Effets combinés du rayonnement ultraviolet et du réchauffement climatique sur les coraux Scléractiniaires

Lucile Courtial

▶ **To cite this version:**

Lucile Courtial. Effets combinés du rayonnement ultraviolet et du réchauffement climatique sur les coraux Scléractiniaires. Milieux et Changements globaux. Université Pierre et Marie Curie - Paris VI, 2017. Français. NNT : 2017PA066214 . tel-02301986v2

HAL Id: tel-02301986

https://theses.hal.science/tel-02301986v2

Submitted on 1 Oct 2019

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

UNIVERSITÉ PIERRE ET MARIE CURIE

Effets combinés du rayonnement ultraviolet et du réchauffement climatique sur les coraux Scléractiniaires

Lucile COURTIAL

THÈSE DE L'UNIVERSITÉ PIERRE ET MARIE CURIE

École doctorale des Sciences de l'Environnement

Pour l'obtention du grade de Docteur en des Sciences de l'Environnement

Soutenue publiquement le 29 Septembre 2017

Réalisée au Centre Scientifique de Monaco et à l'IRD Nouméa

Publiée par le
CENTRE SCIENTIFIQUE DE MONACO

ETABLISSEMENT PUBLIC FONDE LE 23 MAI 1960
PAR S.A.S. LE PRINCE RAINIER III

Université Pierre et Marie Curie

Ecole doctorale des Sciences de l'Environnement

Centre Scientifique de Monaco / IRD de Nouméa

Effets combinés du rayonnement ultraviolet et du réchauffement climatique sur les coraux Scléractiniaires

par **Lucile COURTIAL**

Thèse de doctorat des Sciences de l'Environnement

Directrice de recherche : Christine Ferrier-Pagès

Co-encadrant : Fanny Houlbrèque

Présentée et soutenue publiquement le 29 Septembre 2017 à Monaco

Devant un jury composé de :

Pr. Maoz Fine	Rapporteur
Pr. Michael Kühl	Rapporteur
Pr. Paola Furla	Examinateur
Dr. Jean-Pierre Gattuso	Examinateur
Pr. Oren Levy	Examinateur
Dr. Christine Ferrier-Pagès	Directrice de thèse
Dr. Fanny Houlbrèque	Co-encadrante

À mon baboune, ma moune et mon Tatane

Et à toi qui veilles sur moi depuis là-haut…

Remerciements

Et voilà, trois ans de travail et quelques heures de rédaction qui s'achèvent. Bien sûr, cette thèse n'aurait pas pu se faire sans l'aide et le soutien d'un grand nombre de personnes, au travail mais aussi en dehors. Par ces quelques mots, je souhaite vous remercier pour ce que vous avez fait pour moi, en espérant oublier personne.

Je commencerai par remercier l'administration du Centre Scientifique de Monaco et de l'IRD de Nouméa qui m'ont permis de réaliser ma thèse dans leurs laboratoires. Un merci particulier au Professeur Denis Allemand, Directeur Scientifique du CSM, conseiller d'orientation professionnelle mais aussi de randonnées à ses heures perdues ! Merci à tous les membres du CSM et de l'IRD de Nouméa avec qui j'ai interagi pendant ces trois années. La liste est longue mais je pense plus particulièrement à tous ceux qui m'ont aidé dans mes quêtes pour trouver le matériel pour mes manips et à ceux qui m'ont filé un coup de main quand j'en avais besoin. Merci aux aquariologistes du CSM et de l'aquarium des lagons, toujours là pour aider et toujours avec le sourire ! Leur bonne humeur m'a souvent fait du bien dans les moments de galère…

Merci à tous ceux avec qui j'ai directement travaillé à Monaco et à l'autre bout du monde. Un merci particulier à Alex, soutien moral, artistique et technique indispensable ; un exemple de son talent s'illustre par les magnifiques pages de présentation des chapitres de ma thèse ! Merci aux équipes d'Écophysiologie et d'Entropie pour les conseils mais aussi les aventures, dans les labos (hein Cécile !), en mission (Steph, on se souviendra de cette pu**** de température des bacs…) et en congrès (les jurassic parkiens se reconnaîtront !). Merci à la team Biomed de m'avoir accueillie dans leur labo et à Poupinou pour sa patience avec les western blots. Merci Vincent pour les discussions officielles (ou non) sur nos MAPK, pour les apéros clandestins et les balades en mer. On remet ça quand tu veux ! Merci aux copains thésards, postdocs, et étudiants qui ont partagé un bout de chemin avec moi : Leila, Carine, Nath, Jeroen, Vanessa, Virginie, Claire, Tom, Lucas, Flo, Alex, Julie, Germain, Laura, Solène, Raph, Camille, Lucie, Charly… Vous avez tous contribué à faire de ces trois années un vrai bonheur.

