Primary production assessment on eco-engineering infrastructures: English Channel case study

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Abstract. In the Marineff project, experimental eco-engineering dyke modules were immersed in 2020 in the Bay of Cherbourg (English Channel). Eco-engineering and classic dyke modules were compared regarding biodiversity and productivity. This paper presents the monitoring strategy of primary production assessment applied. Primary production assessment was performed by coupling various technics in order to consider all the compartments of primary producers present on the dykes and in the associated surrounding water mass. Seaweeds, microphytobenthos and phytoplankton were studied. Benthic chambers measuring oxygen and pCO_2 under controlled light, and technics based on variable fluorescence were applied on eco-engineering and classic dyke modules. Preliminary results are presented in order to illustrate the expected indicator and methodology improvement and limits were discussed. The whole data set will allow us to explore the relationship between productivity and biodiversity as a function of the sites and of the nature of the infrastructure, eco-engineering or not. At this stage of our study we pointed out that the macroalgae were 7.9 times more productive than biofilm per surface on eco-engineering module whilst only 1.5 times on classic module. Smooth surface appeared to favour biofilm productivity at this stage of colonisation while rough surface drove more quickly the system to a production dominated by macroalgae.

1. Introduction

Coastal artificialisation is a widespread phenomenon that leads to irreversible loss in many ecosystems. Artificialisation is a highly visible footprint of anthropic activities related to commercial exchanges, energy production, fisheries, tourism, leisure activities, etc. A reduction of this footprint is required but is socially and economically complex [1, 2]. Marine eco-engineering infrastructures can limit negative effects and enhance the ecological status of artificialized areas [2]. Artificialisation of shallow coastal ecosystems by marine infrastructures offers new substrates for benthic microalgae and macroalgae which may increase local primary production [1]. Primary production is at the base of trophic network and drives many chemical cycles at local and global scales [3-6]. Oxygenic primary production, which

represents the production of chemical energy in organic compounds by photosynthetic organisms is one of the major ecological functions of ecosystem in photic area [7, 8]. Macroalgae (i.e. seaweeds) which belong to three phyla or classes, Ulvophyceae (Chlorophyta - green algae), Rhodophyta (red algae) and Phaeophyceae (Ochrophyta - brown algae); microalgae which regroups 10 eukaryotic phyla including various taxa like diatoms, dinoflagellates, Prymnesiophyceae [9], seagrasses (Angiosperm) and Cyanobacteria which belong to prokaryotes represent a very large biodiversity of primary producers. The functioning of these ecosystems and the interaction between benthic flora and fauna influence also the phytoplankton of water masses surrounding [2, 10]. An assessment of the whole primary production related to artificial infrastructure is then required to characterize the functioning and the ecological trajectories of these new ecosystems [1, 2]. Methods allowing primary production measurements of the different components of primary producers have to be deployed in parallel. Several technics based on O_2, CO_2, carbon isotopes, or fluorescence variations measurements were commonly used to assess primary production [11, 12]. Coupling different technics allow to consider various organisms and processes as a function of the primary producer size and time scale. In this context, marine eco-engineering infrastructures were developed to improve ecological impacts of artificialized areas [1, 2]. In the European INTERREG Va MARINEFF project, experimental eco-engineering dyke modules and classic modules were immersed in September 2020 in the bay of Cherbourg and a monitoring of primary production dynamics was initiated. In the present paper the selected main protocols are briefly described and preliminary results are presented and discussed.

2. Materials & methods

2.1. Sampling site

Twelve dyke modules were immerged into 10 m depth on sandy soft bottom in the bay of Cherbourg in September 2020. Two types were deployed: six classic modules corresponding to concrete cubes of 1.7 m^3 with smooth external texture (Figure 1a) and six eco-engineering modules corresponding to concrete cube of 1.7 m^3 with rough external texture and various holes, gutters, overhangs, grooves of various sizes and positions to promote biological colonisation (Figure 1b, Figure 2). Eco-engineering modules were designed by MARINEFF project patterns (University of Caen Normandie, TPC - VINCI Construction France, and ESITC) and produced by TPC - VINCI Construction France.

a b

Figure 1. Dyke modules deployed during the MARINEFF project: a: classic module; b: Eco-engineering module.

