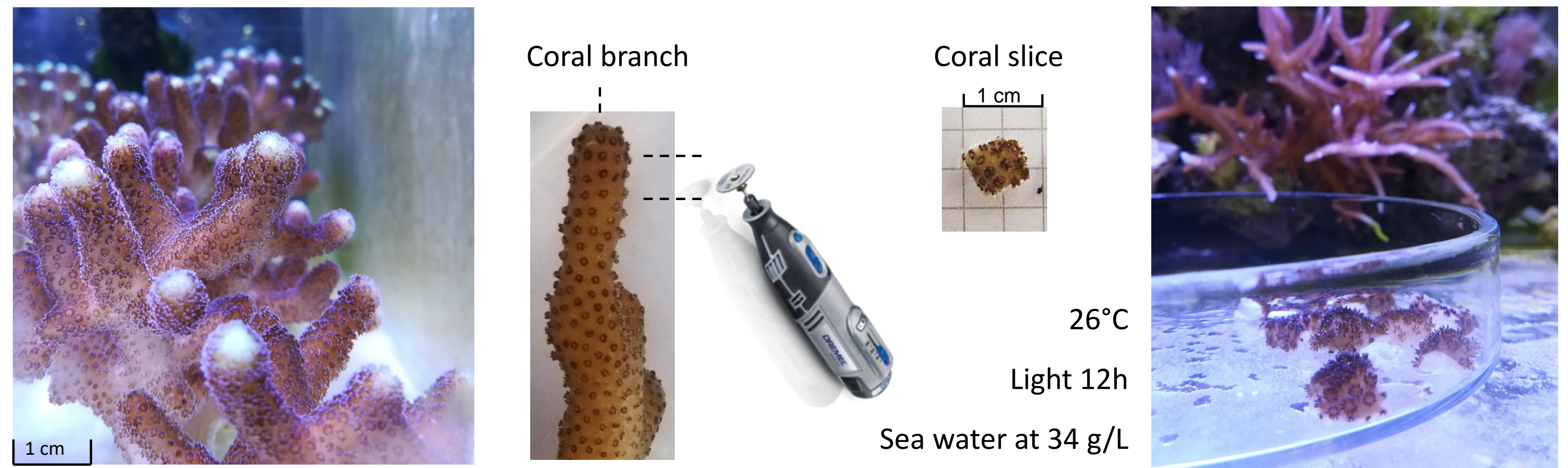


Figure 1. Coral fragmentation to obtain small slices suitable for biophysical analysis.

## I. Photosystem II

Coral slices were obtained from different coral colonies of *S. pistillata* (Figure 1). Their good survival was monitored by following the maximum quantum yield of photosystem II (Fv/Fm) during two days (Figure 2).