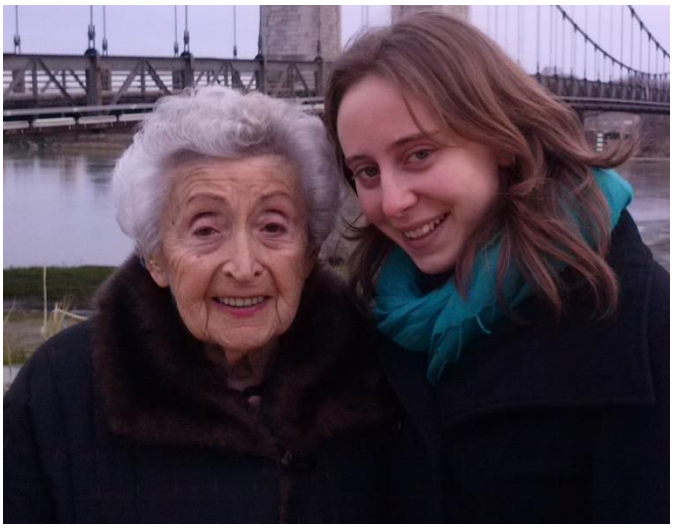
Une pensée aussi pour mes potes d'aventure : Ja, merci, merci, merci pour la découverte de l'escalade et de la rando. Nos périples entre la Norvège, la Calédo et le Mercantour resteront gravés. Leïla, je n'oublierai pas de sitôt nos péripéties en Norvège, à Hawaii et en sortie rando/grimpe. Je te souhaite de trouver ton bonheur où que tu ailles ! Lucas, une merveilleuse rencontre qui m'a menée au fin fond de la Calédo, sur l'eau et sous l'eau. J'attends avec impatience nos retrouvailles avec les calamars ! Merci à mes confidentes et compagnes de camping Flo et Julie avec qui j'ai aussi usé beaucoup de mèches de dremel ! Je n'oublie pas non plus les grimpeurs du CAM qui ont animé presque tous mes weekends cette dernière année. Un clin d'œil plus spécial à Bibi, Dams, Mika, Jean-No, Tom et Alex ; une équipe de choc toujours partante pour de nouvelles découvertes. Je vous aime les copains !

Parce qu'une journée de travail c'est dur, il est indispensable de se détendre. Pour ça, vous pouvez toujours compter sur la joie et la bonne humeur des musicos : Jeff, Vincent, Renaud, Victor et Ben ! Et parce que c'est trop bon, on en redemande encore et toujours! Merci les gars pour ces heures de rigolade, j'espère pouvoir vous casser les oreilles encore un moment !

Merci aussi aux joueurs qui ont partagé mes vendredi soirs : Alex, Carlos, Grover et Nath. Je vous pardonne de m'avoir infligée les heures de lecture des règles de jeu et j'espère pouvoir participer encore longtemps à nos parties jusqu'au bout de la nuit !

Il me reste à remercier les deux personnes qui se sont le plus investies dans la thèse et qui m'ont le plus appris : Christine et Fanny. Christine, comment résumer 3 années en quelques mots ? Tu as toujours été là pour moi tout en me laissant la liberté de faire les choses à ma manière. Tu as su me faire confiance et pour ça, je ne te remercierai jamais assez. Merci aussi pour les innombrables heures que tu as passées à t'arracher les cheveux sur mes écrits, désolée pour tes vacances et weekends, j'ai pourtant vraiment essayé de les épargner ! Fanny, merci de m'avoir si bien accueillie en Calédo, avec un petit tour en bateau pour mon premier weekend. Merci pour les heures passées à chercher des solutions aux problèmes de température et de matériel et à relire les papiers et la thèse. Mon séjour en Calédo n'aurait pas été le même sans ton aide.

Enfin, un inconditionnel merci à ma famille (les tribus Courtial/Eyraud) et mes amis de longue date (Kiki, Méla et Lo). Vous êtes mes racines ; une partie de chacun d'entre vous se trouve dans cette thèse parce que c'est avec vous que je me suis et que je continue à me construire.



Sommaire | Table of contents

Remerciements | Acknowledgments 1

Sommaire | Table of contents 4

Liste des figures | Figure list 8

Liste des tableaux | Table list 10

Liste des encarts | Box list 11

Liste des publications et communications orales | Publications and oral presentations 12