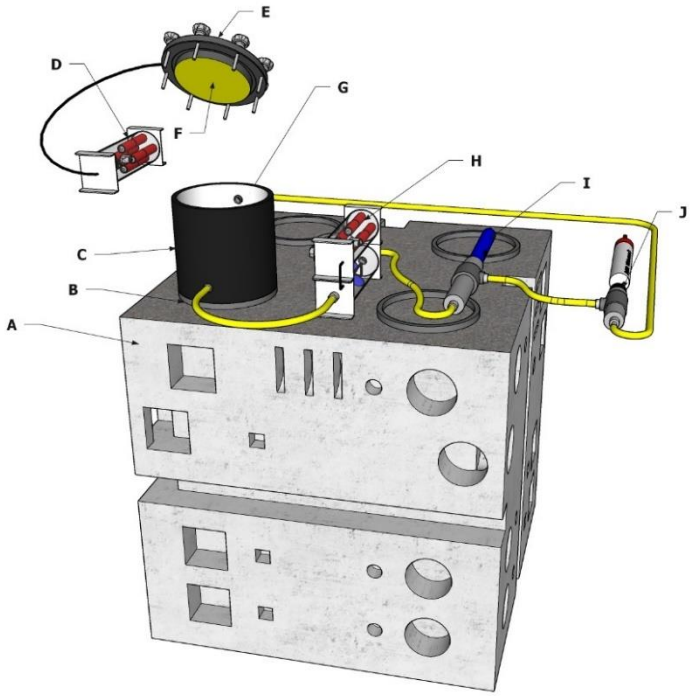

Figure 2. Automated benthic chamber for primary production measurement. (A) MARINEFF eco-engineering dyke module with the mooring ring (B) to fix the benthic chamber (C). Aluminium tape (G) covers the benthic chamber inside. LED panel (F) is fixed to the benthic chamber top (E) which is screwed on the benthic chamber to close it hermetically. The LED panel is driven by the LED controller (D). Water flow is generated by an underwater pump (H). Two sensors, YSI EXO 1 (I) with oxygen probe, and Turner Design C-Sense pCO2 probe (J) driven by the RBR Virtuoso3 logger (J), measures the gas concentration. The whole system is in a water closed circuit connected by pipe.

2.2. Primary production assessments (Figure 3)

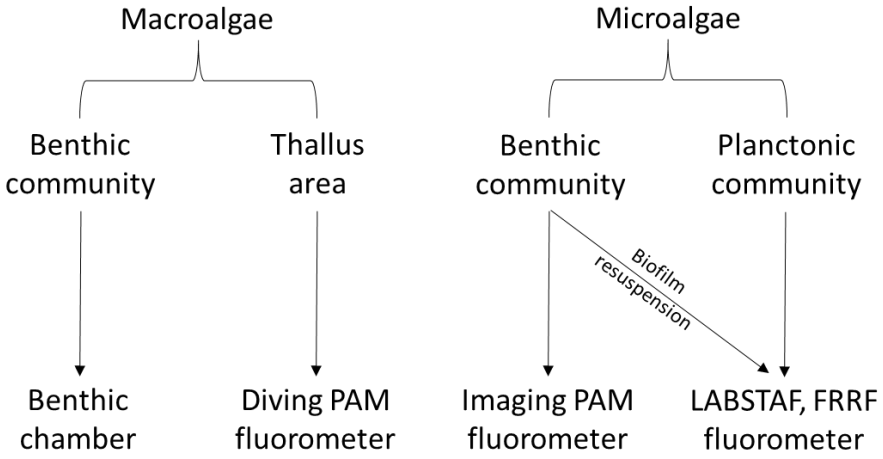

Figure 3. Monitoring strategy deployed for primary production assessment on eco-engineering infrastructures during the MARINEFF project.