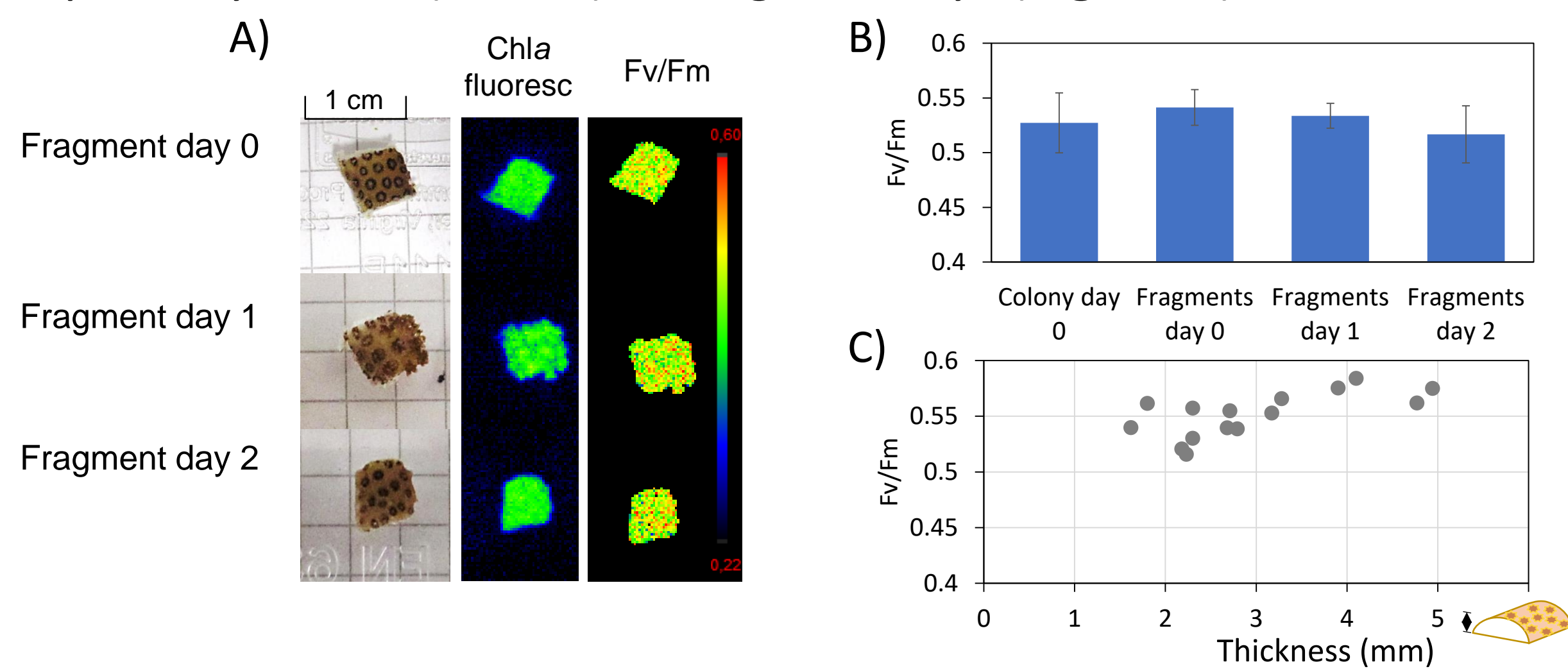


Figure 2. Monitoring of coral slices after fragmentation. A) True color images and false color chlorophyll *a* fluorescence images showing the general appearance of one coral fragment. B) Coral fragments showed no variation in Fv/Fm during two days of cutting compared with a coral colony. C) Fv/Fm of coral fragments of different thickness 1 day after cutting.

Relative electron transport rate of photosystem II (rETR PSII) calculated from chlorophyll *a* fluorescence yield and oxygen evolution were determined at several actinic light intensities (Figure 3). Photosynthesis-irradiance curves were very similar to those previously reported (Sorek and Levy 2012). Both parameters showed a positive linear relationship in contrast to the one obtained with cultured algae (Roberty et al. 2014). Respiration was also measured in the dark, and the average value (21  $\mu\text{mol}/\text{cm}^2/\text{d}$ ) was comparable to the value reported previously for whole coral nubbins (Holcomb et al. 2014).

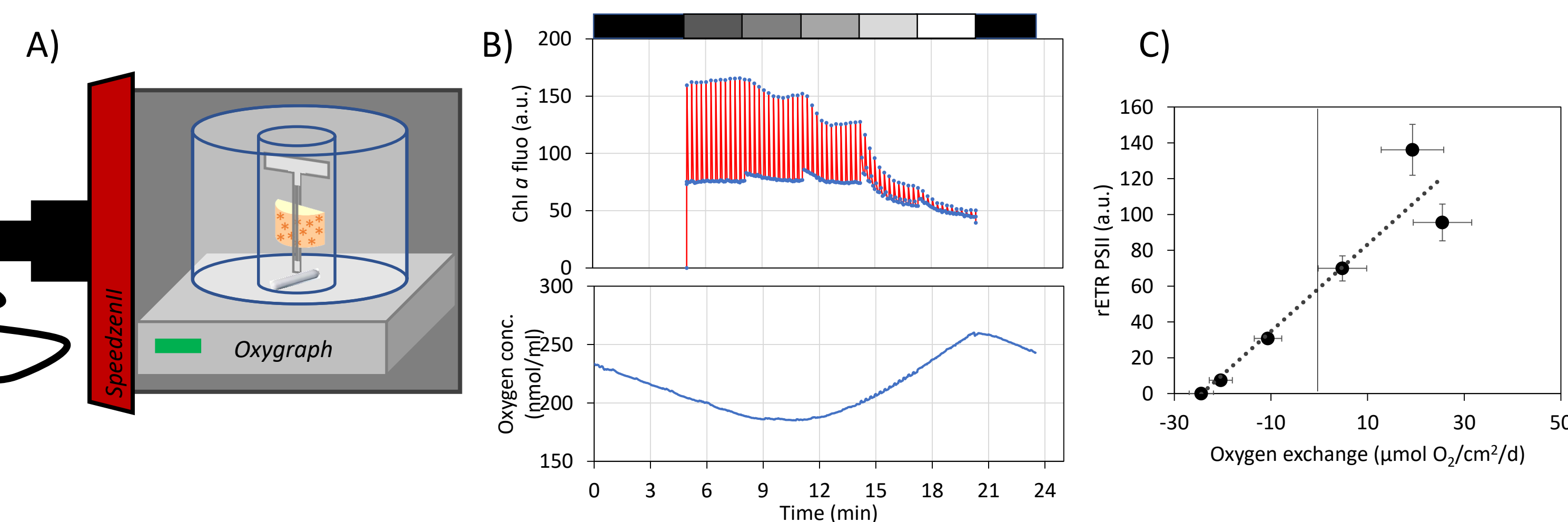


Figure 3. A) Coral slices were set up in a 3 ml translucent chamber for oxygen exchange measurement (Hansatech, UK). The chamber was placed inside the SpeedZen camera (BeamBio, France) for simultaneous measurement of Chl *a* fluorescence yield at different light intensities. B) (up) Example of fluorescence trace; (down) example of oxygen exchange trace. Light intensities are indicated by the grey scale. C) Relative electron transport rate of photosystem II (rETR PSII) in function of oxygen evolution.

## Conclusions

Coral slices survive after being cut from a coral colony of *S. pistillata* in aquarium conditions. The small size of fragments allows a better handling of the sample for the use of typical oxygen exchange measurement systems and spectrophotometer apparatus. Moreover, its optical properties allow absorbance measurement of P700 photo-oxidation. Our method of fragment slices was tested on various coral species from acid and control sites in a coral reef in Palau. Important differences were obtained in PSI activity in different coral species.

## II. Photosystem I

Photosystem I activity was determined by recording P700 absorbance changes at 705 nm with a JTS-10 (BioLogic, France). Coral slices were maintained in a 1 cm square spectrophotometer cuvette (Figure 4). Although the amplitude of absorbance change is low, the signal is stable (no drift) and the noise is low. Light dependent activity of PSI was then tested for different light intensities and compared to PSII activity.