Liste des abréviations majeurs | List of the main abbreviations 13

Chapitre 1 | Introduction 17

I. Les récifs coralliens, un écosystème basé sur la symbiose corallienne 17
I.1. Les récifs coralliens : formation, répartition et importance 17
I. 2. Les coraux Scléractiniaires, des animaux symbiotiques à l'origine des récifs coralliens 20
I. 2. 1. Taxonomie des Cnidaires 20
I. 2. 2. Le corail, anatomie et histologie 22
I. 2. 3. Le corail, un animal en symbiose avec les *Symbiodinium* 23
La symbiose corallienne 23
Les *Symbiodinium*, taxonomie et caractéristiques biologiques 24
Physiologie de la symbiose 25
Bénéfices, coûts et rupture de la symbiose 26
I. 2. 4. Le corail, un holobionte 27
I.3. Le corail, son rôle dans le récif : protection et nutrition 28
I. 3. 1. Le corail, un refuge pour les autres organismes du récif 28
I. 3. 2. Le corail, une source de nutrition 30
Synthèse du mucus, quoi, comment, pourquoi ? 31
L'excrétion de matière dépend de l'espèce et des conditions environnementales 31
Le devenir de la matière organique, son importance pour le récif 32
II. Les coraux, des organismes dans un environnement changeant 32
II.1. Conditions environnementales du récif et variabilité naturelle 33
II. 1. 1. La lumière, définition et conditions sur les récifs 33
II. 1. 2. La température, conditions et variabilité 35
II. 1. 3. Les sels nutritifs 36
II.2. Les effets des paramètres environnementaux sur les coraux 37
II. 2. 1. La lumière 37
Les effets directs des UVs 38
Les effets indirects des UVs : le stress oxydant 39
Les mécanismes de défense 40
II. 2. 2. La température 45
Effets sur la physiologie du corail et mécanismes de défense 45
Conséquences sur les flux de matière organique et de nutriments 46
II. 2. 3. Effets de la disponibilité en sels nutritifs 46
Conséquences d'un manque de sels nutritifs 46
Conséquences d'un excès de sels nutritifs 47
III. Devenir des récifs coralliens, prévision, incertitudes et enjeux 49
III.1. Les récifs coralliens, un écosystème en danger 49

III. 1. 1. Le changement climatique : définition et conséquences au niveau des zones récifales 49
III. 1. 2. Les enjeux de la situation actuelle 55
III.2. Les difficultés des prédictions : les enjeux de la thèse 56
III. 2. 1. Diversité des paramètres biologiques 56
III. 2. 2. La variabilité inter-espèces 57
III. 2. 3. Approche multi-paramètres 58
III. 2. 4. Acclimatation : le rôle des conditions de croissance 59
III. 2. 5. Prise en compte des multi-échelles 60
III. 2. 6. Le déroulement de la thèse 60

Chapter 2 | Materials and methods 67

I. Experimental settings: two different but complementary approaches in Monaco and in New Caledonia 67
I.1. Experimental set up in Monaco 67
I. 1. 1. UVR 67
The choice of UV lamps: the right quality and quantity of UVR 67
Limits of the experimental design: UV dose, UV intensity and duration of the exposure 69
Ecological relevance of the experimental setting 70
I. 1. 2. Thermal stress and nutrients levels 70
I. 2. Experimental set up in New Caledonia 71
I. 2. 1. UVR 71
Light quality and quantity 71
Ecological relevance of the experimental setting 74
I. 2. 2. Temperature: natural variability and thermal stress 74
II. Physiological and organic matter fluxes study 75
II.1. Tissue parameters (symbiont, protein and pigment density) 76
II. 2. Coral metabolism (Photosynthesis, respiration, calcification and coral growth) 77
II. 3. Organic matter fluxes 77
II. 4. Reactive oxygen species concentration 77
III. Micro-organisms associated with the mucus and coral tissue 78
III.1. Population identification 78
III.2. Growth and enzymatic activity 79
IV. Molecular approach: signalling pathways 80
IV.1. Aims and difficulties 80
IV.2. Description of the new steps of the experimental design 80
IV. 2. 1. Sample preparation 80
IV. 2. 2. Antibody validation 81
Paper n° 1 – Validation of commercial ERK antibodies against the ERK orthologue of the scleractinian coral *Stylophora pistillata* 82

Chapter 3 | Effects of UVR, temperature and nutrient condition on coral physiology - Summary of the results 99

I. UVR and global warming 99
I. 1. Coral response to a UVR stress is species specific 99
I. 2. Coral response to UVR stress depends on the nutritional status 101
I. 3. Effect of natural UVR exposure on the physiology of acclimated corals and seasonal variability 102
I. 4. UVR worsens the thermal stress effects 103
I.4. 1. Effect of thermal stress 103

I.4. 2. UVR and thermal stress 103
II. JNK signalling pathway and oxidative stress defenses 104

Chapter 4 | Combined effects of UVR, temperature, seasonality and nutrient level on mucus production and coral microbiome 109

I. Organic matter fluxes, UVR, global warming, nutrient level and seasonal variability 109
II. Mucus associated bacterial activities: effect of UVR, global warming, nutrient level and seasonality 110
II. 1. Effects of UVR 110
II. 2. Effects of high temperature 110
II. 3. Matter degradation 110
III. Coral and mucus associated bacteria: effect of seasonality and UVR exposure 111
III. 1. Mucus associated microbiome 111
III. 2. Coral associated microbiome 113

Chapter 5 | General discussion and perspectives 119

I. UVR and corals 119
I. 1. Changes in coral physiology and in coral-associated microbes between corals shaded from UVR and those exposed to natural UVR 119
I. 2. Coral response to an increased UVR level: is it species-specific? Is there an impact on mucus release and degradation? 120
I. 3. Effects of UVR on coral response to global warming, eutrophication and nutrient depletion 121
I. 4. Molecular pathways involved in the coral response to increased UVR 123
II. Temperature effects on corals 123
II. 1. Temperature, the main factor influencing coral physiology 124
II. 2. Temperature variation and changes in the organic matter 125
II. 3. