

TABLE DES MATIERES

CHAPITRE I : INTRODUCTION GENERALE	1
I. LA MANCHE, UN ECOSYSTEME COTIER ANTHROPISE.....	3
I.1. <i>Situation géographique et paramètres environnementaux</i>	3
I.2. <i>Une mer productive et exploitée</i>	5
II. LES RECIFS ARTIFICIELS, MECANISMES DE FONCTIONNEMENT ET BENEFICES ECOLOGIQUES ET SOCIETAUX 11	
II.1. <i>Généralités et intérêts écologiques, sociétaux et économiques</i>	11
II.2. <i>Mécanismes de fonctionnement des récifs artificiels.....</i>	12
III. LES PROJETS MARINEFF ET RECIF	14
III.1. <i>Le projet RECIF</i>	14
III.2. <i>Le projet MARINEFF</i>	14
III.3. <i>Sites d'études et description des modules expérimentaux</i>	17
III.3.1. <i>Sites d'études des projets MARINEFF et RECIF</i>	17
III.4. <i>Sites d'études dans le cadre de cette thèse</i>	18
III.5. <i>Description des infrastructures marines immergées</i>	21
IV. LES BIOFILMS MARINS.....	24
IV.1. <i>Généralités</i>	24
IV.2. <i>La formation du biofilm.....</i>	25
V. LES PRODUCTEURS PRIMAIRES	27
V.1. <i>Classification phylogénétique.....</i>	27
V.2. <i>Classification écologique</i>	31
V.2.1. <i>Producteurs primaires planctoniques.....</i>	31
V.2.2. <i>Producteurs primaires benthiques</i>	31
VI. LA PRODUCTION PRIMAIRE ET SES MECANISMES DE REGULATION.....	34
VI.1. <i>Généralités</i>	34
VI.2. <i>Techniques de mesure.....</i>	40
VI.3. <i>Mécanismes de régulation.....</i>	42
VII. PROBLEMATIQUE ET OBJECTIFS.....	45
CHAPITRE II : MATERIEL ET METHODES	51
I. SITES D'ETUDES.....	53
II. MODELES D'ETUDES ET STRATEGIES D'ECHANTILLONNAGES	53
II.1. <i>Dallettes expérimentales (MI)</i>	53
II.2. <i>Récifs artificiels (projet RECIF).....</i>	56
III. ECHANTILLONNAGE <i>IN SITU</i>	60
IV. BIOMASSE MICROPHYTOBENTHIQUE	60
V. PARAMETRES PHOTOSYNTHETIQUES ET PRODUCTION PRIMAIRE	60
V.1. <i>Imaging-PAM</i>	60
V.2. <i>Diving-PAM.....</i>	65

V.3.	<i>Coefficient d'absorption spécifique de la chlorophylle a (a*)</i>	65
V.4.	<i>Production primaire et respiration – Incubations</i>	66
VI.	RUGOSITE DE SURFACE – CAMERA 3D	67
VII.	SUBSTANCES POLYMERIQUES EXTRACELLULAIRES (EPS).....	68
VIII.	BIODIVERSITE	69
VIII.1.	<i>Microscopie électronique à balayage</i>	69
VIII.2.	<i>Identification des espèces de macroalgues et calculs de biomasses</i>	69
VIII.3.	<i>Identification des espèces de faune benthique et calculs de biomasses</i>	69
IX.	SELS NUTRITIFS ET PARAMETRES ABIOTIQUES	71
CHAPITRE III : MARINE ARTIFICIAL REEFS, A META-ANALYSIS OF THEIR DESIGN, OBJECTIVES AND EFFECTIVENESS		73
RESUME DE LA PUBLICATION		77
ABSTRACT		82
KEYWORDS		83
ABBREVIATIONS		83
I.	INTRODUCTION	84
II.	METHODOLOGY	85
III.	RESULTS	89
III.1.	<i>Graphic representation of global data and dominant variables</i>	90
III.2.	<i>Multiple correspondence analysis (MCA) representations with clusters</i>	92
III.3.	<i>Effectiveness analysis of relevant variables</i>	94
III.4.	<i>Evaluation of artificial reef purposes</i>	94
III.5.	<i>Variable correlations according to the purpose</i>	95
III.5.1.	Fisheries enhancement purpose.....	95
III.5.2.	Experimental purpose.....	95
III.5.3.	Biocenosis protection purpose.....	96
III.5.4.	Biocenosis restoration purpose.....	96
IV.	DISCUSSION	96
IV.1.	<i>Design, location and effectiveness of artificial reefs</i>	96
IV.2.	<i>Assessment of artificial reef purpose</i>	97
IV.3.	<i>Assessment of monitoring techniques</i>	99
V.	CONCLUSIONS.....	100
VI.	ACKNOWLEDGEMENTS.....	101
CHAPITRE IV : INFLUENCE DE LA STRUCTURE ET DU NIVEAU D'EUTROPHISATION SUR LE DEVELOPPEMENT MICROPHYTOBENTHIQUE.....		103
PARTIE IV-A : INFLUENCE OF INFRASTRUCTURE MATERIAL COMPOSITION AND MICROTOPOGRAPHY ON MARINE BIOFILM GROWTH AND PHOTOBIOLOGY		105
RESUME DE LA PUBLICATION		109
ABSTRACT		112
I.	INTRODUCTION	113
II.	MATERIALS & METHODS	114

II.1.	<i>Experimental setup</i>	114
II.2.	<i>Experimental monitoring</i>	117
II.2.1.	Scanning Electron Microscopy observations	117
II.2.2.	Photosynthetic parameters.....	117
II.2.3.	3D camera acquisitions	119
II.2.4.	Chlorophyll a extractions	119
II.3.	<i>Data treatment and analysis</i>	120
III.	RESULTS	121
III.1.	<i>Environmental conditions and species identification</i>	121
III.2.	<i>Biological parameters varied with the substratum</i>	123
III.2.1.	Chl a biomass	123
III.2.2.	Photosynthetic parameters.....	123
III.3.	<i>Biofilm colonisation depending on the microtopography</i>	127
III.3.1.	Microphytobenthic biomass distribution	127
III.3.2.	Distribution of microphytobenthic photosynthetic indicators	127
III.3.3.	Correlation between microtopography and MPB biomass or photosynthetic indicators	130
IV.	DISCUSSION	133
IV.1.	<i>Influence of the substrate on biofilm development and its physiological quality</i>	133
IV.2.	<i>Influence of microtopography on biofilm colonisation and several physiological indicators</i>	135
V.	CONCLUSION.....	137
VI.	ACKNOWLEDGMENTS	137
	PARTIE IV-B : INFLUENCE OF NUTRIENT ENRICHMENT ON GROWTH AND PHOTOSYNTHETIC PARAMETERS OF HARD SUBSTRATE MARINE MICROPHYTOBENTHOS	139
	RESUME DE LA PUBLICATION	143
	ABSTRACT	146
	KEYWORDS	146
I.	INTRODUCTION	147
II.	MATERIALS & METHODS	148
II.1.	<i>Experimental setup</i>	148
II.2.	<i>Experimental design</i>	149
II.3.	<i>Abiotic parameters and inorganic nutrient analyses</i>	151
II.4.	<i>Chl a biomass</i>	151
II.5.	<i>Photosynthetic parameters</i>	152
II.6.	<i>Extracellular polymeric substances</i>	154
II.7.	<i>3D camera recordings</i>	155
II.8.	<i>Scanning Electron Microscopy</i>	155
II.9.	<i>Data treatment and analysis</i>	155
III.	RESULTS	156
III.1.	<i>Experimental conditions</i>	156
III.2.	<i>Chlorophyll a biomass, growth rate of extracellular polymeric substances</i>	157
III.3.	<i>Photosynthetic parameters</i>	159

III.4.	<i>Correlation between microtopography versus biofilm biomass and photosynthetic indicators</i>	166
III.5.	<i>Species identification and diversity</i>	167
IV.	DISCUSSION	167
IV.1.	<i>Influence of the nutrient conditions on biofilm development and EPS secretion</i>	168
IV.2.	<i>Influence of the nutrient condition on biofilm photochemistry and photosynthetic performances</i> 170	
IV.3.	<i>Influence of microtopography on biofilm colonisation and physiological indicators</i>	172
IV.4.	<i>Conclusion</i>	174
V.	ACKNOWLEDGMENTS	175
CHAPITRE V : DYNAMIQUE DE COLONISATION D'INFRASTRUCTURES MARINES IN SITU		177
PARTIE V-A : COLONISATION OF ARTIFICIAL STRUCTURES BY PRIMARY PRODUCERS: INTERSPECIES COMPETITION AND PHOTOSYNTHETIC BEHAVIOUR		
	RÉSUMÉ	183
	ABSTRACT	186
	KEYWORDS	186
	ABBREVIATIONS	186
I.	INTRODUCTION	187
II.	MATERIAL AND METHODS.....	189
II.1.	<i>Sampling sites</i>	189
II.2.	<i>Experimental monitoring</i>	190
II.2.1.	Abiotic parameters and inorganic nutrients analyses	190
II.2.2.	Identification of macroalgae.....	191
II.2.3.	Photosynthetic parameters.....	191
II.2.4.	Chlorophyll a measurements.....	193
II.3.	<i>Data treatment and analysis</i>	193
III.	RESULTS	195
III.1.	<i>Environmental parameters</i>	195
III.2.	<i>Microphytobenthic biomass</i>	196
III.3.	<i>Photosynthetic parameters</i>	197
III.4.	<i>Macroalgae biomass and diversity</i>	202
III.5.	<i>Encrusting algae</i>	204
III.6.	<i>Principal component analysis (PCA) on biotic and abiotic variables</i>	207
IV.	DISCUSSION	209
IV.1.	<i>Microphytobenthic biofilm growth, photobiology, and drivers</i>	209
IV.2.	<i>Development and photobiology of encrusting alga and competition with microphytobenthic biofilm</i> 211	
IV.3.	<i>Macroalgal diversity and structural–functional relationships</i>	213
V.	CONCLUSION.....	214
VI.	ACKNOWLEDGMENTS	214

PARTIE V-B : DIVERSITY, STRUCTURE ASSEMBLAGES, PRODUCTION AND PHOTOSYNTHESIS OF BENTHIC COMMUNITIES ON ARTIFICIAL REEFS, A COMPARATIVE CASE STUDY IN THE ENGLISH CHANNEL.....	217
RESUME DE LA PUBLICATION	221
ABSTRACT	224
KEYWORDS.....	225
ABBREVIATIONS	225
I. INTRODUCTION	225
II. MATERIALS & METHODS.....	227
II.1. <i>Sampling sites</i>	227
II.2. <i>Sample collection</i>	229
II.2.1. Benthic communities and production.....	229
II.2.2. Photosynthetic parameters.....	230
II.2.3. Abiotic parameters and inorganic nutrient analyses	231
II.3. <i>Data treatment and analysis</i>	231
III. RESULTS	232
III.1. <i>Biotic indices and structure assemblages</i>	232
III.2. <i>Primary production and macroalgae photosynthetic parameters</i>	242
III.3. <i>Principal component analysis (PCA) of biotic and abiotic variables</i>	246
IV. DISCUSSION	248
V. CONCLUSION.....	254
CONFLICT OF INTEREST	254
FUNDING.....	254
ACKNOWLEDGMENTS	254
CHAPITRE VI : SYNTHESE, DISCUSSION GENERALE ET PERSPECTIVES.....	257
I. APPROCHE EN CONDITIONS CONTROLEES SUR DES INFRASTRUCTURES MARINES EXPERIMENTALES .	259
I.1. <i>Influence de la nature du substrat et de la rugosité micro-échelle sur le développement microphytobenthique et sa photosynthèse</i>	260
I.2. <i>Influence du niveau d'eutrophisation sur le développement microphytobenthique et sa photosynthèse</i>	262
I.3. <i>Evaluation de la diversité microphytobenthique et amélioration des techniques de mesures photobiologiques</i>	263
II. APPROCHE <i>IN SITU</i> SUR DES INFRASTRUCTURES MARINES EXPERIMENTALES.....	265
II.1. <i>Structure des communautés benthiques</i>	265
II.2. <i>Développement et photosynthèse des producteurs primaires, influence des sites expérimentaux et de la rugosité micro-échelle</i>	266
III. APPROCHE <i>IN SITU</i> SUR DES RECIFS ARTIFICIELS (PROJET RECIF) PUIS SUR LES INFRASTRUCTURES MARINES ECOCONÇUES MARINEFF	268
III.1. <i>Macrofaune benthique : structure des communautés, influence des sites expérimentaux et des infrastructures</i>	268
III.2. <i>Producteurs primaires : structure des communautés, influence des sites expérimentaux et des infrastructures</i>	270
III.3. <i>Production primaire et photochimie : influence des sites expérimentaux et des infrastructures</i>	272

III.4. <i>Efficacité des infrastructures écoconçues</i>	275
IV. LIMITES DES ETUDES	277
V. PERSPECTIVES DE RECHERCHE ET APPLICATIONS	278
BIBLIOGRAPHIE	281
ANNEXES	319

LISTE DES FIGURES

FIGURE 1 – CARTE DE LA MER DE LA MANCHE AVEC REPRESENTATION DES PRINCIPAUX BASSINS. ISSUE DE DAUVIN 2012.	3
FIGURE 2 – CARTE DES COURANTS RESIDUELS EN MANCHE D'APRES SALOMON ET BRETON, 1991.....	4
FIGURE 3 – CARTE REPRESENTANT LES CINQ TYPES MAJEURS DE SEDIMENTS SUPERFICIELS EN MER DE LA MANCHE (D'APRES LARSONNEUR ET AL., 1982 DANS DAUVIN, 2019).....	5
FIGURE 4 – LOCALISATION DE LA ZONE MARINEFF AVEC L'ENSEMBLE DES ACTEURS DU PROJET. MODIFIE DE WWW.CIRIA.ORG/MARINEFF	16
FIGURE 5 – LOCALISATION DES DEUX SITES EXPERIMENTAUX DANS LE CADRE DE CES TRAVAUX DE THESE. LES ETOILES ROUGES REPRESENTENT LES SITES D'IMMERSION DES RECIFS ARTIFICIELS.	18
FIGURE 6 – SITE D'IMMERSION DES MODULES DIGUES EN RADE DE CHERBOURG. LES TRIANGLES REPRESENTENT L'EMPLACEMENT DES CORPS MORT DELIMITANT LA ZONE D'IMMERSION (PROJET RECIF), LES RECIFS DU PROJET RECIF IMMERGES DEPUIS 2015 SONT REPRESENTES PAR DES RECTANGLES (RECIF 1, 2 ET 3), LES CARRES REPRESENTENT L'EMPLACEMENT DES MODULES DIGUES MARINEFF EN ZONE SUBTIDALE, LES BLOCS TEMOINS SONT APPELES CN, LES BLOCS EXPERIMENTAUX CR. LE TRIANGLE ROUGE REPRESENT LA ZONE D'INSTALLATION DES MODULES DE DIGUES EN MILIEU INTERTIDAL.	19
FIGURE 7 – CARTE DES DIFFERENTS TYPES DE SEDIMENTS DE LA RADE DE CHERBOURG MODIFIEE DEPUIS BAUX ET AL. 2017.	20
FIGURE 8 – SITE D'IMMERSION DES MODULES DIGUES AU LARGE DE BERNIERES-SUR-MER. LES CARRES ORANGE REPRESENTENT L'EMPLACEMENT DES TROIS RECIFS IMMERGES DEPUIS 2015 (BLOC 1, 2 ET 3 ; PROJET RECIF), LES CARRES VERTS (BR01, BN01, ETC) REPRESENTENT L'EMPLACEMENT DES MODULES DIGUES, LES BLOCS TEMOINS SONT APPELES CN, LES BLOCS EXPERIMENTAUX CR.	21
FIGURE 9 – DESIGN DES MODULES DE DIGUES.....	22
FIGURE 10 – PHOTOGRAPHIES DE BLOCS EXPERIMENTAUX (A) ET CLASSIQUES (B) AVANT LEUR IMMERSION SUR LE SITE DE LA RADE DE CHERBOURG. TPC®	22
FIGURE 11 – LES ETAPES DE LA FORMATION D'UN BIOFILM (1 A 6) ET SON EVOLUTION EN MILIEU MARIN (7 A 9). MODIFIE DEPUIS GRZEGORCZYK ET AL., 2018.....	26
FIGURE 12 – REPRESENTATION SCHEMATIQUE DE L'ARBRE PHYLOGENETIQUE DES EUCARYOTES. LES TYPES DE PLASTES AINSI QUE LEUR REMPLACEMENT ET LEUR PERTE SONT PRECISES. LA PERTE DE LA PHOTOSYNTHESE EST EGALEMENT INDIQUEE. DEPUIS SIBBALD & ARCHIBALD, 2020.	29
FIGURE 13 – DIAGRAMME REPRESENTANT LE COMPLEXE D'ADHESION D'UNE DIATOME PENNEE RAPHIDE ET SUGGERANT UN MODELE DE MOBILITE (WETHERBEE ET AL., 1998; WANG ET AL., 2013).	30
FIGURE 14 – RELATIONS PHYLOGENETIQUES ENTRE LES DIFFERENTS GROUPES D'ALGUES ET LEURS APPAREILS PHOTOSYNTHETIQUES RESPECTIFS. LES CERCLES TRADUISENT LES RELATIONS PHYLOGENETIQUES ENCORE DEBATUES.	35
FIGURE 15 – SCHEMA REPRESENTANT UNE SECTION TRANSVERSALE DE MEMBRANE DE THYLAKOÏDE MONTRANT L'ORGANISATION ET CERTAINS DES PRINCIPAUX COMPOSANTS DE L'APPAREIL PHOTOSYNTHETIQUE. LE TRANSPORT DES ELECTRONS (E ⁻) EST REPRESENTE PAR LES FLECHES ROUGES, CELUI DES PROTONS (H ⁺) PAR LES FLECHES VIOLETES. LES ELECTRONS SONT CAPTES DANS LE MILIEU EXTERIEUR PAR LE PHOTOSYSTEME II (PSII) ET SONT TRANSFERES AU CYTOCHROME b ₆ /f (CYT b ₆ /f). ILS SONT ENSUITE TRANSFERES AU PHOTOSYSTEME I (PSI) PAR LA PLASTOCYANINE (PC) OU ILS SONT UTILISES POUR REDUIRE LE NADP EN NADPH.	36
FIGURE 16 – ORGANISATION DU PHOTOSYSTEME II (PSII) AVEC VISUALISATION DE LA CHAINE DE TRANSPORT DES ELECTRONS. FIGURE ISSUE DE SUGGET ET AL. (2010).....	38
FIGURE 17 – PROTEINES LHC (A) ET PIGMENTS (B) DANS LES DIFFERENTS GROUPES D'EUCARYOTES. LES PARANTHESES INDIQUENT LA PRESENCE DU GENE MAIS PAS DE LA PROTEINE. LES CERCLES E2 ET E3 REPRESENTENT LE DEGRE D'ENDROSYMBIOSE. D'APRES BÜCHEL ET AL., 2015.	39
FIGURE 18 – SCHEMA REPRESENTANT LES TRANSFERTS D'ENERGIE AU SEIN DU PSII. LA LUMIERE INCIDENTE EST ABSORBEE PAR LES ANTENNES COLLECTRICES (LHC) AVEC UNE SECTION D'ABSORPTION (Σ_{PSII}). LA	

CHLOROPHYLLE EXCITEE VA MIGRER PAR RESONNANCE JUSQU'AU CENTRE REACTIONNEL (RC). S'IL EST OUVERT (A), P_{680} VA S'OXYDER (P_{680}^{+}) ET LE PREMIER ACCEPTEUR D'ELECTRON (Q_A) SERA REDUIT (Q_A^{-}). LA FLUORESCENCE EMISE SERA MINIMALE (F_0). SI AU MOMENT OU LE PHOTON EST ABSORBE, Q_A EST REDUIT (B), L'ENERGIE ABSORBEE PEUT ETRE RENVOYEE SOUS FORME DE FLUORESCENCE, AUGMENTANT CELLE-CI JUSQU'AU NIVEAU MAXIMAL (F_M). LA VALEUR DE FLUORESCENCE F OBSERVEE A UN NIVEAU D'IRRADIANCE E EST UNE MOYENNE DE F_0 ET F_M PONDEREE PAR LA FRACTION DE RC OUVERTS ET FERMES ET CORRESPOND AU QUENCHING PHOTOCHIMIQUE (Q_P). MODIFIE A PARTIR DE KOLBER & FALKOWSKI (1993).	41
FIGURE 19 – (A) EFFET DE L'INTENSITE LUMINEUSE DE CROISSANCE SUR LE COEFFICIENT D'ABSORPTION DE LA LUMIERE NORMALISE PAR LA CHLOROPHYLLE A ET (B) SCHEMA DECRIVANT LE PHENOMENE D'AUTO-OMBRAGE. D'APRES FALKOWSKI ET RAVEN (2007).	44
FIGURE 20 – REPRESENTATION SCHEMATIQUE DES DIFFERENTES EXPERIMENTATIONS ET SUIVIS REALISES CONSTITUANT LES DIFFERENTS ARTICLES DE CETTE THESE.	48
FIGURE 21 – PHOTOGRAPHIES DES DIFFERENTS TYPES DE DALLETTES (RUGUEUSE, LISSE ET PVC) UTILISES.	54
FIGURE 22 – PHOTOGRAPHIE DES DALLETTES IMMERGEES SUR LE SITE DE LA RADE DE CHERBOURG EN FEVRIER 2020 (ENVIRON CINQ MOIS APRES LEUR INSTALLATION).	56
FIGURE 23 – LOCALISATION DES RECIFS ARTIFICIELS (ETOILES ROUGES), DE LA BOUEE INSTRUMENTEE SMILE (POINT ROUGE) ET REPRESENTATION SCHEMATIQUE D'UN MODULE DE CHERBOURG (GAUCHE) ET DE BAIE DE SEINE (DROITE).	57
FIGURE 24 – SCHEMA REPRESENTANT LE DEROULEMENT DE L'EXPERIMENTATION SUR LES PARPAINGS DANS LE BUT DE DECRIRE L'ETAT INITIAL DES SITES D'ETUDES.	58
FIGURE 25 – EXEMPLES DE PHOTOGRAPHIES REALISEES DURANT LES PLONGEES SUR LE SITE DE LA RADE DE CHERBOURG. ICI DES ALGUES ROUGES (A) ET LAMINAIRES (B) SONT EN SURFACE D'UN MODULE. UN TOURTEAU (B) AINSI QU'UNE BLENNIE ET UN LABRIDE (D) SONT ABRITES DANS LES ESPACES LIBRES CREEES PAR LES PARPAINGS.	59
FIGURE 26 – EXEMPLE DE DONNEE OBTENUE PAR L'IMAGING-PAM AVEC LA ZONE D'INTERET (CERCLE BLANC). 61	61
FIGURE 27 – PRINCIPE DE LA FLUORESCENCE MODULEE PAM. SOUS UNE TRES FAIBLE LUMIERE DETECTRICE (LD), L'ACTIVITE PHOTOSYNTHETIQUE EST INSIGNIFIANTE. LE PREMIER ACCEPTEUR D'ELECTRON DU PSII, Q_A , EST ALORS COMPLETEMENT OXYDE, LA FLUORESCENCE EMISE EST PAR CONSEQUENT MINIMALE. CE NIVEAU DE FLUORESCENCE OBTENU APRES UN PASSAGE A L'OBSCURITE EST APPELE LE NIVEAU MINIMUM DE FLUORESCENCE F_0 . ENSUITE UN FLASH LUMINEUX DE HAUTE INTENSITE EST EMIS. LE PSII EST SATURE, Q_A EST COMPLETEMENT REDUIT. LA FLUORESCENCE ATTEINT ALORS UN MAXIMUM (F_M). UNE GAMME DE LUMIERE ACTINIQUE (LA_1 , LA_2 ETC.) D'INTENSITES CROISSANTES EST APPLIQUEE SUR L'ECHANTILLON. CHAQUE INTENSITE LUMINEUSE (FLUX DE PHOTONS) EST APPLIQUEE PENDANT 30 S DANS CETTE ETUDE. POUR CHAQUE INTENSITE, UNE FLUORESCENCE DE BASE STABLE F_S EST ATTEINTE. TOUTES LES 30 SECONDES UN FLASH DE LUMIERE SATURANTE (LS) EST EMIS ET F_M' EST ALORS DETERMINEE.	62
FIGURE 28 – EXEMPLE DE COURBE P/E OBTENUE POUR UN PIXEL D'UN ECHANTILLON.....	64
FIGURE 29 – EXEMPLE DE FIGURE OBTENUE (F_V/F_M) APRES TRAITEMENT DES DONNEES BRUTES PAR LA ROUTINE MATLAB.	64
FIGURE 30 – REPRESENTATION SCHEMATIQUE DU SYSTEME D'INCUBATION POUR LES MESURES DE PRODUCTION PRIMAIRES ASSOCIEES AUX PARPAINGS.	67
FIGURE 31 – DIFFERENTES FRACTIONS D'EPS PRODUITS PAR LES DIATOMEES (MODIFIE DEPUIS SHNYUKOVA AND ZOLOTARIOVA, 2015).....	68
FIGURE 32 – GRAPHIC REPRESENTATION OF THE PERCENTAGE OF ARTIFICIAL REEF SITES IN TERMS OF THREE CATEGORIES OF VARIABLES: AR TYPE AND PURPOSE (1), ENVIRONMENTAL VARIABLES (2) AND MONITORING METHODS (3).	91
FIGURE 33 – MULTIPLE CORRESPONDENCE ANALYSIS (MCA) OF ALL THE DATA WITH ALL THE MEASURED VARIABLES THAT CONTRIBUTE MORE THAN 1% TO THE TWO FIRST DIMENSIONS. CLUSTERS ARE REPRESENTED (A TO E) IN DIFFERENT COLOURS.....	93
FIGURE 34 – GRAPHIC REPRESENTATION OF THE PERCENTAGE OF AR SITES IN TERM OF THREE EFFECTIVENESS CATEGORIES (WEAK, PARTIAL AND STRONG) FOR EACH RELEVANT VARIABLE (N = 20) SELECTED FOR THE MCA REPRESENTATION.	94

FIGURE 35 – GRAPHIC REPRESENTATION OF ASSESSED EFFECTIVENESS FOR EACH AR PURPOSE.	95
FIGURE 36 – EXPERIMENTAL SETUP DESIGN. MARINE INFRASTRUCTURES WERE PLACED BY THREE IN 20 MICROCOSMS (SEPARATED BY THE DASHED LINES) PLACED IN A LARGE MESOCOSM (PICTURE A). MICROCOSMS (PICTURE B) WERE RANDOMLY DISPOSED IN THE MESOCOSM TO GUARANTEE THE TOTAL INDEPENDENCE BETWEEN SAMPLED UNITS. ARTIFICIAL LIGHTNING AND CIRCULATION PUMP (300L PER HOUR) WERE ADDED FOR THE ENTIRE MESOCOSM. AIR BUBBLING WERE ADDED IN EACH MICROCOSM. ALL MICROCOSMS WERE ENTIRELY IMMERSSED AT THE SAME LEVEL IN THE MESOCOSM. THE AVERAGE LIGHT WAS $113.83 \mu\text{MOL PHOTONS M}^{-2} \text{S}^{-1}$ FOR 8 H (FROM 8 AM TO 4 PM) AND THE AVERAGE TEMPERATURE WAS OF 20°C	116
FIGURE 37 – DIAGRAM OF THE SPLIT-PLOT ANOVA DESIGN. FOR EACH OF THE FOURTH INVESTIGATED PARAMETERS, THE SAME DESIGN WAS APPLIED WITH ROUGHNESS AND TYPE OF CONCRETE IN THE MAIN PLOTS AND AGE FACTOR IN THE SUBPLOTS.	121
FIGURE 38 – SCANNING ELECTRON MICROSCOPY OBSERVATIONS OF MICROPHYTOBENTHIC BIOFILMS SAMPLED ON ONE ROUGH MI AT THE END OF THE EXPERIMENT. AMPHORA SP. (A.); THALASSIOSIRA SP. (B.); ENTOMONEIS SP. (C. AND E.); CHOANOFLAGELLATE (D.); HANTZSCHIA SP. IN THE CENTRE (F.); HIGH DENSITIES OF CELLS EMBEDDED IN A MATRIX OF EPS (G. AND H.); THALASSIOSIRA SP. (I.); MICROTABELLA SP. (J.); CYLINDROTHECA CLOSTERIUM (K.); CYLINDROTHECA CLOSTERIUM IN THE CENTRE (L.).	122
FIGURE 39 – BIOFILM BIOMASS (A, $\mu\text{G CHL A CM}^{-2}$) AND PHOTOSYNTHETIC INDICATORS F_v/F_m (B), RETR_{MAX} (C, RELATIVE UNIT), E_k (D, $\mu\text{MOL PHOTONS M}^{-2} \text{S}^{-1}$), α (E, $\mu\text{MOL ELECTRONS } (\mu\text{MOL PHOTONS})^{-1}$) AND NPQ (F, RELATIVE UNIT) ASSESSMENT THROUGHOUT THE COURSE OF THE 6-DAY EXPERIMENT ON THE THREE DIFFERENT TYPES OF STRUCTURE (PVC, ROUGH AND SMOOTH).	126
FIGURE 40 – SPATIAL DISTRIBUTION OF BIOFILM BIOMASS (F_0) AND SELECTED PHOTOSYNTHETIC INDICATORS (F_v/F_m , RETR_{MAX} (RELATIVE UNIT), α ($\mu\text{MOL ELECTRONS } (\mu\text{MOL PHOTONS})^{-1}$) AND NPQ (RELATIVE UNIT)) AND MICROTOPOGRAPHY (MM) ON ONE ROUGH BMI (N°8) DURING THE MONITORING PERIOD.	128
FIGURE 41 – SPATIAL DISTRIBUTION OF BIOFILM BIOMASS (F_0) AND SELECTED PHOTOSYNTHETIC INDICATORS (F_v/F_m , RETR_{MAX} (RELATIVE UNIT), α ($\mu\text{MOL ELECTRONS } (\mu\text{MOL PHOTONS})^{-1}$) AND NPQ (RELATIVE UNIT)) AND MICROTOPOGRAPHY (MM) ON ONE SMOOTH BMI (N°30) DURING THE MONITORING PERIOD. ..	129
FIGURE 42 – CORRELATION COEFFICIENT (R) BETWEEN BIOFILM BIOMASS AND RUGOSITY LEVEL (A) OR PHOTOSYNTHETIC INDICATORS AND RUGOSITY LEVEL: F_v/F_m (B), RETR_{MAX} (C), E_k (D), α (E) AND NPQ (F).	132
FIGURE 43 – EXPERIMENTAL SETUP.	150
FIGURE 44 – CHANGES IN CHL A BIOMASS ($\mu\text{G.CM}^{-2}$) (A) AND ASSOCIATED GROWTH RATE KINETICS (μ) (B) AT DAYS 3, 7, 10, 16, 24 AND 28 AFTER THE BEGINNING OF THE EXPERIMENT ON MI. VARIATION IN THE EPS:CHL A RATIO ($\text{MGGEQ}/\mu\text{GCHL A}$) ON MI (C) AND IN THE CONCENTRATION OF B-EPS ($\mu\text{GGEQ CM}^{-2}$) (D) DURING THE FIRST 24 DAYS OF THE EXPERIMENT ON MI.	158
FIGURE 45 – VARIATIONS IN CHL A BIOMASS ($\mu\text{G.CM}^{-2}$) (A) AND ASSOCIATED GROWTH RATE KINETICS (μ) (B) AT DAYS 3, 7, 10, 16, 24 AND 28 AFTER THE BEGINNING OF THE EXPERIMENT ON PVC. VARIATION IN THE EPS:CHL A RATIO ($\text{MGGEQ}/\mu\text{GCHL A}$) ON MI (C) AND IN THE CONCENTRATION OF B-EPS ($\mu\text{GGEQ CM}^{-2}$) (D) DURING THE FIRST 24 DAYS OF EXPERIMENTATION ON PVC.	159
FIGURE 46 – BIOFILM PHOTOSYNTHETIC INDICATORS F_v/F_m (A), RETR_{MAX} (B, RELATIVE UNIT), NPQ (C, RELATIVE UNIT AT GROWTH IRRADIANCE) AND $\text{ETR}_{\text{MAX}}^a$ (D, $\text{MMOL E}^- (\text{MG CHL A H})^{-1}$) DURING THE EXPERIMENT IN THE THREE DIFFERENT CONDITIONS (CONTROL, MEDIUM AND HIGH ENRICHMENT CONDITIONS) ON MI.	162
FIGURE 47 - BIOFILM PHOTOSYNTHETIC INDICATORS F_v/F_m (A), RETR_{MAX} (B, RELATIVE UNIT), NPQ (C, RELATIVE UNIT AT GROWTH IRRADIANCE) AND $\text{ETR}_{\text{MAX}}^a$ (D, $\text{MMOL E}^- (\text{MG CHL A H})^{-1}$) DURING THE EXPERIMENT IN THE THREE DIFFERENT CONDITIONS (CONTROL, MEDIUM AND HIGH ENRICHMENT CONDITIONS) ON PVC.	164
FIGURE 48 – CORRELATION COEFFICIENTS (R) BETWEEN MICROTOPOGRAPHY AND CHL A BIOMASS OR BIOFILM PHYSIOLOGICAL INDICATORS F_v/F_m , RETR_{MAX} , α AND NPQ IN THE THREE DIFFERENT CONDITIONS (CONTROL, MEDIUM AND HIGH ENRICHMENT CONDITIONS) AT THE END OF THE EXPERIMENT (DAY 28).	167
FIGURE 49 – LOCATION OF THE EXPERIMENTAL SITES (RED STARS) AND THE SMILE BUOY (RED DOT) IN THE ENGLISH CHANNEL.	190

FIGURE 50 – EXAMPLE OF MI PICTURES (ROUGH AND SMOOTH) IN JULY 2020. BLACK LINES DISCRIMINATE PHYMATOLITHON PURPUREUM ZONE, THE ASSOCIATE SCHEMATIC REPARTITION OF “INSIDE” AND “OUTSIDE” PHYMATOLITHON PURPUREUM ZONE WAS DEFINED AND USED TO CALCULATE THE ASSOCIATED PHOTOSYNTHETIC PARAMETERS AND BIOMASS.	195
FIGURE 51 – CHLOROPHYLL A BIOMASS FOR EACH RUGOSITY ON THE BAY OF CHERBOURG (A) AND BAY OF SEINE (B).....	197
FIGURE 52 – CHANGES IN PHOTOSYNTHETIC PARAMETERS F_v/F_m (A), $RETR_{MAX}$ (B, REL. UNIT), α (C, $\mu\text{MOL ELECTRONS } (\mu\text{MOL PHOTONS})^{-1}$) AND NPQ (D, RELATIVE UNIT) DURING THE SURVEY OF THE MI IN THE BAY OF CHERBOURG.	200
FIGURE 53 – CHANGES IN PHOTOSYNTHETIC PARAMETERS F_v/F_m (A), $RETR_{MAX}$ (B, REL. UNIT), α (C, $\mu\text{MOL ELECTRONS } (\mu\text{MOL PHOTONS})^{-1}$) AND NPQ (D, RELATIVE UNIT) DURING THE SURVEY OF THE MI IN THE BAY OF SEINE.....	201
FIGURE 54 – PERCENTAGE COVER OF INCRUSTING ALGAE PER MI BETWEEN FEBRUARY 2020 AND APRIL 2021 IN THE BAY OF CHERBOURG.	204
FIGURE 55 – EXAMPLE OF MI PICTURES (ROUGH AND SMOOTH) IN JULY 2020. BLACK LINES IDENTIFY THE PHYMATOLITHON PURPUREUM ZONE, THE ASSOCIATED SCHEMATIC DISTRIBUTION OF “INSIDE” AND “OUTSIDE” THE PHYMATOLITHON PURPUREUM ZONE WAS DEFINED AND USED TO CALCULATE THE ASSOCIATED PHOTOSYNTHETIC PARAMETERS (F_v/F_m , α AND $RETR_{MAX}$) AND BIOMASS (F_0).	206
FIGURE 56 – PRINCIPAL COMPONENT ANALYSIS (PCA) OF BIOTIC AND ABIOTIC VARIABLES IN THE BAY OF CHERBOURG AND THE BAY OF SEINE. ABIOTIC VARIABLES FOR BOTH SITES WERE TEMPERATURE, SALINITY, NO_{TOT} , SILICATE, PHOSPHATE, CHL A AND TURBIDITY. BIOTIC VARIABLES WERE MPB BIOMASS, $RETR_{MAX}$, F_v/F_m AND α FOR MPB AND ENCRUSTING ALGA. PLOTS A AND B REPRESENT ALL VARIABLES. INDIVIDUALS ARE REPRESENTED ON THE OTHER PLOTS: B AND C FOR THE BAY OF CHERBOURG, E AND F FOR THE BAY OF SEINE. B AND E PLOTS REPRESENT THEM ACCORDING TO THE SAMPLING TIME FACTOR, C AND F PLOTS REPRESENT THEM ACCORDING TO THE SURFACE RUGOSITY FACTOR.	208
FIGURE 57 – (A) LOCATION OF THE ARTIFICIAL REEFS IN THE BAY OF CHERBOURG AND IN THE BAY OF SEINE. SAMPLING SITES ARE REPRESENTED BY RED STARS. THE LOCATION OF THE SMILE BUOY IS SHOWN BY A RED DOT. (B) ARTIFICIAL REEF SCHEMA: SAMPLES WERE COLLECTED FROM THE CINDER BLOCKS IN THE UPPER PART OF THE AR MODULE.	228
FIGURE 58 – ABUNDANCE (A, B) AND PERCENTAGE OF TOTAL AFDW BIOMASS PER SEASON (C, D) OF TROPHIC GROUPS IN THE BAY OF SEINE (A, C) AND THE BAY OF CHERBOURG (B, D) IN EACH SEASON. TROPHIC GROUPS: GRAZERS (Gr), FILTER FEEDERS (FF), PREDATORS (Pr), OMNIVOROUS SCAVENGERS (O/Scv), SUB-SURFACE DEPOSIT FEEDERS (ssDF) AND SURFACE DEPOSIT FEEDERS (sDF).	241
FIGURE 59 – GROSS PRIMARY PRODUCTION (GPPMAX) AND RESPIRATION INCUBATION IN MG C M-2 D-1 PER CINDER BLOCK AT LIGHT SATURATION AND IN EACH SEASON IN THE BAY OF SEINE (A, C) AND THE BAY OF CHERBOURG (B, D).	243
FIGURE 60 - PHOTOSYNTHETIC PARAMETERS (F_v/F_m (A, B, C AND D) AND $RETR_{MAX}$ (E, F, G AND H)) MEASURED ON MACROALGAE SAMPLED ON ARTIFICIAL REEFS IN THE BAY OF CHERBOURG IN SUMMER (A, E), AUTUMN (B, F), WINTER (C, G) AND SPRING (D, H). RHODOPHYCEAE ARE IN RED, PHAEOPHYCEAE IN BROWN AND ULVOPHYCEAE IN GREEN.	245
FIGURE 61 – PRINCIPAL COMPONENT ANALYSIS (PCA) OF BIOTIC AND ABIOTIC VARIABLES IN THE BAY OF SEINE AND THE BAY OF CHERBOURG. BIOTIC VARIABLES ARE BENTHIC INVERTEBRATE BIOMASS AND ABUNDANCE PER TROPHIC GROUP AND MACROALGAE BIOMASS PER CLASS. BIOTIC INDICES ARE ALSO GIVEN (H': SHANNON-WIENER DIVERSITY INDEX; S: TAXONOMIC RICHNESS (TR); J': PIÉLOU'S EVENNESS INDEX; A: ABUNDANCE; B: BIOMASS). THE ABIOTIC VARIABLES FOUND IN BOTH SITES ARE INORGANIC NUTRIENTS, SALINITY, AND TEMPERATURE. THE INSTRUMENTED BUOY (SMILE) ALLOWED US TO ANALYZE MORE VARIABLES IN THE BAY OF SEINE: DISSOLVED OXYGEN (OXY_SMILE), pH, MES (MES_SMILE), FLUORESCENCE (FFU_SMILE) AND PAR (PAR_SMILE). AUTOCORRELATED VARIABLES ARE NOT REPRESENTED FOR THE BAY OF SEINE, Pr_BIOMASS WAS CORRELATED WITH N_{TOT} AND MES_SMILE; Gr_BIOMASS WITH Gr_ABUNDANCE; pH WITH OXY_SMILE; Pr_ABUNDANCE WITH ssDF_BIOMASS AND FF_ABUNDANCE WITH sDF_BIOMASS, ssDF_ABUNDANCE, TEMPERATURE AND sDF_ABUNDANCE. FOR THE BAY OF CHERBOURG, Gr_BIOMASS WAS CORRELATED WITH Gr_ABUNDANCE AND Pr_ABUNDANCE WITH ssDF_BIOMASS, Scv.O_ABUNDANCE, sDF_BIOMASS, FF_ABUNDANCE AND ssDF_ABUNDANCE.	247

FIGURE 62 – PHOTOGRAPHIE D’UN BLOC DE DIGUE EXPERIMENTAL AVEC L’INSTALLATION DE MESURE DE PRODUCTION PRIMAIRE (CHAMBRE BENTHIQUE). PHOTOGRAPHIE REALISEE EN JUIN 2021..... 273

FIGURE 63 – CLOCHE BENTHIQUE AUTOMATISEE POUR LES MESURES DE PRODUCTION PRIMAIRE. (A) MODULE DE DIGUE EXPERIMENTAL MARINEFF AVEC LES ANNEAUX DE FIXATIONS SUR LA FACE SUPERIEURE (B) AFIN D’Y ATTACHER LA CLOCHE BENTHIQUE (C). REVETEMENT EN ALUMINIUM (G) RECOUVRANT L’INTERIEUR DE LA CHAMBRE BENTHIQUE. PANNEAU LED (F) VISSE SUR LE DESSUS DE LA CHAMBRE BENTHIQUE (E) AFIN DE RENDRE LE SYSTEME ETANCHE. LE PANNEAU LED EST DIRIGE PAR UN CONTROLEUR (D). LE FLUX D’EAU EST GENERE PAR UNE POMPE IMMERGEABLE (H). DEUX SONDES, YSI EXO 1 (I) AVEC UN CAPTEUR D’OXYGENE ET UNE TURNER DESIGN C-SENSE pCO_2 (J) DIRIGEE PAR UN LOGGER RBR VIRTUOSO2 (J) PERMETTENT DE MESURER LA CONCENTRATION DES GAZ. LE SYSTEME ENTIER EST IMMERGE SUR SITE ET EST ENTIEREMENT CLOS. D’APRES CLAQUIN ET AL., 2022..... 273

LISTE DES TABLEAUX

TABLEAU 1 – FORMULATIONS RETENUES POUR LA FABRICATION DES BMI (PROPORTIONS DONNEES EN KG M ⁻³ DE BETON).....	53
TABLE 2 – ENVIRONMENTAL VARIABLES (FOUR CATEGORIES) WITH THEIR CODE USED IN THE STATISTICAL ANALYSES.	86
TABLE 3 – ARTIFICIAL REEF VARIABLES (FOUR CATEGORIES) WITH THEIR CODE USED IN THE STATISTICAL ANALYSES.	87
TABLE 4 – MONITORING VARIABLES (THREE CATEGORIES) AND ARTIFICIAL REEF EFFECTIVENESS VARIABLES, WITH CODES USED IN THE STATISTICAL ANALYSES.	88
TABLE 5 – CONCRETE TYPE FORMULATIONS DETAILS. THE FOUR DIFFERENT CONCRETE TYPE (1 TO 4) WERE COMPOSED OF TWO DIFFERENT CEMENTS (CEM I OR II) AND OF THE ADDITION OR NOT OF 20% OF OYSTER SHELLS.	115
TABLE 6 – SUMMARY OF THE POSITIVE OR NEGATIVE EFFECTS OF BOTH VARIABLES (RUGOSITY AND CONCRETE FORMULATION) ON THE BIOFILM BIOMASS (CHL A) AND ITS ASSOCIATED PHOTOSYNTHETIC PARAMETERS. THE REFERENCE USED FOR THE RUGOSITY VARIABLE WERE PVC PLATES, THE REFERENCE USED FOR THE CONCRETE FORMULATION VARIABLE WERE CEMENT TYPE I.	135
TABLE 7 – A* IN M ² MG CHL A ⁻¹ AND α^{a*} MMOL E ⁻ MG CHL A H ⁻¹ (μMOL PHOTONS) ⁻¹ ON MARINE INFRASTRUCTURES (MI) AND PVC DURING THE SURVEY IN EACH CONDITION.....	166
TABLE 8 – ENVIRONMENTAL PARAMETERS AT THE TWO EXPERIMENTAL SITES DURING THE SURVEY.....	195
TABLE 9 – MACROALGAE SPECIES RECORDED ON THE MI IN THE BAY OF SEINE AND THE BAY OF CHERBOURG DURING THE SURVEY.	203
TABLE 10 – AFDW BIOMASS IN GC M ⁻² FOR EACH MACROALGAE SPECIES IDENTIFIED IN THE BAY OF SEINE AND THE BAY OF CHERBOURG FOR EACH CAMPAIGN.....	233
TABLE 11 – AFDW BIOMASS IN GC M ⁻² FOR EACH MACROALGAE CLASS (ULVOPHYCEAE, PHAEOPHYCEAE AND RHODOPHYCEAE) IDENTIFIED IN THE BAY OF SEINE AND THE BAY OF CHERBOURG IN EACH CAMPAIGN... ..	235
TABLE 12 – MAIN CHARACTERISTICS OF MACROFAUNA WITH DIFFERENT UNIVARIATE INDICES IN THE TWO SITES AND IN ALL SEASONS. H': SHANNON-WIENER DIVERSITY INDEX; TR: TAXONOMIC RICHNESS; J': PIÉLOU'S EVENNESS INDEX; A: ABUNDANCE (NUMBER OF INDIVIDUALS PER M ⁻² AND B: BIOMASS (G AFDW M ⁻²). SIGNIFICANT DIFFERENCES (P-VALUE < 0.05) ARE INDICATED BY LETTERS (A, B).	235
TABLE 13 – RESULTS OF THE NON-PARAMETRIC KRUSKAL-WALLIS TEST ON FIVE PARAMETERS IN THE TWO SITES. H': SHANNON-WIENER DIVERSITY INDEX; TR: TAXONOMIC RICHNESS; J': PIÉLOU'S EVENNESS INDEX; A: ABUNDANCE; B: BIOMASS (G AFDW M ⁻²); DF: DEGREES OF FREEDOM; F: F-RATIO SCORE; BS: BAY OF SEINE; BC: BAY OF CHERBOURG.	236
TABLE 14 – MACROFAUNA TAXA ABUNDANCE PER M ² AND ASSOCIATED AFDW BIOMASS (GC M ⁻²) FOR EACH CAMPAIGN IN THE BAY OF SEINE AND THE BAY OF CHERBOURG.	236
TABLE 15 – PRIMARY PRODUCTIVITY OF MACROALGAE COMMUNITIES (IN GC GC D ⁻¹).	242

LISTE DES ABREVIATIONS

α : Efficacité photosynthétique

α^{a^*} : Efficacité photosynthétique calculée à partir du a^*

β : Photoinhibition

a^* : Section d'absorption fonctionnelle de la Chl a

AFDW : Poids sec libre de cendres (Ash-Free Dry Weight)

Chl a : Chlorophylle a

E (ou I) : Irradiance

E_k : Intensité de saturation lumineuse

EPS : Substances Polymériques Extracellulaires (Extracellular Polymeric Substances)

(r)ETR_(MAX) : Taux (relatif) (maximum) de transport des électrons au niveau du PSII

ETR_{max}^{a*} : Taux de transport des électrons au niveau du PSII calculé à partir du a^*

F_0 : Fluorescence minimale

FF : Filtreurs (Filter feeders)

F_M : Fluorescence maximale

F_S : Fluorescence stable

F_v/F_M : Rendement quantique maximal du PSII

Geq : Equivalent glucose

GPP : Production primaire brute (Gross Primary Production)

Gr : Brouteurs (Grazers)

H' : Indice de diversité de Shannon-Wiener

J' : Indice d'équitabilité de Pielou

LHC : Antennes Collectrices de Photons (Light Harvesting Complexes)

MI : Marine Infrastructure (Infrastructure marine)

MPB : Microphytobenthos

NPP : Production primaire nette (Net Primary Production)

NPQ : Quenching non-photochimique

PAM : Pulse Amplitude Modulated (fluorescence)

PAR : Radiation Photosynthétique Active (Photosynthetic Active Radiation)

Pr : Prédateurs (Predators)

PSI, PSII : Photosystèmes I et II

RA : Récifs Artificiels

Scv/O : Détritivores / Omnivores (Scavengers / Omnivorous)

sDF : Dépositivores de surface (surface Deposit Feeders)

ssDF : Dépositivores sub-surface (sub-surface Deposit Feeders)

PREAMBULE

Ce travail de thèse s'inscrit dans un contexte d'anthropisation des milieux marins et côtiers et plus spécifiquement celui de la mer de la Manche. Cet espace est soumis à de nombreuses contraintes comme l'artificialisation de certains habitats naturels entraînant la perte de certains services écosystémiques. L'implantation de récifs artificiels représente une réponse adaptée permettant de recréer des habitats artificiels favorables au développement de la vie marine. En suivant ces principes, la considération écologique des infrastructures marines nouvellement implantées représente un enjeu majeur. Les projets RECIF et MARINEFF sont nés dans ce contexte avec l'objectif de développer de nouvelles solutions permettant la valorisation, d'un point de vue écologique, de l'artificialisation de certains espaces marins. L'éco-conception de ces infrastructures marines pourrait permettre d'associer développement humain et valorisation environnementale. Afin d'évaluer l'impact de ce type d'infrastructure sur l'écosystème, il est nécessaire de mesurer différents indicateurs liés à la biodiversité (faune/flore) et aux fonctions écologiques associées. Parmi les fonctions fondamentales des écosystèmes, la fonction de production primaire qui permet la transformation de l'énergie lumineuse en énergie chimique utilisable par le vivant est particulièrement importante à appréhender à différentes échelles. Cette problématique est explorée dans ce manuscrit qui est constitué d'une introduction générale, d'un matériel et méthodes décrivant les approches déployées puis de cinq articles publiés ou soumis regroupés sous trois chapitres. Un premier article présente une méta-analytique d'un corpus bibliographique s'intéressant aux récifs artificiels. Les autres articles constituent deux chapitres différents qui traitent respectivement du développement de biofilms microphytobenthiques sur des échantillons de béton (dalles) en conditions contrôlées et de suivis *in situ* sur différentes infrastructures marines et récifs artificiels. Cette dernière partie s'intéresse aux indicateurs de diversité et de production tout en comparant deux sites d'études et les différents forçages.

CHAPITRE I : INTRODUCTION GENERALE

I. La Manche, un écosystème côtier anthropisé

I.1. Situation géographique et paramètres environnementaux

La Manche est une mer épicontinentale peu profonde, elle est délimitée par la France au sud et le Royaume-Uni au nord. Elle est connectée par l'ouest à l'océan Atlantique et reliée à la mer du Nord par le détroit du Pas-de-Calais. Sa bathymétrie décroît d'ouest en est et elle se caractérise par une dynamique de marée importante (macrotidale) avec une résiduelle de courant d'ouest en est et la présence de différents tourbillons induits par les îles ou caps se situant sur le littoral (Orbi & Salomon, 1988; Salomon & Breton, 1991; Ménesguen & Gohin, 2006; Coles et al., 2017). Le type de substrat est largement dépendant de ces courants de marées, la majeure partie des fonds est ainsi constituée de cailloutis et de sédiments grossiers, à l'inverse, des sédiments plus fins de type sablo-vaseux sont retrouvés dans les baies et estuaires (Cabioc'h, 1968; Dauvin, 2015).

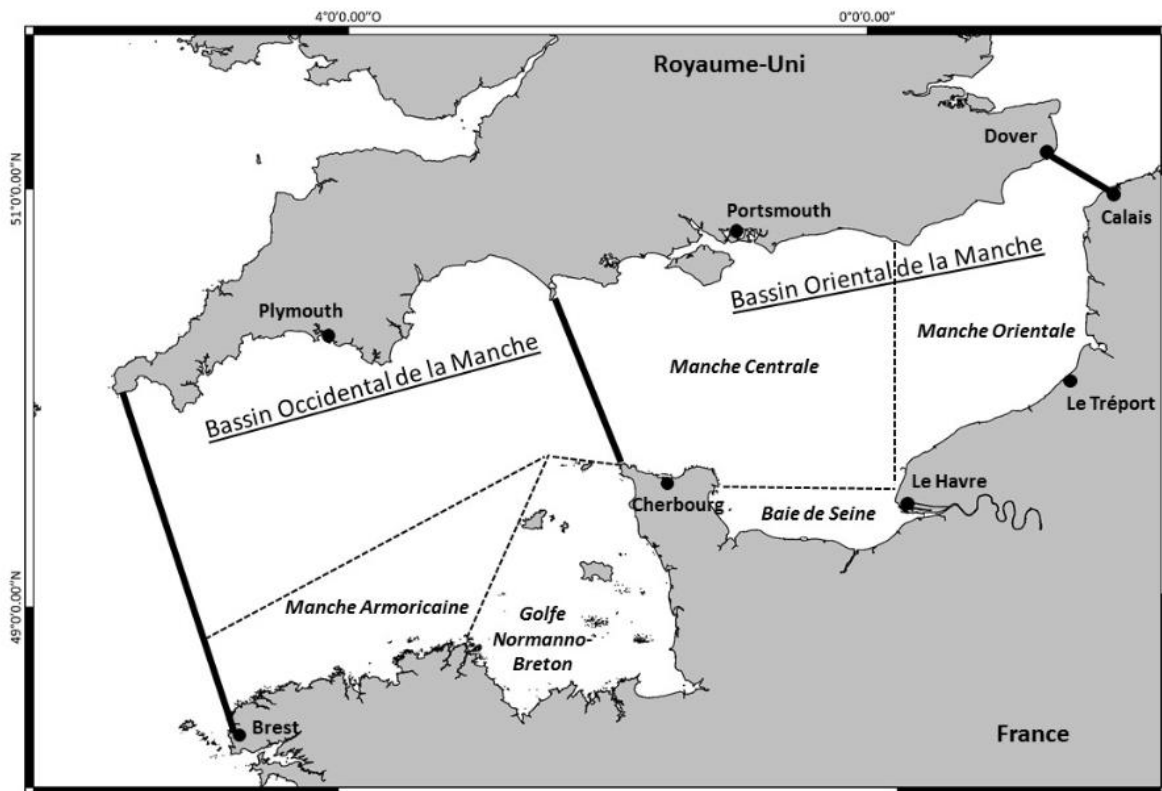


Figure 1 – Carte de la mer de la Manche avec représentation des principaux bassins. Issue de Dauvin 2012.

Les caractéristiques hydrodynamiques de cette mer semi-fermée permettent de la diviser en deux parties (Dauvin, 2012). Bien qu'il y existe des courants induits par les vagues et par le vent, les courants de marée sont dominants. En Manche Est, ces courants de marée sont alternatifs et parallèles à la côte, le flot étant orienté vers le nord-est et le jusant vers le sud-ouest (Figure 2). Cet hydrodynamisme complexe structure la dynamique et la couverture

sédimentaire en Manche. Les eaux y sont continuellement brassées, la turbidité y est donc élevée et il n'y a pas de thermocline. La Manche occidentale est caractérisée par une plus forte bathymétrie sauf sur les côtes, on y observe une augmentation des températures à la fin du printemps et l'apparition d'une thermocline jusqu'à la fin de l'été (Lewis & Allen, 2009; Smyth et al., 2010).

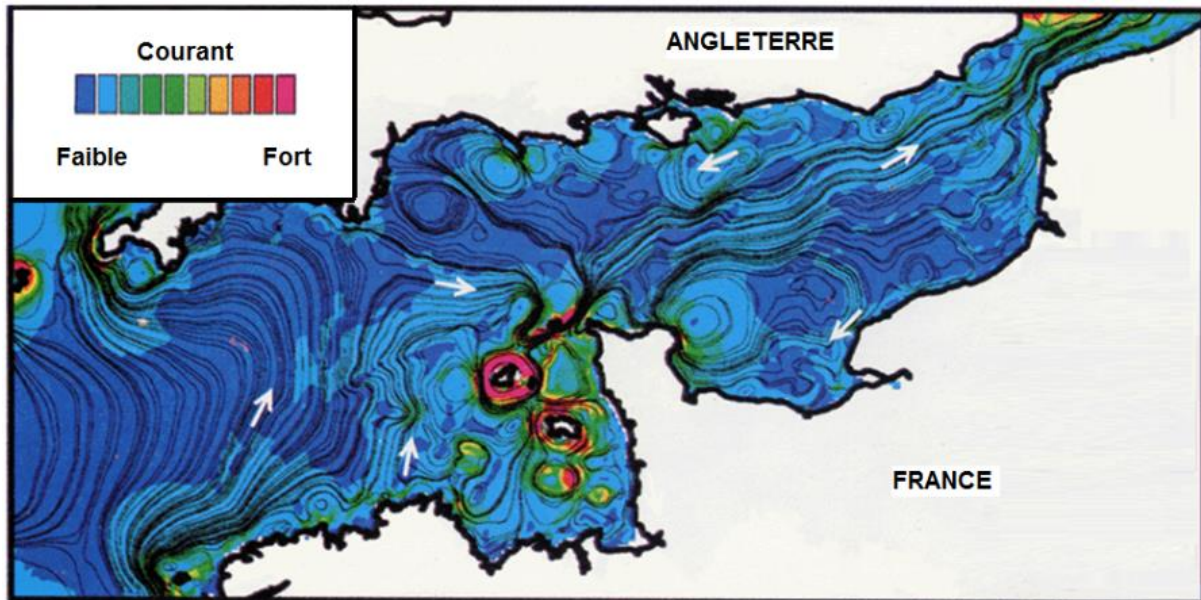


Figure 2 – Carte des courants résiduels en Manche d'après Salomon et Breton, 1991.

Un gradient côte-large fortement marqué s'observe pour plusieurs paramètres environnementaux comme la salinité, la turbidité, la concentration en sels nutritifs mais également pour des paramètres biologiques comme la biomasse phytoplanctonique. En zone côtière de la partie orientale de la Manche le long des côtes françaises, l'influence plus ou moins forte des grands estuaires (Seine, ensemble Escaut-Rhin-Meuse), relayée par des fleuves plus petits (Somme, Canche, Authie) se traduit par une dessalure de la bande littorale dite « fleuve côtier ». Cette différenciation entre les eaux du large et les eaux côtières se retrouvent aussi pour les particules organiques et minérales. Les eaux provenant de l'Atlantique sont peu turbides et faiblement chargées en matières particulaires en suspension (Bodineau et al., 1999). Les apports fluviaux vont augmenter la turbidité (Thomas, 1985) ce qui aura un impact sur la pénétration de la lumière dans la colonne d'eau et affectera la production primaire (Grobbelaar, 1991; Cloern, 1996; Napoléon et al., 2012; Morelle, 2017; Gohin et al., 2019). Les eaux côtières française de la Manche sont riches en sels nutritifs, leur distribution est contrôlée par les mouvements de marées et par les apports d'eau douce fluviaux (Caddy & Bakun, 1994) en particulier de la Seine (Napoléon et al., 2012; Morelle et al., 2017). Leur distribution est aussi impactée par la thermocline saisonnière en Manche occidentale (Lewis & Allen, 2009; Smyth

et al., 2010). Toutefois, la colonne d'eau de la zone côtière est épuisée en sels nutritifs à la fin de la phase de poussée phytoplanctonique en juin-juillet. Un second bloom phytoplanctonique, observé plus ou moins régulièrement en septembre et avec une intensité également variable d'une année sur l'autre, provient de la régénération estivale en sels nutritifs (Gentilhomme & Lizon, 1997; Napoléon et al., 2012; Thorel et al., 2017).

La couverture sédimentaire de la Manche a été décrite dans de nombreuses études (Larsonneur et al., 1982; Reynaud et al., 2003; Dauvin, 2019), une carte synthétique a été fournie par Dauvin (2019) (Figure 3). La Manche orientale est dominée par des sédiments de type gravelo-sableux, elle est séparée de la Manche occidentale par une zone caillouteuse (Manche centrale). La baie de Seine est principalement composée d'un fond sablo-graveleux et de zones sablo-vaseuses à proximité de l'estuaire de la Seine et de la baie de Veys.

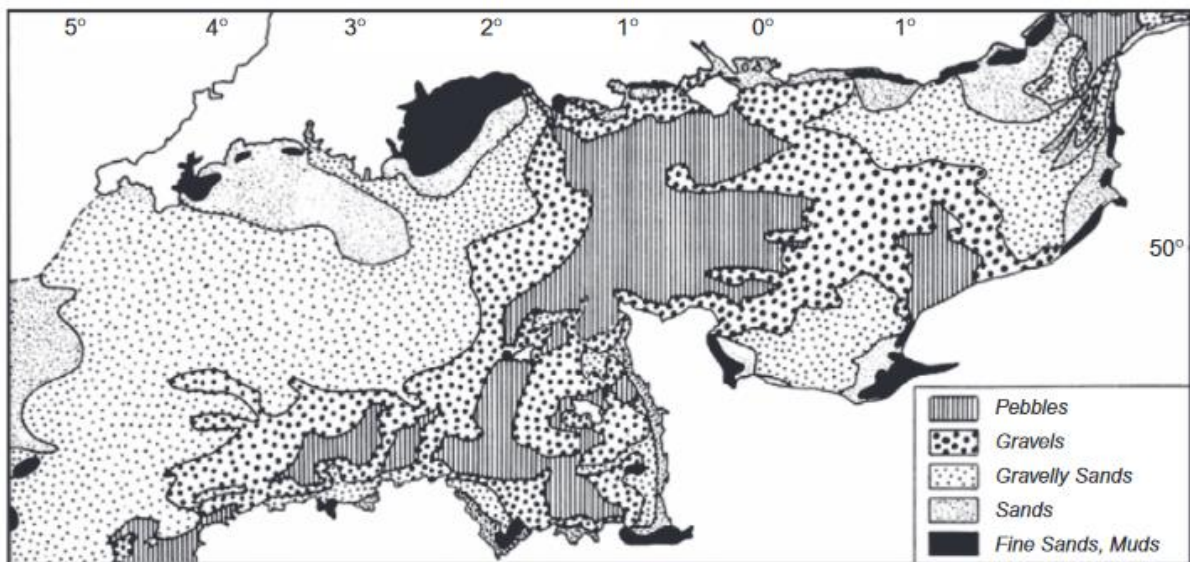


Figure 3 – Carte représentant les cinq types majeurs de sédiments superficiels en mer de la Manche (d'après Larsonneur et al., 1982 dans Dauvin, 2019).

I.2. Une mer productive et exploitée

La production primaire phytoplanctonique en Manche permet de soutenir les compartiments supérieurs du réseau trophique qui sont largement exploités par la pêche et la conchyliculture. Le développement du phytoplancton est contraint par les conditions physico-chimiques du milieu telles que la concentration en nutriments, la pénétration de la lumière ou encore l'hydrodynamisme (Brunet et al., 1996; Cloern, 1996; Napoléon et al., 2013a; Serre-Fredj et al., 2021). En Manche orientale, les efflorescences phytoplanctoniques ont lieu entre mars et juin sur les côtes françaises et dans la baie de Seine (Napoléon et al., 2012) et des rebonds peuvent être observés à l'automne (Thorel et al 2017). Les eaux côtières se distinguent des eaux

du large par des concentrations nutritives plus fortes et des efflorescences phytoplanctoniques plus complexes qu'au large pouvant impacter les communautés benthiques côtières (Desroy & Denis, 2004; Dauvin, 2008; Napoléon et al., 2012; Lefran et al., 2021; Serre-Fredj et al., 2021). Les assemblages phytoplanctonique présentent une plus forte hétérogénéité en été que pendant le reste de l'année (Lefran et al., 2021).

Encart 1

Benthos : ensemble des organismes vivants en étroite relation avec les fonds marins (Gray, 1974), représente une des ressources marines clés. Se déclinant en phytobenthos et zoobenthos, il peut occuper plusieurs strates permettent de définir trois catégories :

- Endobenthos : « totalité des espèces sessiles ou vagiles qui se trouvent dans l'épaisseur, les cavités, fissures ou interstices du substrat » (Pérès, 1961)
- Epibenthos : « totalité des espèces sessiles ou vagiles qui se trouvent à la surface du sédiment ou de la roche » (Pérès, 1961)
- Suprabenthos : « partie de la faune benthique constituée d'animaux de petite taille, surtout des Crustacés, qui tout en étant liés au fond d'une façon ou d'une autre, possèdent de bonnes capacités natatoires et peuvent occuper, pendant des périodes et sur des distances variables, la couche d'eau immédiatement adjacente au fond » (Brunel et al., 1978).

Les « assemblages » écologiques définissent un groupe d'espèces, de populations, vivant ensemble dans un lieu ou un environnement donné sans notion d'interrelation entre les espèces (Pérès, 1982). La « communauté » fait référence à une entité de la biocénose, elle est définie quantitativement par l'abondance, la dominance ou la structure fonctionnelle. Elle se définit par un groupe d'organismes rencontrés dans un environnement particulier, interagissant entre eux et avec l'environnement (Mills, 1969). Enfin, la « biocénose » définit un groupement d'organismes vivants, liés par des relations d'interdépendance dans un biotope dont les caractéristiques dominantes sont relativement homogènes (Karl August Möbius, 1877). La « biocénose » est inséparable du « biotope » qui se définit comme les conditions physico-chimiques d'une biocénose. Un « habitat » est synonyme de « biotope » et combine les facteurs abiotiques (environnement physique) et biotique (communautés). Un « peuplement » correspond à l'ensemble des populations d'un même niveau taxonomique vivant dans un même biotope.

Les communautés macrobenthiques peuvent être modifiées par des perturbations anthropiques, de nature biologique, physique ou chimique et par des processus naturels. La majorité de ces perturbations se situent en zone côtière et sont le résultat des activités humaines (Gray, 1997). La Manche une zone de forte productivité aquacole et halieutique (Fritsch et al., 2007; Carpentier et al., 2009). On y exploite de nombreuses espèces d'intérêt commercial et la pêche est l'une des activités économiques principale. L'aquaculture représente aussi un secteur important de cet espace avec notamment l'ostréiculture et la mytiliculture.

Sa position géographique en fait une voie privilégiée pour les flux maritimes commerciaux. Près de 20% du trafic mondial passe par le détroit du Pas de Calais pour emprunter la Manche. Il s'y ajoute les trafics transmanche (France – Royaume Uni), Roscoff – Plymouth à l'Ouest et Calais – Douvres à l'Est. Le tourisme constitue aussi une activité économique majeure dans chacun des régions littorales de cet espace et contribue largement au développement d'activités saisonnières comme les sports nautiques. L'exploitation des ressources naturelles qui y sont abondantes constitue aussi un des principaux secteurs économiques de la région. L'extraction de granulats marins comme les granulats siliceux ou calcaires, y est très développée (Boyd & Rees, 2003; Dauvin, 2019). On recense également de nombreuses centrales nucléaires sur le littoral de la Manche aussi bien du côté français qu'anglais. Le rejet dans le milieu marin des eaux de refroidissement, réchauffées et chlorées, des centrales nucléaires crée des taches thermiques chaudes qui favorisent l'installation d'espèces plus thermophiles et stimulent le développement bactérien cependant leurs effets restent très limités en surface ce qui en fait un impact local (Poornima et al., 2005; Saravanan et al., 2008; Lin et al., 2018). De plus, plusieurs projets d'implantation d'éolienne off-shore sont en cours de développement aussi bien sur les côtes anglaises que françaises (Raoux et al., 2021). Des ports d'importance internationale sont répartis le long des côtes comme Dunkerque, Le Havre, Rouen ou Southampton, plusieurs milliers de passagers et de véhicules y transitent chaque année (Carpentier et al., 2009). L'installation et l'entretien de ces aménagements perturbent les écosystèmes en place. En effet, les travaux de construction portuaire peuvent modifier les conditions hydrodynamiques et sédimentaires locales, ce qui perturbe les communautés existantes. L'aménagement du nouveau bassin du grand port maritime du Havre par exemple (projet Port 2000), a engendré des modifications des communautés (Dauvin et al., 2006). Les bassins portuaires ainsi que leurs chenaux d'accès doivent être régulièrement entretenus par dragages lorsque le trafic maritime y est intense. Les sédiments dragués sont rejetés en mer dans des zones de clapage où en général l'énergie hydrodynamique permet la dispersion des sédiments de dragage (Alzieu et al., 1999;

Coulon, 2014), ce qui peut modifier le milieu comme cela a été montré en Manche (Baux et al., 2019). De plus, des particules fines sont remises en suspension lors des différentes étapes de l'entretien des ouvrages portuaires et peuvent ainsi modifier la sédimentologie à la fois des zones côtières situées juste à la sortie des ports et des zones de clapage (Coulon, 2014). Les sédiments rejetés sont riches en matière organique et la plupart du temps anoxiques mais également porteurs de polluants divers, qui peuvent perturber les communautés benthiques en place. Toutefois, ces perturbations sont restreintes à la zone de dépôt et l'environnement proche (quelques centaines de mètres) (Marmin, 2013; Baux, 2018).

La somme de ces activités peut entraîner des pollutions physiques, chimiques et biologiques (Boughriet et al., 1994; Desprez, 2000; Desprez et al., 2010; Garnier et al., 2019; Pezy et al., 2019). Les pollutions chroniques, comme les rejets d'effluents pollués en mer, constituent un facteur important de la dégradation des biocénoses marines côtières (Dauvin, 1997). Ces rejets peuvent entraîner des conséquences variables telles que :

- La dégradation de la matière organique par les processus bactériens va induire une plus forte demande en oxygène, ce qui occasionne une diminution de la concentration en oxygène dans l'eau au niveau du fond et des modifications des processus d'oxydoréduction des sédiments.
- Un enrichissement en sels nutritifs localisé pouvant entraîner une efflorescence phytoplanctonique menant à un phénomène d'eutrophisation (Rabalais, 2009).
- Un enrichissement en métaux lourds pouvant altérer les métabolismes et la reproduction de certains organismes (Langston, 1990; Baker et al., 2014).

Les pollutions diffuses concernent les rejets issus de l'aquaculture par exemple, ces pollutions impactent positivement la quantité de matières organiques et peuvent aussi engendrer des contaminations pharmacologiques (Ottinger et al., 2016). Les pollutions accidentelles, comme les accidents pétroliers, sont également présentes en Manche. Ces pollutions ont un effet immédiat sur les communautés biologiques avec des conséquences sur la structure et la production des écosystèmes touchés à long terme (Cabioch et al., 1978; Dauvin, 1987, 1998). Enfin, les pollutions biologiques représentent un risque élevé et également très étudié en mer de la Manche. Les introductions d'espèces marines exotiques par l'homme (eaux de ballast, coques des navires) sont nombreuses. Plus de 150 espèces (faune et flore) introduites ont été recensées en Normandie et certaines comme la crépidule (*Crepidula fornicata*) ou encore *Hemigrapsus sanguineus*, un crabe originaire du Japon qui entre en compétition avec le crabe

vert européen (*Carcinus maenas*) ont des impacts sur le fonctionnement des écosystèmes littoraux et côtiers. Toutefois, l'ensemble de ces espèces ne représente pas un risque : en Normandie, seulement 10 espèces introduites ont un caractère invasif (Baffreau et al., 2018).

Encart 2

Les conséquences du changement climatique sur les écosystèmes marins et côtiers sont difficiles à prédire et à mesurer. Le dernier rapport du groupe d'experts intergouvernementaux sur l'évolution du climat (GIEC) met en avant des conclusions claires sur le sujet en appuyant sur le fait que les émissions de gaz à effet de serre n'ont jamais été aussi élevées qu'actuellement et que les conséquences sur la température moyenne de l'atmosphère et des océans sont sans équivoques. Sans enrayment franc de cette dynamique, les conséquences futures sur les écosystèmes humains et naturels seront sévères et irréversibles (Pachauri et al., 2014). Ceci entraîne des modifications de la répartition des précipitations et des températures à l'échelle du globe, d'où des changements dans les systèmes climatiques régionaux. L'augmentation des événements météorologiques exceptionnels (tempêtes, cyclones) serait peut-être également liée à ce changement climatique. Les conséquences de ces événements climatiques et météorologiques se feront ressentir sur les écosystèmes côtiers, notamment avec une augmentation des apports sédimentaires fluviaux dans la baie de Seine par exemple (Fettweis et al., 2012). Ces enrichissements sédimentaires impacteront les communautés marines et notamment les communautés benthiques en modifiant les habitats. L'ensemble des habitats marins seront impactés par le changement climatique, les communautés benthiques (macro et microorganismes) seront affectées par les changements environnementaux induits par l'activité humaine (Rombouts et al., 2012; Pachauri et al., 2014; Dobretsov et al., 2019). Les communautés microbiennes phototrophes seront également impactées par les modifications de température et d'acidité (Mazière et al., 2022).

La mer de la Manche est soumise à une augmentation constante de la température moyenne des eaux de surface (Saulquin & Gohin, 2010; Gohin et al., 2019), les données du suivi SOMLIT (Service d'Observation en Milieu Littoral) réalisé en baie de Seine indiquent une augmentation moyenne de 0,6°C par an. L'augmentation du niveau de la mer pourrait atteindre 1,1 m par rapport au niveau actuel d'ici 2100. Les conséquences sur les habitats naturels continentaux, littoraux et marins seront importantes et impacteront la diversité et les services rendus à la société Normande. Les conséquences sur la répartition des espèces seront majeures

et impacterons le secteur de la pêche mais également l'aquaculture (Travers-Trolet et al., 2020). La macrofaune benthique sera fortement impactée par le changement climatique dans la mer de la Manche, des modélisations indiquent une réduction significative des habitats à horizon 2050 (Rombouts et al., 2012). Sur le long terme, le déplacement des isothermes pourrait modifier la répartition des espèces, ce qui induirait un changement dans la capacité de résilience des écosystèmes. Ainsi, parmi les effets les plus marquants et les mieux documentés de l'impact du changement climatique sur les écosystèmes marins, il convient de mentionner les changements observés dans la phénologie et la distribution des espèces qui se traduisent principalement par un avancement de la période de reproduction ou de bloom des organismes planctoniques (Edwards & Richardson, 2004) et par une migration des espèces en relation avec les changements de la distribution des températures vers les pôles ou vers des eaux plus profondes (Perry et al., 2005; Sunday et al., 2012). A l'échelle de la Manche, un déplacement de certaines espèces d'affinité boréale vers des isothermes plus froids et une augmentation du nombre d'occurrences des espèces chaudes est déjà observé (Gaudin, 2017). L'augmentation des concentrations de CO₂ dans les océans aura également des conséquences sur l'acidification de ces derniers et influera sur les organismes contenant du carbonate de calcium (Mostofa et al., 2016).

Les trajectoires d'acclimation des organismes marins en milieu côtier sont difficiles à appréhender. En effet, à l'inverse des systèmes océaniques, les milieux côtiers sont soumis à des variations importantes des paramètres environnementaux (Stock et al., 2015; Carstensen & Duarte, 2019). La température, le pH, la salinité ou la luminosité sont soumis à des variations quotidiennes et saisonnières, ainsi, les organismes vivant dans ces écosystèmes sont connus pour leur forte adaptation. Leur capacité d'acclimation aux changements climatiques globaux et lents sont par conséquent difficile à prévoir (Bernhardt & Leslie, 2013; Koppel et al., 2015; Battisti, 2021). Par exemple, sur les 10 dernières années, les communautés phytoplanctoniques estuariennes de la baie de Seine montrent une forte stabilité (Lefran et al., 2021). Les communautés benthiques côtières sont également décrites comme fortement résiliente face à ces variations de pressions environnementales (Steneck et al., 2002; Douglas et al., 2017; Martinelli et al., 2017). Ainsi, dans ce contexte climatique particulier, en tenant compte du risque de diminution des habitats naturels marins et de la nécessité de préserver les services rendus à la société Normande, il est nécessaire d'étudier les trajectoires écosystémiques et d'acclimation des espèces marines tout en considérant l'ensemble des solutions adaptées pour limiter ces impacts. Les récifs artificiels constituent ainsi une réponse adaptée aux

problématiques futures et actuelles permettant de contrer la perte d'habitat naturels rocheux, de protéger certains écosystèmes voir de valoriser la ressource halieutique.

II. Les récifs artificiels, mécanismes de fonctionnement et bénéfices écologiques et sociétaux

II.1. *Généralités et intérêts écologiques, sociétaux et économiques*

L'immersion de structures artificielles en mer peut apporter de nombreux bénéfices, à la fois écologiques, sociétaux mais aussi économiques (Salaün et al., 2020). L'histoire des récifs artificiels remonte au XVII^e siècle avec l'immersion volontaire de structures archaïques afin d'augmenter les captures de pêches au Japon. C'est aussi dans ce pays que l'on répertorie le plus grand nombre de récifs artificiels à ce jour (Bortone, 2006). Un récif artificiel (RA) est défini comme une structure immergée ou partiellement exposée aux marées de manière volontaire avec le but de mimer certaines fonctions d'un récif naturel comme l'apport d'une protection, d'un habitat ou d'une nourricerie pour certaines espèces marines. Cette structure doit s'intégrer dans un écosystème naturel comme un habitat supplémentaire sans effet négatif (FAO et al., 2015). Cette définition exclue donc les structures de défense côtière comme les digues mais aussi les îles artificielles, les plateformes off-shore ou les mouillages. Nous parlerons d'infrastructures marines pour définir l'ensemble des structures artificielles réalisées par l'Homme et implantées en milieu marin (digue, plateformes off-shore...). Ces infrastructures marines représentent un enjeu écologique important en constituant l'opportunité de créer un nouvel habitat marin tout en préservant la structure de sa fonction initiale : c'est l'éco-conception comme défini par Pioch et Souche, (2021).

Le principal objectif concernant l'implantation de récifs artificiels est l'augmentation des captures de pêches (Pioch, 2008; Koeck et al., 2011; Kasim et al., 2013; Vivier et al., 2021b). Ces structures peuvent aussi remplir un rôle de restauration écologique par la création de nouveaux habitats et ainsi favoriser la recolonisation d'un écosystème (Seaman, 2000; Krohling et al., 2006; Liversage & Chapman, 2018). Ils peuvent aussi permettre de protéger un espace contre la pêche ou toute autre activité illégale (Silva et al., 2016). Les RA peuvent aussi remplir un rôle d'éducation à l'environnement et de loisir en suscitant notamment l'intérêt des plongeurs et en permettant la mise en place d'opérations de communication avec le but de sensibiliser le grand public face aux problématiques écologiques (Ditton et al., 2002). Enfin, les structures artificielles immergées sont aussi exploitées scientifiquement et permettent de mieux comprendre et interpréter les différents mécanismes écologiques impliqués dans le

fonctionnement global des réseaux trophiques associés à ces écosystèmes (Baine, 2001; Danovaro, 2002; Relini et al., 2002; Bortone, 2006; Koeck et al., 2011; Leitão, 2013). Elles permettent aussi de tester différentes hypothèses scientifiques en comparant différents traitements expérimentaux à une structure identique (Hixon & Beets, 1989; Charbonnel et al., 2002).

II.2. *Mécanismes de fonctionnement des récifs artificiels*

Il existe une grande variété de récifs artificiels dans le monde, ils diffèrent par leur formes, matériaux, taille etc (Jensen et al., 2000; Vivier et al., 2021b). A l'échelle unitaire, le RA se définit par différentes caractéristiques. L'architecture est un élément clé de leur spécificité, elle est liée à la fonction première du récif et doit tenir compte de son interaction avec l'environnement. Elle intègre la rugosité, la porosité, l'hétérogénéité ou encore l'élévation de la structure. Les principaux matériaux retrouvés sont la roche, le bois, le métal et les bétons classiques ou transformés. On peut aussi retrouver des structures en terre cuite, polymères ou cordages. Le volume et le poids de chaque module est aussi un paramètre important pouvant influencer sur l'efficacité du récif.

L'ensemble de ces caractéristiques, propre à chaque structure, va influencer sur le fonctionnement écologique du récif. Chaque structure va ainsi permettre de reconstituer un habitat plus ou moins complexe dans une zone qui en est dépourvue. Il va permettre de recréer les fonctions écologiques principales comme la reproduction, l'abri face à la prédation, la mise en place d'une colonisation primaire et d'une succession écologique plus complexe aboutissant sur une structure trophique propre à cet écosystème. Il existe un débat scientifique autour de la réelle capacité d'un récif artificiel à être producteur de biomasse. C'est un débat qui existe depuis les premières études scientifiques sur le sujet et qui oppose deux concepts concernant le fonctionnement écologique de ces structures : l'attraction ou la production (Bohnsack, 1989; Grossman et al., 1997; Pickering & Whitmarsh, 1997; Brickhill et al., 2005).

Le premier concept, celui de l'attraction, considère que ni l'habitat, ni l'alimentation, ne sont des facteurs limitants et que les habitats artificiels et naturels sont comparables qualitativement. Cette hypothèse implique donc que les RA sont des structures de concentration de biomasse entraînant ainsi une diminution de cette biomasse dans les zones naturelles environnantes. L'enrichissement mesuré sur le récif serait donc contre-productif dans un contexte de restauration environnementale. Il permettrait aussi une capture plus facile de la biomasse halieutique et favoriserait son exploitation en les concentrant de la même manière que

les dispositifs de concentration de poissons utilisés pour capturer les grands pélagiques comme le thon. Il est donc indispensable de mettre en place des mesures de gestion des récifs artificiels (Salaün et al., 2020) et des indicateurs d'évaluation des effets des RA sur les écosystèmes marins (Taormina et al., soumis). Le second concept, celui de la production, considère que l'immersion de récifs artificiels permet d'augmenter la qualité du milieu via une augmentation de la disponibilité de nourriture ou de l'habitat. Cet apport permet une augmentation de la biomasse locale sans impacter la biomasse des milieux naturels environnants et donc permet d'augmenter la biomasse totale du milieu. L'effet récif diffère selon l'espèce et le niveau trophique étudié (Brickhill et al., 2005; Leitão, 2013). La fonction de production est difficile à démontrer pour chaque niveau trophique et il est évident que les premières espèces colonisant une structure artificielle sont le résultat d'une attraction et non d'une production propre au récif (ex : les poissons), toutefois, les producteurs primaires et certaines espèces de la macrofaune sessile ou vagile vont coloniser les RA selon une succession écologique et donc vont créer une biomasse et ainsi générer une production secondaire.

Toutefois, l'intérêt écologique de telles structures est important, de plus, la mise en place d'une succession écologique et la structuration d'un réseau trophique complexe va dépendre de la capacité du module immergé à accueillir efficacement des espèces pionnières ou ingénieures (Sempere-Valverde et al., 2018). Ces espèces sont elles-mêmes dépendantes de la qualité et de la quantité d'organismes microphytobenthiques ayant colonisé le substrat en formant un biofilm (Salta et al., 2013). Cette couche fine composée d'organismes unicellulaires diversifiés va constituer un apport alimentaire nécessaire aux consommateurs primaires (Underwood, 1984; Anderson, 1995).

III. Les projets MARINEFF et RECIF

III.1. *Le projet RECIF*

Le projet Interreg IVa France (Manche) – Angleterre « RECIF » (2013 – 2015) a été conçu avec l'objectif de créer des récifs artificiels en béton intégrant des coproduits coquilliers recyclés. Les objectifs de ce projets étaient de :

- a) Apporter une valeur ajoutée à certains produits issus de la pêche ou de l'aquaculture considérés comme déchets comme les coquilles d'huîtres en les intégrant au processus de fabrication du béton pour la réalisation des récifs artificiels
- b) Développer des matériaux innovant pour la réalisation de récifs artificiels
- c) Déployer des récifs artificiels en mer afin d'améliorer la production et la biodiversité de l'écosystème associé.

Les structures immergées dans le cadre du projet RECIF en 2015 entrent donc bien dans la définition d'un récif artificiel donnée précédemment puisque leur seul et unique objectif était la création d'un nouvel habitat.

Le projet RECIF rassemble 8 partenaires français et britanniques :

- Ecole Supérieur d'Ingénieurs des Travaux de la Construction de Caen – ESITC Caen
- Vinci Construction Maritime et Fluvial – VCMF
- University of Exeter – UE
- Muséum national d'Histoire naturelle – MNHN
- University of Southampton – US
- Plymouth Marine Laboratory – PML
- Université de Caen Normandie – UC
- Travaux Public du Cotentin – TPC

III.2. *Le projet MARINEFF*

Le projet Interreg Va France (Manche) – Angleterre « MARINEFF » (2018 – 2022) a été mis en place avec l'objectif de répondre aux attentes socio-économiques et écologiques sur le littoral de la Manche, il est né à la suite du projet RECIF. En effet, considérant l'ensemble des pressions exercées sur cet écosystème et tenant compte de l'obligation d'artificialiser certains habitats dans certains cas, le projet MARINEFF cherche à apporter une réponse permettant de concilier ce développement humain à la question environnementale. Pour ce faire, des

infrastructures marines écoconçues ont été réalisées. Ces dernières apportent les mêmes avantages en termes de construction ou de protection par exemple que des blocs en béton classiquement utilisés pour les ouvrages marins (digue, enrochement artificiel...) tout en intégrant, dès leur conception, une dimension écologique avec la présence de différentes modifications de surface permettant de recréer des habitats pour la vie marine. La définition d'un récif artificiel donnée précédemment ne correspond pas aux infrastructures immergées dans le cadre du projet MARINEFF car celles-ci répondent à des objectifs supplémentaires de ceux définis par Fabi et al. (2015). Nous parlerons donc d'infrastructures marines plutôt que de récifs artificiels pour décrire les blocs de digues MARINEFF. C'est dans ce contexte que le projet Interreg Va MARINEFF (MARine INFrastructure EFFects, <http://marineff-project.eu/>) s'inscrit, avec comme principal objectif le développement de solutions et d'outils afin d'associer aux infrastructures marines existantes et aux futurs projets une dimension écologique. Le projet vise ainsi à réaliser de nouvelles infrastructures marines ayant comme but la limitation des effets négatifs sur les écosystèmes dus à l'anthropisation des côtes en France et en Grande-Bretagne. Le projet MARINEFF a été sélectionné dans le cadre du Programme européen de coopération transfrontalière INTERREG VA France (Manche) / Angleterre cofinancé par le FEDER et rassemble neuf partenaires français et britanniques (Figure 4) :

- Ecole Supérieure d'Ingénieurs des Travaux de la Construction de Caen – ESITC Caen (porteur du projet)
- University of Southampton – US
- Ports de Normandie – PNA
- Bournemouth University Higher Education Corporation – BU
- University of Exeter – UE
- Université de Caen Normandie – UC
- Muséum national d'Histoire naturelle – MNHN
- Vinci Construction Maritime et Fluvial – VCMF
- Travaux Public du Cotentin – TPC

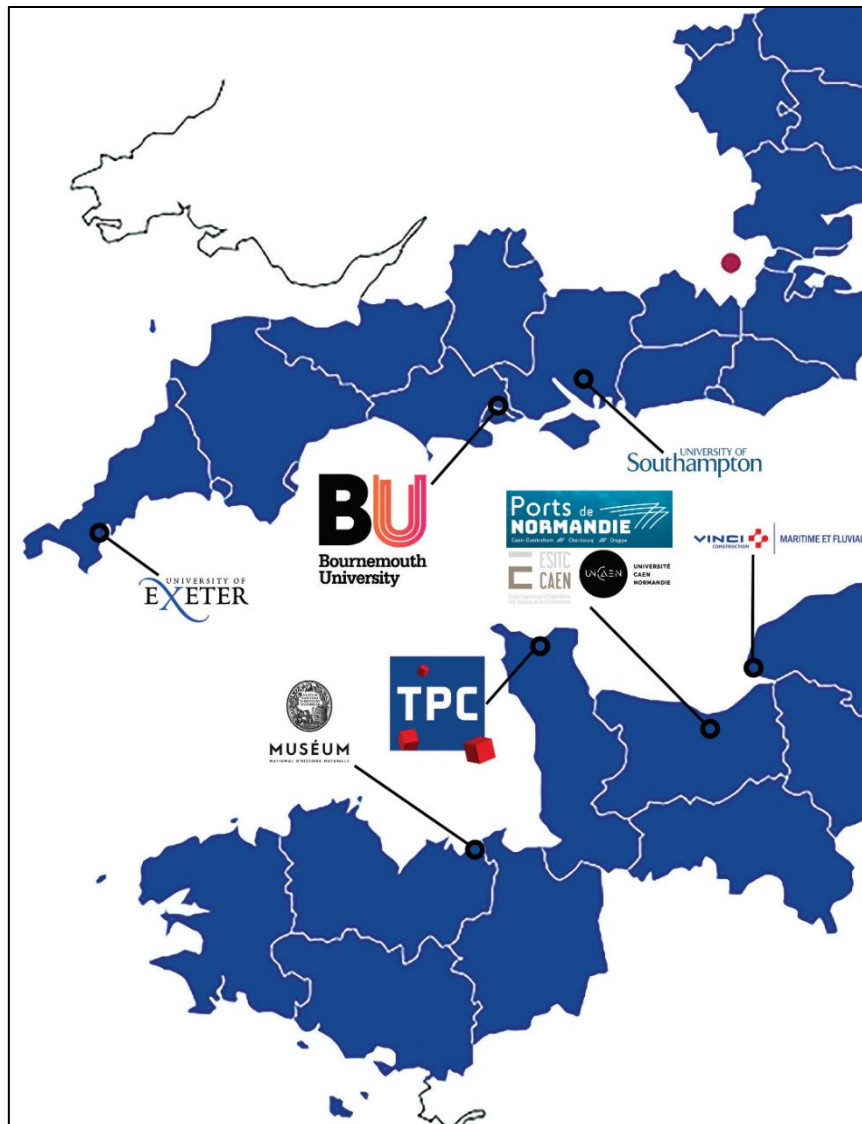


Figure 4 – Localisation de la zone MARINEFF avec l'ensemble des acteurs du projet. Modifié de www.ciria.org/marineff

Ce projet comporte différentes actions, dont l'évaluation de l'impact de structures immergées de type récifs artificiels sur les compartiments écologiques et biologiques du milieu avec notamment l'étude de leur impact sur la biodiversité, la structure du réseau trophique associé et la fonction de production primaire du milieu. C'est dans ce contexte que s'inscrit ce travail de thèse qui s'est donc focalisé sur l'étude de la biodiversité et de la production primaire associés à des substrats durs en zone subtidale dans un contexte d'artificialisation des côtes.

III.3. *Sites d'études et description des modules expérimentaux*

III.3.1. *Sites d'études des projets MARINEFF et RECIF*

Dans le cadre du projet MARINEFF, différents types de structures ont été immergées dans plusieurs sites d'études répartis dans la zone présentée précédemment. L'université de Southampton a travaillé sur des modules destinés à améliorer le statut de l'huître plate (*Ostrea edulis*) en Manche. Une cinquantaine de structures ont été immergées dans une zone d'un hectare accueillant environ 20 000 huîtres plates. Deuxièmement, des infrastructures marines ont été réalisées avec l'objectif de limiter l'impact humain sur le milieu marin lorsque le déploiement d'infrastructures est inévitable. Le travail collaboratif des différents partenaires du projet a permis de réaliser trois types de structures : les modules de digue, les modules de lutte contre l'érosion et les mouillages pour la plaisance. Afin de garantir la transférabilité des résultats dans toute la Manche, ces infrastructures marines ont été déployées sur cinq sites : une opération pilote à Cherbourg et quatre sites expérimentaux à Bournemouth, l'Isle de Wight, la baie de Dinard et la baie de Seine. Compte tenu de la situation sanitaire, les modules de digues MARINEFF ont été immergés sur les sites de Cherbourg et Bernières-sur-Mer (baie de Seine) assez tardivement. L'immersion en rade de Cherbourg a eu lieu le 21 septembre 2020 et en baie de Seine le 9 mars 2021. Compte tenu de ces immersions retardées, ce travail de thèse s'est concentré sur l'étude de infrastructures marines déjà présentes sur les mêmes sites expérimentaux. Ces récifs artificiels ont été immergés en 2015 et ont permis de caractériser les processus de colonisation après cinq ans d'immersion en fonction des sites. Outre l'intérêt propre de ce travail, cette approche sur des structures précédemment immergées est un atout majeur car il permet de mettre en place et de valider des protocoles qui seront appliqués dans le projet MARINEFF et permet d'anticiper la dynamique de colonisation des structures. Contrairement au projet RECIF où les différences de colonisation n'avaient pas pu être suivies, le projet MARINEFF nous apporte cette opportunité. De plus, afin de caractériser les processus de colonisation biologique et l'efficacité des blocs de digues MARINEFF, différentes expérimentations en conditions contrôlées et *in situ* ont été mise en place durant cette thèse. Ces expérimentations ont été réalisées sur de petites infrastructures composées des mêmes matériaux et présentant la même structure que les blocs de digues MARINEFF. Ces points seront détaillés plus largement dans la suite de l'introduction et dans la partie Matériel et Méthodes.

III.4. Sites d'études dans le cadre de cette thèse

Dans le cadre de ces travaux, les deux sites étudiés étaient ceux de la rade de Cherbourg (50) et la baie de Seine (14) (Figure 5).

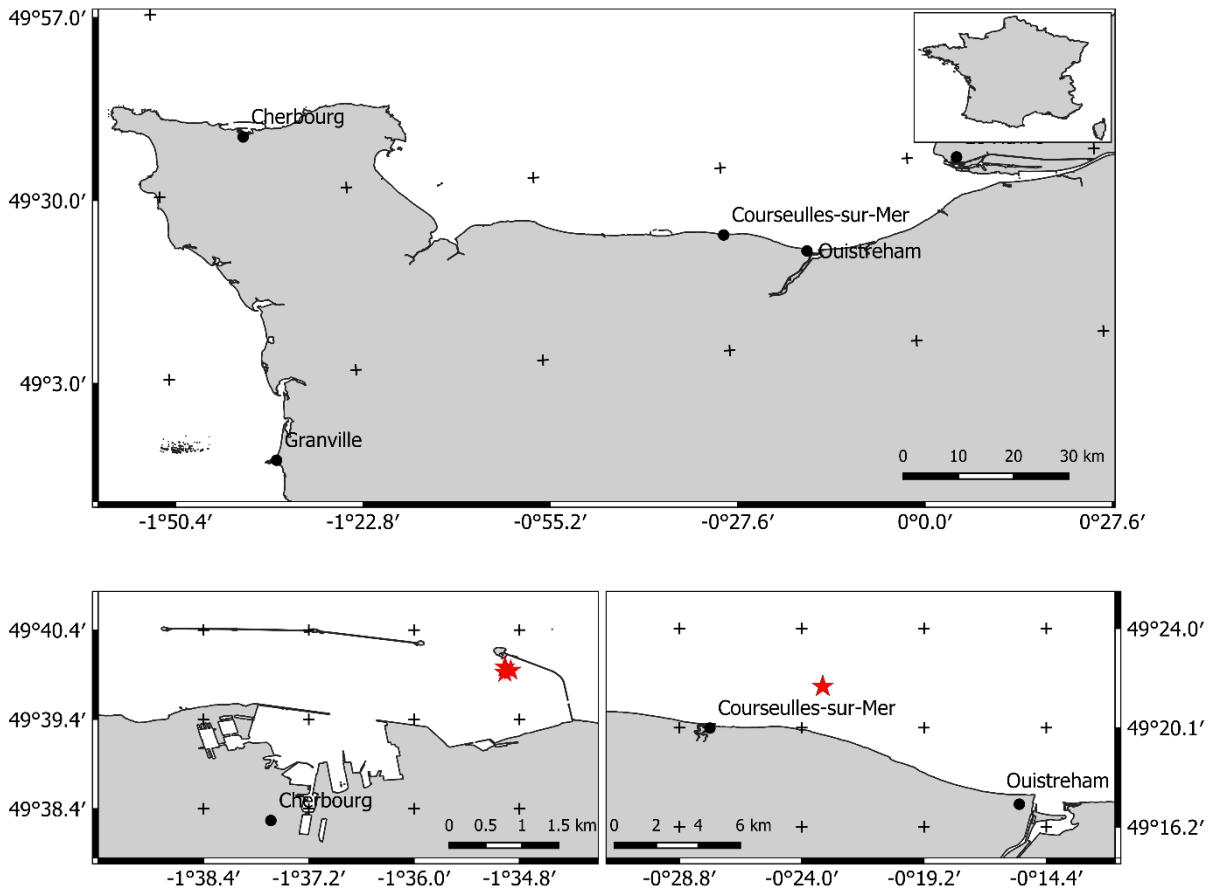


Figure 5 – Localisation des deux sites expérimentaux dans le cadre de ces travaux de thèse. Les étoiles rouges représentent les sites d'immersion des récifs artificiels.

Le premier site d'étude se situe dans la grande rade de Cherbourg (Figure 5), c'est la plus grande rade artificielle au monde avec une superficie d'environ 1 500 ha. La digue du large fait 3 640 mètres de longueur, avec une largeur moyenne de 100 m à sa base et 12 m à son sommet pour une hauteur de 27 m. L'ouverture de la passe de l'Est est de 700 m, celle de l'Ouest est de 1 100 m. Sa profondeur maximale est de 13 m à marée basse. Cet espace semi-fermé conditionne l'hydrodynamisme. La digue influe également sur la distribution des sédiments (Gregoire et al., 2019), et elle habrite partiellement la rade de la houle (Kempf et al., 1997). Les échanges entre les masses d'eau sont conditionnés par le cycle des marées. A marée montante, le « système rade » se remplit par la passe de l'Ouest avec un courant qui circule d'ouest en est. Lorsque l'espace se rétrécit au niveau de la passe de l'Ouest, cela provoque « un effet venturi » ou une accélération du courant. A marée descendante, le fonctionnement de la rade est différent. La rade se vide par la passe de l'Ouest. A l'Est, par la passe de Collignon, l'eau est

aspirée et génère un courant est-ouest, il en est de même pour la passe de l'est où prend naissance le courant principal. Le courant maximal est de 0,55 m/s en vive-eau selon un axe généralement Est-Ouest. Au total, 12 modules de digues sont immergés en zone subtidale et 12 en zone intertidale (six expérimentaux et six classiques pour chaque zone) (Figure 6).

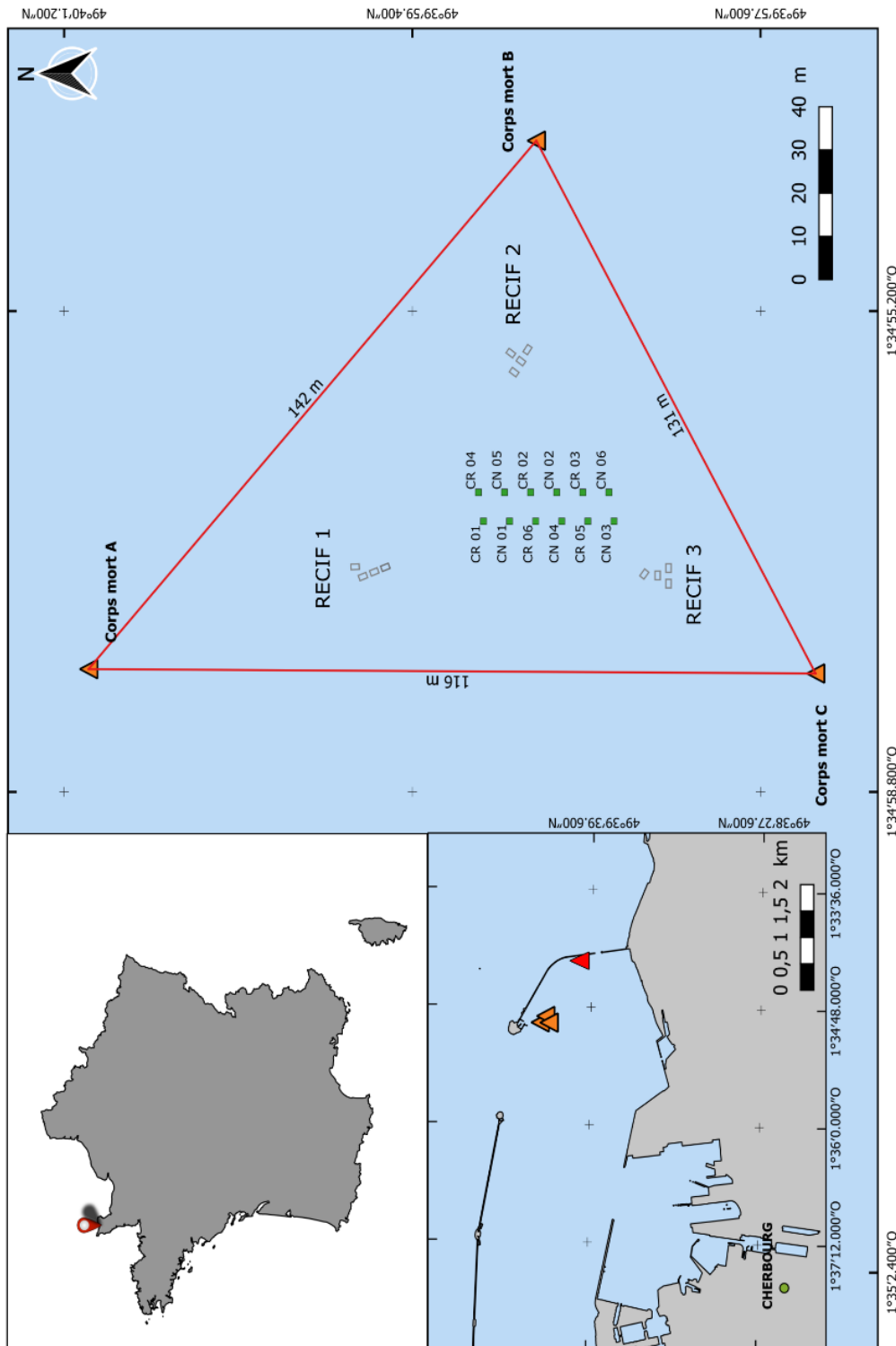


Figure 6 – Site d'immersion des modules digues en rade de Cherbourg. Les triangles représentent l'emplacement des corps mort délimitant la zone d'immersion (projet RECIF), les récifs du projet RECIF immergés depuis 2015 sont représentés par des rectangles (RECIF 1, 2 et 3), les carrés représentent l'emplacement des modules digues MARINEFF en zone subtidale, les blocs témoins sont appelés CN, les blocs expérimentaux CR. Le triangle rouge représente la zone d'installation des modules de digues en milieu intertidal.

Ces échanges conditionnent la qualité de la masse d'eau et les apports en nutriments et en oxygène des différents milieux se trouvant dans la grande rade. L'hydrodynamisme permet aussi les échanges sédimentaires au sein de la grande rade. Les dépôts de sédiments sont très faibles à l'échelle de la grande rade. Deux principaux types de sédiments sont retrouvés, les sableux et les vaseux. L'Est de la rade est recouvert par des sédiments sableux ainsi que centre-ouest. La dynamique de ces sédiments au sein de la rade est influée par l'hydrodynamisme particulier lui-même conditionné par les digues artificielles (Gregoire et al., 2019). Le reste de la rade est recouvert par des sédiments vaseux. Le site d'immersion pour les infrastructures marines se trouve à l'Est de la grande rade, au Sud du Fort de l'île Pelée (Figure 7). Il a déjà fait l'objet d'un site expérimental pour l'immersion de récifs artificiels dans le cadre du projet RECIF. La bathymétrie moyenne du site est de 4 m à marée basse et le substrat est de type sable fin. Les modules de digues sont immergés à l'intérieur de la zone existante.

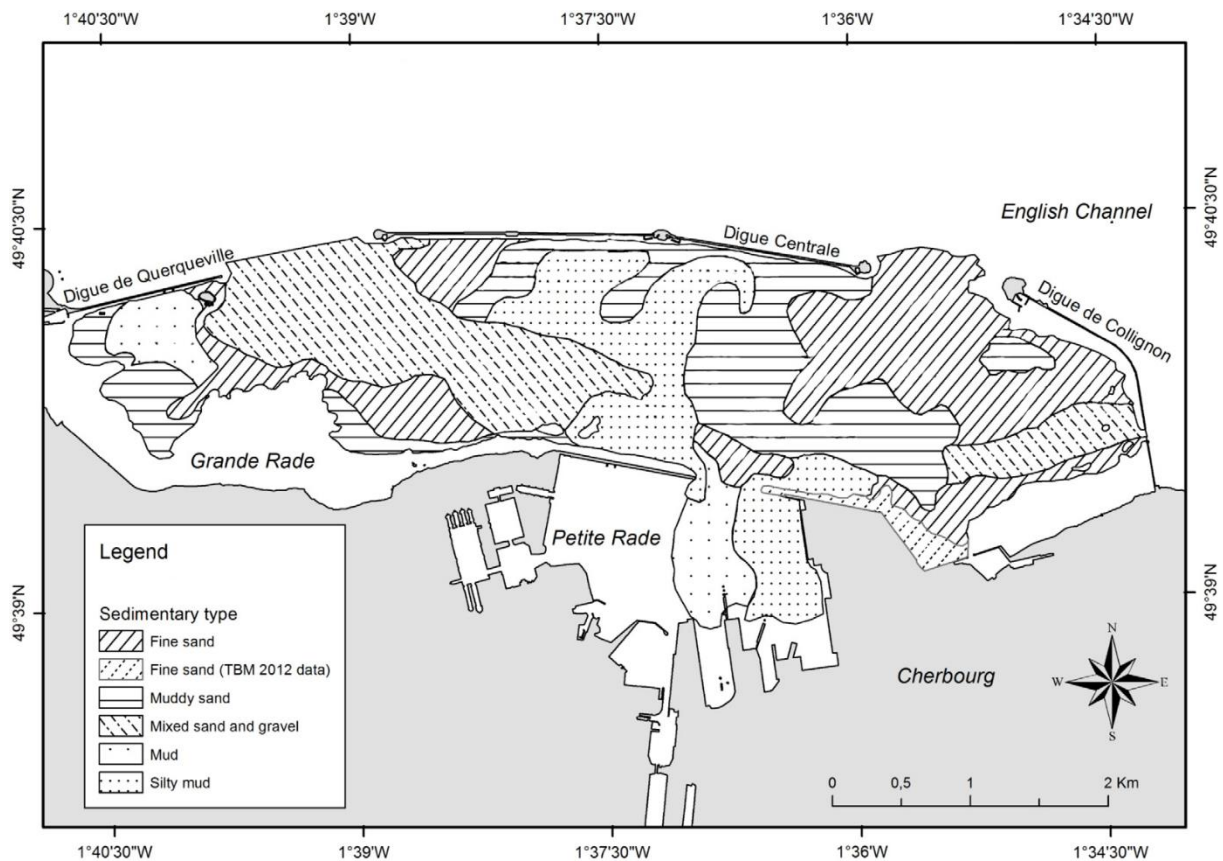


Figure 7 – Carte des différents types de sédiments de la rade de Cherbourg modifiée depuis Baux et al. 2017.

Le site d'étude de la baie de Seine est localisé au large de Bernières-sur-Mer. Ce site présente des paramètres hydrodynamiques différents que le précédent. La bathymétrie moyenne du site est de 4 m à marée basse et il se trouve dans une zone sableuse avec de nombreux platiers rocheux à proximité. Les modules de digues y sont immergés au centre des récifs existants

depuis le projet RECIF (Figure 8). Au total, 12 modules sont immergés (six expérimentaux et six classiques). Sur ce littoral, les courants sont principalement dus à la marée et sont de l'ordre de 1 m/s au maximum du flot et du jusant en vive eau moyenne. Leur direction varie au cours du cycle de la marée mais reste relativement parallèle à la côte (Goulain, 2012; Brivois et al., 2015). L'amplitude des vagues est généralement comprise entre 0,4 m et 1 m. Ce site est donc plus exposé que celui de Cherbourg, soumis à des courants plus importants et des variations environnementales plus drastiques.

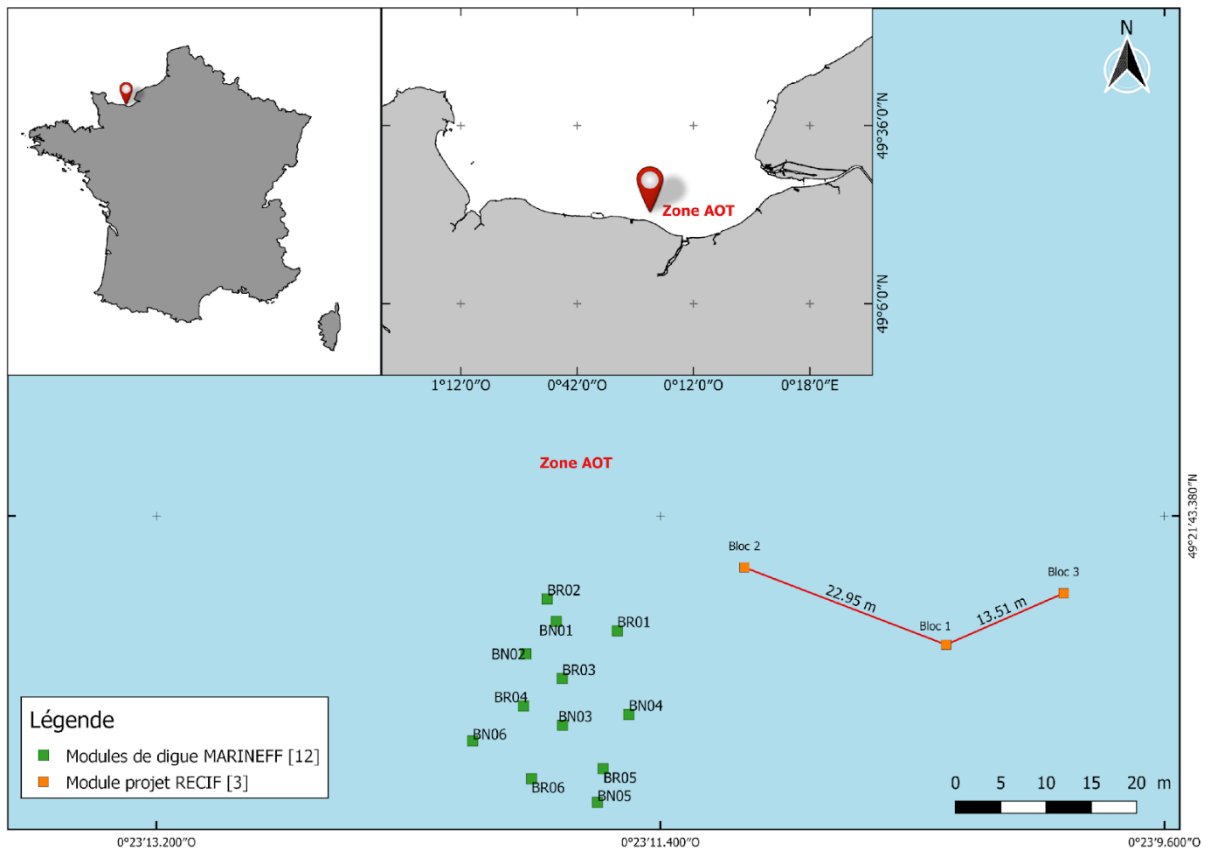


Figure 8 – Site d'immersion des modules digues au large de Bernières-sur-Mer. Les carrés orange représentent l'emplacement des trois récifs immergés depuis 2015 (bloc 1, 2 et 3 ; projet RECIF), les carrés verts (BR01, BN01, etc) représentent l'emplacement des modules digues, les blocs témoins sont appelés CN, les blocs expérimentaux CR.

III.5. Description des infrastructures marines immergées

Les infrastructures marines biomimétiques ou modules digues sont des créations innovantes et originales réalisées dans le cadre du projet MARINEFF et font l'objet d'une enveloppe Soleau. Ces modules sont de formes cubiques et en béton. Deux types de modules ont été créés, des modules expérimentaux présentant différents types de modification de surface et des modules témoins ou classiques ne présentant aucune modification (Figure 10).

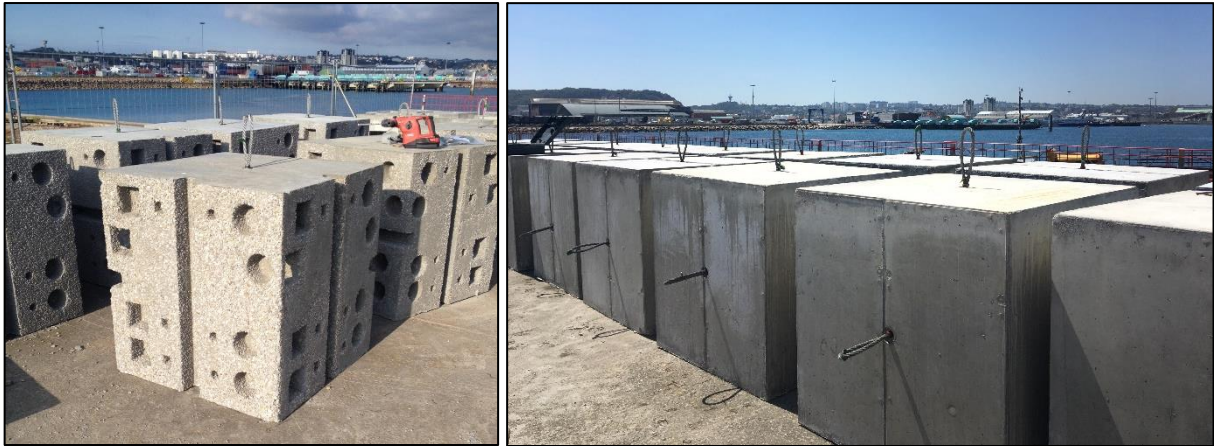


Figure 10 – Photographies de blocs expérimentaux (A) et classiques (B) avant leur immersion sur le site de la rade de Cherbourg. TPC®

Les infrastructures expérimentales possèdent des réservations avec différentes géométries (Figure 9). Le design de ces modifications ont fait l'objet d'une étude bibliographique approfondie durant les premiers mois de ce travail de thèse de manière à optimiser leur efficacité, le but premier étant de créer une diversité d'habitats maximale afin d'améliorer la colonisation sur ces blocs. De plus, l'incorporation de coproduits coquillés issus de l'aquaculture ou de la pêche permet de diminuer la proportion de graviers dans les bétons et donc de limiter l'impact environnemental.

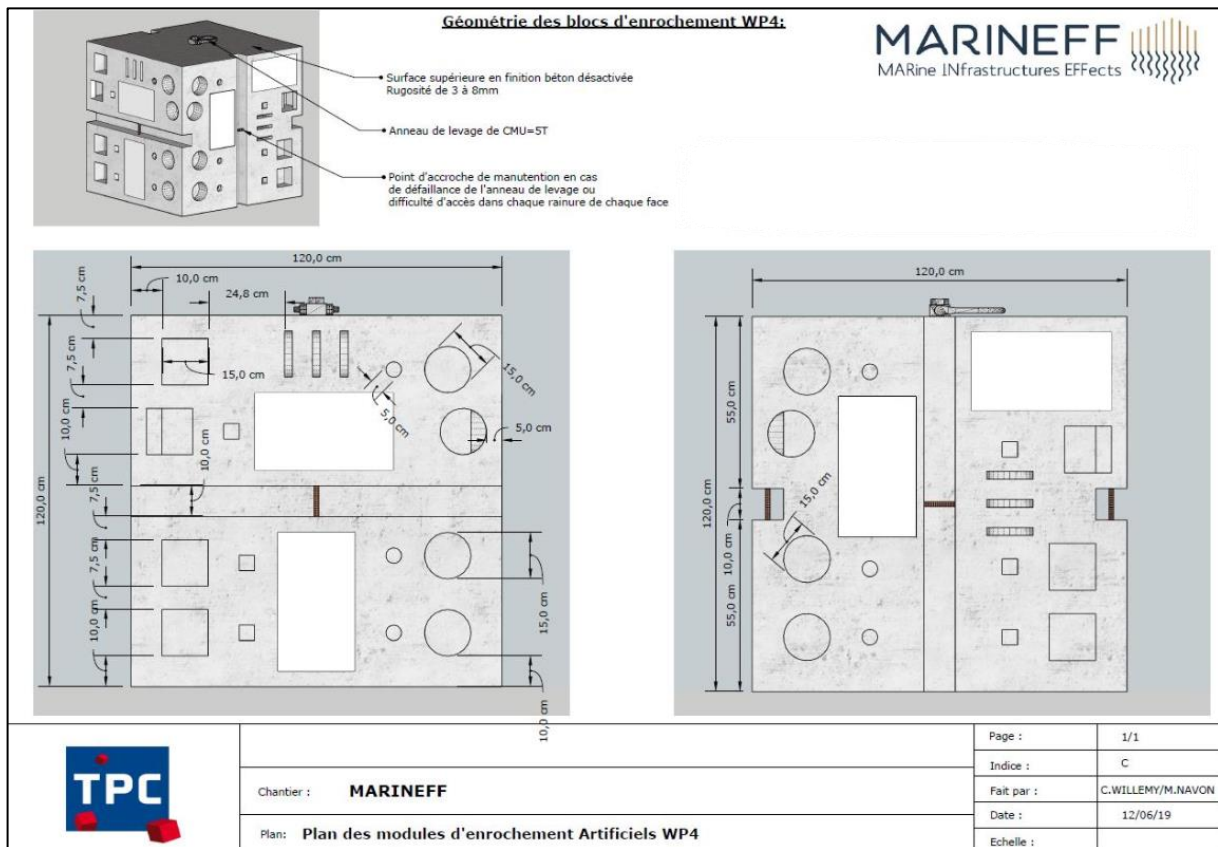


Figure 9 – Design des modules de digues.

La conception des modules de digues expérimentaux MARINEFF a fait l'objet d'un travail collaboratif entre les différents membres du projet. Les modules expérimentaux présentent une masse de 3,3 T, les modules témoins ou classiques ont une masse de 3,9 T. Les infrastructures expérimentales présentent diverses modifications de surfaces, leur fabrication a été réalisée par la société TPC (Figure 9). Les modules de digues expérimentaux doivent respecter certaines contraintes techniques. Les réservations (*i.e.* modifications du relief) sont principalement en surface afin de garantir la stabilité d'ensemble du bloc et de faciliter le coffrage. Les réservations présentent un fruit (*i.e.* diminution de l'épaisseur), celui nécessaire au décoffrage. La profondeur des réservations n'est pas supérieure à la plus petite des distances suivantes : largeur, longueur. La position des réservations est calepinée (*i.e.* alignée) de manière à garantir l'enrobage minimal en tout point.

Le béton utilisé pour la mise en œuvre des modules de digue doit satisfaire les exigences suivantes :

- Classe d'exposition : XS3
- Classe de résistance : C35/45
- Classe de consistance : S4

Pour la réalisation de ce béton, différents matériaux granulaires ont été utilisés. Un sable 0/2 siliceux lavé roulé, des gravillons 4/10 siliceux concassés et des gravillons 10/20 siliceux concassés. Deux types de ciments ont été utilisés de type prise mer. Les surfaces latérales et supérieures des modules expérimentaux sont désactivées (3 à 8 mm) de manière à accentuer la rugosité de surface et favoriser la colonisation par les organismes. Des zones non modifiées, en blanc sur la Figure 9, sont conservées sur chaque face des blocs expérimentaux pour permettre un suivi photographique par quadrats. L'objectif final de ces modules de digues expérimentaux et de pouvoir ajouter un bénéfice écologique par l'augmentation de la qualité et de la diversité des habitats qui y sont associés dans un contexte de développement des énergies renouvelables off-shore, de consolidation des ouvrages portuaires ou de défense du trait de côte.

Ce travail de thèse s'est donc concentré sur l'étude des écosystèmes associés à des structures artificielles et plus particulièrement à la biodiversité et la production primaire benthique. Lorsqu'une infrastructure marine ou un récif artificiel est immergé, la première étape de la colonisation biologique est la formation du biofilm.

IV. Les biofilms marins

IV.1. Généralités

Le terme de biofilm définit le mode de vie sessile de micro-organismes adhérant à une surface s'opposant donc à un mode de vie planctonique (Costerton et al., 1978). Il est estimé que 80% de la biomasse microbienne sur Terre vit sous cette forme (Richards & Melander, 2009). Ils sont généralement décrits comme des agrégats de cellules bactériennes, de microalgues (chlorophytes, diatomées), de champignons ou d'invertébrés attachés à une surface et enrobés d'une matrice polymérique extracellulaire d'origine microbienne (Costerton et al., 1978; Wetzel, 1983; Hall-Stoodley & Stoodley, 2009). Cette matrice extracellulaire apporte aux cellules une intégrité structurelle et fonctionnelle (Wingender et al., 1999).

Schématiquement, les biofilms peuvent être décrits comme des systèmes microbiens dynamiques et structurés sous forme de patches séparés par des pores, des espaces dans lesquels l'eau va pouvoir circuler et transporter les nutriments, les macromolécules et l'oxygène et ainsi atteindre l'ensemble des cellules (Costerton et al., 1995). Une multitude de facteurs vont influencer sur cette architecture comme l'hydrodynamisme, la profondeur (*i.e.* la lumière), le cisaillement, la concentration en nutriments, le type de micro-organismes, leur mobilité, la communication intra-cellulaire ou les polysaccharides et protéines sécrétées (Flemming & Wingender, 2010). Ce mode de vie benthique apporte de nombreux bénéfices à ces organismes comme le piégeage des éléments nutritifs ou la protection des cellules face à de nombreux stress extérieurs tels que l'érosion, la dessiccation, etc (Hillebrand et al., 2000; Staats et al., 2000; Battin et al., 2003). Dans les milieux aquatiques, on utilise différents qualificatifs désignant le type de support colonisé : l'épiphyton pour ce qui colonise les plantes, l'épisammon pour ce qui colonise les grains de sable, l'épipélon pour ce qui colonise les sédiments fins et la vase, l'épixylon pour ce qui colonise le bois et enfin, l'épilithon pour la pierre (McIntire, 1973; De Nardi & Pontié, 2012; Orvain et al., 2012). Nous nous intéresserons ici aux biofilms colonisant des surfaces solides comme les pierres ou tout type de substrat dur, il s'agira donc de biofilm épilithique.

Les biofilms marins constituent une source d'énergie importante pour les niveaux trophiques supérieurs, les autotrophes et hétérotrophes du biofilm épilithique utilisent l'azote et le phosphore (Kindaichi et al., 2004). Les autotrophes pour constituer leur propre biomasse et les hétérotrophes pour dégrader la matière organique. Leur association au sein du biofilm permet un recyclage efficace des nutriments (Teissier et al., 2007). Nous utiliserons le terme de

microphytobenthos lorsque nous nous intéresseront uniquement aux organismes phototrophes comme les microalgues benthiques (eucaryotes) ou les cyanobactéries.

IV.2. *La formation du biofilm*

Tout matériau immergé ou au contacté d'eau sera recouvert après quelques jours ou semaines d'un biofilm. Les mécanismes d'adhésion et de développement de ce biofilm restent encore en partie incompris malgré une bibliographie consistante à ce sujet (Vieira et al., 1993; Battin et al., 2003; Salta et al., 2013; Crouzet et al., 2014). Le biofilm se forme en plusieurs étapes selon un modèle établi puis, en milieu marin, il va évoluer vers un biofilm dit secondaire composés d'une multitude d'espèces appartenant à différents niveaux trophiques (Grzegorzczuk et al., 2018) (Figure 11).

La première étape amenant la formation d'un biofilm en milieu marin est le conditionnement de la surface qui sera colonisée. Il s'agit d'une adsorption de composés du milieu (molécules organiques et ions), elle se déroule dans les premières heures suivant l'immersion de la surface en question (La Barre & Haras, 2007; Palmer et al., 2007). Afin d'adhérer au substrat, les bactéries vont devoir s'en approcher. Les interactions entre bactéries et substrat seront favorisées par un milieu plus turbulent ou ouvert, à l'inverse un milieu statique avec peu de flux sera défavorable aux interactions et favorisera la locomotion bactérienne autonome en mode planctonique (Thomas et al., 2010; Pierre et al., 2014). Lorsque les bactéries s'approchent à environ 50 nm du substrat, elles peuvent interagir avec la surface *via* une succession d'interactions physico-chimiques, c'est l'adhésion microbienne. Elle peut être divisée en deux étapes principales : l'adhésion réversible et l'adhésion irréversible (Busscher et al., 1995; Busscher & Mei, 2006). Lorsque les bactéries sont attachées de manière irréversible au substrat, leur multiplication va conduire à la formation de colonies qui vont recouvrir celle-ci de manière partielle ou totale. La croissance exponentielle des bactéries durant cette phase va finir par former un film hétérogène en trois dimensions. Des canaux vont ainsi se former au sein de ce biofilm et permettent la circulation de l'eau et des nutriments mais aussi l'évacuation des cellules mortes et des déchets (Decho, 1990; Costerton et al., 1995; Neu et al., 2003). La première phase de cette étape consiste à la régulation génétique permettant la différenciation phénotypique des organismes unicellulaire en biofilm. Après cette régulation génétique, la seconde phase comprend la synthèse active de la matrice extracellulaire. Cette matrice est constituée d'EPS (substance polymérique extracellulaire) comprenant diverses molécules organiques (Staats et al., 2000; Xiao & Zheng, 2016). Le détachement des bactéries constituant le biofilm permet un retour d'une partie des organismes à l'état planctonique et assure un

renouvellement génétique permettant d'augmenter la diversité microbienne. Cette dernière étape peut s'effectuer passivement (via l'action du flux ou des forces de cisaillement) ou activement.

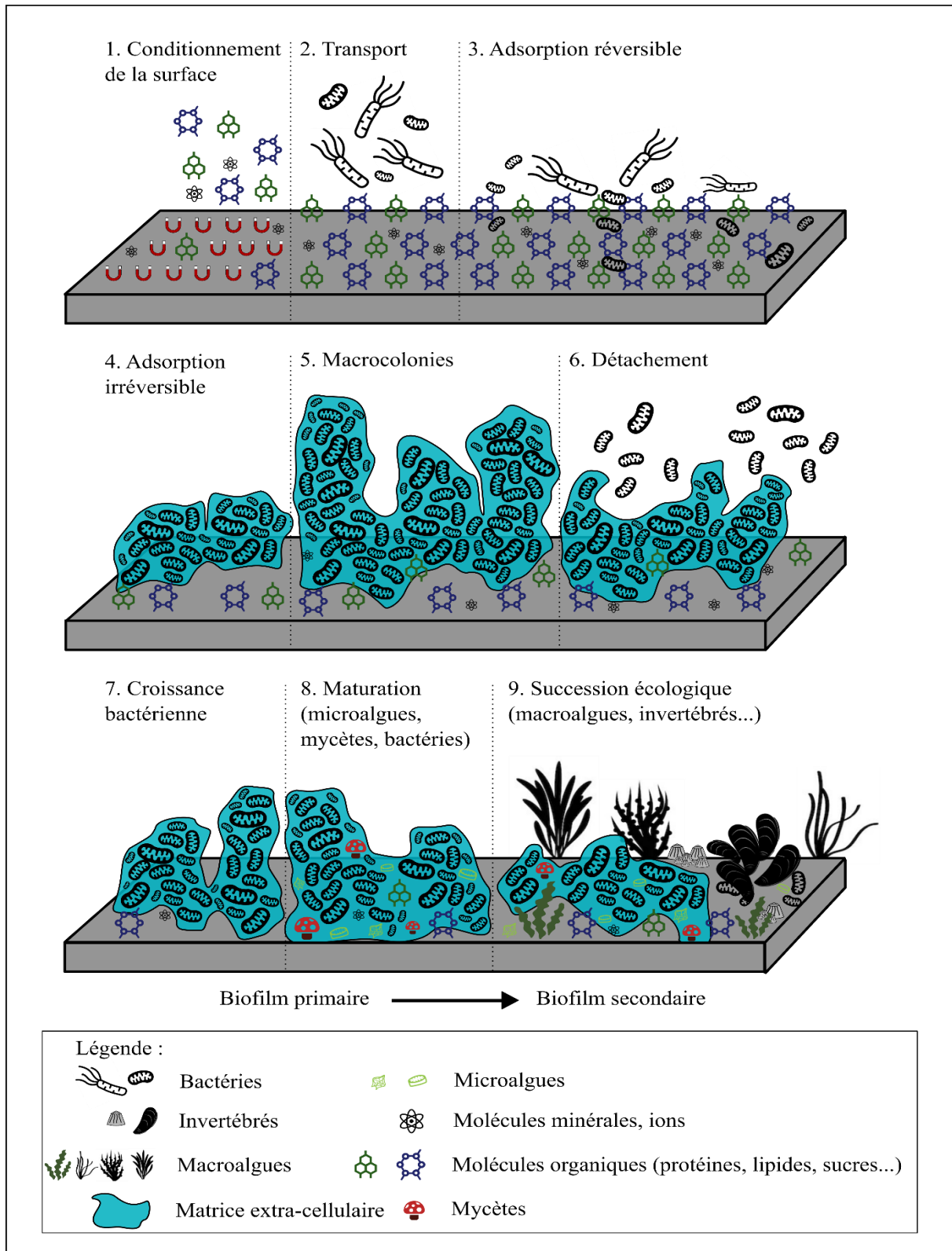


Figure 11 – Les étapes de la formation d'un biofilm (1 à 6) et son évolution en milieu marin (7 à 9). Modifié depuis Grzegorzczuk et al., 2018.

Encart 3

La matrice extracellulaire joue un rôle central dans l'architecture tridimensionnelle du biofilm. Elle contient différents exopolymères comme des polysaccharides, des protéines, des acides nucléiques ou des lipides (Flemming & Wingender, 2010; McDougald et al., 2012). Cette matrice peut représenter jusqu'à 90% de la matière sèche totale du biofilm et elle constitue l'environnement direct des cellules. La formation et le maintien structurel d'un biofilm est essentiellement dépendant des polymères sécrétés (Sutherland, 2001). Cette matrice polymérique extracellulaire offre différents bénéfices aux cellules constituant le biofilm comme un rôle structurant, de défense ou encore de source nutritive (Flemming & Wingender, 2010). Cependant, la majeure partie des interactions moléculaires au sein de ce compartiment reste encore inconnue et fait l'objet de nombreux travaux de recherche. La matrice polymérique extracellulaire présente aussi un intérêt plus large que celui propre au biofilm et aux cellules qui le composent. Cette matière permet aussi un apport conséquent de nutriments pour les consommateurs primaires et favorise la mise en place d'une succession écologique pour ce qui concerne les biofilms marins. Les biofilms constituent donc un élément clé dans la mise en place et la pérennité des réseaux trophiques côtiers.

Comme décrit précédemment, les biofilms marins sont composés de différents organismes et notamment des producteurs primaires. Le biofilm primaire sera composé de microalgues benthiques notamment, un biofilm secondaire sera également composé de macroalgues. Ces organismes sont au cœur des travaux réalisés dans le cadre de ma thèse.

V. Les producteurs primaires

V.1. *Classification phylogénétique*

Dans les écosystèmes aquatiques, les producteurs primaires réalisant la photosynthèse oxygénique sont représentés par des organismes multicellulaires tels que les angiospermes ou les macroalgues, et des organismes unicellulaires, procaryotes (*i.e.* les cyanobactéries) et eucaryotes (*i.e.* les microalgues). Il est important de noter que le terme « algue » n'a aucune réalité phylogénétique et qu'il regroupe l'ensemble des organismes photosynthétiques eucaryotes qui ont un appareil végétatif rudimentaire (thalle) ayant un besoin absolu d'eau pour réaliser leur cycle de vie. Les cyanobactéries sont à l'origine de la photosynthèse oxygénique et tous les chloroplastes des algues ont pour origine phylogénétique une cyanobactérie (Sibbald and Archibald 2020). Dans les écosystèmes tempérés comme celui de la Manche le genre *Synechococcus* est le plus abondant. Les algues sont très diversifiées d'un point de vue

taxonomique et sont représentées par de nombreuses lignées eucaryotes (Figure 12) (Baldauf, 2008; Sibbald & Archibald, 2020). Les algues vertes et rouges issues de l'endosymbiose primaire (Archaeplastida) appartiennent respectivement aux phyla des Chlorobionta et des Rhodophyta. Les macroalgues brunes, qui sont issues d'une endosymbiose secondaire, appartiennent à la lignée des Straménopiles, et forment la classe des Phaeophyceae.

Les microalgues présentent une diversité phylogénétique plus importante que celle des macroalgues avec des organismes qui présentent des plastes issus de l'endosymbiose primaire mais également d'endosymbioses secondaires et tertiaires (sans omettre les taxons ou groupes qui ont perdus secondairement leur plaste (Sibbald & Archibald, 2020)). Il est difficile d'évaluer le nombre d'espèce de microalgues, (Norton et al., 1996) estime un chiffre entre 200 000 et quelques millions. Les microalgues les plus abondantes en Manche sont les diatomées (Bacillariophycées, Straménopila), les dinoflagellés (Alveolata), les prymnésiohyccées (haptophycées, Haptista) et le cryptophycée (Cryptista).

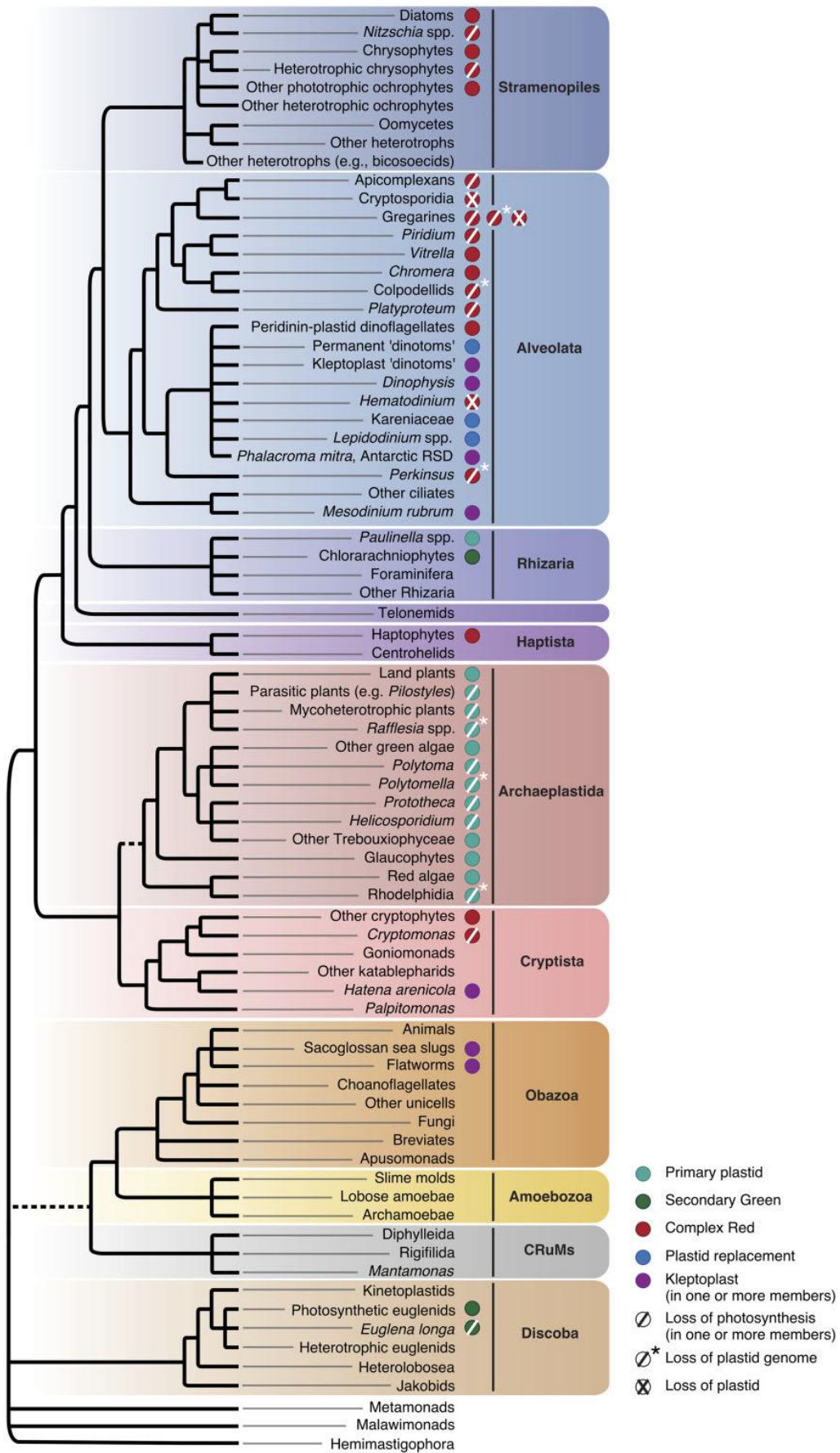


Figure 12 – Représentation schématique de l'arbre phylogénétique des eucaryotes. Les types de plastes ainsi que leur remplacement et leur perte sont précisés. La perte de la photosynthèse est également indiquée. Depuis Sibbald & Archibald, 2020.

Encart 4

Les diatomées ou Bacillariophyta appartiennent aux stramenopiles, elles sont composées de trois classes : Coscinodiscophyceae, Mediophyceae et Bacillariophyceae (Medlin, 2016), qui compte environ 100 000 espèces (Mann & Vanormelingen, 2013). Les diatomées peuvent être marines, dulçaquicoles et terrestres, elles sont planctoniques et benthiques et se caractérisent par la présence d'un frustule formé de valves et bandes intercalaires siliceuse (silice biogène). Les diatomées présentent deux grands types morphologiques, les centriques et les pennées. Certaines diatomées pennées possèdent un raphé qui est impliqué dans la mobilité des cellules (Wetherbee et al., 1998; Wang et al., 2013)

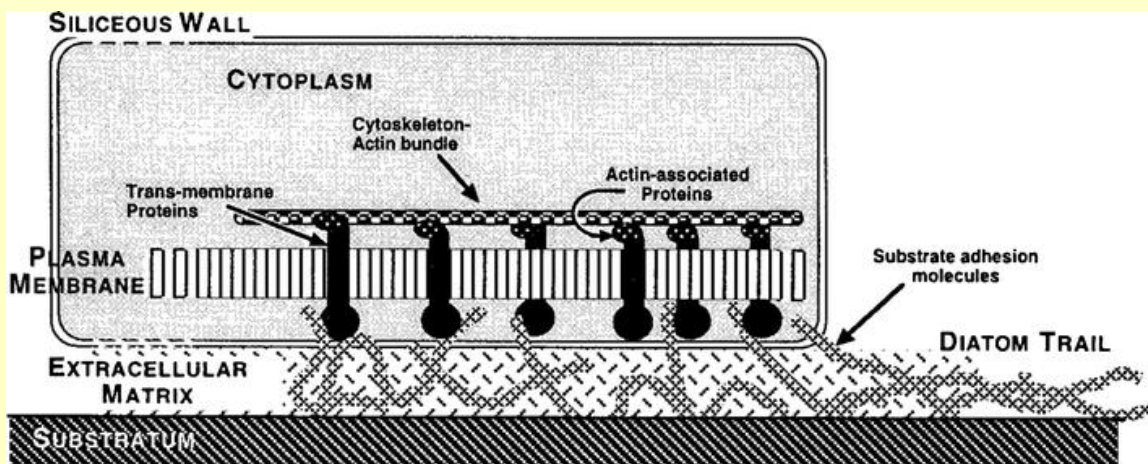


Figure 13 – Diagramme représentant le complexe d'adhésion d'une diatomée pennée raphide et suggérant un modèle de mobilité (Wetherbee et al., 1998; Wang et al., 2013).

La majorité des diatomées benthiques sont des pennées raphides capables de se déplacer activement. L'adhésion aux substrats et la mobilité des cellules impliquent des EPS (Underwood & Paterson, 2003; Shnyukova & Zolotarova, 2015; Chen et al., 2019). Leur mobilité est fonction des paramètres environnementaux comme l'accès à la lumière (Serôdio et al., 2006), l'hydrodynamisme (Kingston, 1999), la température (Cohn et al., 2003), les nutriments (Svensson et al., 2014), etc.

Les diatomées présentent une composition pigmentaire particulière. À côté de la chlorophylle *a*, de la chlorophylle *c* de la fucoxanthine, on trouve des caroténoïdes comme le β -carotène et des xanthophylles (fucoxanthine, diatoxanthine, diadinoxanthine...).

V.2. *Classification écologique*

V.2.1. *Producteurs primaires planctoniques*

D'un point de vue écologique, la classification des producteurs primaires peut se définir selon différentes variables comme la nature de l'habitat qui permet de distinguer les producteurs primaires benthiques des producteurs primaires planctonique. Le terme plancton désigne ici, par opposition au necton, l'ensemble des organismes vivant dans un environnement aquatique et qui ne présentent pas de mouvement propre sur l'horizontal. Leurs déplacements sont donc dominés par un transport passif, sous l'action des courants (les mouvements actifs existent au sein du plancton ; via la migration verticale, la présence de flagelles mais ne sont pas majoritaires). Les producteurs primaires planctoniques, appelé phytoplancton, sont composés essentiellement de microalgues de différentes lignées ou classes comme les diatomées, les dinoflagellés ou encore les haptophytes. Cependant des macroalgues comme certaines espèces de sargasses ou d'ulves sont également planctoniques. Le phytoplancton contribue largement à la production primaire marine (41,4% de la production primaire nette marine), il dépasse en terme de production annuelle les deux écosystèmes terrestres les plus productifs : les forêts tropicales et la savane (Geider et al., 2001).

V.2.2. *Producteurs primaires benthiques*

Le second habitat marin occupé par les producteurs primaires est l'habitat benthique. Ce sont ces producteurs primaires qui ont été étudiés dans le cadre de ma thèse. Les producteurs primaires benthiques en milieu marin et côtier sont représentés par des microalgues, des macroalgues et des phanérogames marines.

Les macroalgues jouent un rôle essentiel dans le fonctionnement de la zone euphotique des écosystèmes côtiers, où elles constituent les principaux producteurs primaires (Gattuso et al., 2006). Ces macrophytes servent de réservoir de carbone et approvisionnent ainsi les écosystèmes marins voisins par une forte exportation de leur production sous forme de carbone organique particulaire et dissous. Les macroalgues constituent également une source de nourriture pour de nombreux représentants de la faune vagile, en se positionnant à la base des réseaux trophiques côtiers (Riera & Hubas, 2003; Bittick et al., 2019). Les macroalgues ont également un rôle d'espèce ingénieure en procurant des habitats pour de nombreux invertébrés benthiques ou pour des juvéniles de poissons (Wilson et al., 2014). Elles représentent également un support privilégié pour la fixation de larves d'invertébrés et offrent une protection contre la

prédation (Bégin et al., 2004). Les macroalgues peuvent également protéger les côtes de la houle et ainsi atténuer leur érosion.

Le microphytobenthos (étymologiquement microalgues marines) correspond aux microorganismes photosynthétiques qui se développent sur le substrat ou dans le sédiment. Il se compose essentiellement de cyanobactéries, chlorophycées et en majorité de diatomées (MacIntyre et al., 1996). Le microphytobenthos, dont le développement se manifeste par exemple sous forme de tâches plus ou moins brunâtres à la surface du sédiment ou du substrat montre une grande richesse d'adaptation aux conditions abiotiques, tant physiologique que morphologique, d'où une multiplicité d'assemblages colonisant tous les substrats. Il est nécessaire de distinguer le microphytobenthos de substrat meuble et celui de substrat dur, ainsi que le microphytobenthos des zones intertidales et subtidales la composition des communautés microphytobenthiques sera affectée par le type de substrat colonisé (Longphuir et al., 2006; Jesus et al., 2009; Pennesi & Danovaro, 2017).

En zone intertidale, le microphytobenthos est soumis à de nombreuses contraintes environnementales telles que la dessiccation, les variations de températures, de salinité ou encore de lumière (Decho, 2000; Orvain et al., 2012, 2014; Juneau et al., 2015; Méléder et al., 2020; Savelli et al., 2020). En zone intertidale, les substrats meubles apportent la possibilité aux cellules microphytobenthiques d'effectuer des migrations verticales afin de se protéger des stress environnementaux comme un excès de lumière (Longphuir et al., 2009; Orvain et al., 2012; Barnett et al., 2020).

En milieu subtidal et sur substrat meuble, le microphytobenthos contribue de manière significative à la production primaire en milieu estuarien et dans les eaux peu profondes (Longphuir et al., 2006; Chatterjee et al., 2013). En zone subtidale, les migrations verticales du microphytobenthos seront également influencées par le cycle de marée (Longphuir et al., 2006). Cette capacité de motilité est favorisée par la production d'EPS qui vont modifier les propriétés physiques du sédiment (Tolhurst et al., 2002). Le cycle de marée aura donc un impact sur le rythme de vie du microphytobenthos est réglera son activité photosynthétique (Serôdio & Catarino, 1999; Longphuir et al., 2006; Méléder et al., 2020; Savelli et al., 2020; Ribeiro et al., 2021). En milieu subtidal et sur fond meuble, le cycle de marée aura un impact direct sur les paramètres photosynthétiques et la production microphytobenthique (Longphuir et al., 2006). Toutefois, le microphytobenthos est également capable de coloniser des substrats durs en zone intertidale (Hutchinson et al., 2006). Sur les substrats durs (microphytobenthos épilithique) ces migrations sont évidemment impossibles, des processus physiologiques

entrerons en jeu de manière à protéger les cellules contre les effets néfastes d'un excès d'irradiance par exemple (Müller et al., 2001; van de Poll et al., 2009; Deore et al., 2020). Ces processus seront abordés en détail dans la partie suivante de l'introduction. Le microphytobenthos en zone subtidale a été relativement peu étudié en comparaison au milieu intertidal (Androuin et al., 2018). Toutefois des études récentes ont mis en évidence que le microphytobenthos contribuait significativement à la biomasse photosynthétique benthique subtidale (Chatterjee et al., 2013), à la production primaire (Ní Longphuirt et al., 2007) et à l'ensemble du réseau trophique (Rigolet et al., 2014). Concernant le microphytobenthos subtidal de substrat dur, seulement quelques études ont été réalisées afin d'évaluer l'effet d'un enrichissement nutritif sur la structure de ces communautés (Hillebrand & Sommer, 2000) ou de caractériser les processus d'adhésion des microalgues benthique en fonction de la nature du substrat (Sekar et al., 2004; Siboni et al., 2007; Pennesi & Danovaro, 2017). Les variations saisonnières de ces communautés en terme d'abondance, biomasse et de structure des communautés ont également été étudiées (Totti et al., 2007). En revanche, les processus photosynthétiques associés au microphytobenthos de substrat dur en zone subtidale n'ont pas été étudié à notre connaissance.

L'organisation en biofilms permet aux micro-organismes d'adhérer efficacement à des substrats pour y fonder une architecture biologique complexe leur assurant, notamment grâce à la matrice d'EPS (Sutherland, 2001; Xiao & Zheng, 2016). De plus, les fonctionnalités écologiques des différents micro-organismes sont souvent complémentaires, permettant une exploitation optimale des ressources disponibles (Marsh & Bowden, 2000; Sutherland, 2001; Antunes et al., 2019). Le microphytobenthos épilithique est très exposé aux perturbations et est donc soumis à une alternance de phases de croissances et de décrochages. Le microphytobenthos et son importance écologique ne se restreint pas uniquement au substrat colonisé. Grâce à la remise en suspension des cellules et le niveau d'abrasion, de résistance, du substrat colonisé, le microphytobenthos ne peut être considéré isolément de la colonne d'eau. La sécrétions de polysaccharides, composant la matrice extracellulaire, constitue également une fonction importante associée au microphytobenthos. La forte densité cellulaire et la valeur nutritive élevée (protéines, lipides, sucres) font du microphytobenthos un élément essentiel de l'alimentation des consommateurs secondaires (Majdi, 2011; Androuin et al., 2018). Les diatomées sont des organismes riches en acides gras et sont une source de nourriture privilégiée des dépositivores (Lopez & Levinton, 1987; López-Figueroa & Niell, 1988). Enfin, le microphytobenthos est désormais reconnu comme un compartiment essentiel au

fonctionnement des écosystèmes côtiers et contribue significativement à la production primaire de ces environnements (Kromkamp et al., 1995; MacIntyre et al., 1996; Barranguet & Kromkamp, 2000; Hutchinson et al., 2006; Murphy et al., 2006; Vivier et al., 2021a).

VI. La production primaire et ses mécanismes de régulation

VI.1. Généralités

La production primaire représente le stock de carbone organique produit par unité de temps et de surface à la suite de la fixation de carbone inorganique *via* la photosynthèse (Falkowski & Raven, 2007). La production primaire annuelle nette des écosystèmes marins représente 46% du total net mondial (Geider et al., 2001). Elle constitue un processus clé des cycles biogéochimiques et a un rôle central dans la structure des réseaux trophiques (Cloern, 1996; Cloern et al., 2014; Bharti et al., 2017). La totalité des niveaux trophiques marins reposent sur les organismes autotrophes capables de réaliser la photosynthèse, il est donc essentiel d'estimer cette production notamment dans les écosystèmes côtiers comme la Manche (Napoléon & Claquin, 2012). Chez les eucaryotes photosynthétiques, les thylakoïdes sont localisés au sein des chloroplastes, organites cellulaires nés de l'endosymbiose, ceinturés de deux, trois ou quatre membranes en fonction des lignées et du degré d'endosymbiose (primaire, secondaire ou tertiaire) (Figure 14). Des protéines membranaires complexes et des pigments (chlorophylles, caroténoïdes) se retrouvent sur les membranes des thylakoïdes et composent l'appareil photosynthétique. La photosynthèse oxygénique nécessite la coopération des photosystèmes I et II (PSI et PSII) qui se trouvent au niveau des membranes des thylakoïdes (Schubert 1998) (Figure 15 modifiée depuis Suggett et al., (2010)).

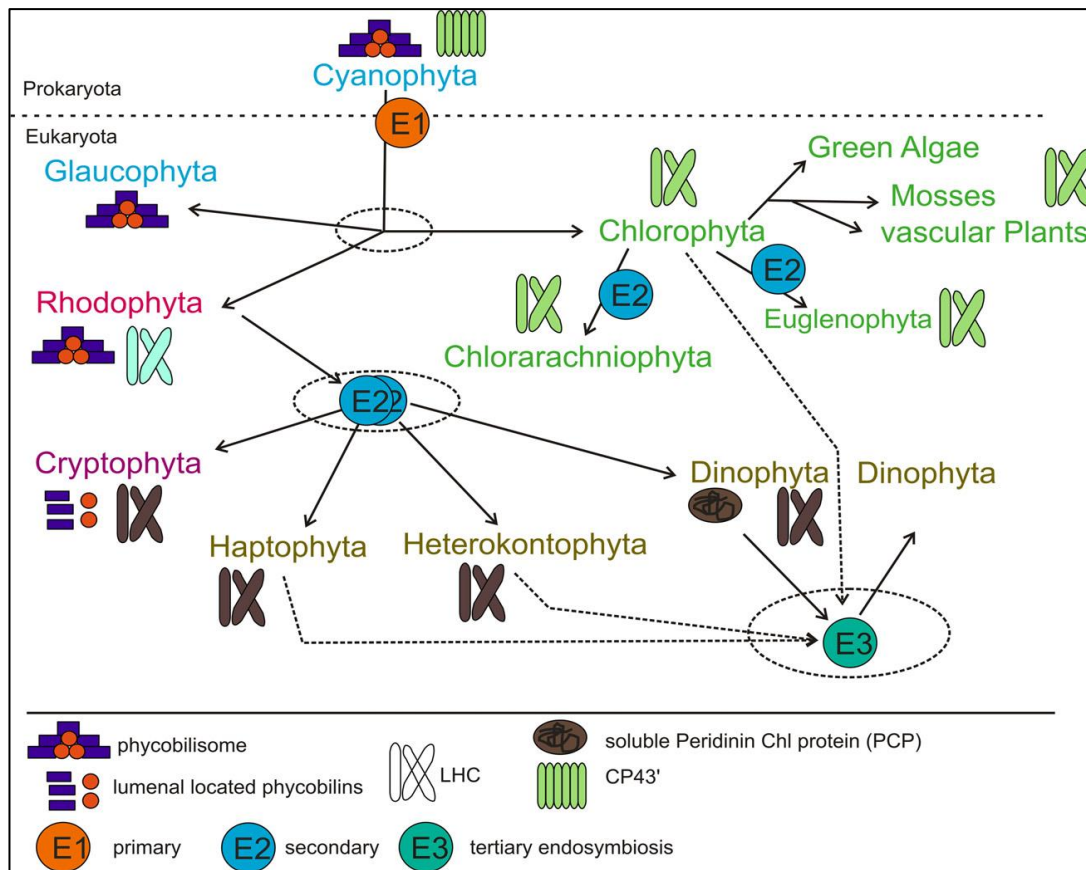
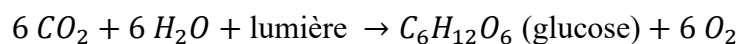


Figure 14 – Relations phylogénétiques entre les différents groupes d'algues et leurs appareils photosynthétiques respectifs. Les cercles traduisent les relations phylogénétiques encore débattues.

La photosynthèse se déroule en deux phases. Une première phase « claire » pendant laquelle l'énergie lumineuse est utilisée pour briser les molécules d'eau (H_2O) en oxygène (O_2), protons (H^+) et en électrons (e^-) au niveau du PSII (Figure 15). Les protons sont utilisés pour la formation d'une coenzyme permettant de stocker l'énergie chimique, l'ATP (Adénosine triphosphate), les électrons parcourent la chaîne de transfert entre les deux photosystèmes et permettent la production d'une coenzyme réductrice, le NADPH (nicotinamide adénine dinucléotide phosphate réduit). Lors de la phase « sombre », ces deux coenzymes sont utilisées pour l'absorption et la réduction du carbone. Ces deux étapes permettent la transformation du CO_2 en glucose, molécule indispensable pour la formation des autres molécules organiques. La photosynthèse peut s'écrire selon l'équation suivante :



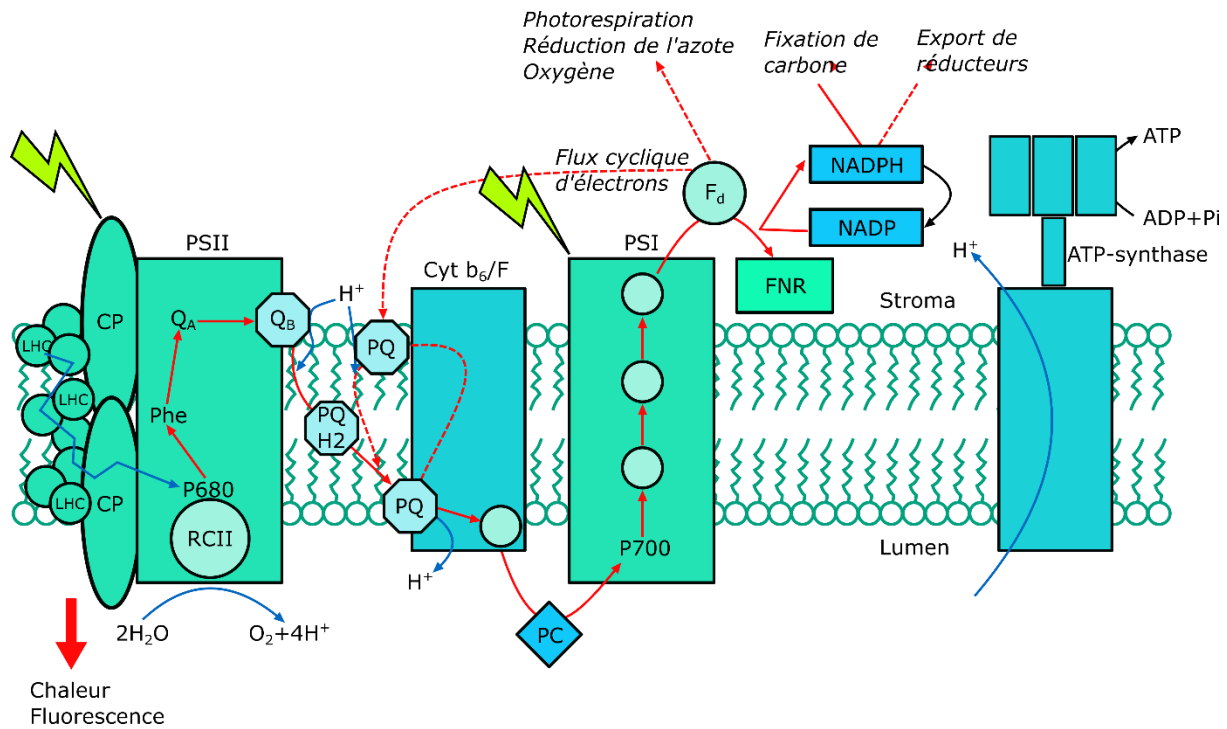


Figure 15 – Schéma représentant une section transversale de membrane de thylakoïde montrant l'organisation et certains des principaux composants de l'appareil photosynthétique. Le transport des électrons (e^-) est représenté par les flèches rouges, celui des protons (H^+) par les flèches violettes. Les électrons sont captés dans le milieu extérieur par le photosystème II (PSII) et sont transférés au cytochrome b_6/f (Cyt b_6/f). Ils sont ensuite transférés au photosystème I (PSI) par la plastocyanine (PC) où ils sont utilisés pour réduire le NADP en NADPH.

Abréviations : LHC : antennes collectrices de photons (représentés en éclairs jaunes) ; Phe : molécule de phéophytine a ; Q_A et Q_B : quinones liées ; PQ : plastoquinones libres ; F_d : ferrédoxine ; FNR : ferrédoxine / NADP réductase ; Pi : phosphate inorganique. Modifié depuis Sugget et al. (2010).

Le PSII peut être comparé à une enzyme capable de catalyser la photo-oxydation d'une molécule d'eau (Figure 16). Le PSII permet aux électrons d'entrer dans la chaîne de transfert et apporte une partie de l'énergie libre nécessaire pour la réduction du carbone inorganique. Le PSII apporte aussi les premiers accepteurs d'électrons au sein de cette chaîne. Il se compose de plusieurs protéines auxquelles des centaines de molécules pigmentaires sont liées selon une configuration très précise (Suggett et al., 2010). Celle-ci peut être influée et régulée par les conditions environnementales. Le PSII se compose donc de plusieurs éléments :

- Un complexe périphérique de pigments et de protéines (LHCII) qui servent essentiellement de récepteur de photons ($h\nu$) formant ce qu'on appelle l'antenne collectrice.
- Une antenne interne formé de complexe pigment-protéine appelé « CP » pour « Chlorophyll Protein » tels queles CP24, CP26 et CP29 chez les Chloronbionta, dont le rôle principal est le transfert et la régulation de l'énergie absorbée depuis l'antenne collectrice périphérique vers le noyau protéique (CP43 et CP47).
- Un noyau complexe de quatre protéines (D1, D2, CP47 et CP43). Les protéines D1 et D2 lient les premiers accepteurs d'électrons, le site de séparation de l'eau et le site de séparation des charges (cluster de chlorophylles P680) alors que CP47 et CP43 agissent principalement dans le transfert d'énergie depuis l'antenne interne vers le site de séparation des charges.
- Quelques protéines (représentées en gris clair sur la Figure 16) se situent au niveau du lumen, du côté de la réaction d'oxydation de l'eau. Elles ont une fonction structurante et permettent une oxydation efficace de l'eau.
- Plusieurs protéines de faible poids moléculaire (gris foncé sur la Figure 16) sont également présentes, la majorité d'entre-elles ont des fonctions inconnues. L'une d'entre-elles cependant, la protéine PsbS (en vert), joue un rôle important dans les processus de dissipation de l'excès d'énergie et dans la régulation du transfert d'énergie depuis l'antenne vers le noyau.

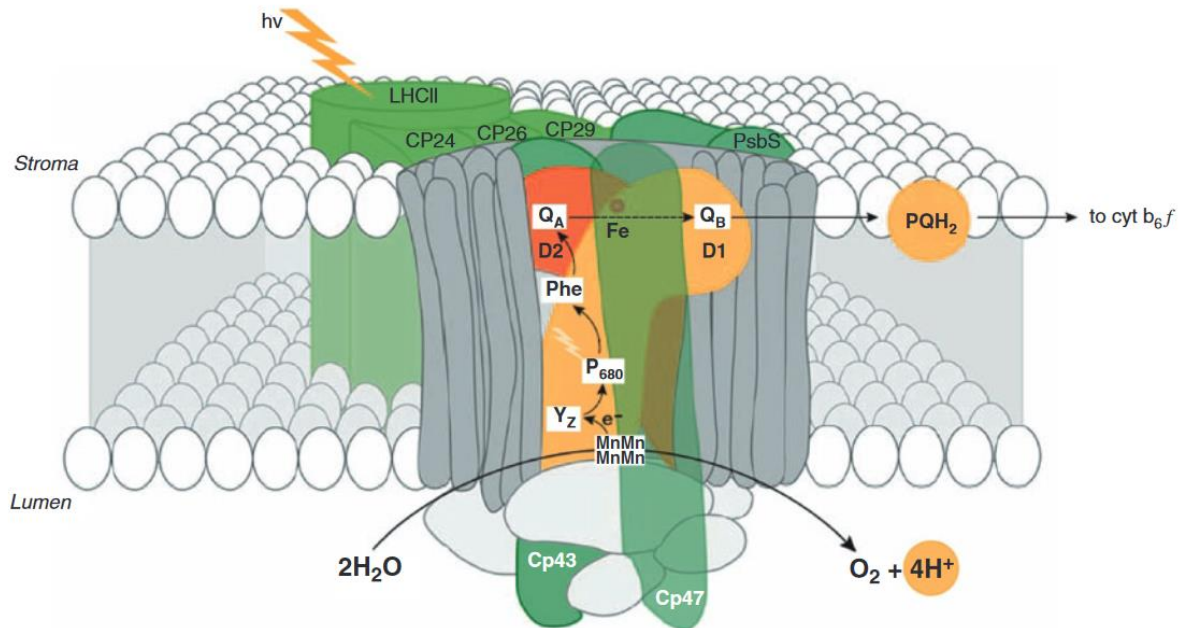


Figure 16 – Organisation du photosystème II (PSII) avec visualisation de la chaîne de transport des électrons. Figure issue de Sugget et al. (2010).

Il est important de noter qu'il existe des différences de structures de l'appareil photosynthétique en fonction de la phylogénie (Wilhelm et al., 2006). Au cours des dernières années, de nombreux génomes d'algues ont été entièrement séquencés, alimentant un travail considérable sur l'évolution, mais aussi sur la fonction des antennes collectrices et des protéines associées (Hoffman et al., 2011; Büchel, 2015). Les antennes collectrices de photons (*Lhcs* pour *Light harvesting complexes* en anglais) appartiennent à deux grands groupes d'un point de vue génétique : les gènes codants pour les protéines de liaison à la Chl *a/b* chez les lignées vertes comme les algues vertes, les mousses ou les plantes supérieures. Ces gènes peuvent être séparés en deux groupes : les gènes *Lhca* codant pour les protéines associées au PSI et les gènes *Lhcb* codant pour les protéines *Lhcs* associées au PSII. Le groupe restant peut à son tour être subdivisé en *Lhcf*, *Lhcr*, *Lhcx* (anciennement *LI1818* et également nommé *LhcSR*, en particulier dans la lignée verte, et donc appelé *LhcSR* ici aussi) et *Lhcz*. Le groupe entier se trouve dans les taxons dérivés d'une endosymbiose secondaire impliquant une cellule algale rouge, et seuls les gènes *Lhcx/LhcSR* et *Lhcz* se trouvent également dans la lignée verte. Plus précisément, les cryptophytes contiennent des gènes *Lhcr* et *Lhcz* (Dittami et al., 2010; Neilson & Durnford, 2010), les dinoflagellés expriment *Lhcf* et *Lhcr* (Hoffman et al., 2011), et certains d'entre eux, qui ont obtenu leurs plastes par une endosymbiose tertiaire à partir d'haptophytes, contiennent

également des *Lhcx*. Les haptophytes eux-mêmes contiennent des gènes qui codent pour les protéines *Lhcf*, *Lhcr*, *Lhcz* et *Lhcx* (Neilson & Durnford, 2010) et il en va de même pour les algues brunes (Cock et al., 2010) et les diatomées (Armbrust et al., 2004; Bowler et al., 2008) (Figure 17A). La capacité de récolte de la lumière dans les *Lhcs* est principalement déterminée par les pigments, qui sont liés de manière non covalente à l'échafaudage protéique. Ceux-ci montrent une variété beaucoup plus large que les séquences protéiques elles-mêmes: Dans le cas des chlorophylles, les Chl *a*, Chl *b* et Chl *c* peuvent être liés, couvrant une large gamme d'hydrophobicité avec la Chl *a* étant plutôt hydrophobe mais la Chl *c* étant extrêmement hydrophile (Figure 17B). Ces différences structurales de l'appareil photosynthétique entre les différentes classes d'algues vont avoir un impact sur la mesure de la photosynthèse. L'efficacité de telle ou telle technique d'estimation de photosynthèse sera affectée par ces différences structurales. Par exemple, la fluorimétrie de type PAM sera moins efficace pour évaluer les performances photosynthétiques chez les algues rouges (Luimstra et al., 2018).

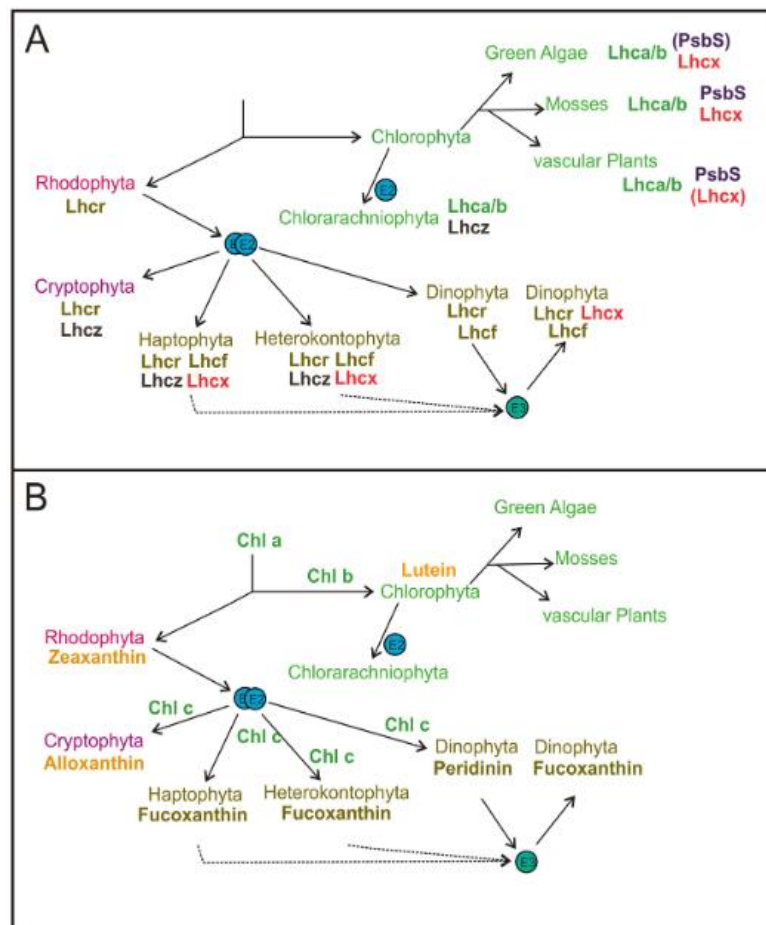


Figure 17 – Protéines Lhc (A) et pigments (B) dans les différents groupes d'eucaryotes. Les parenthèses indiquent la présence du gène mais pas de la protéine. Les cercles E2 et E3 représentent le degré d'endosymbiose. D'après Büchel et al., 2015.

VI.2. *Techniques de mesure*

La production primaire aquatique peut se mesurer de différentes manières. Deux méthodes traditionnelles de mesure directe ont été développées pour estimer la production primaire. La plus utilisée historiquement est la méthode d'incorporation d'un isotope radioactif du carbone, le ^{14}C , introduite par Nielsen, (1952) et modifiée par Marcel et al., (1994). Pour pallier aux contraintes de radioprotection, un isotope stable du carbone, le ^{13}C peut également être utilisé (Napoléon & Claquin, 2012; Morelle & Claquin, 2018). Cette méthode permet de mesurer l'incorporation de carbone inorganique dans la matière organique. C'est une technique qui nécessite des temps d'incubations relativement longs (plusieurs heures). Une autre approche est basée sur le suivi de l'évolution de l'oxygène produit lors du processus de la photosynthèse. Cependant, cette méthode nécessite également des temps d'incubation longs et sa sensibilité s'est avérée faible. Elle est plus adaptée à des mesures en laboratoire (Falkowski & Raven, 2007). Les longs temps d'incubation de ces deux méthodes engendrent des données à faible fréquence d'acquisition et donc spatialement et temporellement espacées, ne permettant pas d'appréhender les variations à court-terme des processus photosynthétiques.

Des méthodes de mesure des processus photosynthétiques sont apparues pendant les années 1970 et se basent sur la fluorescence chlorophyllienne des cellules photosynthétiques (Samuelsson & Öquist, 1977; Cullen & Renger, 1979; Schreiber et al., 1986). Afin de comprendre ce principe de fluorescence, il est nécessaire de détailler le mécanisme de photosynthèse expliqué plus tôt. Les pigments chlorophylliens des centres réactionnels (RC) des photosystèmes émettent des électrons en réponse à une excitation par un photon. Cette émission d'électrons leur permet de revenir à leur état fondamental. Il existe deux autres voies de désexcitation qui aboutissent à une réémission de l'énergie absorbée sous forme de chaleur et de fluorescence. La fluorescence provient principalement du PSII (Krause & Weis, 1991). L'ensemble des voies de désexcitation sont dépendantes, donc, toute variation de fluorescence traduit une modification du transport des électrons. A l'obscurité, on admet que les RC du PSII sont ouverts. Lorsque la molécule de Chl *a* P680, se situant au centre du RC du PSII, est excitée par un photon, elle transmet un électron au premier accepteur, la quinone A (Q_A), qui est réduite et le RC est alors fermé, n'acceptant plus d'électrons. L'électron est alors transmis au second accepteur, la quinone B (Q_B), qui va rejoindre un pool de plastoquinones. Dans la voie acyclique des électrons, les électrons produits au niveau du PSII iront vers le PSI et aboutiront à la réduction du NADP en NADPH (pouvoir réducteur). Quand Q_B est réduite, Q_A est oxydée, le centre réactionnel est à nouveau ouvert. La fermeture des centres entraîne une augmentation de

la fluorescence, une fluorescence minimale (F_0) est mesurée lorsque tous les centres sont ouverts et une fluorescence maximale (F_M) lorsqu'ils sont tous fermés (Kolber & Falkowski, 1993) (Figure 18).

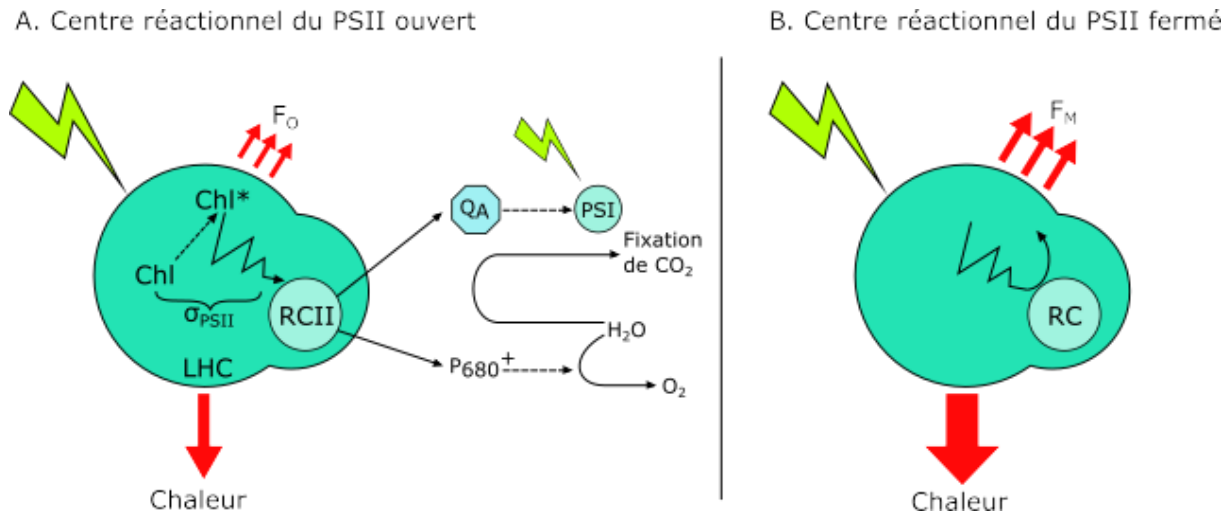


Figure 18 – Schéma représentant les transferts d'énergie au sein du PSII. La lumière incidente est absorbée par les antennes collectrices (LHC) avec une section d'absorption (σ_{PSII}). La chlorophylle excitée va migrer par résonance jusqu'au centre réactionnel (RC). S'il est ouvert (A), P_{680} va s'oxyder (P_{680}^+) et le premier accepteur d'électron (Q_A) sera réduit (Q_A^-). La fluorescence émise sera minimale (F_0). Si au moment où le photon est absorbé, Q_A est réduit (B), l'énergie absorbée peut être renvoyée sous forme de fluorescence, augmentant celle-ci jusqu'au niveau maximal (F_M). La valeur de fluorescence F observée à un niveau d'irradiance E est une moyenne de F_0 et F_M pondérée par la fraction de RC ouverts et fermés et correspond au quenching photochimique (q_p). Modifié à partir de Kolber & Falkowski (1993).

Il existe deux méthodes principales permettant de mesurer la variation de fluorescence du PSII. La méthode MT « Multi Turnover » est utilisée dans les fluorimètres types PAM (Pulse Amplitude Modulated). Elle utilise un flash long (≈ 600 ms) et puissant qui réduit les accepteurs primaires, Q_A , mais également les accepteurs secondaires, Q_B , et le pool de plastoquinones menant à un F_M plus important. La méthode ST pour « Single Turnover » est utilisée dans les fluorimètres de types « FRRF » (Fast Repetition Rate Fluorometers). Elle réduit progressivement le pool de Q_A de F_0 jusqu'à un niveau F_M , à l'aide d'une succession de flashes courts d'une durée de quelques microsecondes ($\approx 2 \mu s$) et d'intensité modérée. Ces méthodes permettent d'estimer le rendement quantique maximal du PSII (F_V/F_M). Ce rendement représente pour partie l'état physiologique des organismes étudiés (Parkhill et al., 2001; Schreiber, 2004), il diminue avec l'augmentation des intensités lumineuses en raison de l'équilibre qui s'installe entre l'énergie allouée pour la photosynthèse (i.e. quenching photochimique [q_p]), et celle allouée pour la dissipation de l'excès d'énergie (i.e. quenching non-photochimique [q_N ou NPQ]). Associées à des mesures de a^* (coefficient d'absorption de la lumière normalisé par la chlorophylle a) correspondant à la section d'absorption optique du

PSII, ces méthodes vont permettre de calculer le taux de transport des électrons (ETR ; Electron Transport Rate).

Le couplage de ces différentes méthodes de mesure des paramètres photosynthétiques à partir de la fluorescence ou de l'oxygène a permis d'estimer la production primaire de manière précises à différentes échelles d'espace (conditions contrôlées, *in situ*) et de temps. Enfin, considérant les différences de structures de l'appareil photosynthétique en fonction de la phylogénie comme détaillé dans la partie précédente, il est important de considérer que les techniques de mesures de la photosynthèse par fluorescence seront dépendantes de ces variations structurelles, les résultats obtenus devront être discutés en fonction de la phylogénie.

VI.3. Mécanismes de régulation

Les producteurs primaires vont réagir aux variations des paramètres physicochimiques (nutriments, température, lumière...) de leur environnement par des modifications physiologiques, morphologiques et moléculaires qui vont impacter la photosynthèse et donc la biomasse et la production. Ces régulations vont impacter l'ensemble des compartiments du réseau trophiques qui dépendent des producteurs primaires, on parle de cascade « bottom-up ».

La disponibilité en nutriments va affecter les paramètres photosynthétiques (Behrenfeld et al., 2004; Mangoni et al., 2009; Endo et al., 2017). Les sels nutritifs les plus limitant en milieu marin sont l'azote, le phosphore et le silicate. L'azote est l'élément le plus important dans ces limitations. La silice est un élément important car nécessaire pour la formation du frustule chez les diatomées. C'est donc un facteur déterminant pour la production primaire (Martin-Jézéquel et al., 2003; Cox et al., 2010). En ce qui concerne les limitations en azote et phosphore, ces dernières affecteront la capacité de synthèse protéique, les phospholipides membranaires et les métabolismes énergétiques de manière générale affectant ainsi les capacités de photoacclimatation des microalgues (Kolber et al., 1988; Geider et al., 1997; Behrenfeld et al., 2004; Napoléon et al., 2013b) et les macroalgues (Pedersen, 1995; Davison et al., 2007).

La température va aussi influencer sur les paramètres photosynthétiques des microalgues (Claquin et al., 2008) et des macroalgues (Wiencke et al., 1993; Hanelt et al., 2003; Necchi, 2004). Les producteurs primaires montrent une grande variété de réponses physiologiques face aux variations de ce paramètre (Davison, 1991; Hanelt et al., 2003; Bernard & Rémond, 2012). Les macrophytes marins vivent dans un environnement très variable en ce qui concerne la lumière, le rayonnement UV, la température et aussi la salinité. Au cours de l'évolution, leur métabolisme s'est adapté à ces conditions fortement changeantes, en particulier dans la zone

littorale supérieure. Dans la zone subtidale inférieure, l'appareil photosynthétique s'est adapté aux conditions de faible luminosité, pour absorber au maximum les photons incidents et pour utiliser l'énergie absorbée avec une efficacité élevée. En zone subtidale, le microphytobenthos sera soumis à certaines pressions environnementales qui affecteront ses performances photosynthétiques comme la lumière (Grobbelaar, 1991; Wiencke et al., 1993; Mangoni et al., 2009), le cycle de marée (Longphuir et al., 2006) ou encore la température (Morris & Kromkamp, 2003; Di Pippo et al., 2012). Dans des conditions de température et de nutriments optimales, la production primaire est directement liée à l'intensité lumineuse incidente et à la photopériode (Behrenfeld et al., 2004). Les variations de production à l'échelle journalière sont expliquées par les variations de lumière à cette même échelle (Prézelin, 1992). La capacité photosynthétique maximale (P_{MAX}) et l'efficacité photosynthétique (α) sont largement contrôlées par la lumière incidente. L'hydrodynamisme, la turbidité la marée (variation de la profondeur) vont contrôler les paramètres photosynthétiques (Behrenfeld et al., 2002; Mangoni et al., 2009) par la mise en place de processus de photoacclimatation (Mangoni et al., 2009; van de Poll et al., 2009).

La photoacclimatation permet aux organismes autotrophes de modifier leur appareil photosynthétique et donc de moduler leur photosynthèse pour s'adapter aux variations de lumière (Dubinsky & Stambler, 2009). Ce mécanisme se met en place en régulant le contenu de chlorophylle par cellule (Richardson et al., 1983; Falkowski & Raven, 2007) et des pigments accessoires. Lors d'acclimations à de fortes intensités lumineuses, la concentration en Chl *a* par cellule diminue. Les cellules sont aussi capables de modifier la taille de leurs antennes collectrices, le nombre de photosystèmes actifs ou de réorganiser la position des thylakoïdes dans le chloroplaste contribuant à ce qui est nommé le « package effect » (Figure 19). D'une manière plus générale le « package effect » est dû au fait que lors de l'augmentation de la taille du chloroplaste ou de la cellule à quantité de pigment constant, le volume augmente plus vite que la surface ce qui affecte (régule) les propriétés d'absorption des microalgues (Lehmuskero et al., 2018). Enfin, la dissipation de l'excès d'énergie sous forme de chaleur constitue d'autres processus de photoacclimatation regroupés sous le terme de quenching non photochimique (Müller et al., 2001). Ces mécanismes permettent de limiter les dommages causés à l'appareil photosynthétique des cellules durant de fortes expositions lumineuses ou, à l'inverse, d'optimiser la photosynthèse durant des expositions à de faibles intensités. Cependant, ils sont très coûteux en énergies et vont impacter la croissance des cellules (Anning et al., 2000; Douglas et al., 2003; Moore et al., 2006; Kirilovsky, 2020).

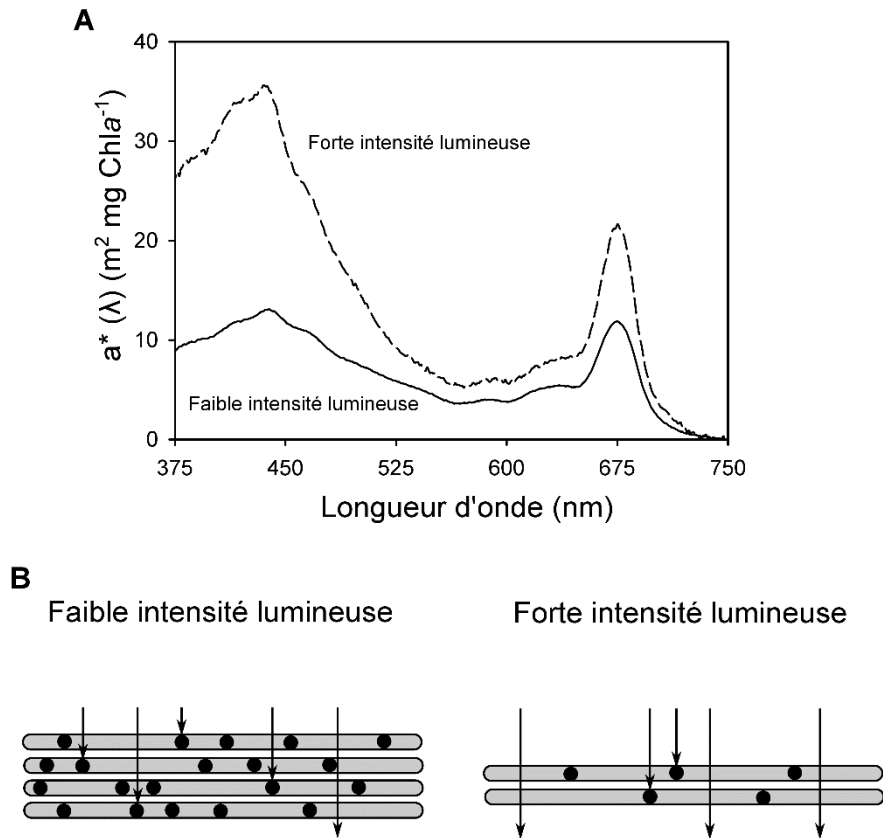


Figure 19 – (A) Effet de l'intensité lumineuse de croissance sur le coefficient d'absorption de la lumière normalisé par la chlorophylle a et (B) schéma décrivant le phénomène d'auto-ombrage. D'après Falkowski et Raven (2007).

Les producteurs primaires peuvent aussi être affectés par des cascades « top-down ». Elles décrivent une régulation des organismes autotrophes par les compartiments trophiques supérieurs (Sterner, 1989). De cette manière, une forte quantité de consommateurs primaires comme des copépodes peut limiter la croissance du phytoplancton, de la même manière, des brouteurs en forte abondance vont limiter le développement du microphytobenthos. Certains virus peuvent également être inclus dans ce type de régulation (Suttle et al., 1990).

VII. Problématique et objectifs

L'accroissement des pressions anthropiques sur les écosystèmes marins et côtiers, en particulier en Manche, provoquent des modifications des habitats, des écosystèmes et des différentes fonctions écosystémiques associées. La construction d'ouvrages maritimes et côtiers tels que les digues ou des ouvrages portuaires ainsi que l'émergence de plusieurs projets d'implantation d'éoliennes off-shore sont en augmentation constante sur les côtes de la Manche française. Avec ces différents projets, la bétonisation de certains habitats devient inévitable, il est donc primordial d'évaluer leurs impacts écosystémiques, et, si possible, de les limiter. Le projet MARINEFF est né dans ce contexte, il vise à concilier développement humain et bénéfice écologique. En effet, le développement de ces nombreuses infrastructures côtières peut aussi permettre de créer de nouveaux habitats. Le but n'est pas d'améliorer un écosystème naturel existant, qui par nature constitue déjà un biotope complexe indépendamment du type d'habitat et de la valeur de certains indicateurs comme la diversité spécifique, mais de profiter de cette artificialisation locale pour recréer un nouvel habitat propice à la mise en place de services écosystémiques. En effet, le maintien ou l'amélioration des paramètres biologiques et écologiques locaux peuvent apporter des bénéfices de diverses natures (pêche, cycle des éléments nutritifs, régulation et stockage du carbone, prévention de l'érosion, tourisme...). C'est à travers ce prisme qu'ont été conçues les infrastructures marines du projet MARINEFF et notamment les blocs de digues expérimentaux décrits précédemment. Ce travail de thèse s'inscrit dans cette réflexion et a pour objectif d'évaluer, de caractériser, l'impact de ces infrastructures sur l'écosystème et de décrire les processus de colonisation biologique mis en jeu après leur immersion. Le suivi environnemental de récifs artificiels immergés depuis cinq années sur les mêmes sites d'études (projet RECIF) permet d'évaluer un écosystème similaire après une demi-décennie qui à ce stade tend vers un état écologique évolué (Charbonnel & Bachet, 2010). De plus, le développement de structures, à petite échelle, identiques à la composition et à la structure de surface des digues MARINEFF a permis d'étudier la colonisation biologique à différentes échelles de temps et sous différentes conditions expérimentales. Ces petites infrastructures seront appelées MI (Marine Infrastructures) ou dallettes dans le reste du manuscrit. Enfin, tenant compte de l'immersion retardée des blocs de digues MARINEFF, des résultats préliminaires issus des suivis environnementaux *in situ* seront présentés à la fin de mon mémoire.

Le second chapitre présente l'ensemble des stratégies d'échantillonnage, des expérimentations réalisées et des techniques déployées au cours de cette thèse. Le troisième chapitre correspond à un article scientifique qui aborde la problématique générale sur les récifs artificiels. L'objectif de ce chapitre était d'identifier, sur un corpus de 127 articles scientifiques traitant de récifs artificiels, l'efficacité de ces derniers en fonction de différents facteurs tels que leur design, les techniques de suivi environnementales déployées pour leur évaluation, les conditions de milieu dans lesquelles ces structures se trouvent, etc. De plus, cette synthèse s'intéresse au degré d'efficacité des structures artificielles en fonction de leurs objectifs initiaux. Le sujet est traité par une approche novatrice pour ce type d'étude avec l'emploi d'outils statistiques afin de garder le maximum d'objectivité. De plus, cette étude apporte un certain nombre de constatations concernant la conception des RA, la complémentarité des techniques de suivi déployées pour leur évaluation et apporte quelques recommandations afin d'améliorer l'efficacité de ce type de projet.

Les principaux résultats de ce travail et leur discussion s'articulent ensuite sous la forme d'articles scientifiques. Ainsi, le quatrième chapitre de cette thèse traite de la colonisation d'infrastructures marines en conditions expérimentales par des biofilms microphytobenthiques et des facteurs conditionnant ce développement. La partie IV-A s'intéresse à la colonisation de dallettes (MI) par un biofilm microphytobenthique en conditions contrôlées (dalles 1 dans la Figure 20). Lors de cette étude, l'influence de la composition de différents bétons biosourcés et de la rugosité de surface de ces derniers sur la croissance et les paramètres photophysologiques du biofilm ont été étudiés. Afin de mesurer les indicateurs de croissance et les paramètres photosynthétiques associés au biofilm, différentes techniques ont été utilisées comme des mesures de biomasses chlorophyllienne et des mesures de fluorimétrie PAM. L'appareil utilisé est un Imaging-PAM, il permet de caractériser spatialement les différents indices photobiologiques que la fluorimétrie chlorophyllienne modulée permet de calculer. Associé à des mesures spatialisées de la rugosité de surface des dallettes en béton ayant servi de support pour la croissance des biofilms, des calculs plus précis et notamment des corrélations entre le niveau de rugosité et l'indicateur sélectionné ont pu être mis au point. Cette étude a permis de mettre en lumière l'influence de la structure à micro-échelle du substrat sur la croissance d'un biofilm microphytobenthique et sur ses paramètres photosynthétiques.

La partie IV-B s'intéresse à l'influence de la rugosité de surface de dalles en béton sur le développement et la photosynthèse du biofilm microphytobenthique (dalles 2 dans la Figure 20). Cependant, cette expérimentation en conditions contrôlées inclue également un autre facteur qui est la disponibilité en sels nutritifs dans le milieu. Des approches par fluorimétrie PAM ont également été utilisées pour caractériser la croissance et la photosynthèse des biofilms colonisant ces structures. D'autres indicateurs comme la dynamique des excréments de carbone sous forme de polysaccharides (EPS) constituant ce qu'on appelle la matrice extracellulaire ont également été mesurés. De plus, des mesures d'absorption spécifique de la chlorophylle *a* (a^*) ont permis de calculer des paramètres photosynthétiques comme la capacité maximale de production ($ETR_{max}^{a^*}$) ou encore l'efficacité photosynthétique (α^{a^*}). Cette étude a permis de mettre en avant les interactions entre différents facteurs comme la rugosité à micro-échelle de la surface de la structure et l'influence d'un gradient d'eutrophisation sur les dynamiques de colonisation microphytobenthiques et les performances photosynthétiques associées.

Le cinquième chapitre de ce manuscrit traite de la colonisation biologique d'infrastructures marines en situation *in situ*. La partie V-A s'intéresse aux dynamiques de colonisations biologiques de dalles en béton (dalles 3 dans la Figure 20). Cette étude s'intéresse cette fois à une approche *in situ* afin d'évaluer et de caractériser les différentes étapes de cette colonisation benthique. Une comparaison inter-sites et un suivi saisonnier régulier a permis de mettre au jour des différences spatiales et temporelles pour différents indicateurs comme la croissance du biofilm microphytobenthique et sa photochimie ou encore le développement d'un biofilm dit secondaire constitué de faune sessile et de macroalgues. L'utilisation de dalles composées du même matériau que les blocs de digues MARINEFF et présentant la même structure de surface a permis d'évaluer l'efficacité de ces derniers.

La partie V-B de cette thèse traite du suivi *in situ* des récifs artificiels immergés depuis 2015 dans le cadre du projet RECIF sur les deux sites d'études (récifs artificiels dans la Figure 20). Différents indicateurs biologiques comme la diversité et la biomasse d'espèces en faune et en flore, la production primaire globale du récif et les paramètres photosynthétiques des différentes espèces de macroalgues ont été mesurés pendant plus d'une année. Cette étude permet d'apporter de résultats nouveaux et des notions importantes concernant la relation entre la biodiversité et la production, elle souligne aussi deux fonctionnements écosystémiques bien distincts et met en avant les bénéfices écologiques associés à ce type de structures.

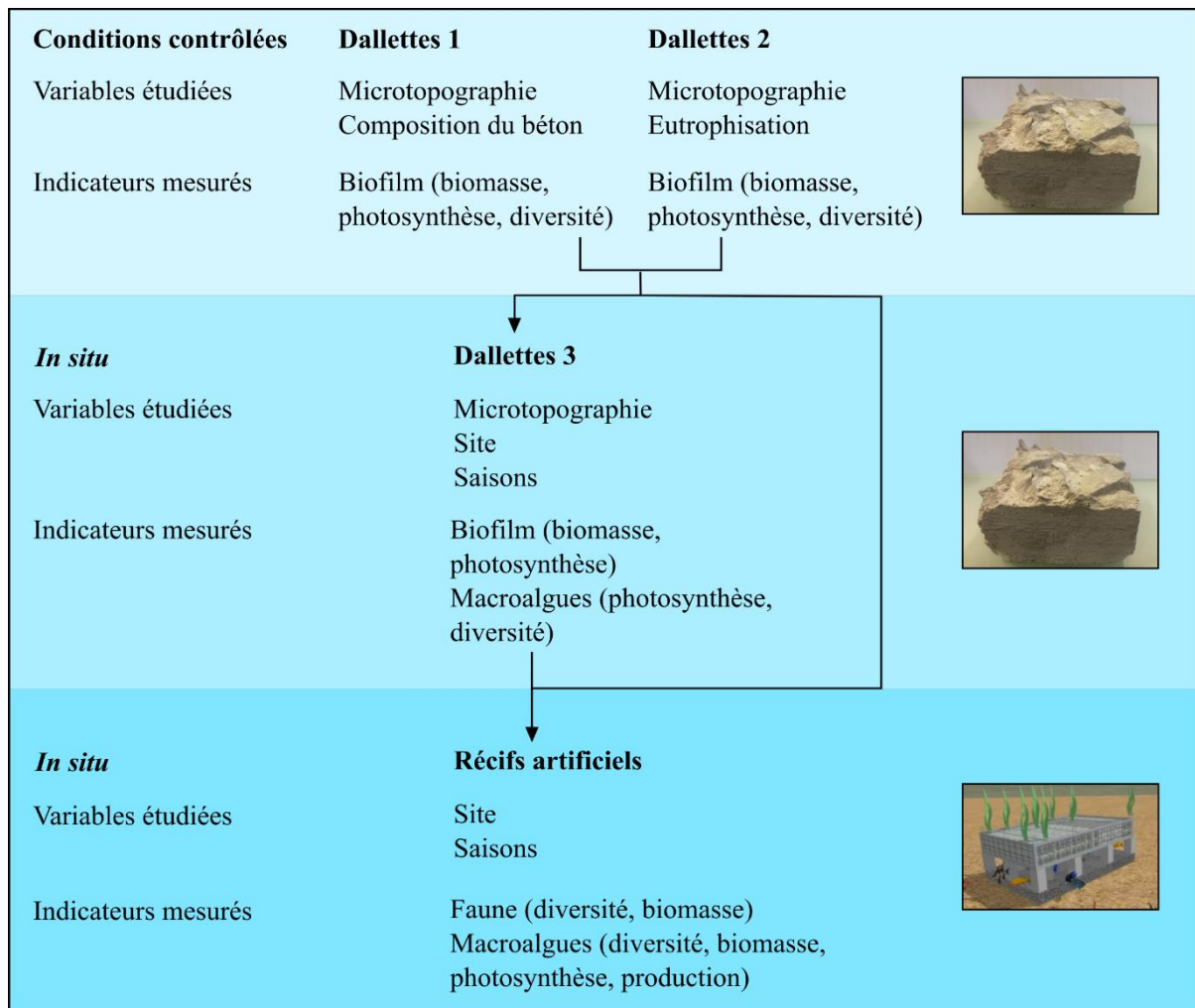


Figure 20 – Représentation schématique des différentes expérimentations et suivis réalisés constituant les différents articles de cette thèse.

CHAPITRE II : MATERIEL ET METHODES

I. Sites d'études

Les sites d'études ont été décrits dans la partie III.4 de l'introduction.

II. Modèles d'études et stratégies d'échantillonnages

II.1. *Dallettes expérimentales (MI)*

Différents modèles de récifs artificiels ont été étudiés au cours de ces travaux. Pour l'ensemble des expérimentations en conditions contrôlées et pour un des suivi *in situ*, des dalles expérimentales en béton ont été réalisées. Pour la première expérimentation (Partie IV-A) 50 MI (infrastructures marines) (5 cm×5 cm×3 cm ; L×l×h) ont été réalisées selon quatre formulations (Tableau 1) et deux types de ciments afin de tester l'influence de ces différentes compositions sur le développement et les paramètres photosynthétiques du biofilm. La conception des matériaux et la réalisation de ces structures ont été assurés par l'ESTIC, partenaire du projet MARINEFF. Les deux formulations de ciments sélectionnées étaient : CEM I/A-LL 42.5 R CE PM-CP2 NF et CEM II/A (S-V) 32.5 N-LH CE PM-ES-CP1 NF (Calcia). Ce sont des formulations adaptées à l'utilisation en milieu marin. Du sable siliceux de classe granulaire 0/2 mm était utilisé. Des agrégats alluviaux de deux tailles étaient aussi utilisés (4/10 mm et 10/20 mm). Un remplacement de 20% des agrégats (4/10 mm) par des coquilles d'huîtres broyées (6/12,5 mm) était réalisé. Les dalles présentaient une face lisse et une rugueuse (Figure 21). Des témoins en PVC étaient utilisés pour comparer les données, ce matériau est souvent utilisé pour le captage larvaire en milieu marin. Les bétons étaient alors réalisés en accord avec la norme NF EN 206-1 selon les formulations suivantes :

Tableau 1 – Formulations retenues pour la fabrication des BMI (proportions données en kg m⁻³ de béton)

Formulations Composés	Type 1 : CEM I sans coquilles	Type 2 : CEM I avec 20% de coquilles	Type 3 : CEM II sans coquilles	Type 4 : CEM II avec 20% de coquilles
Ciment	350	350	350	350
Sable	800	800	800	800
Graviers 4/10	600	479	600	479
Graviers 10/20	500	500	500	500
Coquilles	-	121	-	121
Eau	175	175	175	169
Superplastifiant	4,5	7	4,5	7

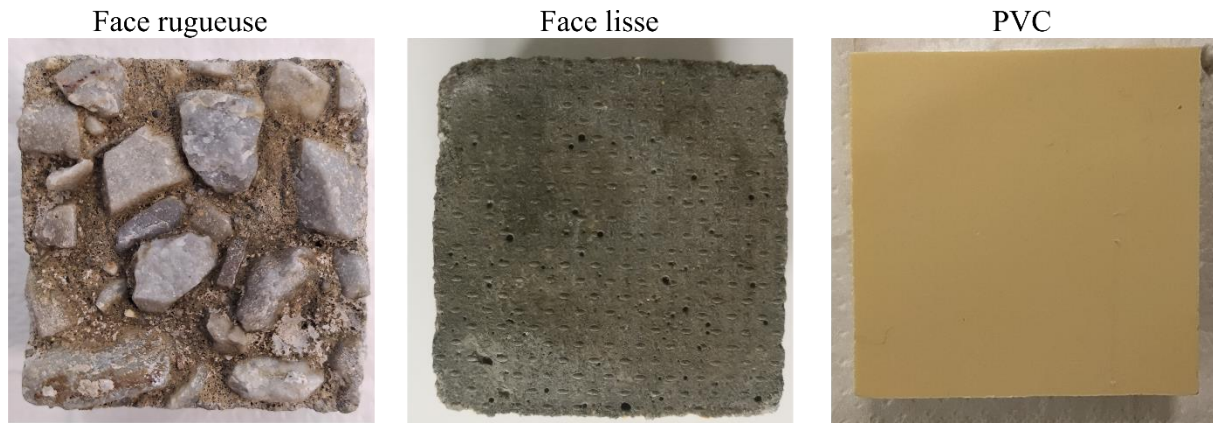


Figure 21 – Photographies des différents types de dallettes (rugueuse, lisse et PVC) utilisés.

Pour cette première expérimentation en conditions contrôlées, l'ensemble des MI était ensuite disposé dans un bac avec de l'eau de mer afin d'effectuer la marénisation du béton, ce processus permet de nettoyer le matériau de ces impuretés. Les faces verticales de chaque MI étaient ensuite paraffinées afin de prévenir leur éventuelle colonisation par les micro-organismes et donc que ces faces entrent en compétition avec les faces d'intérêt (les deux principales). Vingt microcosmes (16,2 cm × 11,2 cm × 11,7 cm ; L × l × h) ont été réalisés afin d'optimiser les conditions environnementales et d'améliorer le développement du biofilm. Les murs internes de ces microcosmes ont eux aussi été paraffinés. Les MI étaient ensuite placés par lot de trois dans chaque microcosme, eux-mêmes disposés dans un mésocosme (650 L) alimenté avec de l'eau de mer stérile et des pompes de brassage. Des bulleurs d'air étaient ajoutés dans chaque microcosme, chaque microcosme était immergé au même niveau dans le mésocosme. Un éclairage artificiel était assuré et la stérilisation de l'eau de mer était réalisée avec un traitement UV (JBL AquaCristal UV-C 36W) pendant 48 h. Les biofilms microphytobenthiques ont été échantillonnés sur des rochers prélevés sur l'estran de Luc-sur-mer au niveau de l'infralittoral. Ils ont d'abord été mis en culture dans un grand bassin alimenté en eau de mer à la station marine du CREC. Plusieurs parpaings, composé du même matériau que celui utilisé pour les MI de cette expérience, ont été disposés dans ce bassin et les biofilms ayant colonisé les surfaces de ces derniers ont été prélevés après deux semaines d'incubation. Les biofilms ont été prélevés soigneusement à l'aide d'une brosse à dent, ils ont ensuite été dilués dans 1 L d'eau de mer. Enfin, cette solution mère a été ajoutée au mésocosme une semaine après l'installation des MI dans les microcosmes. Une série d'indicateurs ont été mesurés sur une période de six jours après l'ajout des biofilms dans les microcosmes. La température et le pH ont été mesurés pendant le suivi. La distance entre l'éclairage LED et les MI était de 60 cm. L'intensité de l'éclairage était mesurée à une minute d'intervalle grâce à une sonde Onset Hobo UA-002 Pendant light/temperature[®] placée dans le mésocosme.

Pour la seconde expérimentation (Partie IV-B), 60 MI (5 cm×5 cm×3 cm ; L×l×h) ont été réalisées selon la formulation suivante : CEM II/A-LL 42.5 R CE PM-CP2 NF. 45 dalles en PVC ont été réalisées pour servir de témoins pour cette expérimentation. Lors de cette expérience, seule la face rugueuse des structures en béton (MI) était utilisée. Vingt MI et quinze PVC étaient disposés aléatoirement dans trois différents mésocosmes (45 cm×32 cm×25 cm ; L×l×h). Les trois mésocosmes étaient alimentés avec de l'eau de mer filtrée ($\varnothing$ 100 μ m) par un système semi-ouvert. Le volume total de chaque mésocosme était de 44 L et le taux de renouvellement d'environ 10% du volume total toutes les quatre heures. Deux mésocosmes étaient enrichis en nutriments selon un ratio Redfield/Brzezinski (C:N:P = 106:16:1) pendant tout le suivi grâce à une pompe péristaltique (une dose par heure). Une solution mère unique était réalisée pour les deux conditions enrichies respectivement à + 30 μ mol L⁻¹ (enrichissement moyen ou C2) et + 100 μ mol L⁻¹ (enrichissement élevé ou C3). La condition témoin n'était pas enrichi (C1). Des pompes de brassage étaient disposées dans chaque mésocosme, elles étaient activées pendant 15 minutes toutes les deux heures. Un éclairage artificiel était mis en place 10 heures par jour (150 μ mol photon m⁻² s⁻¹). Plusieurs paramètres étaient mesurés pendant 28 jours après l'ajout des biofilms. Les biofilms ont été prélevés sur des rochers issus de l'estran à Luc-sur-mer le 29 juin 2020. Un inoculum de 100 mL à 2975 cellules par mL a été ajouté dans chaque mésocosme au début du suivi.

Enfin, pour le suivi *in situ* (Partie V-A) un total de 96 MI a été réalisé, des dalles ont été immergées sur les deux sites expérimentaux (rade de Cherbourg et baie de Seine) en septembre 2019 de manière à réaliser un suivi saisonnier (quatre prélèvements par an) durant deux ans sur chacun des sites (Figure 22). Les dalles immergées sont identiques à celles décrites de la seconde expérimentation. Elles ont été immergées par lot de six avec une alternance de face rugueuse et lisse orientées vers la surface. Lors de chaque prélèvement, un lot de six dalles était prélevé en plongée puis ramené en surface dans un tube hermétique permettant de conserver les échantillons dans l'eau du milieu sans modification des paramètres physico-chimiques. Une fois au laboratoire, les dalles étaient alors identifiées (trois rugueuses et trois lisses) et conservées dans des caisses individuelles avec eau de mer. L'utilisation d'infrastructures identiques pour ces différentes expérimentations en conditions contrôlées et pour le suivi *in situ* a permis de comparer les processus de colonisation observés et de réaliser un transfert d'échelle (conditions contrôlées vers *in situ*). L'étude *in situ* avec ces dalles

expérimentales a également permis de réaliser un transfert d'échelle entre les MI et les récifs artificiels immergés sur ces mêmes sites.

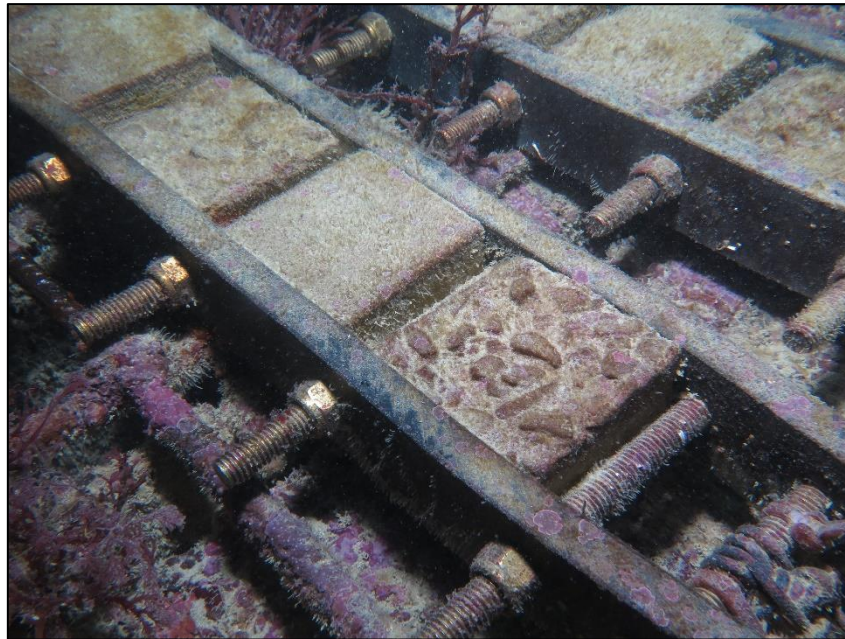


Figure 22 – Photographie des dalles immergées sur le site de la rade de Cherbourg en février 2020 (environ cinq mois après leur installation).

II.2. Récifs artificiels (projet RECIF)

Les récifs artificiels étudiés dans l'avant dernière partie (Partie V-B) ont été immergés en 2015 dans le cadre du projet Interreg Européen RECIF (Boutouil et al., 2015; Claquin et al., 2015). Ces récifs ont été déployés sur les deux sites expérimentaux de cette thèse, en rade de Cherbourg et en baie de Seine (Figure 23).

Dans la rade de Cherbourg, les récifs artificiels sont constitués de douze modules regroupés en trois clusters créant un triangle. Chaque module est identique et mesure 3 m de long, 2 m de large et 1,35 m de hauteur ($8,1 \text{ m}^3$ par module – $32,4 \text{ m}^3$ par cluster – $97,2 \text{ m}^3$ au total dans la rade de Cherbourg). Les modules sont constitués de trois niveaux, le premier niveau à un rôle structurant et permet au module de ne pas s'ensabler. Le deuxième niveau est constitué de piliers en béton et de vide. Le dernier niveau est constitué d'un gabion métallique à l'intérieur duquel des parpaings sont disposés sur plusieurs étages. Les dimensions de chaque parpaing sont de 0,4 m de long, 0,2 m de large et 0,2 m de hauteur. Chaque parpaing est constitué de béton poreux. En baie de Seine, les récifs artificiels sont uniquement constitués de trois modules de taille plus réduite (1 m^3) constitués des mêmes structures élémentaires (parpaings) que le site de Cherbourg positionné dans des gabions dont la face supérieure a été enlevée. Les

parpaings de surface et des couches inférieures des RA de Cherbourg et de la Baie de Seine ont ainsi la même position.

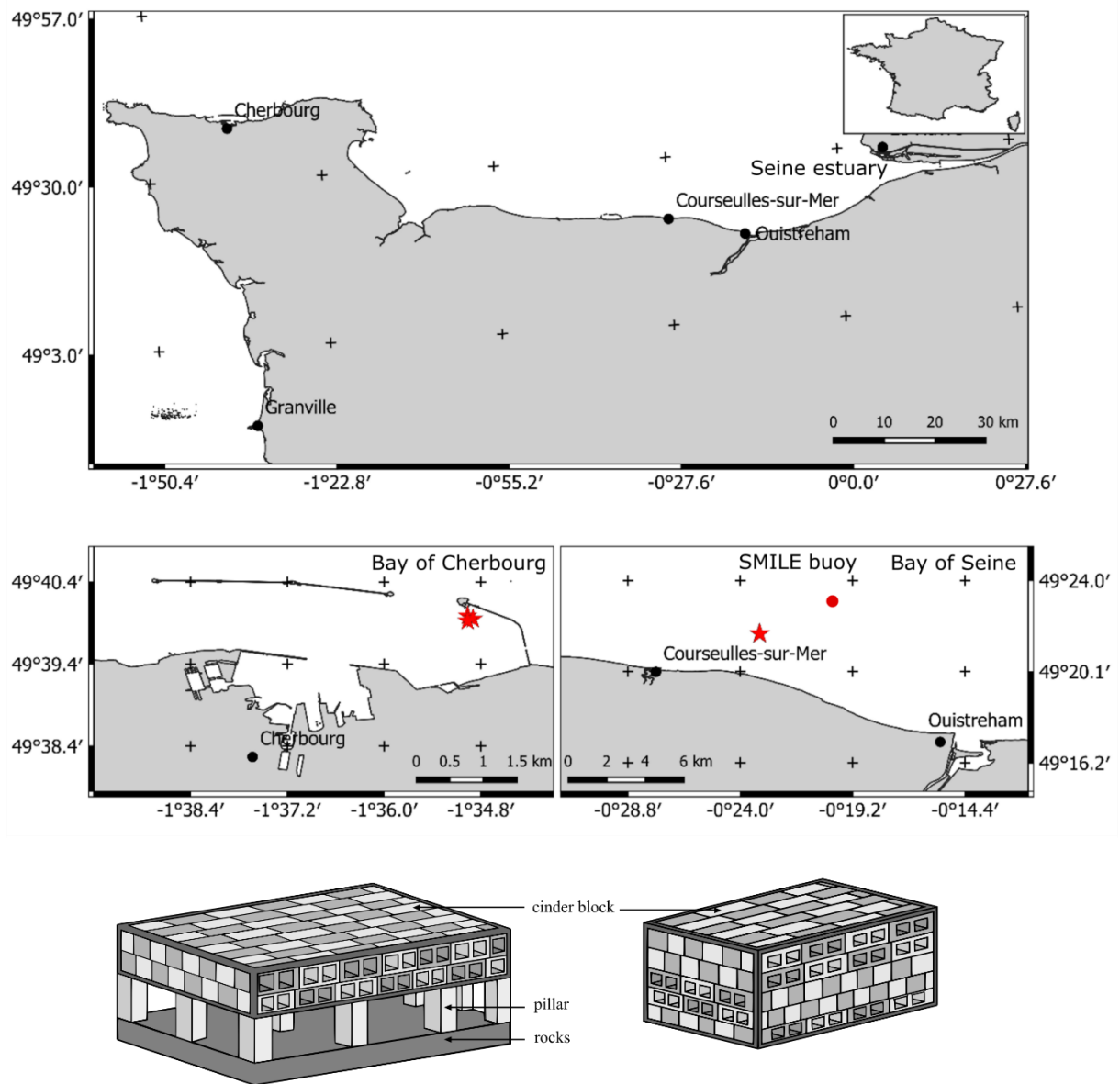


Figure 23 – Localisation des récifs artificiels (étoiles rouges), de la bouée instrumentée SMILE (point rouge) et représentation schématique d'un module de Cherbourg (gauche) et de baie de Seine (droite).

L'échantillonnage s'est déroulé entre 2019 et 2021 et a été fait de façon saisonnière sur les deux sites. Trois campagnes ont été réalisées en baie de Seine et quatre en rade de Cherbourg. Lors de chaque échantillonnage, les paramètres physico-chimiques du milieu ont été mesurés. A chaque échantillonnage, six parpaings étaient prélevés aléatoirement sur la face supérieure du récif, ils étaient remontés en surface à l'intérieur d'un filet à plancton (maille de 500 μm de $\varnothing$) fermés et conservés dans des bidons remplis d'eau de mer jusqu'au retour au laboratoire (Figure 24). Les parpaings étaient ensuite conservés dans des bacs alimentés par un système ouvert d'eau de mer. Les parpaings étaient ensuite incubés individuellement pour mesurer la production primaire, ensuite, des calculs de diversités et de biomasses en algue et en faune étaient réalisés (Figure 25).

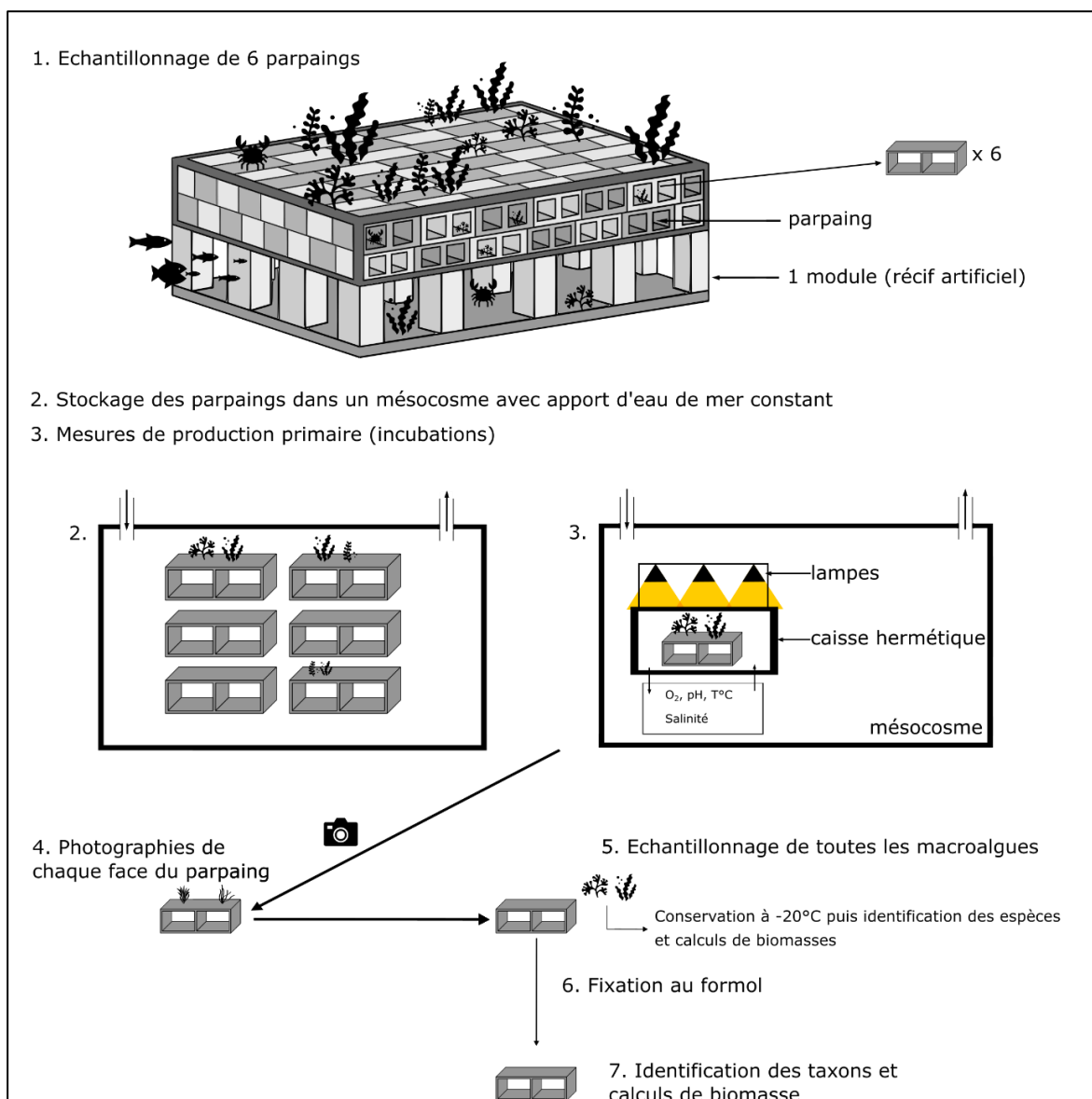


Figure 24 – Schéma représentant le déroulement de l'expérimentation sur les parpaings dans le but de décrire l'état initial des sites d'études.

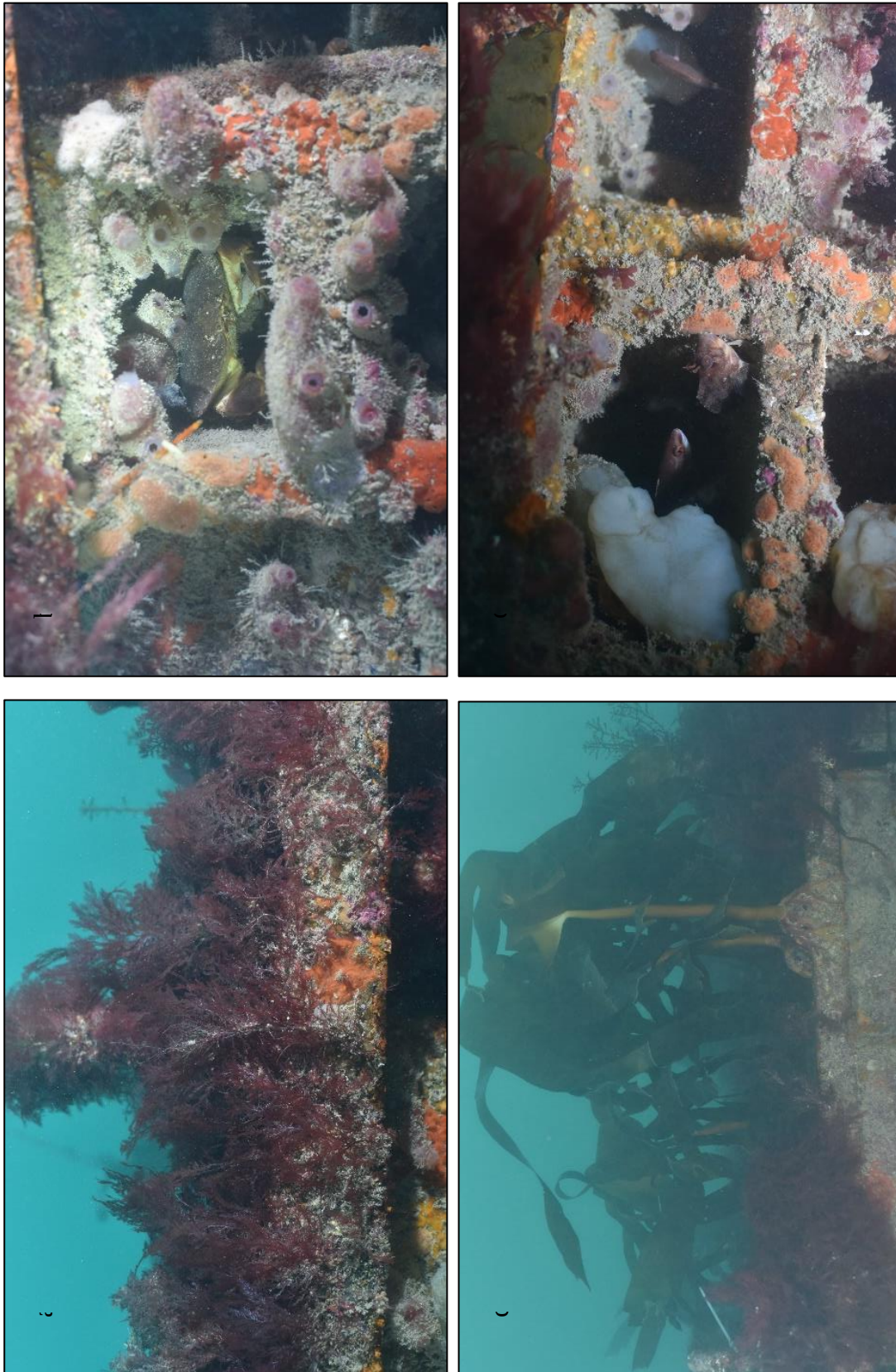


Figure 25 – Exemples de photographies réalisées durant les plongées sur le site de la rade de Cherbourg. Ici des algues rouges (a) et laminaires (b) sont en surface d'un module. Un tourteau (b) ainsi qu'une blennie et un labridé (d) sont abrités dans les espaces libres créés par les parpaings.

III. Echantillonnage *in situ*

Différents moyens ont été nécessaires afin de réaliser le suivi scientifique *in situ*. Lors des suivis réalisés sur le site de la baie de Seine, les moyens nautiques de la station marine du CREC ont été utilisés. Pour les opérations de remontée en surface des parpaings, deux embarcations étaient nécessaires compte tenu de l'espace nécessaire pour les plongeurs et les échantillons. La majeure partie des suivis réalisés en rade de Cherbourg ont été fait avec l'aide des moyens nautiques du Pôle Plongée Normandie (PPN) de Cherbourg. Le partenariat réalisé avec PPN a permis d'éviter le déplacement des embarcations du CREC jusqu'à Cherbourg. Le bateau mis à disposition par PPN (Sepia) a également permis de travailler efficacement et avec confort. Enfin, la majeure partie des données hydrologiques ont été acquise grâce au suivi SOMLIT (Service d'Observation en Milieu Littoral) assuré par la station marine de Luc-sur-Mer. L'ensemble des échantillonnages et des mesures a été fait en plongée par les six plongeurs (CAH 1B ou 2B) de l'Université de Caen Normandie (CREC, BOREA, SUAPS) (Pascal Claquin, Léo Chasselin, Maël Deloor, Maxime Navon, Jean-Paul Chenal et Baptiste Vivier).

IV. Biomasse microphytobenthique

La biomasse microphytobenthique est estimée en chlorophylle *a* (Chl *a*) par unité de surface. Cet indicateur est mesuré pour les expérimentations sur les dalles en conditions contrôlées et le suivi *in situ*. Le biofilm est échantillonné à l'aide d'une brosse à dent et remis en suspension dans 250 mL d'eau de mer filtrée (Stericup GV Millipore, Ø 0.22 µm). Un sous-échantillon de 150 mL est ensuite filtré sur un filtre en fibre de verre (Whatman, GF/F, 47 mm, 0.7 µm) et immédiatement conservé à -20°C jusqu'à analyse dans les jours suivants. 10 mL d'acétone (90%) sont ajoutés pour extraire les pigments chlorophylliens et l'échantillon est ensuite incubé à 4°C pendant 12 heures. Après deux centrifugations de 5 min à 1700 g, la concentration de Chl *a* des extraits est mesurée grâce à un fluorimètre Trilogy (Turner Designs, Sunnyvale, USA) selon la méthode de Strickland & Parsons (1968).

V. Paramètres photosynthétiques et production primaire

V.1. *Imaging-PAM*

Les paramètres photosynthétiques du biofilm microphytobenthique sont mesurés à l'aide d'un IMAGING-PAM Chlorophyll Fluorometer (Walz, Effeltrich, Germany). Cet outil est associé à une dalle LED-Array Illumination Unit IMAG-MAX/L (44 high-power royal-blue (450 nm) LED-lamps) et à une caméra CCD Camera IMAG- K7. Il permet de mesurer les

paramètres photosynthétiques précédemment décrits en deux dimensions et d'observer les hétérogénéités spatiales de l'activité photosynthétique. Les mesures sont réalisées à une distance fixe de 18,5 cm par rapport à l'échantillon, une acclimatation à l'obscurité de 5 min est réalisée en amont. Pour chaque échantillon, une zone d'intérêt (AOI, area of interest) est choisie à l'endroit où la fluorescence F_0 est maximale (Figure 26). La fluorescence minimale (F_0) était mesurée après l'excitation de l'algue par une faible lumière bleue (470 nm). La fluorescence maximale (F_M) était obtenue après application d'un pulse de lumière saturante (470 nm de $4500 \mu\text{mol photon m}^{-2} \text{s}^{-1}$ pendant 0,8 secondes à une fréquence de 8 Hz) permettant de réduire en totalité le pool de Q_A (Figure 27). Chaque échantillon est exposé à huit intensités de lumières actiniques croissantes : 0, 21, 111, 281, 396, 531, 611 et $701 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ d'une durée de 30 secondes chacune.

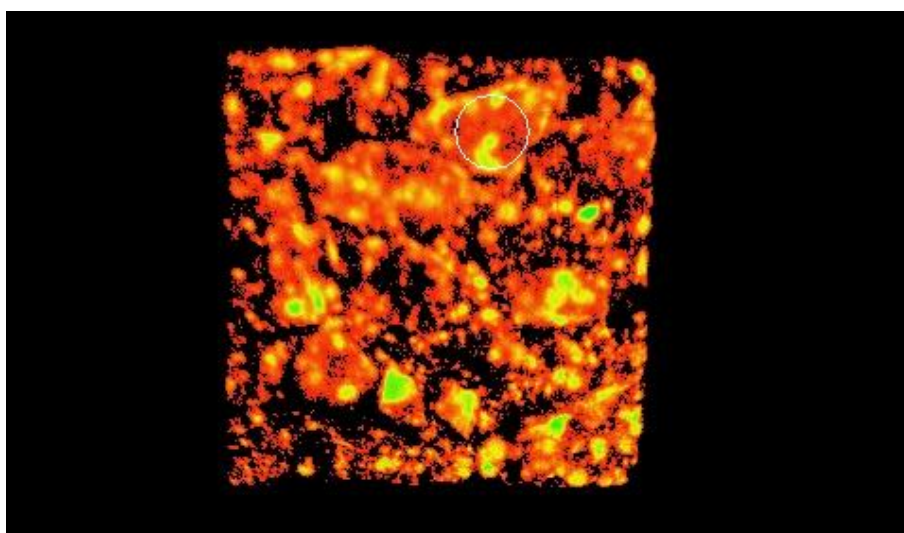


Figure 26 – Exemple de donnée obtenue par l'Imaging-PAM avec la zone d'intérêt (cercle blanc).

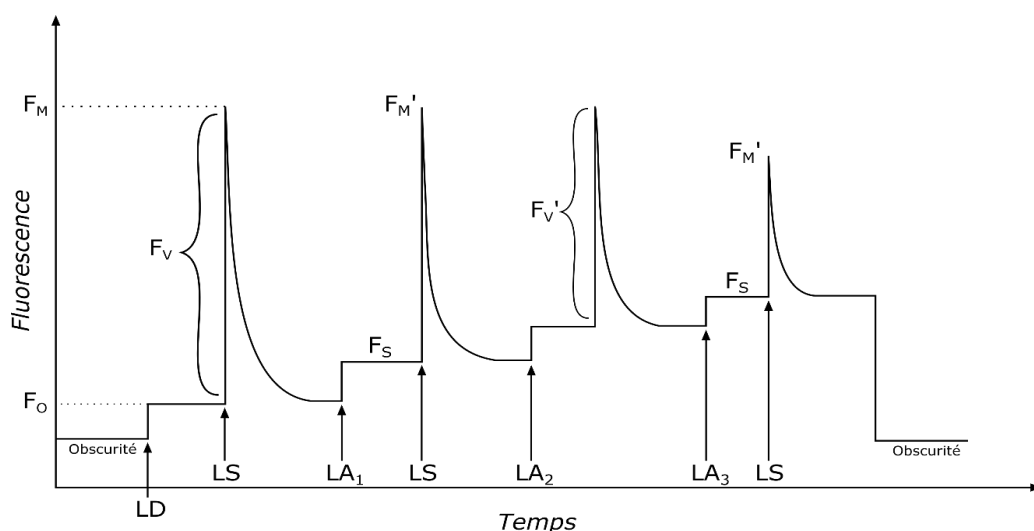


Figure 27 – Principe de la fluorescence modulée PAM. Sous une très faible lumière détectrice (LD), l'activité photosynthétique est insignifiante. Le premier accepteur d'électron du PSII, Q_A , est alors complètement oxydé, la fluorescence émise est par conséquent minimale. Ce niveau de fluorescence obtenu après un passage à l'obscurité est appelé le niveau minimum de fluorescence F_0 . Ensuite un flash lumineux de haute intensité est émis. Le PSII est saturé, Q_A est complètement réduit. La fluorescence atteint alors un maximum (F_M). Une gamme de lumière actinique (LA_1 , LA_2 etc.) d'intensités croissantes est appliquée sur l'échantillon. Chaque intensité lumineuse (flux de photons) est appliquée pendant 30 s dans cette étude. Pour chaque intensité, une fluorescence de base stable F_S est atteinte. Toutes les 30 secondes un flash de lumière saturante (LS) est émis et F_M' est alors déterminée.

L'efficacité quantique maximale du PSII pour chaque irradiance est calculée selon l'équation suivante (Genty et al., 1989) :

$$\frac{F_V}{F_M} = \frac{(F_M - F_0)}{F_M} \quad (1)$$

Le taux relatif maximal de transport d'électrons ($rETR_{MAX}$, unité relative) est estimé selon le modèle Webb (1974), α , l'efficacité photosynthétique ($\mu\text{mol électrons } (\mu\text{mol photons})^{-1}$) et E_k l'intensité de saturation lumineuse ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$) est ensuite estimés avec la pente initiale de la RLC (rapid light response curve) et E_k :

$$rETR = \alpha \times E_k \times (1 - e^{-\frac{E}{E_k}}) \quad (2)$$

Le second modèle applicable est celui d'Eilers & Peeters (1988). Ce modèle contrairement au modèle de Webb permet d'estimer la photoinhibition (β). Il s'agit d'un modèle mécanistique qui demande la détermination de trois paramètres a , b et c permettant de calculer le $rETR_{MAX}$ et l'efficacité photosynthétique (α) et la photoinhibition (β).

$$rETR = \frac{E}{(aE^2 + bE + c)} \quad (3)$$

$$\beta = \frac{b}{\sqrt{ac}} \quad (4)$$

Le $rETR_{MAX}$ était alors calculé selon l'équation suivante :

$$rETR_{MAX} = \alpha_{webb} \times Ek_{webb} \quad (5)$$

ou,

$$rETR_{MAX} = \frac{1}{(b + 2\sqrt{ac})} \quad (6)$$

α était calculé comme suit :

$$\alpha = \frac{1}{c} \quad (7)$$

Enfin, le quenching non-photochimique (NPQ) est calculé :

$$NPQ = \frac{F_M - F_{M'}}{F_{M'}} \quad (8)$$

Afin d'estimer les paramètres photosynthétiques comme l'efficacité photosynthétique (α) ou le taux relatif maximal de transport d'électrons ($rETR_{MAX}$) pour chaque pixel, un modèle de régression non linéaire était appliqué sur les courbes RLC en utilisant la méthode de Nelder & Mead (1965). L'Imaging-PAM permet de mesurer la fluorescence émise par le microphytobenthos sur l'ensemble de la dalle colonisée et donc de calculer des RLC pour chaque pixel de l'échantillon. Selon le profil des courbes, le modèle le plus adéquat entre Webb *et al.* (1974) et Eilers & Peeters (1988) est automatiquement choisi par l'algorithme (Figure 28). Dans le cas exceptionnel où le $rETR_{MAX}$ est plus grand que le plus fort $rETR$ mesuré, un modèle linéaire est appliqué pour déterminer α et le plus fort $rETR$ est considéré comme $rETR_{MAX}$ afin d'éviter une surestimation de l' $rETR_{MAX}$. Dans les cas où (1) la première valeur de la RLC est la plus forte, (2) les valeurs de la RLC étaient toutes nulles et (3) la sélection ne comprend pas plus de trois valeurs positives, les paramètres photosynthétiques étaient considérés comme nuls.

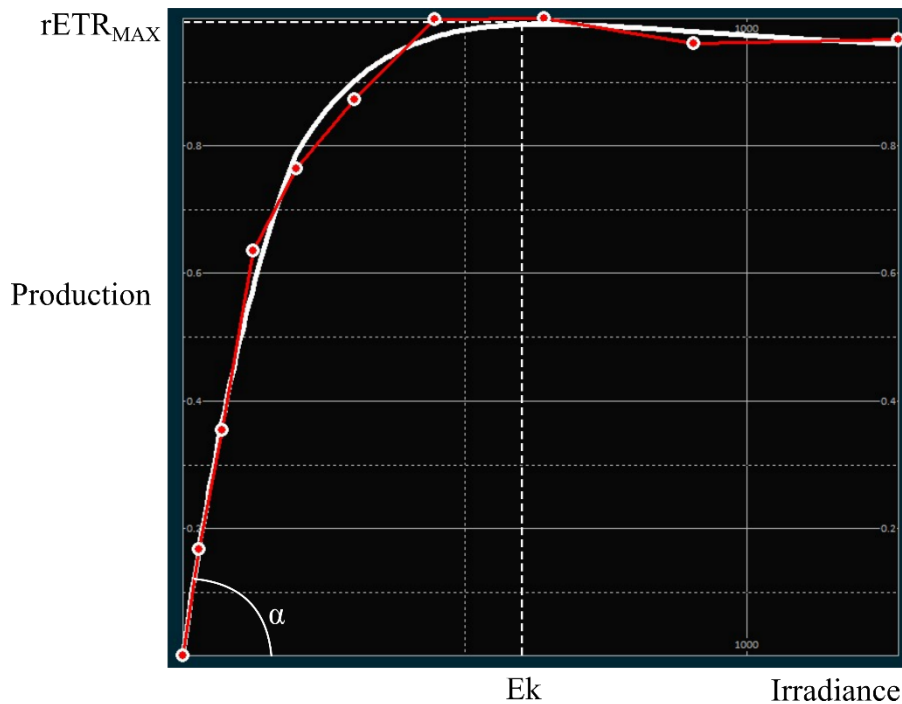


Figure 28 – Exemple de courbe P/E obtenue pour un pixel d'un échantillon.

Une routine MATLAB[®] a été développée afin d'obtenir les valeurs numériques de fluorescence à partir des images obtenues par l'IMAGING-PAM. Elle a permis de déterminer pour chaque pixel les valeurs de F_0 , F_M , F_S (fluorescence à l'état stable) et F_M' . Le F_V/F_M est calculé pour chaque pixel selon l'équation présentée précédemment (Figure 29). Afin d'éliminer le bruit de fond, les valeurs de F_M inférieures à 0,048 ont été considérées comme non valides. En effet, sous ce seuil les valeurs acquises peuvent être considérées comme trop faible pour être fiable (Heinz Walz GmbH 2014). Enfin, pour chaque pixel et chaque intensité de lumière (E), le rETR est estimé selon l'équation n°3, des courbes rETR-I étaient réalisées et la lumière d'acclimatation (E_k) était estimée.

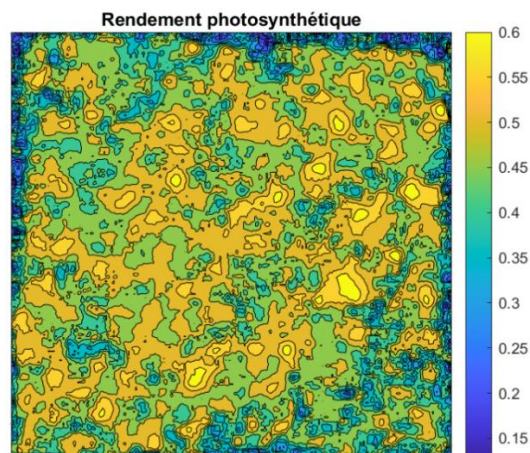


Figure 29 – Exemple de figure obtenue (F_V/F_M) après traitement des données brutes par la routine MATLAB.

V.2. *Diving-PAM*

Lors de certaines campagnes en rade de Cherbourg, les paramètres photosynthétiques des macroalgues étaient ont été mesurés *in situ* à l'aide d'un DIVING-PAM-II fluorometer (Heinz Walz GmbH, Germany). Les paramètres photosynthétiques des macroalgues étaient mesurés grâce à un DIVING-PAM-II fluorometer (Heinz Walz GmbH, Germany). Ces mesures sont réalisées sur un maximum d'échantillon en fonction des contraintes liées à la plongée (profondeur, température, consommation d'air etc.) représentatifs de permettant de représenter la diversité d'algues sur le site. Les paramètres mesurés et les équations appliquées sont les mêmes que celle décrite pour l'IMAGING PAM. Cependant la mise en œuvre des mesures, les réglages de l'appareil et les longueurs d'onde appliqués sont spécifiques pour le diving PAM. Cet outil permettait de mesurer la productivité primaire via divers indicateurs comme le rendement quantique maximal du PSII (F_v/F_m). Après 5 min d'acclimatation au noir permettant l'oxydation du pool de Q_A , le thalle de l'algue est placé dans une chambre opaque à l'obscurité pendant 3 min à l'aide d'un clips aimantés fournis avec l'équipement permettant les mesures de fluorescence avec une fibre optique. Les flashes de lumière saturante étaient de $4000 \mu\text{mol photon m}^{-2} \text{s}^{-1}$ pendant 0,6 s. Afin de réaliser des RLC, l'échantillon a ensuite est exposé à onze irradiances (E) de lumière actinique de 0 à $1490 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ séparées entre-elles par une durée dépendant 20 s pour chaque intensité. La fluorescence minimale (F_0) était est mesurée après l'excitation de l'algue par une faible lumière bleue (470 nm). La fluorescence maximale (F_m) était obtenue après application d'un pulse de lumière saturante (470nm de $4000 \mu\text{mol photon m}^{-2} \text{s}^{-1}$ pendant 0,6 s.) permettant de réduire en totalité le pool de Q_A (Figure 27).

V.3. *Coefficient d'absorption spécifique de la chlorophylle a (a^*)*

Le coefficient d'absorption spécifique de la chlorophylle (a^* , $\text{m}^2 \text{mg Chl } a^{-1}$) a été calculé pour l'expérimentation en conditions contrôlées simulant un gradient d'eutrophisation. Il était obtenu après filtration de 50 mL de l'échantillon remis en suspension (250 mL) qui avait servi à la Chl a . La densité optique *in vivo* de la totalité des particules entre 380 et 750 nm est mesurée directement sur le filtre avec un spectrophotomètre (UV-2600i Shimadzu) équipée d'une sphère intégrante (IR-2600 Shimadzu). Du méthanol (MeOH) est ensuite ajouté à l'échantillon afin de détruire les pigments (Babin et al., 2003). Après une heure, la densité optique *in vivo* des particules non photosynthétiques est mesurée directement sur le filtre après rinçage avec de l'eau de mer stérile. Le coefficient d'absorption spécifique de la chlorophylle (a^* , $\text{m}^2 \text{mg Chl } a^{-1}$) était calculé selon l'équation de Johnsen and Sakshaug, (2007) :

$$\alpha^* = \frac{A \times S \times 2.3}{V \times [Chl\ a]} \quad (9)$$

avec A la moyenne de la densité optique de l'échantillon entre 380 et 750 nm, S la surface du filtre (295,6 mm²), V le volume filtré en mL et la concentration de Chl a en mg m⁻³.

Le taux de transport maximal des électrons (mmol e⁻ (mg Chl a h)⁻¹)⁻¹ était ensuite calculé selon l'équation suivante :

$$ETR_{max}^{a^*} = rETR_{MAX} \times \alpha^* \times fAQ_{PSII} \times 3.6 \quad (10)$$

avec fAQ_{PSII} la fraction d'énergie quantique absorbée par le PSII, chez les diatomées, 74% des photons sont utilisés par la chaîne de photo-réaction dans le PSII (Johnsen & Sakshaug, 2007; Napoléon et al., 2013b; Morelle & Claquin, 2018).

Enfin, le α^{a^*} (μmol e⁻ (μmol photons)⁻¹) était calculé selon l'équation suivante :

$$\alpha^{a^*} = \frac{ETR_{max}^{a^*}}{Ek} \quad (11)$$

V.4. Production primaire et respiration – Incubations

Pour le suivi sur les récifs artificiels, la production primaire des parpaings est mesurée une fois ramenés au laboratoire. Chaque parpaing est alors incubé individuellement dans une caisse hermétique avec un contrôle de l'intensité lumineuse afin de mesurer la production primaire sous différentes conditions d'éclairage (Figure 30). Pendant chaque incubation, le PAR est mesuré avec une sonde RBR solo³ PAR connectée à un Li-COR "Underwater Quantum Sensor" LI-192. L'oxygène dissous est aussi mesuré avec une sonde EXO Optical Dissolved Oxygen Sensor hermétiquement connectée au système de mesure. Une pompe de brassage permettait de maintenir un flux constant d'eau dans le système clos. Chaque incubation consiste en sept différentes conditions d'éclairage de 20 minutes (dark 1 ≈ 0 μmol photon m⁻² s⁻¹ – PAR 1 ≈ 100 μmol photon m⁻² s⁻¹ – PAR 2 ≈ 200 μmol photon m⁻² s⁻¹ – PAR 3 ≈ 360 μmol photon m⁻² s⁻¹ – PAR 4 ≈ 430 μmol photon m⁻² s⁻¹ – PAR 5 ≈ 750 μmol photon m⁻² s⁻¹ – dark 2 ≈ 0 μmol photon m⁻² s⁻¹). Pour chaque condition la production ou la consommation d'oxygène est calculée selon la pente de la régression linéaire appliquée à l'évolution de la concentration en oxygène, elle est alors exprimée en μmol O₂ m⁻² h⁻¹ après intégration du volume d'eau contenu dans la caisse (58,9 L) et de la surface du parpaing étudiée. La production primaire brute (gross primary production ou GPP) est alors calculée pour chaque condition comme l'addition de la respiration

(condition dark 2) et de la production primaire nette (net primary production ou NPP). Ces données sont ensuite converties en équivalent carbone tel décrit par Cesbron et al., (2019) en appliquant le ratio de Redfield ($C:O_2 = 106:138$) et exprimée en $gC\ m^{-2}\ jour^{-1}$. GPP_{max} et NPP_{max} sont obtenus à lumière saturante après transformation selon un modèle de Webb et al., (1974). La productivité primaire est calculée comme le ratio de la biomasse sur GPP_{max} et exprimée en $gC\ gC\ jour^{-1}$.

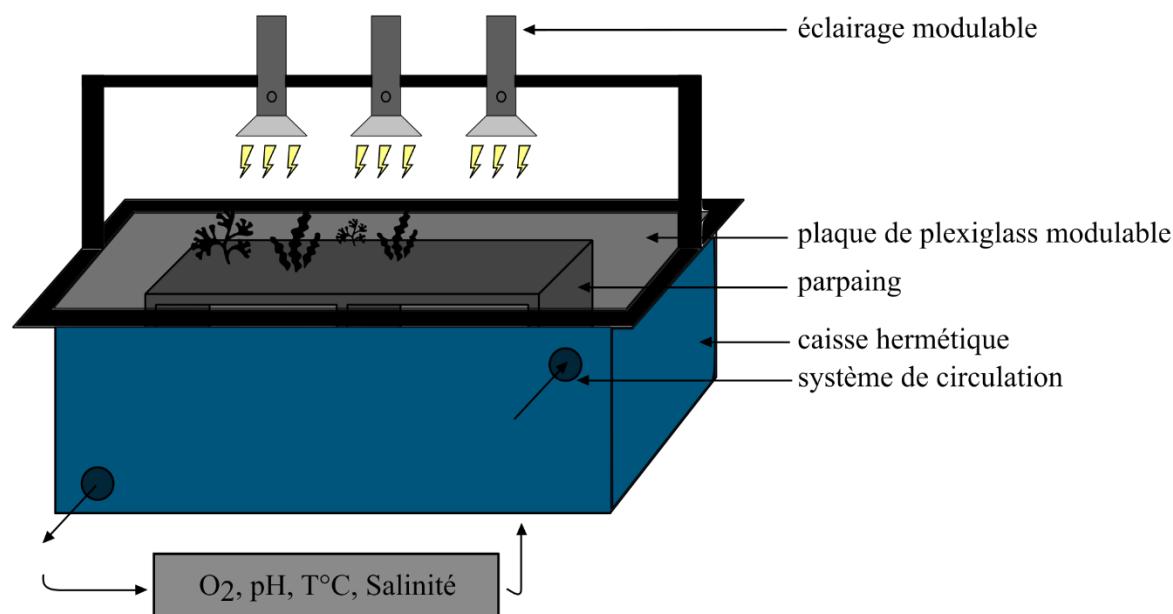


Figure 30 – Représentation schématique du système d'incubation pour les mesures de production primaire associées aux parpaings.

VI. Rugosité de surface – Caméra 3D

Des mesures de la topographie de surface de l'échantillon étaient réalisées à l'aide d'une caméra 3D Gocator 3110. Cet outil permet de scanner à haute fréquence (5 Hz) la surface de l'échantillon et de produire un relief numérique. Les données brutes étaient ensuite exploitées par une routine MATLAB® afin de calculer un coefficient de corrélation (R) entre le niveau de rugosité et la biomasse de biofilm ou différents indices photosynthétiques (F_v/F_m , $rETR_{MAX}$, E_k , α et NPQ). Ces scripts ont été développés par Francis Orvain et retravaillés par Baptiste Vivier. Un traitement en amont a été effectué afin de corriger la potentielle inclinaison de la surface supérieure de la MI grâce à cette équation :

$$Z_{interp} = a \times X + b \times Y + c \times X.Y + d \quad (12)$$

avec X et Y les limites inférieures de la structure. Les paramètres a, b, c et d étaient obtenus grâce à la méthode de minimisation par simplex permettant la détermination d'une meilleure équation (plus fort R^2) passant par le plan de surface (Z_0). Afin de déterminer exactement la rugosité de surface sans l'interférence de l'inclinaison globale de la structure, il était nécessaire de retirer cette dernière. La différence entre les valeurs était alors calculée :

$$Z_{final} = Z_0 - Z_{interp} \quad (13)$$

VII. Substances polymériques extracellulaires (EPS)

Les EPS totaux ont été mesurés pour l'expérimentation en condition contrôlées selon un gradient d'eutrophisation. La Figure 31 représente les différentes fractions des EPS produits par les diatomées (Shnyukova & Zolotariova, 2015). Un sous-échantillon de 10 mL était prélevé sur l'échantillon de biofilm remis en suspension (250 mL). Cet échantillon de 10 mL était ensuite centrifugé à 3000 rpm pendant 20 min à 20°C, le culot obtenu était immédiatement congelé à -20°C. Pour l'analyse, 1 mL d'eau MilliQ était ajoutée, puis 1 mL de phénol à 5% et 5 mL d'acide sulfurique à 96% étaient ajoutés (DuBois et al., 1956). Les échantillons étaient ensuite incubés pendant 45 min à température ambiante et les mesures étaient réalisées avec un spectromètre (Ultrospec 1000, Pharmacia Biotech) à 485 nm. Du glucose était utilisé comme standard pour la courbe de calibration. Les EPS étaient exprimés en $\mu\text{gGeq cm}^{-2}$. Une fois divisé par la concentration en Chl *a*, le ratio EPS:Chl *a* était calculé et exprimé en $\text{mgGeq}/\mu\text{gChl } a$.

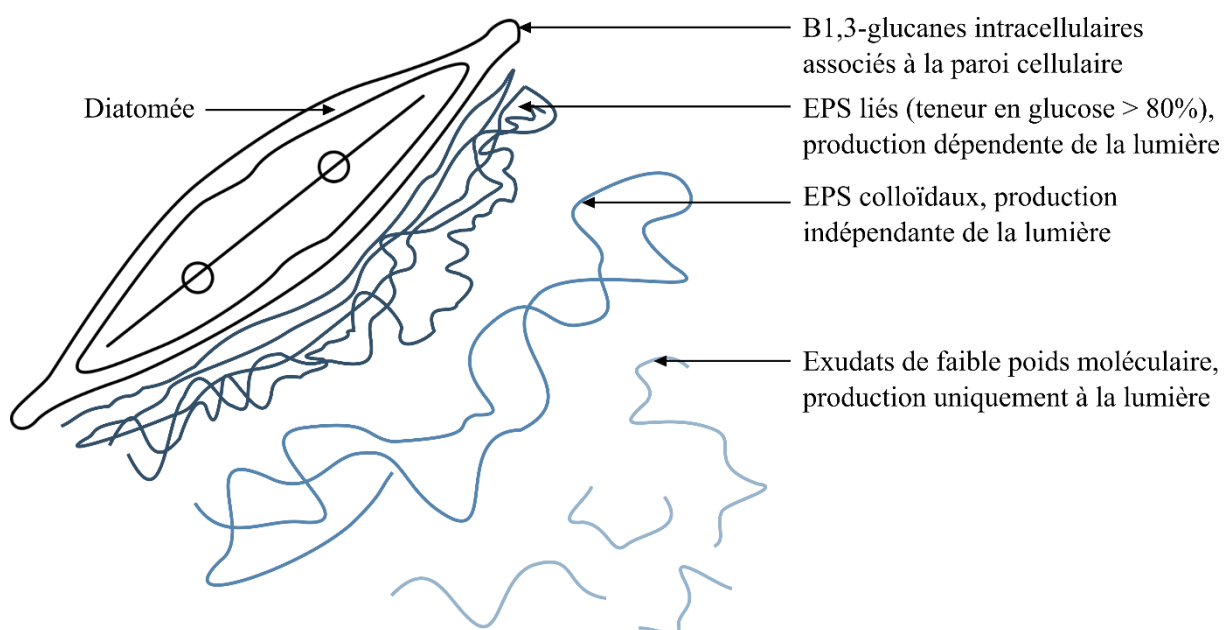


Figure 31 – Différentes fractions d'EPS produits par les diatomées (Modifié depuis Shnyukova and Zolotariova, 2015).

VIII. Biodiversité

VIII.1. *Microscopie électronique à balayage*

Des observations de microscopie électronique à balayage (MEB) ont été réalisées dans le cadre des expérimentations en conditions contrôlées. Ces mesures ont été réalisées avec le CMAbio (Centre de Microscopie Appliquée à la biologie) de l'université de Caen Normandie. Des échantillons de biofilms ont été prélevés soigneusement à l'aide d'une brosse à dent sur la surface des MI (25 cm²). Les échantillons ont été ensuite fixés pendant une nuit avec du glutaraldéhyde 2,5% dans un tampon de cacodylate de sodium (0,2 M) avec 7% de saccharide. La sédimentation des cellules était réalisée sur des bandes Thermanox™. Les échantillons ont été ensuite rincés dans des bains de tampon et déshydratés dans des bains progressifs d'éthanol. Enfin, les échantillons étaient séchés avec un CPD 030 LEICA®. Les échantillons étaient ensuite métallisés au platine avec un JFC 1200 JEOL®. Enfin, les observations étaient réalisées avec un MEB Supra 55 ZEISS®.

VIII.2. *Identification des espèces de macroalgues et calculs de biomasses*

Pour les suivis *in situ* sur les dalles et les récifs artificiels, les espèces de macroalgues dressées étaient identifiées et leur biomasse calculée. Les macroalgues dressées étaient prélevées, identifiées et conservées à -20°C pour les calculs de biomasses. La biomasse était déterminée et exprimée en poids sec libre de cendres (Ash-Free Dry Weight, AFDW en anglais) par m² (perte de poids sec après 5h à 50°C). La biomasse AFDW était ensuite convertie en carbone par un facteur de conversion de 0,35 (Surif & Raven, 1990; Pihl et al., 1996). Les espèces d'algues étaient ensuite assignées à leur classes respectives (Ulvophyceae, Pheophyceae ou Rhodophyceae). Pour le suivi *in situ* sur les dalles, les macroalgues encroûtantes étaient identifiées et des calculs de recouvrement ont été réalisés.

VIII.3. *Identification des espèces de faune benthique et calculs de biomasses*

Pour le suivi sur les récifs artificiels, la macrofaune benthique a été identifiée et les biomasses calculées. Les parpaings prélevés ont été découpés puis conservés dans du formaldéhyde 10% en vue des identifications de macrofaune benthique (surface totale d'un parpaing : 1,8 m²). Un rinçage du parpaing et une filtration ont ensuite été réalisés (sur un tamis de maille de 0,5 mm de Ø). Les organismes présents sur chaque face du parpaing ainsi que dans l'eau de rinçage ont été échantillonnés puis conservés dans de l'éthanol 70%. L'identification se faisait jusqu'au rang taxonomique le plus bas. L'abondance et la biomasse de chaque groupe

ont ensuite été déterminés. La biomasse était exprimée en poids sec libre de cendre (AFDW après 5h à 500°C) puis convertie en contenu carbone grâce à un facteur de conversion de 0,518 utilisé pour les invertébrés benthiques (Brey, 1999). Ces invertébrés ont ensuite été assignés à des groupes trophiques : grazers (Gr), filter feeders (FF), predators (Pr), scavengers – omnivorous (Scv/O), sub-surface deposit feeder (ssDF) et surface deposit feeder (sDF) selon la classification de (Pezy & Dauvin, 2021). L'analyse de la structure de la communauté était basée sur différents indices comme la richesse taxonomique, l'indice de diversité de Shannon-Wiener (H') et l'indice d'équitabilité de Pielou (J').

L'indice de diversité de Shannon-Wiener (H') était calculé comme suit :

$$H' = - \sum \left(\frac{n_i}{N} \right) \times \log_2 \left(\frac{n_i}{N} \right) \quad (14)$$

avec n_i l'abondance du taxon i et N l'abondance totale de l'échantillonnage considéré.

L'indice d'équitabilité de Pielou (J') était alors calculé comme suit :

$$J' = \frac{H'}{H'_{max}} \quad (15)$$

avec H'_{max} la diversité maximale mesurée pour l'échantillonnage considéré.

Pour le suivi *in situ* sur les dalles expérimentales, les espèces de macrofaune fixée ont également été identifiées. Une estimation du pourcentage de recouvrement de la dalle par des algues encroûtantes et l'abondance de faune fixée a été réalisée. Ces indicateurs ont été uniquement mesurés pour les échantillons provenant de la rade de Cherbourg avec le logiciel ImageJ2.

IX. Sels nutritifs et paramètres abiotiques

Des analyses de sels nutritifs ont été réalisées lors de l'expérimentation en conditions contrôlées selon un gradient d'eutrophisation mais aussi sur des échantillons d'eau provenant des sites expérimentaux pour les suivis *in situ*. Un échantillon d'eau était prélevé dans chaque condition et filtré sur filtre d'acétate de cellulose (ClearLine, CA, 33mm, 0.45 μm) dans un falcon 50 mL. Ce dernier était ensuite conservé à -20°C . Les analyses étaient réalisées avec un système Seal Analytical AA-3 (Aminot & Kérouel, 2007). Les limites de quantification étaient de $0,02 \mu\text{mol L}^{-1}$ pour PO_4^{3-} et $0,05 \mu\text{mol L}^{-1}$ pour NO_3^- et Si(OH)_4 . NH_4^+ était analysé par fluorimétrie selon le protocole d'Oriol et al. (2014).

Pour les expérimentations en conditions contrôlées, les paramètres abiotiques et l'intensité lumineuse ont été mesurés durant tout le suivi avec une sonde RBRsolo T et une sonde RBR solo³ PAR connectée à un Li-COR "Underwater Quantum Sensor" LI-192. La salinité, le pH, l'oxygène dissous et la concentration en nutriments inorganiques ont été mesurés à intervalles de temps régulier.

Pour les suivis *in situ*, la bouée instrumentée SMILE située en baie de Seine ($0^{\circ}19'41.00''\text{O}$ $49^{\circ}21'14.00''\text{N}$) permet de réaliser des mesures haute fréquence (données disponibles : <https://doi.org/10.17882/53689>). La conductivité, la température (sonde tetracon, WTWTM), la turbidité (Seapoint turbidity meter, Seapoint Sensor), l'oxygène dissous (AADI Oxygen optode, Anderraa) et la fluorescence (Cyclops-6K, Turner Design) sont mesurés par un système de mesure automatique (MP7, NKE Instrumentation[®]). Le PAR était mesuré avec une sonde Saltantics.

**CHAPITRE III : MARINE ARTIFICIAL REEFS, A META-ANALYSIS OF
THEIR DESIGN, OBJECTIVES AND EFFECTIVENESS**

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Marine artificial reefs, a meta-analysis of their design, objectives and effectiveness



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Résumé de la publication

Cette première étude vise à évaluer le succès des récifs artificiels en fonction de leur objectif initial et également selon les différentes variables associées à leur conception, leur environnement et les moyens mis en place pour leur évaluation scientifique. Ils présentent une grande diversité de formes et de matériau. Ces structures sont aussi immergées avec des objectifs qui peuvent être bien distincts et les techniques de suivi scientifique mises en place pour l'évaluation de leurs réussites sont aussi très diverses (Baine, 2001; Salaün et al., 2020). Dans ce contexte, il apparaissait nécessaire d'évaluer l'efficacité de ces structures en fonction de différentes variables caractérisant leur design, le suivi mis en place et tenant compte de l'objectif initial du récif. Notre synthèse a donc porté sur un corpus de 127 articles scientifiques au sein duquel 67 variables ont été identifiées afin de caractériser ces études.

Premièrement, le corpus de publications scientifiques sélectionné pour cette étude n'est pas exhaustif et ne reflète donc pas la répartition mondiale des RA, ni même l'effort scientifique complet déployé pour leur suivi mais il regroupe des études permettant de caractériser un nombre suffisant de critères à analyser. Le corpus est composé de 69 RA en Europe, 37 en Asie et 26 en Amérique du Nord. Les récifs identifiés pour l'étude sont composés de 11 matériaux différents avec une dominance de béton (60% des AR) et de 14 formes. La part importante de structures en béton s'explique par le taux de réussite élevé de ce type de récif et par leur longévité (Baine, 2001; Ido & Shimrit, 2015; Sempere-Valverde et al., 2018). Ces premiers résultats justifient le choix de ce matériau pour la réalisation des infrastructures marines du projet MARINEFF. De plus, le développement de béton biosourcé comme celui développé pour ce projet, permet de réduire considérablement l'empreinte environnementale en réduisant les besoins de sables. Ce type de matériau peut apporter un bénéfice en terme de colonisation biologique (Perkol-Finkel et al., 2018a; Pioch et al., 2018; Lima et al., 2019b; Hayek et al., 2020). Le design d'un RA va impacter de manière conséquente les processus de colonisation ainsi que leur vitesse. Notre étude a montré que le volume influe positivement sur le taux de réussite. Plus le récif est volumineux, plus il sera capable de supporter une production importante (Bortone et al., 2011). Le niveau de complexité de la structure, en particulier l'hétérogénéité de la surface du récif, va également influencer sur son efficacité. De manière générale, plus la structure sera complexe, plus elle permettra à une diversité d'espèces importante de s'y installer et plus elle sera efficace (Paalvast, 2015; Loke & Todd, 2016; Pioch et al., 2018). De nombreux autres facteurs vont aussi influencer sur les processus de colonisation, la biomasse et la diversité d'organismes vivants associés aux RA. Les facteurs

environnementaux telles que les courants, la disponibilité en nutriments dans le milieu, la quantité de lumière ou encore la température constituent des variables importantes qui vont également conditionner la dynamique de colonisation du récif (Baine, 2001; Boaventura et al., 2006; Loke & Todd, 2016; Komyakova & Swearer, 2018).

Les RA peuvent être immergés pour différentes raisons pouvant être associées à quatre catégories : l'augmentation des prises de pêches, la protection physique d'un habitat, la restauration écologique d'un milieu ou l'expérimentation scientifique. Notre étude a mis en évidence le taux d'efficacité élevé des récifs ayant un objectif de promotion halieutique. Nous avons aussi mis en avant que ces récifs sont composés d'une faible variété de formes et de matériaux. Ces récifs à vocation « halieutique » sont également corrélés avec la plus faible quantité de protocoles scientifiques pour leur suivi. Bien que l'objectif de production halieutique de ces structures soit atteint, ils peuvent également avoir des impacts néfastes sur l'écosystème. Une attraction locale de poissons sans bénéfice positif sur la production locale peut entraîner une surpêche (Jensen et al., 2000; Whitmarsh et al., 2008). En comparaison, des récifs ayant un but expérimental vont être associés à une grande variété de formes, de matériaux et de techniques de suivi. Ils constituent également les récifs avec la plus faible efficacité ce qui semble logique par leur nature expérimentale. Enfin, une variable qui influe fortement sur l'efficacité mesurée d'un récif est le temps d'immersion de ce dernier. Plus le temps d'immersion de la structure sera important, plus l'écosystème associé se rapprochera d'un état stable, d'un climax. Cette étude a mis en avant le fait que les récifs avec un but halieutique sont ceux qui restent le plus longtemps en mer.

Cette étude a également considéré les protocoles scientifiques déployés pour évaluer les récifs artificiels considérés. La durée du suivi scientifique a également été prise en compte. La complémentarité et le degré de précision des protocoles scientifiques utilisés dans ces études va énormément influencer sur le niveau de réussite qui sera associé au récif artificiel. Nous pouvons distinguer deux grandes catégories de suivi écologique : les suivis d'écologie structurelle et les suivis d'écologie fonctionnelle. Une part importante des études scientifiques répertoriées utilisent exclusivement des suivis d'évaluation structurelle. Certaines études n'ont pas de réel protocole scientifique permettant une évaluation correcte de l'impact des récifs sur l'environnement ou n'utilisent que quelques techniques (Gregg, 1995; Kasim et al., 2013). Ces problématiques ont par ailleurs déjà été soulevées (Koeck et al., 2011). En ce qui concerne les récifs avec un objectif de promotion de la biomasse halieutique, la diversité et la complémentarité des techniques déployées est insuffisante pour obtenir une vision détaillée de

leur impact (Cresson et al., 2014). Les techniques de suivi dites de processus (réseau trophique, flux de carbone, cycles de l'azote, productivité, photosynthèse...) sont essentielles si l'on veut comprendre les mécanismes et les interactions complexes entre les organismes vivants et leur environnement. Enfin, l'intégration des facteurs socio-économiques aux variables environnementales et écologiques sont également à prendre en compte afin d'optimiser la gestion des RA (Lima et al., 2019a; Salaün et al., 2020).

Le manque de protocoles de suivi efficaces ou leur utilisation inadaptée met en avant l'importance d'une réflexion pour sélectionner des variables complémentaires afin d'évaluer correctement la performance du récif artificiel. Il est évident que l'utilisation de protocoles de suivi permettant l'évaluation de l'objectif premier de la structure est indispensable, cependant, un plan d'évaluation complet doit prendre en compte une évaluation écologique avant et après l'immersion de la structure sur une longue durée et avec une fréquence d'échantillonnage maximale (appropriée aux compartiments étudiés) (Seaman & Sprague, 1991; Seaman, 2000).

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Abstract

Artificial Reefs (AR) show a wide diversity and vary in their construction materials, shape and purpose, as illustrated by the present analysis of 127 scientific papers. AR have been deployed for different purposes, including fisheries improvement, ecological restoration of marine habitats, coastal protection or purely scientific research. Statistical analyses using 67 variables allow us to characterize the design, objectives and monitoring strategies used for AR. An effectiveness indicator comprised of three categories (low, moderate and high) was adapted from previous studies and applied to the present dataset in terms of the objectives defined in each scientific paper. The effectiveness of various monitoring approaches was investigated and recommendations are formulated regarding environmental parameters and the assessment of ecological processes as a function of AR type. These analyses showed that inert materials like concrete associated with biomimetic designs increase the benefits of reefs to the local environment. This study also compared effectiveness between the different economic, ecological or scientific objectives of AR projects and reveals that fisheries projects showed the highest efficiencies but points out the weakness of environmental assessments for this type of project. In conclusion, the analyses presented here highlight the need to use a panel of complementary monitoring techniques, independently of the initial purpose of the artificial structures, to properly assess the impact of such structures on the local environment. It is recommended to adopt approaches that associate structural and functional ecology. An improved characterisation of the role of AR should be integrated into future assessments, taking into account the complex framework of ecosystem structure and trophic relationships.

Keywords

Artificial reefs; Designs; Objectives; Effectiveness; Monitoring; Review

Abbreviations

AHC: Ascending Hierarchical Classification

AR: Artificial Reefs

ARMS: Autonomous Reef Monitoring Structures[®]

Eco: Ecological

MA: Material

Obj: Objectives

MCA: Multiple Correspondence Analysis

TC: Techniques

I. Introduction

Artificial reefs (AR) are man-made structures emplaced in aquatic environments that serve as habitats or shelters for organisms. AR have long been used to attract fish and the development of these structures has been intensified over the three last decades (Baine, 2001; Santos et al., 2011; Lowry et al., 2014). In the present study, we consider an artificial reef as a structure intentionally placed on the seabed aiming to mimicking natural reef functions that are able to protect, regenerate, concentrate and enhance populations of marine organisms (Guillen et al., 1994; Hunter & Sayer, 2009; Walles et al., 2016). This includes the protection (Silva et al., 2016) and regeneration of habitats (FAO et al., 2015) or the fisheries enhancement (Hackradt et al., 2011). However, AR with a defined objective such as ecosystem protection may have both positive and negative impacts on the local environment (Brickhill et al., 2005; Firth et al., 2016). This term excludes artificial islands, cables, pipelines, platforms, mooring and structures for coastal defence (e.g. breakwaters and dykes) which were primarily constructed for other purposes, as well as devices developed for fish aggregation that were used simply to attract fish in certain fishing areas, and wrecks that are accidentally present on the sea bed (FAO et al., 2015).

Despite the number and the diversity of projects and objectives of AR, their overall effectiveness have rarely been fully demonstrated. The assessment of AR performance is complex because of the multivariable factors and co-variation among descriptors to be taken into consideration. There is a tendency for research to obscure the main objectives of AR placement and many examples can be cited where self-assessment appears to be lacking in terms of evaluating performance (Baine, 2001). Monitoring is often limited to only a few species and/or the immediate environment with no complementary techniques using approaches such as process or structural ecology (Baine, 2001). Moreover, monitoring periods are highly variable, ranging from days to years. Research on AR involving studies of ecosystem functioning need to respond to the increased development of more sophisticated and complex techniques based on isotopes, organic indicators or molecular biology (Lima et al., 2019a). Ecological studies can be divided into two categories: structural ecology describes the ecosystem (species diversity, distribution, etc.) and functional ecology refers to the flow of energy and cycling of materials through structural components of the ecosystem (productivity, production, trophic web, carbon fixation, etc.) (Gómez et al., 2004; Villéger et al., 2008). There is also a great interest in investigating the coupling between function and diversity in the case of submerged AR.

These structures are usually expected to produce an overall increase in species richness by protecting some species, and also an increase and diversification of trophic contributions (Hixon & Beets, 1989; Piazza et al., 2005; Bodilis et al., 2011). The improvement in habitat is generally reflected in greater food availability and more shelter against predators, as well as new recruitment areas for juveniles of various species (benthic invertebrates or fish), which explains the increase in organism biomass associated with these structures after their installation (Sherman, 2002; Perkol-Finkel et al., 2018b). The numerous reviews in the literature dealing with AR mainly concern management (Claudet & Pelletier, 2004; Becker et al., 2018; Lima et al., 2019a), recommendations and priorities (Bohnsack & Sutherland, 1985; Lima et al., 2019a), but also deal with social aspects (Carral et al., 2018; Lima et al., 2019a) as well as overall assessment with performance evaluation (Baine, 2001; Lee et al., 2018; Lima et al., 2019a).

In the present paper, a statistical approach is adopted and combine the results of multiple scientific studies in order to improve the AR performance evaluation. This meta-analysis investigated the effectiveness of 162 AR by using several parameters including the shape and composition of materials used for their construction, the monitoring techniques, and the original purpose of the artificial reef in question. We define effectiveness as the performance of the structure in relation to the purpose of its emplacement or research objectives. Our analysis is based on a simplify Reef Performance Scale proposed by Baine (2001). This approach does not yield an absolute qualitative assessment but is based rather on the information and data provided by selected papers and reflects the interpretations of various different authors. The main objectives of this study were to bring impartial information on AR effectiveness according to their design, monitoring and purposes. Considering the large variety of AR studies, the lack of recent literature review and the necessity of an updated meta-analysis, the present study provide recommendations to improve the AR success rate by enhancing their performance assessment using appropriate monitoring techniques consistent with the initial purpose of the structure.

II. Methodology

This non-exhaustive analysis examines 126 papers dated from 1973 to 2019 covering 162 AR sites (Appendix A). When several AR sites are discussed in a given paper, each site is considered independently in our analysis. The bibliographic corpus was selected from 5,771 publications listed in Web of Science from January to September 2019 using the following keywords: artificial reef and marine infrastructure. Relevant papers were selected based on

three criteria: (1) scientific monitoring of the AR had to be included; (2) the AR had to have been intentionally deployed and (3) the structure had to be submerged in a marine or estuarine mesohaline environment, with a specified objective.

Each of the 126 papers was thoroughly analysed to identify the specific AR design, local environment, location and monitoring techniques employed for the assessment as well as the initial objectives of the AR. This led to the establishment of 67 variables belonging to 12 categories, which were then separated into four groups: (1) environmental categories, (2) AR type, (3) monitoring categories and (4) AR effectiveness. Each of the 12 categories are independent and groups 1, 2 and 3 are strictly representative of the bibliographic corpus information. Some of these variables correspond to several sub-variables that can be grouped together into categories (Appendix B). Tables 1 to 3 present the 12 categories along with the 67 associated variables. Environmental categories correspond to the variables: geographic location, seabed type, salinity and AR depth of immersion (Table 2). AR categories correspond to AR facility volume, AR shape, AR material and AR purpose (Table 3). The AR purpose category is divided into four variables. Fisheries enhancement corresponds to the sole purpose of improving fishing. Biocenosis protection corresponds to the immersion of an AR to physically protect an existing ecosystem against a threat. Biocenosis restoration corresponds to the immersion of an AR to rebuild an ecosystem by creating a new habitat. Finally, experimental purpose corresponds to the immersion of the AR as part of a scientific experiment and biocenosis restoration means reconstructing an ecosystem, *i.e.*, a habitat, to promote its colonization by the living organisms associated with the original habitat. In the case of an AR fitted more than the variable (e. g. concrete and metal), it was considered in all of the relevant variables.

Table 2 – Environmental variables (four categories) with their code used in the statistical analyses.

Environmental categories							
Geographic location		Seabed substrate		Salinity		AR immersing depth	
Tropical	Climate_Tropical	Sandy	Seabed_Sandy	> 30	Salinity_Inf30	Intertidal	Depth_Inter
Temperate	Climate_Temperate	Hard	Seabed_Hard	< 30	Salinity_Sup30	0.1 to 10 m	Depth_10
Subarctic	Climate_Subartic	Muddy	Seabed_Muddy			11 to 20 m	Depth_20
		Corals	Seabed_Corals			21 to 50 m	Depth_50
		Seagrass	Seabed_Seagrass			> to 51 m	Depth_51
		Artificial	Seabed_Artificial				

Chapitre III : Marine artificial reefs, a meta-analysis of their design, objectives and effectiveness

Table 3 – Artificial Reef variables (four categories) with their code used in the statistical analyses.

Artificial Reef variables							
AR facility volume		AR shape		AR material		AR purpose	
0 to 100 m ³	Volume_100	ARMS	AR_ARMS	ECONcrete®	MA_ECO	Fisheries enhancement	Obj_Fisheries
101 to 1000 m ³	Volume_1000	Bags	AR_bags	Shells	MA_Shells	Biocenosis protection	Obj_BiocenosisP
> to 1001 m ³	Volume_1001	Blocs	AR_blocs	Wood	MA_Wood	Biocenosis restoration	Obj_BiocenosisR
		Cages	AR_Cages	Plastics	MA_Plastics	Experimental	Obj_Experimental
		Cubics	AR_Cubics	Rocks	MA_Rocks		
		Pyramidal	AR_Pyram	Tires	MA_Tires		
		Vertical	AR_Vert	Asbestos	MA_abest		
		Multipods	AR_Multi	Ceramic	MA_Ceramic		
		Tires	AR_tires	Concrete	MA_Concrete		
		ReefBalls®	AR_ReefB	Metal	MA_Metal		
		Vehicle	AR_Vehi	Fiberglass	MA_Fiberglass		
		Plates	AR_Plates				
		Cylindrical	AR_Cyl				
		Other	AR_Other				

The third group (Table 4) corresponds to AR monitoring variables including monitoring techniques, ecological monitoring and immersion time. The ecological monitoring category provides information about the ecological nature of the monitoring techniques used. Ecological monitoring may be structural or functional. It also included biodiversity measurements and ecological process. Structural ecology mostly involves descriptive techniques; functional ecology refers to relations, dealing with the fluxes between different trophic compartments. Ecological process corresponds to production measurements, including trophic and non-trophic interactions.

Table 4 – Monitoring variables (three categories) and artificial reef effectiveness variables, with codes used in the statistical analyses.

Monitoring variables						AR effectiveness variables	
Monitoring techniques		Ecological monitoring		Immerging time length		AR effectiveness	
Scrapings	TC_Scrap	Functional	Eco_Functional	Short (< 1 months)	Time_Initial	Low	Eff_Weak
Spectrophotometry	TC_Spectro	Strucutral	Eco_Structural	Intermediate (< 1 year)	Time_Intermediate	Moderate	Eff_Partial
CPCe software	TC_CPCE	Biodiversity measurements	Eco_Biodiv	Long (> 1 year)	Time_Stable	High	Eff_Strong
Microscope observations	TC_Micro	Ecological process	Eco_Process				
Visual observations	TC_Visual						
SCUBA diving	TC_SCUBA						
Environmental parameters	TC_Env						
Pelagic measurements	TC_Fish						
Pictures	TC_Pic						

Submersion time corresponds to the duration of immersion of the AR according to the monitoring period. “Short Time” corresponds to periods between a day and a month, “Intermediate Time” to periods of between a month and a year and “Long Time” to periods longer than a year.

AR effectiveness is evaluated according to the interpretations of each paper with respect to the stated objectives. This evaluation is based on the Reef Performance Scale drawn up by Baine (2001). The Reef Performance Scale is an indicator of reef performance in relation to its given purpose. Our intention is not to provide an absolute assessment of AR effectiveness but rather to reflect the author’s interpretation. Baine’s scale is composed of seven levels from -3 to +3, with negative levels corresponding to AR which fail in their objectives and which have a negative impact on the surrounding environment (-3), which yield no useful data (-2) or which produce questionable results (-1). Level 0 corresponds to AR with inconclusive performance showing negative and positive effects. Finally, positive levels correspond to AR which succeed in their objectives. Level +1 is attributed to AR with a limited success and which provide limited useful data; these AR require some changes and management to improve their effectiveness. Level +2 corresponds to AR that succeed partly in meeting their objectives and which require some minor changes of design or management. Level +3 corresponds to ARs that fully succeed in their objectives and which require no change. These AR provide useful data for the assessment of reef performance and management. Out of the 30 case studies exploited by Baine (2001), 29 yield an effectiveness distributed between 0 and +3.

Our AR effectiveness evaluation is composed of three levels: low, moderate and high (Table 4). Low effectiveness corresponds to AR with extremely limited success or none. This level corresponds to level 0 on the Reef Performance Scale (Baine, 2001). These AR might have some negative impacts and require some management and design changes. Moderate effectiveness corresponds to AR with a limited success in their objectives, but which might have some other positive effects. This level corresponds to +1 on the Reef Performance Scale (Baine, 2001). These AR require some management and changes to increase their success. High effectiveness corresponds to AR that have succeeded in their objectives for a major part. This level corresponds to +2 and +3 on the Reef Performance Scale (Baine, 2001). These AR confer benefits on the surrounding environment and require little or no modifications to improve their effectiveness.

Statistical analyses were performed with R i386 3.5.1 (R Development Core Team, 2008), with FactoMineR (Lê et al., 2008) and ggplot2 (Wickham, 2009). The data frame is made up of a binary presence or absence (1 or 0) matrix with observations in rows and the sum of variables in columns. The matrix is presented in supplementary material (<https://doi.org/10.1016/j.gecco.2021.e01538>). Ascending hierarchical classification (AHC) was performed on the total data base. The optimal number of clusters was calculated using the Gap statistical method. AHC provides information on the similarities and dissimilarities of selected papers. This technique is widely used in ecological sciences and data analysis (Dolan & Parker, 2005; Azzag et al., 2006; Cullis et al., 2018). Associated dendrograms are available in the supplementary material (appendix C). Correlation coefficients are calculated using Pearson's method to identify those variables which are correlated together. A correlation is considered significant when $P < 0.05$. These correlation coefficients are calculated between all variables from the total data set. Data analysis with coefficient correlations is commonly applied in the biological and ecological sciences (Obayashi & Kinoshita, 2009; Rupp et al., 2012). A graphic representation and the full set of results (R coefficients and p-values) is available in the supplementary material.

III. Results

The selected AR show a worldwide distribution: 69 in Europe, 37 in Asia, 26 in North America, 10 in Central America, 5 in South America, 3 in Africa, 11 in Australia and 2 in the Pacific Ocean. However, this selection is not representative of the actual distribution of AR across the world.

III.1. *Graphic representation of global data and dominant variables*

The Figure 32 provides information on the main variables. The main shapes are cubic, cylindrical, plate, pyramidal and cage. The main construction material used is concrete. The main purposes of AR submersion are biocenosis restoration, fisheries enhancement and purely experimental (Figure 32A). The main geographic locations are temperate and tropical. The selected depths for artificial structures are between 0.1 and 50 m. The main seabed substrate is sandy followed by muddy and hard bottoms (Figure 32B). The main tools for monitoring are biodiversity assessment, SCUBA diving and visual observations with ecological structural monitoring (Figure 32C).

Chapitre III : Marine artificial reefs, a meta-analysis of their design, objectives and effectiveness

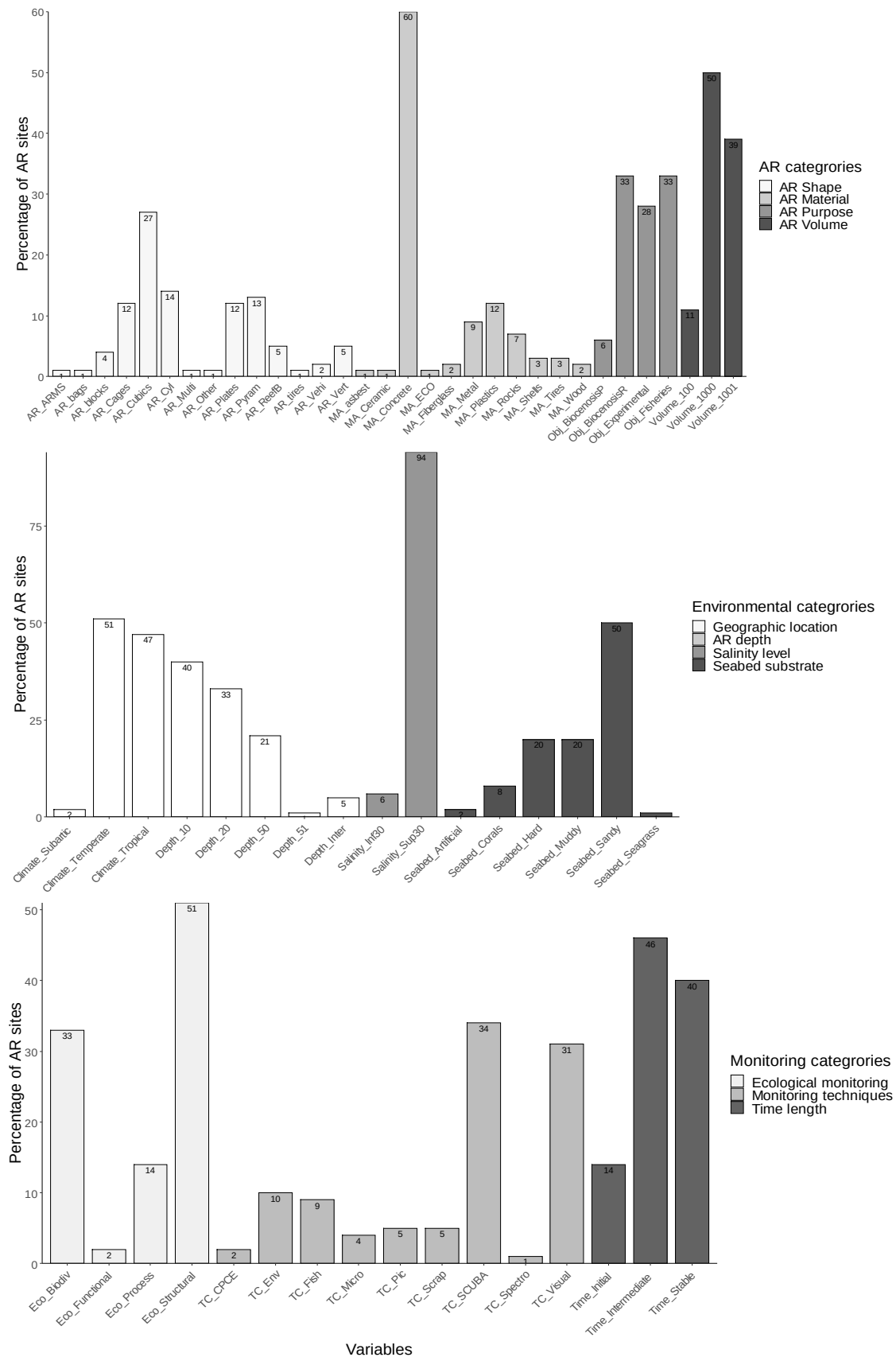


Figure 32 – Graphic representation of the percentage of artificial reef sites in terms of three categories of variables: AR type and purpose (1), environmental variables (2) and monitoring methods (3).

III.2. *Multiple correspondence analysis (MCA) representations with clusters*

MCA (Figure 33) was performed to identify those variables that could be used to distinguish the selected AR. The MCA represents all relevant variables that contribute more than 1% to the two first dimensions. Clusters groups (A to E) are represented as follows: group A is composed of 22 AR sites, B of 33, C of 11, D of 36 and E of 60. The two first dimensions explain 21.8% of the scatter plot. Dimensions 3 and 4 explain 7.7% and 7.3% of the data. Eigen values are available in supplementary data. The weak cumulative variance of the two first MCA axes associated with the number of clusters reflects the disparity of the data due to the diversity of AR projects worldwide with different purposes, in different ecosystems, using various shapes, materials and monitoring methods.

Clusters A and B seemed close and mostly comprised of elements such as experimental purpose; structural and functional ecology analysis; plastics, rocks, and EConcrete[®] materials; depth of submersion between 0 and 20 meters; short and medium immersion periods. The group C dendrogram is composed of 11 AR studied by Kasim et al. (2013) in the tropical Indian Ocean. Cluster C describes the same variables as cluster D, situated between the dominant variable strong AR effectiveness and fisheries enhancement. Finally, cluster E is characterized by fisheries enhancement and biocenosis protection purposes, long immersion period, AR facility volume volumes higher than 1,000 m³, cages shape, concrete construction material, visual census, SCUBA diving and pelagic measurement monitoring techniques.

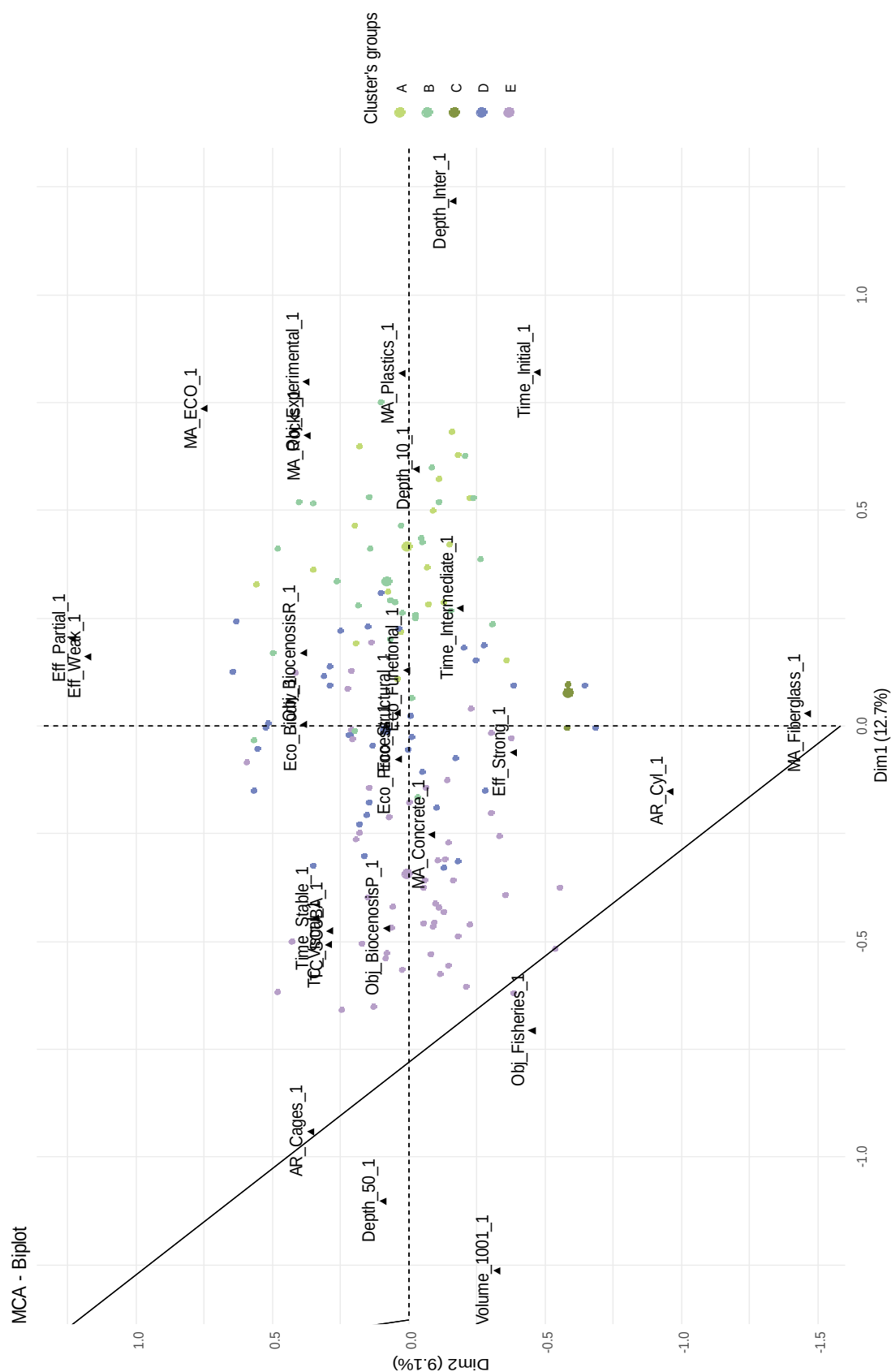


Figure 33 – Multiple correspondence analysis (MCA) of all the data with all the measured variables that contribute more than 1% to the two first dimensions. Clusters are represented (A to E) in different colours.

III.3. *Effectiveness analysis of relevant variables*

The proportion of AR sites in term of effectiveness categories (weak, partial and strong) was calculated for the relevant variables selected for the previous MCA analysis (Figure 34). Cages and cylindrical shapes were the most effective ones (70% in strong effectiveness). Depth of submersion between 0 and 10 (75% in strong effectiveness) or intertidal (64.7% in strong effectiveness) were the most efficient ones. Concrete was the most effective material (79% in strong effectiveness), in comparison, plastics and rocks material seemed less efficient with respectively 35.7% in medium effectiveness and 7.7% in weak effectiveness. Long immersion period was the most effective (72.7% in strong effectiveness) in comparison with short one (60% in strong effectiveness).

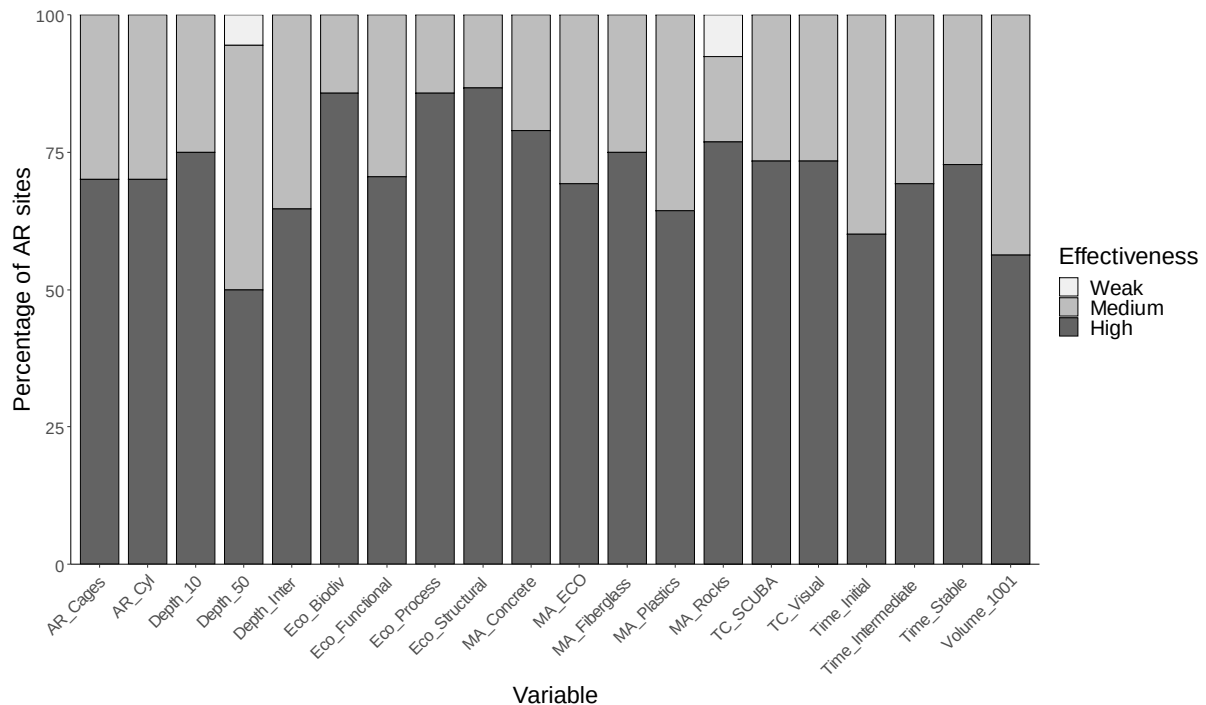


Figure 34 – Graphic representation of the percentage of AR sites in term of three effectiveness categories (weak, partial and strong) for each relevant variable (n = 20) selected for the MCA representation.

III.4. *Evaluation of artificial reef purposes*

The Figure 35 shows the percentage of assessed effectiveness for each AR purpose. For each of the four different AR purpose, the main assessed effectiveness was high (always more than 73% of the AR sites). Moderate effectiveness represented between 14.9% for fisheries enhancement AR and 23.4% for experimental AR. Low effectiveness represented between 1.4% for fisheries enhancement AR and 7.7% for biocenosis protection AR.

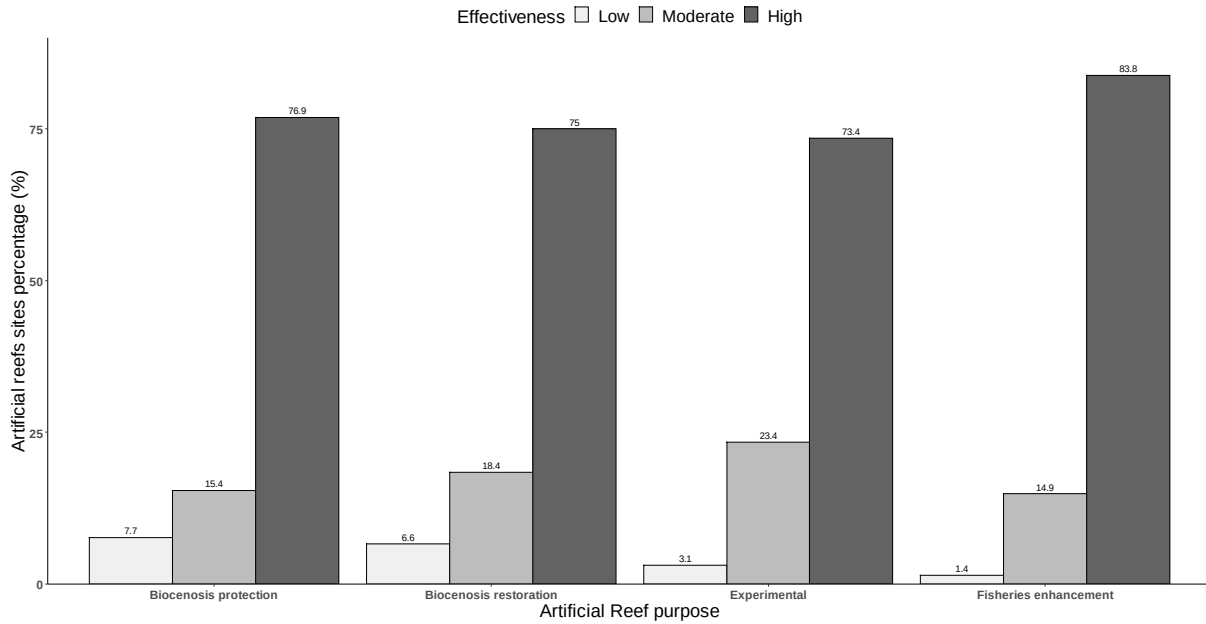


Figure 35 – Graphic representation of assessed effectiveness for each AR purpose.

III.5. Variable correlations according to the purpose

III.5.1. Fisheries enhancement purpose

The objective of improving fisheries is correlated with a high level of efficiency ($R = 0.25$, $P = 0.038$) as well as visual census ($R = 0.36$, $P = 0.002$), SCUBA diving ($R = 0.34$, $P = 0.005$) and pelagic measurements ($R = 0.42$, $P < 0.001$). This objective is also correlated with depths of submersion between 10 to 20 m ($R = 0.33$, $P = 0.006$) and 21 to 50 m ($R = 0.59$, $P < 0.001$), sandy substratum seabed ($R = 0.32$, $P = 0.007$), salinity > 30 ($R = 0.26$, $P = 0.033$) and a temperate climate ($R = 0.29$, $P = 0.018$). Fisheries enhancement purpose is also correlated with several AR variables, such as cages ($R = 0.49$, $P < 0.001$), cylindrical shapes ($R = 0.46$, $P < 0.001$), pyramid shapes ($R = 0.57$, $P < 0.001$), cubic shapes ($R = 0.74$, $P < 0.001$) but also the concrete construction material ($R = 0.58$, $P < 0.001$).

III.5.2. Experimental purpose

The experimental purpose is correlated with microscopic analysis ($R = 0.57$, $P < 0.001$), CPCE software ($R = 0.49$, $P < 0.001$) and biodiversity analysis ($R = 0.44$, $P < 0.001$). This objective is also correlated with a structural ecology approach ($R = 0.27$, $P = 0.024$), submersion depths from 0 to 10 m in the intertidal zone ($R = 0.51$, $P < 0.001$ and $R = 0.41$, $P < 0.001$), artificial seabed ($R = 0.35$, $P = 0.004$) and short or medium immersion periods ($R = 0.49$, $P < 0.001$ and $R = 0.32$, $P = 0.008$). Experimental purpose is correlated with the following AR construction materials: plastic ($R = 0.41$, $P < 0.001$), ceramic ($R = 0.28$, $P < 0.022$), shells ($R = 0.27$, $P = 0.031$) and rocks ($R = 0.44$, $P < 0.001$); and with shapes made of bags ($R = 0.24$,

$P = 0.048$), plates ($R = 0.76$, $P < 0.001$), and ARMS® ($R = 0.43$, $P < 0.001$). It is also correlated with the biocenosis restoration purpose ($R = 0.25$, $P = 0.029$).

III.5.3. *Biocenosis protection purpose*

The biocenosis protection purpose is correlated with a long immersion period ($R = 0.34$, $P = 0.005$), sandy and seagrass seabed substrates ($R = 0.33$, $P = 0.05$ and $R = 0.44$, $P < 0.001$) and submersion depths between 11 and 20 m ($R = 0.25$, $P = 0.041$). This purpose is correlated with AR volume $> 1,001 \text{ m}^3$ ($R = 0.29$, $P = 0.020$) and with EConcrete® construction material ($R = 0.29$, $P = 0.023$). Finally, biocenosis protection is correlated with visual observations ($R = 0.35$, $P = 0.004$) and SCUBA diving ($R = 0.29$, $P = 0.017$).

III.5.4. *Biocenosis restoration purpose*

The biocenosis restoration purpose is correlated with lower efficiency ($R = 0.23$, $P = 0.06$) and salinity < 30 ($R = 0.28$, $P = 0.027$). It is correlated with biodiversity measurements ($R = 0.24$, $P = 0.041$). The biocenosis restoration purpose is correlated with shapes made up of multipods ($R = 0.25$, $P = 0.041$) and ReefBalls® ($R = 0.44$, $P < 0.001$) and with tyres ($R = 0.35$, $P = 0.040$). Finally, the biocenosis restoration purpose is correlated with the experimental purpose ($R = 0.27$; $P = 0.029$).

IV. Discussion

There is a great diversity of AR worldwide with a large range of different shapes, materials and purposes. In this study, we selected 162 AR from 126 scientific publications. A statistical approach on 67 common variables allowed us to describe the selected AR structures in terms of their design, location, objectives and the monitoring techniques used to assess their effectiveness.

IV.1. *Design, location and effectiveness of artificial reefs*

Although AR are widely used around the world, their scientific monitoring is mainly performed in Europe (69 AR), Asia (37 AR) and North America (26 AR). However, the data set used in this paper does not represent the actual distribution of AR in the world because of the non-exhaustivity of the analysis and the unequal nature of scientific monitoring of AR projects between regions. These structures are composed of different materials ($n = 11$), predominantly concrete (60%). There is also a wide diversity of shapes ($n = 14$), dominated by cubic structures (27%). Concrete AR are widely distributed across all continents. Our analysis shows a significant correlation between concrete material and high AR effectiveness due to the

high fixation rate of marine organisms and the high level of colonization (Baine, 2001; Ido & Shimrit, 2015; Sempere-Valverde et al., 2018; Mos et al., 2019). Beside these performances, concrete AR were selected because they have a lower environmental impact than plastics such as PVC which are toxic and generate micro-plastic particles (Zhang et al., 2020). In this context, the development of biogenic materials such as EConcrete[®], with addition of marine products including oyster shells to replace part of the sand, represent a sustainable solution which strengthen the coherence of AR projects by limiting the environmental footprint (Walles et al., 2016; Perkol-Finkel et al., 2018b; Lima et al., 2019a). Moreover, the design of AR has a significant impact on their effectiveness. According to our analysis, the most efficient shape is cylindrical. However, AR shape has a limited impact on efficiency in comparison to AR size. Our analysis points out that AR facility volume volumes higher than 1,000 m³ are significantly correlated with a high effectiveness. Furthermore, our meta-analysis shows that surface heterogeneity is an important condition affecting the AR efficiency. An increase of surface complexity promotes biodiversity and facilitates colonization (Boaventura et al., 2006; Hageman et al., 2013; Paalvast, 2015; Loke & Todd, 2016). Orientation as a function of currents and/or light are also key factors influencing colonisation by ecosystem engineer species, as well as larval recruitment and benthic biodiversity (Boaventura et al., 2006; Loke & Todd, 2016).

Geographic location does not have a significant influence on AR efficiency in the papers selected for this study. However, AR shape and material are found to differ as a function of latitudes. Tropical climates are correlated with two different AR shapes (vertical and tyres) and four construction materials (ceramic, concrete, plastics and tyres). Temperate climate locations are only correlated with concrete material and two shapes (cubic and cages). This difference could be explained by a discrepancy of objectives of AR projects between low and higher latitudes. Our analysis shows that tropical climate is correlated with experimental purpose, which might lead to the large diversity of AR shapes and materials, while temperate climate is correlated with fisheries enhancement and to a lesser extent to biocenosis protection. It is interesting to note that most artificial structures in temperate climates mainly have an economic objective.

IV.2. *Assessment of artificial reef purpose*

Because the reef effect induces fish aggregation and increases the capture rate (Hackradt et al., 2011; Koeck et al., 2011; Becker et al., 2019), AR projects are found to be highly efficient in promoting fisheries in our analysis. Clear objectives contribute to a rational and scientific approach to AR evaluation. The fisheries enhancement purpose represents 33% of the purpose

variable category. This variable is highly correlated with cluster E, which is the largest group (60 AR) and with cluster C. Group C is different because all the AR originate from the same study (Kasim et al., 2013). These latter authors submerged AR at 11 sites along the Indian coastline with a fisheries enhancement objective. They demonstrated the notable economic benefits of these structures for local fishermen compared with adjacent natural reefs. Many studies have reported that a large number of fisheries actors and the rising interest of the fishing industry in AR projects are relevant factors playing an essential role in the success and economic value of artificial reefs. The fisheries enhancement variable is correlated with a limited number of shapes, construction materials and monitoring techniques, highlighting the fact that AR projects targeting fisheries enhancement reach their objective of attracting fish. However, as reported in the literature, the apparent success of such structures might mask the negative effects on the ecosystem. Attraction local fish biomass without any increase of productivity in the area could lead to overfishing and/or an increase of accidental captures (Jensen et al., 2000; Whitmarsh et al., 2008; Smith et al., 2016).

The experimental purpose is correlated with four different monitoring techniques, five AR shapes and two AR construction materials. In contrast to the fisheries enhancement purpose, the effectiveness of experimental AR seems to be moderate in our analysis even if the correlation between each variable is not highly significant. The difference in effectiveness between these two purposes could be explained by the greater heterogeneity of monitoring techniques, shapes and materials used for experimental AR. For example, Paalvast (2015) used concrete slabs to assess the settlement of algae and macrofauna on a breakwater (Netherlands), while Brown (2005) used four different substrata (wood, rubber, steel and PVC) to record their influence on epifaunal assemblages in Loch Linnhe (Scotland, UK) and Fariñas-Franco et al. (2013) used scallop shells to assess the importance of habitat complexity in recruitment in Strangford Lough (Northern Ireland, UK). Most experimental AR are built to investigate the performance of different materials or shapes, thereby explaining the higher proportion of these variables compared to other purposes, and also the lower effectiveness of experimental reefs compared to other types of AR. We show that biocenosis restoration purpose is correlated with the experimental purpose where many types of shapes and construction materials are used (concrete, plastic, fibreglass, tyres, stone) (Clark and Edwards 1999; Walker et al. 2002; Kotb 2013). As previously mentioned, this diversity of AR types leads to a low effectiveness.

Submersion time and depth are key factors for the success of AR that influence the efficiency according to the purpose of deployment. Submersion time is an important variable

that affects the effectiveness of AR. This variable is correlated with biocenosis restoration and fisheries enhancement purposes. On the contrary, short submersion time is correlated with experimental AR purpose, often because of a strict legislation (London Convention and Protocol, 2009). This highlights the fact that economic AR, such as those targeting fisheries enhancement, are submerged for very long or even unlimited periods (Charbonnel & Bachet, 2010). Short term survey may not be long enough to record the overall influence of an AR on its environment, the survey need to be conducted over several years (Seaman & Sprague, 1991; Seaman, 2000). Another key factor is the submersion depth, which plays an important role in maximizing the efficiency of the reef. In our analysis, submersion depths in the photic zone between 11 and 20 m lead to the highest recorded effectiveness; this result might be explained by the fact that these depths correspond to the depths of AR targeting fisheries enhancement.

IV.3. *Assessment of monitoring techniques*

The assessment of monitoring techniques considers the scientific protocols deployed and the survey duration. For example, for fisheries assessment, long-term monitoring is required to prevent any undesirable consequences such as overfishing (Ajemian et al., 2015; Smith et al., 2016). Otherwise, this approach involves protocols with trusted communication between the local fishermen and the AR project managers to maximize the efficiency and longevity of the monitoring and the structures (Keller et al., 2017; Lima et al., 2019b; Ramos et al., 2019). Pelagic measurements including gill and trammel nets to make catch per unit effort estimations are largely used, combining with visual observations these techniques provided a relatively accurate image of fish assemblages (Seaman & Sprague, 1991). Several AR projects did not have any real monitoring protocol or used only a small range of techniques to a limited extent (Gregg, 1995; Kasim et al., 2013); this point was already made by Koeck et al. (2011). This was the case in some AR projects dealing with protection areas or fish concentration, which were usually poorly documented. However, most of the AR projects cited in the present study were evaluated using the structural ecology approach which provides information such as biodiversity or species abundance for a variable number of ecosystem compartments. In many projects focused on fisheries, only a partial vision of the AR-associated ecosystem is considered (Cresson et al., 2014). Ecological process monitoring techniques (trophic fluxes, non-trophic interaction including ecosystem engineering, carbon and nitrogen cycles, productivity, etc.) are essential to understand the mechanisms and interactions between living organisms and their environment. This type of monitoring allows us to study functional ecology on AR sites. In our analysis, the functional ecology approach includes all papers that deal with

trophic fluxes (Carvalho et al., 2013; Mazzei & Biber, 2015). For example, Cresson et al. (2014) pointed out the importance of integrating functional ecology techniques to allow a satisfactory evaluation of biomass production associated with AR in the bay of Marseilles. In their study, Cresson et al. (2014) used stable isotopic ratios to characterize the trophic network associated with the AR, thus providing an integrative view of trophic relationships. Surprisingly, in our analyses, the functional ecology approach appears to be correlated with low AR effectiveness. This result is probably due to the small number of the selected papers dealing with this topic (2%) as well as the caution shown by authors in their interpretations in comparison, for example, to fisheries or area protection assessment.

The lack of efficient monitoring techniques, or perhaps their suboptimal utilization, underlines the importance of choosing an appropriate complementary technique (*i.e.* functional and structural ecology approaches) for the evaluation of AR performance. As highlighted by this study, trophic food web, primary productivity and associated assemblages are also key indicators allowing a complete assessment of the AR function (Seaman, 2000). Seaman (2000) has published recommendations on AR evaluation, stressing the importance of defining the AR objectives and the success criteria used to optimize the monitoring techniques and evaluation plan. The choice of monitoring techniques to evaluate the structure performance must fit its primary objective. However, it is not sufficient to focus just on the primary objective. A study plan should integrate an evaluation before and after the AR deployment with an important frequency of sampling (Seaman & Sprague, 1991; Seaman, 2000). As mentioned above, simply monitoring AR design to enhance fisheries or physically protect an area are frequently associated with incomplete monitoring protocols (Piazza et al., 2005). Even if a positive effect is recorded with respect to the original purpose of the AR, it is difficult to ensure sustainable management of the structures and, consequently, any long-lasting positive impact, without comprehensive monitoring techniques using the functional ecology approach.

V. Conclusions

The binary nature (presence or absence) of the data frame might explain the massive loss of detailed information in the scientific literature. While the 67 studied variables are the most representative of the selected papers, there are many other aspects not included in this statistical analysis such as the surrounding environment or non-indigenous species. Our study nevertheless represents an objective view of the quantitative and qualitative aspects of artificial

reefs. It reveals the wide variety of artificial reefs in the world. Independently of any purpose, the effectiveness of AR depends to a great extent on the properties of these structures.

Concrete with high roughness is by far the most widely used material and seems to be one of the most efficient. The rugosity of the substrate would increase the surface available for settlement and can consequently increase the biomass, the coverage percentage and the primary production of the structure. Further studies should investigate the microscale topography influence of the AR surface on the first colonisation steps after its immersion. In addition, this material ensures high resistance and does not produce pollutants. The construction material seems to be more important than the shape of the structure, but our results nevertheless suggest that cylindrical or cubic designs are best.

The selection of monitoring techniques is an important factor for the assessment of AR effectiveness. That means selecting the appropriate monitoring techniques must be carefully thought out and include complementary methods. To limit potential negative impacts, and ensure sustainable management and a proper assessment, appropriate monitoring techniques including functional analysis may be necessary to obtain a full understanding of the AR and its impact. Optimal monitoring should be seasonal and continue for at least five years. It is also important to assess different trophic groups to correctly evaluate the effect of the AR on the associated ecosystem and at least on the primary producers, primary consumers, benthic fauna and secondary consumers. Strategically, the selection of the monitoring techniques should be correctly divided between structural and functional approach. Biological indicators like diversity and abundance should be measured by visual census, picture analysis and microscopic observations. Ecological processes (*i.e.* trophic webs, productivity, production, etc.) should be assessed by isotopic analysis, primary productivity and production measurements. In addition, environmental parameters such as temperature, nutrients, depth or light and surrounding ecosystem should also be assessed in order to evaluate the impact of AR on it.

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**CHAPITRE IV : INFLUENCE DE LA STRUCTURE ET DU NIVEAU
D'EUTROPHISATION SUR LE DEVELOPPEMENT MICROPHYTOBENTHIQUE**

Chapitre IV : Influence de la structure et du niveau d'eutrophisation sur le développement microphytobenthique

Partie IV-A : Influence of infrastructure material composition and microtopography on marine biofilm growth and photobiology

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Influence of infrastructure material composition and microtopography on marine biofilm growth and photobiology

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Résumé de la publication

Afin d'explorer les processus de développement microphytobenthique sur substrat dur et d'évaluer l'influence de la nature du substrat colonisé et de sa rugosité de surface, une expérimentation en conditions contrôlées a été réalisée avec des dalles en bétons lisses et rugueuses (MI) ainsi que des structures en PVC. La croissance microphytobenthique et les paramètres photosynthétiques associés ont été évalués pendant six jours. Cette étude a montré une différence importante de biomasse microphytobenthique entre les structures en PVC et en béton tout au long de l'expérience. La biomasse élevée sur les structures en PVC a confirmé que ce matériau peut être facilement colonisé par le MPB. Deux jours après le début de l'expérience, la biomasse de Chl *a* sur les structures en béton (rugueuses et lisses) est plus de deux fois supérieure à celle des structures en PVC. La biomasse est également toujours plus élevée sur les surfaces rugueuses que sur les lisses et a augmenté de manière significative après le troisième jour. Ces résultats confirment l'influence de la rugosité sur la colonisation du biofilm avec une croissance favorisée par une topographie plus complexe (Almaguer-Flores et al., 2012; Souche et al., 2016). L'augmentation uniforme et rapide de la biomasse de MPB sur les structures en PVC par rapport à celle sur les MI est un indicateur de la faible capacité des matériaux en PVC à soutenir l'établissement progressif et durable du biofilm.

L'efficacité quantique maximale du PSII (F_v/F_m) est associée à l'état physiologique et au niveau d'acclimatation du MPB (Kromkamp et al., 1998; Morris & Kromkamp, 2003; Jesus et al., 2005). Des valeurs du rapport F_v/F_m supérieures à 0,5 traduisent un bon état physiologique et/ou une bonne acclimatation (Kromkamp et al., 1998; Morris & Kromkamp, 2003; Napoléon et al., 2013a). Le F_v/F_m mesuré sur les structures en PVC était significativement inférieur à celui mesuré sur les structures en béton. La croissance très rapide du biofilm sur les PVC n'a pas abouti à un biofilm résilient, en effet une baisse des paramètres physiologiques a rapidement été mesurée. Le MPB colonisant les structures en béton présente un meilleur état physiologique avec des valeurs du rapport F_v/F_m proches de 0,5. Cette différence pourrait s'expliquer par la biomasse plus élevée de MPB mesurée sur les structures en PVC conduisant à une augmentation de la compétition pour l'accès à la lumière (auto-ombrage) ou/et pour les sels nutritifs ce qui aboutit à des stress physiologiques. Ces stress physiologiques sont également mis en avant par des valeurs élevées du quenching non-photochimique (NPQ). D'autre part, les légères différences observées entre les valeurs de F_0 et celles des rapports F_v/F_m entre les structures en béton rugueux et lisse tout au long de l'expérience peuvent également s'expliquer par les micro-habitats créés par la rugosité du substrat. En effet, une microtopographie complexe permet de

Chapitre IV : Influence de la structure et du niveau d'eutrophisation sur le développement microphytobenthique

créer des petits creux où les diatomées peuvent s'abriter contre un excès de lumière ou des facteurs de stress hydrodynamiques (forces érosives). Ainsi protégé, les cellules peuvent mieux faire face aux conditions environnementales stressantes, comme le révèle l'augmentation continue de leurs performances photosynthétiques. Il a déjà été décrit dans la bibliographie que le stress induit par l'excès de lumière a un impact sur la croissance et les performances photosynthétiques des diatomées comme *Cylindrotheca closterium* (Rijstenbil, 2003; Roncarati et al., 2008). Les aspérités du support peuvent donc expliquer les meilleurs paramètres physiologiques enregistrés pour les biofilms microphytobenthiques sur les MI rugueuses et le NPQ plus élevé mesuré sur les PVC et les MI lisses.

Afin d'expliquer plus en détail l'influence de la rugosité sur les paramètres photosynthétiques, la microtopographie de chaque structure a été évaluée au cours de l'expérience et comparée aux paramètres photosynthétiques du MPB. La combinaison de ces techniques a permis d'évaluer avec précision, pixel par pixel et sur chaque échantillon, la distribution de chaque indicateur physiologique en fonction du niveau microtopographique. En tenant compte de la bibliographie et de nos résultats, il est clair que le MPB le plus compétitif a été mesuré sur des structures en béton rugueux, par rapport aux structures lisses, et a été influencé par l'amplitude millimétrique de la rugosité de surface et par les microhabitats locaux créés par cette microtopographie plus élevée. À plus grande échelle, comme des projets de restauration des écosystèmes ou d'autres applications, ces résultats doivent être pris en considération. Un biofilm plus compétitif sera plus stable à long terme et fournira un meilleur apport aux consommateurs primaires, ce qui est essentiel pour l'établissement de successions écologiques (Anderson, 1995; Jenkins et al., 2001; Hutchinson et al., 2006).

Influence of infrastructure material composition and microtopography on marine biofilm growth and photobiology

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Abstract

The impact of concrete composition and roughness on the formation of microalgal biofilms and their photobiology were studied on marine infrastructures presenting four different compositions combined with two degrees of roughness (rough and smooth). The structures were first inoculated with a natural microphytobenthic biofilm and immersed in sterilised seawater with a controlled photoperiod for six days. Photosynthetic activity was assessed with an imaging PAM-(Pulse Amplitude Modulated) fluorometer and microtopography was monitored in parallel with a 3-D camera. The results indicated that roughness had an impact on the biofilm biomass, its physiological status and its photosynthetic efficiency and capacity. The assessment of surface roughness indicated that negative reliefs were preferably colonised by MPB (microphytobenthic) cells with better photosynthetic performances. Moreover, MPB biofilms showed better photoacclimation in these microhabitats than on the positive and smooth reliefs. This study confirms the importance of microhabitat for biofilm formation and their photobiology.

Keywords: Biofilm; Hard substrata; Rugosity; Photosynthesis; PAM fluorometry; Biomaterial

The results presented in this article were obtained in the collaborative project Interreg Va MARINEFF, selected under the European Cross-border Programme INTERREG FMA, and co-funded by the ERDF.

I. Introduction

Artificial structures or Artificial Reefs (AR) have been used for decades mainly to attract fish or to protect shallow coastal zones from trawling (Jensen et al., 2000), and their development has intensified over the three last decades (Vivier et al., 2021b). Beyond these targets, such structures are also favourable areas for biodiversity as they act as settlement zones and nurseries for many marine species (Patranella et al., 2017) by providing a novel habitat, by supporting the primary production of organic carbon and its transfer through the trophic network (Charbonnel et al., 2002; Langhamer & Wilhelmsson, 2009; Layman et al., 2016; Komyakova & Swearer, 2018; Komyakova et al., 2019).

In aquatic environments, surfaces are rapidly colonised by micro-organisms (Bakker et al., 2003; Bhosle et al., 2005). Microphytobenthos (MPB) is characterised by the assemblage of photosynthetic microalgae (mostly diatoms), cyanobacteria, protozoa and macrophyte spores which form a biofilm on soft sediment or hard substrate surfaces (Kromkamp et al., 1995; Nagarkar et al., 2004). MPB is one of the most important carbon sources for benthic and pelagic trophic webs on intertidal rocky shores (Bulleri, 2005). MPB is also a pioneer assemblage in the succession of benthic communities, facilitating the settlement of macroalgae and invertebrates (Huang & Boney, 1984; Hung et al., 2007). Biofilms vary in space and over time; they are regulated by environmental and biological parameters like nutrient availability and top-down herbivory control by grazers (Underwood, 1984; Hillebrand et al., 2000; Jenkins et al., 2001; Thompson et al., 2004, 2005). Thus, colonisation of hard substrates by MPB is a crucial step for the following ecological succession of new hard substrata in marine environments. The photo-biological features of these biofilms may be cues that drive subsequent colonisation stages by mediating the bio-adhesion of planktonic metazoan larvae during settlement (Olivier et al., 2000; Tamburri et al., 2008; Hladysz et al., 2011; Whalan & Webster, 2014; Ly et al., 2020).

Reflecting the ecological importance of biofilms in marine environments, their colonisation mechanisms and drivers have been widely studied (Huang & Boney, 1984; Hutchinson et al., 2006; Briand et al., 2012, 2017) on soft substrata (Jesus et al., 2006; Orvain et al., 2012; Morelle et al., 2021) or hard substrata (Anderson, 1995; Bulleri, 2005; Leite et al., 2012). However, only a few authors have investigated the impact of micro-topography, roughness, on biofilm adhesion and colonisation (Chung et al., 2007; Doghri et al., 2015) in marine ecosystems even though these aspects have been widely explored in medical sciences (Oh et al., 2009; Perera-

Costa et al., 2014). Marine biofilms are likely to be affected by the microstructure of the substrate and its degree of roughness (Salta et al., 2013; Souche et al., 2016), and it appears that MPB do not colonise such substrates homogeneously (Anderson & Underwood, 1994; Hutchinson et al., 2006; Salta et al., 2013). Their complex micro-topography creates a wide range of microenvironments, mainly exposure to light, but also nutrient availability. In small crevices on the surface of a rocky shore, microalgae can find refuge from desiccation and light overexposure which could impair cellular organelles like DNA, membrane or protein integrity (Sekar et al., 2004; Perera-Costa et al., 2014). The addition of marine co-produces like shells can also increase substrate porosity and enhance its bio-receptivity (Cuadrado-Rica et al., 2016).

The deployment and management of hard substrates has recently become more important in the current ecological and societal context with the development of immersed marine structures for the purpose of ecological involvement by the creation of novel habitats with expected economic benefits (*i.e.* the construction of offshore wind farms, seawalls, dikes, etc.). Such structures offer innovative solutions with the use of novel materials that increase both their quantitative and qualitative colonisation. The development of marine resources can be promoted by adding coproducts like fragments of oyster shells, which are considered as marine production waste. Shell fragments can also be considered as natural long-term carbon storage that can be exploited to mitigate climate change (Lejart, 2009). This study investigated several photosynthetic indicators of MPB biofilms and how biomass developed on different structures. To this end, marine infrastructures (MI) were built to assess the importance of the composition and roughness of the concrete in MPB colonisation and primary productivity.

II. Materials & Methods

II.1. *Experimental setup*

A total of 50 MI samples were constructed. The MI were shaped like a cobble (5 cm × 5 cm × 3 cm; L × l × h) and for this study, four concrete formulations were designed with two types of cement, Portland cement, CEM I/A-LL 42.5 R CE PM-CP2 NF and CEM II/A (S-V) 32.5 N-LH CE PM-ES-CP1 NF as these two types of cement are suitable for use in seawater. Granular class 0/2 mm siliceous alluvial sand was used. Alluvial aggregates of two sizes, 4/10 mm and 10/20 mm were also used. Twenty percent of the aggregates (4/10 mm) were replaced by oyster shell aggregates to study the effect of this biomineral by-product on the recruitment of oyster larvae. It has been shown that the incorporation of mollusc shell aggregates (6/12.5

mm) could increase the bio-receptivity of concrete because they provide an ideal substrate for the settlement of marine organisms (Graham et al., 2017; Hanlon et al., 2018). Six control samples were made of PVC, as this type of support is suitable and is generally used for oyster larval catchers in shellfish aquaculture.

The absolute density [NF EN 1097-6:2014-01], granular compactness [NF EN 932-2:1999-08] and water absorption coefficient [NF EN 1097-6:2014-01] of the different materials were determined. After the characterisation of the raw materials concrete mixes were formulated in accordance with NF EN 206-1 (NF EN 206-1 2016). Table 5 lists the four concrete formulations tested (type 1 to type 4). Each formulation was composed of the cement I (CEM I) or cement II (CEM II) and of the addition or not of oyster shells. In our study, the control structures were polyvinyl chloride (PVC) structures and corresponded to the type 5.

Table 5 – Concrete type formulations details. The four different concrete type (1 to 4) were composed of two different cements (CEM I or II) and of the addition or not of 20% of oyster shells.

Formulations Components	Type 1: CEM I no shells	Type 2: CEM I with 20% shells	Type 3: CEM II no shells	Type 4: CEM II with 20% shells	Type 5: PVC controls
Cement	350	350	350	350	-
Sand	800	800	800	800	-
Gravel 4/10	600	479	600	479	-
Gravel 10/20	500	500	500	500	-
Shell aggregates	-	121	-	121	-
Water	175	175	175	169	-
Superplasticiser	4.5	7	4.5	7	-

These MI had two different surfaces: one rough and one smooth. The mean gross surface rugosity (R_g) was calculated as the mean of the difference between the five higher and lower points of the structure. The mean R_g by structure type was: 6.19 mm for the rough ones, 2.72 mm for the smooth ones and 1.08 mm for the PVC controls. Each structure was exposed in sterile seawater for one week in order to condition the concrete. Sterilisation were achieved with UV treatment (JBL AquaCristal UV-C 36W) for 48 h (Figure 36). Then, the vertical sides of each MI were paraffined to prevent their colonisation from the external borders. Twenty microcosms (16.2 cm × 11.2 cm × 11.7 cm; L × l × h) were created to optimise the environmental conditions and promote microphytobenthic development. The internal walls of each microcosm were also covered with paraffin to prevent any interaction between the MI during the experiment. The MI were placed in several microcosm by three and all microcosms were disposed in a mesocosm (650 L) with sterilised sea water and alimented with a circulation pump

Chapitre IV : Influence de la structure et du niveau d'eutrophisation sur le développement microphytobenthique
(300 L per hour). Air was bubbled in each microcosm. All microcosms were entirely immersed at the same level in the mesocosm.

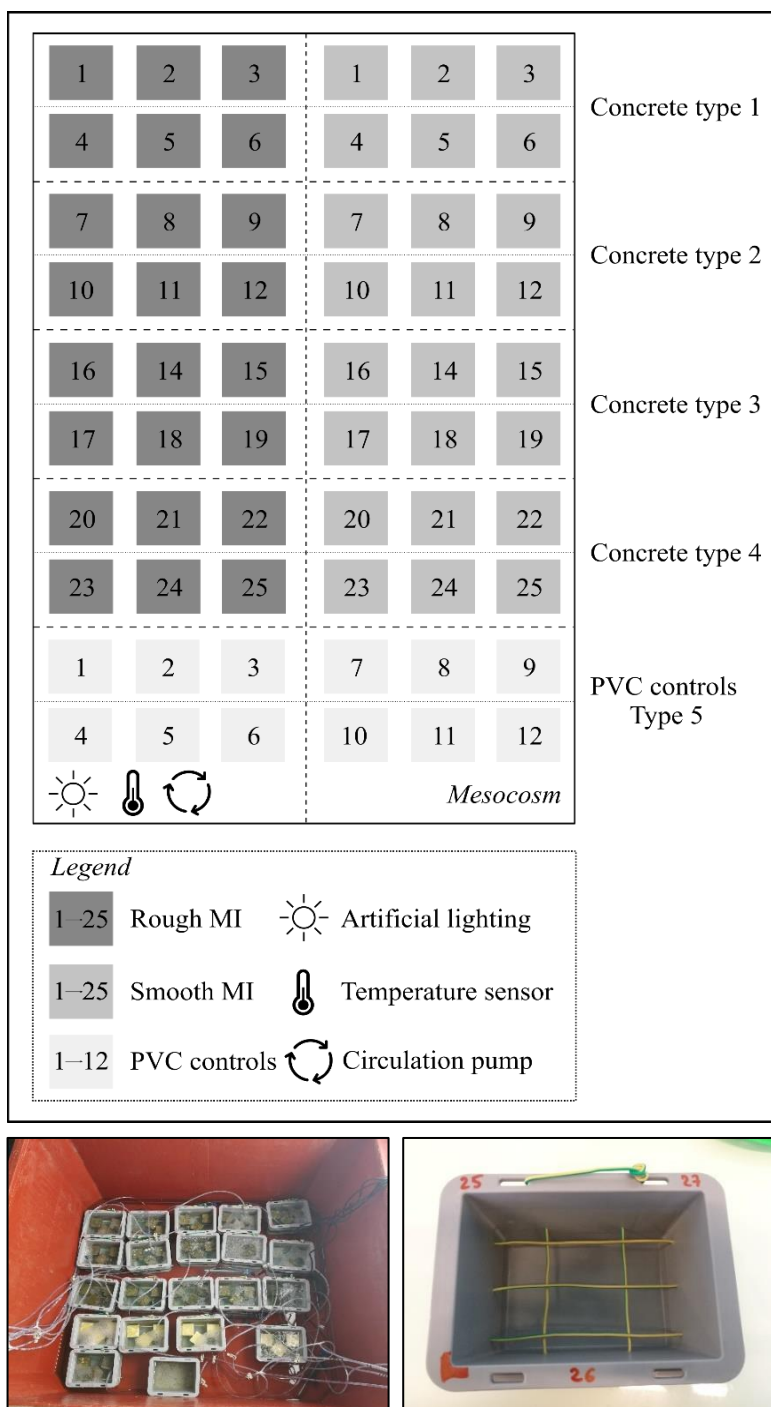


Figure 36 – Experimental setup design. Marine infrastructures were placed by three in 20 microcosms (separated by the dashed lines) placed in a large mesocosm (picture a). Microcosms (picture b) were randomly disposed in the mesocosm to guarantee the total independence between sampled units. Artificial lightning and circulation pump (300L per hour) were added for the entire mesocosm. Air bubbling were added in each microcosm. All microcosms were entirely immersed at the same level in the mesocosm. The average light was $113.83 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ for 8 h (from 8 am to 4 pm) and the average temperature was of 20°C .

II.2. *Experimental monitoring*

Several parameters and indicators were measured over a period of six days after the addition of biofilms. Temperature and pH were recorded throughout the experiment. The distance between the LED and the MI was 60 cm. Light intensities were recorded continuously (*i.e.*, at 1 min intervals) using Onset Hobo UA-002 Pendant light/temperature® data logger placed on specified cores in the tank.

Microphytobenthic biofilms were taken from natural sea samples in tanks with running seawater pumped from the shore in front of the marine station of the University of Caen (CREC) which is located in the Bay of Seine (French-English Channel). Several large MI built with the same material as the sample MI used for this experiment were placed in these large tanks. Dense microphytobenthic biofilms were obtained on these large MI after two weeks of incubation with running seawater. MPB biofilm were sampled gently on the large MI with toothbrush then, biofilms were diluted in 1L of sea water. Then, this stock solution was added in the sterile mesocosm one week after the distribution of MI in each microcosm.

II.2.1. *Scanning Electron Microscopy observations*

Scanning electron microscopy (SEM) observations were performed at the end of the monitoring period. MPB biofilm was carefully sampled from a rough MI with a soft toothbrush at the end of the experiment. The sample was fixed overnight with glutaraldehyde 2.5% in a buffer of sodium cacodylate (0.2 M) with 7% of saccharide during sedimentation on Thermanox™ strips. The samples were then rinsed in this buffer and dehydrated in progressive ethanol baths. Finally, the samples were dried using the critical point bypass method with CPD 030 LEICA®. Samples were metalized with platinum with JFC 1200 JEOL®. Observations were made with the SEM Supra 55 ZEISS®.

II.2.2. *Photosynthetic parameters*

Photosynthetic parameters were measured daily using the Imaging-PAM fluorometer (Walz, Germany). The IMAGING-PAM Chlorophyll Fluorometer was designed to investigate the two-dimensional heterogeneities of photosynthetic activity. Fluorescence measurements were carried out using the Maxi Version of Imaging-PAM Chlorophyll Fluorometer (Walz, Effeltrich, Germany) associated with a LED-Array Illumination Unit IMAG-MAX/L (44 high-power royal-blue (450 nm) LED-lamps) and a CCD Camera IMAG- K7 equipped with a zoom objective lens (640 × 480 pixel resolution). Measurements were performed at a fixed working distance of 18.5 cm. A 5-min dark adaptation period allowed oxidation of the electron acceptor

pools before each measurement. The saturation pulse intensity was 4,500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ for 0.8 s at the surface of the sample and the measuring pulse frequency was 8 Hz. Rapid light curves were performed as follows: samples were exposed to eight incremental intensities of actinic light (E): 0, 21, 111, 281, 396, 531, 611 and 701 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ with 30 s irradiance steps. Numerical values and fluorescence images were extracted using analytical software (Imaging Win; Walz). Auto-fluorescence of each structure was recorded before the experiment in order to apply a correction to F_0 .

The maximum quantum efficiency of PSII (F_V/F_M) was calculated using the following equation from Genty et al. (1989):

$$\frac{F_V}{F_M} = \frac{(F_M - F_0)}{F_M} \quad (16)$$

where F_M is the maximum fluorescence yield measured after a saturating pulse and F_0 the minimum fluorescence yield immediately before the saturating pulse.

The maximum relative electron transport rate ($rETR_{MAX}$, relative units) was estimated by fitting FLC data with the Webb model (1974) to estimate α ($\mu\text{mol electrons } (\mu\text{mol photons})^{-1}$) and Ek ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$) with α the initial slope of the FLC and Ek the light saturation index:

$$rETR = \alpha \times Ek \times (1 - e^{-\frac{E}{Ek}}) \quad (17)$$

$rETR_{MAX}$ was then calculated as:

$$rETR_{MAX} = \alpha_{webb} \times Ek_{webb} \quad (18)$$

The other option was the Eilers & Peeters (1988) model with a , b and c the equation coefficients to calculate the $rETR_{MAX}$ and the photosynthetic efficiency (α).

$$rETR = \frac{E}{(aE^2 + bE + c)} \quad (19)$$

or,

$$rETR_{MAX} = \frac{1}{(b + 2\sqrt{ac})} \quad (20)$$

α was calculated as:

$$\alpha = \frac{1}{c} \quad (21)$$

Finally, non-photochemical quenching (NPQ) based on the relative difference between the maximum fluorescence measured in the dark-adapted state, F_M , and upon exposure to light, F_M' was calculated:

$$NPQ = \frac{F_M - F_{M'}}{F_{M'}} \quad (22)$$

To estimate photosynthetic α and $rETR_{MAX}$ for each pixel of the fluorescence image, a nonlinear regression model was fitted on RLC curves using the simplex method of Nelder & Mead (1965). According to the curve profile, the algorithm automatically chose the fitting model between that of Webb et al. (1974) and Eilers & Peeters (1988). In the exceptional case where $rETR_{MAX}$ was much higher than the highest $rETR$, a linear model was applied and the highest $rETR$ was considered as $rETR_{MAX}$ to avoid overestimation. In the case where (i) the first value (*i.e.*, first E) was the highest in the RLC, (ii) RLC values were all equal to zero and (iii) the set comprised no more than three positive values, the photosynthetic parameters were considered null.

II.2.3. 3D camera acquisitions

A 3D camera was used to map the micro-topography of MI and the changes it underwent during the course of the experiment. The model used was the Gocator 3110 which allows high frequency analyses (*i.e.* a scan rate of 5 Hz). MI were assessed in pairs, a blue light (465 nm) and a stereo scan were performed of each surface face to build a numerical relief.

II.2.4. Chlorophyll *a* extractions

In order to convert the biomass proxy F_0 into Chl *a* biomass, 15 MI were specially incubated in the same condition than those of this experiment. Then, chlorophyll *a* (Chl *a*) was extracted from MPB biofilms sampled on these MI after 7, 14 and 28 days of incubation. The MPB biofilm was sampled with a soft toothbrush on MI upper faces and then diluted in 250 mL of filtered sea water (Stericup GV Millipore, Ø 0.22 µm). A sub-sample (150 mL) of the diluted sample was filtered through a glass-fibre filter (Whatman, GF/F, 47 mm, 0.7 µm) and immediately frozen at -20 °C until analysis. A 10-mL aliquot of 90% acetone (v/v) was added for pigment extraction and the sample was then left at 4 °C in the dark for 12 h. After two 5-

min centrifugations at 1,700 g, the Chl *a* concentration of the extracts was measured using a Trilogy fluorimeter (Turner Designs, Sunnyvale, USA) according to Strickland & Parsons (1968). These data allowed us to estimate a factor between F_0 and Chl *a* calculated for 15 samples. The conversion factor calculated was:

$$\frac{F_0}{chl\ a} = 0.362 \quad (23)$$

with Chl *a* expressed in $\mu\text{g cm}^{-2}$, $R^2 = 0.365$ and $n = 99$.

II.3. *Data treatment and analysis*

We developed a MATLAB[®] routine (available upon request) to determine the values of each level of fluorescence (F_0 , F_M , F_S (steady state fluorescence) and $F_{M'}$) for each pixel. For each sample, auto-fluorescence was deducted and the images corresponding to all successive actinic light per RLC were nested in a 3D matrix. A colour value was assigned to each gray level and, using a conversion index (based on the fluorescence-color scale provided by PAM software), each pixel was converted into a numerical value. The quantum efficiency of PSII charge separation ($\Delta F/F_{M'}$; fluorescence ratio) was calculated for each pixel in each image as $(F_{M'} - F_S)/F_{M'}$. To avoid noise bias during imaging-PAM measurements, all F_M values below 0.048 were previously considered invalid. Indeed, below this threshold, the values acquired were too weak to be reliable (Heinz Walz GmbH 2014). Subsequently, for each pixel and actinic light (E ; $\mu\text{mol photons m}^{-2} \text{s}^{-1}$), the relative electron transport rate was estimated ($rETR = \Delta F/F_{M'} \times E$), $rETR$ -I curves were performed and the acclimatisation light (E_k , $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) was estimated. Microtopography data obtained with the 3D camera were also exploited with a MATLAB[®] routine in order to calculate the correlation between several indicators (F_0 , F_v/F_M , $rETR_{MAX}$, E_k , α and NPQ) and the rugosity level. An upstream treatment allowed us to correct the potential inclination of the MI upper face with the followed polynomial equation:

$$Z_{interp} = a \times X + b \times Y + c \times X.Y + d \quad (24)$$

with X and Y the 2-dimansion axes of the top surface of the concrete. The parameters a , b , c and d were obtained with a simplex minimisation method allowing the determination of the better equation (higher R^2) passing through the surface plan (Z_0). In order to determine the exact surface microtopography without possible inclination plan interferences, it was necessary to remove it. The difference between these values was calculated for each pixel as followed:

$$Z_{final} = Z_0 - Z_{interp} \quad (25)$$

Data analysis was performed with R i386 3.5.1 (R Development Core Team, 2008). Factors were organised in a partial hierarchical design with three fixed factors: age (2, 3, 4 or 6 days), roughness (rough, smooth or PVC), type of concrete (1 to 5). Data normality was tested (Shapiro-Wilk normality test) and, if necessary, data were transformed with the boxcox function of the MASS package on R. A split-plot ANOVA design was performed involving crossed factors see Potvin (1993) for a detailed description (Figure 37) and supplementary material. Tukey tests were performed to distinguish any significant differences between several variables of a factor. Pearson correlation tests were also performed between the degree of roughness and each physiological indicator. Imaging-PAM and 3D camera analyses were performed 2, 3, 4 and 6 days after the beginning of the experiment on every MI.

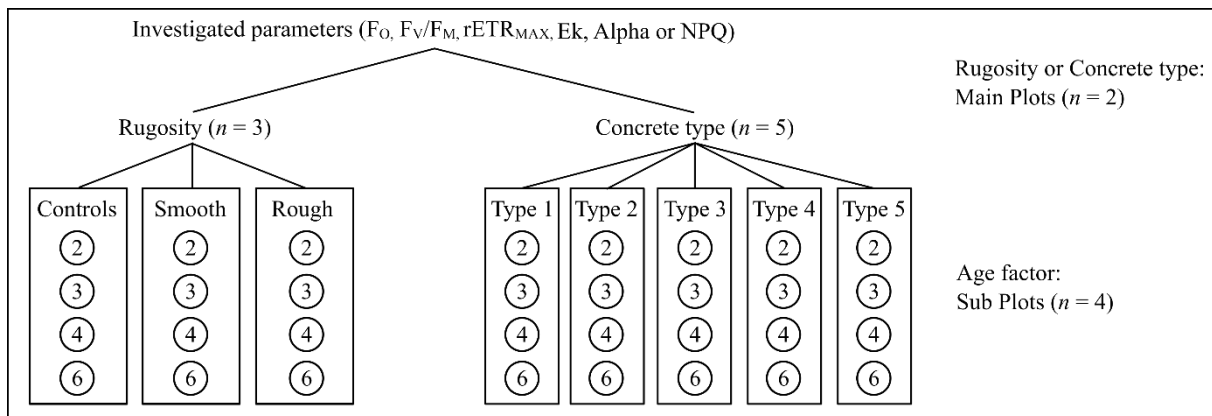


Figure 37 – Diagram of the split-plot ANOVA design. For each of the fourth investigated parameters, the same design was applied with roughness and type of concrete in the main plots and age factor in the subplots.

III. Results

III.1. Environmental conditions and species identification

Mesocosm temperature varied slightly from 18.6 °C to 21.4 °C from day 2 to 6 while the pH (+/- 8.3) remained constant throughout the experiment. The light intensity at the surface of the MI was on average 113.83 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ for 8 h (from 8 am to 4 pm) every day of the survey.

SEM observations revealed a wide diversity of MPB species in different proportions (Figure 38). *Cylindrotheca closterium* was abundant. Many MPB biofilm fragments with high densities of cells embedded in a matrix of EPS were found with a wide range of genera including *Amphora* sp., *Cylindrotheca closterium*, *Entomoneis* sp., *Hantzschia* sp., *Microtabella* sp., *Thalassiosira* sp., and some choanoflagellates.

Chapitre IV : Influence de la structure et du niveau d'eutrophisation sur le développement microphytobenthique

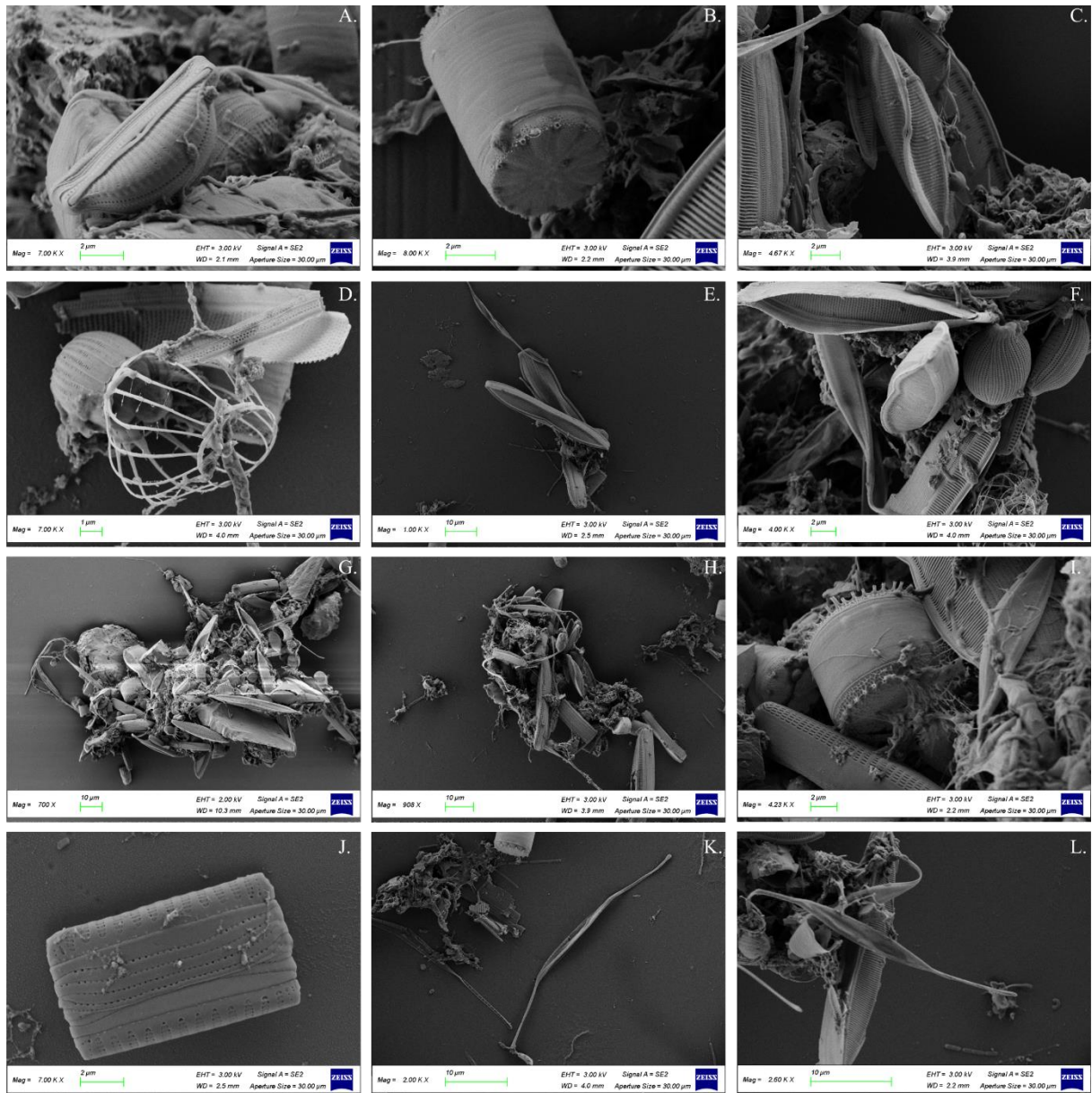


Figure 38 – Scanning electron microscopy observations of microphytobenthic biofilms sampled on one rough MI at the end of the experiment. *Amphora* sp. (A.); *Thalassiosira* sp. (B.); *Entomoneis* sp. (C. and E.); *Choanoflagellate* (D.); *Hantzschia* sp. in the centre (F.); High densities of cells embedded in a matrix of EPS (G. and H.); *Thalassiosira* sp. (I.); *Microtabella* sp. (J.); *Cylindrotheca closterium* (K.); *Cylindrotheca closterium* in the centre (L.).

III.2. *Biological parameters varied with the substratum*

III.2.1. *Chl a biomass*

The averaged value of Chl *a* ($\mu\text{g cm}^{-2}$) was calculated for each MI (PVC, rough and smooth) at each sampling point, from day 2 to day 6 after the beginning of the experiment (Figure 39A). On PVC structures, Chl *a* showed a slight increase from $0.0235 \pm 0.0039 \mu\text{g cm}^{-2}$ (standard deviation) to $0.0273 \pm 0.0083 \mu\text{g cm}^{-2}$ between day 2 and 6 ($+ 0.0038 \mu\text{g cm}^{-2}$). On smooth MI, it increased slightly from 0.0047 ± 0.0021 and $0.0113 \pm 0.0042 \mu\text{g cm}^{-2}$ between day 3 and 6 ($+ 0.0066 \mu\text{g cm}^{-2}$). On the rough MI, it increased from 0.0091 ± 0.0028 and $0.0148 \pm 0.0027 \mu\text{g cm}^{-2}$ ($+ 0.0057 \mu\text{g cm}^{-2}$). According to the split-plot ANOVA (supplementary material), there was a significant effect of roughness, type of concrete and age on the MPB biomass. An HSD Tukey test (supplementary material) revealed that each roughness differed significantly from the other ones. There was no significant difference between the types of concrete except for PVC controls. In both cases, the age factor had a significant effect on Chl *a* after day 3.

III.2.2. *Photosynthetic parameters*

Several photosynthetic parameters were analysed with Imaging-PAM by also providing an estimate of their spatial variations. F_v/F_m (Figure 39B) of the PVC structures remained constant during the first three days (between 0.35 ± 0.02 and 0.36 ± 0.03), it decreased on the fourth day to 0.30 ± 0.02 and increased to 0.42 ± 0.04 on the last two days. F_v/F_m for rough and smooth surfaces faces showed the same dynamics, remaining constant for the first three days, decreasing slightly on day 4 and finally increasing until day 6. On the rough surfaces, photosynthetic yields F_v/F_m reached 0.52 ± 0.02 at the end of the experiment. A value of F_v/F_m of 0.51 ± 0.02 was measured on smooth surfaces at day 6. According to the split plot ANOVA (supplementary material), there was a significant effect of roughness, type of concrete, and age on F_v/F_m , but not of the interaction between age and roughness, meaning that the temporal dynamics were comparable between the two surfaces and also with the PVC control. An HSD Tukey test (supplementary material) revealed that each degree of roughness differed significantly from the other ones. There was a significant effect of age during the monitored biofilm growth, but no significant difference was found between the types of concrete except for PVC controls, and the interaction term between type and age was not significant.

$rETR_{MAX}$ (Figure 39C) increased slightly on the PVC controls from 10.35 ± 9.81 relative unit to 31.64 ± 10.98 relative unit between day 2 and 3, decreased to 19.89 ± 20.83 relative

unit at day 4 and increased again to reach 57.02 +/- 30.49 relative unit at the end of the experiment. On rough structures, $rETR_{MAX}$ increased progressively throughout the experiment from 7.69 +/- 13 relative unit at the first sampling point to 98.51 +/- 25.95 relative unit at day 6. Finally, $rETR_{MAX}$ of smooth structures remained very low for four days (below 1.75 +/- 4.03 relative unit) and reached 81.89 +/- 45.93 relative unit on the last day. According to the split-plot ANOVA (supplementary material), there was a significant effect of roughness, the type of concrete, and age on $rETR_{MAX}$. An HSD Tukey test (supplementary material) revealed that smooth surfaces differed significantly from the other ones. There was no significant difference between the types of concrete except for PVC controls. The age factor had a significant effect on $rETR_{MAX}$ at day 6.

E_k (Figure 39D) values for PVC structures decreased slightly from 4.28 +/- 7.41 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ to 3.68 +/- 6.80 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ between day 2 and day 4 before increasing to reach 9.16 +/- 11.71 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ at day 6. On rough structures, E_k remained stable between 2.77 +/- 3.71 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ and 3.90 +/- 5.11 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ until day 4 before increasing to reach 6.56 +/- 8.67 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ at day 6. Finally, E_k decreased slightly on smooth structures with between 3.28 +/- 3.65 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ and 1.65 +/- 2.71 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ between day 2 and day 4 before reaching 5.08 +/- 6.27 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ at day 6. There was no significant effect of roughness or of the type of concrete on E_k , only a significant effect of age was found on E_k (split-plot ANOVA, $P < 0.001^{***}$). The HSD Tukey test indicated that the age factor had a significant effect, with a difference that was particularly apparent on the last day. Interaction terms with time were not significant, whatever the factor (roughness or type of material).

α (Figure 39E) showed a slight increase on PVC structures from 0.0175 +/- 0.0158 $\mu\text{mol electrons } (\mu\text{mol photons})^{-1}$ to 0.18 +/- 0.107 $\mu\text{mol electrons } (\mu\text{mol photons})^{-1}$ between days 2 and 4. α was almost null during the first three days on smooth MI and reached 0.228 +/- 0.115 $\mu\text{mol electrons } (\mu\text{mol photons})^{-1}$ on the last day. On rough MI, it increased continuously from 0.028 +/- 0.049 $\mu\text{mol electrons } (\mu\text{mol photons})^{-1}$ to 0.266 +/- 0.0745 $\mu\text{mol electrons } (\mu\text{mol photons})^{-1}$ between days 2 and 6. There was a significant effect of roughness, the type of concrete and age on α (split-plot ANOVA, $P < 0.001^{***}$). HSD Tukey test (supplementary material) revealed that α differed significantly on rough and smooth MI. Age also affected α , there were significant differences between each day except between days 3 and 4. The type of concrete type also had an impact on α , there was a significant difference between type 4 and 5.

The NPQ was determined under the experimental light conditions ($113 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) (Figure 39F) and showed a strong increase on PVC from 0.427 ± 0.210 to 0.921 ± 0.103 between days 2 and 4 before falling to 0.033 ± 0.033 on the last day. On smooth MI, it increased from 0.111 ± 0.057 to 0.234 ± 0.075 between days 2 and 4 before reaching 0.021 ± 0.014 at day 6. On rough MI, NPQ increased from 0.110 ± 0.077 to 0.167 ± 0.079 during the first four days then fell to 0.0161 ± 0.009 on day 6. There was a significant effect of roughness and age on NPQ (split-plot ANOVA, $P < 0.001^{***}$). An HSD Tukey test (supplementary material) showed that NPQ on PVC always differed significantly from smooth and rough NPQ. Age also affected NPQ: there were significant differences between each day except between days 2 and 3. Finally, the type of concrete did not affect the NPQ except for type n°3 and n°5 (PVC).

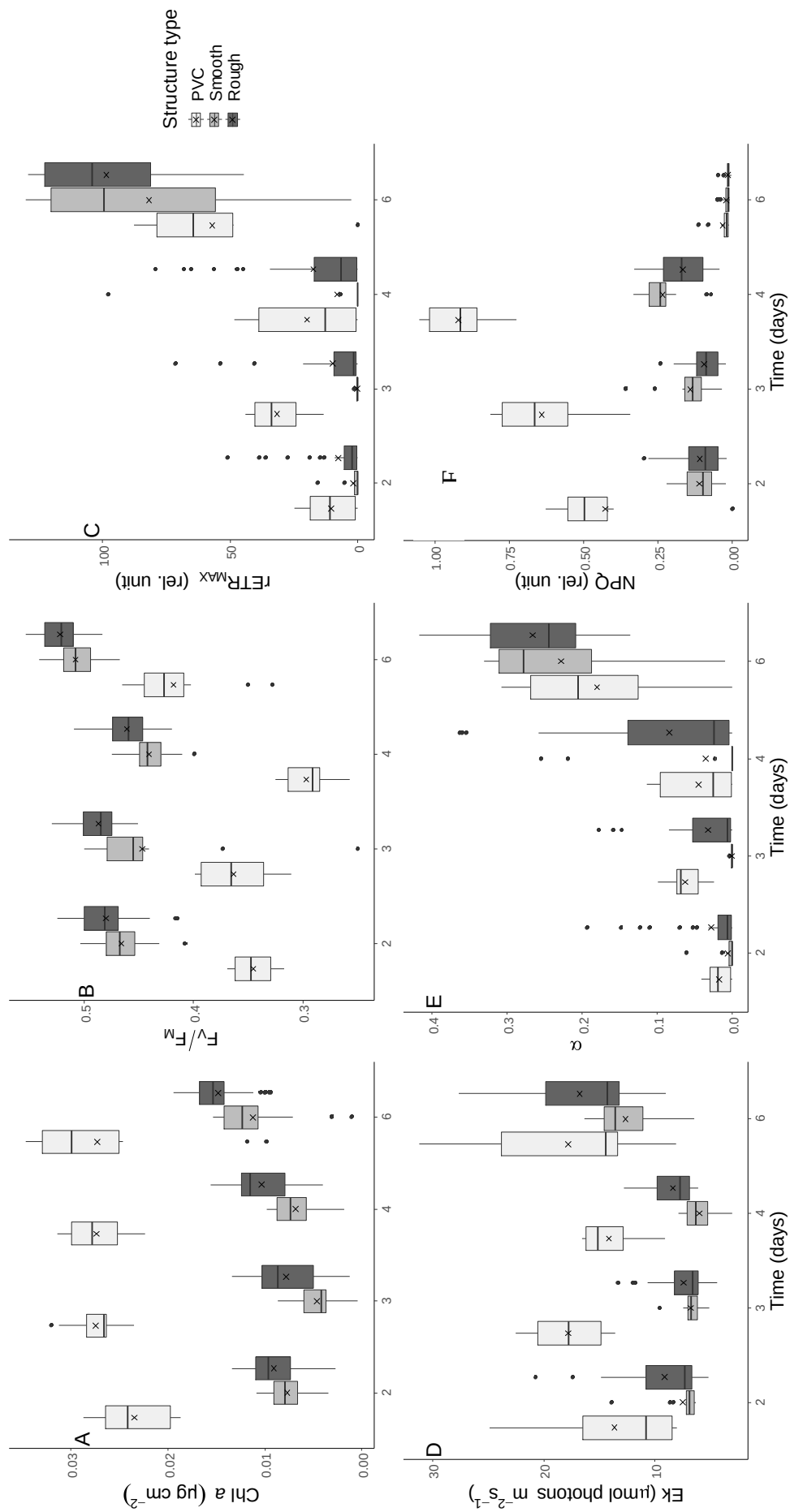


Figure 39 – Biofilm biomass (A, $\mu\text{g Chl a cm}^{-2}$) and photosynthetic indicators F_v/F_m (B), $rETR_{MAX}$ (C, relative unit), E_k (D, $\mu\text{mol photons m}^{-2}\text{s}^{-1}$), α (E, $\mu\text{mol electrons } (\mu\text{mol photons})^{-1}$) and NPQ (F, relative unit) assessment throughout the course of the 6-day experiment on the three different types of structure (PVC, rough and smooth).

III.3. *Biofilm colonisation depending on the microtopography*

III.3.1. *Microphytobenthic biomass distribution*

The spatial distribution of F_0 was very heterogenous on the rough surfaces. On the second day, there was no MPB biomass on the high positive relief of the rough surfaces. During the entire experiment, there were more MPB cells on the negative reliefs than on the positive ones (millimetric scale). On the last day of the experiment, MPB biomass was present on positive reliefs, but the quantities were smaller than on the negative ones (Figure 41). The MPB biomass was distributed differently between the smooth and rough structures at the same sampling point. The spatial distribution of F_0 was less heterogeneous on the smooth MI than on the rough ones throughout the survey (Figure 41).

III.3.2. *Distribution of microphytobenthic photosynthetic indicators*

The F_v/F_m distribution was also notably heterogeneous on the rough MI (Figure 40) compared with the smooth one (Figure 41). Its distribution seemed to be affected by the relief. $rETR_{MAX}$ and α were also affected by the surface roughness on the rough MI, while these parameters were not correctly graphically represented on smooth MI. The NPQ also seemed to be affected by the MI topography, on rough MI, NPQ appeared was on high positives reliefs. On smooth ones, NPQ seemed to be more homogenously distributed.

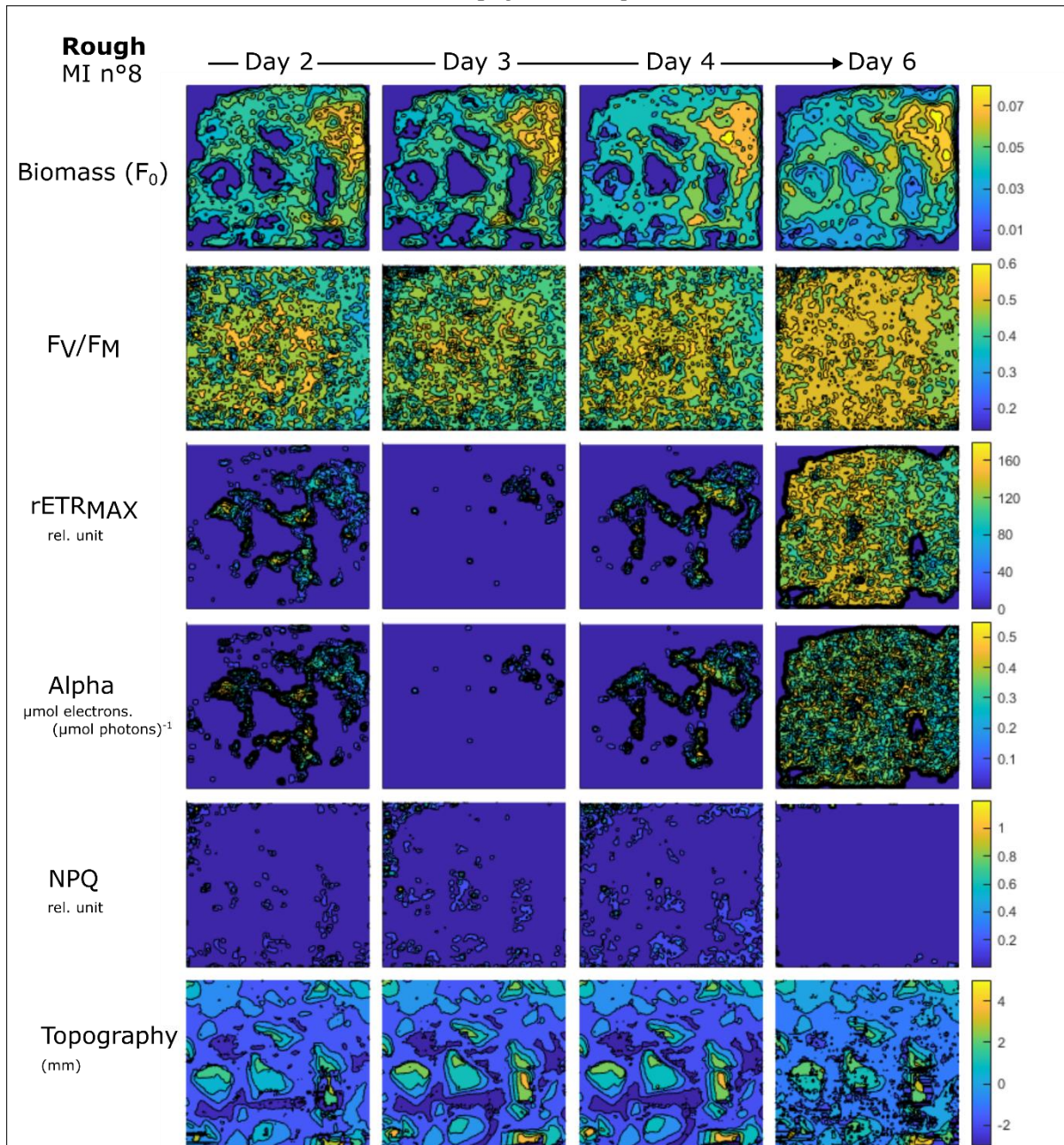


Figure 40 – Spatial distribution of biofilm biomass (F_0) and selected photosynthetic indicators (F_V/F_M , rETR_{MAX} (relative unit), Alpha ($\mu\text{mol electrons} (\mu\text{mol photons})^{-1}$) and NPQ (relative unit)) and microtopography (mm) on one rough BMI (n°8) during the monitoring period.

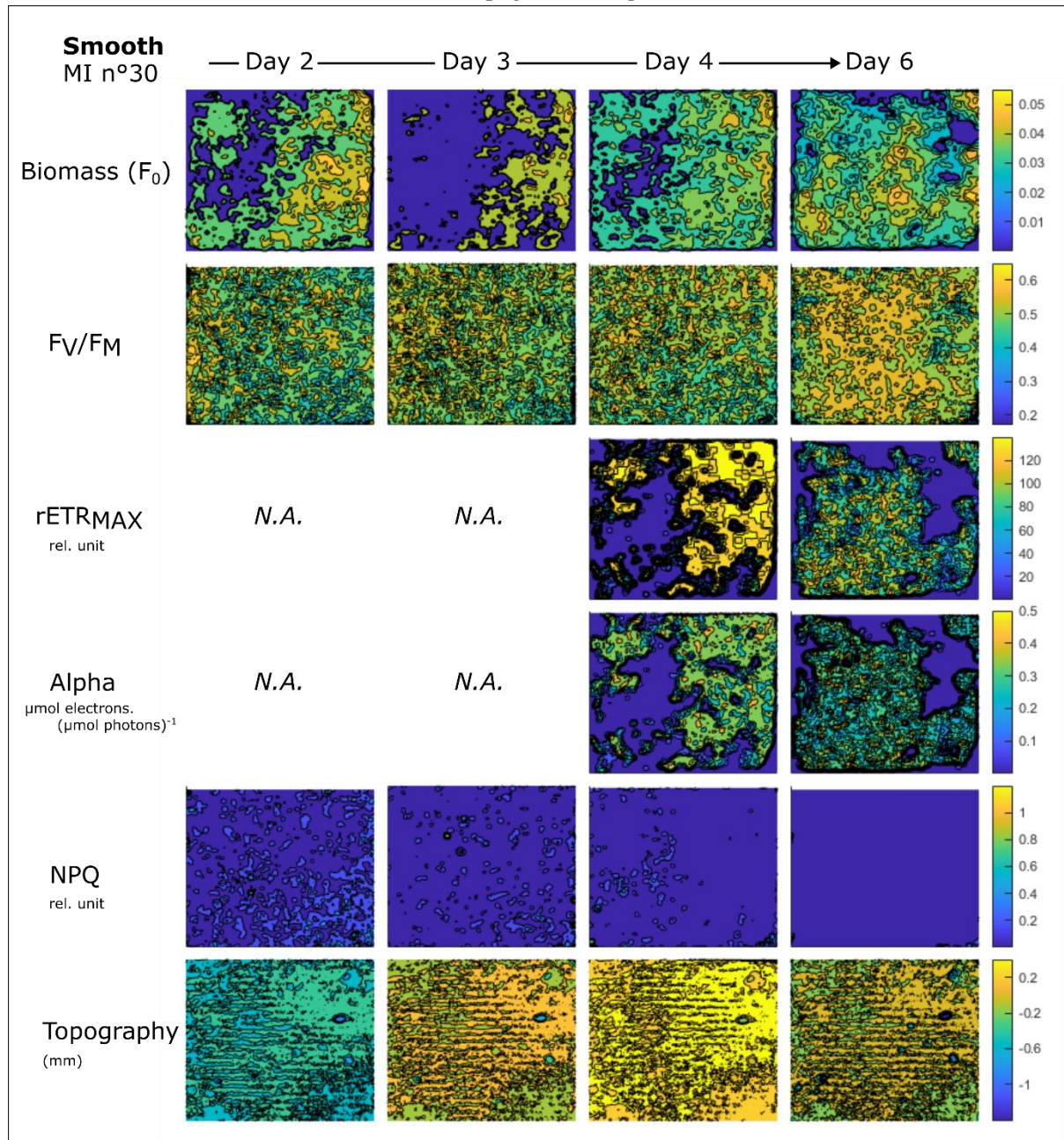


Figure 41 – Spatial distribution of biofilm biomass (F_0) and selected photosynthetic indicators (F_v/F_m , $rETR_{MAX}$ (relative unit), Alpha ($\mu\text{mol electrons} / (\mu\text{mol photons})^{-1}$) and NPQ (relative unit)) and microtopography (mm) on one smooth BMI (n°30) during the monitoring period.

III.3.3. *Correlation between microtopography and MPB biomass or photosynthetic indicators*

Correlation coefficients (R) were calculated between the level of microtopography (negative or positive) and the F_0 , F_v/F_m , $rETR_{MAX}$, E_k , α and NPQ. $R > 0.1$ or < -0.1 were considered as significantly positive or negative (critical values in a Student's t test with this sample pixel number). A positive R indicated that the indicator was mostly measured on positive reliefs and, conversely, a negative R indicated that the indicator was mostly measured on negative ones.

The correlation between roughness and surface Chl *a* biomass was calculated (Figure 42A). Correlation coefficients were always higher than 0 on PVC or smooth structures throughout the survey. In these cases, the correlation was quite similar throughout the survey. On rough structures, correlation coefficients were significantly lower than 0 and varied between -0.17 ± 0.2 at day 2 and -0.083 ± 0.17 on the last day. On rough structures, the correlation coefficient also differed significantly from that on the two other structures ($P < 0.01^{**}$).

The correlation between roughness and F_v/F_m was calculated (Figure 42B). The R coefficient on PVC or smooth structures was always positive throughout the experiment and its dynamics was almost the same in the two cases. It increased slightly from 0.068 ± 0.1 to 0.082 ± 0.11 between day 2 and day 6 on smooth structures. On PVC structures, it increased slightly from 0.067 ± 0.087 to 0.14 ± 0.05 between day 2 and the last day of experiment. The R coefficient on rough structures was notably lower than 0 and varied between -0.13 ± 0.15 at day 2 and -0.05 ± 0.16 on the last day. The correlation coefficient on rough structures also differed significantly different from that of the two other structures ($P < 0.01^{**}$).

The correlation between roughness and $rETR_{MAX}$ was calculated (Figure 42C). There were no significant differences between smooth and control structures. The correlation coefficient for the controls ranged between 0.057 ± 0.093 and 0.075 ± 0.083 from day 2 until the last day of the experiment. On smooth structures, it varied between 0.046 ± 0.095 and 0.048 ± 0.11 over the same period. There was a significant difference between the rough surface and the two other surfaces ($P < 0.001^{***}$). The correlation coefficient varied between -0.035 ± 0.27 and -0.005 ± 0.13 between day 2 and the last day of the experiment.

The correlation between roughness and E_k was calculated (Figure 42D). Its dynamics was similar in the three types of structure and no significant difference was found between them.

The correlation between α and surface roughness was calculated (Figure 42E). On rough MI, it was significantly lower than on the others ($P < 0.001^{***}$). There also was a significant difference between the first two days and the two last days of the experiment. On rough MI, the coefficient varied from -0.15 ± 0.17 and -0.056 ± 0.16 between day 2 and day 6. On smooth MI, it varied between 0.021 ± 0.041 and 0.057 ± 0.13 during the experiment. On PVC, it varied from 0.059 ± 0.079 and 0.093 ± 0.073 between day 2 and 6.

Finally, the correlation between the NPQ and the roughness was calculated (Figure 42F). There was a significant impact of roughness ($P < 0.001^{***}$) and age ($P < 0.001^{***}$) on NPQ. NPQ was always significantly higher on rough MI than on the other ones. It was higher than 0 during the first three days on rough MI and varied between 0.12 ± 0.17 and -0.028 ± 0.12 from day 2 to day 6. On smooth MI, NPQ varied from -0.078 ± 0.095 and -0.091 ± 0.1 between day 1 and day 6. On PVC, it varied between -0.017 ± 0.059 and -0.066 ± 0.07 between day 2 and day 6.

This analysis revealed that the correlation between the level of microtopography and F_0 , F_V/F_M and $rETR_{MAX}$ was significantly lower on the rough structures than on the smooth ones and on the controls. Moreover, this correlation coefficient was also negative. On controls and smooth structures, the correlation coefficient was close to zero and on all structures the R coefficient showed a slight increase on the last day of experiment.

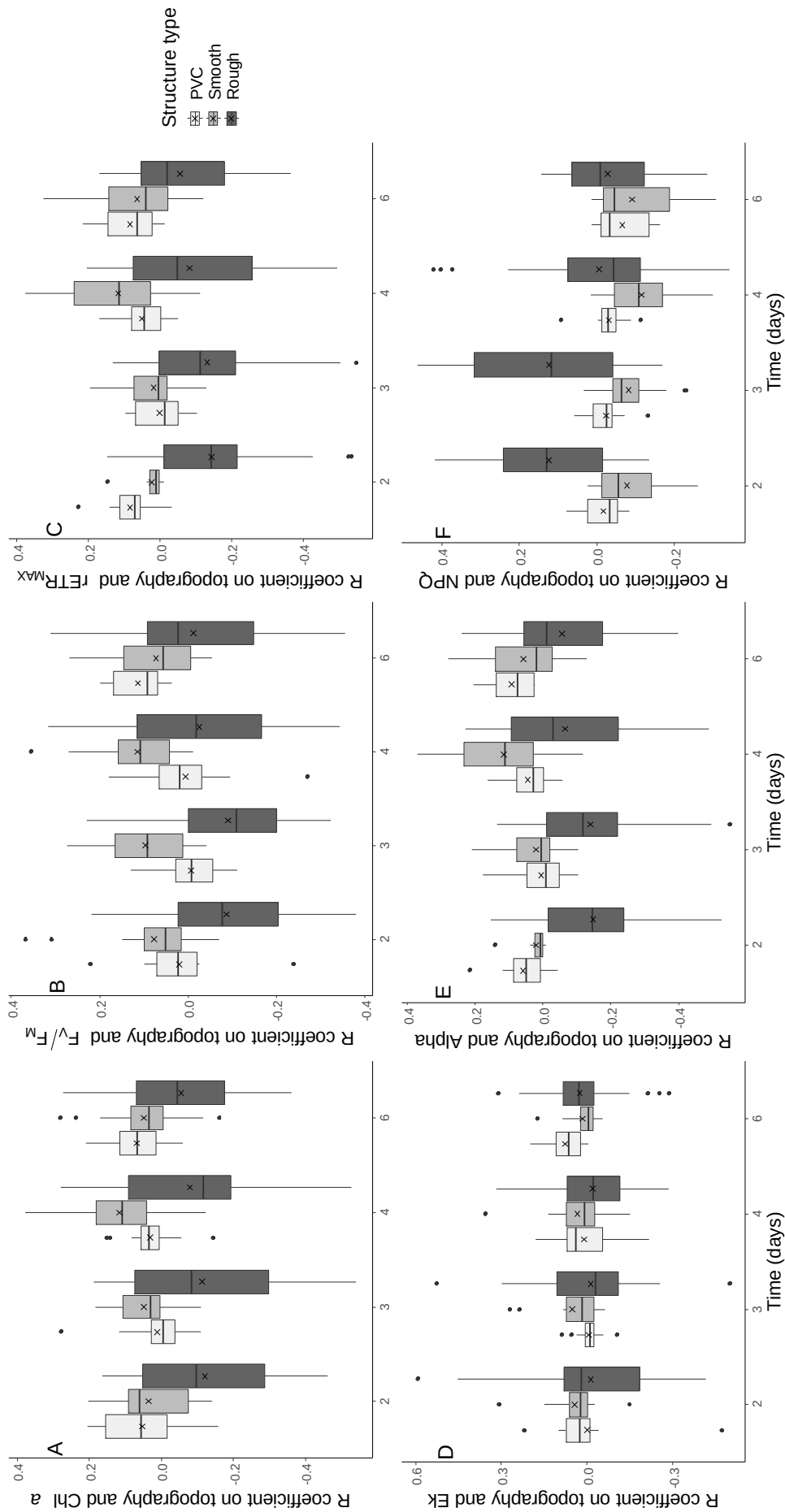


Figure 42 – Correlation coefficient (R) between biofilm biomass and rugosity level (A) or photosynthetic indicators and rugosity level: F_v/F_m (B), $rETR_{MAX}$ (C), E_k (D), α (E) and NPQ (F).

IV. Discussion

IV.1. *Influence of the substrate on biofilm development and its physiological quality*

In dark adapted microalgae, most of the F_0 originates from the Chl a , a correlation between F_0 and Chl a is often used in oceanography and in biofilms studies (Barranguet & Kromkamp, 2000; Stock, 2019). Our results showed a large and significant difference in Chl a concentrations between PVC and concrete structures throughout the experiment. The MPB biomass on these structures remained constant throughout the experiment. This observation confirmed that this material can be easily and efficiently colonised by MPB. These first colonisation steps are primordial before the establishment of subsequent ecological successions (Fonsêca-Genevois et al., 2006; Sokołowski et al., 2017). Two days after the beginning of the experiment, the volume of Chl a biomass was more than two times lower on concrete (rough and smooth) structures than on the PVC ones. Behind the fact that concrete led to slower growth of the MPB biofilm compared to PVC, Chl a biomass was also always higher on rough surfaces than on the smooth ones and increased significantly after the third day. However, these results seemed to confirm the influence of roughness on biofilm colonisation with slower growth on more complex topography (Almaguer-Flores et al., 2012; Souche et al., 2016). The rapid uniform increase in MPB biomass on PVC structures compared with that on rough MI was an indicator of the poor ability of PVC material (*i.e.* smooth) to support gradual and sustainable biofilm establishment.

The maximum quantum efficiency of PSII (F_V/F_M) was associated with the physiological state of the MPB (Kromkamp et al., 1998; Morris & Kromkamp, 2003; Jesus et al., 2005). F_V/F_M higher than 0.5, considered to be a good physiological status, have frequently been measured in marine benthic and phytoplankton microalgae (Kromkamp et al., 1998; Morris & Kromkamp, 2003; Napoléon et al., 2013a). The F_V/F_M measured on PVC structures was significantly lower than on the concrete structures. The very rapid growth of biofilm on PVC did not result in a resilient biofilm: microalgae rapidly showed some evidence of poor physiological status. The MPB colonising the concrete structures were in a better physiological state, confirmed by F_V/F_M values close to 0.5. This difference could be explained by the higher density of MPB measured on PVC structures leading to an increase in competition for light or nutrients and may have caused stress. Decreases in F_V/F_M have also been associated with high NPQ values. At the same time, the slight difference in F_0 and F_V/F_M values between rough and

smooth concrete structures throughout the experiment could also be explained by the microhabitats created by the roughness. Indeed, in complex microtopography, small crevasses were observed where diatoms found refuge from excess light or hydrodynamical stressors (erosive forces). There, they can better cope with stressful environmental conditions, as revealed by the gentle and continuous increase in their photosynthetic performance. It is also known that excess light induced stresses impacted the growth and photosynthetic performances of diatoms like *Cylindrotheca closterium* (Rijstenbil, 2003; Roncarati et al., 2008) and may explain the better physiological parameters recorded for MPB biofilms on rough MI and the higher NPQ induction measured on PVC and smooth MI.

One expected consequence of a lower F_V/F_M would be a parallel decrease of $rETR_{MAX}$ and the photosynthetic efficiency (*i.e.* α) because the energy captured by the PSII is diverted from photochemistry to non-photochemical quenching (Ralph et al., 2002). However, our results suggest better electron transport rate during the first three days of the experiment on the PVC structures. $rETR_{MAX}$ measurements also confirmed the better photosynthetic capacity of MPB on rough structures than on smooth ones, and this was also the case of photosynthetic efficiency. The falling NPQ values on the last day of experiment were concomitant with increasing $rETR_{MAX}$ values and could be explained by the settlement of a well photo-acclimatised MPB biofilm. Additional measurements of a^* (Chl *a* specific absorption coefficient) and σ_{PSII} (functional absorption cross-section of PSII) would be useful to interpret the $rETR_{MAX}$ in more detail.

A dense MPB biomass with low photosynthetic parameters was recorded on PVC structures in contrast to concrete structures. Rough structures seemed to be more suitable for colonisation by a photo-synthetically efficient biofilm. After day 6 of the experiment, some of the structures were kept in the tank with a photoperiod and the biofilm on the PVC detached itself into the water (visual observation on day 10). Even if these results were not acquired until the senescent phase on PVC, our data clearly confirmed the weak efficiency of the PVC structures to be colonised by a competitive MPB compared with concrete ones. The Table 6 summarise the qualitative effect of all variables on the biofilm. MPB biofilm colonising rough MI was dominated by benthic diatoms (*Amphora* sp., *Cylindrotheca closterium*, *Entomoneis* sp., *Hantzschia* sp. and *Microtabella* sp.) corresponding to native species and attesting to the potential of these structures to promote MPB biofilm development. The large number of cells embedded in the matrix of EPS observed by SEM confirmed the high physiological quality of these MPB biofilms. One of the most important criteria was the capacity of a structure to be

colonised by a perennial biofilm leading to high photosynthetic performances and strong physical stability.

*Table 6 – Summary of the positive or negative effects of both variables (rugosity and concrete formulation) on the biofilm biomass (Chl *a*) and its associated photosynthetic parameters. The reference used for the rugosity variable were PVC plates, the reference used for the concrete formulation variable were cement type I.*

	Rugosity			Concrete formulation		Shell addition
	Control PVC	Smooth	Rough	Cement type I	Cement type II	
Chl <i>a</i>	Reference	--	-	Reference	=	=
F _V /F _M	Reference	+	++	Reference	=	=
rETR _{MAX}	Reference	+	+	Reference	=	=
E _k	Reference	=	=	Reference	=	=
α	Reference	+	++	Reference	=	=
NPQ	Reference	-	--	Reference	=	=

IV.2. Influence of microtopography on biofilm colonisation and several physiological indicators

In order to explain the influence of roughness on these photosynthetic parameters in more detail, the microtopography of each structure was assessed over the course of the experiment and compared with MPB photosynthetic parameters. The combination of these techniques made it possible to accurately assess, pixel by pixel and on every sample, the distribution of each physiological indicator in accordance with the micro-topographic level.

Analyses of microphytobenthic communities can provide important insights for the evaluation of environmental status. Hard substrate marine biofilms have already been studied, these biofilms have been shown to be very diversified and abundant (Bulleri, 2005; Bellou et al., 2012). Biofilms are influenced by several biotic and abiotic factors such as temperature (Di Pippo et al., 2012; Jackson et al., 2013), seasonality (Jackson et al., 2013; Orvain et al., 2014), grazing (Anderson, 1995), hydrodynamical forces (Battin et al., 2003), depth (Bellou et al., 2012) and light (Di Pippo et al., 2012). The structure and composition of the substrata may also affect the development of MPB but only a few studies have investigated these aspects to date. Autonomous Reef Monitoring Structures (ARMS) have been shown to be relatively easily colonised by MPB. Even if these structures had a smooth surface, they provide protection for biofilms and consequently facilitated colonisation (Pennesi & Danovaro, 2017).

Many authors have examined the importance of roughness in the formation of biofilm, especially in medical sciences (Schwarz et al., 2007; Oh et al., 2009; Almaguer-Flores et al., 2012). Conversely, there have been few investigations of the impact of roughness on the formation of marine biofilms. In this study, the negative correlation between Chl *a* biomass, F_V/F_M , α and $rETR_{MAX}$ and the degree of roughness on rough structures indicated that the MPB preferred microhabitats. This statement was supported by the fact that the correlation rates for these parameters were also differed significantly between rough structures and smooth structures or controls. On the smooth surfaces of the concrete slabs, we observed colonisation by the biofilm edging towards the inside of the structure leading to a uniform biofilm at the scale of the surface. On the rough surfaces, we highlighted colonisation by aggregation. These foci of microphytobenthic colonisation appeared at the level of negative micro-relief anomalies. During the growth of the biofilms, the higher areas were gradually colonized towards the end of growth. No significant difference was found in the correlation coefficients between E_k and the level of roughness. Moreover, the assessment of NPQ indicated that photo-inhibition was higher on the high positive relief of rough MI and also on smooth faces of MI or PVC controls. These results show that roughness does affect the MPB biomass, its physiological state (F_V/F_M), the $rETR_{MAX}$ and photosynthetic efficiency. Photo-inhibition processes could have been boosted by the high Chl *a* concentration and the absence of relief on smooth and PVC structures. It has been shown that diatoms and cyanobacteria abundance are favourably affected by surface roughness on intertidal rock surfaces (Hutchinson et al., 2006). Souche et al. (2016) used a biomaterial similar to the concrete used in this study. They showed that increased roughness or the addition of bio-component like shells had a positive impact on algae colonisation. The influence of substrate topography or instability on MPB biomass is well known especially in intertidal mudflat systems where the diatom cover and biomass decrease in areas exposed to physical disturbances (Weerman et al., 2010). Taking the literature into account and considering our results, it is clear that the most competitive MPB was assessed on rough concrete structures, compared with the smooth structures, and was influenced by the millimetric amplitude of surface roughness and by the local microhabitats created by this higher micro-topography range. At a larger scale, e.g., ecosystem restoration projects or other applications, these results need to be taken into consideration. A more competitive biofilm will be more stable in the long run and will provide a better input for primary consumers, which is essential for the establishment of ecological successions (Anderson, 1995; Jenkins et al., 2001; Hutchinson et al., 2006).

V. Conclusion

This study showed that concrete is a more suitable material for colonisation by a biofilm than PVC. The microhabitats represent micro-niches which were more colonised than the rest of the structure due to easier photoacclimation of the MPB in this microenvironment. In natural environments, the colonisation of MI by a competitive and physiologically active biofilm may promote the establishment of an ecological succession. These results are innovative for marine biofilms on hard substrates and confirm the importance of the addition of microhabitats for the creation of marine artificial structures designed for environmental restoration projects.

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The authors have no competing interests to declare.

Chapitre IV : Influence de la structure et du niveau d'eutrophisation sur le développement microphytobenthique

Partie IV-B : Influence of nutrient enrichment on growth and photosynthetic parameters of hard substrate marine microphytobenthos

This work is under review in *Hydrobiologia*

Résumé de la publication

Compte tenu de la précédente expérimentation et des résultats obtenus concernant l'influence de la rugosité de surface sur le développement microphytobenthique et sa photosynthèse, une seconde expérimentation a été réalisée afin d'évaluer ces mêmes indicateurs sur des structures identiques en fonction d'un gradient d'eutrophisation.

La disponibilité des nutriments est l'un des principaux moteurs de la croissance des microalgues planctoniques et benthiques pouvant entraîner une eutrophisation des écosystèmes côtiers lorsque les apports sont excessifs (Rabalais, 2009). Les phénomènes d'eutrophisation sont généralement associés à une biomasse de phytoplancton élevée. Les effets de l'eutrophisation sur le biofilm sont également étudiés (Fricke et al., 2016; Lawes et al., 2016). Contrairement au phytoplancton, plusieurs études mettent en évidence l'influence modérée ou nulle des apports de nutriments sur la biomasse du biofilm sur les substrats rocheux (Sanz-Lázaro et al., 2011; Lawes et al., 2016). La principale différence entre les habitats du phytoplancton et des biofilms est l'espace disponible pour leur développement. Dans la colonne d'eau, la disponibilité de l'espace ne constitue pas un facteur limitant, même si une biomasse élevée peut entraîner une limitation de la lumière (Zhang et al., 1995). Notre étude n'a pas montré d'augmentation significative de la biomasse du biofilm lorsque la concentration en nutriments est plus élevée ce qui est en accord avec Sanz-Lázaro et al., (2011).

Les EPS sont connus pour protéger les cellules contre divers stress environnementaux tels que les substances toxiques ou les agents microbiens, les EPS sont également importants pour les processus de colonisation (adhérence du substrat, agrégation des cellules) (Orvain et al., 2003; Flemming & Wingender, 2010). Nos résultats concernant ce compartiment ont permis de confirmer l'importance des EPS pour l'adhérence des diatomées dans les premières étapes du développement du biofilm (Sutherland et al., 1998; Orvain et al., 2003). Nos résultats ont établi la relation positive entre l'exsudation d'EPS et la disponibilité des nutriments en raison de la quantité plus élevée d'EPS mesurée à la fin de l'expérimentation dans la condition fortement enrichie en sels nutritifs (Staats et al., 2000; Yallop et al., 2000; Orvain et al., 2003).

Les paramètres photosynthétiques associés au microphytobenthos ont également été évalué grâce à un IMAGING-PAM, des mesures d' a^* ont également été réalisées et ont permis de calculer le ETR_{max}^a . Nos résultats ont indiqué que l'apport nutritif peut avoir un impact positif sur le statut physiologique du microphytobenthos. La disponibilité des nutriments, en particulier de l'azote, constitue un facteur clé contrôlant la croissance des diatomées et leur photobiologie

Chapitre IV : Influence de la structure et du niveau d'eutrophisation sur le développement microphytobenthique

(Van der Grinten et al., 2004; Sanz-Lázaro et al., 2011; Napoléon et al., 2013b; Fricke et al., 2016; Lawes et al., 2016; Brodersen et al., 2020; Serre-Fredj et al., 2021). L'utilisation de la fluorimétrie PAM permet une estimation non invasive, rapide et simple du paramètre photosynthétique relatif mais avec le risque de surestimer ces indicateurs (comme cela pourrait être le cas dans notre étude). Cette technique devrait donc être utilisée en association avec d'autres méthodes (a^*) et ainsi permettre la caractérisation des processus d'acclimatation face aux stress environnementaux, comme l'excès, le manque de lumière ou de disponibilité des nutriments, conduisant à une réorganisation et/ou à une réduction de la teneur en pigments pouvant affecter l'efficacité photosynthétique (Morelle & Claquin, 2018).

L'impact de la microtopographie de surface sur la biomasse du biofilm a déjà été étudié. Dans notre étude, une corrélation positive entre la biomasse de Chl *a* et le degré de rugosité a été mesurée dans la condition non enrichie en sels nutritifs en fin de suivi. Cette corrélation était plus faible dans la condition intermédiaire et négative dans la condition fortement enrichie en sels nutritifs. Ces résultats indiquent que les apports de nutriments peuvent être impliqués dans le processus de colonisation du biofilm et confirment l'implication de la rugosité de surface sur la biomasse du biofilm et ses performances photosynthétiques.

Influence of nutrient enrichment on growth and photosynthetic parameters of hard substrate marine microphytobenthos

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Abstract

The influence of nutrient enrichment on the formation of microalgal biofilm on concrete from marine infrastructures compare and on PVC structure was studied. The structures were immersed in mesocosms where a nutrient gradient was applied. Biomass, photosynthetic parameters, microtopography, and extracellular polymeric substances (EPS) of microphytobenthos were measured over a period of 28 days. Imaging PAM measurements were performed on the whole surface of the structure associated with the chlorophyll-specific absorption cross section (a^*), enabling estimation of the electron transport rate from PSII (ETR). In the first three days of the experiment, the EPS:Chl a ratio reached its highest level in all conditions, underlining the importance of EPS for the adhesion of the diatoms in the initial stages of biofilm development. The Chl a biomass was not significantly affected by nutrient availability and the impact of nutrient availability on photosynthetic capacity (ETR_{MAX}) and efficiency (α) was limited. The negative correlation between the level of rugosity and the Chl a biomass and several photosynthetic indicators observed in the enriched conditions underlined the importance of surface rugosity for biofilm development and photoacclimation. The present study showed that eutrophication conditions had only a minor impact on the microalgal biofilm growth and its photobiology.

Keywords

Microphytobenthos; Marine infrastructures; Photosynthesis; Eutrophication; Pulse Amplitude Modulated (PAM) fluorimetry ; Extracellular polymeric substances (EPS)

I. Introduction

In aquatic environments, surfaces rapidly become colonised by micro-organisms (Bakker et al., 2003; Bhosle et al., 2005). Microphytobenthos is characterised by the assemblage of photosynthetic microalgae (mostly diatoms), cyanobacteria, protozoa and macrophyte spores that form a biofilm at the surface of the soft sediment or hard substrate (Kromkamp et al., 1995; Nagarkar et al., 2004). After macroalgae, microphytobenthos is one of the main sources of carbon for benthic and pelagic trophic webs along intertidal rocky shores (Bulleri, 2005). Microphytobenthos is also a pioneer assemblage in the succession of the benthic communities and facilitates the settlement of macroalgae and invertebrates (Huang & Boney, 1984; Hung et al., 2007). Biofilms vary in space and over time and are regulated by environmental and biological parameters including grazers and nutrient availability (Underwood, 1984; Hillebrand et al., 2000; Jenkins et al., 2001; Thompson et al., 2004, 2005). Thus, the colonisation of hard substrates by microphytobenthos is a crucial step in the succession of new hard substrata in the sea. The photobiological features of these biofilms can provide cues concerning the subsequent stages of colonisation by mediating the bio-adhesion of planktonic metazoan larvae during recruitment (Olivier et al., 2000; Tamburri et al., 2008; Hladysz et al., 2011; Whalan & Webster, 2014).

The mechanisms behind -and the drivers of- colonisation have been the subject of intensive studies, reflecting the ecological importance of biofilms in marine environments (Huang & Boney, 1984; Hutchinson et al., 2006; Briand et al., 2012, 2017). Many of these studies focussed on the development patterns of soft-substrate microphytobenthic biofilms (Jesus et al., 2006; Orvain et al., 2012) and hard substrate biofilms (Anderson, 1995; Bulleri, 2005; Leite et al., 2012). The influence of nutrient concentrations on the development of hard substrate biofilm has rarely been investigated and the few studies that have been conducted mainly focussed on species diversity or biomass indicators (Flothmann & Werner, 1992; Hillebrand & Sommer, 1997, 2000). Considering the increase in coastal urbanisation and rural runoff, understanding how biofilms respond to nutrient enrichment is pertinent. Indeed, anthropogenic disturbance are widespread in estuarine and coastal systems. Runoff can introduce contaminants and increase nutrient inputs in the natural habitat (Kennish, 2002). Elevated nutrient inputs can have a variety of effects including increased primary production, causing eutrophication, anoxia, or hypoxia and harmful algal blooms (Rabalais, 2009; Serre-Fredj et al., 2021). At moderate levels, eutrophication may promote the development of marine biofilms whereas at higher levels, eutrophication may reduce biomass and diversity, thereby favouring opportunistic

species (Moran & Grant, 1989). However, the potential implications of the concentration of nutrients on the photosynthesis of hard substrate biofilm communities have been poorly investigated.

Hard substrates have become more important given the ecological and societal questions concerning the increasing use of immersed marine structures for ecological purposes through the creation of novel habitats with expected economic benefits (e.g., the construction of offshore windfarms, seawalls, or dikes). Such innovative structures use novel materials that may increase both the quantity and quality of colonisation. However, the environmental conditions, especially nutrient concentrations, differ markedly in coastal or harbour systems and in the open sea. The main objective of this study was to evaluate the influence of inorganic nutrient availability on the development and photosynthesis of marine epilithic biofilm. To this end, marine infrastructures (MI) were built and immersed in different controlled conditions and the influence of surface rugosity of the structure on the biofilm growth and its photosynthetic activity was evaluated. The photobiology of the primary producers' biofilms was also investigated by combining different techniques including measuring chlorophyll fluorescence and performing chlorophyll-specific absorption cross sections to calculate the ETR as a function of the nutrient conditions and the surface micro-scale rugosity.

II. Materials & Methods

II.1. *Experimental setup*

A total of 60 cobble-shaped marine infrastructures were built (5 cm × 5 cm × 3 cm; L × l × h). The type of cement used was CEM II/A-LL 42.5 R CE PM-CP2 NF, which is suitable for use in sea waters. Siliceous alluvial sand was in granular class 0/2 mm. Alluvial aggregates of two sizes, 4/10 mm and 10/20 mm, were also used. After characterisation of the raw materials, concrete mixes were formulated according to NF EN 206-1, 2016. The formulation was 350 kg m⁻³ of cement, 800 kg m⁻³ of sand, 600 kg m⁻³ of gravel (4/10 mm), 500 kg m⁻³ of gravel (10/20 mm), 175 kg m⁻³ of water and 4.5 kg m⁻³ of superplasticizer.

A total of 45 polyvinyl chloride (PVC) plates had already been used as controls in a previous experiment to investigate the influence of different concrete formulations and rugosity on biofilm development (Vivier et al., 2021a). This type of support has also been shown to be suitable and is widely used in shellfish aquaculture as oyster larval catchers. The mean gross surface rugosity (R_g) was calculated as the mean of the difference between the five highest and lowest points of the structure. The mean R_g per structure type was 6.42 ± 1.15 mm for the MI

and 0.64 ± 0.03 mm for the PVC controls. Twenty MI and 15 PVC plates were randomly placed in three separate mesocosms ($45 \text{ cm} \times 32 \text{ cm} \times 25 \text{ cm}$; $L \times l \times h$).

II.2. *Experimental design*

Three mesocosms were set up and filled with filtrated sea water ($\text{Ø } 100 \text{ }\mu\text{m}$), each connected to a semi-open sea water system. The total volume of each mesocosm was approximately 44 L and the renewal rate was 10% of the total volume every four hours. Throughout the experiment, two mesocosms were enriched with a Redfield/Brzezinski ratio solution (C:N:P = 106:16:1) by a peristaltic pump (one dose per hour). A single mother solution was prepared and the two mesocosms were enriched, one at a rate of $+ 30 \text{ }\mu\text{mol L}^{-1}$ (medium enrichment condition or C2) and the other at $+ 100 \text{ }\mu\text{mol L}^{-1}$ (high enrichment condition or C3). The control treatment was not enriched (C1). A circulation pump was placed in each mesocosm to homogenise each condition and ran for 15 minutes every two hours. Artificial lighting ($150 \text{ }\mu\text{mol photon m}^{-2} \text{ s}^{-1}$ (Figure 43) was switched on 10 hours per day. Biofilms were taken from natural hard substrate sea samples at the Luc-sur-Mer marine station (France) (CREC) on the 29th of June 2020. An inoculum of 100 mL at $\sim 3000 \text{ cells mL}^{-1}$ was added to each mesocosm on the same day (day 0). The experiment lasted from the 29th of June to the 27th of July 2020. Each mesocosm was directly connected to the pumping system of the CREC marine station, this body of sea water is monitored at high frequency by the instrumented SMILE buoy ($0^{\circ}19'41.00''\text{O } 49^{\circ}21'14.00''\text{N}$, data available doi.org/10.17882/53689). The choice of this period of the year to set up this

experiment was supported by the fact that phytoplankton growth was shown to be limited by inorganic nutrient availability in this season in the Bay of Seine (Napoléon et al., 2012).

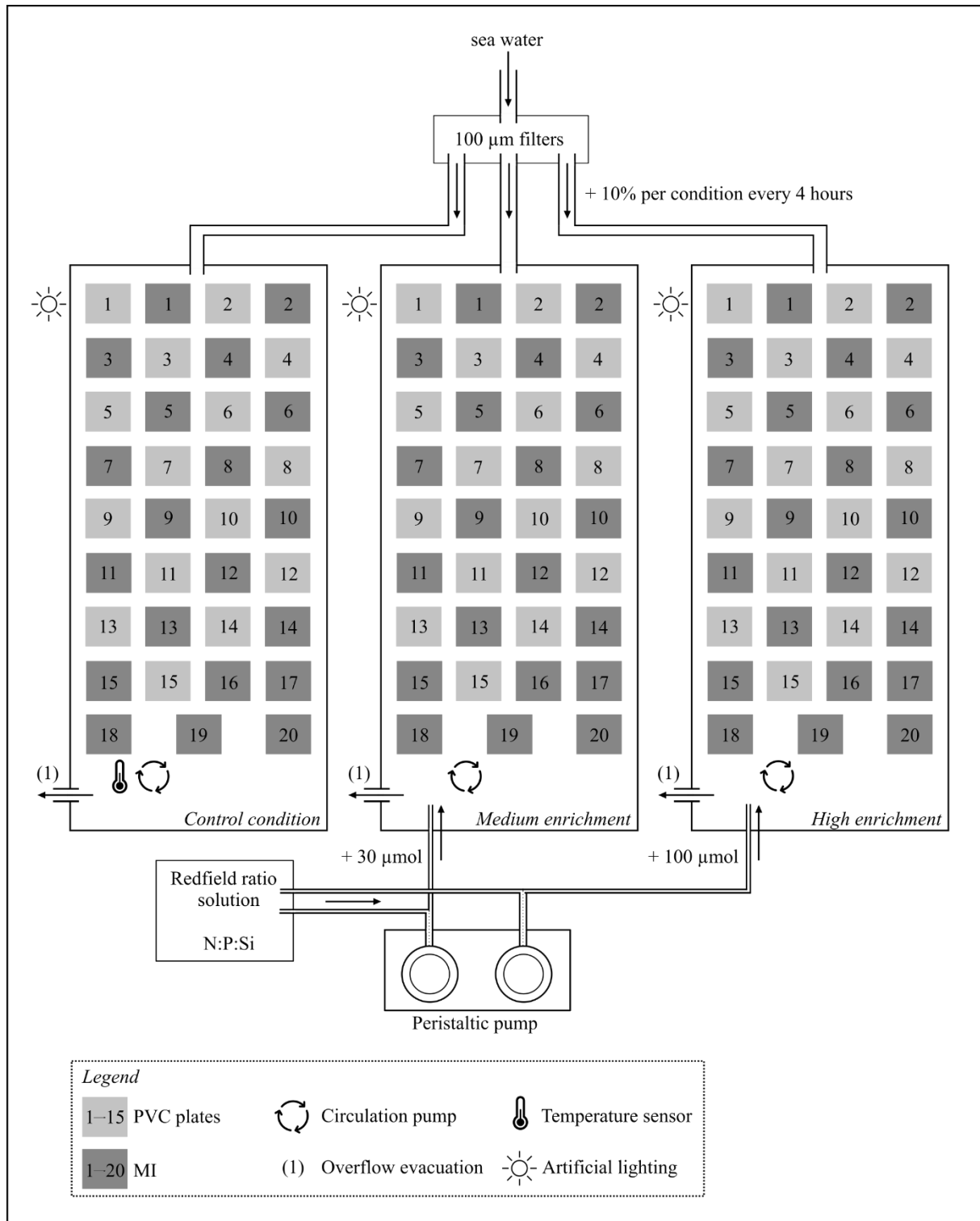


Figure 43 – Experimental setup

II.3. *Abiotic parameters and inorganic nutrient analyses*

Water temperature and light intensity were recorded throughout the experiment with an RBRsolo T logger and a RBR solo³ PAR logger connected to a Li-COR “Underwater Quantum Sensor” LI-192. Salinity, pH, dissolved oxygen, and nutrient concentrations were recorded and analysed at regular interval during the course of the experiment.

For the analyses of inorganic nutrients, a sample of water was collected from each treatment and filtered through a cellulose acetate filter (ClearLine, CA, 33 mm, 0.45 µm) in 50 mL Falcon tubes and frozen (-20 °C). Analyses were conducted using a Seal Analytical AA-3 system (Aminot & K  rouel, 2007). The limits of quantification were 0.02 µmol L⁻¹ for PO₄³⁻ and 0.05 µmol L⁻¹ for NO₃⁻ and Si(OH)₄. NH₄⁺ were analysed by fluorometric measurements according to the protocol of Oriol et al. (2014).

II.4. *Chl a biomass*

Chl *a* was extracted from the samples at days 3, 7, 10, 16, 24 and 28. Soft toothbrush was used to carefully sample biofilm from MI upper surfaces, which was then diluted in 250 mL of filtrated sea water (Stericup GV Millipore, Ø 0.22 µm). The diluted sample was used for the extraction of Chl *a*, microscopic observations, and for the extraction of extracellular polymeric substances. A sub-sample (150 mL) of the diluted sample was filtered through a fibreglass filter (Whatman, GF/F, 47 mm, 0.7 µm) and immediately frozen (-20 °C) until analysis. Aliquots (10 mL of 90% acetone, v/v) were added for pigment extraction then the samples were left for 12 h in the dark at 4 °C. After being centrifuged for 5 min at 1700 g twice, the Chl *a* concentration of the extracts was measured using a Trilogy fluorimeter (Turner Designs, Sunnyvale, USA) according to the method of Strickland and Parsons (1968).

Growth rates (μ , Ln([Chl *a*] (µg cm⁻² day⁻¹))) were calculated for each experimental condition based on changes in the concentration of chlorophyll using the following equation:

$$\mu = \frac{\ln(N_t) - \ln(N_{t-1})}{\Delta t} \quad (26)$$

where N_t is the Chl *a* concentration at time t , N_{t-1} is the Chl *a* concentration at the previous time step and Δt is the difference in days between t and $t-1$.

II.5. *Photosynthetic parameters*

The photosynthetic parameters of each biofilm on every MI and PVC plate were assessed at days 3, 6, 7, 10, 14, 18, 21, 24 and 28 after the addition of the biofilm inoculum. Photosynthetic parameters were measured daily using the Imaging-PAM fluorometer (Walz, Germany). The IMAGING-PAM Chlorophyll Fluorometer is designed for the study of two-dimensional heterogeneities of photosynthetic activity. Fluorescence measurements were carried out using the Maxi Version of Imaging-PAM Chlorophyll Fluorometer (Walz, Effeltrich, Germany) associated with a LED-Array Illumination Unit IMAG-MAX/L (44 high-power royal-blue (450 nm) LED-lamps) and a CCD Camera IMAG- K7 equipped with a Zoom objective (640 × 480 pixel resolution). Measurements were performed at a fixed working distance of 18.5 cm. A 5-min dark adaptation period was respected before each measurement to allow oxidation of the electron acceptor pools. The saturation pulse intensity was 4,500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ for 0.8 s and the measuring pulse frequency was 8 Hz. For rapid light curves were performed as follows: samples were exposed to 11 incremental intensities of actinic light (E): 0, 73, 216, 370, 554, 658, 774, 896, 1018, 1166 and 1336 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ with 30-s irradiance steps. Numerical values and fluorescence images were extracted using analytical software (Imaging Win; Walz). The autofluorescence of each structure was recorded before the experiment began in order to apply a correction to F_0 .

The maximum quantum efficiency of PSII (F_V/F_M) was calculated using the following equation from Genty et al. (1989):

$$\frac{F_V}{F_M} = \frac{(F_M - F_0)}{F_M} \quad (27)$$

where F_M is the maximum fluorescence measured after a saturating pulse and F_0 is the minimum fluorescence immediately before the saturating pulse.

The maximum relative electron transport rate ($rETR_{MAX}$, rel. unit) was estimated by fitting FLC data with the Webb model (1974) to estimate α ($\mu\text{mol electrons } (\mu\text{mol photons})^{-1}$) and E_k ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$) with α the initial slope of the FLC and E_k the light saturation index:

$$rETR = \alpha_{webb} \times E_{k_{webb}} \times (1 - e^{-\frac{E}{E_{k_{webb}}}}) \quad (28)$$

Then $rETR_{MAX}$ was calculated as:

$$rETR_{MAX} = \alpha_{webb} \times Ek_{webb} \quad (29)$$

The other option, in the case of visible photoinhibition, was the Eilers & Peeters (1988) model with a , b and c the equation coefficients to calculate the $rETR_{MAX}$ and the photosynthetic efficiency ($\alpha_{E.P.}$).

$$rETR = \frac{E}{(aE^2 + bE + c)} \quad (30)$$

or,

$$rETR_{MAX} = \frac{1}{(b + 2\sqrt{ac})} \quad (31)$$

$\alpha_{E.P.}$ was calculated as:

$$\alpha_{E.P.} = \frac{1}{c} \quad (32)$$

Finally, non-photochemical quenching (NPQ) which was based on the relative difference between the maximum fluorescence measured in the dark-adapted state, F_M , and upon exposure to light ($150 \mu\text{mol photon m}^{-2} \text{s}^{-1}$), F_M' was calculated:

$$NPQ = \frac{F_M - F_M'}{F_M'} \quad (33)$$

To estimate photosynthetic α and $rETR_{MAX}$ on each pixel of the fluorescence image, a nonlinear regression model was fitted on RLC curves using the simplex method of Nelder & Mead (1965). According to the curve profile, the best fitting model was automatically chosen by the algorithm between Webb et al. (1974) and Eilers and Peeters (1988). In the exceptional case where $rETR_{MAX}$ was much higher than the highest $rETR$, a linear model was applied and the highest $rETR$ was considered as $rETR_{MAX}$ to avoid overestimation. In the case where (i) the first value (*i.e.* first E) was highest in the RLC, (ii) RLC values were all equal to zero and (iii) the set comprised no more than three positive values, while the photosynthetic parameters were considered null.

The chlorophyll-specific absorption cross section (a^* , $\text{m}^2 \text{mg Chl } a^{-1}$) was obtained by filtering 50 mL of the diluted sample (250 mL) used for the Chl a extraction. The *in vivo* optical density of total particles from 380 to 750 nm was measured directly on the filter using a

spectrophotometer (UV-2600i Shimadzu) and an integrating sphere (IR-2600 Shimadzu). The sample was recovered with methyl alcohol (MeOH) to extract diatom pigments (Babin et al., 2003). After one hour, the *in vivo* optical density of non-algal particles was measured directly on the filter rinsed with filtered seawater. The chlorophyll-specific absorption cross section (a^* ; $\text{m}^2 \text{mg Chl } a^{-1}$) was calculated using the equation of Johnsen and Sakshaug, (2007) :

$$a^* = \frac{A \cdot S \cdot 2.3}{V \cdot [\text{Chl } a]} \quad (34)$$

where A is the average optical density of biofilms between 380 and 700 nm, S is the clearance area of the filter (295.6 mm^2), V is the filtered volume in mL and the chlorophyll a concentration is in mg m^{-3} .

The $ETR_{max}^{a^*}$ in $\text{mmol e}^- \text{mg Chl } a \text{ h}^{-1}$ was then calculated according to (Morelle & Claquin, 2018):

$$ETR_{max}^{a^*} = rETR_{MAX} \times a^* \times fAQ_{PSII} \times 3.6 \quad (35)$$

where fAQ_{PSII} is the fraction of absorbed quanta to PSII assuming that, for diatoms, 74% of the absorbed photons were allocated to photoreactions in the PSII (Johnsen & Sakshaug, 2007; Napoléon et al., 2013b), and 3.6 a factor to express the unit in “ $\text{mmol e}^- \text{mg Chl } a \text{ h}^{-1}$ ” by transforming $\mu\text{mol e}^-$ to mmol e^- and seconds to hours.

The α^{a^*} in $\text{mmol e}^- \text{mg Chl } a \text{ h}^{-1} (\mu\text{mol photons})^{-1}$ was then calculated as follows:

$$\alpha^{a^*} = \frac{ETR_{max}^{a^*}}{Ek} \quad (36)$$

II.6. Extracellular polymeric substances

Water samples (10 mL) were sub-sampled in the diluted biofilm (250 mL). The sub-sample was centrifuged at 3000 rpm for 20 min at 20 °C and the pellet was immediately frozen at -20 °C. For carbohydrate analysis, 1 mL of MilliQ water was added, followed by 1 mL 5% phenol and 5 mL of 96% sulphuric acid (DuBois et al., 1956). Samples were incubated for 45 min at ambient temperature and measurements were made using a (Ultrospec 1000, Pharmacia Biotech) spectrometer at 485 nm using glucose (G) as standard for the calibration curve. EPS are expressed in $\mu\text{gGeq cm}^{-2}$. After being divided by the Chl a concentration, the EPS:Chl a ratio was calculated and is expressed in $\text{mgGeq}/\mu\text{gChl } a$.

II.7. *3D camera recordings*

3D camera recordings were acquired in order to map the microtopography of MI at the end of the experiment. The Gocator 3110 (LMI Technology) device was used as it allows high frequency analyses (*i.e.* a scan rate of 5 Hz). MI was assessed by pair, a blue light (465 nm) and a stereo scan were performed on each both surfaces to build a numerical relief (for more information, see (Vivier et al., 2021a)).

II.8. *Scanning Electron Microscopy*

SEM observations were performed on the samples at days 3, 7, 10, 16, 24 and 28. The biofilm was carefully sampled with a smooth toothbrush and filtered on a fibreglass filter (Whatman, GF/F, 47 mm, 0.7 μm). The samples were fixed overnight with 2.5% glutaraldehyde in a buffer of sodium cacodylate (0.2 M) with 7% sucrose during sedimentation on Thermanox™ strips. The samples were then rinsed in the same buffer, dehydrated in progressive ethanol baths, then dried using the critical point bypass method with CPD 030 LEICA®. Samples were metalized with platinum with JFC 1200 JEOL®. Observations were made with the SEM Supra 55 ZEISS® from CRISMAT.

II.9. *Data treatment and analysis*

To obtain numerical values of fluorescence spatial heterogeneity from images, we developed a MATLAB® routine (Orvain, pers. com.) to determine the values of each level of fluorescence (F_0 , F_M , F_S (steady state fluorescence) and $F_{M'}$) for each pixel. For each sample, autofluorescence was deducted and the images corresponding to all successive actinic light per RLC were nested in a 3D matrix. A colour value was assigned for each gray level using a conversion index (based on the fluorescence-colour scale provided by PAM software), and each pixel was converted into a numerical value. The quantum efficiency of PSII charge separation ($\Delta F/F_{M'}$; fluorescence ratio) was calculated for each pixel of each image as $(F_{M'} - F_S)/F_{M'}$. To avoid noise bias during PAM imaging measurements, all F_M values below 0.048 were considered invalid. Indeed, below this threshold, the values acquired were too weak to be reliable (Heinz Walz GmbH 2014). For each pixel and actinic light (E ; $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$), the relative electron transport rate was estimated ($rETR = \Delta F/F_{M'} \times E$), $rETR$ -I curves were carried out and acclimatization light (E_k , $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) was estimated.

Data analysis was performed with R i386 3.5.1 (R Development Core Team, 2008). Factors were organised according to a partially hierarchical design with two fixed factors: time and

nutrient condition (control, medium and high enrichment). Because measurements were repeated at different times, the time factor was considered as the “within-plot” factor, and nutrient condition was the main factor to test differences among pseudo-replicates, since only one mesocosm was tested for each nutrient condition. Data normality was tested using the Shapiro-Wilk normality test and, if necessary, data were transformed with the boxcox function of the MASS package on R. A split-plot analysis of variance (ANOVA) was performed involving crossed factors (see Potvin (1993) for a detailed description) and supplementary material (appendix D). Tukey tests were performed to distinguish the significant differences between several variables of a factor. Split-plot ANOVA results are available in supplementary material. Pearson correlation tests were also performed between the level of roughness and each physiological indicator. PAM imaging and 3D camera analyses were performed at 3, 6, 10, 14, 18, 21, 24 and 28 days on five MI after the beginning of the experiment. Chl *a* biomass was analysed at 3, 7, 10, 16, 24 and 28 days after the beginning of the experiment. A non-linear regression was applied on these data and a three-parameter logistic curve was fitted using the “aomisc” package. A Kruskal-Wallis test was performed on model parameters to calculate potential significant differences between conditions.

III. Results

III.1. *Experimental conditions*

The water temperature in the mesocosms varied between 18.6°C and 21.5°C during the experiment whereas the pH (+/- 8.3) remained constant. The average light intensity at the surface of MI between 8 am and 6 pm was 150 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ each day of the survey.

Nutrient concentrations (NO_3^- , NH_4^+ , PO_4^{3-} and Si(OH)_4) were calculated at several sampling points throughout the experiment (appendix E). NO_3^- was constant throughout the experiment at $4.5 \pm 1.11 \mu\text{mol L}^{-1}$ in the control (C1), $48.81 \pm 10.8 \mu\text{mol L}^{-1}$ under medium enrichment (C2) and $117.48 \pm 21.39 \mu\text{mol L}^{-1}$ under high enrichment (C3). In C1, NH_4^+ increased slightly between day 3 and 4, from 4.54 to $6.86 \mu\text{mol L}^{-1}$ before decreasing until the end of the monitoring to reach $0.48 \mu\text{mol L}^{-1}$. In C2, the ammonium concentration decreased from $6.86 \mu\text{mol L}^{-1}$ to $0.35 \mu\text{mol L}^{-1}$ between day 3 and 23. Finally, in C3, NH_4^+ increased from $14.92 \mu\text{mol L}^{-1}$ to $17.88 \mu\text{mol L}^{-1}$ between day 3 and 4 then decreased strongly to 5.87 at day 8 and reached $0.67 \mu\text{mol L}^{-1}$ on day 23.

In C1, PO_4^{3-} was constant around $0.11 \pm 0.06 \mu\text{mol L}^{-1}$, in C2 at around $2.86 \pm 0.71 \mu\text{mol L}^{-1}$, and in C3 at around $6.44 \mu\text{mol L}^{-1}$. Finally, Si(OH)_4 was constant in C1 around $9.93 \pm$

3.89 $\mu\text{mol L}^{-1}$. Phosphate concentration decreased slowly between 57.46 $\mu\text{mol L}^{-1}$ and 35.95 $\mu\text{mol L}^{-1}$ in C2. In C3, PO_4^{3-} increased slowly from 105.4 $\mu\text{mol L}^{-1}$ until 112.53 $\mu\text{mol L}^{-1}$ between day 3 and 23 with a lower value of 80.71 $\mu\text{mol L}^{-1}$ on day 22.

III.2. *Chlorophyll a biomass, growth rate of extracellular polymeric substances*

In C1, Chl *a* biomass increased to 3.09 $\pm$ 0.66 $\mu\text{g cm}^{-2}$, in C2 reached 6.70 $\pm$ 0.67 $\mu\text{g cm}^{-2}$, and in C3 to 4.89 $\pm$ 0.62 $\mu\text{g cm}^{-2}$ (Figure 44A). The logistic curve applied to these data showed no significant differences between conditions (Kruskal-Wallis test). Growth rate dynamics were similar whatever the condition under MI (Figure 44B). No significant differences were observed between C2 and C3 during the whole experiment and after day 10 in any of the conditions. Growth rates reached a plateau on day 10 which persisted until the end of the experiment.

EPS:Chl *a* ratio was calculated in each condition under MI (Figure 44C). There was a significant effect of time (Kruskal-Wallis test, $P < 0.001^{***}$) but no significant effect of condition. In C1, the EPS:Chl *a* ratio decreased strongly from 1382.7 $\pm$ 316.7 to 228.2 $\pm$ 38.4 between day 3 and 7 and remained stable at around 54.7 $\pm$ 16.7 and 26 $\pm$ 5.5 until the end of the experiment. In C2, the ratio decreased from 241.1 $\pm$ 116.2 to 21.7 $\pm$ 5.6 between day 3 and 10 then remained stable at around 19.1 $\pm$ 4.9 until day 24. In C3, the ratio decreased from 439.5 $\pm$ 134.2 to 27.3 $\pm$ 9 between day 3 and 10. The concentration of B-EPS was calculated in each condition under MI (Figure 44D). In C1 the concentration was stable during the first 16 days of the experiment and then varied between 19.5 $\pm$ 1.3 $\mu\text{gGeq cm}^{-2}$ and 21.8 $\pm$ 2.5 $\mu\text{gGeq cm}^{-2}$ between day 3 and 16 before reaching 72.6 $\pm$ 4.8 $\mu\text{gGeq cm}^{-2}$. In C2, it was stable (between 11.8 $\pm$ 2.3 and 22.2 $\pm$ 2.8 $\mu\text{gGeq cm}^{-2}$) until day 16 and reached 92.6 $\pm$ 14.5 $\mu\text{gGeq cm}^{-2}$ at day 24. In the high enrichment condition, it varied ranged from 18.3 $\pm$ 3.7 $\mu\text{gGeq cm}^{-2}$ and 23.4 $\pm$ 4.1 $\mu\text{gGeq cm}^{-2}$ and reached 69.9 $\pm$ 8.7 $\mu\text{gGeq cm}^{-2}$ on the last day.

Chapitre IV : Influence de la structure et du niveau d'eutrophisation sur le développement microphytobenthique

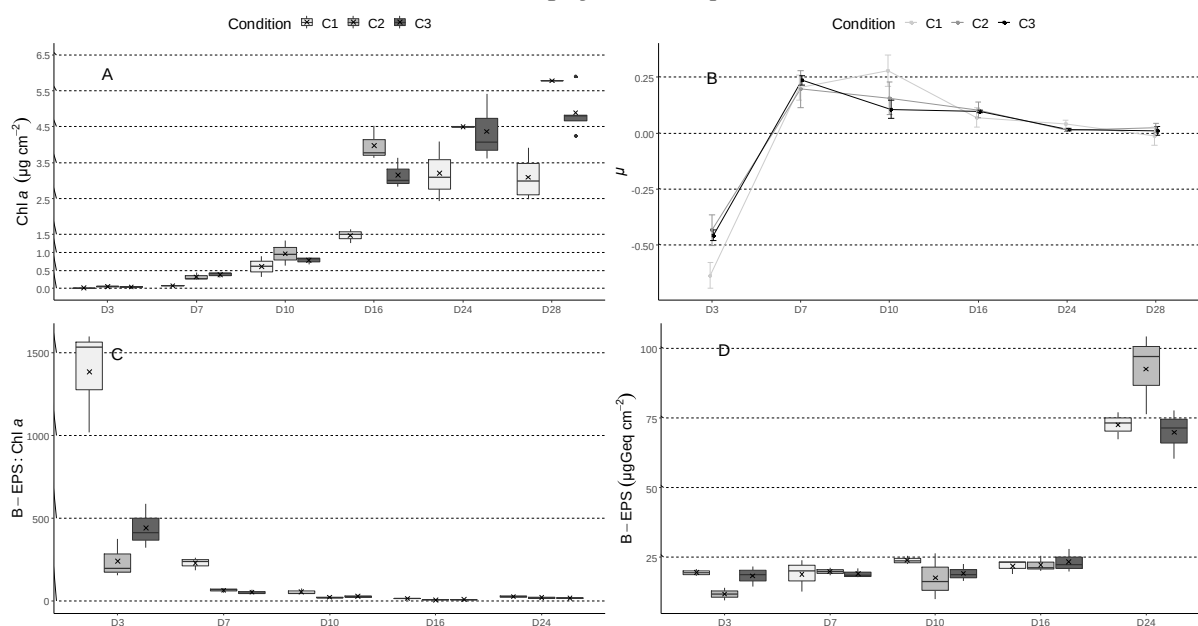


Figure 44 – Changes in Chl *a* biomass ($\mu\text{g.cm}^{-2}$) (A) and associated growth rate kinetics (μ) (B) at days 3, 7, 10, 16, 24 and 28 after the beginning of the experiment on MI. Variation in the EPS:Chl *a* ratio ($\text{mgGeq}/\mu\text{gChl a}$) on MI (C) and in the concentration of B-EPS ($\mu\text{gGeq cm}^{-2}$) (D) during the first 24 days of the experiment on MI.

Chl *a* growth dynamic on PVC structures was less rapid (Figure 45A), in C1, Chl *a* biomass increased until $3.39 \pm 0.22 \mu\text{g cm}^{-2}$ at day 24. In C2, Chl *a* biomass increased to $4.16 \pm 0.50 \mu\text{g cm}^{-2}$ and in C3, to $4.25 \pm 0.43 \mu\text{g cm}^{-2}$. The logistic curve applied to these data showed no significant differences between conditions (Kruskal-Wallis test). Growth rates (Figure 45B) were similar in C2 and C3 with fast and positive dynamics in the first 10 days, after which growth rates were levelled off. In C1, growth dynamics resembled those in C2 and C3 during the first seven days before levelling off until day 10 and then increased strongly from day 10 to 24. Growth rates did not differ significant between MI and PVC in each condition (Student's *t* test).

The EPS:Chl *a* ratio was calculated in each condition on PVC (Figure 45C). A significant effect of time was observed (Kruskal-Wallis test, $P < 0.001^{***}$) but no significant effect of condition. Its global dynamics was approximately the same as on MI (Student's *t* test), the ratio decreased strongly in the first 7-10 days in each condition and then remained stable until the end of the experiment. The EPS concentration (Figure 45D) remained stable in all conditions and increased to $57.2 \pm 10.3 \mu\text{gGeq cm}^{-2}$ on the last day in C1, to $69.6 \pm 10.1 \mu\text{gGeq cm}^{-2}$ in C2, and to $105.5 \pm 35.4 \mu\text{gGeq cm}^{-2}$ in C3.

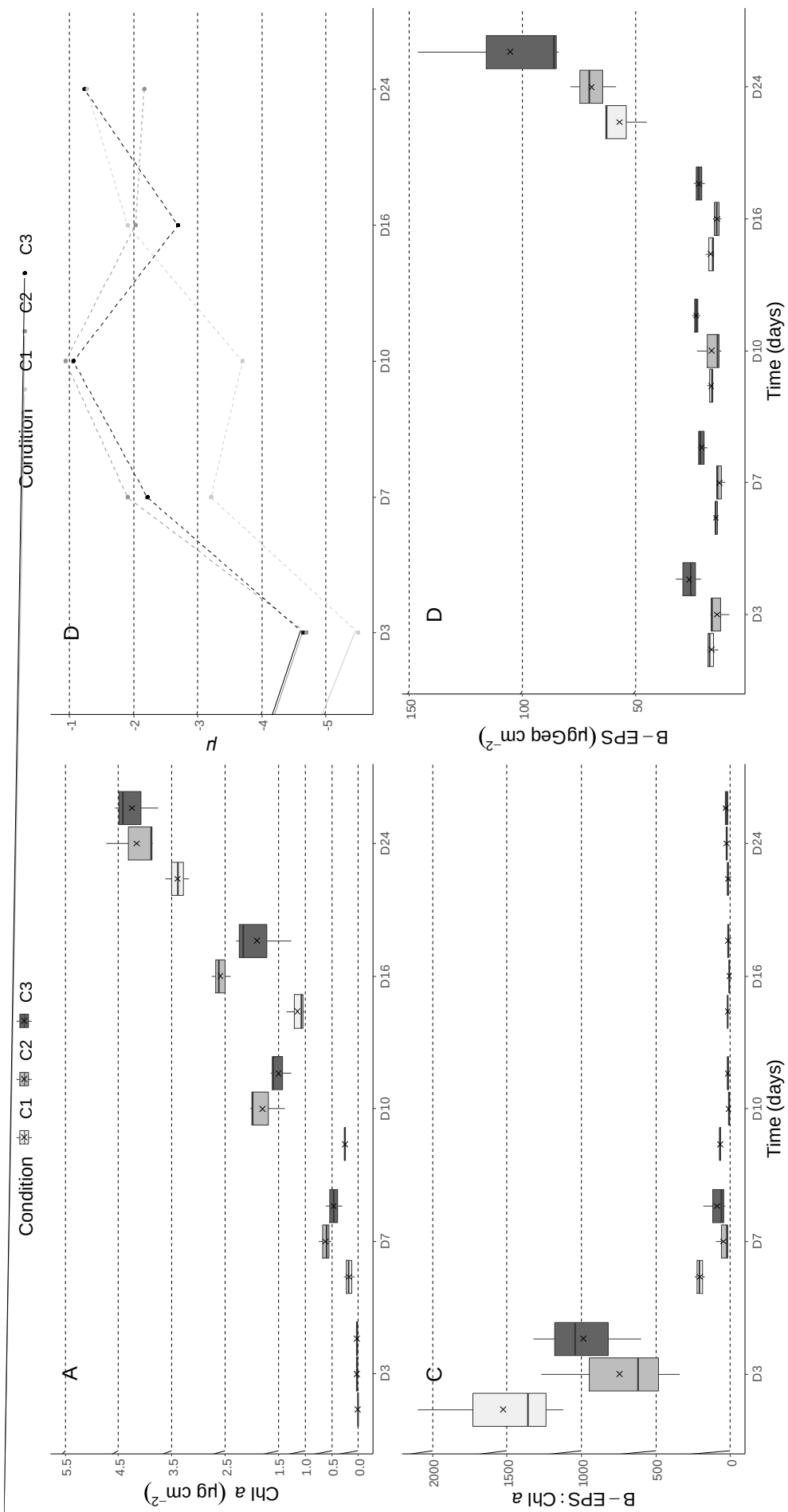


Figure 45 – Variations in Chl a biomass ($\mu\text{g cm}^{-2}$) (A) and associated growth rate kinetics (μ) (B) at days 3, 7, 10, 16, 24 and 28 after the beginning of the experiment on PVC. Variation in the EPS:Chl a ratio ($\text{mgGeq}/\mu\text{gChl a}$) on MI (C) and in the concentration of B-EPS ($\mu\text{gGeq cm}^{-2}$) (D) during the first 24 days of experimentation on PVC.

The averaged value of F_V/F_M was calculated for each MI in each condition at each sampling point (Figure 46A). The split-plot ANOVA showed a significant effect of condition and time on F_V/F_M values ($P < 0.001^{***}$). In C1, F_V/F_M increased continuously from 0.29 ± 0.01 to 0.55 ± 0.01 between day 3 and day 28, but between day 10 and day 18, levelled off at 0.41 ± 0.08 . In C2, F_V/F_M increased continuously from 0.30 ± 0.02 to 0.54 ± 0.004 between day 3 and 14, then decreased to 0.46 ± 0.01 at day 18 and finally reached 0.61 ± 0.004 on the last day. In C3, F_V/F_M increased continuously from 0.29 ± 0.02 to 0.46 ± 0.05 between day 3 and 10, then decreased to 0.44 ± 0.01 and finally reached 0.58 ± 0.008 on the last day. In C3, the coefficient of variation (CV) was higher on day 6 (CV = 11.5%) than on day 28 (CV = 1.3%). In C1, the CV was also higher on day 10 (CV = 11.7%) than on day 28 (CV = 1.7%) indicating that, except on day 3, variability was higher in the first days of the experiment.

The averaged value of $rETR_{MAX}$ was calculated for each MI in each condition at each sampling point (Figure 46B). No significant differences were observed in the three conditions (ANOVA). In C1, $rETR_{MAX}$ increased from 25.9 ± 6.1 rel. unit to 63.5 ± 4.7 rel. unit from day 7 to 24. In C2, $rETR_{MAX}$ decreased from 77.4 ± 9.7 rel. unit to 58.3 ± 4.1 rel. unit between day 7 to 24. In C3, it increased from 84.5 ± 16.2 rel. unit to 105.4 ± 70.2 rel. unit between day 7 and 24.

The NPQ (Figure 46C) was determined under experimental light conditions ($150 \mu\text{mol photons m}^{-2} \text{s}^{-1}$). According to the split-plot ANOVA, there was a significant effect of condition and time on NPQ values. In C1, NPQ showed a strong decrease from 1.32 ± 0.22 to 0.19 ± 0.19 between day 3 and 10. On day 14, it was almost null (0.051 ± 0.008) and remained so for the rest of the experiment. In C2, the NPQ value decreased from 0.95 ± 0.18 to 0.062 ± 0.017 between day 3 and 10. In C3, it decreased from 1.27 ± 0.25 to 0.10 ± 0.02 between day 3 and 10.

The averaged values of ETR_{max}^{a*} (Figure 46D expressed in $\text{mmol e}^- (\text{mg Chl } a \text{ h})^{-1})^{-1}$ were calculated on MI as a function of the a^* . No significant differences were observed between conditions (ANOVA). However, time had a significant effect on these data (ANOVA, $P < 0.01$). In C1 and C2 and on the MI, the dynamics were similar and the values were low at the end of the survey. On the MI in C1, it decreased from $5.42 \pm 3.46 \text{ mmol e}^- (\text{mg Chl } a \text{ h})^{-1})^{-1}$ to $2.54 \pm 0.30 \text{ mmol e}^- (\text{mg Chl } a \text{ h})^{-1})^{-1}$ between day 7 and 24. In C2, its value decreased from $6.15 \pm 2.24 \text{ mmol e}^- (\text{mg Chl } a \text{ h})^{-1})^{-1}$ to $0.54 \pm 0.11 \text{ mmol e}^- (\text{mg Chl } a \text{ h})^{-1})^{-1}$ between day 7 and 24. In C3, the dynamic were different, ETR_{max}^{a*} decreased from $5.39 \pm 0.31 \text{ mmol}$

e^- (mg Chl *a* h)⁻¹)⁻¹ and 2.27 +/- 0.59 mmol e^- (mg Chl *a* h)⁻¹)⁻¹ from day 7 to 16 and reached 5.66 +/- 4.1 mmol e^- (mg Chl *a* h)⁻¹)⁻¹ at day 24. Except in C2, rETR_{MAX} dynamics differed from ETR_{max}^a dynamics.

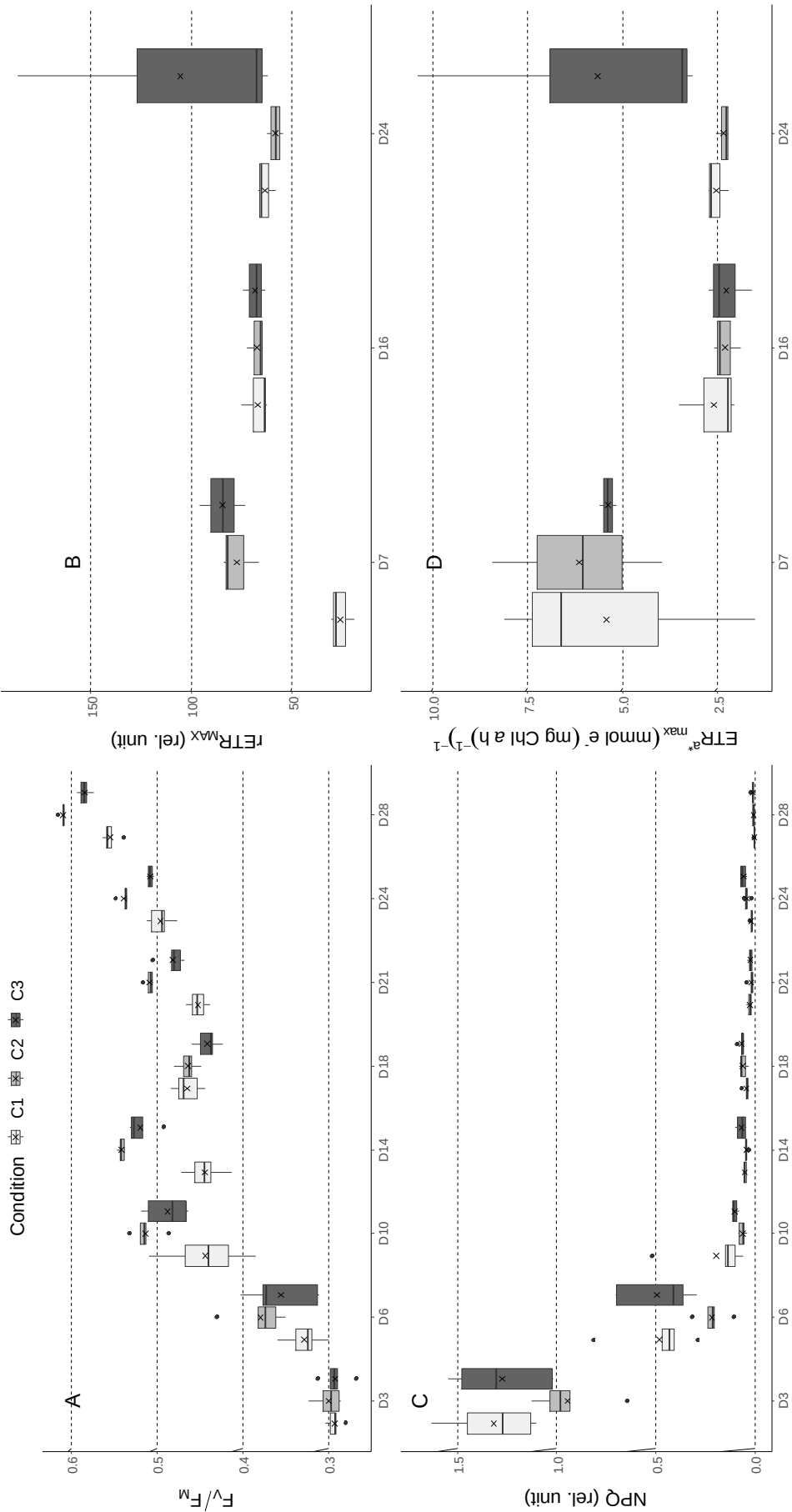


Figure 46 – Biofilm photosynthetic indicators F_v/F_m (A), $rETR_{MAX}$ (B, relative unit), NPQ (C, relative unit at growth irradiance) and $ETR_{a_{max}}^{a_{max}}$ (D, mmol e⁻ (mg Chl a h⁻¹)) during the experiment in the three different conditions (control, medium and high enrichment conditions) on MI.

The averaged value of F_V/F_M was calculated for each PVC in each condition at each sampling point (Figure 47A). According to the split-plot ANOVA, there was a significant effect of condition and time on F_V/F_M values ($P < 0.001^{***}$). In all three conditions, F_V/F_M increased continuously until reaching a plateau on day 10. The highest values were reached on the last day, 0.38 ± 0.01 in C1, 0.52 ± 0.01 in C2 and 0.48 ± 0.01 in C3. The coefficient of variation (CV) was higher in C3 on day 6 (CV = 28.8%) than on day 24 (CV = 1.6%). The CV was also higher in C1 on day 6 (CV = 5.7%) than on day 21 (CV = 0.6%) indicating that, except on day 3, variability was higher in the first days of the experiment.

The averaged value of $rETR_{MAX}$ was calculated for each MI in each condition at each sampling point (Figure 47B). Significant differences were observed within conditions (ANOVA, $P < 0.05^*$). C3 differed significantly from the C1 and C2. In C1, $rETR_{MAX}$ increased from 40.1 ± 7.2 rel. unit to 49.6 ± 6.3 rel. unit from day 7 to 24. In C2, $rETR_{MAX}$ increased from 40.1 ± 3.2 rel. unit to 66.0 ± 1.3 rel. unit between day 7 to 24. In C3, it increased from 56.8 ± 9.3 rel. unit to 70.1 ± 10.8 rel. unit between day 7 and 16 before reaching 50.6 ± 1.0 rel. unit on the last day.

The NPQ (Figure 47C) was determined under experimental light conditions ($150 \mu\text{mol photons m}^{-2} \text{s}^{-1}$). According to the split-plot ANOVA, there was a significant effect of condition and time on NPQ values. In C1, NPQ decreased strongly from 0.36 ± 0.19 to 0.05 ± 0.02 between days 3 and 10. It was almost null from day 14 (0.001 ± 0.002) to the end of the experiment. In C2, it decreased from 0.19 ± 0.05 to 0.01 ± 0.001 between days 3 and 10. In C3, it decreased from 0.22 ± 0.03 to 0.02 ± 0.01 between days 3 and 10 but showed a slight increase (0.10 ± 0.12) on the last day.

The average values of $ETR_{max}^{a^*}$ (Figure 47D expressed in $\text{mmol e}^- (\text{mg Chl } a \text{ h})^{-1}$) were calculated on PVC as a function of the a^* . Significant differences were observed between nutrient conditions (ANOVA, $P < 0.001^{***}$). C2 differed significantly from C1 and C3 (Tukey test). The interaction between time and nutrient condition also had a significant effect (ANOVA, $P < 0.01$). In C1, it decreased from $3.24 \pm 1.76 \text{ mmol e}^- (\text{mg Chl } a \text{ h})^{-1}$ to $1.19 \pm 0.26 \text{ mmol e}^- (\text{mg Chl } a \text{ h})^{-1}$ between day 7 and 16 but increased to reach $4.19 \pm 0.59 \text{ mmol e}^- (\text{mg Chl } a \text{ h})^{-1}$ on the last day. In C2, $ETR_{max}^{a^*}$ showed a slight decrease from $1.38 \pm 0.17 \text{ mmol e}^- (\text{mg Chl } a \text{ h})^{-1}$ to $0.54 \pm 0.11 \text{ mmol e}^- (\text{mg Chl } a \text{ h})^{-1}$ between day 7 and 24. In C3, $ETR_{max}^{a^*}$ remained constant at $2.1 \pm 0.56 \text{ mmol e}^- (\text{mg Chl } a \text{ h})^{-1}$ between day 7 and 16 and decreased slightly to $1.7 \pm 0.69 \text{ mmol e}^- (\text{mg Chl } a \text{ h})^{-1}$ on the last day.

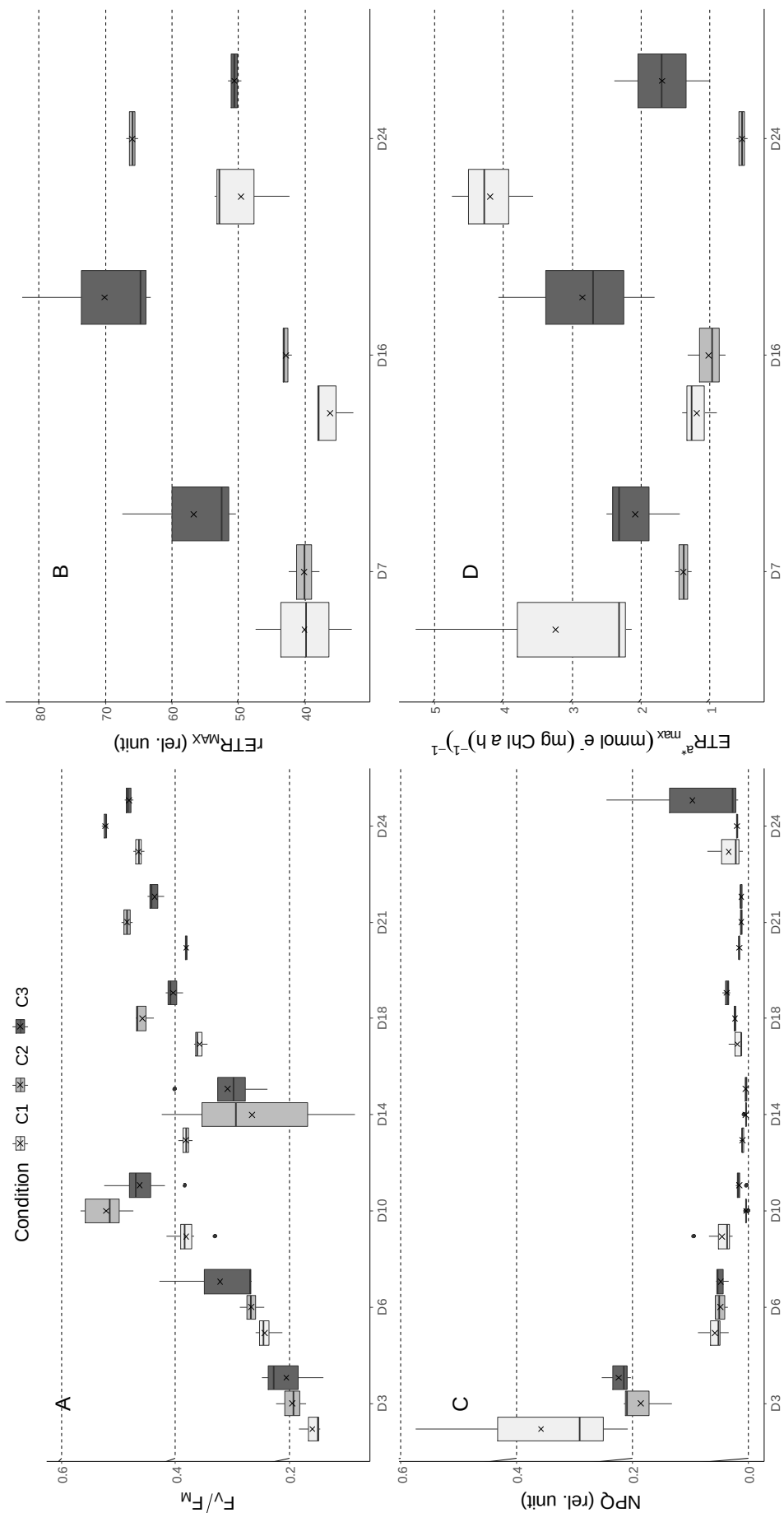


Figure 47 - Biofilm photosynthetic indicators F_v/F_m (A), $rETR_{MAX}$ (B, relative unit), NPQ (C, relative unit at growth irradiance) and $ETR_{a^{max}}$ (D, mmol e^- (mg Chl a h) $^{-1}$) during the experiment in the three different conditions (control, medium and high enrichment conditions) on PVC.

The averaged values of a^* (Table 7, expressed in $\text{m}^2 \text{mg Chl } a^{-1}$) on rough concrete structures (MI) revealed an overall decrease in each condition. Significant differences were observed over time (ANOVA, $P < 0.01^{**}$) but no significant differences were observed between conditions. A marked decrease was observed between day 7 and 10, particularly in C1 (from $0.084 \pm 0.057 \text{ m}^2 \text{mg Chl } a^{-1}$ to $0.014 \pm 0.002 \text{ m}^2 \text{mg Chl } a^{-1}$). In C2, the a^* was lower than in the control at the beginning of the survey ($0.031 \pm 0.015 \text{ mg Chl } a^{-1}$ on day 7). In C3, the a^* was lower on day 7 ($0.023 \pm 0.004 \text{ mg Chl } a^{-1}$). On the PVC structures (Table 7), the a^* dynamics observed in all conditions were comparable to the dynamic observed on the MI. Significant differences were observed between conditions (ANOVA, $P < 0.001^{***}$), a^* values in the control differed significantly from the values in the other conditions. a^* was highest in the early days of monitoring, especially in C1 ($0.033 \pm 0.024 \text{ m}^2 \text{mg Chl } a^{-1}$ at day 7). In C2, a constant decrease was observed between day 7 and 24, from $0.015 \pm 0.003 \text{ m}^2 \text{mg Chl } a^{-1}$ to $0.002 \pm 0.001 \text{ m}^2 \text{mg Chl } a^{-1}$. In C3, the a^* remained stable at around $0.015 \text{ m}^2 \text{mg Chl } a^{-1}$ throughout the survey. In C1, a marked increase reaching $0.032 \pm 0.006 \text{ m}^2 \text{mg Chl } a^{-1}$ was observed on day 24. The averaged values of α^{a^*} (Table 7, expressed in $\text{mmol } e^- \text{mg Chl } a \text{ h}^{-1} (\mu\text{mol photons})^{-1}$) was calculated on MI and PVC structures as a function of the a^* . No significant differences were found between conditions. On PVC structures, there were a significant effect of nutrient condition (ANOVA, $P < 0.01^{**}$) and of the interaction between time and nutrient conditions (ANOVA, $P < 0.01$) C2 differed significantly from C1 and C3. In C1, α^{a^*} values remained stable at $0.008 \pm 0.001 \text{ mmol } e^- \text{mg Chl } a \text{ h}^{-1} (\mu\text{mol photons})^{-1}$ until day 10 before reaching a plateau at $0.015 \pm 0.003 \text{ mmol } e^- \text{mg Chl } a \text{ h}^{-1} (\mu\text{mol photons})^{-1}$ on day 24. In C2, α^{a^*} values were higher than in C1 in the first 10 days ($0.013 \pm 0.002 \text{ mmol } e^- \text{mg Chl } a \text{ h}^{-1} (\mu\text{mol photons})^{-1}$ on day 10) before decreasing to $0.009 \pm 0.002 \text{ mmol } e^- \text{mg Chl } a \text{ h}^{-1} (\mu\text{mol photons})^{-1}$ on day 24. In C3, α^{a^*} decreased slightly decrease $0.014 \pm 0.005 \text{ mmol } e^- \text{mg Chl } a \text{ h}^{-1} (\mu\text{mol photons})^{-1}$ to $0.010 \pm 0.002 \text{ mmol } e^- \text{mg Chl } a \text{ h}^{-1} (\mu\text{mol photons})^{-1}$ between day 7 and 16 before reached $0.022 \pm 0.018 \text{ mmol } e^- \text{mg Chl } a \text{ h}^{-1} (\mu\text{mol photons})^{-1}$ on day 24.

Table 7 – a^* in $m^2\ mg\ Chl\ a^{-1}$ and α^* $mmol\ e^{-}\ mg\ Chl\ a\ h^{-1}\ (\mu mol\ photons)^{-1}$ on marine infrastructures (MI) and PVC during the survey in each condition.

Structure	Condition	Date	a^*		α^*	
			Mean	SD	Mean	SD
MI	C1	D7	0.084	0.057	0.014	0.009
		D16	0.014	0.003	0.019	0.002
		D24	0.015	0.001	0.016	0.007
	C2	D7	0.031	0.015	0.018	0.006
		D16	0.013	0.003	0.016	0.005
		D24	0.015	0.002	0.014	0.009
	C3	D7	0.023	0.004	0.016	0.002
		D16	0.012	0.003	0.01	0.002
		D24	0.02	0.001	0.02	0.008
PVC	C1	D7	0.033	0.024	0.015	0.008
		D16	0.012	0.002	0.021	0.002
		D24	0.032	0.006	0.039	0.016
	C2	D7	0.015	0.003	0.016	0.001
		D16	0.009	0.002	0.012	0.003
		D24	0.002	0.001	0.003	0.001
	C3	D7	0.014	0.006	0.016	0.008
		D16	0.015	0.004	0.011	0.003
		D24	0.013	0.005	0.016	0.008

III.4. Correlation between microtopography versus biofilm biomass and photosynthetic indicators

Correlation coefficients (R) were calculated between the level of microtopography (negative or positive) and the Chl *a* biomass, F_v/F_m , $rETR_{MAX}$, α and NPQ. $R > 0.1$ or < -0.1 were considered significantly positive or negative (critical values in a Student's t test with the sample pixel frequency). A positive R means the positive values of the biofilm variable were found on top of small relief irregularities while, a negative R indicated that the positive values of the measured biofilm variable were found in the small holes and crevices (negative microrelief). Regarding the correlation with Chl *a* biomass, the relationship was significantly more positively significant in C1 than in C2 and C3 (ANCOVA, $P < 0.001^{***}$). R was positive in C1 (0.16 +/- 0.05), null in C2 and C3 (0.0098 +/- 0.032 and -0.034 +/- 0.053). Regarding the correlation with F_v/F_m , no significant differences were found between the three conditions,

negative correlation values found for each condition. There was no significant difference in the correlation between rugosity and $rETR_{MAX}$ in the three conditions (Figure 48). The correlation with α was significantly higher in C1 (0.098 ± 0.047), than in C2 and C3, with a negative R in C2 and C3 (-0.025 ± 0.074 and -0.043 ± 0.042). The differences between nutrient conditions were significant (ANCOVA, $P < 0.01^{**}$). Finally, the correlation between rugosity and NPQ was calculated (Figure 48) and no significant differences were found between conditions.

III.5. Species identification and diversity

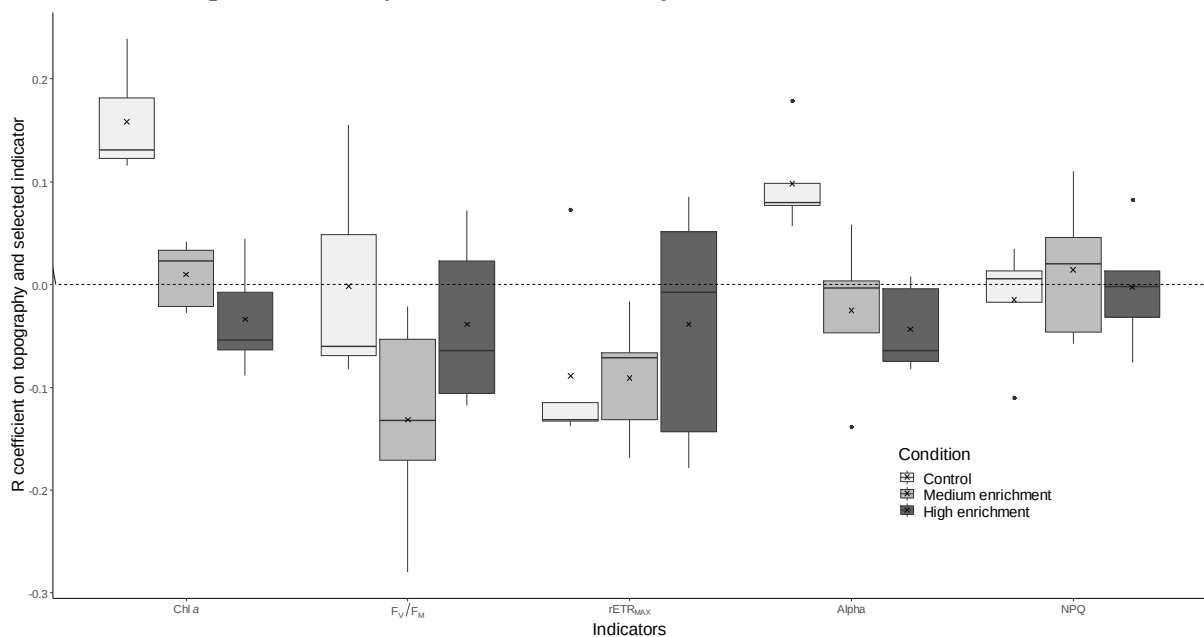


Figure 48 – Correlation coefficients (R) between microtopography and Chl a biomass or biofilm physiological indicators F_v/F_m , $rETR_{MAX}$, Alpha and NPQ in the three different conditions (control, medium and high enrichment conditions) at the end of the experiment (day 28).

SEM observations revealed a wide range of micro-organism species in diverse proportions (appendix F). Pennate diatoms, *Cylindrotheca closterium*, *Diploneis* sp. and *Entomoneis* sp. were abundant. Many biofilm fragments with high densities of cells embedded in a matrix of EPS were found with diverse species such as *Amphora* sp., *Thalassiosira* sp., *Cylindrotheca closterium*, *Diploneis* sp. and *Entomoneis* sp. Several solitary and rosette colonies of choanoflagellates were found with some protists (*Paulinella ovalis*).

IV. Discussion

The biofilm mainly comprised pennate diatoms associated with a few protozoans as usually observed in epilithic biofilm on rocky shores (Arndt et al., 2003). Some plankton species like *Thalassiosira* sp. were also observed due to their sedimentation. Thanks to their raphe, pennate

diatoms can adhere to the hard substrates with capsule EPS and are also able to move over the surface (Round et al., 1990) making them the dominant group of many biofilms on different substrates (Patil & Anil, 2005; Wigglesworth-Cooksey & Cooksey, 2005; Salta et al., 2013).

IV.1. *Influence of the nutrient conditions on biofilm development and EPS secretion*

Nutrient availability is one of the main drivers of plankton and benthic microalgae growth, which can lead to the eutrophication of coastal ecosystems in the case of excessive inputs (Rabalais, 2009). Eutrophication phenomena are usually associated with high phytoplankton biomass. The effects of eutrophication on the biofilm have been investigated more rarely. However, nutrient run-offs can modify the assemblage of species of microorganisms that comprise the biofilm (Fricke et al., 2016; Lawes et al., 2016). Changes in Bacterial and diatom assemblage can influence succession and lead to a different assemblage at equilibrium point (Fricke et al., 2016). In contrast to phytoplankton, several studies highlight the moderate or null influence of nutrient inputs on biofilm biomass on rocky shores (Sanz-Lázaro et al., 2011; Lawes et al., 2016). The main difference between phytoplankton and biofilm habitats is the space available for community development. In the water column, the availability of space is not a restricting factor even if high biomass can limit access to light. On hard substrates, this factor is often identified as the major driver of primary producers (Zhang et al., 1995). Our study showed no significant enhancement of biofilm biomass when the nutrient concentration was higher. Indeed, the growth rate kinetics and the total Chl *a* biomass was not significantly affected in the nutrients enriched conditions. Even if the total biofilm biomass at a given time differed between conditions, the growth rate of the biofilm was not significantly affected by the nutrient inputs. In the control condition, biofilm biomass reached a stationary phase at day 24 but was still growing in the two enriched conditions. Belando *et al.*, (2017) showed that biofilm biomass growing on glass plates was not significantly enhanced by nutrient enrichment alone. Sanz-Lázaro *et al.*, (2011) showed that biofilm biomass remained constant under an *in situ* eutrophic gradient. Our results are in accordance with the latter study and suggest that another limiting factor influenced biofilm growth. Considering the generally favourable environmental conditions that prevailed during the experiment, the main limiting factor may be the size of the colonized structures. Indeed, the absence of a positive effect of nutrient enrichment on diatom growth can be explained by strong competition for the available space. Even though our results revealed no limitation in the two nitrogen enriched conditions, the stationary phase was reached earlier in the control condition than in the two other conditions, although there could be a slight

effect of nutrient enrichment during the first stages related to colonisation. Biofilm growth was also concomitant with the strong decrease in NH_4^+ decrease in all three conditions indicating a strong denitrification process in the early stages of biofilm development. The NH_4^+ assimilation differed as a function of the NO_3^- enrichment conditions. Preferential use of NH_4^+ over other sources of nitrogen has already been demonstrated on hard substrate marine biofilms (Magalhães et al., 2005).

EPS are known to protect cells against environmental stress such as toxic substances or microbial agents. EPS are also important for the colonisation processes (substrate adhesion, cell aggregation) (Flemming & Wingender, 2010) and are even more important for the formation of the biofilm (Orvain et al., 2003). The EPS:Chl *a* ratio was high in the first three days of the experiment in all three conditions, underlining the importance of EPS for diatom adhesion in the first stages of biofilm development (Sutherland et al., 1998; Orvain et al., 2003). Moreover, numerous cells embedded in the matrix of EPS were observed by SEM, confirming the high exudation recorded (appendix G). However, no differences were observed between the different nutrient conditions in terms of diversity or abundance using this technique. Moreover, the elevated EPS:Chl *a* ratio in the early days of the survey in the control condition may be due to the ability of EPS to increase the adsorption capacity of biofilms by providing potential binding sites for inorganic nutrients (Sabater et al., 2002; Li et al., 2016). EPS exudation must play a role in the efficiency of benthic diatoms to capture EPS (de Brouwer et al., 2001, Orvain et al., 2003). Our results confirmed the relationship between EPS exudation and nutrient availability because the larger quantity of EPS recorded at the end of monitoring in the high enrichment condition (C3) must correspond to a senescent phase after the plateau (Staats et al., 2000; Yallop et al., 2000; Orvain et al., 2003). Biofilm can easily be detached by tidal currents in such a senescent phase with high levels of EPS stimulating bacterial hydrolytic processes ((Yallop et al., 2000; Orvain et al., 2003). On MI and during the first days of the experiment, this ratio was higher in the control condition (C1) than in the nutrient enrichment ones (C2 and C3), combined with the lower biofilm biomass in the first two weeks in the control condition, with a drastic increase between day 16 and 24. This result demonstrates that nitrogen limitation may be responsible of higher EPS secretion rates, thereby underlining the key role of nutrient availability in adhesion cell processes related to glycoproteins (Staats *et al.*, 2000; Orvain *et al.*, 2003, Gerbersdorf et al. 2009, Orvain et al., 2014). This phenomenon was even clearer on PVC structures with higher concentrations of EPS than on MI, probably due to the absence of surface roughness. Indeed, it has been shown that surface microtopography plays a determining role in

biofilm adhesion (Hutchinson et al., 2006; Salta et al., 2013; Ditsche et al., 2014, 2017; Vivier et al., 2021a). On a smooth surface, like that of the PVC structures, a larger quantity of EPS is probably necessary to enable adhesion of diatoms on the surface. Our results confirmed that concrete material can be efficiently colonised by diatoms and underline the advantages of such structures for the establishment of ecological succession. This could be related to the fact that rough structure provides a larger contact surface for binding EPS within biofilms, and a smaller quantity of EPS is thus required to embed all the cells that comprise the biofilm.

IV.2. *Influence of the nutrient condition on biofilm photochemistry and photosynthetic performances*

The maximum quantum efficiency of PSII (*i.e.* F_v/F_m) was associated with the physiological state of the biofilm (Kromkamp et al., 1998; Morris & Kromkamp, 2003; Jesus et al., 2005). F_v/F_m higher than 0.5 is considered to be a good physiological status and frequently assessed in marine benthic diatoms and phytoplankton (Morris & Kromkamp, 2003; Napoléon et al., 2013b). In our experiment, F_v/F_m was always higher in the medium enrichment condition (+ 30 $\mu\text{mol L}^{-1}$ N:P:Si) (C2) than in the two other ones (C1 and C3). F_v/F_m was higher than 0.5 on day 10 in the medium enrichment condition (C2) versus day 14 in the high enrichment condition (C3) and on day 24 in the control condition (C1). Our results show that, in limited amounts, nutrient inputs may have a positive impact on the physiological status of the diatoms. Indeed, the physiological status of the diatoms in the medium enrichment condition (C2) was better than in the high enrichment condition (C3). Nutrient availability, in particular nitrogen availability, is a key driver of diatom growth and photobiology (Van der Grinten et al., 2004; Sanz-Lázaro et al., 2011; Napoléon et al., 2013b; Fricke et al., 2016; Lawes et al., 2016; Brodersen et al., 2020; Serre-Fredj et al., 2021). Like in the case of biofilm biomass, our results showed no positive impact of an increase in nutrient availability on the physiological status of the diatoms. The elevated ETR_{max}^* on MI at the end of the experiment in the high enrichment condition (C3) pointed to higher photosynthetic capacity in these diatoms. The slight decrease in F_v/F_m between day 14 and 18 may be due to nutrient limitation (Parkhill et al., 2001; Sheng et al., 2011). Indeed, nutrient stress can lead to a physiological response of the diatoms, which in turn, affects PSII efficiency and the rest of the photosynthetic apparatus, leading to a decrease of the F_v/F_m as already documented in phytoplankton and which we observed on biofilms (Napoléon et al., 2013b). However, this temporary limitation seemed to have a minor impact on the biofilm biomass and photosynthesis because of the quick recovery after day 18. Under certain circumstances, regulation of excess light is necessary to balance the utilisation of energy

and minimise potential damage, for example through NPQ (Ralph et al., 2010). This photo-protective mechanism is one of the most important mechanisms for diatoms, especially on hard substrates because of the impossibility of vertical migration. The diatom *Cylindrotheca closterium* was dominant in all three conditions, suggesting potentially excess light may have affected its photosynthetic performances and growth, as already documented (Rijstenbil, 2003; Roncarati et al., 2008). The NPQ felt measured, in all three growing conditions during the first six days on MI and PVC corresponded to the light acclimation period. During this period, the biofilm in the medium enrichment condition (C2) appeared to be better photo-acclimated than in the other two conditions and tends to confirm previous observations of the higher photosynthetic performances of these diatoms. Another important mechanism of acclimation to light or to nutrient availability is the variation in the chlorophyll-specific optical-absorption cross section (a^*) (Kromkamp et al., 2008; Napoléon et al., 2013b). Our results showed significant differences in a^* after seven days of survey. A lower a^* was observed in the medium and high enrichment conditions in comparison with the control. However, these differences disappeared after 10 days of monitoring. On the PVC structures, an elevated value of a^* was recorded in the control condition (C1) and, in lower proportions, in the high enrichment one (C3). These results are in agreement with those of previous studies on the effect of nutrient limitation on phytoplankton photobiology. Indeed, several studies already reported an increase in a^* under nutrient limitation (Geider et al., 1998; Napoléon et al., 2013b). As explained by Falkowski and Raven, (2007) high irradiance leads to an increase in a^* due to a decrease in the package effect. The specific dynamic of a^* values recorded on the PVC plates at the end of the survey may thus be due to the higher amount of light received by the diatoms on this surface than on MI, where rugosity offers shelter from excess light. In addition, with the lowest amount of nutrient available, the higher photo-inhibition recorded in the control condition reduced photosynthesis and growth. Differences in diatom physiological status between control and nutrient enriched conditions could also explain the differences in the concentrations of EPS at the end of the experiment. Indeed, EPS exudation also plays an important role in the physiology and photosynthesis performance of algae, as already demonstrated (Staats *et al.*, 2000, Underwood et al., 2004). The estimation of $ETR_{max}^{a^*}$ at several sampling points revealed a higher electron transport rate from PSII for diatoms colonising the MI, especially in the high enrichment condition (C3). Considering the elevated biomass in the medium enrichment condition, the decrease of the $ETR_{max}^{a^*}$ over time and the low a^* at the end of the survey was unexpected. These results may be due to an acclimation process. Indeed, the increase in biomass in the medium enrichment condition over the course of the experiment reduced light penetration

and increased the self-shading mechanisms in thick biofilms and the availability of light for photosynthesis (Huisman & Weissing, 1994). This phenomenon could lead to an increase in the Chl *a* concentration in the PSII antenna (Falkowski & LaRoche, 1991; MacIntyre et al., 2002). This regulation has been associated with an internal increase in pigment (Chl *a*) self-shading thereby reducing the chlorophyll-specific absorption cross section (α^*) and has already been reported in a phytoplankton experiment under nutrient enrichment (Serre-Fredj et al., 2021). The α^* did not show a clear pattern on MI during our experiment. In comparison, the α obtained with the imaging-PAM measurements (appendix F) revealed a constant increase in all three conditions over the course of the experiment. The use of variable fluorometry (such as PAM fluorometry) allows non-invasive, fast and simple estimation of relative photosynthetic parameter but with the risk of overestimating these indicators (as could be the case in our study). It should therefore be used in association with another method such as chlorophyll-specific absorption cross section measurements for the evaluation of ETR_{max}^* and α^* , which enable the characterisation of acclimation versus environmental stress, for example, excess light or lack of light or nutrient availability, leading to reorganisation and/or a reduction in pigment content which can affect photosynthetic efficiency (Morelle & Claquin, 2018).

IV.3. *Influence of microtopography on biofilm colonisation and physiological indicators*

The biofilm formation process is a complex phenomenon involving several species like bacteria, diatoms, fungi, protozoans, etc. This process is also influenced by environmental conditions including nutrient availability, temperature, light, and pH (Rijstenbil, 2003; Jackson et al., 2013; Mangwani et al., 2016). The availability and layout of micro-habitats is also a key factor in biofilm development (Vivier et al., 2021a). Increased roughness may also promote microbial diversity (Štifterová & Neustupa, 2017). In order to explain the influence of roughness on biofilm development and photosynthesis, the microtopography of each structure was assessed at the end of the experiment and compared with biofilm photosynthetic parameters or biomass. The microtopography of the surface of the substrate has already been shown to play an important role in the adhesion of biofilm cells. However this phenomenon is poorly documented for marine biofilms (Hutchinson et al., 2006; Souche et al., 2016; Vivier et al., 2021a).

The impact of surface microtopography on biofilm biomass has already been studied. Indeed, it has been shown that diatom and cyanobacteria abundance are favourably affected by

surface roughness on intertidal rock surfaces (Hutchinson et al., 2006). The influence of substrate topography or instability on biofilm biomass is well known particularly in intertidal mudflat systems where the diatom cover and biomass decrease in areas exposed to physical disturbances (Weerman et al., 2010). On hard substrates, the effect of micrometric roughness on the bacterial attachment is almost instantaneous (Magin et al., 2010). A previous study by our team? on comparable structures revealed a significant relationship between surface roughness and biofilm biomass. Indeed, a strong negative correlation was recorded between the level of rugosity and the Chl *a* biomass (Vivier et al., 2021a). What is more, other authors have already investigated the impact of surface micro-scale rugosity on biofilm growth (Giannantonio et al., 2009; Sweat & Johnson, 2013). However, these studies did not consider potential nutrient inputs and their implication in biofilm formation on hard rough substrates. In the present study, a positive correlation was found between Chl *a* biomass and the degree of roughness in the control condition (C1) at the end of the experiment. This correlation was lower in the medium enrichment condition (C2) and negative in the high enrichment condition (C3). At the same time, patchy biofilm colonization was visually observed in all three conditions. These results suggest that nutrient inputs could be involved in the biofilm colonization process. Moreover, the assessment of the correlation between surface rugosity and F_v/F_m or $rETR_{MAX}$ was also negative, independent of the condition. A stronger negative correlation was recorded for F_v/F_m in the medium enrichment condition than in the two other conditions. These results confirm the involvement of surface rugosity in biofilm biomass and its photosynthetic performance. Indeed, even if the biomass on positive reliefs was highest in the control condition, this biomass did not correspond to diatoms with a good physiological status (*i.e.* with elevated photosynthetic performances). This result is supported by the ETR_{max}^{a*} , which highlighted the low productive capacity and by the weak photosynthetic efficiency estimated with a^* (α^{a*}) in this same condition on the MI at the end of the experiment. However, this is moderated by the strong positive correlation in the control condition between α and the surface microtopography, indicating that the high biofilm biomass on the elevated relief of the MI showed strong photosynthetic efficiency. Our results show that nutrient enrichment can strongly affect the relationship between a biofilm and substrate rugosity by enhancing the colonization process in negative reliefs. The strong negative correlation between F_v/F_m and the level of rugosity in the medium condition confirms that, in limited amounts, nutrient input can have positive impact on the physiological status of biofilm cells as previously discussed. The same process was observed in the high enrichment condition (C3) with a negative correlation between some photosynthetic indicators (F_v/F_m and $rETR_{MAX}$) indicating the benefits of a

negative relief for biofilm development and photobiology. This result is also supported by the elevated $ETR_{max}^{a^*}$ and α^{a^*} in C3 at the end of the experiment. The assessment of NPQ showing that photo-inhibition was higher on neutral – positive reliefs revealed more photo-acclimated cells in microhabitats. The interactions between substrate structure and nutrient availability play a key role in the acclimation process of the benthic diatoms, as supported by the elevated productivity capacity ($ETR_{max}^{a^*}$) and efficiency (α^{a^*}) in the high enrichment condition (C3) at the end of the experiment. Considering previous results and statements concerning the lack of influence of nutrient inputs on biofilm growth and photobiology, microtopography analyses are a determining indicator to understand the limiting factors. The present study showed that more biofilm biomass was present in the negative reliefs of MI than on PVC structures, and that this biofilm was also well acclimated and photosynthetically more active. In the context of eutrophication, the addition of surface rugosity to the structure to be colonized will create favourable conditions and enhance the biofilm photoacclimation.

Briefly, surface rugosity combined with nutrient inputs had a significant impact on biofilm biomass and its photosynthetic performance. Indeed, the negative correlation between the rugosity level and the Chl *a* biomass or other photosynthetic indicators in the enriched conditions (C3) confirm the importance of the surface of the structure and the creation of microhabitats to promote the development of biofilm. At a larger scale, e.g., ecosystem restoration projects or other applications in a nutrient rich environment like harbours, these results clearly show that the surface roughness of the structure is a key factor which improved the quality of the biofilm. A more competitive biofilm will be more stable and will provide a better source of nutrition for the primary consumers and will promote the establishment of ecological succession (Jenkins et al., 2001; Hutchinson et al., 2006).

IV.4. Conclusion

The present study showed that rough concrete is an efficient material for colonization by biofilms. The growth and photobiology of the biofilm were not significantly influenced by the availability of inorganic nutrients. The combination of imaging-PAM and 3D camera measurements provided reliable data on the influence of micro-scale rugosity of the surface on the growth and photo-chemistry of the biofilm and these results underline the benefits of rugosity on these indicators. The a^* measurements enabled characterisation of maximum production capacity ($ETR_{max}^{a^*}$) and photosynthetic efficiency (α^{a^*}) and showed better production capacity of biofilms growing in nutrient enriched conditions, especially the biofilm

on the rough concrete structures (MI). These results underline the importance of chlorophyll-specific absorption cross section measurements for the evaluation of ETR_{max}^a and α^a which enabled the characterisation of the acclimation process. This study provides innovative results on the influence of the surface structure on biofilm establishment and photosynthesis. This study also provides interesting results on the absence of significant influence of nutrient inputs on the biofilm growth and photosynthesis. These results will be useful for further study or environmental restoration projects in anthropized environments and highlight the importance of surface structure in promoting the development of biofilms in such environments. Indeed, at larger scale, these results suggest that eutrophication may have limited impacts on the biofilm establishment. Our investigation of the relationship between the influence of nutrient availability and surface rugosity provides reliable data showing that in the case of elevated eutrophication, growth and photosynthesis of biofilm is enhanced in the negative reliefs of the colonized structure. These results once again emphasize the importance of the design of the surface of artificial reefs or marine infrastructure in the global context of anthropogenic run-offs and coastal eutrophication.

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CHAPITRE V : DYNAMIQUE DE COLONISATION D'INFRASTRUCTURES
MARINES *IN SITU*

Partie V-A : Colonisation of artificial structures by primary producers:
interspecies competition and photosynthetic behaviour

This work is under review in Ecological Engineering

Résumé

A la suite des premières expérimentations réalisées en conditions contrôlées sur des petites infrastructures expérimentales (dallettes), une approche *in situ* sur les mêmes modèles a été réalisée. L'objectif était d'étudier la dynamique de colonisation benthique sur les mêmes structures dans un contexte environnemental complexe et sur les mêmes sites d'études que ceux utilisés pour les blocs de digues MARINEFF. La croissance microphytobenthique, la diversité en macroalgues et les paramètres photosynthétiques associés à ces producteurs primaires ont été mesurés entre septembre 2019 et avril 2021.

La complexité de surface d'une structure artificielle peut fortement influencer le développement du biofilm épilithique marin (Hutchinson et al., 2006; Souche et al., 2016) et la structure ou la diversité des communautés benthiques en général (Baine, 2001; Bellou et al., 2012; Jaxion-Harm & Szedlmayer, 2015; Vivier et al., 2021a). Nos résultats ont montré de fortes différences entre les deux sites concernant la croissance microphytobenthique, cependant, aucune différence significative concernant cet indicateur n'a été observée entre les dalles lisses et rugueuses à l'inverse des expérimentations précédentes. L'absence de dynamique claire entre les conditions de rugosité dans notre étude pour la biomasse du biofilm peut s'expliquer par les conditions environnementales et l'échelle de temps importante entre les échantillonnages. En termes de photosynthèse et à l'échelle de l'ensemble de la dalle, la dynamique globale du F_v/F_m , $rETR_{MAX}$ et α est semblable à celle de la biomasse microphytobenthique. Ces résultats ont mis en évidence la relation étroite entre la dynamique de la biomasse et des paramètres photosynthétiques du biofilm en milieu naturel. La biomasse microphytobenthique est significativement plus faible en baie de Seine qu'en rade de Cherbourg. La faible biomasse enregistrée pour la baie de Seine MI peut s'expliquer par des facteurs abiotiques importants comme la turbidité élevée de l'eau et l'hydrodynamisme plus important. Les indicateurs photosynthétiques (F_v/F_m , α et $rETR_{MAX}$) sont globalement plus faibles sur les dalles rugueuses que sur les lisses. De plus, le NPQ plus élevé sur les structures rugueuses en baie de Seine indique une photoacclimatation plus forte pour ces biofilms.