2.2.1. *Benthic chamber*. A fully automated system for measurements of photosynthetic oxygen and CO_2 exchange was developed in the MARINEFF project (Figure 2). The benthic chamber allows generating light gradient between 0 to 900 µmol photons m^{-2} s^{-1} produced by white LEDs with maximum pics at 450 nm and 600 nm (Figure 4). Light intensity and the duration of each light step are

controlled by a programmable controller unit. Photoactive radiation (PAR) intensity of each step was measured using a RBR solo3 PAR logger connected to a Li-COR "Underwater Quantum Sensor" LI-192. Dissolved oxygen was recorded with an EXO Optical Dissolved Oxygen Sensor and pCO2 by a Turner Designs C-sense pCO_2 probe every 30 seconds. Temperature and pH were measured at the same frequency in the chamber. A pump guarantied the water homogenisation during the incubations. In the present experiment, 7 light steps of 15 min each from 0 to 750 µmol photons m^{-2} s^{-1} were applied. A dark phase was applied after the last light step in order to estimate the respiration rate. Because O_2 evolution consumption in the light, may differ from dark O_2 consumption, the respiration rate was estimated during 5 min just after the last light phase [13]. O_2 measurements were used to estimate respectively net primary production (NPP) and benthic community respiration (BCR) of the phyto- and zoo-benthos community [14]. GPP was then calculated from NPP and BCR measurements (GPP = NPP + BCR). pCO_2 measurements have to be associated to alkalinity and/or DIC measures at the beginning and at the end of incubations to be properly exploited [15]. This step of the experiments is not presented in this paper.

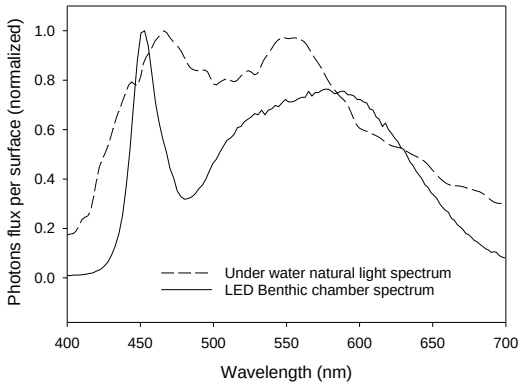

Figure 4. LED spectrum used in the automated benthic chamber and under water natural light spectrum.

2.2.2. Variable fluorescence measurements

2.2.2.1. *Chlorophyll- a fluorescence induction pulse amplitude modulation (PAM).* Diving and imaging PAM (WaltzTm, Effeltrich, Germany) are used in the assessment protocol. Imagining PAM is not present in this paper. See Vivier et al. [16] for more details. The maximum quantum yield (Fv/Fm in the dark) and the effective photochemical efficiency (Fq'/Fm' in the light), were measured on the module by scuba divers on several species of macroalgae using a Diving PAMII (WaltzTm, Effeltrich, Germany) [17].The macroalgae sample placed in the dark was excited by a weak blue measuring light (1 µmol m^{-2} s^{-1}, 474 nm, frequency 0.6 kHz) to record minimum fluorescence (F_0). The optical fiber of the PAM was positioned exactly 5 mm from the surface of the macroalgae. Maximum fluorescence (Fm) was measured using a multiple turnover flash method [17]. We used a saturating blue light pulse (0.6 s, 4000 µmol m^{-2} s^{-1} at 474 nm) produced by a LED allowing reduction of the quinone A (QA), quinone B (QB) and part of plastoquinone (PQ) pools.

Fv/Fm was calculated according to the following equation [18]:

$$\frac{F_v}{F_m} = \frac{(F_m - F_0)}{F_m}$$

The samples were exposed to 10 irradiances (E) from 0 to 1000 µmol photons m^{-2} s^{-1} at 474 nm for 20 seconds at each step. Steady state fluorescence (Fs) and maximum fluorescence (Fm') were measured. The effective quantum efficiency of PSII for each irradiance was determined as follows [18]:

$$\frac{\Delta F}{F_{m'}} = \frac{(F_{m'} - F_s)}{F_{m'}}$$

The electron transport rate (ETR, µmol electrons m^{-2} s^{-1}) was calculated for each irradiance as described by Figueroa et al. [19]. ETR is a measure of the rate of linear electron transport through photosystem II, which is correlated with the overall photosynthetic performance of the algae. In order to properly estimate the ETR, light absorption (A) has to be measured in laboratory on sample collected on dyke modules.

$$ETR(E) = \mathrm{AQ}_\lambda \times FII \times \frac{\Delta F}{F_{m'}}$$

Where AQ_λ is the absorbed quanta calculated as the product of the integration of the spectral absorptance (A_λ) between 400-700 nm and spectral irradiance of the light source (E_λ), FII is the fraction of AQ directed to PS II including its light harvesting complexes. FII for Rhodophyta is about 0.15 and 0.5 for Ulvophyceae and Phaeophyceae [19-21]. The absorptance (A_λ) was determined for each 0.5 nm between 400 and 700 nm by means of an integrating sphere installed in a Shimadzu UV2600i spectrophotometer [19]. Fluorescence excitation spectra from 400 to 700 nm with emission measured at 730 nm was performed using a Shimadzu Spectrofluorometer RF-6000 [19].

2.2.2.2. *LabSTAF fluorometer*. Photosynthetic parameters of the phytoplankton and of the resuspended microalgal biofilm were determined by using a LabSTAF (Chelsea Technologies, UK) fluorimeter. The microalgal biofilm was collected by scuba divers using a syringe of 1.66 L specially designed for this sampling. 25 cm^2 were resuspended using toothbrush and drawn into the syringe. The LabSTAF allows measurements at different wavelengths. A single turnover (ST) saturation phase is delivered with a solid flash if 100 µs to measure minimal and maximum fluorescence as described in Boatman et al. (2019) [22]. Samples were exposed to 12 intensities of increasing PAR (0 – 1,500 µmol photon. $s^{-1}.m^{-2}$) with 30-second intervals in order to obtain fluorescent-light curve (FLC). The Labstaf enables to measure the electron flux from the PSII per volume unit (JV_{PII}, mmol electrons m^{-3} h^{-1}) which allowed to estimate the oxygen production (GO_{PII}) in mmol O_2 m^{-2} h^{-1}.

$$JV_{PII} = a_{LHII} \times rETR$$

With a_{LHII} the absorption coefficient of PSII in m^{-1} (see Boatman et al. (2019) [22] for details). The LabSTAF also allows to measure the absorption cross section of PSII, $Sigma_{PSII}$ ($nm2.PSII^{-1}$) at different wavelengths. The same measurements were performed on water samples around modules in order to estimate the phytoplankton production. A correction was then applied on microalgal biofilm measurements which are slightly contaminated by phytoplankton during sampling.

2.2.3. Production / Light curves. The GPP, ETR and GO_{PII} were plotted against light (PAR) produced respectively by the benthic chamber, the Diving PAM and the LabSTAF. To estimate photosynthetic parameters, the mathematical model of Webb et al. (1974) [23] was applied to the data:

$$X(PAR) = Xmax \times e^{\left(1-\frac{PAR \times \alpha_X}{Xmax}\right)}$$

where X (PAR) is GPP (PAR), ETR (PAR) or GO_{PII} (PAR). Xmax is the maximum photosynthetic capacity measured with the O_2 incubation method (GPP_{max} in mmol O_2 m^{-2} h^{-1}), the PAM method (ETRmax in µmol electrons m^{-2} s^{-1}) or with LabSTAF (GO_{PIImax}, mmol O_2 m^{-2} h^{-1}), α is the photosynthetic efficiency which corresponds to the slope of each Production / Light curve.