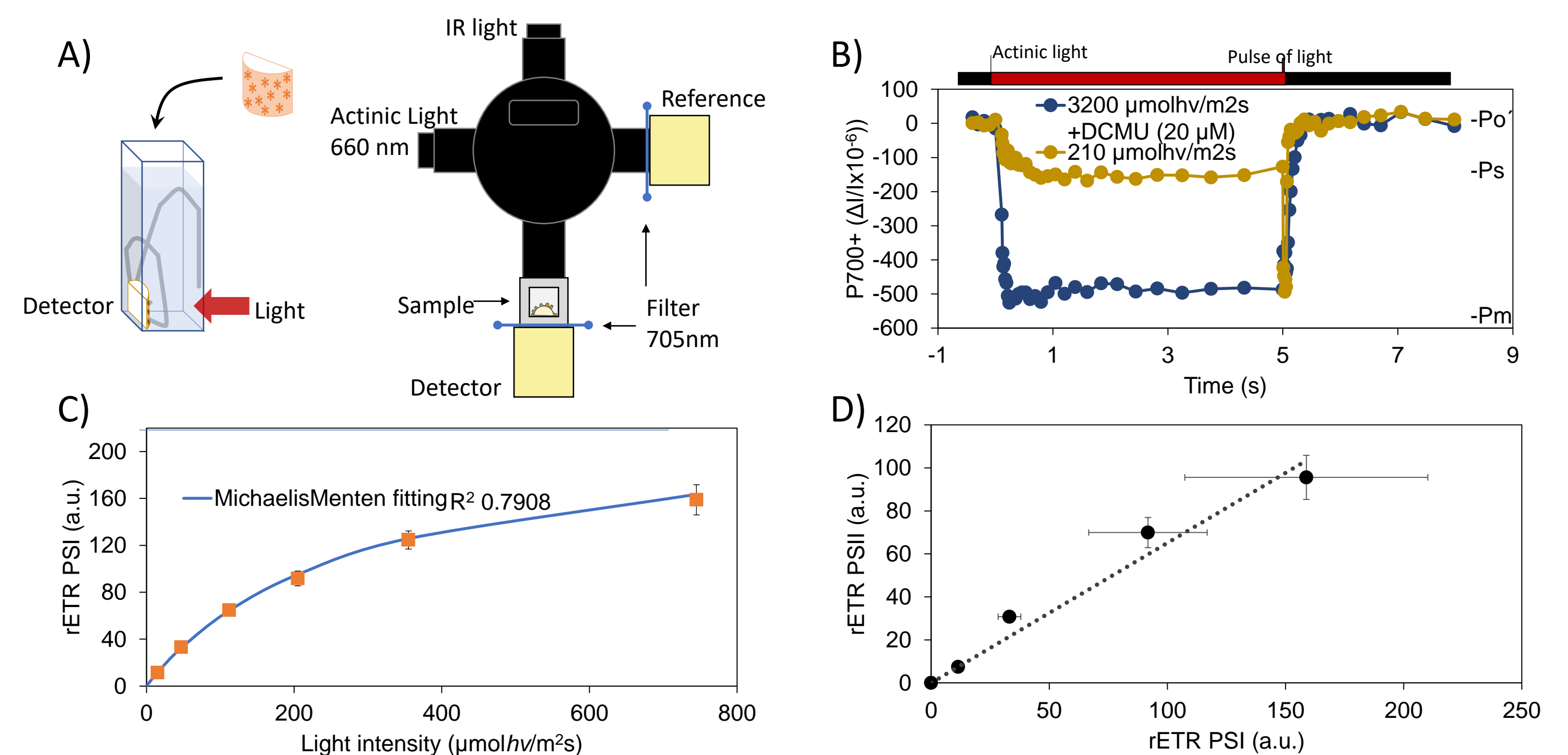


Figure 4. P700 absorbance changes at 705 nm. A) Coral slice maintained inside a cuvette for measurement in the JTS-10 system. B) Examples of absorption changes upon light to dark changes in presence or absence of DCMU. C) Relative electron transport rate of photosystem I (rETR PSI) under different light intensities. D) PSI and PSII activity comparison.

## III. Photosynthesis of corals from the field

Coral colonies (*Pocillopora*, *Porites*, *Pachyseris* and *Acropora* sp.) from Uchelbeluu reef (pH 8.05) and Nikko bay (acidic site, pH 7.84; Barkley et al. 2015) in Palau (Western Indo-Pacific Ocean) were sampled by scuba diving at 3-5 m depth. Small fragments were prepared and analyzed for chlorophyll *a* fluorescence yield and P700 absorbance change as described here above (Figure 5).

When comparing PSI activity under high light for the same species between the two sampling sites, we observed higher values for two species from acidic site (*Pachyseris* and *Acropora*) and for one species of control site (*Porites*). The values were identical for *Pocillopora* sampled in the two sites.

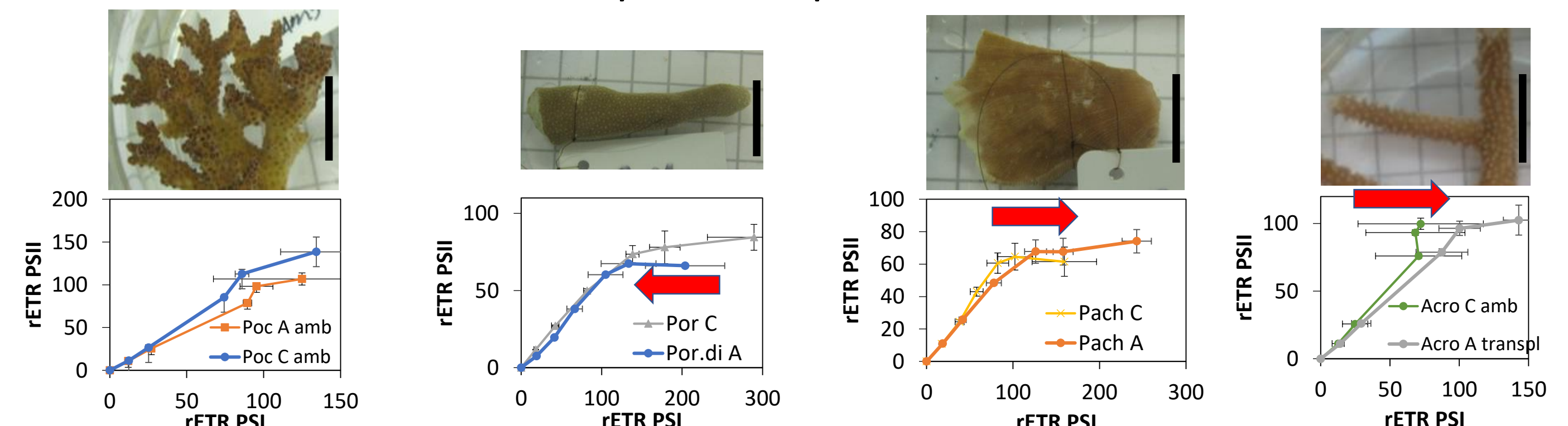


Figure 5. PSI and PSII activity of corals from Palau reefs. Poc - *Pocillopora*, Por - *Porites*, Pach - *Pachyseris* and Acro - *Acropora*, were obtained from Uchelbeluu reef (control site) and Nikko bay (acidic site). PSI and PSII activity were measured and compared. Red arrows indicate the difference in PSI activity between the samples of acidic (A) and control sites (C). Bar: 1 cm.

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