La colonisation par une algue encroûtante subtidale *Phymatolithon purpureum* sur les dalles de la rade de Cherbourg a été observée et sa phase de croissance s'est étendue de février à septembre 2020 pour atteindre environ 75% de la surface de la dalle en termes de recouvrement. Le pourcentage de couverture sur les dalles lisses est légèrement plus élevé que sur les dalles rugueuses et semble indiquer un meilleur taux de réussite pour les surfaces

de structures non modifiées. La biologie de *Phymatolithon purpureum* et des algues encroûtantes de climat tempéré en général est mal documentée, leur physiologie et leur photobiologie dans des environnements comparables n'ont pas été étudiées. L'utilisation de la fluorométrie de type IMAGING-PAM a permis la spatialisation des paramètres photosynthétiques. Connaissant les zones colonisées par *Phymatolithon purpureum*, il a été possible de discriminer les mesures à l'intérieur et à l'extérieur de cette zone. L'étude de la répartition spatiale de plusieurs indicateurs photosynthétiques et de la biomasse photosynthétiquement active a été réalisée à trois points d'échantillonnage représentatifs de la dynamique de croissance de cette algue (colonisation précoce, exponentielle et stabilisée). Ces résultats ont montré un schéma clair entre l'algue encroûtante et les autres zones. Au cours de la phase précoce de la colonisation et de la croissance exponentielle (de février à juillet 2020), le F_V/F_M , le $rETR_{MAX}$ et α étaient significativement plus élevés à l'extérieur de la zone de *Phymatolithon purpureum* qu'à l'intérieur de celle-ci. Ces résultats indiquent la faible efficacité photosynthétique et la faible capacité de production de *Phymatolithon purpureum* par rapport au microphytobenthos présent en dehors des zones colonisées. Compte tenu de la faible biomasse microphytobenthique en juillet 2020, ces résultats ont tendance à montrer la forte concurrence entre les biofilms phototrophes et l'algue encroûtante pour l'espace disponible et sous l'influence de facteurs environnementaux tels que la lumière et les nutriments disponibles.

Colonisation of artificial structures by primary producers: interspecies competition and photosynthetic behaviour

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Abstract

In photic subtidal marine environments, the colonisation of artificial structures by primary producers is an important determinant of their success and of ecological succession. In this context, colonisation by microphytobenthic biofilm and macroalgae and photosynthesis need to be studied as a function of abiotic pressures. Benthic colonisation by micro and macro-organisms, the associated photosynthesis and primary production were studied on 96 sample marine infrastructures (MI) immersed for one year at two different sites on the French side of the English Channel. The MI samples had one smooth and one rough surface to assess the influence of the surface micro-scale rugosity on benthic development. Our results revealed significant differences in biofilm biomass and physiology at the two sites. Benthic macroalgal diversity and biomass also differed significant at the two sites with higher diversity in the Bay of Seine than in the Bay of Cherbourg. MI colonisation by a red encrusting alga, *Phymatolithon purpureum*, was recorded in the Bay of Cherbourg, and its photosynthesis was spatially separate from the rest of the structure (*i.e.* only colonised by microphytobenthos). These results highlight the ability of this alga to colonise up to 75% of the MI cover in the early stages of immersion despite weak photosynthetic parameters. The dynamics of microphytobenthic biofilm differed, with low biomass but high photosynthetic parameters. Beyond assessing the properties of concrete required to promote primary productivity in engineering projects, we explored the interactions between macro and microalgae and the photobiological regulation of a hard substrate.

Keywords

Benthic communities; Photosynthesis; Microphytobenthos; Encrusting algae; Marine infrastructures

Abbreviations

α : Photosynthetic efficiency

Ek: Acclimation light

F_v/F_m : maximum quantum efficiency of PSII

MI: Marine Infrastructure

MPB: Microphytobenthos

NPQ: Non-Photochemical Quenching

rETR_{MAX}: maximum of relative Electron Transport Rate

I. Introduction

Surfaces in aquatic environments are rapidly colonised by microorganisms (Bakker et al., 2003; Bhosle et al., 2005). Microphytobenthic biofilms are characterised by assemblages of photosynthetic microalgae (mostly diatoms), cyanobacteria, protozoa and macrophyte spores which form a biofilm mainly on the surfaces of hard substrates (Kromkamp et al., 1995, 2020; Nagarkar et al., 2004). Microphytobenthos is one of the main sources of carbon for benthic and pelagic trophic webs on intertidal rocky shores (Bulleri, 2005). Microphytobenthos is also a pioneer assemblage in the succession of benthic communities and facilitates the establishment of macroalgae and invertebrates (Huang & Boney, 1984; Hung et al., 2007). Biofilms vary in space and over time; they are regulated by environmental and biological parameters including nutrient availability and top-down control of herbivory by grazers (Underwood, 1984; Hillebrand et al., 2000; Jenkins et al., 2001; Thompson et al., 2004, 2005). Thus, colonisation of hard substrates by biofilms is crucial for the subsequent ecological succession of new artificial hard substrata in marine environments. The photobiological features of these biofilms may provide cues influence subsequent colonisation stages by mediating the bio-adhesion of planktonic metazoan larvae during settlement (Olivier et al., 2000; Tamburri et al., 2008; Hladyz et al., 2011; Whalan & Webster, 2014; Ly et al., 2020).

The possibly competitive relationship between microphytobenthic biofilms and encrusting algae has rarely been studied to date (Guasch & Subater, 1995). These photosynthetic organisms share the same habitat and competition for space determines the abundance and distribution of marine organisms on hard substrata (Paine, 1984). Red encrusting algae have been described as poor competitors for space (Breitburg, 1984); however, they compete well with epiphytes and canopy algae. Indeed, positive effects of extensive canopy on colonisation and photosynthesis of encrusting algae have already been described (Irving et al., 2004). Excess light may bleach the encrusting algae from red to white, due to photoinhibition associated with a reduction in their productivity

(Littler, 1973; Figueiredo et al., 2000; Irving et al., 2004). This has mainly been explained by the change in local conditions (*i.e.* desiccation, light intensity) in intertidal and shallow subtidal hard substrate habitats (Figueiredo et al., 2000; Irving et al., 2004).

Artificial structures had been used for decades mainly to attract fish or to protect shallow coastal zones from trawling (Jensen et al., 2000; Salaün et al., 2020), and their development has intensified over the three last decades (Vivier et al., 2021b). Beyond these targets, such structures also favour biodiversity as they act as settlement zones and nurseries for many marine species (Patranella et al., 2017) by providing a novel habitat, by supporting the primary production of organic carbon and its transfer through the trophic food web (Charbonnel et al., 2002; Langhamer & Wilhelmsson, 2009; Layman et al., 2016; Komyakova & Swearer, 2018; Komyakova et al., 2019).

The English Channel is an epicontinental sea with a maximum depth of 174 m (Dauvin, 2019). It is also a macrotidal system with strong hydrodynamics (Orbi & Salomon, 1988). The English Channel is one the most exploited seas in the world due to its high productivity and richness (fishing, aquaculture, granular extraction, etc.) (Boyd & Rees, 2003; Fritsch et al., 2007; Carpentier et al., 2009; Dauvin, 2019). It is also a major route for maritime traffic with several large harbors, and several million people live along its coasts. The combination of all these pressures on the ecosystems is responsible for their degraded ecological quality, and natural resources, biodiversity, and ecosystem services are also seriously affected (Desprez, 2000; Loizeau et al., 2001; Boyd and Rees, 2003; Molfese et al., 2014; Dauvin, 2019).

The Bay of Cherbourg, a highly anthropised area of the English Channel, is a semi-enclosed ecosystem located in Normandy (France). The Bay of Cherbourg is characterised by a sandy bottom while the second site, the Bay of Seine, is characterised by sandy-rocky bottom. The Bay of Cherbourg is also under human pressure which affects both ecosystem functions and ecological services (Boyd & Rees, 2003; Molfese et al., 2014; Dauvin et al., 2020). Environmental conditions are harsh at the second site, the Bay of Seine: the hydrodynamics are strong and nutrient availability, turbidity, and salinity are influenced by freshwater inputs mainly from the Seine estuary which influence ecosystem functioning including primary productivity and the composition of the benthic communities (Dauvin, 2012; Lemesle, 2015; Dauvin et al., 2020; Serre-Fredj

et al., 2021). The low tide bathymetry at both sites is about 4 m. Under such human pressure, artificial reefs are one possible way to restore or create new habitats to improve or at least protect local biodiversity and production (Bortone, 2006; Bodilis et al., 2011; Layman et al., 2016).

Given the current ecological and societal context, the use and management of hard substrates has recently expanded thanks to the use of immersed marine structures for the purpose of creating novel habitats with expected economic benefits (offshore wind farms, seawalls, dikes, etc.). These innovative solutions use novel materials that increase both their quantitative and qualitative colonisation potential. The two aims of the present study were to measure microphytobenthic growth and photosynthesis on sample marine infrastructures (MI) at two sites in the English Channel, and to investigate benthic colonisation by macroalgae and associated photosynthesis. The comparison of identical structures at two different sites along the French coasts of the English Channel confirms the success of such projects in restoring affected ecosystems. The comparison of two types of surface rugosity provided reliable data on the influence of the nature of the structure on the development of biofilms and other benthic communities. Finally, this study advances our understanding of the relationship between biodiversity and production, particularly the interaction between microphytobenthic and encrusting algal communities.

II. Material and methods

II.1. *Sampling sites*

A total of 96 MI cobble shaped samples were constructed and immersed in series comprising six modules on top of artificial reefs in the Bay of Cherbourg and the Bay of Seine (Figure 49) in early September 2019. The type of cement used was CEM II/A-LL 42.5 R CE PM-CP2 NF, which is suitable for use in sea water. Siliceous alluvial sand (granular class 0/2 mm) and alluvial aggregates (4/10 mm and 10/20 mm) were also used. Following characterisation of the raw materials, concrete mixes were formulated in accordance with NF EN 206-1, 2016. The formulation used was 350 kg m⁻³ of cement, 800 kg m⁻³ of sand, 600 kg m⁻³ of gravel (4/10 mm), 500 kg m⁻³ of gravel (10/20 mm), 175 kg m⁻³ of water and 4.5 kg m⁻³ of superplasticizer. The MI were placed in series comprising six modules with an alternating rough surface and a smooth surface facing

upwards. The study lasted from November 2019 to April 2021. Six samplings were carried out in the Bay of Cherbourg and four in the Bay of Seine. At each sampling date, a batch of six MI were collected by scuba divers and conserved in a hermetic tube during transport from the sampling site to the laboratory.

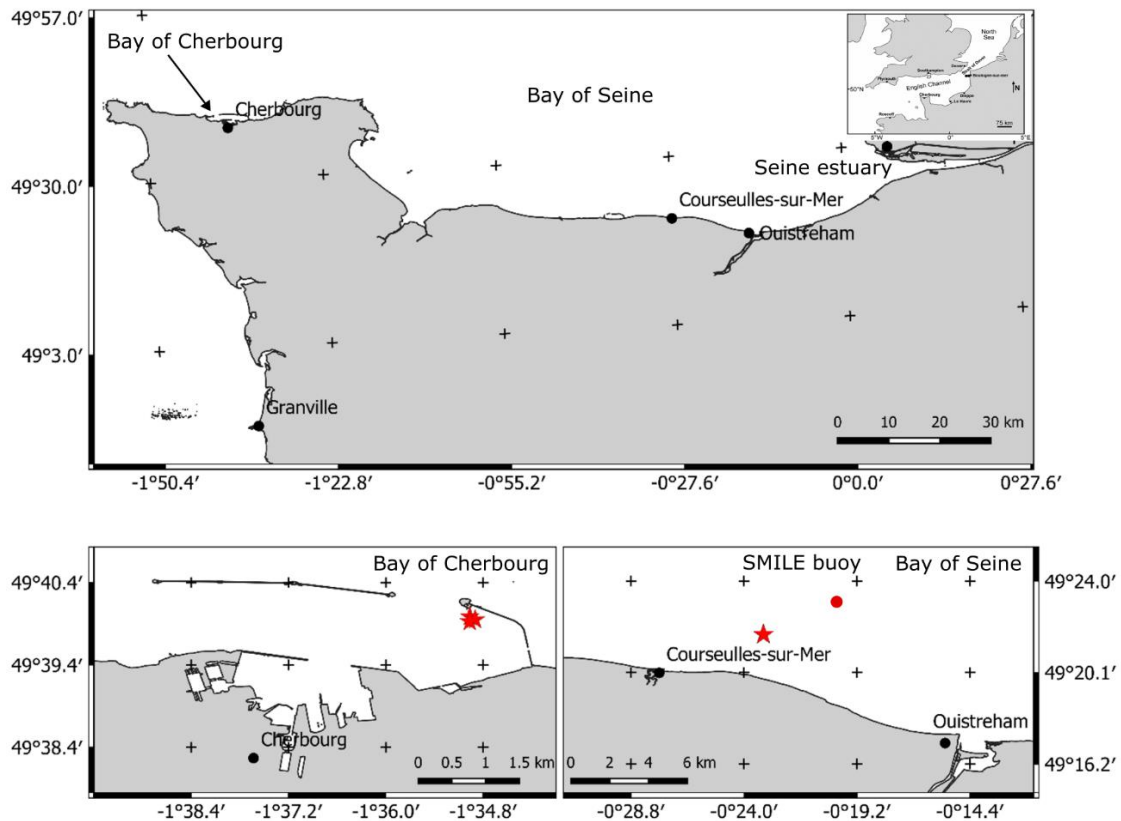


Figure 49 – Location of the experimental sites (red stars) and the SMILE buoy (red dot) in the English Channel.

II.2. Experimental monitoring

II.2.1. Abiotic parameters and inorganic nutrients analyses

High frequency measurements made by the SMILE instrumented buoy located in the Bay of Seine ($0^{\circ}19'41.00''\text{O}$ $49^{\circ}21'14.00''\text{N}$) were used (data available doi.org/10.17882/53689). Conductivity and temperature (WTWTM sensor, TetraCon), turbidity (Seapoint Turbidity Meter), oxygen (AADI Oxygen optode, Anderraa) and fluorescence (Cyclops-6K, Turner Designs) were measured by an automatic NKE instrument (MP7, NKE Instrumentation[®]). PAR (photosynthetically active radiation) was measured with a Saltanics sensor. A water sample was also collected on each sampling

occasion in the Bay of Cherbourg and in the Bay of Seine for analysis of inorganic nutrients. Each sample was filtered through a cellulose acetate filter (ClearLine, CA, 33 mm, 0.45 μm) in 50 mL falcon tubes and frozen at -20 °C. Analyses were conducted using a Seal Analytical AA-3 system (Aminot & K  rouel, 2007). The limits of quantification were 0.02 $\mu\text{mol.L}^{-1}$ for PO_4^{3-} and 0.05 $\mu\text{mol.L}^{-1}$ for NO_3^- , NO_2^- and Si(OH)_4 . NH_4^+ was analysed by fluorometry following the protocol of Oriol et al. (2014). N_{tot} was calculated as follows: $\text{N}_{\text{tot}} = \text{NO}_3^- + \text{NO}_2^- + \text{NH}_4^+$.

II.2.2. *Identification of macroalgae*

All the macroalgae were sampled and conserved at -20 °C for species identification. Macroalgae species were assigned to their class (Ulvophyceae, Rhodophyceae or Phaeophyceae) using Algaebase.

II.2.3. *Photosynthetic parameters*

Photosynthetic parameters were measured daily using an imaging-PAM fluorometer (Walz, Germany). The IMAGING-PAM Chlorophyll Fluorometer is designed to investigate the two-dimensional heterogeneities of photosynthetic activity. Fluorescence measurements were carried out using the Maxi Version of the Imaging-PAM Chlorophyll Fluorometer (Walz, Effeltrich, Germany) associated with a LED-Array Illumination Unit IMAG-MAX/L (44 high-power royal-blue (450 nm) LED-lamps) and a CCD Camera IMAG- K7 equipped with a zoom objective lens (640 $\times$ 480 pixel resolution). Measurements were performed at a fixed working distance of 18.5 cm. A 5-min dark adaptation period was respected before each measurement to allow oxidation of the electron acceptor pools. The saturation pulse intensity was 4,500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ for 0.8 s and the measuring pulse frequency was 8 Hz. Rapid light curves were performed as follows: samples were exposed to 11 incremental intensities of actinic light (E): 0, 73, 216, 370, 554, 658, 774, 896, 1018, 1166 and 1336 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ with 30 s irradiance steps. Numerical values and fluorescence images were extracted using Imaging Win (Walz) analytical software. The autofluorescence of each structure was recorded before the experiment in order to apply a correction to F_0 .

The maximum quantum efficiency of PSII (F_v/F_m) was calculated using the following equation from Genty et al. (1989):

$$\frac{F_V}{F_M} = \frac{(F_M - F_0)}{F_M} \quad (37)$$

where F_M is the maximum fluorescence yield measured after a saturating pulse and F_0 is the minimum fluorescence yield measured immediately before the saturating pulse.

The maximum relative electron transport rate ($rETR_{MAX}$, rel. unit) was estimated by fitting FLC data with the Webb model (1974) to estimate α ($\mu\text{mol electrons } (\mu\text{mol photons})^{-1}$) and E_k ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) with α the initial slope of the FLC, and E_k the light saturation index:

$$rETR = \alpha \times E_k \times (1 - e^{-\frac{E}{E_k}}) \quad (38)$$

$rETR_{MAX}$ was then calculated as:

$$rETR_{MAX} = \alpha \times E_k \quad (39)$$

The other option was the Eilers & Peeters (1988) model with a , b and c the equation coefficients to calculate the $rETR_{MAX}$ and the photosynthetic efficiency (α).

$$rETR = \frac{E}{(aE^2 + bE + c)} \quad (40)$$

or,

$$rETR_{MAX} = \frac{1}{(b + 2\sqrt{ac})} \quad (41)$$

α was calculated as:

$$\alpha = \frac{1}{c} \quad (42)$$

Finally, non-photochemical quenching (NPQ), based on the relative difference between the maximum fluorescence measured in the dark-adapted state, F_M , and upon exposure to light, F_M' was calculated:

$$NPQ = \frac{F_M - F_{M'}}{F_{M'}} \quad (43)$$

To estimate photosynthetic α and $rETR_{MAX}$ for each pixel of the fluorescence image, a nonlinear regression model was fitted on RLC curves using the method of Nelder & Mead (1965). According to the curve profile, choice of the appropriate model between the Webb et al. (1974) and Eilers and Peeters (1988) models was automatically made by the algorithm. In the exceptional case when $rETR_{MAX}$ was much higher than the highest $rETR$, a linear model was applied and the highest $rETR$ was considered as $rETR_{MAX}$ to avoid overestimation. If (1) the first value (*i.e.* first E) was the highest in the RLC, (2) RLC values were all equal to zero, or (3) the set comprised no more than three positive values, all the photosynthetic parameters were considered null.

II.2.4. *Chlorophyll a measurements*

The microphytobenthos (MPB) was carefully sampled on the upper surface of the MI using a smooth toothbrush, then diluted in 250 mL of filtered sea water (Stericup GV Millipore, Ø 0.22 μ m). A sub-sample (150 mL) of the diluted sample was filtered through a glass-fibre filter (Whatman, GF/F, 47 mm, 0.7 μ m) and immediately frozen and stored at -20 °C until analysis. Aliquots (10 mL) of 90% acetone (v/v) were added to extract the pigment, and the samples were then left for 12 h in the dark at 4 °C. After being centrifuged twice for 5 min at 1700 g, the Chl *a* concentration of the extracts was measured using a Trilogy fluorimeter (Turner Designs, Sunnyvale, USA) according to the Strickland and Parsons (1968) method.

II.3. *Data treatment and analysis*

To obtain numerical values of fluorescence spatial heterogeneity from images, we developed a MATLAB[®] routine to determine the value of each fluorescence level (F_0 , F_M , F_S (steady state fluorescence) and $F_{M'}$) for each pixel (Vivier et al., 2021a). For each sample, autofluorescence was deducted and the images corresponding to all successive actinic light per RLC (rapid light response curves) were nested in a 3D matrix. A colour value was assigned to each gray level, and then, using a conversion index based on the fluorescence-colour scale included in PAM software, each pixel was converted into a numerical value. The quantum efficiency of PSII charge separation ($\Delta F/F_M$; fluorescence ratio) was calculated for each pixel of each image as $(F_{M'} - F_S)/F_{M'}$. To avoid noise bias during imaging-PAM measurements, all F_M values below 0.048 were previously considered invalid. Indeed, below this threshold, the values acquired were too weak to be

reliable (Heinz Walz GmbH 2014). Subsequently, for each pixel and actinic light (E ; $\mu\text{mol photons m}^{-2} \text{s}^{-1}$), the relative electron transport rate was estimated ($rETR = \Delta F/F_M \times E$), $rETR-I$ curves were carried out and acclimatization light (E_k , $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) was estimated.

Data analysis was performed with R i386 3.5.1 (R Development Core Team, 2008). The normality of the data was tested (Shapiro-Wilk normality test) and, if necessary, data were transformed with the box cox function of the MASS package in R. Significant differences were tested with an ANOVA test, a significant p-value of 5%. When the box cox function was not sufficient to normalise the data, Wilcoxon or Kruskal-Wallis non-parametric tests were used to identify potential significant differences between conditions (seasons or rugosity).

The last step was estimating the percentage cover of encrusting algae. This parameter was calculated only for the Bay of Cherbourg MI using the ImageJ2 software. In order to define the photosynthetic parameters associated with the encrusting algae, random sampling inside and outside its colonised area was conducted during data treatment on MATLAB®. One rough and one smooth MI were selected, 50 samplings were performed per MI for each parameter. The spatial distribution of the biomass and several photosynthetic parameters were obtained from the imaging-PAM measurements (Figure 50). Figure 50 shows the method used for one rough and one smooth MI in July 2020. This method was applied on three sampling occasions (February 2020, July 2020 and April 2021) representing the beginning of colonisation, the growth phase and the stabilisation of *Phymatolithon purpureum* (P.L. Crouan & H.M. Crouan) Woelk. & L.M.Irvine, 1986. “Inside the area” refers to the area colonised by the encrusting algae and “outside the area” refers to the area not colonised by this alga. The non-colonised area was only colonised per MPB.

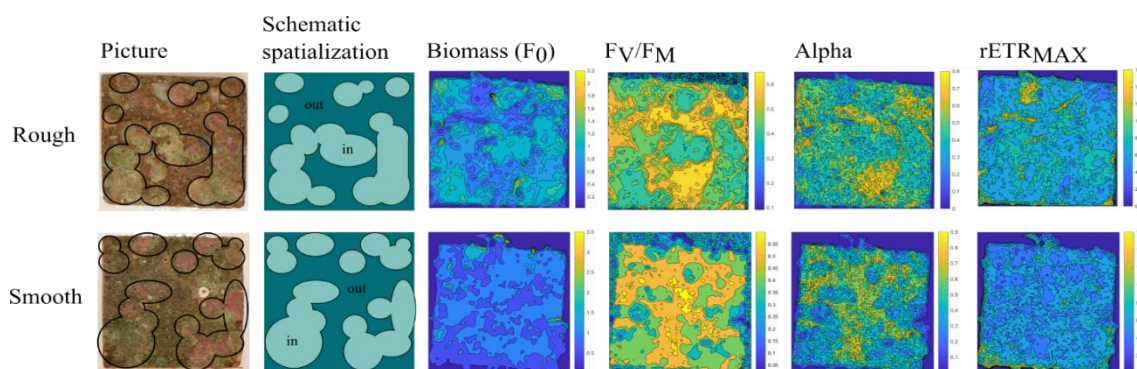


Figure 50 – Example of MI pictures (rough and smooth) in July 2020. Black lines discriminate *Phymatolithon purpureum* zone, the associate schematic repartition of “inside” and “outside” *Phymatolithon purpureum* zone was defined and used to calculate the associated photosynthetic parameters and biomass.

III. Results

III.1. Environmental parameters

The values of the seven environmental parameters recorded at both sites during the survey are listed in Table 8. However, water turbidity in the Bay of Seine in September was considerably higher than in the Bay of Cherbourg. Inorganic nutrient concentrations were also higher in the Bay of Seine than in the Bay of Cherbourg.

Table 8 – Environmental parameters at the two experimental sites during the survey.

	Bay of Seine		Bay of Cherbourg					
	12/2019	5/2020	9/2020	2/2020	6/2020	7/2020	9/2020	4/2021
Temperature (°C)	10	14	20		14	16	17	10.50
Salinity	33.36	33.26	33.39	33.40	34.30	34.60	34.56	34.01
Turbidity (NTU)		5.00	88.40			0.70	0.60	0.15
Chlorophyll <i>a</i> (µg/L)	0.66	4.36	1.05		1.33	1.54	0.87	8.03
NO _{TOT} (µmol/L)	24.88	9.00	2.16	11.81	2.20	0.17	3.31	
PO ₄ ³⁻ (µmol/L)	0.81	0.75	0.12	0.53	0.03	0.03	0.30	
SiOH ₄ (µmol/L)	15.64	1.09	6.11	5.30	1.52	1.41	3.39	0.70

III.2. *Microphytobenthic biomass*

On the smooth MI sampled in the Bay of Cherbourg (Figure 51A), the Chl *a* biomass underwent a marked decrease (from $9.06 \pm 0.6 \mu\text{g cm}^{-2}$ to $2.62 \pm 0.73 \mu\text{g cm}^{-2}$) between February and June 2020. During the remaining monitoring period, the biomass never reached its level in February 2020, reached only five months after immersion of the MI. Between June 2020 and April 2021, the biofilm biomass on the smooth MI varied slightly ranging from a minimum value of $1.26 \pm 0.73 \mu\text{g cm}^{-2}$ in November 2020 to a maximum value of $5.23 \pm 0.93 \mu\text{g cm}^{-2}$ in April 2021. The biomass recorded on rough MI displayed a similar pattern with a maximum value ($8.43 \pm 0.75 \mu\text{g cm}^{-2}$) in February 2020 before decreasing to $2.95 \pm 0.56 \mu\text{g cm}^{-2}$ in June 2020. Like for the smooth MI, the Chl *a* biomass on the rough MI showed slight variations during the rest of the survey. The minimum value on the smooth MI ($1.85 \pm 0.25 \mu\text{g cm}^{-2}$) was reached in November 2020, and the maximum ($4.72 \pm 0.58 \mu\text{g cm}^{-2}$) in September 2020. No significant differences were found between the rough and smooth MI at any time during the survey, while significant differences in Chl *a* biomass were found between seasons (ANOVA, $P < 0.001$). The Chl *a* biomass samples in the Bay of Seine (Figure 51B) underwent a continuous increase between November 2019 and September 2020 followed by a marked decrease in March 2021. The volume of biomass on the smooth MI was lower than on the rough ones at each date; the minimum value ($0.42 \pm 0.18 \mu\text{g cm}^{-2}$) was measured in November 2019, the maximum ($3.75 \pm 0.36 \mu\text{g cm}^{-2}$) in September 2020. Minimum biomass on the rough MI ($0.53 \pm 0.02 \mu\text{g cm}^{-2}$) was recorded in November 2019, and maximum ($4.48 \pm 0.38 \mu\text{g cm}^{-2}$) in September 2020. A significant decrease in biomass on both rough and smooth MI was observed in March 2021. Significant differences in Chl *a* biomass were observed between seasons (ANOVA, $P < 0.001$) and for the different types of rugosity (ANOVA, $P < 0.01$).

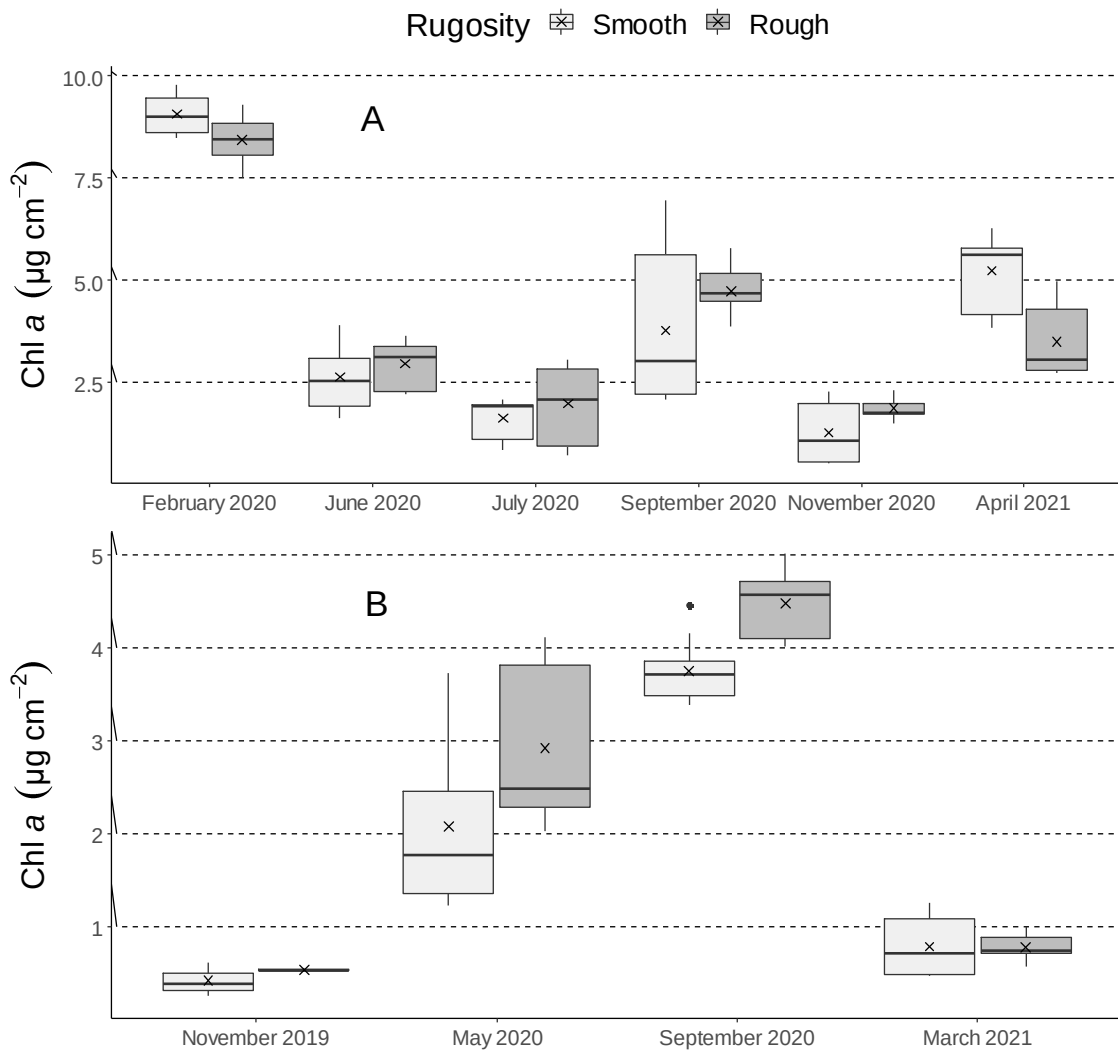


Figure 51 – Chlorophyll a biomass for each rugosity on the Bay of Cherbourg (A) and Bay of Seine (B).

III.3. Photosynthetic parameters

Figure 52 shows the photosynthetic parameters of the samples collected on rough and smooth MI samples in the Bay of Cherbourg and Figure 53 shows the same parameters for samples from the Bay of Seine. These parameters correspond to the entire MI, *i.e.*, microphytobenthos and encrusting algae in samples collected in the Bay of Cherbourg but only the microphytobenthos in samples from the Bay of Seine. In the Bay of Cherbourg, the F_v/F_m (Figure 52A) was high in the early months after immersion of the MI (0.55 ± 0.09 on smooth MI and 0.61 ± 0.01 on rough ones). Rough MI showed a higher F_v/F_m ratio until summer 2020 (0.44 ± 0.07 for rough versus 0.37 ± 0.04 for smooth MI in June 2020). Between September 2020 and April 2021, the F_v/F_m ratio

remained constant with no differences between roughness (around 0.43). Significant differences in F_v/F_m were found between seasons (ANOVA, $P < 0.001$), but no significant differences between the two types of rugosity. The $rETR_{MAX}$ (Figure 52B) of both types of rugosity on MI increased between February and June 2020 to reach 91.63 ± 11.9 rel. unit on the smooth MI. It decreased on both types of rugosity, reaching 59.73 ± 4.56 A.U. in July on smooth MI and approximately 100 rel. unit on all MI in September. Significant differences in $rETR_{MAX}$ were found between seasons (ANOVA, $P < 0.001$), but not between the two types of rugosity. α (Figure 52C) was high in February 2020, and higher on rough MI ($0.63 \pm 0.04 \mu\text{mol electrons } (\mu\text{mol photons})^{-1}$ versus $0.49 \pm 0.12 \mu\text{mol electrons } (\mu\text{mol photons})^{-1}$ than on smooth MI. In June, α decreased to $0.22 \pm 0.07 \mu\text{mol electrons } (\mu\text{mol photons})^{-1}$ on smooth MI before increasing to $0.41 \pm 0.10 \mu\text{mol electrons } (\mu\text{mol photons})^{-1}$ in July, and then remained constant at around $0.25 \mu\text{mol electrons } (\mu\text{mol photons})^{-1}$ until April 2021. Significant differences in α were found between seasons (Kruskal-Wallis, $P < 0.001$), but not between rugosity types. Finally, the NPQ (Figure 52D), showed a global and constant increase on both types of MI over the entire monitoring period (increasing from 0.004 ± 0.002 in February 2020 to 0.18 ± 0.07 in April 2021 on rough MI). Significant differences in NPQ were found between seasons (ANOVA, $P < 0.05$), but not between rugosity types. In the Bay of Seine, the F_v/F_m (Figure 53A) was high in the early months of monitoring (0.46 ± 0.08 on rough MI and 0.51 ± 0.01 on smooth ones in November 2019). There was a marked decrease in the F_v/F_m (down to 0.28 ± 0.05) on rough MI in May 2020 and to 0.52 ± 0.03 in March 2021 on rough MI. Significant differences were found between seasons (Kruskal-Wallis, $P < 0.01$), but not between rugosity types. $rETR_{MAX}$ (Figure 53B) remained constant on smooth MI throughout the monitoring period (increasing from 99.92 ± 27.88 rel. unit in November 2019 to 106.49 ± 1.04 rel. unit in March 2021). Data recorded on rough MI resembled data recorded on smooth MI with a significant decrease down to 83.9 ± 17.16 rel. unit recorded in May 2020. No significant differences were found between either seasons or rugosity. α (Figure 53C) was always higher on smooth MI, but decreased from $0.32 \pm 0.10 \mu\text{mol electrons } (\mu\text{mol photons})^{-1}$ to $0.21 \pm 0.07 \mu\text{mol electrons } (\mu\text{mol photons})^{-1}$ between November 2019 and May 2020 and finally reached $0.32 \pm 0.05 \mu\text{mol electrons } (\mu\text{mol photons})^{-1}$ in March 2021 on smooth MI. Significant differences in α were found between seasons (ANOVA, $P < 0.01$), but not between rugosity types. The

NPQ (Figure 53D) increased continuously on both types of MI from 0.11 ± 0.06 to 0.29 ± 0.01 on smooth ones between November 2019 and September 2020. It decreased to 0.07 ± 0.01 on smooth MI and to 0.10 ± 0.09 on rough MI in March 2021. Significant differences in NPQ were found between seasons (Kruskal-Wallis, $P < 0.05$), but not between rugosity types.





Figure 52 – Changes in photosynthetic parameters F_v/F_m (A), $rETR_{MAX}$ (B, rel. unit), α (C, $\mu\text{mol electrons (}\mu\text{mol photons)}^{-1}$) and NPQ (D, relative unit) during the survey of the MI in the Bay of Cherbourg.

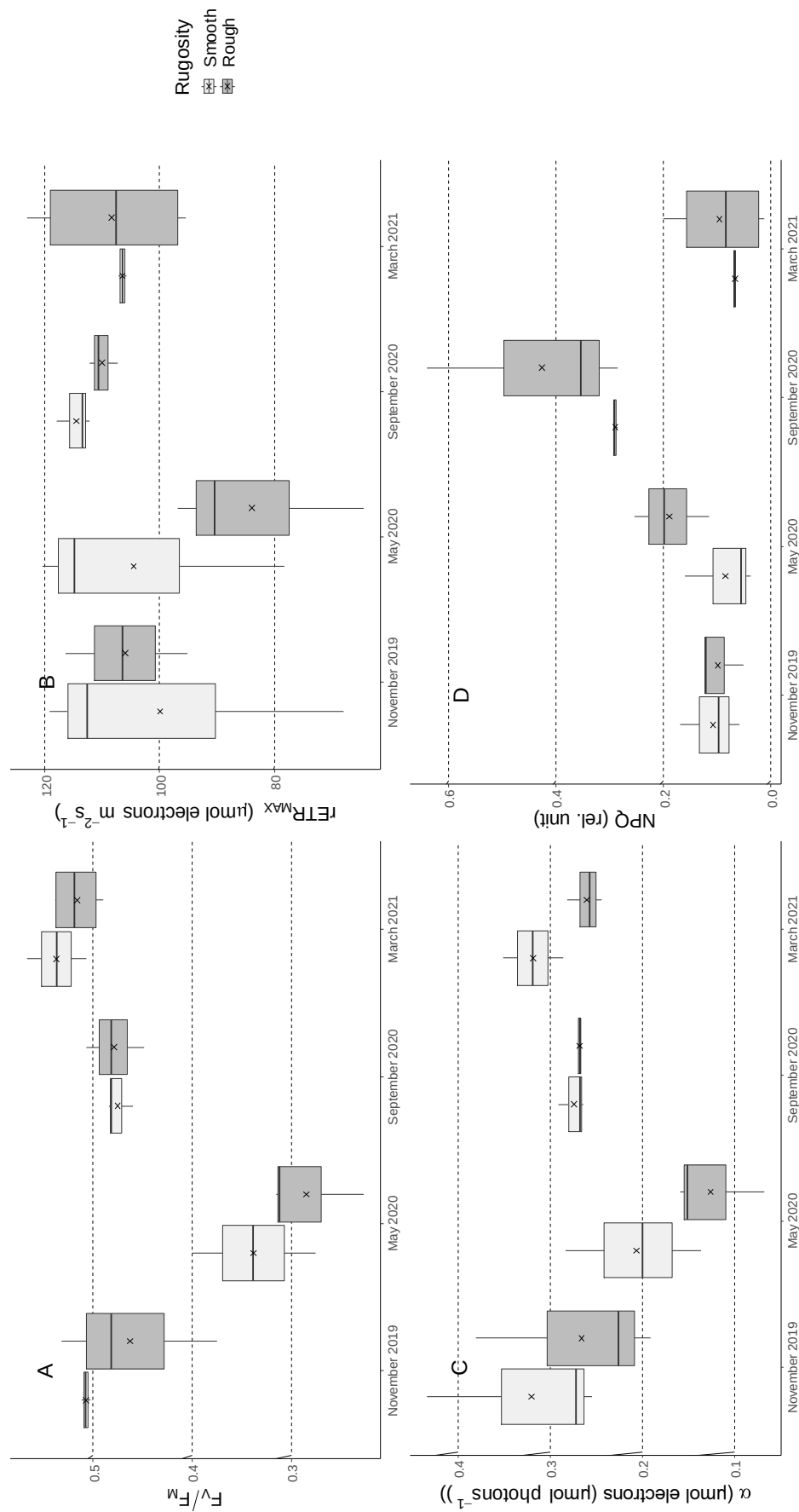


Figure 53 – Changes in photosynthetic parameters F_v/F_m (A), $rETR_{MAX}$ (B, rel. unit), α (C, $\mu\text{mol electrons } (\mu\text{mol photons})^{-1}$) and NPQ (D, relative unit) during the survey of the MI in the Bay of Seine.

III.4. *Macroalgae biomass and diversity*

A total of 12 different species of macroalgae were recorded, 10 on the MI samples collected from the Bay of Seine and only three on the MI collected from the Bay of Cherbourg (Table 9). *Cryptopleura ramosa* (Hudson) L.Newton 1931, *Kallymenia reniformis* (Turner) J.Agardh 1842, *Sphondylothamnion multifidum* (Hudson) Nägeli 1862, *Chondria dasyphylla* var. *intermedia* (Grunow) P.C.Silva 1996, *Plocamium cartilagineum* (Linnaeus) P.S.Dixon 1967, *Callithamnion tetragonum* (Withering) S.F.Gray 1821, *Hypoglossum hypoglossoides* (Stackhouse) Collins & Hervey 1917, *Pterosiphonia parasitica* (Hudson) Falkenberg 1901, *Heterosiphonia plumosa* (J.Ellis) Batters 1902 and *Sphaerococcus coronopifolius* Stackhouse 1797 for the Rhodophyceae species. *Ulva* sp. for the Ulvophyceae and *Cladostephus spongiosus* (Hudson) C.Agardh 1817 for the Phaeophyceae.

Table 9 – Macroalgae species recorded on the MI in the Bay of Seine and the Bay of Cherbourg during the survey.

Site	Season	Rugosity	Class	Species
Bay of Seine	September 2020	Rough	Rhodophyceae	<i>Cryptopleura ramosa</i>
				<i>Kallymenia reniformis</i>
				<i>Sphondylothamnion multifidum</i>
				<i>Chondria dasyphylla</i>
				<i>Plocamium cartilagineum</i>
			Ulvophyceae	<i>Ulva</i> sp.
		Smooth	Rhodophyceae	<i>Cryptopleura ramosa</i>
				<i>Kallymenia reniformis</i>
				<i>Callithamnion tetragonum</i>
				<i>Chondria dasyphylla</i>
				<i>Hypoglossum hypoglossoides</i>
				<i>Pterosiphonia parasitica</i>
	March 2021	Rough	Phaeophyceae	<i>Cladostephus spongiosus</i>
			Ulvophyceae	<i>Ulva</i> sp.
			Rhodophyceae	<i>Cryptopleura ramosa</i>
				<i>Chondria dasyphylla</i>
Bay of Cherbourg	November 2020	Rough	Rhodophyceae	<i>Heterosiphonia plumosa</i>
				<i>Ulva</i> sp.
		Smooth	Rhodophyceae	<i>Ulva</i> sp.
				<i>Cryptopleura ramosa</i>
				<i>Hypoglossum hypoglossoides</i>
				<i>Ulva</i> sp.
	April 2021	Rough	Rhodophyceae	<i>Cryptopleura ramosa</i>
				<i>Sphaerococcus coronopifolius</i>
		Smooth	Rhodophyceae	<i>Cryptopleura ramosa</i>
				<i>Hypoglossum hypoglossoides</i>

III.5. *Encrusting algae*

The percentage cover of incrusting *Phymatolithon purpureum* was calculated for each MI sample in Bay of Cherbourg (Figure 54). On smooth MI, percentage cover increased strongly from 9.2 $\pm$ 4.6% to 78.1 $\pm$ 8.6% between February and September 2020, then remained stable (78.9 $\pm$ 11.6%) until April 2021. On rough MI, the percentage cover of incrusting *Phymatolithon purpureum* showed a similar pattern but with lower values. Percentage cover increased from 6.3 $\pm$ 1.7% to 71.2 $\pm$ 7.6% between February and September 2020 before levelling off at 74.8 $\pm$ 4.5% until April 2021. No significant differences were found between the two types of rugosity, but significant differences were found between seasons (ANOVA, $P < 0.001$).

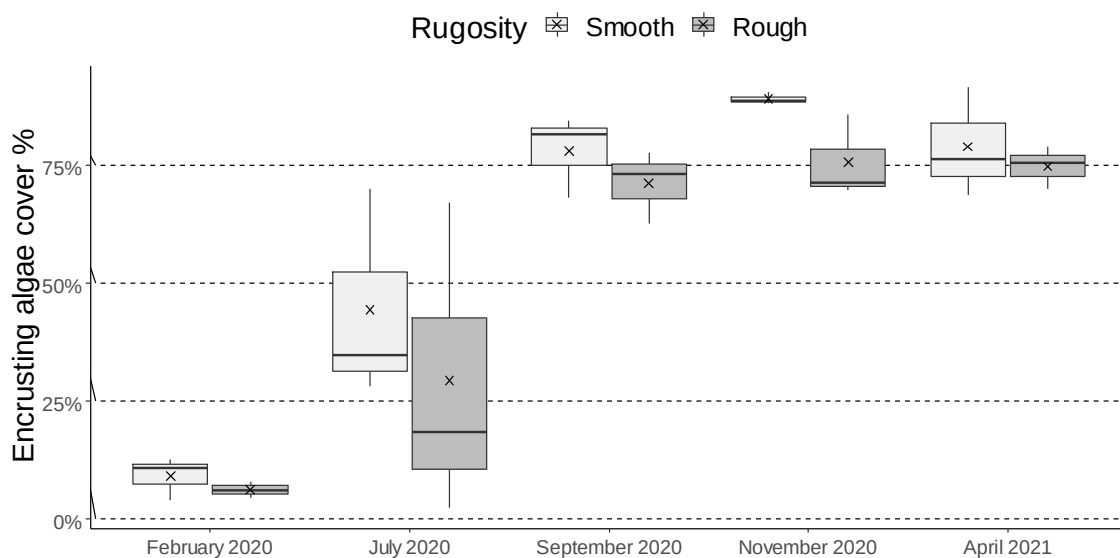


Figure 54 – Percentage cover of incrusting algae per MI between February 2020 and April 2021 in the Bay of Cherbourg.

The spatial distribution of biomass and several photosynthetic parameters were obtained from the imaging-PAM measurements which enabled the comparison of these parameters inside and outside the area colonised by *Phymatolithon purpureum* (Figure 55). Significant differences were found in the biomass indicator (ANOVA, $P < 0.01$), the F_v/F_m (ANOVA, $P < 0.01$) and the α (ANOVA, $P < 0.05$) between rugosity conditions. No significant differences were found between rugosity types for the $rETR_{MAX}$. On the smooth MI, significant differences between encrusting alga and other areas were found in biomass, F_v/F_m , α and $rETR_{MAX}$ (ANOVA, $P < 0.001$). The biomass of the encrusting alga area was significantly higher than that in the other areas (1.52 $\pm$ 0.61 versus 1.3 $\pm$ 0.38 on average). The F_v/F_m in the other areas was significantly higher on every sampling occasion (0.34 $\pm$ 0.07 in the encrusting alga

area versus 0.52 ± 0.06 in the other areas). The same pattern was observed for α with the maximum value of 0.75 ± 0.08 in the other areas reached in July 2020. The average value of $rETR_{MAX}$ in the other areas (93.6 ± 28.1 rel. unit) was significantly higher than in the areas colonised by the encrusting algae (71.5 ± 32.5 rel. unit). However, the highest value was reached in April 2021 and no significant differences were found between areas on that occasion. On the rough MI, significant differences were found in biomass, F_V/F_M , α and $rETR_{MAX}$ (ANOVA, $P < 0.001^{***}$) between encrusting alga area and other areas. The biomass indicator of the other areas was significantly lower than that of the encrusting alga area (0.68 ± 0.38 versus 1.51 ± 0.57 on average). The F_V/F_M for the other areas was significantly higher on every sampling occasion (0.57 ± 0.07 for the encrusting alga area versus 0.41 ± 0.06 for the other areas). The same dynamics was observed for α with a maximum value reached in July 2020, at 0.68 ± 0.07 for the other areas. The average value of $rETR_{MAX}$ for the other areas was significantly higher (105.3 ± 30.1 rel. unit) than for the encrusting alga areas (66.1 ± 25.3 rel. unit). A plateau was reached for the other area in July 2020 and no significant differences were found between the different areas in April 2021.

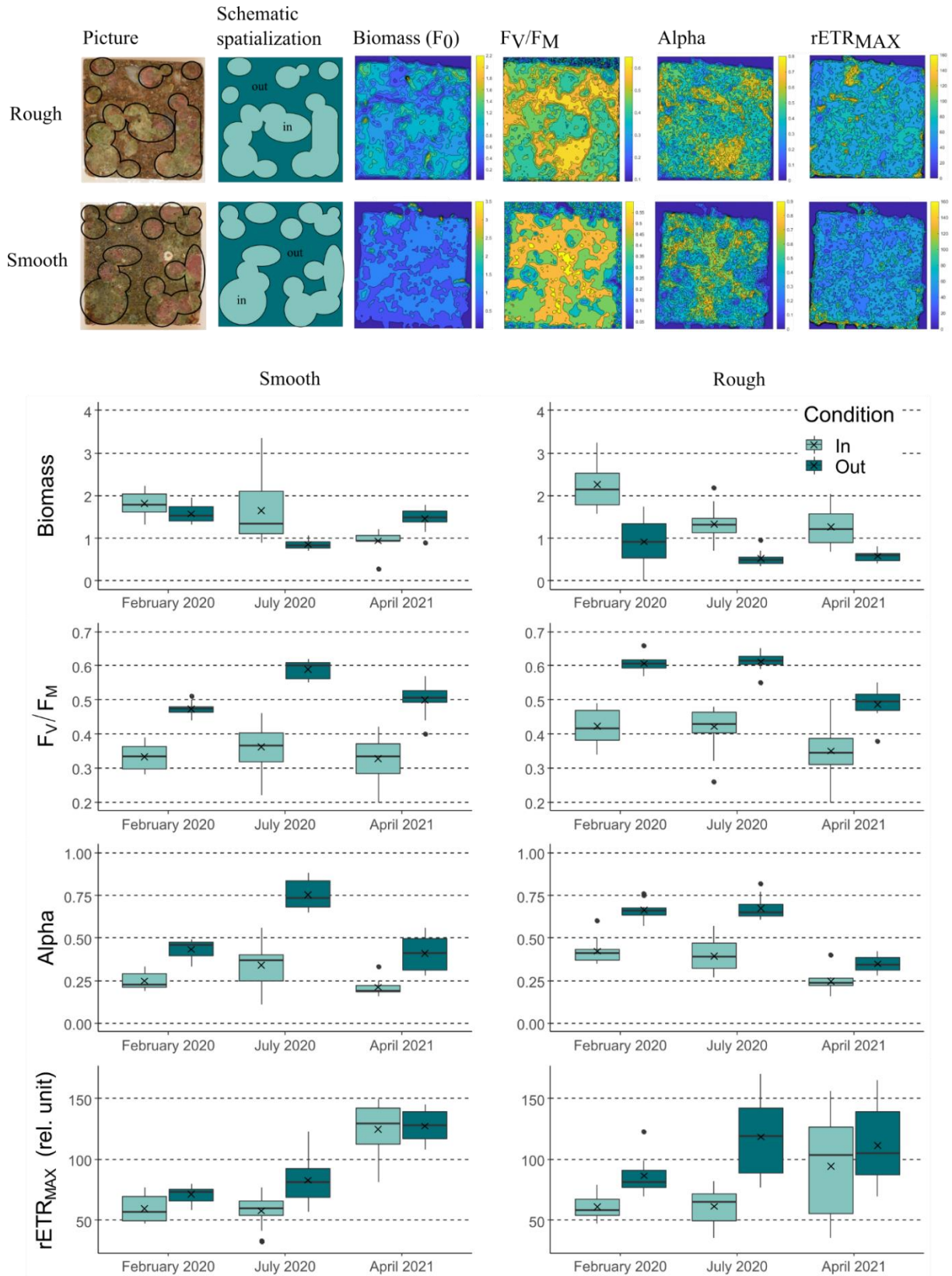


Figure 55 – Example of MI pictures (rough and smooth) in July 2020. Black lines identify the *Phymatolithon purpureum* zone, the associated schematic distribution of “inside” and “outside” the *Phymatolithon purpureum* zone was defined and used to calculate the associated photosynthetic parameters (F_v/F_m , alpha and $rETR_{MAX}$) and biomass (F_0).

III.6. *Principal component analysis (PCA) on biotic and abiotic variables*

A PCA (Figure 56) was performed to identify the variables that explained the influence of seasonality at the two sites. Supplementary quantitative variables are shown in grey in Figure 56. The first PCA was performed on data from the Bay of Cherbourg and the two first dimensions explained 81.5% of the scatter plot. The second PCA was performed on data from the Bay of Seine and the two first dimensions explained 92.5% of the scatter plot. Concerning the samples collected from the Bay of Cherbourg, data concerning the first MI sampled in February 2020 were correlated with MPB biomass and inorganic nutrients. These dynamics evolved in July 2020 when a strong correlation was observed with F_V/F_M and α associated with MPB and the encrusting alga. This period was also correlated with water turbidity and temperature. The end of the survey (April 2021) was correlated with the $rETR_{MAX}$ of the MPB and the encrusting alga, this period was also correlated with the high percentage cover of the MI per unit of encrusting alga. The distance between rough and smooth MI was high at the beginning of the survey (February 2020), rough MI were F_V/F_M and α of MPB and encrusting alga associated, the smooth MI were correlated with MPB biomass. The distance between the rugosity condition was reduced during the rest of the monitoring period, and, at the end of the survey, smooth MI were more correlated with the percentage alga cover and associated $rETR_{MAX}$ than with the rough MI. In the Bay of Seine, the beginning (November 2019) and the end (March 2021) of the survey were close and correlated with inorganic nutrients and MPB associated F_V/F_M and α . Samples collected in September 2020 were correlated with MPB biomass. Rough and smooth MI remained similar throughout the survey except in May 2020, when smooth MI were closer to the rest of the samples than rough MI.

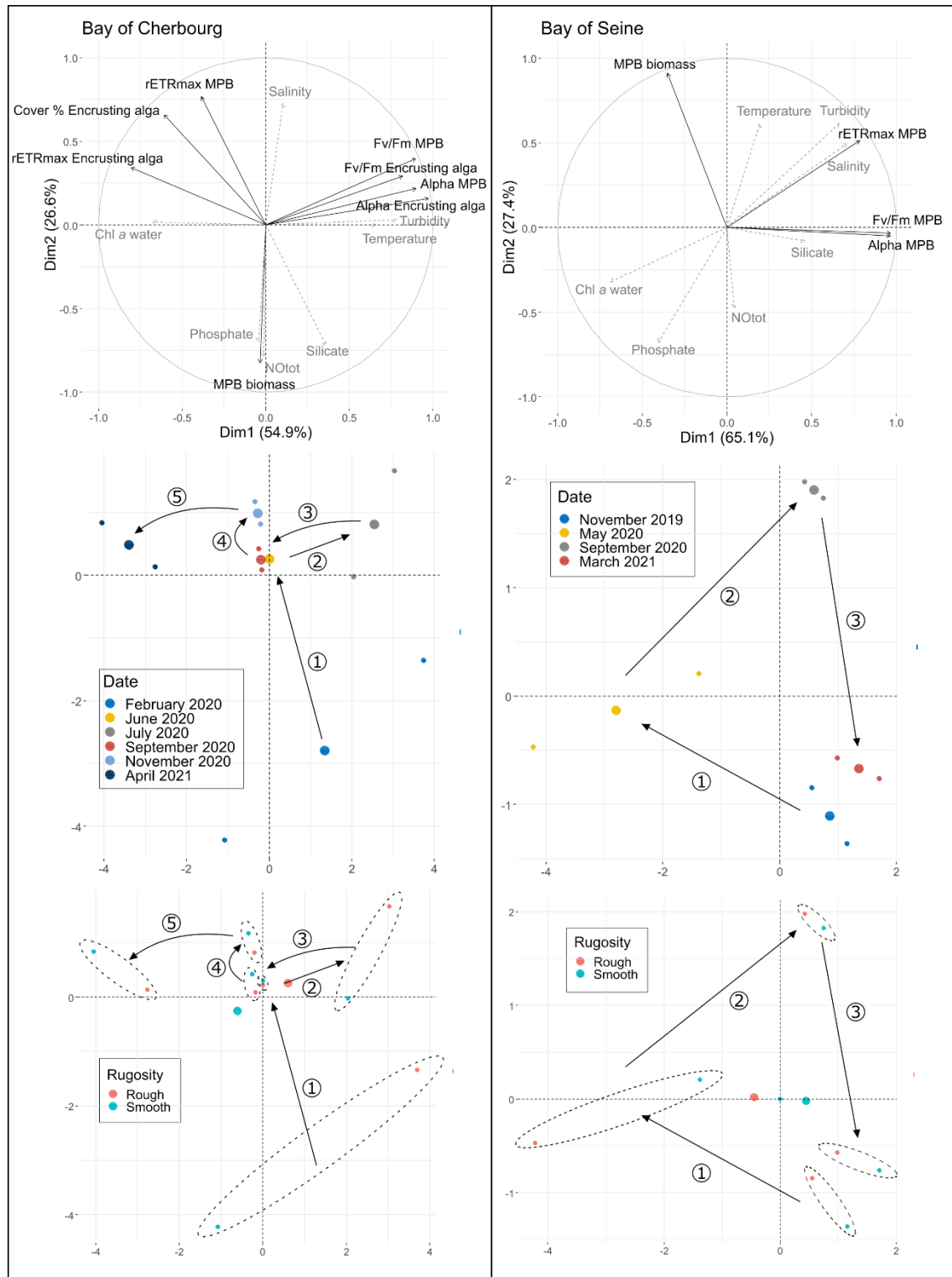


Figure 56 – Principal component analysis (PCA) of biotic and abiotic variables in the Bay of Cherbourg and the Bay of Seine. Abiotic variables for both sites were temperature, salinity, NOtot, silicate, phosphate, Chl a and turbidity. Biotic variables were MPB biomass, $rETR_{MAX}$, F_v/F_m and alpha for MPB and encrusting alga. Plots A and B represent all variables. Individuals are represented on the other plots: B and C for the Bay of Cherbourg, E and F for the Bay of Seine. B and E plots represent them according to the sampling time factor, C and F plots represent them according to the surface rugosity factor.

IV. Discussion

Firstly, the two experimental sites are characterised by marked environmental differences including tide amplitude, current velocity, and water turbidity. Indeed, the Bay of Cherbourg is hydrodynamically much more protected than the Bay of Seine thanks to a long dike. One of the consequences of this man-made structure is that it has modified the hydrodynamics and the influence of the tide on local currents, while the Bay of Seine is an open system. Secondly, water parameters also differed between the two sites. The Bay of Seine is under riverine influence, mainly freshwater inputs from the Seine and the Orne rivers where abiotic parameters revealed marked seasonal spatio-temporal variations, while the Bay of Cherbourg is directly connected to offshore water masses. Water turbidity was much higher in the Bay of Seine than in the Bay of Cherbourg particularly in September 2020 (more than 147 times higher in the Bay of Seine), concentrations of inorganic nutrients were also much higher in the Bay of Seine than in the Bay of Cherbourg (Table 8). Water turbidity has a direct impact on the quantity and quality (spectrum) of the light, which is a determinant of the growth and photobiology of primary producers (Behrenfeld et al., 2002; Douglas et al., 2003). Given these disparities, comparing several biotic variables on similar artificial structures immersed in the two sites since September 2019 is extremely relevant.

IV.1. *Microphytobenthic biofilm growth, photobiology, and drivers*

The surface complexity of an artificial structure can greatly influence the development of marine epilithic biofilm (Hutchinson et al., 2006; Souche et al., 2016) and the structure or the diversity of benthic communities in general (Baine, 2001; Bellou et al., 2012; Jaxion-Harm & Szedlmayer, 2015; Vivier et al., 2021a). In the Bay of Cherbourg, biofilm growth increased considerably between the immersion of the MI samples and the first sampling occasion in February 2020. Total Chl *a* biomass increased significantly in June 2020 and remained stable until 2021. The absence of a significant difference in this indicator between the rough and the smooth MI is surprising and differed from previous studies on the same structures in controlled conditions (Vivier et al., 2021a). Indeed, the surface structure is an important variable which may significantly affect the development of biofilm (Hutchinson et al., 2006; Giannantonio et al., 2009; Salta et al., 2013). The absence of clear pattern in biofilm biomass between the two rugosity conditions in our study can be explained by the environmental conditions and the long monitoring period. Environmental conditions in the Bay of Cherbourg were relatively favourable for communities of primary producers (Vivier et al, under review), indeed the fact

that water turbidity was relatively low in this area led to a high available PAR. Moreover, the Bay of Cherbourg is encircled by a long dike which protects the site, including from the main major currents. In terms of photosynthesis and at the scale of the entire MI, the global dynamics of F_V/F_M , $rETR_{MAX}$, and α were similar to those of biomass over the course of the survey. These results highlight the close relation between the dynamics of biomass and of photosynthetic parameters of biofilm in natural environments. However, an increase in F_V/F_M and α was observed in July 2020 in parallel with a decrease in $rETR_{MAX}$. Like for biomass, each photosynthetic parameter appeared to remain stable after July 2020, once again highlighting the influence of environmental conditions and biofilm acclimation over time. These observations were confirmed by the PCA showing the strong correlation with the MPB photosynthetic parameters.

In the Bay of Seine, biofilm colonisation of the MI over time differed considerably from that in the Bay of Cherbourg. The lower biomass recorded on the MI in the Bay of Seine can be explained by abiotic factors including high water turbidity at this site and high hydrodynamics. These results correspond to those of another study conducted at the same sites which identified a highly productive system in the Bay of Cherbourg and a consumer system in the Bay of Seine (Vivier et al., under review). In the Bay of Seine, the photosynthetic parameters of the biofilm did not reflect biomass dynamics. Even if the statistical analysis revealed no significant differences between the two types of rugosity, the general trend was more pronounced than in the Bay of Cherbourg. F_V/F_M , α and $rETR_{MAX}$ were globally lower on the rough MI than on the smooth ones. Moreover, the NPQ was higher on the rough structures in the Bay of Seine MI indicating higher photoacclimation of these biofilms. These results are surprising because, as mentioned above, they differed from the results of previous studies conducted on identical structures in controlled conditions. Indeed, a significant impact of added rugosity on the biofilm biomass and its photosynthesis have already been reported (Hutchinson et al., 2006; Sweat & Johnson, 2013; Vivier et al., 2021a). The tougher environmental conditions in the Bay of Seine compared with those in the Bay of Cherbourg and increased competition with various species like barnacles may explain our results. High rates of colonisation by benthic faunae, particularly barnacles, were observed in the Bay of Seine MI (unpublished data). Combined with the local environmental conditions, this colonisation may explain the lower biofilm biomass and the differences observed between rough and smooth MI. Increased colonisation of smooth MI may reproduce the inherent rugosity of the rough MI and hence create favourable conditions for the development of biofilm (Olivier et al., 2000).

IV.2. *Development and photobiology of encrusting alga and competition with microphytobenthic biofilm*

Colonisation by a subtidal encrusting alga was observed and recorded only in the Bay of Cherbourg MI during our survey. The only species recorded was *Phymatolithon purpureum* and during its growth phase between February and September 2020, it covered approximately 75% of the surface of the MI. The percentage cover on the smooth MI was slightly higher than on the rough MI, suggesting a better success rate on the unmodified surface of the structure. On both types of structure, stable state was reached after one year of immersion. The biology of *Phymatolithon purpureum* and encrusting algae in general under temperate climates is poorly documented, and their physiology and photobiology in comparable environments has not been studied to date. However, several studies conducted in tropical waters highlighted the low photosynthetic activity and the elevated photoinhibition of red coralline algae (Irving et al., 2004; Burdett et al., 2014) and their adaptation to low light conditions in general (Irving et al., 2004; Underwood, 2006). Several studies have already measured the growth rate of encrusting algae to evaluate their response to environmental conditions (Leukart, 1994) or other pressures including herbivory or competition between coral species (Figueiredo et al., 1997). The use of imaging-PAM fluorimetry allowed the spatialisation of the photosynthetic parameters. Knowing the areas colonised by *Phymatolithon purpureum*, it was possible to assess photosynthesis inside and outside this zone. The spatial distribution of several photosynthetic indicators and the photosynthetically active biomass were investigated on three sampling occasions representative of the growth dynamics of this alga (early, exponential and stabilised colonisation). These results revealed a clear pattern between the area covered by the encrusting alga and the other areas. During the early phase of colonisation and exponential growth (from February to July 2020), the F_V/F_M , $rETR_{MAX}$ and α were significantly higher outside the *Phymatolithon purpureum* area than inside it. These results revealed the poor photosynthetic efficiency and capacity of *Phymatolithon purpureum* compared with those of the microphytobenthos present outside the colonised areas. Considering the low microphytobenthic biomass in July 2020, these results tended to show the strong competition between phototrophic biofilms and the encrusting alga for available space plus under the influence of environmental factors like available light and nutrients. Such competition is also supported by the results of the PCA which revealed an anti-correlation between MPB biomass and the percentage cover by the encrusting alga. The high percentage cover by the encrusting alga after July 2020 may explain the decrease in several photosynthetic parameters like F_V/F_M or α observed during the

same period and until the end of the survey. This explanation is supported by the results of the PCA which showed an anti-correlation between the percentage cover of the encrusting alga and the F_V/F_M or α . Moreover, this period also corresponded to a significant increase in the NPQ, reflecting significant photoinhibition, which can also be explained by the encrusting alga (Irving et al., 2004; Burdett et al., 2014). These hypotheses were also supported by the dynamics of these indicators on the MI in the Bay of Seine. Indeed, in contrast to samples collected in the Bay of Cherbourg, there was an increase in or stabilisation of the photobiological parameters of samples collect on the MI immersed in the Bay of Seine after September 2020. The absence of encrusting algae at this site may explain the higher photosynthetic effectiveness and capacity. *Phymatolithon purpureum* has already been shown to grow more rapidly laterally than in thickness (Steneck & Paine, 1986; Dethier, 1994) which also explains its rapid expansion on the MI. In April 2021, during the stabilised colonisation phase of *Phymatolithon purpureum*, the gap between the photosynthetic parameters of the area colonised by the encrusting algae and other areas was smaller than in the early phase of colonisation. This was particularly the case of maximum production capacity ($rETR_{MAX}$). Moreover, and in contrast to the F_V/F_M or α , the $rETR_{MAX}$ was higher in the late colonisation phase than in the early and exponential phases. This result was supported by the PCA which revealed a significant correlation between the percentage cover of the encrusting alga and $rETR_{MAX}$. The elevated microphytobenthic biomass (Chl *a*) at this period may explain the reduced difference in photosynthetic parameters between the area colonised by the encrusting alga and other areas. The decrease in the photosynthetic efficiency (α) in the late colonisation phase of the encrusting alga can be explained by the higher microphytobenthic biomass during this period. Indeed, other studies have already reported a negative correlation between biomass and photosynthetic efficiency, highlighting the importance of self-shading in epilithic biofilm photosynthesis (Guasch & Subater, 1995). Knowing the low light preferendum of red encrusting algae (Irving et al., 2004; Burdett et al., 2012, 2014), this phenomenon may also contribute to strong colonisation by *Phymatolithon purpureum* and its stabilisation from early winter to spring. However, these results should be interpreted with caution because chlorophyll fluorescence techniques that use blue light, like imaging-PAM, are not perfectly suited for Rhodophyceae species like *Phymatolithon purpureum*. Blue light is not absorbed by the phycobilisomes and thus creates an imbalance between photosystems with excess energy at PSI and a deficiency at the PSII side of the electron transfer chain (Hanelt et al., 1992; Luimstra et al., 2018) which can influence photosynthetic efficiency and capacity (Korbee et al., 2005). This phenomenon may partly explain the low photosynthetic parameters recorded for areas colonised by *Phymatolithon purpureum*.

IV.3. *Macroalgal diversity and structural–functional relationships*

The first non-encrusting macroalgae were recorded in September and November 2020, after one year of immersion. Strong dominance of Rhodophyceae was observed (10 species out of the 12 described). The abundance of Rhodophyceae in the Bay of Seine suggests that these species are well acclimated to turbid conditions and high hydrodynamics, but the high concentrations of inorganic nutrients in this site may also explain the dominance of Rhodophyceae and the high species richness. Preference for low light conditions and potential photoinhibition in presence of excess irradiance have already been documented, in particular for Rhodophyceae algae (Dring et al., 1996; Nielsen & Nielsen, 2008). The dominance of Rhodophyceae species, particularly in the Bay of Seine, can be explained by the elevated water turbidity which reduces the available PAR for the primary producers. The marked difference in species richness between the two sites was surprising. Indeed, previous observations on large artificial structures at the same sites revealed higher macroalgae richness in the Bay of Cherbourg (Vivier et al., under review). The results presented here and the higher species diversity in the Bay of Seine could be due to the difference in the sampling periods (November in the Bay of Cherbourg and September in the Bay of Seine).

These results support our previous statement concerning the stabilisation of the ecosystem associated with the MI after one year of immersion. Productive ecosystems are not always a synonym of biodiversity, the relationship between those indicators varies across diversity dimensions and external constraints (Brun et al., 2019). For example, high diversity may increase phylogenetic diversity in ecosystems with few external constraints by promoting complementary strategies, but in the presence of external constraints, the best competitors may be selected and diversity consequently reduced (Zobel & Pärtel, 2008; Brun et al., 2019). What is more, this ecology principle varies with the spatial scale considered. At global scale, unimodal diversity–productivity relationships dominate in temperate regions, whereas positive relationships are more common in the tropics (Zobel & Pärtel, 2008). In marine ecosystems, the scale dependence was suggested by more prevalent diversity–productivity relationships at smaller (local, landscape) than larger (regional, continental) spatial scales (Witman et al., 2008). Our study reinforces these ecology concepts and is a good illustration of the potential negative correlation between diversity and productivity of small-scale ecosystems.

V. Conclusion

This study provides reliable data on benthic colonisation by micro- and macroalgae at two different sites in the English Channel. Our results confirm the advantages of using concrete to improve the efficiency of artificial structures. Concrete promotes the development of marine biofilms as observed in this study during the first months of the survey. What is more, the photosynthetic parameters of the biofilm were elevated, pointing to good acclimation to the environment. Except in the Bay of Seine, our results revealed no clear differences between the two surface rugosity conditions (rough and smooth). This can be explained by the strong influence of environmental conditions on the colonisation process. Macroalgae diversity was higher in the Bay of Seine, the associated biomass was higher in the Bay of Cherbourg. The unmodified (smooth) surface of the structure seemed to promote colonisation by the encrusting alga. High colonisation by *Phymatolithon purpureum* seemed to affect microphytobenthic growth and photosynthesis. Indeed, investigation of the spatialisation of several photosynthetic parameters on the MI samples revealed a large gap between the physiological quality and the photosynthetic effectiveness and capacity both inside and outside the area colonised by *Phymatolithon purpureum*: the photosynthetic parameters of the encrusting alga were lower than those of microphytobenthos alone. These results highlight the strong capacity of this alga to colonise artificial hard substrata (Loke et al., 2016). Overall, these results support previous reports of the high efficiency of concrete in promoting benthic colonisation independently of the site and environmental conditions. The ecosystem associated with the MI seemed to reach a first stabilisation phase after one year of immersion. The comparison of identical structures at two different sites along the French coast of the English Channel provided further proof of the high success rate of such projects in restoring affected ecosystems.

VI. Acknowledgments

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Partie V-B : Diversity, structure assemblages, production and photosynthesis of
benthic communities on artificial reefs, a comparative case study in the
English Channel

This work is under review in Sciences of the Total Environment

Résumé de la publication

Après avoir étudié les dynamiques de colonisation benthiques et les fonctions écologiques associées à différentes échelles (microphytobenthos et macroorganismes) sur des infrastructures expérimentales (dalles) en conditions contrôlées et *in situ*, la dernière étude de ces travaux s'est concentrée sur des récifs artificiels de grande taille immergés depuis plusieurs années (2015) sur les mêmes sites expérimentaux que ceux sélectionnés pour l'immersion des blocs de digues MARINEFF.

Cette étude s'est donc concentrée sur les RA du projet RECIF, les communautés benthiques et les fonctions écologiques associées ont été étudiées entre fin 2019 et fin 2020. Des différences significatives entre les deux sites ont été observées concernant la structure des communautés. Les récifs artificiels en rade de Cherbourg ont montré une diversité de producteurs primaires plus forte qu'en baie de Seine (15 espèces contre 8 espèces). Des variations saisonnières importantes ont également été décrites entre les deux sites avec des diversités plus élevées en été/automne. Ces différences peuvent s'expliquer par certains facteurs comme la turbidité, la quantité de lumière disponible ou encore les concentrations en nutriments inorganiques. Afin d'explorer la fonction de production associée à ces structures, des incubations ont été réalisées sur les parpaings constituant les RA. Les récifs artificiels de la rade de Cherbourg sont les plus productifs avec un niveau élevé de production primaire en été. Cette saison est également celle à laquelle biomasse et diversité de macroalgues sont les plus élevées. Les résultats obtenus pour les récifs artificiels de la baie de Seine sont comparables à ceux d'autres études sur des substrats durs (Golléty et al., 2008). Toutefois, pour la rade de Cherbourg, les valeurs sont nettement plus élevées. Certaines études similaires réalisées sous des climats tropicaux montrent des valeurs de production inférieures (Cesbron et al., 2019). Ces résultats ont mis en avant un écosystème fortement productif (rade de Cherbourg) et un fortement consommateur (baie de Seine). La faible biomasse de producteurs primaires en baie de Seine n'est pas suffisante pour compenser la forte consommation (respiration) mesurée sur ce site. Dans un écosystème comparable, dans la même mer, à la même profondeur, la même zone mais dans deux masses d'eau différentes, deux types de fonctionnement associés à ces récifs artificiels ont été observés. L'un peut être décrit comme un récif « producteur » riche en macroalgues, c'est celui de la rade de Cherbourg. L'autre comme un récif « consommateur » utilisant le phytoplancton comme source trophique, celui de la baie de Seine.

Les performances photosynthétiques des communautés de macroalgues sur les récifs artificiels de la rade de Cherbourg ont également été étudiées. Des mesures *in situ* à l'aide d'un DIVING-PAM ont permis de mettre en avant un résultat majeur : la capacité maximale de production ($rETR_{MAX}$) a montré une augmentation graduelle pour toutes les espèces entre l'été et le printemps. Le statut physiologique (F_V/F_M) des algues rouges est plus faible que pour les autres espèces. Les valeurs de F_V/F_M mesurées pour les algues vertes et brunes indiquent des organismes en bonne santé et bien adaptés à leur environnement. Les valeurs plus faibles obtenues pour les algues rouges peuvent s'expliquer par la composition pigmentaire spécifique chez ces espèces (JuHyoung et al., 2019). En été, des valeurs plus faibles de $rETR_{MAX}$ peuvent s'expliquer par un NPQ saisonnier (Franklin & Forster, 1997). En effet, dans des environnements intertidaux ou subtidaux avec des profondeurs faibles, la quantité de lumière disponible dans le milieu (PAR) peut souvent se retrouver en excès et devenir saturant pour la photosynthèse (Müller et al., 2001; Gevaert et al., 2002).

Enfin, les communautés de macrofaune benthique ayant colonisé les RA ont également été étudiées. La communauté de macrofaune associée aux récifs artificiels de la rade de Cherbourg est plus diversifiée que dans la baie de Seine (H'). Toutefois, la biomasse totale est supérieure dans la baie de Seine. L'abondance totale maximale a été atteinte en automne pour les deux sites. Les espèces identifiées ont été classées selon leur groupe trophique, cette approche a permis de mettre en avant deux structures trophiques distinctes en fonction des sites mais également d'observer des variations saisonnières. Le réseau trophique associé aux récifs de la rade de Cherbourg semble plus diverse et complexe, présentant des variations saisonnières marquées. Les groupes trophiques dominants sont différents entre les deux sites pour la même saison. Ainsi, à l'automne, la faune benthique de la baie de Seine est dominée par des filtreurs et dans la rade de Cherbourg, une structure trophique plus diversifiée a été évaluée avec dépositivores, des filtreurs et des prédateurs. Cinq ans après leur immersion, les écosystèmes associés aux récifs artificiels peuvent être considérés comme stabilisés. Considérant que les structures immergées sont les mêmes, les différences enregistrées de structure trophique de la macrofaune entre les sites peuvent être induites par des facteurs abiotiques (hydrodynamisme, salinité, température...), par les communautés environnantes présentes avant et après l'immersion des récifs artificiels ainsi que par les relations proies / prédateurs.

Photosynthesis, primary production, structure, and diversity of macrobenthic flora and fauna assemblages on artificial reefs, a comparative case study in the English Channel

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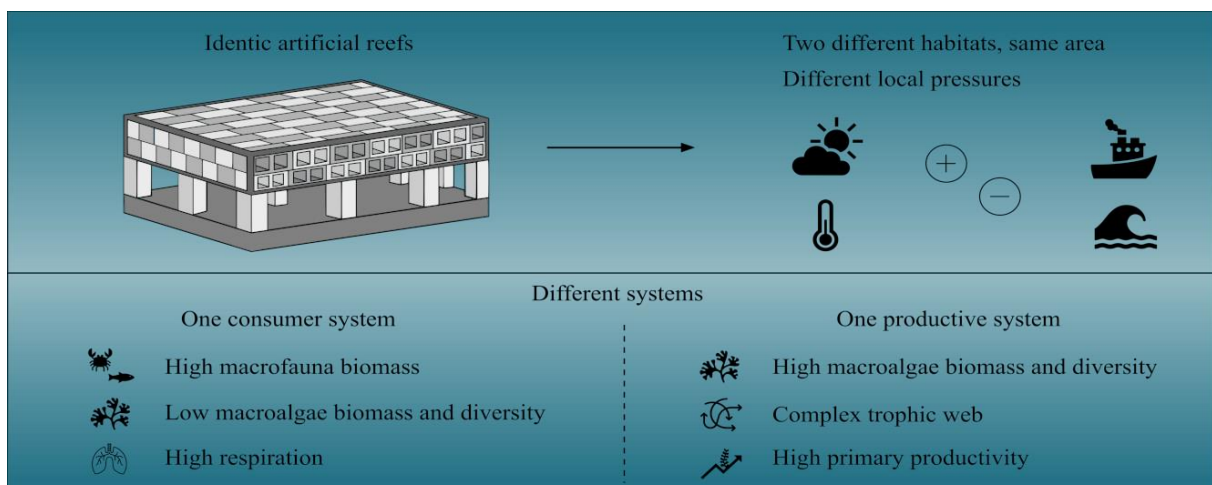
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Abstract

Several biotic indicators of macroalgae and macrobenthic communities present on artificial reefs immersed for five years were monitored over a full year. Comparison of two different sites located along the French coast of the central part of the English Channel, highlighted the relationship between the location and the efficiency of artificial reefs. Macroalgae community was more diverse and primary production higher in the Bay of Cherbourg than in the Bay of Seine and was dominated by Rhodophyceae species. Macroalgae communities in the Bay of Cherbourg were in good physiological condition throughout the year ($F_V/F_M > 0.5$). The diversity index of the macrobenthic fauna was classified based on trophic group and linked to biomass. Benthic fauna in the Bay of Seine was more abundant and more diversified. These results indicate different trophic webs in the two sites with a more complex system with distinct seasonal changes in the Bay of Cherbourg. Moreover, the dominant trophic groups differed in the same season at the two sites. Even though the structures were identical, the ecosystem functions and the associated benthic communities differed significantly in two sites with similar tide amplitude and climate. Our results revealed the efficiency of artificial reefs in creating novel habitats and promoting the biomass and diversity of associated live communities. In the same sea, at the same depth, but in different water masses, two different types of artificial reefs both function: in the Bay of Cherbourg, it is a “primary producer reef” rich in macroalgae, and in the Bay of Seine it is a “primary consumer reef” where production is based on phytoplankton resources. More generally, our results underline that predicting the environmental impact of an artificial structure is complex and the transposition of studies carried out on nearby sites is not sufficient. It is necessary to consider all the environmental constraints.



Keywords

Artificial Reefs; Primary production; Photosynthesis; Benthic communities; Biodiversity

Abbreviations

Ek: Acclimatation light

F_V/F_M : maximum quantum efficiency of PSII

PAM: Pulse Amplitude Modulated

PAR: Photoactive Radiation

PSII: Photosystem II

rETR: relative Electron Transport Rate

RLC: Rapid Light Curve

I. Introduction

Artificial reefs are man-made structures emplaced in aquatic environments that serve as habitats or shelters for organisms. Artificial reefs have long been used to attract fish and the number of such structures has increased over the three last decades (Baine, 2001; Santos et al., 2011; Lowry et al., 2014). Artificial reefs are usually expected to produce an overall increase in species richness by protecting some species, and also an increase and diversification of trophic contributions (Hixon & Beets, 1989; Piazza et al., 2005; Bodilis et al., 2011). The improvement in habitat is generally reflected in greater food availability and increased shelter from predators, as well as new recruitment areas for juveniles of various species (benthic invertebrates and fish), which explains the increase in organism biomass after their installation (Sherman, 2002; Perkol-Finkel et al., 2018b). The ecological value of artificial structures as habitats for native species can vary in relation to many structural and environmental factors (Bortone, 2006; Perkol-Finkel et al., 2008; Vivier et al., 2021b).

The English Channel is an epicontinental sea with a maximum depth of 174 m (Dauvin et al., 2020). It is also a macrotidal system with strong hydrodynamics (Orbi & Salomon, 1988). The English Channel is one the most exploited seas in the world due to its high productivity and richness (fishing, aquaculture, granular extraction, etc.) (Boyd & Rees, 2003; Fritsch et al., 2007; Carpentier et al., 2009; Dauvin et al., 2020). It is also a major route for maritime traffic with several large harbors, and several million people live along its coasts. The combination of

all these pressures on the ecosystems is responsible for their degraded ecological quality, and natural resources, biodiversity, and ecosystem services are also strongly impacted (Desprez, 2000; Loizeau et al., 2001; Boyd and Rees, 2003; Molfese et al., 2014; Dauvin, 2019). The Bay of Cherbourg, a highly anthropized area, is located on the north coast of the Cotentin Peninsula (Normandy, France). The area is surrounded by a long dike that creates a semi-protected habitat (Baux et al., 2017). The Bay of Cherbourg is dominated by soft sediments and is surrounded by artificial hard substrates (Baux et al., 2017). The area was recently designated as a main construction site for French Marine Renewable Energy Development (MRED). In comparison, the rest of the English Channel, particularly the eastern part is under tough environmental conditions. Indeed, the hydrodynamics are strong and water conditions such as nutrient availability, turbidity, and salinity are influenced by freshwater inputs mainly from the Seine estuary that influence ecosystem functions including primary productivity and the composition of the benthic communities (Dauvin, 2012; Lemesle, 2015; Dauvin et al., 2020; Serre-Fredj et al., 2021). Despite the many differences in habitat and environmental conditions between the eastern Bay of Seine and the Bay of Cherbourg, several aspects including the nature of the seabed and the general climatic conditions are comparable. The Bay of Cherbourg is also under human pressure that impact both ecosystem functions and ecological services (Boyd & Rees, 2003; Molfese et al., 2014; Dauvin et al., 2020). Under such human pressure, artificial reefs are one possible way to restore or create new habitats to improve or at least to protect local biodiversity and production (Bortone, 2006; Bodilis et al., 2011; Layman et al., 2016). Comparing identical structures immersed in sites with contrasted environmental conditions advances our knowledge of local ecosystem functions and of the efficiency of the artificial structures (Bombace et al., 1994; Kasim et al., 2013).

Primary production is an integral aspect of ecosystem functioning and an efficient indicator of ecosystem health. Measuring dissolved oxygen is one of the most widely used ways to assess primary production in marine ecosystems and hence to calculate rates of production and respiration. Benthic chambers are widely used to measure macroalgae or biofilm production in benthic systems (Gevaert et al., 2002; Hubas & Davoult, 2006; Randall et al., 2019), but these techniques are influenced by several uncontrollable factors such as the weather or water turbidity that affect solar radiation and make it difficult to replicate measurements. Similar techniques with controlled lighting may make it possible to consolidate the production data and compare them between different sampling sites or over time. PAM fluorimetry is a non-invasive way to monitor the functional state of photosynthetic organisms and as such, a good

way to investigate the physiology and the acclimation of these organisms to their environment (Gevaert et al., 2002; Claquin et al., 2008; Napoléon et al., 2013a). PAM fluorimetry has enabled the measurements of chlorophyll *a* fluorescence associated with photosystem II (PSII) as a proxy of photosynthetic potential (Kromkamp et al., 1998). These measurements provide considerable photosynthetic information on algae photobiology. Establishing rapid light curves (RLC) by calculating the relative electron transport rate (rETR) provides information on the maximum electron transport rate or photosynthetic capacity (rETR_{MAX}), for example. Other indicators like the maximum quantum efficiency of PSII (F_V/F_M) reflect the health and acclimation status of the algae.

The aim of the present study was to investigate the primary production, the photosynthetic parameters of primary producers, the biodiversity and the trophic assemblages associated with artificial reefs. By comparing identical structures in two different sites along the French coast of the English Channel, we aimed to assess:

1. the success rate of artificial reefs in restoring two different affected ecosystems;
2. the relationship between biodiversity and production by investigating the diversity and biomass of benthic fauna and macroalgae and the associated maximum primary production using benthic chambers in controlled conditions;
3. the photobiology and environmental acclimation of the macroalgae communities that colonize these artificial reefs using PAM measurements;
4. the seasonal variations in several biological and environmental parameters over a full year after five years of immersion, as this provides reliable data on the benthic communities (producers and consumers) associated with a stable hard-substrate ecosystem under a temperate climate.

II. Materials & Methods

II.1. *Sampling sites*

Artificial reefs (Figure 57) were installed in 2015 in two different sites (the Bay of Cherbourg and the Bay of Seine) as part of the European RECIF project and were used as a support in the European Interreg Va project MARINEFF (2018-2022). The first site in the Bay of Cherbourg is characterized by a sandy bottom while the second site in the Bay of Seine is characterized by sandy-rocky bottom. In both sites, the low tide bathymetry is about 4 m. In the Bay of Cherbourg, the artificial reef comprised 12 modules organized in three clusters creating a triangle structure, while only three modules were used in the Bay of Seine. Each module was

3 m long, 2 m wide and 1.35 m high (8.1 m³ per module, 32.4 m³ per cluster, giving a total of 97.2 m³ in the Bay of Cherbourg). The modules comprised three levels, the first level was a supporting base to prevent sinking; the second level was made up of solid concrete pillars and empty concrete drains; the third level was a metal cage filled with cinder blocks. The cinder blocks were made of porous concrete and each block was 0.4 m long, 0.2 m wide and 0.2 m high.

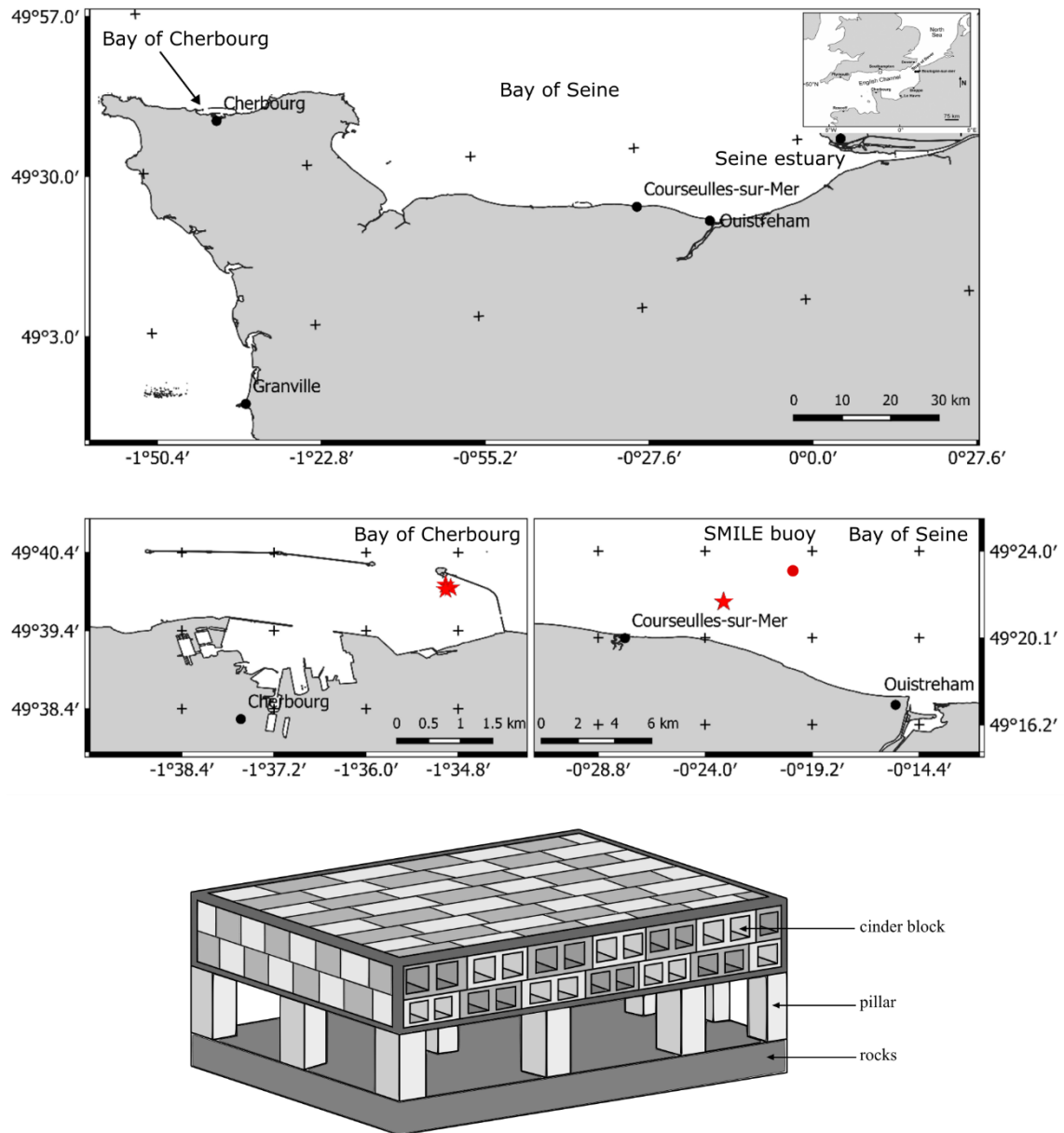


Figure 57 – (A) Location of the artificial reefs in the Bay of Cherbourg and in the Bay of Seine. Sampling sites are represented by red stars. The location of the SMILE buoy is shown by a red dot. (B) Artificial reef schema: samples were collected from the cinder blocks in the upper part of the AR module.

II.2. *Sample collection*

II.2.1. *Benthic communities and production*

Benthic communities were sampled from December 2019 to September 2020. Three campaigns were conducted in the Bay of Seine and four in the Bay of Cherbourg. In the Bay of Seine, samples were collected in winter (Dec 3, 2019), spring (May 26, 2020) and autumn (Sept 15, 2020). In the Bay of Cherbourg, samples were collected in winter (Feb 5, 2020), spring (June 2, 2020), summer (July 1, 2020), and autumn (Sept 29, 2020). In each campaign, six cinder blocks that formed the top of the modules were sampled randomly and the samples transported to the laboratory for analysis. To prevent any loss of fauna, each cinder block was carefully placed in a zooplanktonic net (500 μm) closed and brought to the boat. Each cinder block was conserved separately in a tank until the transport to the Luc-sur-mer marine station, where each block was conserved in its zooplanktonic net in one of several large basins with water renewed from the open sea. Each cinder block was first incubated in a hermetic box with controlled lighting still kept in the large basin to record production by the cinder block. During each incubation, photoactive radiation (PAR) was recorded with a RBR solo³ PAR logger connected to a Li-COR LI-192 Underwater Quantum Sensor. Dissolved oxygen ($\text{mg O}_2 \text{ L}^{-1}$) was recorded with an EXO Optical Dissolved Oxygen Sensor hermetically connected to the system. Each incubation consisted in seven different light conditions (dark 1 = 0 $\mu\text{mol photon m}^{-2} \text{ s}^{-1}$ – PAR 1 = 100 $\mu\text{mol photon m}^{-2} \text{ s}^{-1}$ – PAR 2 = 200 $\mu\text{mol photon m}^{-2} \text{ s}^{-1}$ – PAR 3 = 360 $\mu\text{mol photon m}^{-2} \text{ s}^{-1}$ – PAR 4 = 430 $\mu\text{mol photon m}^{-2} \text{ s}^{-1}$ – PAR 5 = 750 $\mu\text{mol photon m}^{-2} \text{ s}^{-1}$ – dark 2 = 0 $\mu\text{mol photon m}^{-2} \text{ s}^{-1}$). Each condition lasted 20 minutes. A circulating pump fixed to the box maintained the flow of water. Oxygen production was calculated as the slope of the linear regression of O_2 concentration versus the incubation time per light condition after incorporating the volume of water contained in the box (58.9 L) and the surface area of the cinder block, is expressed in $\mu\text{mol O}_2 \text{ m}^{-2} \text{ h}^{-1}$. Gross primary production (GPP) was calculated for each PAR condition as the addition of respiration (dark condition 2) and net primary production (NPP) of the selected condition. These data were converted into carbon equivalent assuming a Redfield ratio of 106:138 and are expressed in mass units as $\text{g C m}^{-2} \text{ d}^{-1}$ (Cesbron et al., 2019). GPP_{max} at saturation light was obtained using the (Webb et al., 1974b) model. Primary productivity was then calculated as the biomass: GPP_{max} ratio and expressed in gC gC d^{-1} .

$$X(\text{PAR}) = X_{\text{max}} \times e^{\left(1 - \frac{\text{PAR} \times \alpha_X}{X_{\text{max}}}\right)} \quad (44)$$

where $X(\text{PAR})$ is GPP (PAR) or rETR (PAR), X_{max} is the maximum photosynthetic capacity measured with the O_2 incubation method (GPP_{max} in $\text{g C m}^{-2} \text{d}^{-1}$) or the PAM method (rETR_{MAX} , rel. unit) and α is the photosynthetic efficiency that corresponds to the slope of each Production / Light curve.