3. Results & Discussion

This paper presents the strategy deployed to measure the primary production of the different component of artificial infrastructure installed in a photic zone of a macrotidal epicontinental sea. The benthic chamber which was developed in this context allowed to standardize light exposure of the macroalgae community during incubation. Traditionally transparent benthic chambers were used [24]

and the results obtained were highly dependent on the daily irradiance which could change from one day to the next and between seasons [14, 24]. The automated benthic chamber used in this study is easily installed by scuba divers and enabled reproductible experiments regarding light exposure. The figures 5a and 5b showed that light energy of the benthic chamber allowed to reach a saturation of GPP which was required to properly fit a Webb model in order to estimate the GPPmax and the photosynthetic efficiency alfa. This preliminary result showed a higher maximal production rate (GPP_{max} = 14.18 ± 2.19 mmol O_2 m^{-2} h^{-1}) and a higher alfa (0.12 ± 0.03 mmol O_2 m^{-2} h^{-1} (µmol photons m^{-2} s^{-1})$^{-1}$) for Eco-engineering modules than for classic module (GPP max = 8.88 ± 0.62 mmol O_2 m^{-2} h^{-1}; alfa = 0.06 ± 0.008 mmol O_2 m^{-2} h^{-1} (µmol photons m^{-2} s^{-1})$^{-1}$) (Figure 5a). Biomass measurements confirmed a higher covering on eco-engineering modules (405.8 g m^{-2}) than on classic modules (256.0 g m^{-2}). The productivity per biomass unit is comparable between both types of modules, with GPP_{max} per weight of 34.96 ± 6.04 µmol O_2 g^{-1} h^{-1} and alfa 0.32 ± 0.09 for eco-engineering modules and GPP_{max} per weight of 34.63 ± 2.44 µmol O_2 g^{-1} h^{-1} and alfa 0.25 ± 0.03 for classic modules (Figure 5b). The equal value of productivity firstly validated our protocol with this new benthic chamber and secondly showed that the advantage of engineering modules is related to fixation success of macroalgae and on the efficiency of the colonisation on rough substrate instead of smooth one [25]. The measurements using benthic chambers will be performed 4 times per year in triplicate on both module types.

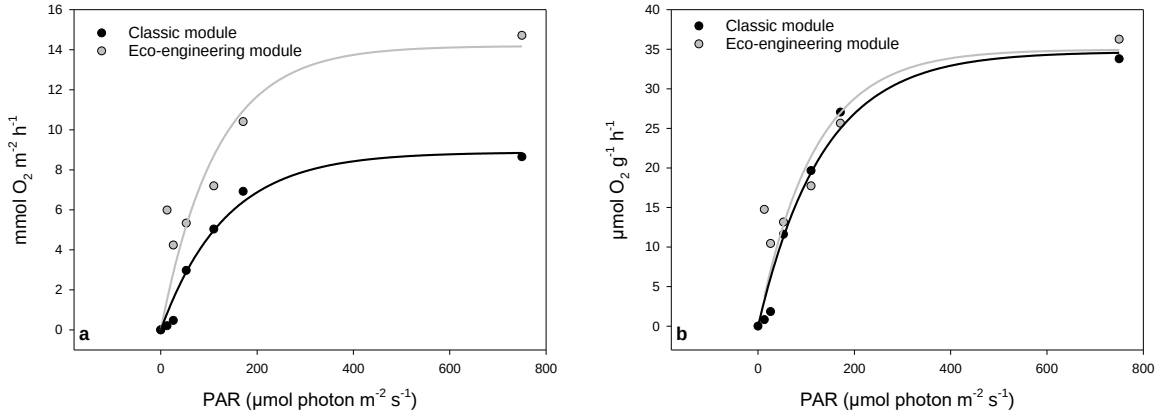

Figure 5. Example of Production / Light curves measured on MARINEFF eco-engineering dyke module and classic dyke module in the Bay of Cherbourg in April 2021. Dots show the GPP measurements at each light intensity (PAR) and the lines show the Webb model fits. a: GPP expressed as a function of subtract surface and time; b: GPP expressed as a function of fresh weight of macroalgae and time.

In addition to benthic chamber experiments, diving PAM measurements were used to estimate photosynthetic parameters *in situ*. GPP estimation gave a production capacity of the whole community while PAM measurements allowed to measure photosynthetic parameters and productivity capacity individually for each species [26] and/or for different parts of thallus for massive species. PAM techniques provide a non-destructive way of assessing the photo-physiology of a range of organisms, including phytoplankton, macroalgae and higher plants. A number of works have used chlorophyll- a fluorescence techniques to study the photosynthetic characteristics of macroalgae, both in the field and in the laboratory [21, 27-30]. In the present study, several species of green, brown and red algae were measured (*Saccorhiza polyschides, Dictyota dichotoma, Gracilaria gracilis, Palmaria palmata, Cryptopleura ramosa, Ulva lactuca, Bryopsis plumosa*) and regrouped as a function of their phylum. Comparison of PAM measurements from different species should be conducted with care, as the irradiance absorption of a photosynthetic organism may change, affecting ETR. As shown in the example presented in figure 6, absorption spectra are highly variable as a function of pigment composition. Absorption spectrum are then required to properly estimate ETR as presented in figure

7a. This absorption spectrum can be improved by using excitation spectrum measurements [19]. According to the literature FII can vary between 0.15 for red algae to 0.5 for green algae [30]. ETR and alfa were significantly higher in brown algae than in red and green. The data were spectrally corrected and brown algae showed a high absorption at 474 nm with high efficiency for the major light-harvesting complex of photosystem II at this wavelength while red algae had a poor light transfer to PSII at this wavelength.

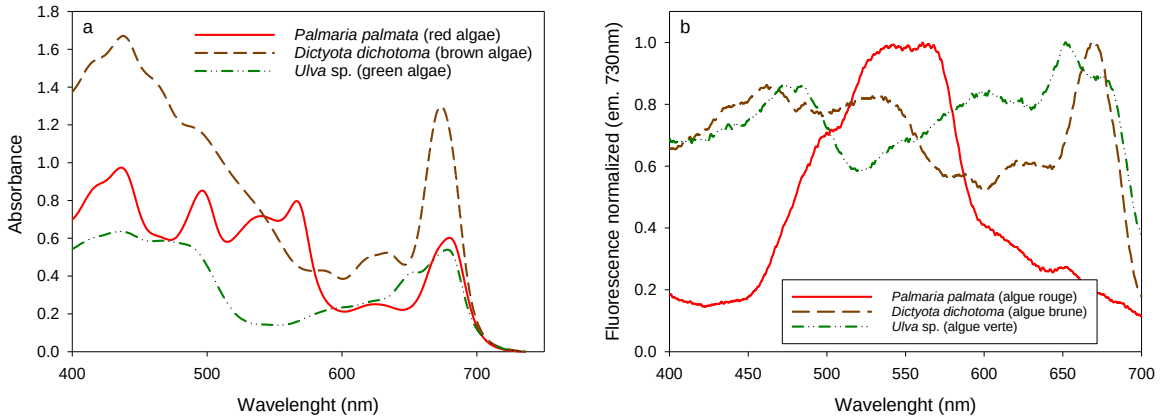

Figure 6. a: Example of spectral absorptance (A_λ) between 400-700 nm and b: Example of fluorescence excitation spectra between 400-700 nm with an emission measured at 730 nm performed on Chlorophyta (green algae), Rhodophyta (red algae) and Phaeophyceae (brown algae) from the Bay of Cherbourg.

The value of ETR are in accordance with literature [30] but calculation of this parameter is very heterogeneous in literature. Beside relative ETR (rETR) [31] measurements, calculation of ETR per unit surface as a function of time are conducted in different ways in literature [26, 27, 30, 32]. The way how to calculate the absorption can change or a standard factor can be applied [32], FII can be considered as variable as a function of pigment groups ([17, 19, 20, 30] or constant for all groups [26, 32]. According to our knowledge the approach proposed by Figueroa et al [19] followed the same principle as described by Johnsen & Sakshaug [33] for phytoplankton. This method which required a determination of absorption factor using an integrative sphere and a determination of FII by using spectrofluorimetry measurements will be applied in our monitoring strategy. Beside producing indicator, such an approach allows to explore ecophysiological regulation of light absorption as a function of environmental parameters. The Fv/Fm obtained for green and brown algae are found in healthy algae, well acclimated to their environment. The lowest value obtained on red algae is probably partly due to pigment composition and is in accordance with literature [32]. The response of red algae to PAM fluorometry techniques can be different from responses in other algae groups due to the presence of phycobilisomes in the photosynthetic apparatus [34, 35]. State transition mechanisms frequently observed in red algae can influence the Fv/Fm [32, 36-38]. We did not exempt to have differences on photosynthetic parameters between the same species collected from eco-engineering modules or classic modules at this stage of the monitoring. If biomass remained significantly higher for longer, the shading due to algae canopy would lead to a discrepancy of algae photoacclimation and possibly of biodiversity and community structures between both types of module.

The microalgal biofilm was also studied in this project. Colonisation of hard substrates by microalgal biofilm is a crucial step for the ecological succession of new hard substrata [16, 39]. Imaging PAM is used to follow the colonisation on small samples of module fixed on dyke modules which are regularly collected [16]. This approach is not detailed in the present paper. As described in the Material and Method section, 25 cm^2 of biofilm were resuspended and analysed using a LabSTAF (Figure 7b).

Contrary to macroalgae, the biofilm of classic modules was more productive. The GO_{IImax}, which correspond to GPP max of macroalgae, was 5.89 ± 0.65 mmol O_2 m^{-2} h^{-1} for classic module and 1.79 ± 1.07 mmol O_2 m^{-2} h^{-1} for eco-engineering module. Biofilm from classic modules also presented a higher Fv/Fm than biofilm from eco-engineering modules. We pointed out that de absorption cross-section of the PSII was also different, which indicated variation of photoacclimatation process or difference on community structure [40, 41]. Analysis of the biofilm community structure is still in process by coupling microscopic and molecular approaches. The higher biomass observed on eco-engineering module probably influenced the biofilm production capacities and its acclimation as a function of light and nutrients. The interactions between microalgal biofilm and macroalgae as well as the temporal variations on these interactions will be a major point of the future data analyses. These first data allowed to compare the primary production of macroalgae and microalgal biofilm. The macroalgae were 7.9 times more productive than biofilm per surface on eco-engineering module whilst only 1.5 times on classic module. Smooth surface appeared to favour biofilm productivity at this stage of colonisation while rough surface drove more quickly the system to a production dominated by macroalgae.