After incubation, all the macroalgae were sampled and conserved at -20°C for species identification and biomass estimation expressed ash-free dry weight (AFDW) per m^2 (loss of weight of dry organisms after 5 h at 500°C). Ash-free dry weight biomass was converted into carbon content using a conversion factor of 0.35 (Surif & Raven, 1990; Pihl et al., 1996). Macroalgae species were assigned to their class (Ulvophyceae, Rhodophyceae or Phaeophyceae). The cinder block was then fixed in 10% formaldehyde, rinsed, and carefully scraped to sample all animal organisms. The samples were preserved in 70% ethanol, identified to the lowest possible taxonomic level and counted (sieved at 0.5 mm). The biomass of each taxonomic group was determined and is expressed in AFDW per m^2 (loss of weight of dry organisms after 5 h at 500°C). Ash-free dry weight biomass was converted into carbon content using a conversion factor of 0.518 (Brey, 1999). Benthic invertebrates were assigned to six trophic groups: grazers (Gr), filter feeders (FF), predators (Pr), scavengers – omnivorous (Scv/O), sub-surface deposit feeders (ssDF) and surface deposit feeders (sDF) according to (Pezy et al., 2020). Community structure analysis was based on taxonomic richness, the Shannon-Wiener diversity index H' and the Pielou evenness index J' . Taxonomic richness corresponded to the total number of taxa recorded on the six cinder blocks sampled in each campaign. Both indices, H' using $\log_2$ and J' were also calculated for each campaign.

II.2.2. *Photosynthetic parameters*

For some of these campaigns, photosynthetic parameters of the macroalgae were assessed by SCUBA diving with a DIVING-PAM-II fluorometer (Heinz Walz GmbH, Germany). This tool allowed us to measure the primary productivity by several indicators as the maximum quantum efficiency of PSII (F_v/F_m). A dark adaptation of 5 min allowing oxidation of the electron acceptor pools was respected before each measurement. The saturation pulse intensity was $4,000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ for 0.6 s. For rapid light curves were performed as follow: samples were exposed to 11 incremental intensities of actinic light (E) from 0 to $1,490 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ with irradiance steps of 20 s. Numerical values were extracted using analytical software (WinControl V3.30; Walz).

The maximum quantum efficiency of PSII (F_V/F_M) was calculated using the following equation from Genty et al. (1989):

$$\frac{F_V}{F_M} = \frac{(F_M - F_O)}{F_M} \quad (45)$$

where F_M is the maximum fluorescence yield measured after a saturating pulse and F_O the minimum fluorescence yield measured immediately before the saturating pulse.

The maximum relative Electron Transport Rate ($rETR_{MAX}$, rel. unit) was estimated by fitting FLC data with the Webb model (1974) to estimate α ($\mu\text{mol electrons } (\mu\text{mol photons})^{-1}$) and E_k ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) with α the initial slop of the FLC and E_k the light saturation index as described earlier.

The $rETR_{MAX}$ was calculated as:

$$rETR_{MAX} = \alpha \times E_k \quad (46)$$

II.2.3. Abiotic parameters and inorganic nutrient analyses

High frequency measurements were taken by the SMILE instrumented buoy located in the Bay of Seine (0°19'41.00"O 49°21'14.00"N) (data available doi.org/10.17882/53689). Conductivity and temperature (tetracon sensor, WTWTM), turbidity (Seapoint turbidity meter, Seapoint Sensor), oxygen (AADI Oxygen optode, Anderraa) and fluorescence (Cyclops-6K, Turner Design) were combined in an automatic NKE instrumentation (MP7, NKE Instrumentation[®]). PAR (photosynthetically active radiation) was measured with a Saltantics sensor. For the inorganic nutrient analyses, a sample of water was collected in the Bay of Cherbourg and in the Bay of Seine during each campaign. Each sample was filtered using cellulose acetate filters (ClearLine, CA, 33 mm, 0.45 μm) in 50 mL falcon tubes and frozen (-20 °C). Analysis were conducted using a Seal Analytical AA-3 system (Aminot & K  rouel, 2007). The limits of quantification were 0.02 $\mu\text{mol L}^{-1}$ for PO_4^{3-} and 0.05 $\mu\text{mol L}^{-1}$ for NO_3^- , NO_2^- and Si(OH)_4 . NH_4^+ were analyzed by fluorometric measurements according to the protocol of Oriol et al. (2014). N_{tot} was calculated as follows: $\text{N}_{\text{tot}} = \text{NO}_3^- + \text{NO}_2^- + \text{NH}_4^+$.

II.3. Data treatment and analysis

Data analysis was performed with R i386 3.5.1 (R Development Core Team, 2008). A non-parametric Kruskal-Wallis test was used to identify differences in benthic macrofauna between

sites and seasons in terms of species richness, abundance, biomass, Shannon-Wiener diversity index H' and Pielou's evenness index J' . A principal component analysis was performed on data using the FactoMineR package.

III. Results

III.1. Biotic indices and structure assemblages

Counting all the campaigns, 18 different macroalgae species were recorded, nine in the Bay of Seine and 15 in the Bay of Cherbourg (Table 10). Table 10 summarizes the ash-free dry weight biomass (gC m^{-2}) for each macroalgae species in the two sites. On each sampling occasion in the Bay of Seine, the species with the highest biomass was *Cryptopleura ramosa* (between 1.091 and 1.314 gC m^{-2}). Higher diversity was recorded in autumn with eight different species including six Rhodophyceae, one Ulvophyceae and one Phaeophyceae. In the Bay of Cherbourg, the species with the highest biomass in winter were *Sphaerococcus coronopifolius* (8.052 gC m^{-2}) and *Kallymenia reniformis* (3.167 gC m^{-2}). *Cryptopleura ramosa* and *Plocamium cartilagineum* were recorded in the same proportion as in the Bay of Seine at the same period (respectively 1.118 and 0.336 gC m^{-2}). In spring, the species with the highest biomass were *Sphaerococcus coronopifolius* (11.539 gC m^{-2}), *Kallymenia reniformis* (6.255 gC m^{-2}) and *Bornetia secundiflora* (6.813 gC m^{-2}). *Cryptopleura ramosa* represented 10 times less biomass than in the Bay of Seine in the same period. In summer, marked diversity was observed with 11 different species. The species with the highest biomass were still *Kallymenia reniformis* (36.924 gC m^{-2}) and *Sphaerococcus coronopifolius* (20.979 gC m^{-2}). In autumn, diversity was lower than in the other seasons (three species) and significantly lower than the diversity recorded in the Bay of Seine at the same period. *Sphaerococcus coronopifolius* (36.189 gC m^{-2}) was still the species with the highest biomass. At the end of autumn (November), the diversity recorded (seven species) was lower than in winter (February). *Sphaerococcus coronopifolius* (23.396 gC m^{-2}), *Plocamium cartilagineum* (7.674 gC m^{-2}) and *Kallymenia reniformis* (6.300 gC m^{-2}) were dominant. Finally, a non-indigenous species, *Sargassum muticum*, was recorded in the bay of Cherbourg between spring and autumn with a maximum of biomass reached in summer (4.223 gC m^{-2}). This invasive species was not observed in the Bay of Seine.

Table 10 – AFDW biomass in gC m⁻² for each macroalgae species identified in the Bay of Seine and the Bay of Cherbourg for each campaign.

Site	Season	Class	Species	AFDW biomass (gC m ⁻²)
Bay of Seine	Winter	Rhodophyceae	<i>Cryptopleura ramosa</i>	1.091
			<i>Plocamium cartilagineum</i>	0.349
			<i>Sphondylothamnion multifidum</i>	0.024
	Spring	Rhodophyceae	<i>Cryptopleura ramosa</i>	1.314
			<i>Hypoglossum hypoglossoides</i>	0.104
	Autumn	Rhodophyceae	<i>Cryptopleura ramosa</i>	1.176
			<i>Heterosiphonia plumosa</i>	0.013
			<i>Hypoglossum hypoglossoides</i>	0.015
			<i>Kallymenia reniformis</i>	0.011
			<i>Plocamium cartilagineum</i>	0.026
			<i>Sphaerococcus coronopifolius</i>	0.012
		Ulvophyceae	<i>Ulva</i> sp.	0.292
		Phaeophyceae	<i>Dictyota dichotoma</i>	0.162
Bay of Cherbourg	Winter (February)	Rhodophyceae	<i>Ahnfeltiopsis devoniensis</i>	0.010
			<i>Bornetia secundiflora</i>	1.136
			<i>Cryptopleura ramosa</i>	1.118
			<i>Hypoglossum hypoglossoides</i>	0.005
			<i>Kallymenia reniformis</i>	3.167
			<i>Plocamium cartilagineum</i>	0.336
			<i>Sphaerococcus coronopifolius</i>	8.052
			<i>Vertebrata byssoides</i>	0.792
	Spring	Phaeophyceae	<i>Sargassum muticum</i>	1.069
		Rhodophyceae	<i>Bornetia secundiflora</i>	6.813
			<i>Cryptopleura ramosa</i>	0.132
			<i>Kallymenia reniformis</i>	6.255
			<i>Plocamium cartilagineum</i>	0.066
			<i>Sphaerococcus coronopifolius</i>	11.539
			<i>Vertebrata byssoides</i>	0.556
		Phaeophyceae	<i>Sargassum muticum</i>	0.283
	Summer	Rhodophyceae	<i>Bornetia secundiflora</i>	0.858

		<i>Calliblepharis jubata</i>	0.036
		<i>Cryptopleura ramosa</i>	1.552
		<i>Halurus flosculosus</i>	0.001
		<i>Kallymenia reniformis</i>	36.924
		<i>Plocamium cartilagineum</i>	2.197
		<i>Sphaerococcus coronopifolius</i>	20.979
		<i>Sphondylothamnion multifidum</i>	0.028
		<i>Vertebrata byssoides</i>	2.031
		<i>Vertebrata fruticulosa</i>	0.138
	Phaeophyceae	<i>Sargassum muticum</i>	4.223
Autumn	Rhodophyceae	<i>Bornetia secundiflora</i>	0.386
		<i>Sphaerococcus coronopifolius</i>	36.189
	Phaeophyceae	<i>Sargassum muticum</i>	0.493
Winter (November)	Rhodophyceae	<i>Bornetia secundiflora</i>	0.411
		<i>Cryptopleura ramosa</i>	3.349
		<i>Kallymenia reniformis</i>	6.300
		<i>Plocamium cartilagineum</i>	7.674
		<i>Sphaerococcus coronopifolius</i>	23.396
		<i>Cladophora pellucida</i>	0.011
	Phaeophyceae	<i>Laminaria digitata</i>	0.478

Table 11 summarizes the ash-free dry weight (AFDW) biomass (gC m^{-2}) for each class of macroalgae in the Bay of Cherbourg and in the Bay of Seine. Total biomass was higher in the Bay of Cherbourg than in the Bay of Seine in all seasons. The macroalgae community in the Bay of Seine was dominated by Rhodophyceae (between 1.253 and 1.464 gC m^{-2}) with some Ulvophyceae and Phaeophyceae recorded in autumn (respectively 0.292 and 0.162 gC m^{-2}). The macroalgae community in the Bay of Cherbourg was also dominated by Rhodophyceae but with higher-biomasses (between 14.617 gC m^{-2} in winter and 64.743 gC m^{-2} in summer). Phaeophyceae were also recorded in all the campaigns whereas some Ulvophyceae were only recorded in early winter (0.011 gC m^{-2}).

Table 11 – AFDW biomass in gC m⁻² for each macroalgae class (Ulvophyceae, Phaeophyceae and Rhodophyceae) identified in the Bay of Seine and the Bay of Cherbourg in each campaign.

Site	Season	AFDW biomass (gC m ⁻²)		
		Ulvophyceae	Phaeophyceae	Rhodophyceae
Bay of Seine	Winter	0	0	1.464
	Spring	0	0	1.418
	Autumn	0.292	0.162	1.253
Bay of Cherbourg	Winter (February)	0	1.069	14.617
	Spring	0	0.283	25.360
	Summer	0	4.223	64.743
	Autumn	0	0.493	36.575
	Winter (November)	0.011	0.478	41.130

Counting all the campaigns, a total of 40 macrofauna taxa were recorded, 35 in the Bay of Seine and 37 in the Bay of Cherbourg. Results showed that H' and J' were significantly higher in the Bay of Cherbourg than in the Bay of Seine in each season, showing that the Bay of Cherbourg site hosted more diversified assemblages than the Bay of Seine (H'). Conversely, total biomass was significantly higher in the Bay of Seine in all surveys. No significant differences in taxonomic richness and abundance were found between sites or in the different campaigns (Table 12 and Table 13).

Table 12 – Main characteristics of macrofauna with different univariate indices in the two sites and in all seasons. H': Shannon-Wiener diversity index; TR: Taxonomic richness; J': Pielou's evenness index; A: Abundance (number of individuals per m⁻² and B: biomass (g AFDW m⁻²). Significant differences (p-value < 0.05) are indicated by letters (a, b).

		Bay of Cherbourg				Bay of Seine		
		Winter	Spring	Summer	Autumn	Winter	Spring	Autumn
Macrofauna	H'	3.95 ^a	4.17 ^a	4.24 ^a	4.45 ^a	3.10 ^b	3.77 ^b	3.69 ^b
	TR	26	27	31	33	28	30	29
	J'	0.84 ^a	0.88 ^a	0.86 ^a	0.88 ^a	0.65 ^b	0.77 ^b	0.76 ^b
	A	400	374	490	1,071	524	1,563	2,078
	B	1.49 ^a	1.06 ^a	1.62 ^a	2.39 ^a	3.75 ^b	4.6 ^b	2.5 ^b

Table 13 – Results of the non-parametric Kruskal-Wallis test on five parameters in the two sites. *H'*: Shannon-Wiener diversity index; *TR*: Taxonomic richness; *J'*: Pielou's evenness index; *A*: Abundance; *B*: Biomass (g AFDW m⁻²); *Df*: Degrees of freedom; *F*: F-ratio score; *BS*: Bay of Seine; *BC*: Bay of Cherbourg.

	Df	p-value	Interpretation
<i>H'</i>	1	0.03389	BC ≠ BS
<i>TR</i>	1	1	BC = BS
<i>J'</i>	1	0.03389	BC ≠ BS
<i>A</i>	1	0.0771	BC = BS
<i>B</i>	1	0.03389	BC ≠ BS

Abundance per m² and AFDW biomass (gC m⁻²) of each macrofauna taxon at each sampling point are listed in Table 14. In the Bay of Seine in winter, the most abundant taxa (> 70 individuals per m²) were the decapoda *Pilumnus hirtellus* (178 per m²), *Pisidia longicornis* (90 per m²) and the crustacean Amphipoda (86 per m²). Spring was dominated by Aphroditidae (268 per m²), Amphipoda (230 per m²), the bristle star Ophiuridae (190 per m²), the decapoda *Pilumnus hirtellus* (148 per m²), the amphipoda Caprellidae (97 per m²), the crustacean Tanaidacea (91 per m²), *Pisidia longicornis* (84 per m²), the polychaeta Sabellidae (79 per m²), Serpulidae (77 per m²) and the crustacean Isopoda (70 per m²). Autumn was dominated by Isopoda (373 per m²), *Pisidia longicornis* (340 per m²), *Pilumnus hirtellus* (270 per m²), Amphipoda (239 per m²), the Mollusca Bivalvia (140 per m²), Ophiuridae (129 per m²), Aphroditidae (102 per m²), Tanaidacea (88 per m²) and Sabellidae (83 per m²). In the Bay of Cherbourg, winter was dominated by *Pisidia longicornis* (70 per m²). Autumn was dominated by Eunicidae (219 per m²), Amphipoda (184 per m²), Isopoda (124 per m²), Bivalvia (121 per m²), the crustacean Balanomorpha (112 per m²), Ectoprocta (96 per m²).

Table 14 – Macrofauna taxa abundance per m² and associated AFDW biomass (gC m⁻²) for each campaign in the Bay of Seine and the Bay of Cherbourg.

		Abundance per m ² and biomass (gC m ⁻²)							
Site	Season	Amphinomidae		Amphipoda		Aphroditidae		Ascidacea	
Bay of Seine	Winter	0	0	86.7	0.008	30	0.0602	1.1	0.0119
	Spring	0	0	230.3	0.0213	267.6	0.1353	1.4	0.3157
	Autumn	0	0	238.6	0.012	101.2	0.1498	0	0
Bay of Cherbourg	Spring	3.6	0.0009	10.9	0.0122	14.5	0.038	0	0
	Summer	0	0	41.5	0.0123	8	0.0434	6.7	0.2877
	Autumn	1.3	0.001	184	0.0116	40.3	0.0902	35.3	0.0871
	Winter	0	0	29.2	0.0019	8.3	0.012	50	0.9067

Chapitre V : Dynamique de colonisation d'infrastructures marines in situ

Site	Season	Asteridae		Balanomorpha		Bivalvia		Capitellidae	
Bay of Seine	Winter	1.1	1.2374	1.1	1.1088	0	0	0	0
	Spring	0	0	1.4	0.5616	0	0	1.4	0.0004
	Autumn	0	0	1.2	1.4476	139.8	0.1307	0	0
Bay of Cherbourg	Spring	1.2	0.3536	8.4	0.0308	30.2	0.0148	0	0
	Summer	10.7	0.1123	1.3	0.1879	6.7	0.0153	1.3	0.001
	Autumn	0	0	112.2	0.1134	121	0.7744	69.3	0.0291
	Winter	0	0	16.7	0.0212	15	0.1235	0	0
Site	Season	Caprellidae		Cirratulidae		Cnidaria		Cumacea	
Bay of Seine	Winter	1.1	0.0021	0	0	0	0	0	0
	Spring	96.6	0.0101	8.3	0.009	0	0	0	0
	Autumn	34.9	0.0041	3.6	0.0006	0	0	0	0
Bay of Cherbourg	Spring	6	0.0004	2.4	0.0011	0	0	0	0
	Summer	18.8	0.0003	33.5	0.0013	0	0	6.7	0.0007
	Autumn	25.2	0.0008	66.8	0.1214	25.2	0.0049	0	0
	Winter	5	0.0004	0	0	0	0	0	0
Site	Season	Decapoda		<i>Dynamene</i>		Ectoprocta		Eunicidae	
Bay of Seine	Winter	6.7	0.0072	24.4	0.0149	0	0	1.1	0.0036
	Spring	4.1	0.0041	0	0	1.4	0.0517	1.4	0.0084
	Autumn	26.5	0.011	13.3	0.0095	1.2	0.0116	1.2	0.013
Bay of Cherbourg	Spring	3.6	0.0071	22.9	0.0056	16.9	0.0144	25.3	0.0316
	Summer	5.4	0.0266	54.9	0.0036	1.3	0.0222	13.4	0.0384
	Autumn	20.2	0.0102	47.9	0.0843	95.8	0.1012	219.3	0.1836
	Winter	0	0	19.2	0.0034	0.8	0.0077	42.5	0.0105
Site	Season	Gastropoda		Holothuria		Isopoda		Lysianassidae	
Bay of Seine	Winter	3.3	0.022	0	0	15.6	0.0038	3.3	0.0026
	Spring	5.5	0.0146	0	0	70.3	0.0111	19.3	0.021
	Autumn	16.9	0.0452	0	0	373.5	0.0313	32.5	0.0052
Bay of Cherbourg	Spring	9.7	0.0085	0	0	47.1	0.0025	4.8	0.0001
	Summer	2.7	0.018	0	0	37.5	0.0046	6.7	0.0063
	Autumn	39.1	0.0151	12.6	0.0059	123.5	0.0748	49.1	0.0051
	Winter	3.3	0.0001	0	0	28.3	0.0044	3.3	0.0005
Site	Season	Nemertea		Nereididae		Nudibranchia		Ophiurida	
Bay of Seine	Winter	2.2	0.0033	4.4	0.0023	0	0	1.1	0.0006

Chapitre V : Dynamique de colonisation d'infrastructures marines in situ

	Spring	45.5	0.0294	8.3	0.0011	0	0	190.3	0.0246
	Autumn	4.8	0.0024	13.3	0.0126	3.6	0.0031	128.9	0.0154
Bay of Cherbourg	Spring	4.8	0.0001	1.2	0.0009	0	0	53.1	0.0037
	Summer	26.8	0.0168	42.9	0.0138	0	0	1.3	0.0027
	Autumn	20.2	0.0022	12.6	0.0045	0	0	55.5	0.0393
	Winter	22.5	0.0007	10.8	0.0022	0	0	64.2	0.0003

Site	Season	Phyllodocidae		<i>P. hirtellus</i>		<i>P. longicornis</i>		Platyhelminthes	
Bay of Seine	Winter	1.1	0.0028	177.8	0.1624	90	0.0706	2.2	0.0047
	Spring	4.1	0.0005	147.6	0.1117	84.1	0.0717	2.8	0.0032
	Autumn	8.4	0.0026	269.9	0.1034	339.8	0.1839	1.2	0.0008
Bay of Cherbourg	Spring	0	0	16.9	0.0523	18.1	0.0278	0	0
	Summer	16.1	0.0013	20.1	0.0074	69.7	0.0273	0	0
	Autumn	30.2	0.0131	59.2	0.1129	47.9	0.0732	0	0
	Winter	4.2	0	1.7	0.0207	15.8	0.0031	0	0

Site	Season	<i>Polydora</i>		Polyplacophora		<i>P. platycheles</i>		Porifera	
Bay of Seine	Winter	0	0	1.1	0.0898	0	0	1.1	0.8249
	Spring	0	0	0	0	52.4	0.1605	1.4	2.7356
	Autumn	0	0	0	0	39.8	0.1679	0	0
Bay of Cherbourg	Spring	0	0	7.2	0.0054	0	0	4.8	0.26
	Summer	6.7	0.0016	0	0	0	0	1.3	0.3025
	Autumn	0	0	0	0	0	0	35.3	0.313
	Winter	0	0	0	0	0	0	2.5	0.1692

Site	Season	Pycnogonida		Sabellaria		Sabellidae		Serpulidae	
Bay of Seine	Winter	6.7	0.0017	1.1	0.0011	31.1	0.0403	18.9	0.0571
	Spring	16.6	0.0016	0	0	78.6	0.1	77.2	0.1589
	Autumn	19.3	0.0047	0	0	83.1	0.0429	57.8	0.0574
Bay of Cherbourg	Spring	27.8	0.0003	0	0	14.5	0.0362	15.7	0.1432
	Summer	13.4	0.001	12.1	0.0265	4	0.0393	13.4	0.3888
	Autumn	6.3	0.0006	0	0	31.5	0.0595	35.3	0.0354
	Winter	0	0	30.8	0.0019	17.5	0.0909	3.3	0.1112

Site	Season	Sipunculidae		Spionidae		Syllidae		Tanaidacea	
Bay of Seine	Winter	0	0	5.6	0.002	3.3	0.0017	1.1	0.0013
	Spring	4.1	0.0008	2.8	0.0006	44.1	0.0045	91	0.0212
	Autumn	3.6	0.0092	19.3	0.0064	13.3	0.0035	88	0.0132

Bay of Cherbourg	Spring	0	0	0	0	0	0	1.2	0.0001
	Summer	0	0	0	0	0	0	2.7	0.0002
	Autumn	12.6	0.0014	18.9	0.0051	6.3	0.0055	7.6	0.0075
	Winter	0.8	0.0012	1.7	0.0001	1.7	0.0003	0	0

Site	Season	Terebellidae	
Bay of Seine	Winter	0	0
	Spring	2.8	0.008
	Autumn	0	0
Bay of Cherbourg	Spring	1.2	0.0078
	Summer	2.7	0.0053
	Autumn	3.8	0.0032
	Winter	0.8	0.0007

The 40 taxa were assigned to six trophic groups. Figure 58 shows the abundance and biomass of these trophic groups in both sites during the survey. In the Bay of Seine, total abundance was higher in autumn than in the rest of the year (Figure 58A). In winter, filter feeders and predators were the most abundant trophic groups (143 and 193 individuals per m²). In spring, the most abundant group was scavengers, omnivorous benthic invertebrates (590 individuals per m²) followed by filter feeders (296 individuals per m²). In autumn, the most abundant group was filter feeders (523 individuals per m²) immediately followed by the surface deposit feeders (507 individuals per m²). Like in the Bay of Seine, benthic invertebrates were most abundant in autumn in the Bay of Cherbourg (Figure 58B). The variations in abundance from winter to summer were not significant in the Bay of Seine. However, the abundance of filter feeders, predators and sub-surface deposit feeders reached respectively 358, 397 and 564 individuals per m² in autumn. Some significant differences were observed between sites, particularly in spring when the abundance of some trophic groups like omnivorous scavengers or filter feeders were three to six times higher than in the Bay of Seine. In terms of biomass, in the Bay of Seine (Figure 58C), filter feeders dominated, contributing 56% of the total AFDW biomass in winter, 84% in spring and 76% in autumn. Predators represented 38% of total AFDW biomass in winter, 10% in spring and 5% in autumn. Some trophic groups, like omnivorous scavengers or surface deposit feeders, represented a small proportion of the total AFDW biomass in comparison with their high abundance. In the Bay of Cherbourg, the main contributors were filter feeders, 27% of the total AFDW biomass in winter, 48% in spring, 56% in summer and 29% in autumn. Predators also represented a large proportion of the total

biomass, 63% in winter, 41% in spring, 38% in summer and 17% in autumn. Sub-surface deposit feeders represented a higher percentage of total biomass in autumn (39%) versus 6% in the Bay of Seine at the same period.

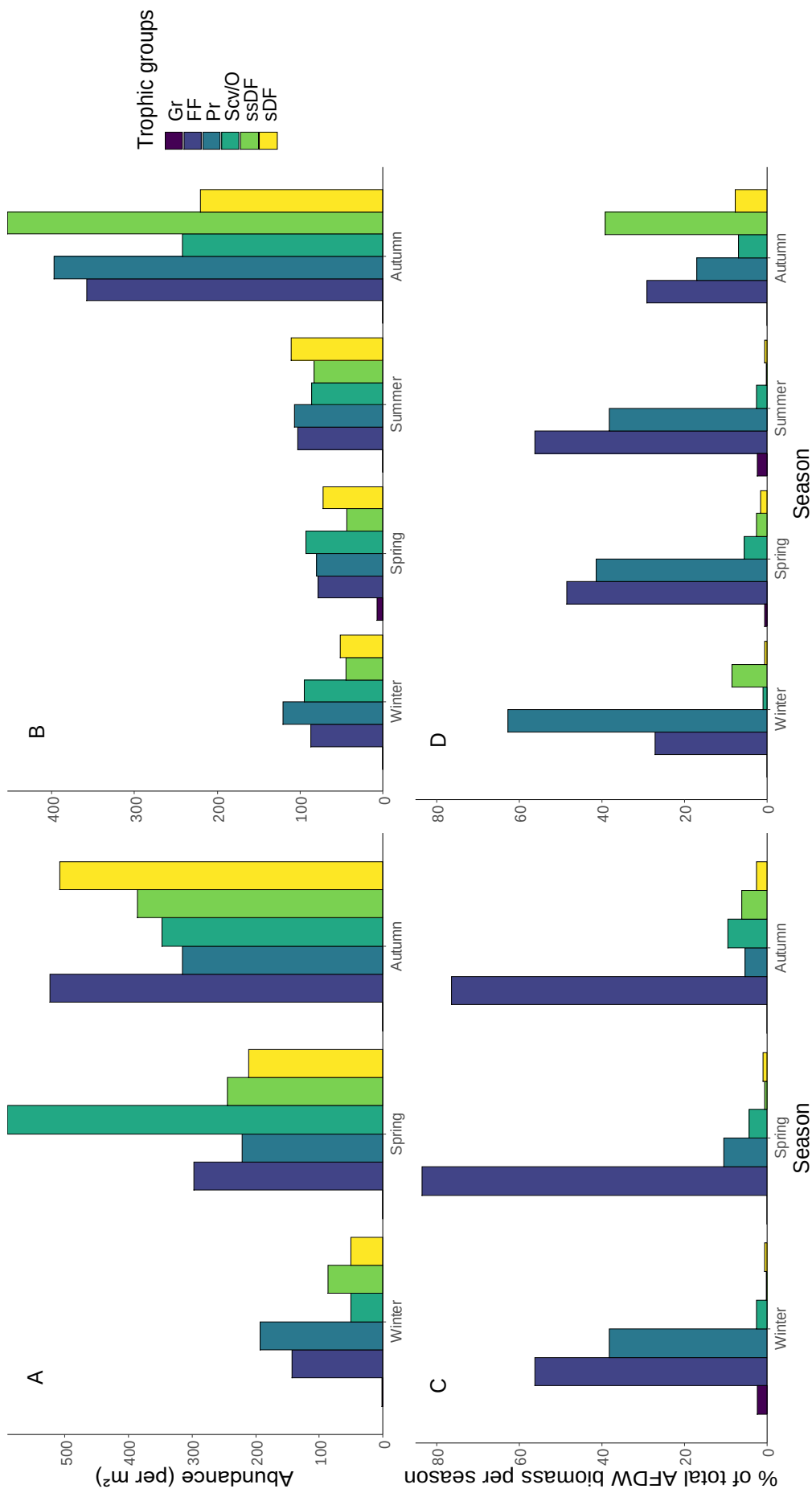


Figure 58 – Abundance (A, B) and percentage of total AFDW biomass per season (C, D) of trophic groups in the Bay of Seine (A, C) and the Bay of Cherbourg (B, D) in each season. Trophic groups: grazers (Gr), filter feeders (FF), predators (Pr), omnivorous scavengers (O/Scv), sub-surface deposit feeders (ssDF) and surface deposit feeders (sDF).

III.2. *Primary production and macroalgae photosynthetic parameters*

Figure 59 shows the results of the incubations. GPP_{max} was calculated at saturation light and respiration was measured in dark conditions at the beginning of each incubation period. The saturation light obtained for the Bay of Seine artificial reef incubation was $795 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ and $680 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ for the Bay of Cherbourg. In the Bay of Seine, total respiration was high with $-514 \pm 163 \text{ mg C m}^{-2} \text{ d}^{-1}$ and $-511 \pm 435 \text{ mg C m}^{-2} \text{ d}^{-1}$ respectively. The consequence of the high oxygen consumption in the Bay of Seine was a low GPP_{max} that decreased from $661 \pm 1,355 \text{ mg C m}^{-2} \text{ d}^{-1}$ in winter to $381 \pm 323 \text{ mg C m}^{-2} \text{ d}^{-1}$ in autumn. No production was measured on any of the six incubated artificial reefs in spring. In the Bay of Cherbourg, total respiration was high in winter ($-2,147 \pm 732 \text{ mg C m}^{-2} \text{ d}^{-1}$) and decreased in spring ($-324 \pm 172 \text{ mg C m}^{-2} \text{ d}^{-1}$) to reach $-1,503 \pm 902 \text{ mg C m}^{-2} \text{ d}^{-1}$ in summer and $-1,860 \pm 591 \text{ mg C m}^{-2} \text{ d}^{-1}$ in autumn. The GPP_{max} in the bay of Cherbourg was high. It decreased from $2,214 \pm 1,025 \text{ mg C m}^{-2} \text{ d}^{-1}$ in winter to $398 \pm 138 \text{ mg C m}^{-2} \text{ d}^{-1}$ in spring. It increased to $3,664 \pm 1,173 \text{ mg C m}^{-2} \text{ d}^{-1}$ in summer and remained stable at $2,450 \pm 630 \text{ mg C m}^{-2} \text{ d}^{-1}$ in autumn. The associated primary productivity is listed in Table 15. The results showed elevated primary productivity in the Bay of Seine ($0.45 \text{ gC gC d}^{-1}$ in winter). In the Bay of Cherbourg, the maximum was reached in autumn ($0.07 \text{ gC gC d}^{-1}$).

Table 15 – Primary productivity of macroalgae communities (in gC gC d^{-1}).

Site	Season	Primary productivity (gC gC d^{-1})
Bay of Seine	Winter	0.45
	Autumn	0.22
Bay of Cherbourg	Winter	0.05
	Spring	0.02
	Summer	0.05
	Autumn	0.07

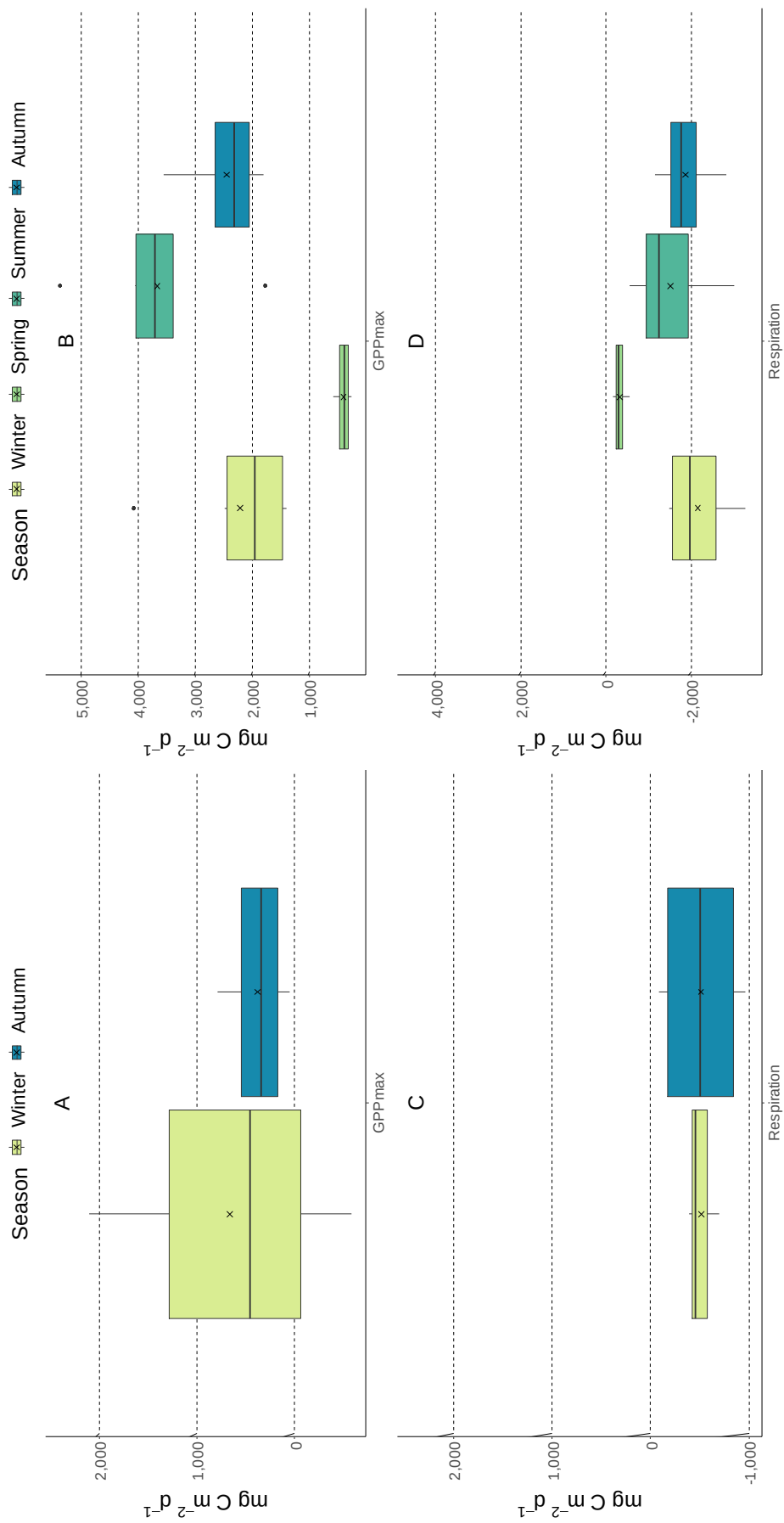


Figure 59 – Gross primary production (GPPmax) and respiration incubation in $\text{mg C m}^{-2} \text{d}^{-1}$ per cinder block at light saturation and in each season in the Bay of Seine (A, C) and the Bay of Cherbourg (B, D).

Photosynthetic parameters were measured in the dominant macroalgae species on the artificial reef in the Bay of Cherbourg (Figure 60). In summer, the lowest F_V/F_M was recorded for the Rhodophyceae species (between 0.48 ± 0.11 for *Calliblepharis jubata* and 0.62 ± 0.01 for *Vertebrata byssoides*). Phaeophyceae species showed an elevated F_V/F_M between 0.58 ± 0.05 for *Laminaria digitata* and 0.73 ± 0.02 for *Dictyota dichotoma*. The non-indigenous species *Sargassum muticum* showed a ratio of 0.62 ± 0.002 . In autumn, the lowest F_V/F_M was always recorded in Rhodophyceae species (between 0.42 ± 0.004 for *Bornetia secundiflora* and 0.53 ± 0.009 for *Cryptopleura ramosa*). *Laminaria digitata* and *Ulva* sp. showed respectively a F_V/F_M of 0.66 ± 0.09 and 0.75 ± 0.03 . In winter, Rhodophyceae species still showed the lowest ratios (between 0.45 ± 0.03 for *Sphaerococcus coronopifolius* and 0.51 ± 0.02 for *Grateloupia turuturu*). *Laminaria digitata* and *Ulva* sp. showed respectively a F_V/F_M of 0.76 and 0.8 . In spring, the F_V/F_M of Phaeophyceae species (0.72 ± 0.022) was higher than that of Rhodophyceae (0.5 ± 0.08). The highest F_V/F_M in Rhodophyceae was 0.62 ± 0.046 recorded in *Calliblepharis jubata*. The maximum relative electron transport rate was also calculated; it showed a global increase for each macroalgae species between summer and spring. In summer, it was similar in all Rhodophyceae species (between 24.51 ± 12.53 rel. unit in *Plocamium cartilagineum* and 32.94 rel. unit in *Cryptopleura ramosa*). It was significantly lower in Phaeophyceae species (between 17.6 ± 8.15 rel. unit in *Sargassum muticum* and 24.27 ± 2.44 rel. unit in *Laminaria digitata*). In autumn, the highest $rETR_{MAX}$ was recorded in *Bornetia secundiflora* (63.98 ± 4.91 rel. unit). *Laminaria digitata* and *Ulva* sp. showed respective $rETR_{MAX}$ values of 42.24 ± 4.32 rel. unit and 44.42 ± 2.68 rel. unit. In winter the $rETR_{MAX}$ values were higher than in the other seasons. Rhodophyceae species had the highest values with 64.31 ± 21.12 rel. unit in *Grateloupia turuturu* and 68.7 ± 25.98 rel. unit in *Sphaerococcus coronopifolius*. *Laminaria digitata* and *Ulva* sp. had $rETR_{MAX}$ values of 59.94 rel. unit. and 48.1 rel. unit. Finally, in spring, Phaeophyceae species had the highest $rETR_{MAX}$ values and Rhodophyceae species had $rETR_{MAX}$ values between 70.36 ± 5.16 rel. unit. Recorded in *Plocamium cartilagineum* 79.88 ± 10.21 rel. unit. in *Cryptopleura ramosa*.

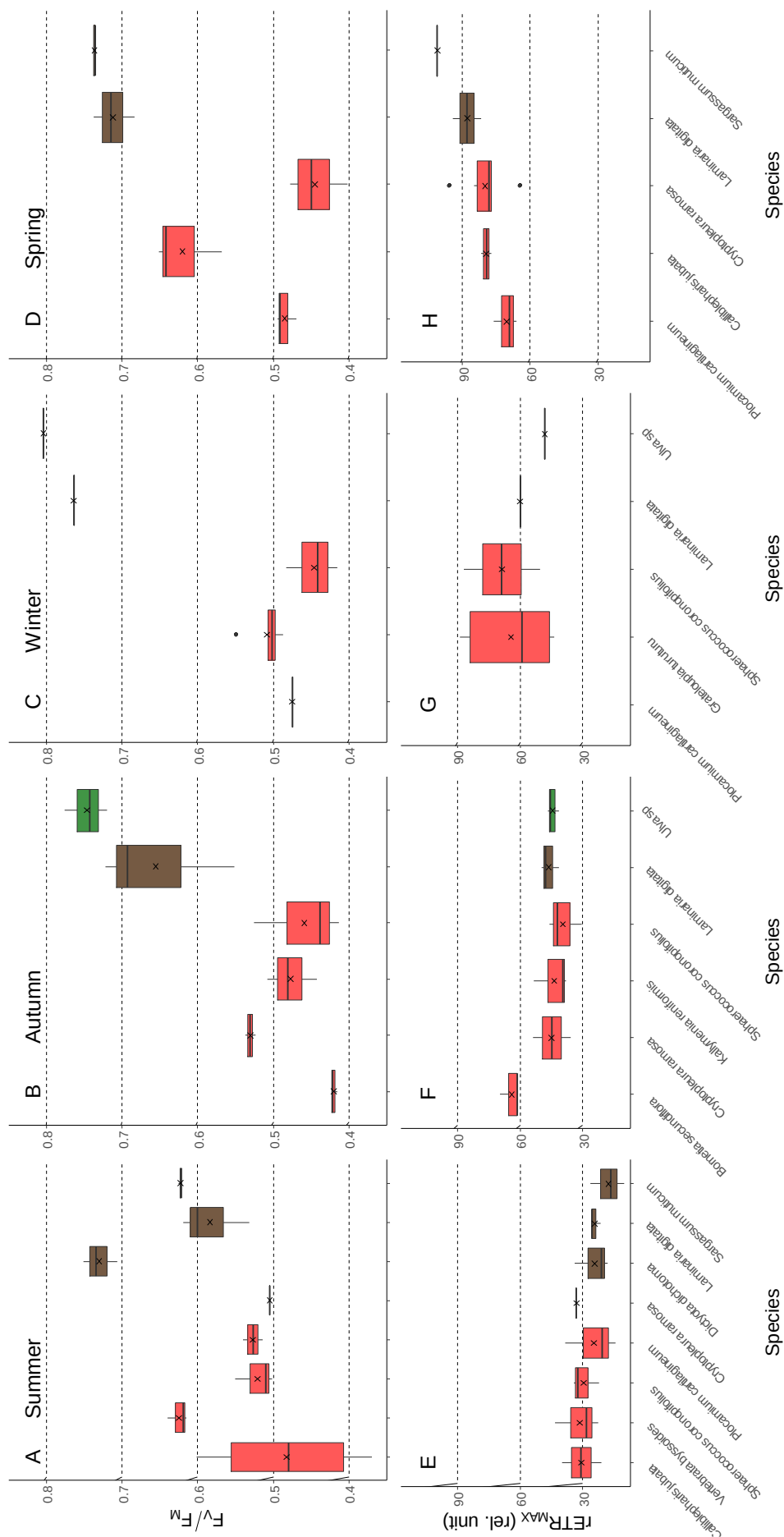


Figure 60 - Photosynthetic parameters (F_v/F_m (A, B, C and D) and $rETR_{max}$ (E, F, G and H)) measured on macroalgae sampled on artificial reefs in the Bay of Cherbourg in summer (A, E), autumn (B, F), winter (C, G) and spring (D, H). Rhodophyceae are in red, Phaeophyceae in brown and Ulvophyceae in green.

III.3. *Principal component analysis (PCA) of biotic and abiotic variables*

Several abiotic parameters were measured to characterize the ecosystems in the Bay of Cherbourg and the Bay of Seine. PCA (Figure 61) was performed to identify the variables that explained the influence of seasonality in the two sites. Supplementary quantitative variables are shown in grey in fig 64. The first PCA was performed on the Bay of Seine data and the two first dimensions explained 100% of the scatter plot. The second PCA was performed on the Bay of Cherbourg data and the two first dimensions explained 93.3% of the scatter plot. In the Bay of Seine, autumn influenced the abundance of filter feeders, sub-surface deposit feeders, surface deposit feeders and predators and surface and sub-surface deposit feeder biomass. Autumn was also correlated with the variables temperature, total fauna abundance and richness (A and S). Phaeophyceae and Ulvophyceae biomasses were strongly correlated with autumn. Spring was strongly correlated with phytoplanktonic biomass (FFU_smile and Chla variables). Spring also influenced filter feeder biomass and omnivorous scavenger species. More generally, autumn and spring in the Bay of Seine were correlated with the Shannon-Wiener diversity index (H') and Piélou's evenness index (J'). Finally, winter was correlated with predator biomass and grazer biomass and abundance. It was also correlated with several abiotic variables including N_{tot} , MES and Si. Rhodophyceae biomass, phosphate, pH and dissolved oxygen variables were located between winter and spring. In the Bay of Cherbourg, autumn influenced temperature, salinity and several biotic variables as illustrated by the correlation with the fauna richness (S). Autumn was correlated with the abundance of surface and sub-surface deposit feeders' abundances and biomass, omnivorous scavengers and biomass and with predators and filter feeders. Spring and summer were highly correlated and influenced phytoplankton biomass (Chla), and the abundance and biomass grazers, Phaeophyceae and Rhodophyceae biomass, fauna abundance and filter feeder biomass. Finally, winter was correlated with predator biomass and N_{tot} . Silicate, phosphate, Piélou's evenness index (J') and the Shannon-Wiener diversity index (H') variables were correlated with autumn and winter.

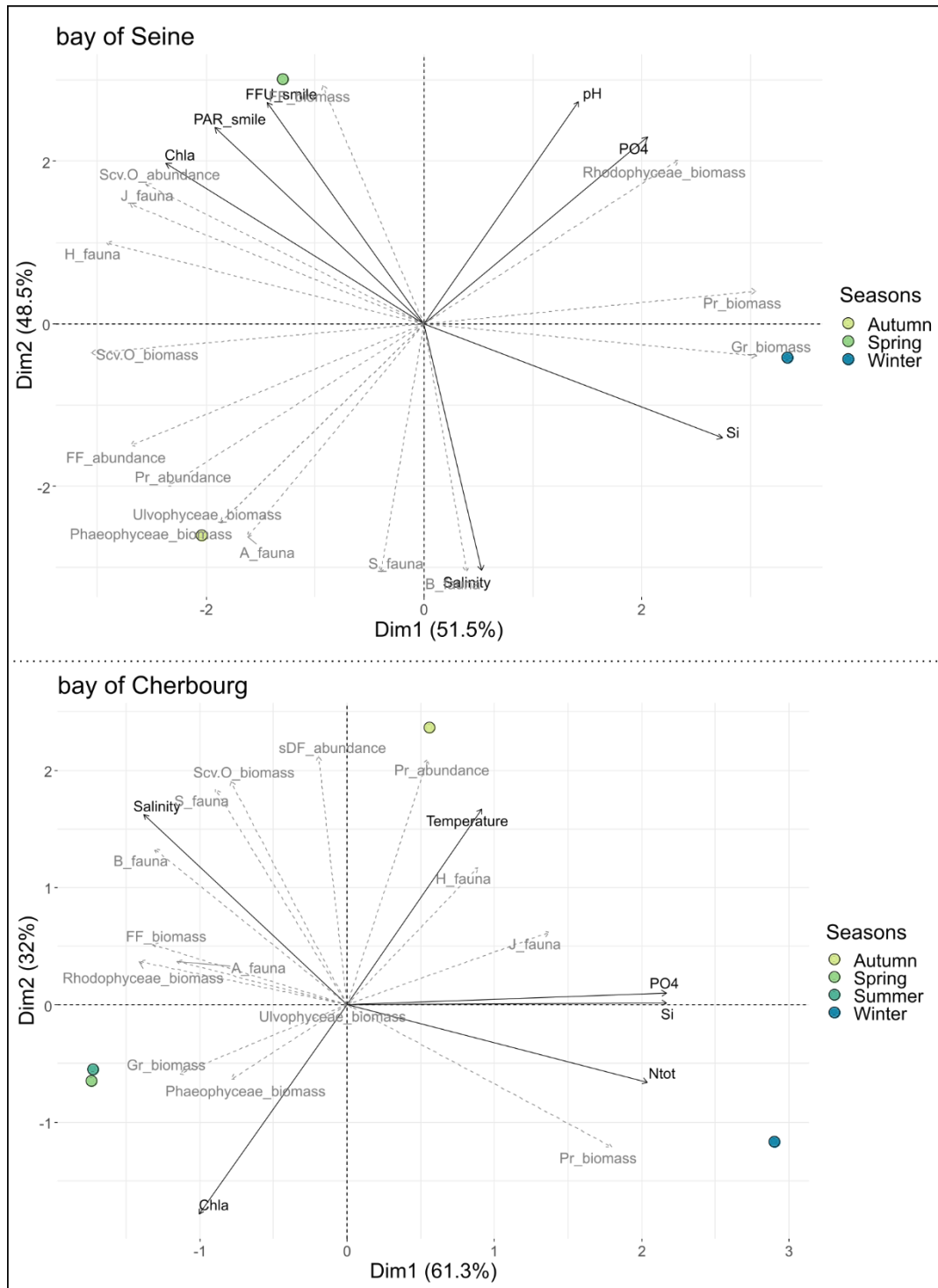


Figure 61 – Principal component analysis (PCA) of biotic and abiotic variables in the Bay of Seine and the Bay of Cherbourg. Biotic variables are benthic invertebrate biomass and abundance per trophic group and macroalgae biomass per class. Biotic indices (H': Shannon-Wiener diversity index; S: Taxonomic richness (TR); J': Pielou's evenness index; A: Abundance; B: Biomass). The abiotic variables found in both sites are inorganic nutrients, salinity, and temperature. The instrumented buoy (SMILE) allowed us to analyze more variables in the Bay of Seine: dissolved oxygen (Oxy_smile), pH, MES (MES_smile), fluorescence (FFU_smile) and PAR (PAR_smile). Autocorrelated variables are not represented for the Bay of Seine, Pr_biomass was correlated with N_{tot} and MES_smile; Gr_biomass with Gr_abundance; pH with Oxy_smile; Pr_abundance with ssDF_biomass and FF_abundance with sDF_biomass, ssDF_abundance, temperature and sDF_abundance. For the bay of Cherbourg, Gr_biomass was correlated with Gr_abundance and Pr_abundance with ssDF_biomass, Scv.O_abundance, sDF_biomass, FF_abundance and ssDF_abundance.

IV. Discussion

Firstly, marked environmental differences were found between the two experimental sites such as tide amplitude, current velocity and water turbidity. Indeed, the Bay of Cherbourg is hydrodynamically much more protected than the Bay of Seine thanks to the long dike. One of the consequences of this manmade structure is that it has modified the hydrodynamics and the influence of the tide on local currents, while the Bay of Seine is an open system. Secondly, water parameters also differed between the two sites. The Bay of Seine is under riverine influence mainly freshwater inputs from the Seine and the Orne rivers where abiotic parameters revealed marked seasonal spatio-temporal variations, while the Bay of Cherbourg is directly connected to offshore water masses. Water turbidity is much higher in the Bay of Seine than in the Bay of Cherbourg, concentrations of inorganic nutrients are also much higher in the Bay of Seine than in the Bay of Cherbourg. Given these disparities, comparing several biotic variables on similar artificial reefs immersed in the two sites since 2015 was highly relevant. Several structural factors such as depth, orientation, age and surface complexity may influence the structure and the diversity of benthic communities (Baine, 2001; Bellou et al., 2012; Jaxion-Harm & Szedlmayer, 2015; Vivier et al., 2021b).

Relations between macrobenthic fauna and macroalgae in artificial reef systems have been poorly investigated (Yang et al., 2018). However, interactions between primary producers and consumers are a key to understanding ecosystem functions and how they change over time. The results of the analysis of macroalgae communities revealed significantly higher diversity in the Bay of Cherbourg than in the Bay of Seine (a total of 15 versus 8 species). Diversity and total biomass were higher in the Bay of Seine in autumn than in the rest of the year. Ulvophyceae and Phaeophyceae species were only recorded in autumn in this site. In the Bay of Cherbourg, total biomass and diversity were higher in summer, and Ulvophyceae species were only recorded in winter. These results indicated that the artificial reef in the Bay of Cherbourg AR supports a more diversified community of primary producers' community, which is also true for benthic fauna. The structural differences between macroalgae communities in the two sites may be explained by abiotic factors like turbidity, available PAR, or concentrations of inorganic nutrients. Although the concentrations of inorganic nutrients, particularly N_{tot} were higher in the Bay of Seine than in the Bay of Cherbourg, higher biomass and diversity of macroalgae were recorded in the Bay of Cherbourg, which can be explained by the lower PAR due to the high water turbidity and the strong tidal currents in the Bay of Seine (Cailleaud et al., 2009; Baux et al., 2019). Like macrobenthic assemblages, macroalgae may be influenced by the

structure, material and surface heterogeneity of the artificial reef, but this cannot be the case here because the composition of the artificial reefs is the same in the two sites (Christie et al., 2007; Wahl, 2009). Another possible explanation for the lower primary producer biomass in the Bay of Seine is the accumulation of sandy sediment on some of the upper surfaces of the artificial reef, which may have influenced macroalgae recruitment by modifying the nature of the substrate and creating abrasive effects. Macroalgae communities on the artificial reef in the Bay of Seine were strongly dominated by *Cryptopleura ramosa* and by *Kallymenia reniformis* and *Sphaerococcus coronopifolius* in the Bay of Cherbourg. *Sphaerococcus coronopifolius* is a perennial species that reproduces in summer thus explaining its higher biomass in summer and autumn in the Bay of Cherbourg. *Kallymenia reniformis* and *Sargassum muticum* are also perennial species, which may explain their greater biomass in the Bay of Cherbourg throughout the monitoring period. *Cryptopleura ramosa* is a perennial species that is fertile in summer and autumn. Its dominance in the bay of Seine suggests that this Rhodophyceae species is well acclimated to turbid conditions with high hydrodynamics. Several species recorded during the survey, such as *Calliblepharis jubata*, *Heterosiphonia plumosa* and *Sacchoriza polyschides* in the Bay of Cherbourg are indicative of protected habitats in Atlantic reef ecosystems. However, the dominant species correspond to those found in exposed parts of Atlantic reef ecosystems (i.e. *Kallymenia reniformis*) (Bensettiti et al., 2004). The long dike that forms the *Rade de Cherbourg* creates a semi-protected area and a long rocky substrate ecosystem all around the bay. This large rocky-habitat may support a perennial macroalgae community thereby explaining the abundance of hard substrate macroalgae species on the artificial reef immersed in this area. Moreover, the Rhodophyceae biomass in the Bay of Cherbourg was highly correlated with total macrofauna abundance and biomass in summer pointing to a productive and diverse system in this season. In comparison, Rhodophyceae biomass was anti-correlated with the abundance, biomass, and richness of macrofauna in the Bay of Seine. Indeed, in this site, Rhodophyceae biomass was correlated with winter and spring, which were extremely poor in benthic fauna richness. In the Bay of Seine, the most diversified season in terms of fauna was autumn, which was also correlated with Ulvophyceae and Phaeophyceae biomass (despite their very low total biomass in this site). These original results for this area need to be taken into consideration in future environmental assessments in the Bay of Cherbourg and the dominant species recorded in this study may be considered as determinant ones. Generally, our results tend to confirm the efficiency of artificial reefs in creating novel habitats because the macroalgae communities we recorded correspond to those observed in nearby rocky areas (Lemesle, 2015; Burel, 2020). The establishment of non-indigenous species in ecosystems

associated with artificial reefs has been widely studied and the immersion of such structures may promote their colonization (Glasby et al., 2007; Morris et al., 2018). *Sargassum muticum* originated from Asia and is already well implanted in the English Channel (Strong et al., 2006; Kim et al., 2019a). Its presence in the Bay of Cherbourg all year round and its absence in the Bay of Seine was surprising and difficult to explain because of its abundance along the English Channel coast. This absence may be explained by the local bathymetry or by the lack of data in summer.

The primary production results obtained by incubation (at light saturation) revealed significant differences between sites and seasons. Not surprisingly because of the higher macroalgae biomass, the artificial reefs in the Bay of Cherbourg were the most productive and the highest production was recorded in summer, the season with the highest macroalgae biomass and diversity in this site: GPP_{max} reached $3,664 \pm 1,173 \text{ mg C m}^{-2} \text{ d}^{-1}$ in summer compared with a maximum of GPP_{max} in the Bay of Seine reached in winter. The data we collected in the Bay of Seine are comparable with the results of another study on benthic primary production on natural reefs in a comparable environment (Goll  ty et al., 2008). However, production in the Bay of Cherbourg was much higher. By way of comparison, (Cesbron et al., 2019) investigated benthic primary production on artificial structures in tropical environments and their data were still below the production we recorded on the artificial reef in the Bay of Cherbourg. In contrast to production, primary productivity was higher in the Bay of Seine than in the Bay of Cherbourg (approximately 3 times higher in autumn, for example). Other studies have already demonstrated that macroalgae communities are not always the most efficient in terms of primary productivity (Bruno et al., 2005, 2006). Maximum respiration was recorded in summer in the Bay of Cherbourg, the most productive season in this site. In autumn, the artificial reef in the Bay of Cherbourg also showed a high GPP and high oxygen consumption but relatively low macroalgae diversity (only three different species) in autumn. The higher respiration recorded in summer and autumn in the Bay of Cherbourg corresponded to the seasons with the highest diversity index (H') and the highest total benthic fauna biomass. These results showed that the ecosystem associated with the artificial reef in the Bay of Cherbourg was more productive than the ecosystem in the Bay of Seine in all seasons. The relatively poor macroalgae biomass in the Bay of Seine was not sufficient to offset the oxygen consumed, especially in winter when respiration was strongly negative. The lower saturation light in the bay of Cherbourg incubations ($680 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$) compared with in the Bay of Seine ($795 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$) showed that the communities in the Bay of Cherbourg were

more able to perform photosynthesis at lower PAR. These results may be due to the large size of the Rhodophyceae community in the Bay of Cherbourg, indeed, these macroalgae are known for high photosynthetic efficiency at low light levels and may contribute to the high oxygen production measured in this site (Weykam & Wiencke, 1996; Eggert & Wiencke, 2000). These results suggested a better photo-acclimation by the communities of primary producers' in the Bay of Cherbourg and the need for higher PAR in the Bay of Seine. In the same sea, at the same depth, in the same area, but in different water masses, two types of functioning by artificial reefs were highlighted: a "primary producer reef" rich in macroalgae, the Bay of Cherbourg, and the other a "primary consumer reef" where production is based on phytoplankton resources, in the Bay of Seine.

The present study also provided interesting results concerning the photosynthetic performances of the macroalgae community assessed on the artificial reef in the Bay of Cherbourg. Indeed, *in situ* measurements revealed that the maximum production capacity ($rETR_{MAX}$) increased gradually in all species between summer and spring (from approximately 30 to 90 rel. unit). F_v/F_m were lower in Rhodophyceae (between 0.4 and 0.55 except for *Calliblepharis jubata*) species than in Phaeophyceae species (between 0.58 and 0.73) in every season. The lowest F_v/F_m were recorded in Rhodophyceae species in autumn and winter, while a marked increase (+ 0.14) was recorded in *Calliblepharis jubata* between winter and spring. Generally, all recorded species showed relatively high F_v/F_m , especially Phaeophyceae and *Ulva* sp. These results are in agreement with measurements of macroalgae chlorophyll fluorescence in several studies in comparable environments (Dring et al., 1996; Nielsen & Nielsen, 2008; Bhagooli et al., 2021). Our results indicated that the physiological status of the communities of primary producers on the artificial reef in the Bay of Cherbourg was relatively good irrespective of the season. The F_v/F_m obtained for green and brown algae were found in healthy algae, well acclimated to their environment. The lowest value obtained in red algae was probably partly due to composition of the pigment in agreement with results in the literature (JuHyoung et al., 2019). The reduced $rETR_{MAX}$ in summer compared with in the other seasons may be explained by a seasonal NPQ of these primary producers (Franklin & Forster, 1997). Indeed, the PAR of macroalgae in intertidal or shallow waters is often in excess of that needed to saturate photosynthesis. Excess irradiance can lead to photo-oxidative damage and, in the long term, to physiological regulation such as the reduction of the size of light-harvesting pigment antennae (Müller et al., 2001; Gevaert et al., 2002). Further investigation may be useful to evaluate the potential photosynthesis of these macroalgae more precisely. Indeed, our results

may be overestimated because we did not measure the light available for photosynthesis in the algae tissue. Marked variations in light absorption by tissues were observed between macrophyte individuals and species (Enríquez et al., 1994). Another factor that may influence the rate of photosynthesis is the age of different tissues in the same individual. Indeed, PAM measurements reflect photosynthesis at a single location (Enríquez & Borowitzka, 2010). To reduce this uncertainty, we made at least three measurements per individual. This has already been described in the literature and our results (rapid light curves) were relatively constant within one individual (Randall et al., 2019).

The macrobenthic fauna recorded in the Bay of Cherbourg was more diverse than that in the Bay of Seine (H'). Total biomass was significantly higher in the Bay of Seine than in the Bay of Cherbourg. To use a functional approach, benthic faunae were assigned to six trophic groups. In both sites, maximum abundance was reached in autumn as shown in Figure 58. In terms of biomass, there were marked differences between sites and seasons. Indeed, in the Bay of Seine, the total biomass per season was always dominated by filter feeders and, in smaller proportions, by predators in winter. In the Bay of Cherbourg, the benthic fauna was also dominated by filter feeders and predators in spring and summer. The benthic assemblage was dominated by sub-surface deposit feeders in autumn (followed by filter feeders and predators) and by predators in winter. These results indicated that the trophic webs in the two sites differed with a more complex system with distinct seasonal changes in the Bay of Cherbourg. Moreover, in the same season, the dominant trophic groups differed between the two sites. In autumn, the benthic fauna in the Bay of Seine was dominated by filter feeders and in the Bay of Cherbourg, a more diversified trophic structure was observed with sub-surface deposit feeders, filter feeders and predators. Five years after their immersion, these artificial ecosystems could be considered as established after their first stage of fauna and flora succession. The only two ways to avoid over-competition between marine organisms is separation of food sources and habitat specialization. Considering that the immersed structures were the same, the differences in macrofauna trophic structure observed between the two sites may be due to abiotic factors (hydrodynamics, salinity, temperature etc.) and to the surrounding communities present before and during immersion of the artificial reef. Water temperature is known to influence the metabolism of a high proportion of marine organisms and may explain the lower abundance of all the trophic groups recorded in both sites in winter (Fraser & Rogers, 2007; Huey & Kingsolver, 2011; Hall et al., 2021). Multivariate analysis provides important information about seasonality and site characteristics. Our results confirmed the high correlation between autumn

or summer and the diversity of benthic fauna diversity. The high filter feeder biomass recorded in spring in the Bay of Seine was explained by the phytoplankton biomass in this season. In both sites, autumn was correlated with several trophic groups of benthic fauna and some biotic indices (TR and H') pointed to high species richness in autumn independently of the site. Predator species were abundant in both sites in winter, but several structural differences in the trophic web were observed. For example, grazers were dominant in spring in the Bay of Cherbourg and in winter in the Bay of Seine. Artificial structures like harbors are known to lead to differences in community composition, density, and biomass from those in natural environments (Airoldi et al., 2009). Several studies highlighted reduced species richness compared to nearby natural communities (Airoldi et al., 2008; Ido & Shimrit, 2015). Like other man-made structures, the composition of macrofauna on artificial reefs often differs from that on natural reefs (Wahl, 2009; Aguilera et al., 2014; Hall et al., 2021). Several studies highlighted the differences in structure composition and trophic function of epibenthic assemblages between artificial and natural reefs (Perkol-Finkel et al., 2006; Carvalho et al., 2013). The high biomass of filter feeders was mainly due to barnacles, as the uneven surface of the artificial reefs favored their establishment. The micro-scale heterogeneity of the colonized surface has already been reported to promote the settlement of barnacle cyprids (Hunter & Sayer, 2009; Paalvast, 2015; Strain et al., 2018). In the Bay of Cherbourg, the high proportion of predators in terms of ash-free dry weight (AFDW) biomass was mainly due to *Pisidia longicornis* and *Pilumnus hirtellus*. The observed abundance of these decapods and barnacles is in agreement with the results of other studies of artificial reefs in similar environmental conditions (Fariñas-Franco & Roberts, 2013). The taxonomic richness recorded in this study was comparable to that in a similar environment at a mature stage of colonization (Taormina et al., 2020). Our results tend to confirm the efficiency of such structures in creating novel habitats and in restoring hard substrate communities. However, it may be important to also investigate trophic assemblages of nearby hard bottom communities to evaluate and compare the communities found on artificial reefs and their similarities or differences from those in natural systems. The artificial reefs in our study have been immersed since 2015 and it has sometimes been suggested that at least 10 years are necessary to observe convergence between natural and artificial reef communities (Perkol-Finkel et al., 2005). However, a high evenness index (J'), which is an indication of an heterogeneous community close to the climax stage, was measured in all the surveys conducted in the Bay of Cherbourg. This result supports the efficiency of artificial structures in creating novel habitats and promoting colonization (Fariñas-Franco & Roberts, 2013).

V. Conclusion

Our study provided results concerning the biodiversity and the trophic structure associated with artificial reefs immersed for several years in two different ecosystems in the English Channel. Our results revealed abundant and diverse benthic fauna communities in both artificial reef sites. The artificial reef in the Bay of Cherbourg hosted higher diversity and biomass of primary producers dominated by Rhodophyceae macroalgae, whereas the artificial reef in the Bay of Seine hosted a more diverse and abundant benthic fauna community. Primary production differed significantly between sites. The artificial reef in the Bay of Cherbourg was more productive than the artificial reef in the Bay of Seine, possibly due to the abundance of Rhodophyceae in this site. This study highlighted the marked impact of local conditions on the structure of the benthic communities. Different ecological functioning was identified on identically structured artificial reefs: a strong “primary producer artificial reef” in the Bay of Cherbourg and a “primary consumer artificial reef” in the Bay of Seine. Despite the fact the structures were identical, the ecosystem functions and the associated benthic communities differed significantly between two sites with comparable tide amplitude and climate. More generally, our results underline that predicting the environmental impact of an artificial structure is complex and the transposition of studies carried out on nearby sites is not sufficient. It is necessary to consider all the environmental constraints.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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CHAPITRE VI : SYNTHÈSE, DISCUSSION GÉNÉRALE ET PERSPECTIVES

Ma thèse s'inscrit au sein du projet européen Interreg Va MARINEFF visant à développer de nouvelles infrastructures marines permettant de limiter les impacts négatifs associés au développement humain sur les écosystèmes marins et côtiers. Considérant l'obligation d'artificialiser certains habitats ou de renforcer certaines infrastructures artificielles comme les digues, le projet MARINEFF cherche à apporter une réponse permettant de concilier ce développement humain à la question environnementale. Pour ce faire, des infrastructures marines écoconçues ont été réalisées. Ces dernières apportent les mêmes avantages en termes de construction ou de protection par exemple que des blocs en béton classiquement utilisés pour les ouvrages marins (digue, enrochement artificiel...) tout en intégrant, dès leur conception, une dimension écologique avec la présence de différentes modifications de surface permettant de recréer des habitats pour la vie marine. Ces différents ouvrages peuvent donc représenter l'opportunité de créer de nouveaux habitats et donc de concilier développement humain et bénéfice écologique. C'est selon cette philosophie qu'ont été conçues les infrastructures marines du projet MARINEFF et notamment les blocs de digues expérimentaux. Les différentes études menées pendant ma thèse ont permis de caractériser les processus associés à la colonisation biologique d'infrastructures marines en zone subtidale et d'évaluer certaines fonctions écosystémiques qui y sont associées et en particulier la fonction de production primaire.

I. Approche en conditions contrôlées sur des infrastructures marines expérimentales

La première partie de ces travaux s'est concentrée sur l'étude de biofilms microphytobenthique de substrats durs en conditions contrôlées. Ces expérimentations ont été réalisées sur des petites infrastructures marines en béton et ont permis de décrire des processus éco-physiologiques fins tels que la croissance et la photobiologie de ces organismes en fonction de différents types forçages (nature du substrat, rugosité de surface, disponibilité en nutriments). Comme montré dans le chapitre III, le béton constitue un matériau de premier choix pour la construction de RA. Il présente aussi un taux de réussite important et permet de favoriser la colonisation benthique marine. Les micro-organismes qui composent ces biofilms marins sont sous l'influence de différents facteurs comme la nature de la surface colonisée ou encore la disponibilité en sels nutritifs (Hillebrand & Sommer, 2000; Hutchinson et al., 2006; Salta et al., 2013; Balqadi et al., 2018). Ces facteurs vont influencer la croissance du biofilm microphytobenthique ainsi que les paramètres photosynthétiques (Kromkamp et al., 1995; Jesus et al., 2005; Stock, 2019) et donc la production primaire.

I.1. Influence de la nature du substrat et de la rugosité micro-échelle sur le développement microphytobenthique et sa photosynthèse

Premièrement, les différents types de bétons testés (différentes formulations et ajout ou non de coquilles d'huîtres broyées) n'ont pas eu d'effet significatif sur le développement du biofilm microphytobenthique et sur sa photobiologie. Cependant, l'intérêt d'intégrer des co-produits coquillés dans la fabrication du béton pour augmenter sa résistance à déjà été démontré (Georges et al., 2021). De plus, cela permet de réduire l'empreinte environnementale du béton en réduisant la proportion de sable nécessaire à sa conception (Dennis et al., 2018). En revanche, des différences notables ont été observées entre les dalles en bétons et celles en PVC (Chapitre IV). La biomasse microphytobenthique était significativement plus forte sur les PVC que sur les dalles en béton, dans le même temps, les paramètres photosynthétiques (F_v/F_m , $rETR_{MAX}$ et α) étaient significativement plus élevés sur les dalles en béton. Des observations visuelles non présentées ici ont permis de constater des détachements importants de biofilm microphytobenthique après une dizaine de jours sur les PVC. La croissance plus rapide sur les structures en PVC et une adhésion plus réduite explique probablement cette mauvaise tenue dans le temps. Cette étude a donc permis de confirmer que le béton constituait un matériau efficace pour favoriser un développement microphytobenthique présentant un meilleur statut physiologique et donc une longévité plus importante.

La nature du substrat n'a pas été le seul facteur étudié, le niveau de rugosité de surface de la structure colonisée sur ces indicateurs microphytobenthiques a été également étudié. Les dalles en béton présentaient une face lisse et une face rugueuse afin d'étudier l'influence de ce facteur sur le développement microphytobenthique. La rugosité à micro-échelle est un facteur clé qui va conditionner le développement du microphytobenthos et sa biomasse (Hutchinson et al., 2006; Souche et al., 2016). Notre étude (Chapitre IV) a permis de confirmer ces hypothèses en mettant en avant une biomasse microphytobenthique plus élevée sur les dalles rugueuses que sur les lisses. Au-delà de l'indicateur de biomasse, l'état physiologique des cellules (F_v/F_m), leur capacité de production ($rETR_{MAX}$) et l'efficacité photosynthétique (α) étaient également plus élevés pour le microphytobenthos des dalles rugueuses. La considération de la rugosité de surface d'une infrastructure marine permet de créer une quantité importante de micro-habitats servant d'abris aux cellules microphytobenthiques en les protégeant d'éventuels stress extérieurs (lumière, force d'érosions, prédation...) ce qui influe positivement sur leur physiologie et améliore leur photoacclimatation (NPQ réduit).

Le couplage des techniques d'imagerie PAM et d'imagerie tridimensionnelle de la structure de surface permet de comparer les indicateurs entre eux et ce, pixel par pixel. Cette approche innovante pour ce type d'étude permet d'obtenir une importante quantité de données permettant une approche statistique robuste. En effet, pour chaque structure, un coefficient de corrélation a pu être calculé entre le niveau de rugosité (calculé par rapport au niveau moyen de la dalle considérée comme nul) et l'indicateur considéré (biomasse ou photosynthétique). Ce coefficient a été calculé pour chaque pixel composant les images (rugosité et biomasse par exemple) soit 640×480 pixels. Un coefficient de corrélation moyen était ensuite calculé pour l'ensemble de l'échantillon considéré. Une corrélation négative indiquait que l'indicateur choisi était corrélé à un niveau de relief négatif. Les conclusions précédentes ont été également appuyées par les calculs de corrélation entre les indicateurs de biomasses ou photobiologiques et le niveau de rugosité indiquant des corrélations négatives pour ces indicateurs sur les dalles rugueuses, à l'exception du NPQ qui était corrélé positivement au relief confirmant ainsi la meilleure photoacclimatation dans les reliefs négatifs. Ces résultats ont donc permis d'expliquer l'avantage écologique engendré par l'addition de rugosité à la surface de la dalle ; ces micro-habitats ont permis au biofilm de se développer par patches dans des conditions favorables. Le biofilm situé dans ces niches présentait un statut physiologique, une capacité de production et une efficacité photosynthétique plus élevées que sur le reste de la structure. Une croissance à l'échelle de la dalle entière a été ensuite observée depuis ces micro-niches vers les reliefs positifs de la structure.

Ces résultats permettent d'appuyer le fait que le béton constitue un élément pertinent pour la construction de récifs artificiels ou d'autres structures marines comme cela a été montré dans le chapitre III. En effet, il représente la majeure partie des récifs artificiels aujourd'hui immergés et constitue également un matériau efficace améliorant les processus de colonisation et l'efficacité des structures (Chapitre III). De plus, notre étude confirme l'importance de l'éco-conception des infrastructures marines à des échelles micro ou millimétriques afin de favoriser le développement microphytobenthique et donc, à plus large échelle, la mise en place de successions écologiques complexes (Olivier et al., 2000; Tamburri et al., 2008; Guo et al., 2021). L'évaluation des paramètres photobiologiques associés au microphytobenthos a permis de quantifier le bénéfice apporté par l'augmentation de la rugosité de surface d'une infrastructure marine sur les premiers organismes à s'y développer. La considération de ce compartiment d'un point de vue écologique est importante puisqu'il est à la base des successions écologiques plus complexes dans un contexte *in situ* et a un rôle prépondérant sur

la structure des réseaux trophiques associés à ces infrastructures (Golléty & Crowe, 2013; Bharti et al., 2017; van der Heijden et al., 2020).

1.2. Influence du niveau d'eutrophisation sur le développement microphytobenthique et sa photosynthèse

La disponibilité des nutriments est l'un des principaux moteurs de la croissance des microalgues planctoniques et benthiques, ce qui peut entraîner l'eutrophisation des écosystèmes côtiers lorsque les apports sont excessifs (Rabalais, 2009). Les phénomènes d'eutrophisation sont généralement associés à une biomasse de phytoplancton élevée. Les effets de l'eutrophisation sur le biofilm sont étudiés dans une moindre mesure (Fricke et al., 2016; Lawes et al., 2016). Considérant le contexte global du projet MARINEFF et les objectifs de restauration de digues ou d'ouvrages portuaires, il était important d'évaluer l'impact d'un gradient d'eutrophisation sur la colonisation microphytobenthique puisque ces habitats sont généralement sous l'influence d'intrants d'origines humaines chargés en nutriments. L'étude réalisée dans ce contexte n'a pas montré d'impact significatif de la concentration en sels nutritifs du milieu sur le développement et la biomasse microphytobenthique (Chapitre IV).

La quantification des EPS produits par le microphytobenthos représente un indicateur pertinent permettant de comprendre les processus de formation des biofilms microphytobenthiques. Les valeurs élevées du ratio EPS:Chl *a* en début de suivi montrent l'importance des EPS pour l'adhérence des diatomées au substrat dans les premières étapes du développement microphytobenthique (Sutherland et al., 1998; Orvain et al., 2003). Nos résultats ont confirmé la relation positive entre l'exsudation d'EPS et la disponibilité des nutriments en raison de la quantité plus élevée d'EPS mesurée à la fin de l'expérimentation dans la condition fortement enrichie en sels nutritifs (Staats et al., 2000; Yallop et al., 2000; Orvain et al., 2003). La structure de la surface colonisée joue également un rôle : des concentrations d'EPS plus fortes ont été mesurées sur les structures lisses que sur les rugueuses indiquant là encore leur rôle important dans la stabilisation du biofilm microphytobenthique.

Les mesures d' α^* réalisées lors de cette expérimentation ont permis le calcul d' ETR_{max}^{a*} ou le α^{a*} c'est-à-dire des valeurs absolues de flux ou d'efficacité énergétiques et non des valeurs relatives et ont permis de d'améliorer et d'affiner les interprétations concernant les processus de photoacclimatation des biofilms microphytobenthiques. Les valeurs plus élevées de ETR_{max}^{a*} en fin d'expérimentation dans la condition fortement enrichie indiquent que la capacité photosynthétique des cellules microphytobenthiques était positivement influée par la

disponibilité en nutriments. Les valeurs élevées de a^* dans la condition non enrichie en sels nutritifs en début de suivi mettent en avant un impact d'une limitation potentielle en sels nutritifs sur la photobiologie des cellules (Geider et al., 1998; Napoléon et al., 2013b). Ces limitations pourraient également expliquer les fortes excrétions d'EPS qui sont souvent considérées comme des puits métaboliques de carbone lorsque la synthèse protéique est limitée (Staats et al., 2000).

I.3. Evaluation de la diversité microphytobenthique et amélioration des techniques de mesures photobiologiques

La diversité microphytobenthique associée aux différentes infrastructures expérimentales (dalles ou PVC) a été évaluée par MEB (Chapitre IV). Cette technique d'observation permet d'obtenir une information qualitative sur la diversité d'espèces présente et elle n'est pas exhaustive. Les espèces identifiées par cette technique lors des différents suivis sont représentative des espèces retrouvées dans l'environnement naturel local sur des substrats durs. Afin d'explorer davantage cette question, des échantillons de biofilm microphytobenthique provenant de l'expérimentation sur les conditions nutritives (Chapitre IV) sont en cours de traitement par des approches moléculaires. La diversité des eucaryotes et des procaryotes sera analysée par métabarcoding. Ces analyses sont réalisées en collaboration Pascal-Jean-Lopez (BOREA, MNHN), elles évalueront de manière détaillée l'éventuel impact de la nature du substrat colonisé (PVC ou béton rugueux) et de la disponibilité en nutriments dans le milieu sur la structure et la diversité des communautés du microbiome.

En ce qui concerne la photobiologie, l'association d'autres techniques de mesures à la fluorimétrie PAM comme des mesures d' a^* et de LabSTAF permettent de calculer des indicateurs de productions absolus à la différence de ceux obtenus par le PAM qui sont relatifs. Le couplage de ces différentes techniques a été réalisé durant la thèse dans le cadre d'un stage de Master 2, ces données ne sont toutefois pas intégrées dans mon mémoire. L'un des objectifs de ce travail était d'améliorer les mesures relatives d'ETR obtenues à l'aide de l'IMAGING-PAM en le couplant à des mesures de LabSTAF et de a^* afin d'estimer une valeur d'ETR (II) absolue permettant ensuite de calculer la production primaire en flux d'oxygène (Boatman et al., 2019) ou de carbone (Napoléon & Claquin, 2012; Morelle & Claquin, 2018). Ces techniques sont déjà appliquées aux communautés phytoplanctoniques mais elles sont plus délicates à réaliser pour le microphytobenthos de substrat dur. Pour ce compartiment, il est nécessaire de remettre les cellules en suspension, un protocole a été mis au point pendant la thèse sur le même

principe que celui réalisé pour les mesures de Chl *a*. Les résultats obtenus dans le cadre de cette expérimentation ont mis en avant une forte corrélation linéaire (coefficient directeur proche de 1) entre les différentes approches (a^* , σ_{PSII} [absorption fonctionnelle du PSII] et a_{LHII} [coefficient d'absorption du PSII]) permettant de calculer l'ETR(II) en utilisant un algorithme basé sur le σ_{PSII} le ou sur l'absorption, a_{LHII} (Oxborough et al., 2012). Ces résultats sont cohérents et permettent de comparer la productivité associée à différents compartiments de producteurs primaires. Il serait pertinent d'effectuer des mesures de ce type à différents endroits du substrat étudiés afin de compléter les informations obtenues par l'IMAGING-PAM sur l'hétérogénéité spatiale de la photobiologie microphytobenthique.

Il serait intéressant d'étudier l'influence d'autres paramètres environnementaux tels que la quantité de lumière, la température, la prédation ou encore l'hydrodynamisme sur la composition des communautés microphytobenthiques de substrat dur et sur leur photobiologie. Ces paramètres environnementaux et leurs variations sont connus comme des facteurs contrôlant la production primaire microphytobenthique et ont déjà fait l'objet d'études scientifiques (Anderson, 1995; Battin et al., 2003; Di Pippo et al., 2012; Juneau et al., 2015; Fricke et al., 2016; Briand et al., 2017; Méléder et al., 2020; Prins et al., 2020). Toutefois, la majeure partie de ces études ont été réalisées en situation *in situ* et sur des biofilms microphytobenthiques de substrat meuble.

Les EPS peuvent être associés à des fractions de poids moléculaires différents. Les EPS colloïdaux correspondent aux molécules liées aux cellules et joue un rôle prépondérant dans l'adhésion et la protection des cellules. Les EPS non liés, avec un poids moléculaire plus faible, seront eux rapidement utilisés par d'autres organismes et représentent ainsi une source importante nutritive (Underwood & Paterson, 2003; Wotton, 2004). Il serait donc intéressant de discriminer les différentes fractions d'EPS lors de futures analyses similaires à celles réalisées pendant ma thèse. Le matériel disponible et le temps imparti n'étaient pas suffisants à la période où cette expérimentation (Chapitre IV) a été réalisée pour évaluer ces différentes fractions. Toutefois, des cinétiques de production différentes peuvent être attendues entre les EPS liés et non liés puisqu'ils n'ont pas les mêmes rôles écologiques et qu'ils ne sont pas utilisés de la même manière par les consommateurs.

L'utilisation de la caméra 3D pour mesurer et reproduire numériquement le relief des dallettes a permis d'étudier finement les relations entre les paramètres biologiques, photosynthétiques et le niveau de rugosité. Toutefois, des expérimentations complémentaires

sont envisageables pour étudier l'influence de cette rugosité de surface sur d'autres indicateurs comme le recrutement larvaire. Il a déjà été démontré que la rugosité influait positivement sur le recrutement larvaire (Olivier et al., 2000), or, il serait possible d'utiliser les données produites par l'analyse en caméra 3D pour identifier un potentiel *preferendum* de recrutement en fonction du degré de rugosité. Enfin, il serait intéressant d'explorer ces relations entre les indicateurs biologiques et photosynthétiques et la rugosité de surface sur un panel plus large d'amplitude de rugosité. Par exemple, il serait envisageable de produire des structures artificielles présentant des rugosités micrométriques jusqu'à des échelles centimétriques et ainsi déterminer la gamme d'amplitude préférentielle pour l'indicateur d'intérêt (biomasse microphytobenthique, paramètres photosynthétiques...). Il serait également pertinent de tester différentes formes de rugosité de surface en utilisant des technologies récentes comme l'impression 3D pour reproduire un pattern bien défini.

II. Approche *in situ* sur des infrastructures marines expérimentales

A la suite des expérimentations réalisées en conditions contrôlées, une étude sur le même modèle expérimental (dallettes) a été réalisée en situation *in situ* sur les deux sites expérimentaux de cette thèse (rade de Cherbourg et baie de Seine, Chapitre V). Compte tenu des résultats obtenus suite aux expérimentations en conditions contrôlées (Chapitre IV), il était pertinent d'évaluer la colonisation biologique de ces petites infrastructures écoconçues dans l'environnement naturel. Ces dalles ont été réalisées avec le même matériau et la même structure que les blocs écoconçus MARINEFF, cette approche a donc également permis d'évaluer le succès écologique de ces blocs à une échelle réduite.

II.1. *Structure des communautés benthiques*

La colonisation par une algue encroûtante, *Phymatolithon purpureum*, a été observée sur les dalles de la rade de Cherbourg (Chapitre V). Une phase de croissance entre février et septembre 2020 a été mesurée jusqu'à l'atteinte d'un plateau à environ 75% de recouvrement des dalles. Le recouvrement était légèrement supérieur sur les dalles lisses et semblerait indiquer une meilleure colonisation pour ce type d'algue en absence de rugosité. La diversité de macroalgues dressées a également été mesurée, 12 espèces ont été recensées avec une diversité plus importante en baie de Seine (10 espèces) qu'en rade de Cherbourg (trois espèces). De manière générale et durant tout le suivi, la diversité associée aux dalles était sensiblement plus forte sur les dalles rugueuses. Les données obtenues lors du suivi de juillet 2021 (non incluses dans la partie V-A) ont permis de mettre en avant une différence importante sur cet

indicateur : environ 50% de recouvrement sur les dalles rugueuses contre 85% sur les lisses. Ce résultat appuie les précédentes observations qui suggéraient une meilleure colonisation de *Phymatolithon purpureum* sur les dalles lisses. La rugosité aurait donc un impact bénéfique sur la diversité de producteurs primaires alors qu'elle aurait un impact négatif sur la biomasse de *Phymatolithon purpureum* et sur la pérennité de cette colonisation. Un autre aspect important à considérer est la période d'immersion des structures artificielles. En effet, la date d'immersion des dalles peut avoir un effet sur les premiers colonisateurs (période de reproduction de certaines espèces) et empêcher le développement d'autres espèces par la suite. Le suivi sur ces dalles se poursuivra pendant le reste du projet MARINEFF et les prochains résultats permettront d'avoir une vision plus large des successions écologiques associées à ces structures sur plusieurs années consécutives. Ceci permettra de confirmer ou non le meilleur succès des dalles lisses pour la colonisation par *Phymatolithon purpureum* et la plus forte diversité sur les rugueuses. De plus, la colonisation par certaines espèces de macrofaune benthique (polychètes et balanes) sur les dalles en rade de Cherbourg a été observée (données non publiées). Le pic d'abondance pour ces organismes a été atteint à la même période où *Phymatolithon purpureum* a atteint son plateau de recouvrement. Ces résultats tendent également à confirmer l'impact positif de ce matériau pour favoriser le recrutement benthique (Anderson & Underwood, 1994; Ditsche et al., 2017; Sedano et al., 2020). La colonisation du béton par le biofilm et les cirripèdes améliorera la durabilité des matériaux et réduira la diffusion potentielle de polluants comme le chlorure (Chlayon et al., 2018).

II.2. *Développement et photosynthèse des producteurs primaires, influence des sites expérimentaux et de la rugosité micro-échelle*

Lors de cette approche *in situ*, aucun effet significatif du niveau de rugosité sur la biomasse microphytobenthique ou sa photobiologie n'a été observé. Ceci pourrait s'expliquer par le cumul des pressions environnementales et leurs variations journalières, saisonnières. L'intérêt écologique lié à l'augmentation de la rugosité de surface de la structure démontré précédemment (Chapitre IV) n'est pas remis en cause pour autant. La différence de fréquence d'échantillonnage entre les approches en conditions contrôlées et *in situ* est importante et pourrait également expliquer l'absence d'observation d'impact significatif de la rugosité de surface des dalles sur la biomasse microphytobenthique. Il est envisageable qu'après plusieurs mois de suivi en conditions contrôlées, l'écart de biomasse entre les deux types de rugosité serait réduit ou inexistant. Enfin, la colonisation des dalles par différents organismes comme les balanes a eu un impact sur la structure de surface : les dalles lisses sont devenues

rugueuses. Ceci pourrait également expliquer l'absence de différence forte entre les deux types de rugosité en ce qui concerne la biomasse microphytobenthique (Olivier et al., 2000).

La biologie de *Phymatolithon purpureum* et des autres algues encroûtantes de climat tempéré est assez peu documentée, la photobiologie de cette espèce n'a jamais été étudiée à notre connaissance. L'utilisation de l'IMAGING-PAM a permis de discriminer spatialement les zones colonisées par l'algue encroûtante et les autres zones. Le biofilm microphytobenthique était présent sur l'ensemble de la surface des dalles, il était donc possible d'évaluer les paramètres photosynthétiques du biofilm seul et du biofilm avec l'algue encroûtante. Cette approche a été réalisée à différents stades du développement de *Phymatolithon purpureum* : en phase de début de croissance, exponentielle et en phase stabilisée. Ces résultats ont permis de mettre en avant des différences significatives entre les zones colonisées par *Phymatolithon purpureum* et le reste de la dalle. Pendant la phase précoce de croissance et la phase exponentielle, les paramètres photosynthétiques (F_v/F_m , $rETR_{MAX}$ et α) étaient significativement plus faibles dans les zones colonisées par l'algue encroûtante qu'en dehors indiquant la faible efficacité et capacité photosynthétique de *Phymatolithon purpureum*. Cette approche a permis d'observer une compétition interspécifique entre le microphytobenthos et *Phymatolithon purpureum*. Les algues rouges encroûtantes ont déjà été décrites comme bien adaptées aux faibles conditions de lumière (Irving et al., 2004; Burdett et al., 2014) ce qui vient appuyer nos observations concernant les faibles paramètres photosynthétiques et la photo-inhibition importante (NPQ élevé) mesurés dans les zones colonisées par *Phymatolithon purpureum*. La réduction importante du taux de recouvrement de *Phymatolithon purpureum* observée en juillet 2021 sur les dalles lisses (de 75% à 50%) pourrait s'expliquer par la quantité de lumière plus importante à cette saison et donc des conditions moins favorables à *Phymatolithon purpureum* qu'au microphytobenthos. Il est cependant important de tenir compte que l'utilisation de la fluorimétrie PAM employant une lumière bleue n'est pas totalement adaptée aux mesures photosynthétiques chez les algues rouges. La lumière bleue est peu absorbée par les phycobilisomes et crée un déséquilibre entre les photosystèmes entraînant des transitions d'état (déplacement des antennes entre les PSI et PSII et réciproquement) (Hanelt et al., 1992; Luimstra et al., 2018) qui peuvent avoir une incidence sur les mesures d'efficacité et la capacité photosynthétiques obtenues (Korbee et al., 2005).

Le suivi de la colonisation benthiques sur ces dalles expérimentales en situation *in situ* a permis de confirmer la bonne efficacité de ce matériau pour favoriser le développement

microphytobenthique et, plus largement, la mise en place de successions écologiques. L'importance de la rugosité de surface mise en avant lors des approches en conditions contrôlées (Chapitre IV) n'était pas aussi marquée lors du suivi *in situ*. Toutefois, ces résultats ont permis d'étudier le développement des producteurs primaire et en particulier la compétition entre le microphytobenthos et *Phymatolithon purpureum*. Ils ont également permis de caractériser spatialement la production primaire et la photobiologie de *Phymatolithon purpureum* pour la première fois.

III. Approche *in situ* sur des récifs artificiels (projet RECIF) puis sur les infrastructures marines écoconçues MARINEFF

Après avoir étudié différents indicateurs fonctionnels associés à la production primaire benthique ainsi que la diversité associée à ces communautés sur des infrastructures marines expérimentales en conditions contrôlées et *in situ*, ces mêmes indicateurs ont été évalués sur des récifs artificiels et des infrastructures marines écoconçues. Ce transfert d'échelle depuis l'expérimental vers l'étude environnementale et écosystémique permet de mesurer l'évolution de ces indicateurs fonctionnels et structurels en fonction de l'infrastructure étudiée et des forçages environnementaux.

III.1. *Macrofaune benthique : structure des communautés, influence des sites expérimentaux et des infrastructures*

L'étude des communautés de producteurs primaires et de macrofaune a été réalisée entre fin 2019 et fin 2020 sur les récifs artificiels immergés en 2015 dans le cadre du projet RECIF (Chapitre V). Les différents taxons de macrofaune benthique ($n = 41$) ont été regroupés par groupes trophiques ($n = 6$). La communauté benthique présente sur les récifs en rade de Cherbourg était plus diversifiée que celle de la baie de Seine qui elle présentait une biomasse totale plus élevée. La communauté benthique en rade de Cherbourg était dominée toute l'année par des filtreurs, des prédateurs et des dépositivores en hiver. En baie de Seine, elle était dominée par des filtreurs toute l'année et également des prédateurs en hiver. Compte tenu du fait que les structures immergées sont identiques entre les deux sites, les différences enregistrées de structure trophique entre les sites peuvent être induites par des facteurs abiotiques (hydrodynamisme, salinité, température, turbidité...), par les communautés environnantes présentes avant et après l'immersion des récifs artificiels ainsi que par les relations proies / prédateurs.

La communauté ichthyologique associée aux RA du projet RECIF (Chapitre V) a été étudiée tardivement et les résultats ne sont pas développés dans mon mémoire. Toutefois, des premiers résultats issus de suivi en plongée ont montré une importante diversité dans la zone d'implantation des RA. Le dernier suivi a été réalisé sur le site subtidal de la rade de Cherbourg le 7 juillet 2021, neuf taxons de poissons (*Trisopterus luscus*, *Pollachius pollachius*, *Dicentrarchus labrax*, *Conger conger*, *Labrus bergylta*, *Symphodus melops*, *Parablennius gattorugine*, *Callionymus lyra*, gobies) et six espèces de crustacés (*Homarus gammarus*, *Cancer pagurus*, *Necora puber*, *Maja tridactyla*, *Palaemon serratus* et *Galathea trigosa*) ont été recensés. Ces résultats sont encore incomplets et le suivi se poursuit sur les deux sites, de plus, des évaluations de biomasses sont en cours d'estimation. Cependant, ces résultats soutiennent les éléments discutés précédemment (Chapitre V) confirmant l'importante diversité biologique associée à ces structures et l'aspect déterminant de l'écoconception afin de favoriser cette diversité (Partie III). Comme pour la macrofaune benthique, des variations saisonnières et inter-sites sont attendues. Ces résultats serviront également de référence pour évaluer le niveau de colonisation biologique associée aux blocs de digues MARINEFF. Les outils de modélisation trophique (Ecopath, Ecosim) permettront d'estimer les dynamiques de production, de diversité et l'évolution du réseau trophique de ces écosystèmes. Ces outils sont déjà utilisés dans de nombreux domaines et servent par exemple à évaluer l'impact qu'engendrerait la construction d'un champ éolien offshore sur les communautés benthiques et pélagiques locales (Raoux et al., 2017; Pezy et al., 2020). Les données obtenues pendant les suivis réalisés dans le cadre de la partie V-B sont actuellement exploitées en collaboration avec des collègues (Jessica Salaün, Aurore Raoux, Nathalie Niquil et Jean-Philippe Pezy) pour évaluer et caractériser la structure trophique associée aux RA grâce à l'outil Ecopath. Les données issues de ces modélisations sont en cours d'analyse et feront l'objet d'une publication scientifique ultérieure.