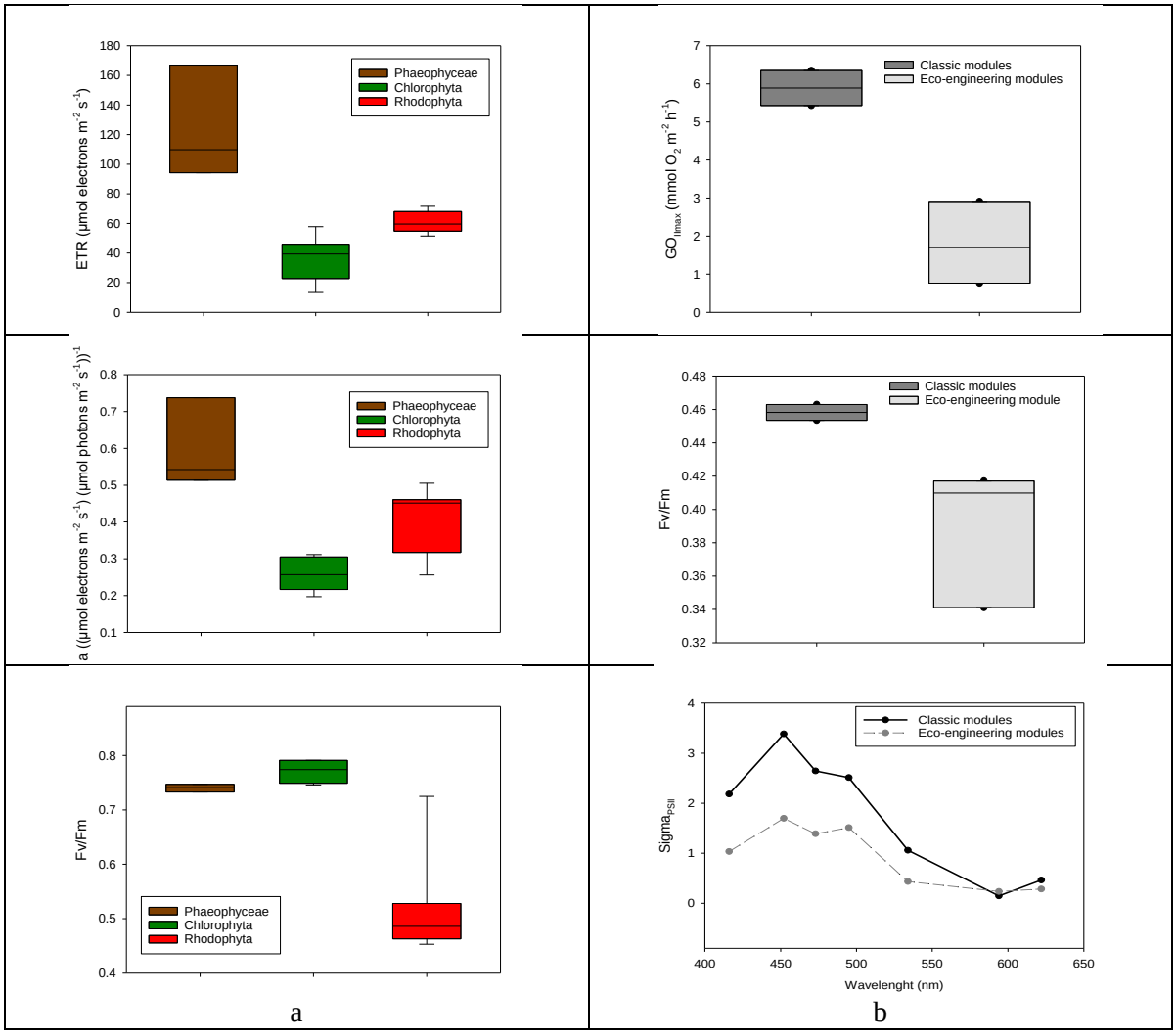

Figure 7. a: ETR, alfa and Fv/Fm measured under water by using Diving PAMII on the dykes modules on Chlorophyta (green algae), Rhodophyta (red algae) and Phaeophyceae (brown algae). b: GO_{IImax},

Fv/Fm, and Sigma PSII measured on microalgal biofilm by using LabSTAF. Measurements were performed in the Bay of Cherbourg in April 2021

4. Conclusion

At the preliminary stage of our monitoring, the sampling and lab protocols deployed were validated. The selection of indicators and their metrics have to be confirmed and some complementary data are required to properly interpret all these first data. We observed differences regarding macro and microalgae primary production. There was no significant difference between productivity of macroalgae ($GPP.g^{-1}.h^{-1}$) measured on eco-engineering and classic modules (ANOVA, $p = 0.967$), because productivity is controlled by environmental parameters like nutrients and light availability while biomass beside productivity capacities also depends on the rate of fixation efficiency. The higher biomass observed on eco-engineering led to a higher GPP per surface unit. For equal surface, at this preliminary stage of our study, eco-engineering modules appeared more productive that classic modules. The monitoring will allow us to increase the replication of these experiments and to confirm or not this trajectory for longer period. The colonization surface of classic module may catch up after several months or years. Competition for space with macrofauna will be also an important point to consider in our monitoring [42]. The primary production estimations will be used in an ecological model. The approach exposed in this work will also allow providing guidelines and efficient protocols to assess primary production in artificial structures and to produce indicators of this main ecology function.

Acknowledgments

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