Il serait intéressant d'évaluer l'impact des structures artificielles (RECIF et MARINEFF) sur l'environnement adjacent. Des échantillonnages du sédiment à différentes distances des structures pourraient permettre d'évaluer l'influence potentielle de ces dernières sur la quantité et la qualité de la matière organique sédimentaire. Des analyses de diversité benthique pourraient également être effectuées et permettraient d'estimer la zone d'influence des RA. On sait que les constructions artificielles comme dans les ports diffèrent des habitats naturels en termes de densité et de biomasse des communautés (Airoldi et al., 2009). Plusieurs études ont mis en évidence une richesse spécifique réduite par rapport aux communautés naturelles

voisines (Airoldi et al., 2008; Ido & Shimrit, 2015) ainsi qu'une composition de macrofaune différente (Wahl, 2009; Aguilera et al., 2014; Hall et al., 2021). Des différences en termes de composition de la structure et de fonction trophique des assemblages épibenthiques entre des habitats artificiels et les récifs naturels ont également été décrites (Perkol-Finkel et al., 2006; Carvalho et al., 2013). Enfin, il sera également important de poursuivre les suivis scientifiques sur les blocs de digues MARINEFF afin de rendre compte de l'évolution sur le long terme des écosystèmes. Ceci permettra d'observer les différentes trajectoires écologiques potentielles entre les blocs classiques et écoconçus. Cette évaluation sur la durée permettra de rendre compte du réel bénéfice apporté par ces structures à l'environnement local. Les résultats obtenus à travers ces études mettent en avant l'intérêt déjà documenté de l'écoconception des infrastructures marines (Perkol-Finkel et al., 2018a; Strain et al., 2018; Pioch & Souche, 2021). Ce type d'approche devrait être davantage considéré à l'avenir afin de limiter les conséquences du développement humain sur les écosystèmes marins et côtiers. A l'échelle de la région normande par exemple, un parc éolien de 50 km² composé de 64 éoliennes verra le jour en 2024 au large de Courseulles-sur-Mer. A un horizon plus lointain, dans le cadre de futurs projets, il sera important de considérer l'ensemble des enjeux écologiques par l'écoconception des fondations de ces éoliennes par exemple.

III.2. *Producteurs primaires : structure des communautés, influence des sites expérimentaux et des infrastructures*

En ce qui concerne les producteurs primaires, une diversité élevée a été observée sur les RA de la rade de Cherbourg (15 espèces) et une diversité moindre sur ceux de la baie de Seine (8 espèces). Ces résultats sont différents de ceux obtenus pour le suivi sur les dalles *in situ* puisqu'une diversité plus forte était observée sur les dalles en baie de Seine. Ceci pourrait s'expliquer par la courte durée d'immersion des dalles en comparaison aux récifs immergés depuis 2015. Les statuts écologiques sont très différents : les écosystèmes associés aux dalles sont en évolution constante alors que ceux associés aux récifs sont presque stabilisés. Pour les deux sites, l'été et l'automne étaient les saisons les plus productives car les biomasses de producteurs primaires étaient élevées à cette période. Les différences de pressions environnementales entre les deux sites (*i.e.* turbidité, lumière disponible, hydrodynamisme, nutriments) peuvent expliquer les différences entre les communautés de producteurs primaires.

Enfin, le début du suivi scientifique sur les blocs de digues MARINEFF a mis en avant des premiers résultats concernant la diversité associée à ces structures. L'effort d'échantillonnage

a été principalement réalisé sur le site de la rade de Cherbourg avec un premier suivi en avril 2021. Lors de ce premier suivi, sept espèces différentes ont été identifiées sur les blocs de digues qui étaient alors immergés depuis sept mois : *Saccorhiza polyschides*, *Dictyota dichotoma*, *Gracilaria verrucosa*, *Palmaria palmata*, *Cryptopleura ramosa*, *Ulva lactuca* et *Bryopsis plumosa*. L'ensemble de ces espèces a été retrouvé sur les blocs expérimentaux et classiques. Un second suivi a été effectué trois mois plus tard en juillet 2021. Neuf espèces ont été identifiées sur les blocs expérimentaux (*Laminaria ochroleuca*, *Calliblepharis jubata*, *Callophylis laciniata*, *Cryptopleura ramosa*, *Vertebrata fruticulosa*, *Ulva* sp., *Dictyotata dichotoma*, *Sacchoriza polyschides* et *Gracilaria* sp.) et 12 sur les blocs classiques incluant toutes celles retrouvées sur les expérimentaux plus *Sphaerococcus corronopifolius*, *Desmarestia aculatea*, *Plocamium cartilageum* et *Chondria capillaris*. Ces résultats préliminaires montrent une augmentation importante de la diversité de producteurs primaires sur les deux types de blocs en seulement trois mois. La grande majorité de ces espèces ont également été retrouvée sur les récifs immergés depuis 2015 (Chapitre V), toutefois, certaines espèces n'étaient pas présentes sur ces récifs comme *Callophylis laciniata*, *Desmarestia aculatea* ou *Chondria capillaris*. Ces premiers résultats sont également différents de ceux obtenus sur le suivi des dallettes *in situ* (Chapitre V) malgré le fait que le matériau composant les dallettes et les blocs de digue soit identique. Ceci pourrait s'expliquer par l'emplacement des dallettes, elles ont été immergées avant l'installation des blocs MARINEFF et sont installées sur les RA du projet RECIF. Toutefois, la communauté de producteurs primaires ayant colonisé les blocs MARINEFF était là encore dominée par des algues rouges (neuf espèces sur les 12) confirmant leur bonne acclimatation aux conditions environnementales locales (Chapitre V). Ce sont des résultats préliminaires et l'absence de certaines algues peut s'expliquer par des périodes de reproduction décalées. Ces résultats sont positifs et tendent à valider l'intérêt écologique des blocs de digues éco-conçus MARINEFF. La diversité recensée sur les structures RECIF est plus élevée du fait de leur ancienneté. Cependant, en considérant les premiers résultats sur les blocs MARINEFF, nous nous attendons à voir l'écart de diversité entre les deux types de structures se réduire avec le temps. Les premiers résultats indiquent une présence importante de laminaires (algues brunes) mais également d'algues rouges (*Sacchoriza polyschides*, *Palmaria palmata*, *Cryptopleura ramosa*, *Rhodophyllis divaricata*) et des ulves. Le pourcentage de recouvrement de la face supérieure est plus important sur les blocs écoconçus (environ 85% contre 60% de la surface), la communauté de macroalgues sur ces blocs écoconçus est également plus homogène (environ 30% de vertes, de rouges et 40% de brunes contre 70% de vertes, 15% de rouges et brunes sur les blocs classiques). Ces résultats sont issus

d'observation réalisées *in situ* sur l'ensemble des blocs immergés, les prochaines plongées permettront de confirmer ou non cette tendance.

III.3. *Production primaire et photochimie : influence des sites expérimentaux et des infrastructures*

La fonction de production primaire et les paramètres photosynthétiques associés aux communautés de producteurs primaires ayant colonisées les structures du projet RECIF ont été caractérisées (Chapitre V). Les mesures de production primaire ont été réalisées grâce à des incubations sous un gradient de lumière. Une forte différence inter-sites et inter-saisons a été observée : les récifs artificiels en rade de Cherbourg sont beaucoup plus productifs qu'en baie de Seine et le niveau de production maximal était atteint en été. Ce résultat s'explique principalement par l'importante biomasse de macroalgue en été à Cherbourg et la très faible biomasse de producteurs primaires toute l'année en baie de Seine ainsi que par la plus forte biomasse de consommateurs en baie de Seine. Les valeurs de production primaire pour les récifs de la rade de Cherbourg sont très élevées par rapport à d'autres études souvent plus proche des valeurs mesurées en baie de Seine (Golléty et al., 2008). Dans un écosystème comparable, dans la même mer, à la même profondeur, la même zone mais dans deux masses d'eau différentes, deux type de fonctionnement associés à ces récifs artificiels ont été observés. L'un peut être décrit comme un récif « producteur » riche en macroalgues, c'est celui de la rade de Cherbourg. L'autre comme un récif « consommateur », celui de la baie de Seine.

Les communautés de macroalgues ayant colonisé les blocs de digues MARINEFF ont été étudiées. Ces résultats préliminaires ont été obtenus lors du suivi en avril 2021 et montrent de fortes différences entre les blocs expérimentaux et classiques (Claquin et al., 2022, annexe H). La chambre benthique développée dans ce contexte a permis de standardiser l'exposition à la lumière de la communauté des macroalgues pendant l'incubation. Des chambres benthiques traditionnellement transparentes sont utilisées (Gevaert et al., 2011) et les résultats obtenus dépendent fortement de l'irradiance quotidienne qui peut changer d'un jour à l'autre et entre les saisons (Hubas & Davoult, 2006; Gevaert et al., 2011). La chambre benthique automatisée utilisée dans cette étude est facilement installée en plongée et a permis de reproduire les mesures en conservant une exposition lumineuse stable (Figure 63 et Figure 62).

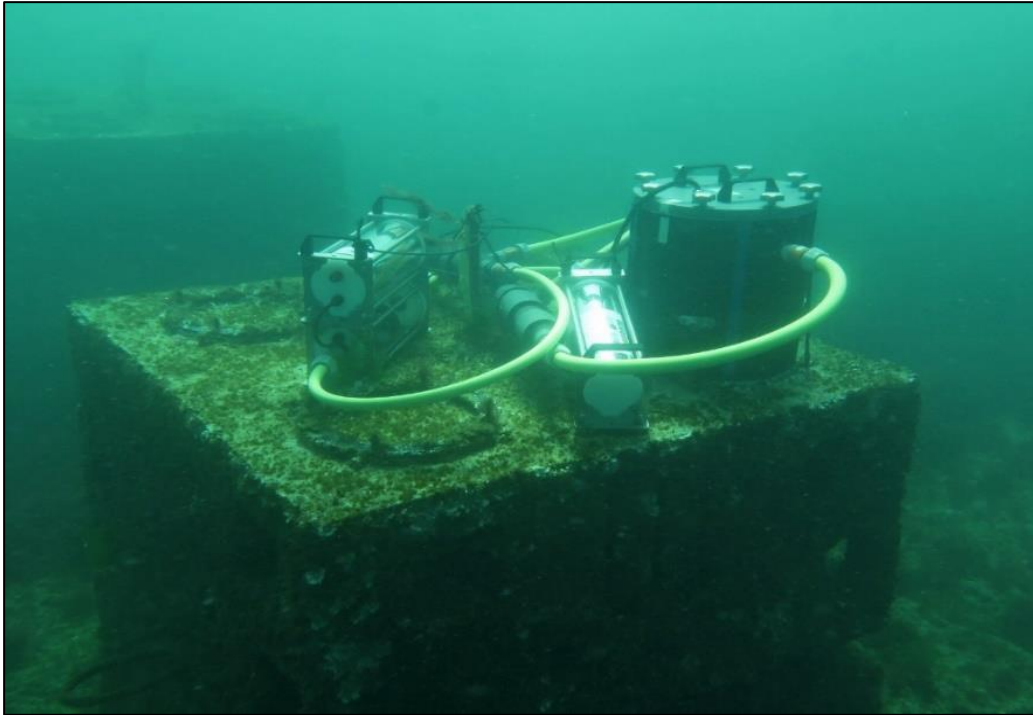


Figure 63 – Photographie d'un bloc de digue expérimental avec l'installation de mesure de production primaire (chambre benthique). Photographie réalisée en juin 2021.

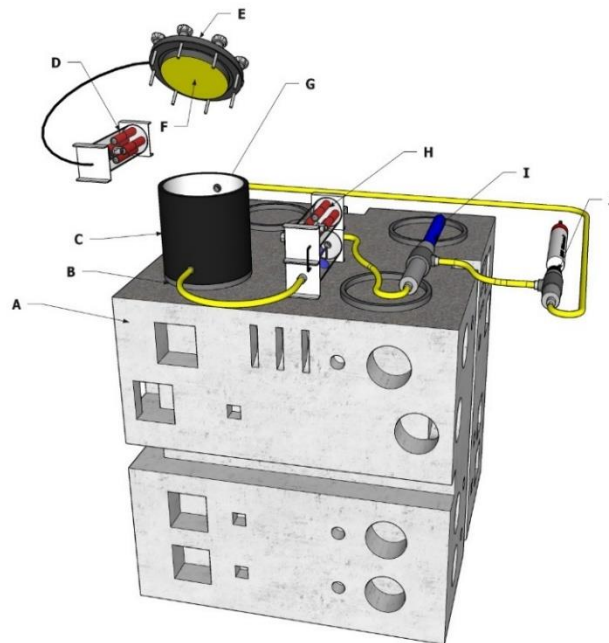


Figure 62 – Cloche benthique automatisée pour les mesures de production primaire. (A) module de digue expérimental MARINEFF avec les anneaux de fixations sur la face supérieure (B) afin d'y attacher la cloche benthique (C). Revêtement en aluminium (G) recouvrant l'intérieur de la chambre benthique. Panneau LED (F) vissé sur le dessus de la chambre benthique (E) afin de rendre le système étanche. Le panneau LED est dirigé par un contrôleur (D). Le flux d'eau est généré par une pompe immergeable (H). Deux sondes, YSI EXO 1 (I) avec un capteur d'oxygène et une Turner Design C-Sense pCO₂ (J) dirigée par un logger RBR Virtuoso2 (J) permettent de mesurer la concentration des gaz. Le système entier est immergé sur site et est entièrement clos. D'après Claquin et al., 2022.

Les résultats concernant ces expérimentations ont fait l'objet d'un acte de colloque (Claquin et al., 2022, annexe H) et ont permis de valider notre protocole expérimental. Ils ont également permis de mettre en avant l'avantage écologique engendré par l'écoconception des modules de digues : la biomasse de macroalgue était environ deux fois supérieure sur ces blocs que sur les classiques, la productivité maximale était également plus élevée sur les blocs éco-conçus. La productivité mesurée par unité de surface sur les blocs écoconçus MARINEFF est largement supérieure (environ 14 fois) à celle mesurée sur les structures RECIF (Chapitre V) pour le même site et à la même saison. Cet écart important pourrait s'expliquer en partie par la structure des communautés de producteurs primaires qui diffère entre les types d'infrastructures marines, toutefois, il semble confirmer l'atout écologique apporté par l'écoconception des infrastructures marines et le bénéfice important en termes de préservation des fonctions écologiques.

Afin d'évaluer la photoacclimatation des différentes espèces de macroalgues et de décrire leur photobiologie, des mesures de DIVING-PAM ont été réalisées sur les deux types de structures (RECIF et MARINEFF) sur le site de Cherbourg. Lors des suivis réalisés dans le cadre du chapitre V, des mesures de fluorescences seules ont été effectuées permettant ainsi l'exploitation de données de production relatives ($rETR_{MAX}$). Un résultat majeur a été mis en avant : la capacité maximale de production ($rETR_{MAX}$) montrait une augmentation graduelle pour toutes les espèces entre l'été et le printemps. Le statut physiologique (F_V/F_M) des algues rouges était plus faible que pour les autres espèces. Des valeurs plus faibles de F_V/F_M ont été mesurées pour les algues rouges, ceci peut en partie s'expliquer par la composition pigmentaire de ces dernières (JuHyoungh et al., 2019). En été, des valeurs plus faibles de $rETR_{MAX}$ peuvent s'expliquer par un NPQ saisonnier (Franklin & Forster, 1997) certainement dû à l'excès de lumière dans ces eaux peu profondes à cette période de l'année en raison de la plus faible turbidité des eaux. Les comparaisons des paramètres photosynthétiques obtenus avec des mesures de PAM doivent être effectuées avec prudence puisque l'absorption de l'énergie lumineuse diffère selon les organismes photosynthétiques ce qui affecte l'ETR.

Dans le cadre du suivi sur les blocs de digues MARINEFF, les spectres d'absorbances et d'excitations des différentes espèces de macroalgues ont été réalisées (Claquin et al., 2022, annexe H). Ces informations ont permis de calculer des valeurs absolues de production d'électrons. Comme pour les blocs RECIF, les valeurs d'ETR et α étaient significativement plus fortes pour les algues brunes que pour les rouges et vertes. De la même manière, le F_V/F_M mesuré pour les algues rouges sur les blocs MARINEFF était faible, ceci peut être en partie dû à la composition pigmentaire de ces algues (Kim et al., 2019b) comme discuté précédemment

pour *Phymatolithon purpureum*. La réponse des Rhodophyceae à la fluorimétrie de type PAM peut être différente de celle des autres classes car les algues rouges possèdent des phycobilisomes dans leur appareil photosynthétique (Kowalczyk et al., 2013; Kaňa et al., 2014). Nous ne nous attendions pas à observer des différences de paramètres photosynthétiques entre des individus d'une même espèce échantillonnés sur des blocs éco-conçus ou classiques. La suite du suivi nous donnera plus d'informations, si la biomasse reste significativement plus élevée sur les modules expérimentaux, l'ombrage généré par la densité d'algues pourrait entraîner des différences de photoacclimatation et potentiellement à des divergences en termes de structures des communautés et de biodiversité entre les deux types de blocs.

III.4. *Efficacité des infrastructures écoconçues*

Les études réalisées dans ce travail portent en partie sur des habitats portuaires et exclusivement sur des substrats durs, ceci représente un intérêt particulier puisque ces habitats sont souvent ignorés. Ce type d'étude apporte un diagnostic écologique dans un environnement potentiellement de mauvaise qualité écologique comme les ports. La majeure partie des études réalisées pour évaluer la qualité écologique d'un milieu sont effectuées sur des sédiments meubles ce qui s'explique par la plus grande facilité d'échantillonnage pour types de substrats (Shim & Singh, 1988; Losi et al., 2013; Baux et al., 2017, 2019). Ceci entraîne un manque de données quantitatives et qualitatives pour les communautés benthiques de substrats durs et rend difficile la création d'indicateurs écologiques comme il en existe beaucoup pour les communautés de substrats meubles (Taormina et al., soumis + annexe I). Le développement actuel des activités humaines en mer entraîne une augmentation significative du nombre d'infrastructures installées comme c'est le cas dans la rade de Cherbourg. Ces infrastructures fournissent de nouveaux habitats de substrat dur à de nombreux organismes marins et contribuent à la modification de la structure et le fonctionnement des écosystèmes côtiers. Afin de mieux évaluer la nature de ces modifications ainsi que les avantages et/ou les inconvénients potentiels générés, il devient essentiel d'élaborer des méthodes d'évaluation qui peuvent être appliquées à une grande variété de sites d'études. Les approches structurales (description des communautés benthiques, diversité et biomasse associées, groupes trophiques) et fonctionnelles (évaluation de la production primaire, photobiologie et photoacclimatation des producteurs primaires benthiques) réalisées dans le cadre de cette thèse permettent de caractériser les écosystèmes associés à ces structures artificielles mais constituent également un indicateur de qualité écologique du milieu différent des indicateurs classiques de substrats meubles. La mise au point d'un système automatisé de mesure de production primaire *in situ* (Figure 63)

représente également une avancée significative pour l'évaluation de cette fonction écologique sur des habitats artificiels. L'étude des producteurs primaires associé aux récifs artificiels est encore trop peu développée (Pennesi & Danovaro, 2017; Dennis et al., 2018; Sheng et al., 2018; Tsiamis et al., 2020). Toutefois, au vu des résultats présentés dans ma thèse, il apparaît essentiel d'étudier davantage les producteurs primaires associés à ces structures artificielles et surtout de caractériser les processus écologiques et en particulier la fonction de production primaire afin de rendre compte du statut écologique de ces structures. Les RA sont assimilés à des substrats durs cependant, il n'est pas possible de comparer directement le fonctionnement de cet écosystème artificiel avec des substrats rocheux naturels. La caractérisation du fonctionnement des récifs naturels proches des structures artificielles étudiées pourrait permettre de comparer les dynamiques de certains indicateurs comme la diversité ou la production primaire et ainsi porter un regard différent sur la capacité des RA à reproduire un écosystème naturel. Cet effort d'échantillonnage conséquent est prévu d'ici 2022, avant la fin du projet MARINEFF, et permettra de comparer la structure des communautés benthiques entre les récifs naturels et artificiels.

Les écosystèmes productifs ne sont pas toujours associés à une biodiversité importante, la relation entre ces indicateurs varie selon les dimensions de la diversité et le niveau des contraintes externes (Brun et al., 2019). Par exemple, la diversité phylogénétique peut être importante dans les écosystèmes avec peu de contraintes externes en promouvant des stratégies complémentaires, mais sous des contraintes externes, les organismes les plus adaptés peuvent être sélectionnés et la diversité sera réduite (Zobel & Pärtel, 2008; Brun et al., 2019). De plus, ce principe écologique varie au sein de l'échelle spatiale considérée. À l'échelle mondiale, les relations unimodales diversité-productivité dominent dans les régions tempérées, tandis que les relations positives sont plus fréquentes sous les tropiques (Zobel & Pärtel, 2008). Dans les écosystèmes marins, la dépendance des relations diversité-productivité à l'échelle spatiale est plus fréquente à petite échelle (locales) que plus grandes (régionales) (Witman et al., 2008). Les études réalisées pendant ma thèse soulignent ces concepts écologiques et constituent un bon exemple de la corrélation négative potentielle entre la diversité et la productivité des écosystèmes à petite échelle (Chapitre V). Toutefois, l'étude réalisée sur le projet RECIF montre la relation inverse à une échelle supérieure : le site présentant une forte productivité est également celui qui présente une forte diversité (*i.e.* rade de Cherbourg).

IV. Limites des études

Les études réalisées durant ma thèse présentent certaines limites. Concernant les protocoles expérimentaux, certains biais peuvent être identifiés. L'expérimentation réalisée dans la partie IV-A présente certaines limites comme le fait que l'eau de mer n'était pas renouvelée mais seulement brassée. C'est un facteur qui a eu un impact sur la durée de l'expérimentation puisque le stock de sels nutritifs n'était pas renouvelé et consommé par le microphytobenthos. Après une semaine de suivi, ces sels nutritifs sont certainement devenus limitant et ceci a pu avoir un impact sur les dynamiques de colonisation des infrastructures et sur les paramètres photosynthétiques. C'est pour cette raison que les données n'ont été considérées que pour les six premiers jours. Cette limite rencontrée dans la partie IV-A a été corrigée lors de l'expérimentation suivante (Partie IV-B) puisqu'un système semi-ouvert d'alimentation en eau de mer filtrée a été mis en place.

Certains biais concernant l'expérimentation *in situ* réalisée sur les dallettes (Partie V-A) sont également identifiables. Les dallettes ont été installées sur les AR du projet RECIF immergés en 2015 et donc déjà fortement colonisés au moment du début de l'expérimentation. Cette forte colonisation a pu être à l'origine de phénomènes d'auto-ombrage entre la communauté de macroalgues s'étant développée sur les AR et celle se développant sur les dallettes. De plus, cette forte diversité à proximité immédiate des dallettes a pu favoriser une colonisation rapide de celles-ci par l'apport de larves, spores et propagules. L'installation des dallettes sur des structures indépendantes et suffisamment éloignées des AR n'était pas cependant pas possible.

Les estimations de diversité à différentes échelles (organismes unicellulaires, macrofaune et macroalgues) souffrent de certaines limites. A l'échelle macroscopique, certains prélèvements sont par nature biaisés. Les grattages réalisés sur les parpaings (Partie V-B) et ceux réalisés en plongée sur les blocs de digues MARINEFF sont non-exhaustifs et les organismes prélevés peuvent être détériorés. De plus, la surface grattée n'est pas exactement égale à la surface théorique prélevée. Ces limites sont bien connues pour ce type de méthode, il faut néanmoins les considérer. De plus, lors des grattages réalisés sur les blocs de digues MARINEFF, une suceuse subaquatique est utilisée afin d'aspirer les organismes grattés et les conserver dans un filet (maille de 500 µm de diamètre). Cette méthode est indispensable pour les grattages *in situ* et comporte certaines limites comme le risque de perte de matériel (non aspiré) ou de surplus (aspiration d'algues épaves par exemple).

L'identification des différentes espèces, taxons, de macrofaune benthique s'est faite avec un choix assumé de ne pas parfaire l'identification systématiquement jusqu'à l'espèce. Ce choix a été appuyé par la limite de temps imparti pour réaliser ces identifications et la nécessité de ne pas impacter les autres travaux mais également par le fait que le raisonnement employé nécessitait d'avoir uniquement les biomasses des groupes trophiques. En effet, l'approche constituait en une question de structure trophique des communautés d'où la possibilité de faire la détermination jusqu'au taxon le plus bas, ce qui était suffisant pour avoir l'information de son mode de nutrition. Il sera également important de caractériser d'éventuels patrons de colonisation en fonction des formes, tailles, des modifications de surfaces apportées aux blocs écoconçus afin de déterminer un design efficace. Enfin, les identifications et comptages des poissons et crustacés réalisés en plongée sont également soumis à certaines limites. Le premier biais est celui de l'observateur et de sa capacité à identifier l'espèce et évaluer la taille de l'organisme. La seconde limite est propre au matériel utilisé et au bruit généré par le plongeur (pas de recycleur), enfin la dernière limite est celle du site (visibilité faible). L'installation de caméras autonomes sur les structures artificielles étudiées est envisageable afin de corriger ces biais.

V. Perspectives de recherche et applications

L'ensemble des études réalisées pendant cette thèse ont permis d'améliorer la compréhension de la mise en place des communautés benthiques associées aux structures artificielles marines. Certaines approches développées pour évaluer la structure de ces communautés et les fonctions écologiques associées pourraient être appliquées à d'autres études sur des systèmes similaires, artificiels ou naturels.

L'utilisation d'un IMAGING-PAM afin d'évaluer la photobiologie associée aux producteurs primaires benthiques (microphytobenthos et algues encroûtantes) permet de spatialiser ces mesures. Comme cela a été montré dans mes travaux, le couplage de cet outil à un appareil de mesure topographique comme une caméra 3D permet de juxtaposer l'information concernant la photobiologie des organismes étudiés à celle de la structure de surface du substrat colonisé. Ces techniques seraient pertinentes pour l'étude du microphytobenthos associé à d'autres types de substrats durs ou meubles. Cette spatialisation des données permet également de connaître les coordonnées associées aux différents paramètres, il serait donc envisageable de cartographier la colonisation benthique par d'autres organismes ou le recrutement larvaire de certaines espèces et d'en tirer des informations concernant des préférences de recrutement

en fonction d'un certain niveau de relief par exemple. D'autre part, comme cela a été montré dans le chapitre V, l'IMAGING-PAM permet également de discriminer les paramètres photosynthétiques associés aux différentes espèces présente sur la surface étudiée.

Concernant la fonction de production primaire associée aux habitats benthiques, le développement de la cloche automatisée réalisé dans le cadre de mes travaux de thèse et du projet MARINEFF représente une avancée conséquente. En effet, ce système déjà très répandu et utilisé pour les mesures de production primaires a été amélioré de manière à homogénéiser les mesures. La cloche a été rendue opaque et un système d'éclairage piloté informatiquement a été développé en ce sens permettant ainsi de reproduire les mêmes conditions d'éclairage quelles que soient les conditions environnementales et météorologiques. L'ensemble de ce système est simple d'utilisation et d'installation, il serait envisageable de l'utiliser plus largement pour d'autres types d'études concernant la production primaire benthique. L'automatisation complète du système permet de réaliser des mesures sur de longues durées et sans nécessité de surveillance. Des applications à d'autres types de substrats durs mais également meubles seraient pertinentes.

Le second système développé durant cette thèse pour évaluer la fonction de production primaire était celui utilisé dans le cadre du chapitre V. Il permet, au même titre que la cloche benthique automatisée, de contrôler la quantité de lumière par un système d'éclairage artificiel. A l'inverse des cloches benthiques classiques, ce système constitué d'une caisse hermétique, d'un système de circulation branché sur les sondes de mesures, permet de réaliser des mesures de production primaire au laboratoire sur des échantillons prélevés *in situ*. Nous l'avons développé et utilisé pour mesurer la production associée aux parpaings constituant les récifs artificiels du projet RECIF, cependant, il serait pertinent de l'utiliser pour mesurer la production primaire associée à d'autres types de substrats durs pouvant être échantillonnés.

Les études réalisées dans le cadre de cette thèse ont permis de mettre en avant l'importance de la considération structurelle des infrastructures marines afin de favoriser leur colonisation. La prise en compte de cette écoconception dès le départ des projets d'artificialisations du milieu marin ou côtier est primordiale pour limiter leur impact environnemental et créer de nouveaux habitats. Cette notion d'écoconception intègre aussi bien la dimension micro ou millimétrique de la surface de l'infrastructure que la dimension centimétrique importante pour la mise en place de successions écologiques complexes.

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ANNEXES

Annexe A (Chapitre III) : Complete table of the references used in this study. Artificial reef numbers correspond to the values in the dendrograms and MCA plots.

Artificial reef number	Reference
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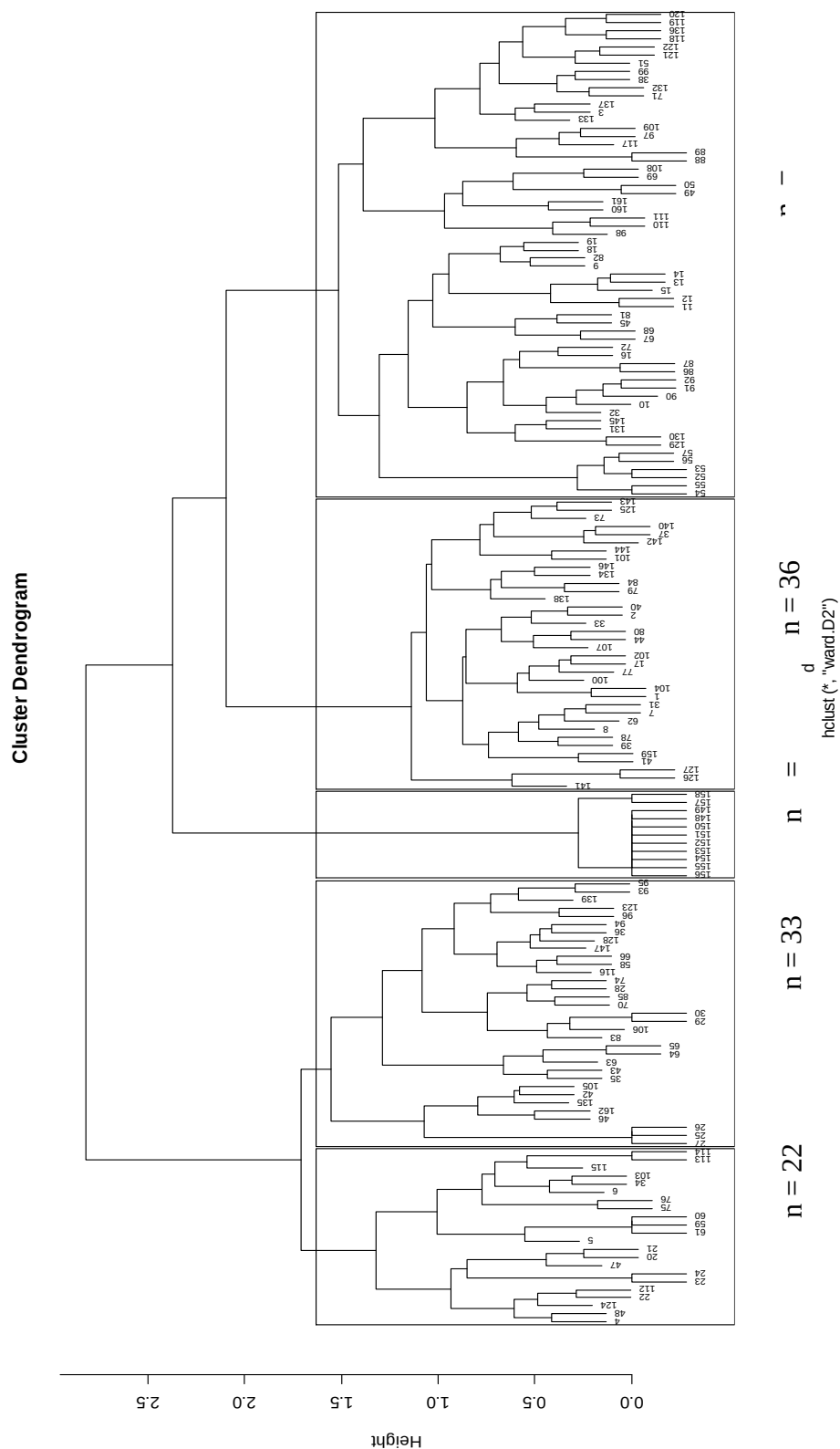
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Annexe B (Chapitre III) : List of final variables used in the study with their respective compositions.

Final variable	Included variables					
AR material wood	Bamboo	Wood				
AR material plastics	Nylon	Plastic	PVC	Polyethylene	Plexiglas	Vinyl cloth
AR material rocks	Clay	Rocks	Limestone	Sandstone	Gabbro	Slate
AR shape cylindrical	Cones	Cylindrical	Pipes	Nozzles		
AR shape plates	Slabs	Plates				
AR shape other	Pan scourers	Shells				
Monitoring techniques pictures	Pictures	Quadrats	Detailed pictures	Video recording	Satellite observations	
Monitoring techniques biodiversity measurements	Cover percentage	Density	Diversity	Abundance	Richness	
Monitoring technique environmental measurements	Physico-chemicals parameters	Water parameters	Organic matter measurements			
Monitoring techniques primary production measurements	Pigments	Pulse Amplitude Modulated (PAM)				
Monitoring techniques pelagic measurements	Acoustic monitoring	Experimental fishing	Fish tagging			
Monitoring techniques ecological process	Survival rates	Biomass	Growth rate	Isotopes	Primary production	

Annexe C (Chapitre III) : Cluster dendrogram of the total data frame. Clusters grouping A to E correspond to the optimal number of clusters (k). Number of artificial reef sites in each group is given below the boxes. Individual numeration corresponds to the AR site (Appendix A).



Annexe D (Partie IV-A) : Résultats des split-plot ANOVA

Table S 1. ANOVA split plot on Chl a - Rugosity

Source of variation	SS	df	MS	F	P
Main plot					
Rugosity	0.026528	2	0.013264	166.3	< 2E-16
Main plot error	0.004467	56	0.00008		
Subplot					
Age	0.028202	3	0.0009401	60.412	< 2E-16
Age × Rugosity	0.0008817	6	0.0001469	9.443	6.23E-09
Subplot error	0.0026143	168	0.0000156		

Table S 2. Associate HSD Tukey test

HSD Tukey - Chl a × Rugosity	
PVC	a
Rough	b
Smooth	c

Table S 3. Associate HSD Tukey test

Tukey - Chl a × Age	
Day 2	a
Day 3	a
Day 4	b
Day 6	c

Table S 4. ANOVA split plot on Chl a - Type

Source of variation	SS	df	MS	F	P
Main plot					
Type	0.0083	1	0.008265	1.145	2.89E-01
Main plot error	0.4041	56	0.007217		
Subplot					
Age	0.05131	3	0.017102	51.863	< 2E-16
Age × Type	0.00049	3	0.000163	0.494	0.687
Subplot error	0.05639	171	0.00033		

Table S 5. Associate HSD Tukey test

HSD Tukey - Chl a × Type	
Type n°1	a
Type n°2	a
Type n°3	a
Type n°4	a
Type n°5	c

Table S 6. Associate HSD Tukey test

Tukey - Chl a × Age	
Day 2	ab
Day 3	a
Day 4	b
Day 6	c

Annexes

Table S 7. ANOVA split plot on FV/FM - Rugosity

Source of variation	SS	df	MS	<i>F</i>	<i>P</i>
Main plot					
Rugosity	0.5111	2	0.25557	205	< 2E-16
Main plot error	0.0411	33	0.00125		
Subplot					
Age	0.12393	3	0.04131	78.996	< 2E-16
Age × Rugosity	0.01735	6	0.00289	5.529	5.64E-05
Subplot error	0.05177	99	0.00052		

Table S 8. Associate HSD Tukey test

HSD Tukey - $F_V/F_M \times \text{Rugosity}$	
PVC	a
Rough	b
Smooth	c

Table S 9. Associate HSD Tukey test

Tukey - $F_V/F_M \times \text{Age}$	
Day 2	a
Day 3	a
Day 4	b
Day 6	c

Table S 10. ANOVA split plot on FV/FM - Type

Source of variation	SS	df	MS	<i>F</i>	<i>P</i>
Main plot					
Type	0.05	1	0.05001	3.381	7.47E-02
Main plot error	0.5029	34	0.01479		
Subplot					
Age	0.12376	3	0.04125	69.337	< 2E-16
Age × Type	0.00828	3	0.00276	4.641	0.00441
Subplot error	0.06009	101	0.00059		

Table S 11. Associate HSD Tukey test

HSD Tukey - $F_V/F_M \times \text{Type}$	
Type n°1	a
Type n°2	a
Type n°3	a
Type n°4	a
Type n°5	b

Table S 12. Associate HSD Tukey test

Tukey - $F_V/F_M \times \text{Age}$	
Day 2	a
Day 3	a
Day 4	b
Day 6	c

Annexes

Table S 13. ANOVA split plot on rETR_{MAX} - Rugosity

Source of variation	SS	df	MS	<i>F</i>	<i>P</i>
Main plot					
Rugosity	63.52	2	31.76	12.225	3.93E-05
Main plot error	145.49	56	2.6		
Subplot					
Age	374.2	3	124.72	77.542	< 2E-16
Age × Rugosity	85.9	6	14.32	8.902	1.95E-08
Subplot error	270.2	168	1.61		

Table S 14. Associate HSD Tukey test

HSD Tukey - rETR _{MAX} - Rugosity	
PVC	a
Rough	a
Smooth	b

Table S 15. Associate HSD Tukey test

HSD Tukey - rETR _{MAX} × Age	
Day 2	a
Day 3	a
Day 4	a
Day 6	b

Table S 16. ANOVA split plot on rETR_{MAX} - Type

Source of variation	SS	df	MS	<i>F</i>	<i>P</i>
Main plot					
Type	11.01	1	11.009	3.085	0.0845
Main plot error	199.85	56	3.569		
Subplot					
Age	374.4	3	124.79	60.42	< 2E-16
Age × Type	2.6	3	0.87	0.42	0.739
Subplot error	353.1	171	2.07		

Table S 17. Associate HSD Tukey test

HSD Tukey - rETR _{MAX} × Age	
Day 2	a
Day 3	a
Day 4	a
Day 6	b

Annexes

Table S 18. ANOVA split plot on Ek - Rugosity

Source of variation	SS	df	MS	<i>F</i>	<i>P</i>
Main plot					
Rugosity	1078.3	2	539.1	39.05	1.06E-10
Main plot error	694.3	49	14.2		
Subplot					
Age	1407.1	3	469	32.236	3.38E-15
Age × Rugosity	245.1	6	40.8	2.807	0.0138
Subplot error	1673.3	115	14.6		

Table S 19. Associate HSD Tukey test

HSD Tukey - Ek × Rugosity	
PVC	a
Rough	b
Smooth	b

Table S 20. Associate HSD Tukey test

HSD Tukey - Ek × Age	
Day 2	a
Day 3	a
Day 4	a
Day 6	b

Table S 21. ANOVA split plot on Ek - Type

Source of variation	SS	df	MS	<i>F</i>	<i>P</i>
Main plot					
Type	3.7	1	3.71	0.111	0.7408
Main plot error	1779.4	53	33.57		
Subplot					
Age	1420.9	3	473.6	32.645	1.95E-15
Age × Type	202.5	3	67.5	4.651	0.00414
Subplot error	1712	118	14.5		

Table S 22. Associate HSD Tukey test

HSD Tukey - Ek × Age	
Day 2	a
Day 3	a
Day 4	a
Day 6	b

Annexes

Table S 23. ANOVA split plot on Alpha - Rugosity

Source of variation	SS	df	MS	<i>F</i>	<i>P</i>
Main plot					
Rugosity	0.8528	2	0.4264	10.3	0.000155
Main plot error	2.3185	56	0.0414		
Subplot					
Age	5.911	3	1.9703	88.36	< 2E-16
Age × Rugosity	0.935	6	0.1559	6.99	1.19E-06
Subplot error	3.746	168	0.0223		

Table S 24. Associate HSD Tukey test

HSD Tukey - Alpha × Rugosity	
PVC	a
Rough	a
Smooth	b

Table S 25. Associate HSD Tukey test

Tukey - Alpha × Age	
Day 2	a
Day 3	b
Day 4	b
Day 6	c

Table S 26. ANOVA split plot on Alpha - Type

Source of variation	SS	df	MS	<i>F</i>	<i>P</i>
Main plot					
Type	0.2026	1	0.20262	3.818	0.0557
Main plot error	2.9717	56	0.05307		
Subplot					
Age	5.909	3	1.9697	73.07	< 2E-16
Age × Type	0.066	3	0.0221	0.82	0.484
Subplot error	4.609	171	0.027		

Table S 27. Associate HSD Tukey test

HSD Tukey - Alpha × Type	
Type n°1	a
Type n°2	b
Type n°3	b
Type n°4	b
Type n°5	ab

Table S 28. Associate HSD Tukey test

Tukey - Alpha × Age	
Day 2	a
Day 3	a
Day 4	a
Day 6	b

Annexes

Table S 29. ANOVA split plot on NPQ - Rugosity

Source of variation	SS	df	MS	<i>F</i>	<i>P</i>
Main plot					
Rugosity	1.3922	2	0.6961	56.314	3.94E-14
Main plot error	0.6922	56	0.0124		
Subplot					
Age	3.741	3	1.2469	112.442	< 2E-16
Age × Rugosity	0.43	6	0.0717	6.465	3.79E-06
Subplot error	1.863	168	0.0111		

Table S 30. Associate HSD Tukey test

HSD Tukey - NPQ × Rugosity	
PVC	a
Rough	b
Smooth	b

Table S 31. Associate HSD Tukey test

Tukey - NPQ × Age	
Day 2	a
Day 3	a
Day 4	b
Day 6	c

Table S 32. ANOVA split plot on NPQ - Type

Source of variation	SS	df	MS	<i>F</i>	<i>P</i>
Main plot					
Type	0.672	1	0.6718	8.832	0.00436
Main plot error	4.26	56	0.0761		
Subplot					
Age	2.936	3	0.9787	39.282	< 2E-16
Age × Type	0.253	3	0.0844	3.389	0.0194
Subplot error	4.261	171	0.0249		

Table S 33. Associate HSD Tukey test

HSD Tukey - NPQ × Type	
Type n°1	ab
Type n°2	ab
Type n°3	ab
Type n°4	ab
Type n°5	b

Table S 34. Associate HSD Tukey test

Tukey - NPQ × Age	
Day 2	a
Day 3	a
Day 4	b
Day 6	c

Annexes

Table S 35. ANOVA split plot on R (FO - Rugosity)

Source of variation	SS	df	MS	<i>F</i>	<i>P</i>
Main plot					
Rugosity	1.248	2	0.624	7.209	0.00164
Main plot error	4.847	56	0.0866		
Subplot					
Age	0.1001	3	0.03338	5.204	0.00184
Age × Rugosity	0.0673	6	0.001121	1.747	0.11297
Subplot error	1.0713	167	0.00642		

Table S 36. Associate HSD Tukey test

HSD Tukey - R (F _O - Rugosity)	
PVC	a
Rough	b
Smooth	a

Table S 37. Associate HSD Tukey test

Tukey - R (F _O - Age)	
Day 2	a
Day 3	a
Day 4	b
Day 6	b

Table S 38. ANOVA split plot on R (FV/FM - Rugosity)

Source of variation	SS	df	MS	<i>F</i>	<i>P</i>
Main plot					
Rugosity	0.933	2	0.4666	7.858	0.00982
Main plot error	3.325	56	0.0594		
Subplot					
Age	0.1502	3	0.05007	9.493	7.96E-06
Age × Rugosity	0.1176	6	0.0196	3.716	0.0017
Subplot error	0.8809	167	0.00527		

Table S 39. Associate HSD Tukey test

HSD Tukey - R (F _V /F _M × Rugosity)	
PVC	a
Rough	b
Smooth	ac

Table S 40. Associate HSD Tukey test

Tukey - R (F _V /F _M × Age)	
Day 2	a
Day 3	a
Day 4	b
Day 6	b

Annexes

Table S 41. ANOVA split plot on R (rETR_{MAX} - Rugosity)

Source of variation	SS	df	MS	<i>F</i>	<i>P</i>
Main plot					
Rugosity	1.3985	2	0.6992	15.501	3.96E-05
Main plot error	2.7966	50	0.0559		
Subplot					
Age	0.1839	3	6.13E-02	6.05	0.000644
Age × Rugosity	0.0708	6	0.0118	1.165	0.328088
Subplot error	1.53	151	0.01013		

Table S 42. Associate HSD Tukey test

HSD Tukey - R (rETR _{MAX} - Rugosity)	
PVC	a
Rough	b
Smooth	a

Table S 43. ANOVA split plot on R (Ek - Rugosity)

Source of variation	SS	df	MS	<i>F</i>	<i>P</i>
Main plot					
Rugosity	0.0628	2	0.03142	0.644	0.53
Main plot error	2.3427	48	0.04881		
Subplot					
Age	0.0288	3	0.009588	0.641	0.59
Age × Rugosity	0.0405	6	0.006749	0.451	0.843
Subplot error	2.0808	139	0.01497		

Table S 44. ANOVA split plot on R (Alpha - Rugosity)

Source of variation	SS	df	MS	<i>F</i>	<i>P</i>
Main plot					
Rugosity	1.3187	2	0.6594	11.938	5.78E-05
Main plot error	2.762	50	0.0552		
Subplot					
Age	0.2404	3	0.08014	7.692	8.11E-05
Age × Rugosity	0.0562	6	0.00936	0.898	0.498
Subplot error	1.5732	151	0.01042		

Annexes

Table S 45. Associate HSD Tukey test

HSD Tukey - R (Alpha × Rugosity)	
PVC	a
Rough	b
Smooth	a

Table S 46. Associate HSD Tukey test

Tukey - R (Alpha × Age)	
Day 2	a
Day 3	a
Day 4	b
Day 6	b

Table S 47. ANOVA split plot on R (NPQ - Rugosity)

Source of variation	SS	df	MS	<i>F</i>	<i>P</i>
Main plot					
Rugosity	0.9468	2	0.4734	9.036	3.97E-04
Main plot error	2.9339	56	0.0524		
Subplot					
Age	0.4505	3	0.15018	23.301	1.19E-12
Age × Rugosity	0.2074	6	0.03457	5.363	4.34E-05
Subplot error	1.0763	167	0.00645		

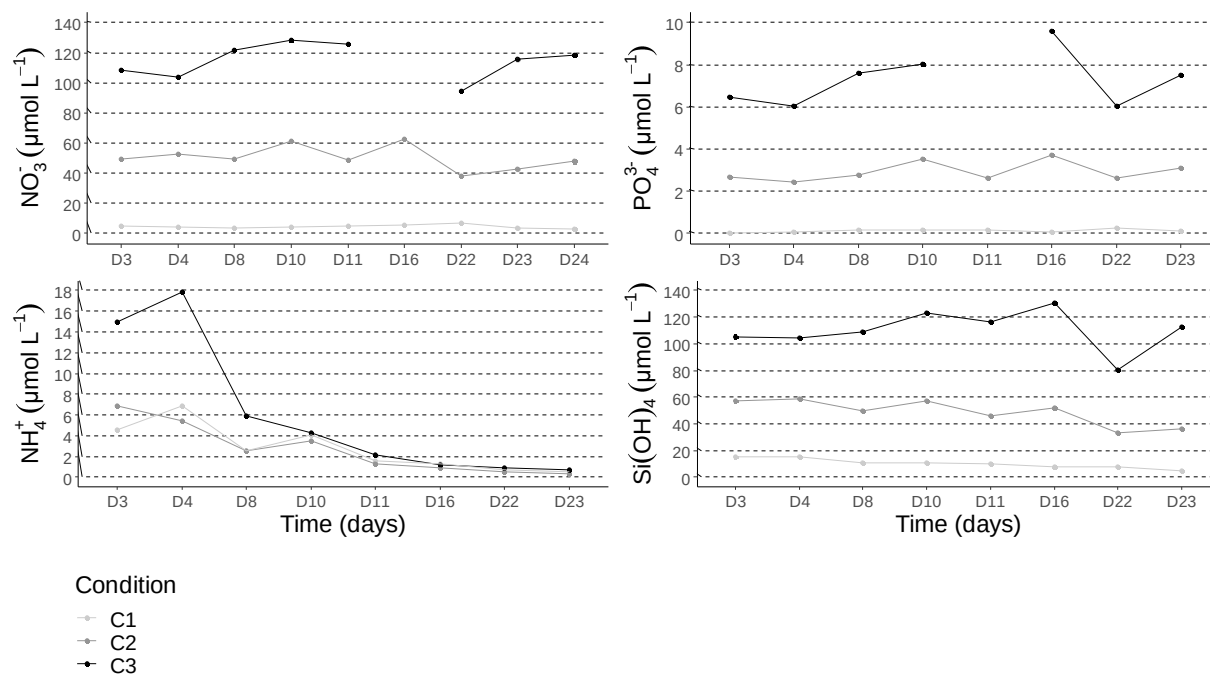
Table S 48. Associate HSD Tukey test

HSD Tukey - R (NPQ × Rugosity)	
PVC	ab
Rough	a
Smooth	b

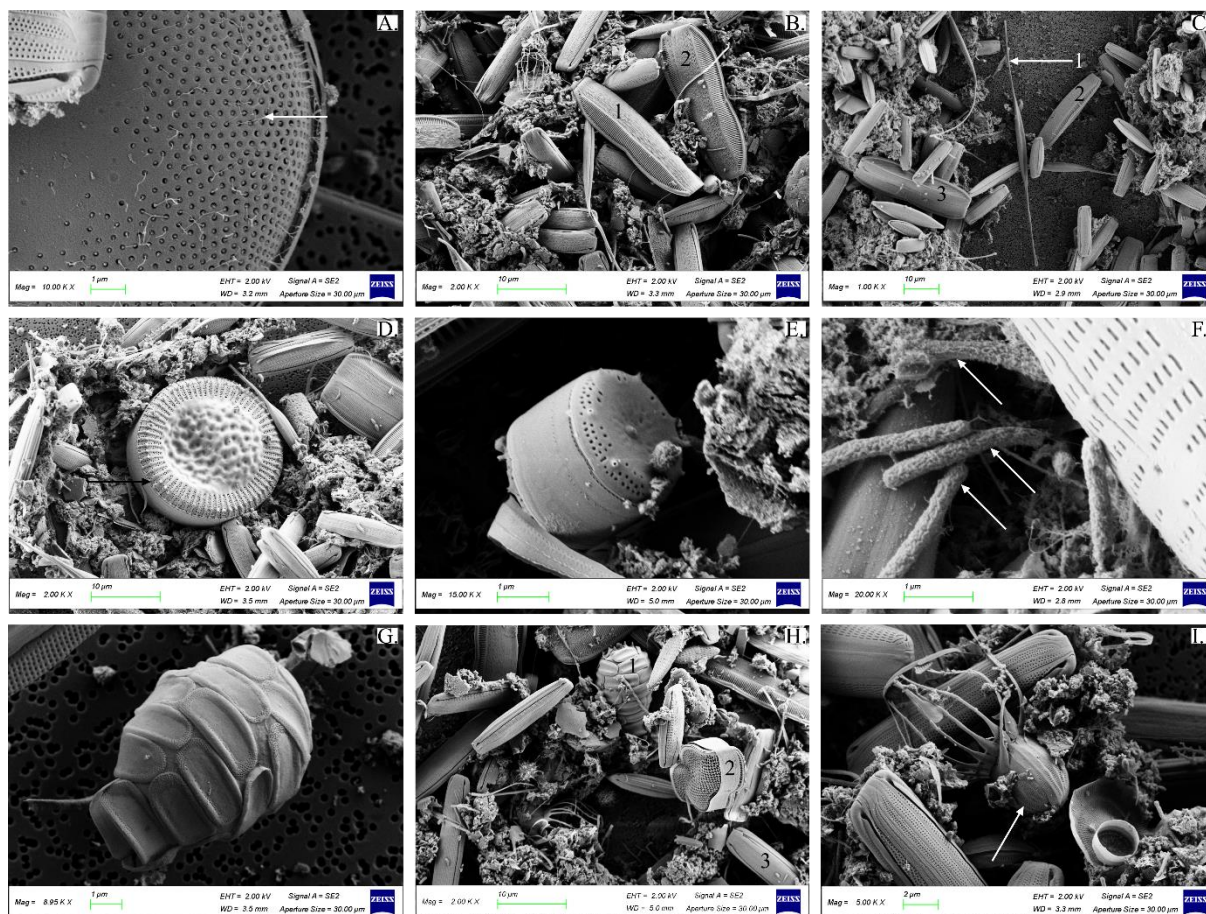
Table S 49. Associate HSD Tukey test

Tukey - R (NPQ × Age)	
Day 2	a
Day 3	a
Day 4	b
Day 6	b

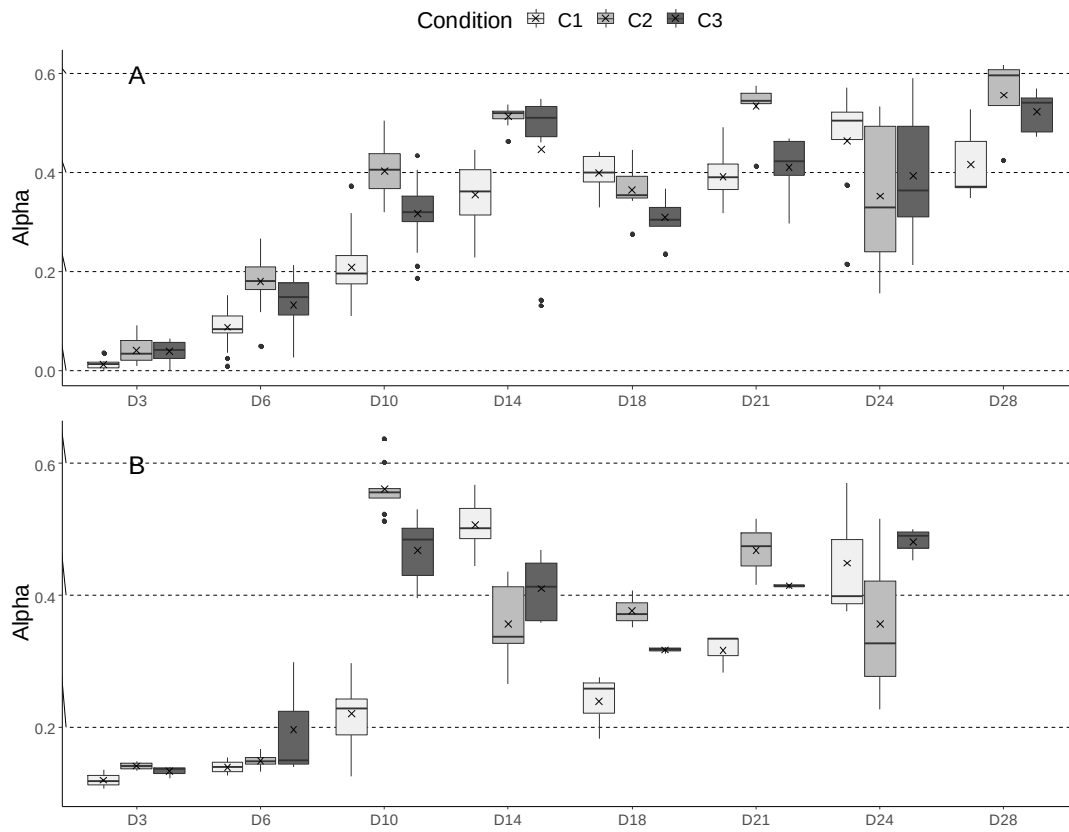
Annexe R (Partie IV-B) : Nutrient concentrations (NO_3^- , NH_4^+ , PO_4^{3-} and Si(OH)_4) evolution in each condition (control, medium and high enrichment) during the experiment in $\mu\text{mol.L}^{-1}$.



Annexe F (Partie IV-B) : Scanning electron microscopy observations of microphytobenthic biofilms sampled on MI during the experiment. *Coscinodiscus* sp. with EPS exudation (A.); *Amphora* sp. (B.1) and *Entomoneis* sp. (B.2); *Cylindrotheca closterium* (C.1), *Amphora* sp. (C.2) and *Entomoneis* sp. (C.3); *Thalassiosira* sp. (E.); bacteria (F.); *Paulinella ovalis* (G.); *Paulinella ovalis* (H.1), *Diploneis* sp. (H.2); Choanoflagellate (I.1).



Annexe G (Partie IV-B) : Alpha dynamic on MI (A) and PVC (B) during the survey.



Annexe H : P Claquin, B Vivier, M Navon, A M Rusig, I Mussio, L Chasselin, M Deloor, J C Dauvin, M Boutouil, F Orvain, 2022. Primary production assessment on eco-engineering infrastructures: English Channel case study. Marineff: from materials and infrastructures to marine ecosystems: interactions and new approaches.

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 pCO₂ 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.

I. 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 regroup 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 O₂, CO₂, 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. Materials & methods

1.1. Sampling site

Twelve dyke modules were immersed 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 m³ with smooth external texture (Figure 1a) and six eco-engineering modules corresponding to concrete cube of 1.7 m³ 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.



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

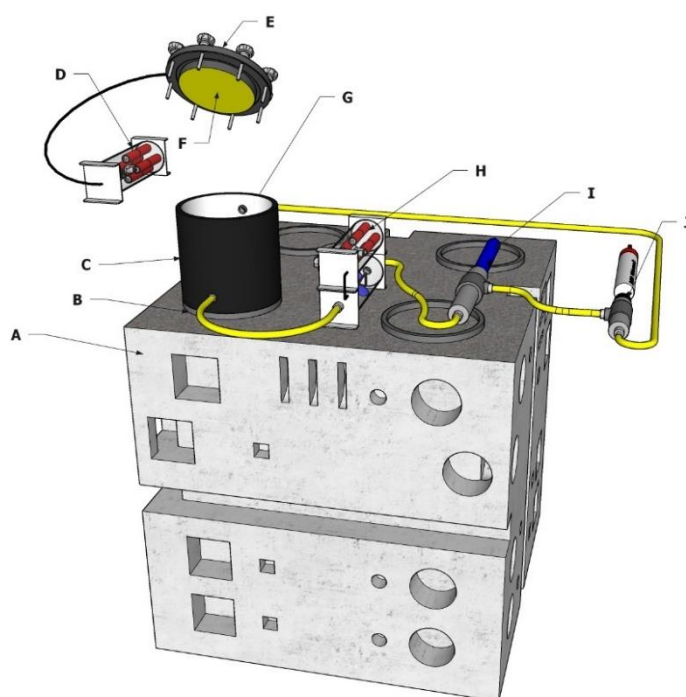


Figure. 2. Automated benthic chamber for primary production measurement. (A) MARINEFF eco-engineering dyke module with the mooring ring (B) to fix the benthic chamber (C). Aluminium tape (G) covers the benthic chamber inside. LED panel (F) is fixed to the benthic chamber top (E) which is screwed on the 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.

I.2. Primary production assessments (Figure 3)

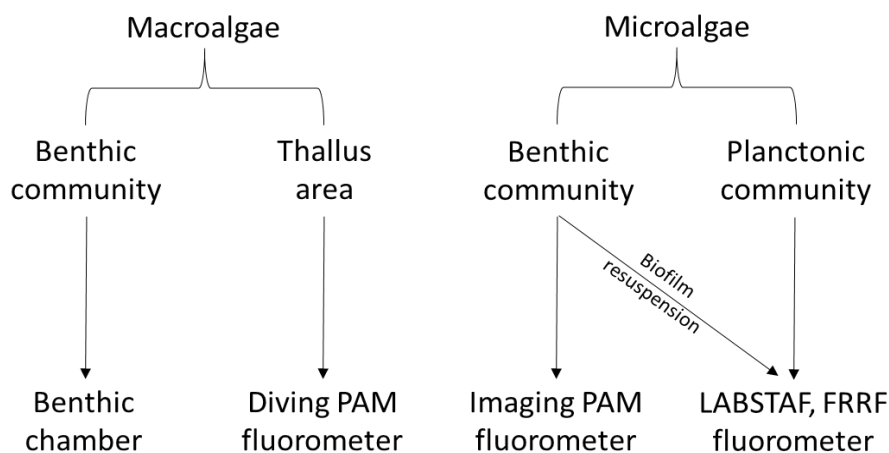


Figure. 3. Monitoring strategy deployed for primary production assessment on eco-engineering infrastructures during the MARINEFF project.

I.3. Benthic chamber

A fully automated system for measurements of photosynthetic oxygen and CO₂ exchange was developed in the MARINEFF project (Figure 2). The benthic chamber allows generating light gradient between 0 to 900 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ 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 pCO₂ probe every 30 seconds. Temperature and pH were measured at the same frequency in the chamber. A pump guaranteed the water homogenisation during the incubations. In the present experiment, 7 light steps of 15 min each from 0 to 750 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ were applied. A dark phase was applied after the last light step in order to estimate the respiration rate. Because O₂ evolution consumption in the light, may differ from dark O₂ consumption, the respiration rate was estimated during 5 min just after the last light phase [13]. O₂ 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 ($\text{GPP} = \text{NPP} + \text{BCR}$). pCO₂ 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.

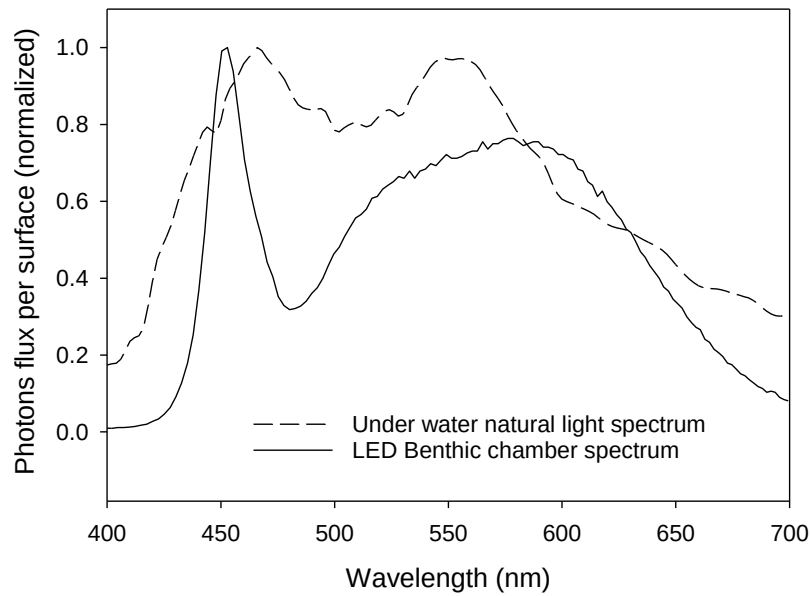


Figure. 4. LED spectrum used in the automated benthic chamber and under water natural light spectrum

I.4. Variable fluorescence measurements

I.4.1. Chlorophyll- *a* fluorescence induction pulse amplitude modulation (PAM)

Diving and imaging PAM (WaltzTm, Effeltrich, Germany) are used in the assessment protocol. Imaging PAM is not present in this paper. See Vivier et al. [16] for more details. The maximum quantum yield (F_v/F_m in the dark) and the effective photochemical efficiency (F_q'/F_m' 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 ($1 \mu\text{mol m}^{-2} \text{s}^{-1}$, 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 (F_m) was measured using a multiple turnover flash method [17]. We used a saturating blue light pulse (0.6 s, $4000 \mu\text{mol m}^{-2} \text{s}^{-1}$ at 474 nm) produced by a LED allowing reduction of the quinone A (QA), quinone B (QB) and part of plastoquinone (PQ) pools.

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

The samples were exposed to 10 irradiances (E) from 0 to $1000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ at 474 nm for 20 seconds at each step. Steady state fluorescence (F_s) and maximum fluorescence

(F_m') 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, $\mu\text{mol electrons m}^{-2} \text{ s}^{-1}$) 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.

$$ETR(E) = AQ_\lambda \times FII \times \frac{\Delta F}{F_m'}$$

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].

I.4.2. *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 cm² 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 of 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 $\mu\text{mol photon. s}^{-1} \text{ m}^{-2}$) 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 O₂ 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, σ_{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.

I.4.3. 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:

$$X(PAR) = X_{max} \times e^{(1 - \frac{PAR \times \alpha}{X_{max}})}$$

where X (PAR) is GPP (PAR), ETR (PAR) or GO_{PII} (PAR). X_{max} is the maximum photosynthetic capacity measured with the O₂ incubation method (GPP_{max} in mmol O₂ m⁻² h⁻¹), the PAM method (ETR_{max} in μ mol electrons m⁻² s⁻¹) or with LabSTAF (GO_{PII}_{max}, mmol O₂ m⁻² h⁻¹), α is the photosynthetic efficiency which corresponds to the slope of each Production / Light curve.

II. 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 reproducible

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 α . This preliminary result showed a higher maximal production rate (GPPmax = 14.18 ± 2.19 mmol O₂ m⁻² h⁻¹) and a higher α (0.12 ± 0.03 mmol O₂ 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 O₂ m⁻² h⁻¹; $\alpha = 0.06 \pm 0.008$ mmol O₂ 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 GPPmax per weight of 34.96 ± 6.04 μmol O₂ g⁻¹ h⁻¹ and α 0.32 ± 0.09 for eco-engineering modules and GPPmax per weight of 34.63 ± 2.44 μmol O₂ g⁻¹ h⁻¹ and α 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.

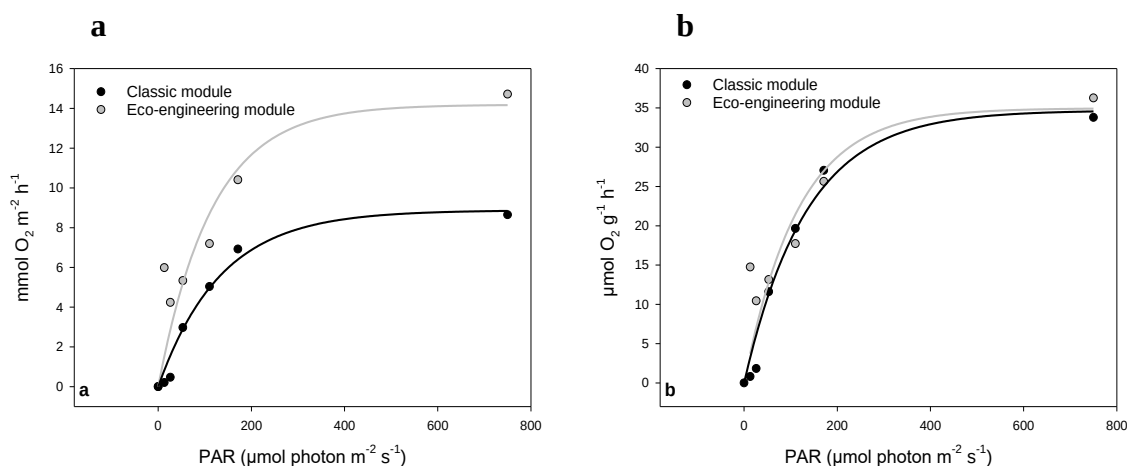


Figure 5. Example of Production / Light curves measured on MARINEFF eco-engineering dyke module and classic dyke module in the Bay of Cherbourg in April 2021. Dots show the GPP measurements at each light intensity (PAR) and the lines show the Webb model fits. a: GPP expressed as a function of substrate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

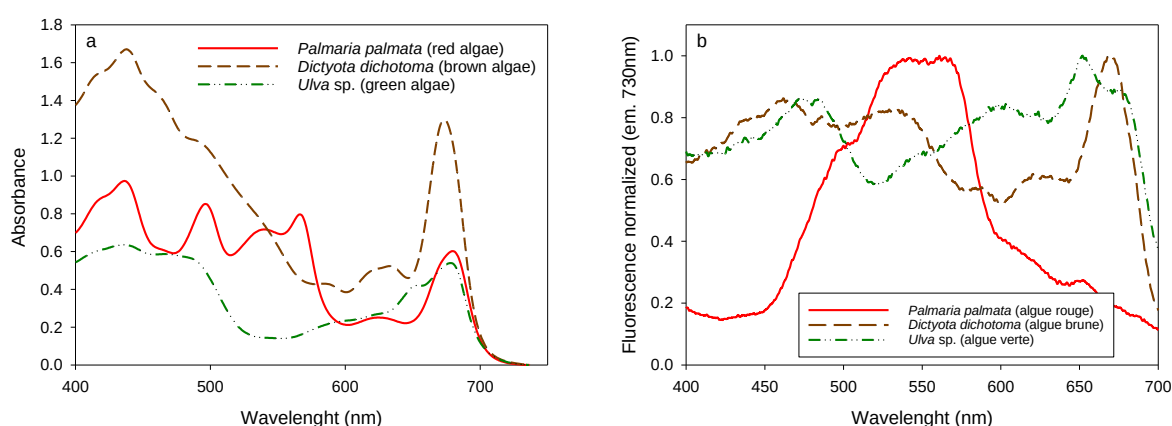


Figure. 6. a: Example of spectral absorbance (A_λ) between 400-700 nm and b: Example of fluorescence excitation spectra between 400-700 nm with an emission measured at 730 nm performed on Chlorophyta (green algae), Rhodophyta (red algae) and Phaeophyceae (brown algae) from the Bay of Cherbourg

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 cm² of biofilm were resuspended and analysed using a LabSTAF (Figure 7b). Contrary to macroalgae, the biofilm of classic modules was more productive. The GOIImax, which correspond to GPP max of macroalgae, was 5.89 ± 0.65 mmol O₂ m⁻² h⁻¹ for classic module and 1.79 ± 1.07 mmol O₂ 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 the absorption cross-section of the PSII was also different, which indicated variation of photoacclimation 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.

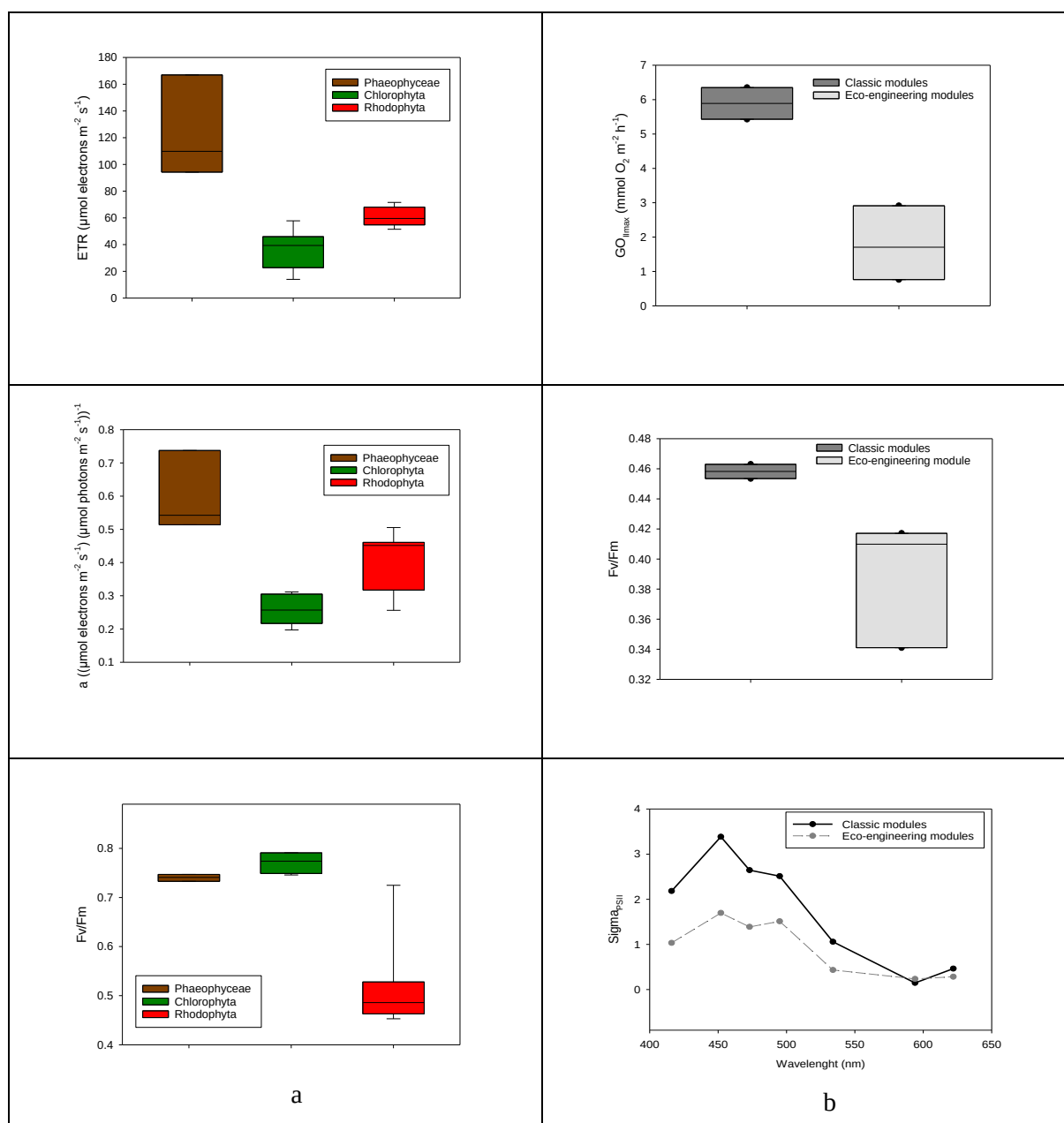


Figure 7. a: ETR, α and Fv/Fm measured under water by using Diving PAMII on the dykes modules on Chlorophyta (green algae), Rhodophyta (red algae) and Phaeophyceae (brown algae). b: GOIImax , Fv/Fm, and $\text{Sigma}_{\text{PSII}}$ measured on microalgal biofilm by using LabSTAF. Measurements were performed in the Bay of Cherbourg in April 2021

III. 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 than 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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Annexe I : Bastien Taormina, Pascal Claquin, Baptiste Vivier, Maxine Navon, Jean-Philippe Pezy, Aurore Raoux and Jean-Claude Dauvin, 2022. A review of methods and indicators used to describe epibenthic communities associated with artificial structures in the marine environment. Marineff: from materials and infrastructures to marine ecosystems: interactions and new approaches.

A review of methods and indicators used to describe epibenthic communities associated with artificial structures in the marine environment

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Abstract

The current development of human activities at sea (*e.g.* land reclamation, maritime activity, marine renewable energy) is leading to a significant increase in the number of infrastructures installed in our ocean. These infrastructures provide new hard-bottom habitats for many marine organisms and can thus modify the structure and functioning of coastal ecosystems. In order to better evaluate the nature of these modifications as well as the potential benefits and/or harms generated, it becomes essential to develop assessment methods that can be applied to a wide variety of study sites from harbours to coastal offshore environments. In this context, the aim of our study is to review the available different methods and indicators available and used in the literature to describe the epibenthic communities associated with artificial structures. Overall, this review highlights an important lack of standardised tools aiming to evaluate ecological quality of epibenthic communities associated with artificial structures. Creation of such a tool will allow stakeholder to identify easily and robustly the performance of eco-engineered structures in order to enhance future artificial structure deployments.

I. Introduction

The current development of human activities at sea (*e.g.* land reclamation, maritime activity, marine renewable energy) is leading to a significant increase in the number of infrastructures installed in our ocean. When submerged in seawater, these artificial structures are invariably colonised by a variety of epibenthic organisms, forming the so-called “biofouling”. This colonisation of a bare substratum by an epibenthic community follows an ecological succession, *i.e.* a sequence of stepwise changes in community composition until it eventually reaches a

persistent stage, called climax [1,2]. In marine environment, the succession begins with the early establishment of a biofilm, allowing afterward the development of pluricellular eukaryote colonisers. Firstly, these eukaryote taxa are mainly constituted of opportunistic species, then gradually replaced by longer-lived morphologically complex species as the colonising community evolves towards its climax [2]. A wide variety of abiotic and biotic factors affects the epibenthic community composition, the characteristics of the ecological succession and time needed to reach a climax state [3]. Biotic factors include facilitation, competition, and trophic interactions between organisms [2]. On the other hand, abiotic factors can be split between local environmental conditions (e.g. temperature, light, pH, salinity, current; [3–5]) and artificial substratum properties (e.g. surface type, material, texture, slope [3]).

Epibenthic communities are highly diverse and usually dominated by marine invertebrates (e.g. Arthropoda, Mollusca, Tunicata, Bryozoa, Annelida, Porifera), and macroalgae (e.g. Chlorophyta, Rhodophyta, and Ochrophyta). These organisms can be divided in two categories: i) sessile taxa which are directly attached to the substratum, and ii) mobile taxa grasping to aforesaid sessile taxa (also called clinging taxa). When artificial structures are installed on soft sediment areas, the development of such community involves the apparition of species previously absent, leading to an overall increase in local diversity [6]. Also, certain epibenthic organisms can create complex tri-dimensional biogenic structures (e.g. kelps, corals) that further increase habitat heterogeneity [7]. Conversely, in comparison with natural hard substrata, epibenthic communities of artificial structures display usually a more important density of non-indigenous species (NIS)[8,9]. For example, artificial structures in the Adriatic sea were shown to display three times more NIS ascidians than natural reefs [8]. Thereby, artificial structures may potentially facilitate the spread of these NIS by creating new connectivity routes via a stepping-stone process [9–11].

While epibenthic communities associated with artificial structures have been widely studied, it currently exists a lack of standardised tool aiming to evaluate their ecological quality. In order to better evaluate the potential benefits and/or harms generated by artificial structures, it becomes essential to develop such tool that can be applied to a wide variety of study sites from harbours to coastal offshore environments. In this context, the aim of our study is 1) to review the available different methods and indicators available in the literature aiming to describe macro-epibenthic communities and 2) to draw up a list of recommendations for suitable ecological indicators.

II. Methods

A literature search was conducted using online databases and internet search tools (*i.e.* Web of Science, Science Direct, Google Scholar, ResearchGate) to create a bibliographic database including peer-reviewed scientific publications, books and PhD thesis. The literature search first focused on publications about generalities on colonisation of artificial structures before targeting specific literature on epibenthic compartment. In this study, we only focused on macro-epibenthic community, *i.e.* part of the epibenthic community whose organisms are larger than 1cm, thus biofilm was not considered here. Giving the lack of available indicators and indices especially developed for artificial structures, documents focussing on natural hard substratum were also considered.

III. Description of epibenthic community

III.1. *Biological structure*

Majority of works aiming to study epibenthic communities associated with artificial structures focus exclusively on their biological structure, *i.e.* these focused on community properties in terms of composition of taxonomic entities [12–14]. Consequently, data used in these studies are commonly a description of the epibenthic community through an inventory of taxa recorded, with associated quantitative information (e.g. number of individuals, density, biomass or coverage [14]). Such inventory are performed with different methods: some are non-destructive like visual-census *in situ* or using underwater imagery, others are destructive with sampling of the epibenthic community and analysis in laboratory [13]. Based on data obtained, number of univariate indices can be calculated in order to inform community characteristics in terms of diversity and evenness (e.g. species or taxonomic richness, Shannon index H' , Pielou index J' etc.). Also, standard similarity indices (e.g. Bray-Curtis or Jaccard similarity indices) and multivariate community analyses [15] are commonly used to evaluate the similarity between different samples and communities, for example to compare epibenthic community of artificial and natural substrata [13].

III.2. *Functional structure*

Instead of focusing on biological structure, a complementary novel approach consists to study the functional structure of communities. Functional structure relies on the individual properties of organisms, also called traits (e.g. morphology, behaviour, feeding strategy), rather than on their taxonomic identity. In the last decade, biological trait analysis and functional

diversity indices (e.g. functional richness, functional divergence, functional evenness and RAO's quadratic entropy) have been exhaustively used to understand response of benthic communities to environmental gradients [16–19]. Nevertheless, these approaches have been little used for epibenthic communities of artificial substrates [20,21] and more broadly of natural hard substratum [22]. Given the growing interest to biological traits approach in marine ecology [17], we suggest that such method must be considered for ecological indicators development aiming to evaluate the ecological value of such epibenthic communities associated with artificial structures

IV. Multimetric indicators

A fashionable approach to inform community characteristics, and more specifically their quality status, is the use of ecological multimetric indices which aim to aggregate diffuse biological data into a single value [23]. Such indices are usually developed in order to be simple to derive, widely applicable and easy for the public to understand [24]. To our knowledge, there is currently no such ecological indices specifically developed to inform ecological quality of epibenthic communities associated with artificial structures. More broadly, even when considering natural habitats, it appears that benthic communities associated with hard substratum suffer from a lack of available ecological tools in contrast with those of soft sediments. Indeed, a wide diversity of methodologies has been developed and intercalibrated for soft-sediment macroinvertebrates communities (e.g. AMBI by Borja et al. [25], BENTIX by Simboura and Zenetos [26], BQI by Rosenberg et al. [27]; BO2A by Dauvin and Ruellet [28]), mainly under the instigation of the European Water Framework Directive in the early 2000s. This disparity between soft and hard substrata can be partly explained by the differences of sampling methods used to describe benthic communities. Indeed, the use of grabs for soft-sediments is much easier to implement across a broad depth range compared to costly methods implying dive work or underwater imagery which are commonly used for hard substratum. Nevertheless, some ecological multimetric indices aiming to describe epibenthic communities of natural hard substratum were still developed in the last decade, their main characteristics are summarised in table 1.

IV.1. *Macroalgae and macrofauna*

Most of reviewed multimetric indices are exclusively based on macroalgae compartment (Table 1): CFR (Quality of Rocky Bottoms index in Spanish) by Juanes et al. [29], MarMAT (Marine Macroalgae Assessment Tool) by Neto et al. [30], CCO (Cover Characteristic

Opportunistic species) by Ar Gall et al. [31], EEI-c (Ecological Evaluation Index continuous formula) by Orfanidis et al. [32] and QiSubMac (Quality Index of Subtidal Macroalgae) by Ar Gall and Derrien-Courtél [33]. To our knowledge, only one index has been developed exclusively for fauna: RMA (Rocky shore Macroinvertebrates Assessment Tool) by Vinagre et al. [34]. This discrepancy can be partly explained because macroalgae are usually dominant in clear shallow water and are considered as good indicators because of their ability to integrate various environmental pressures [35–37]. Nevertheless, as spatial competition, facilitation and trophic interactions commonly occur between fauna and flora on hard substrata [33,38], the use of indicators able to take into account both faunal and floral composition jointly seems more appropriate [39]. In these circumstances, Díez et al. [38] with RICQI (Rocky Intertidal Community Quality Index), like Pinedo and Ballesteros [40] with EGs-I (Indicators of Ecological Groups), developed indicators that took into account not only the macroalgae, but also macrofauna. While Pinedo and Ballesteros [40] showed very similar results when they compared their index with and without fauna, they anyway encourage to consider the simultaneous use of both groups for aforesaid reasons.

IV.2. *Biological metrics*

All the ecological indices reviewed here, whether based on macroalgae or macrofauna, focus exclusively, or in part, on the biological structure of communities. Indeed, some metrics used are indices widely used in community studies, such as species richness (for CFR, MarMAT, CCO, QiSubMac, RICQI; Table 1) or Hurlbert diversity index (for RMA; Table 1). Some of these indicators also relies on the relative occurrence of different ecological groups which are based on the taxa abilities to tolerate environmental pressures (i.e. RMA, EGs-I; Table 1). To do so, taxa are divided into up to four groups according to their tolerance to a stress: 1) sensitive taxa, 2) stress-tolerant taxa, 3) indifferent taxa and 4) opportunistic taxa [40]. Such indicators are actually adapted from previous indicators developed for soft-sediment communities, RMA is based on the BENTIX indicator from Simboura and Zenetos [26]; while EGs-I is an adaptation of the MEDDOC index from Pinedo et al. [41]. While such indicators were proved to be efficient in order to assess ecological status of water body, these are mainly based on taxa tolerance to a particular pressure: organic matter enrichment [42]. As the classification of taxa within different ecological groups is highly pressure-specific, it is important to remind that such indices should not be used unquestioningly in any case-study. Most of the other multimetric indices (i.e. CFR; MarMAT; CCO; EEI-c; QiSubMac; Table 1) rely on another ratio between only two ecological groups: characteristic taxa (i.e. taxa linked to

a particular biotic assemblage; [43]) and opportunistic taxa (i.e. taxa that can quickly exploit new resources or new ecological niches as they become available [43]). Conversely to aforesaid ecological groups which were based on the taxa abilities to tolerate environmental pressures, characteristic and opportunistic taxa are mainly defined by their growth strategies: characteristics being perennial species, and opportunistic being annual species [44]. The popularity of this ratio is essentially due to its versality. Indeed, this ratio is based on the assumption that, as any environmental pressure increases, density of the characteristic species will decrease, while the density of opportunistic species will increase [45]. In the context of artificial structures, metrics based on this ratio can be of particular interest as it can constitute a proxy of the ecological succession stage [2].

IV.3. *Functional metrics*

By focusing exclusively on biological structure, most of the indicators reviewed here tended to neglect the functional structure of epibenthic communities. Indeed, none of the indicators reviewed here are relying on extensive biological trait analysis or functional diversity indices. Instead, some of these indicators focused their attention on particular traits. Thus, EEI-c, QiSubMac and RICQI indices pay a particular attention to the morphology of macroalgae with various metrics: the relative cover of different macroalgae morphology groups (EEI-c) and cover (RICQI) or density (QiSubMac) of structuring macroalgae. Structuring macroalgae are of particular interest as these display important ecosystem functions (e.g. habitat, nursery or feeding functions [46–49]). Only RICQI focuses on another trait: the feeding strategy of benthic fauna by using cover of herbivores and suspension-feeders as metrics.

IV.4. *Non-indigenous species*

Among indices reviewed, one stand out from the crowd because it was developed to detect impact of NIS: the Alien biotic indEX (ALEX; [50]). This index rely on the relative abundance of four different biogeographic groups defined on the basis of species invasiveness: 1) native species (i.e. species naturally occurring in the region), 2) casual NIS (i.e. NIS with only one report in the region), 3) established NIS (i.e. NIS with self-maintaining population) and 4) NIS (i.e. NIS that have overcome biotic and abiotic barriers and are able to expand their geographic range with significant impact on the invaded habitats). While initially developed for soft-bottom macrofauna, ALEX has been tested on epifauna community growing on artificial structures associated with an Italian Mediterranean harbour [51]. In their study, Tempesti et al. [51] highlighted that despite the high number of NIS present within the port, ALEX values were

quite low and identified the port as having high or good environmental status. They explained this result by the fact that ALEX was designed for the Eastern Mediterranean Sea, which hosts highly degraded assemblages with higher cover of NIS. Consequently, despite that biogeographic groups chosen for ALEX seem appropriate; the index calculation may need recalibration in function of the considered bioregion.

V. Recommendation

It exists an important need to develop an ecological multimetric index aiming specifically to assess the ecological quality of epibenthic communities associated with artificial structures. Such tool should be able to allow stakeholder to identify easily and robustly the performance of eco-engineered structures in order to enhance future artificial structure deployments. Multimetric indices described above for epibenthic communities of natural substratum should lay the foundations for such a tool. More specifically, we highlight that in addition to classical community analyses focusing on biological structure (*i.e.* diversity, evenness and similarity of communities) complementary approaches focusing on biological traits should be employed. Also, it appears that the use of a ratio between the abundance of opportunistic and characteristics species (like CFR; MarMAT; CCO; EEI-c; QiSubMac) is particularly adapted to the case of artificial substrata as it constitutes a proxy of the ecological succession stage. Finally, considering the important concerns about the potential ability of artificial structures to facilitate the spread of NIS, a focus on taxa invasiveness (like ALEX) should be a priority.

To summarise, such a multimetric index should be composed of a set of indicators that must be able: 1) to consider both flora and fauna simultaneously, 2) to summarise the structural and functional diversity of the epibenthic community; 3) to estimate the stage of the ecological succession, 4) the ability of the artificial structure to harbour key-stone specie and, 5) and its ability to prevent the establishment of non-indigenous species.

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Table 1. Characteristics of nine multimetric indices aiming to evaluate the ecological quality of epibenthic communities of natural hard substrata. Crosses (X) indicate that at least one metric of the index (column) is related to the associated category (row).

Index	CFR	MarMAT	CCO	EEL-c	QISubMac	Egs-I	RICQI	ALEX	RMAT
Authors	Guinda et al. 2014 / Juanes et al. 2008	Neto et al. 2012	Ar Gal et al. 2016	Orfanidis et al. 2011	Ar Gal et Derrien- Courtet 2015	Pinedo et al. 2019	Diez et al. 2012	Piazzi et al. 2015 / Tempesti et al. 2020	Vinagre et al. 2017
Target compartment	Macroalgae	Macroalgae	Macroalgae	Macroalgae	Macroalgae	Macroalgae+Fauna	Macroalgae+Fauna	Macroalgae+Fauna	Macrofauna
Area	Antlantic	Antlantic	Antlantic	Mediterranean Sea	Antlantic	Mediterranean Sea	Antlantic	Mediterranean Sea	Antlantic
Zone	Intertidal/Subtidal	Intertidal	Intertidal	Subtidal	Subtidal	Subtidal	Intertidal	Subtidal	Intertidal
Data	Coverage	Coverage	Coverage	Coverage	Density	Coverage	Coverage	Coverage	Density/Biomass
Number of metrics	3	7	3	5	14	4	8	4	4
Biological structure	X	X	X	X	X	X	X	X	X
Functional structure				X	X		X		
Non-indigenous taxa								X	
Structuring taxa					X		X		
Physiological status					X				
Ecological group									
Characteristic taxa	X	X	X	X	X				X
Opportunistic taxa	X	X	X	X	X	X			X
Sensitive taxa					X	X	X		X
Tolerant taxa						X			X
Indifferent taxa						X			

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Rôle de la colonisation d'infrastructures marines sur la production et la biodiversité des écosystèmes côtiers en Manche

Résumé

L'accroissement des pressions anthropiques sur les écosystèmes marins et côtiers, en particulier en Manche, provoquent des modifications des habitats, des écosystèmes et des différentes fonctions écosystémiques associées. La construction d'ouvrages maritimes et côtiers tels que les digues ou des ouvrages portuaires ainsi que l'émergence de plusieurs projets d'implantation d'éoliennes off-shore est en augmentation constante sur les côtes de la Manche française. Avec ces différents projets, la bétonisation de certains habitats devient inévitable, il est donc primordial d'évaluer leurs impacts écosystémiques, et, si possible, de les limiter. Le projet MARINEFF est né dans ce contexte, il vise à concilier développement humain et bénéfice écologique. Le développement de ces nombreuses infrastructures côtières crée de nouveaux habitats. L'objectif de ces travaux a été d'évaluer les processus de colonisation biologique à différentes échelles ainsi que certaines fonctions écologiques comme la production primaire sur différents types d'infrastructures marines en conditions contrôlées et en situation *in situ* dans deux sites La Rade de Cherbourg et la baie de Seine. Les mesures de fluorescence modulée (PAM) réalisées sur les différents compartiments de producteurs primaires benthiques ont permis d'évaluer la fonction de production primaire et son évolution en fonction des forçages testés ou observés. Une part importante de ce travail s'est concentrée sur l'étude du microphytobenthos de substrat dur, sa croissance, sa photobiologie en relation avec le type de substrat colonisé, sa structure de surface et l'impact de différents forçages sur ces paramètres. A une plus grande échelle, le développement des communautés benthiques a également été caractérisé sur les deux sites et sur différentes infrastructures. Des mesures de production primaire *in situ* associées à des mesures de biodiversité et de PAM ont permis de décrire les processus de colonisation d'infrastructures artificielles et d'évaluer leur efficacité. Ces travaux apportent une nouvelle vision à l'écologie benthique associée aux substrats durs artificiels et apportent des estimations fiables de l'impact de ces structures sur l'écosystème.

Mots clés : Infrastructures marines ; production primaire ; biodiversité ; communautés benthiques ; photosynthèse ; microphytobenthos

Marine infrastructure colonisation impact on the production and biodiversity of the English channel coastal ecosystems

Abstract

Increasing anthropogenic pressures on marine and coastal ecosystems, particularly in the English Channel, are causing changes in habitats, ecosystems and the various associated ecosystem functions. The construction of marine and coastal structures such as dikes as well as the emergence of several projects to install offshore wind turbines is constantly increasing on the French coasts of the English Channel. With these different projects, the concretization of certain habitats becomes inevitable, so it is essential to assess their ecosystem impacts, and, if possible, to limit them. The MARINEFF project was born in this context, it aims to reconcile human development and ecological benefit. The development of these many coastal infrastructures can also make it possible to create new habitats. The objective of this PhD is to assess biological colonization processes at different scales as well as certain ecological functions such as the primary production on different types of marine infrastructures under controlled conditions and *in situ* situations on two site the Rade of Cherbourg and the Bay of Seine. Modulated fluorescence (PAM) measurements carried out on the different compartments of benthic primary producers made it possible to evaluate the primary production function and its evolution according to the forcing's tested or observed. An important part of this PhD focused on the study of hard substrate microphytobenthos, its growth, its photobiology in relation to the type of colonized substrate, its surface structure and the impact of different forcing's on these parameters. On a larger scale, the development of benthic communities has also been characterized for both sites and on different infrastructures. *In situ* primary production measurements combined with biodiversity and PAM assessment have made it possible to describe the colonization processes of artificial infrastructures and to assess their effectiveness. This work brings a new vision to the benthic ecology associated with artificial hard substrates and makes it possible to provide reliable estimates of the impact of these structures on the ecosystem.

Keywords : Marine infrastructures; primary production; biodiversity; benthic communities; photosynthesis; microphytobenthos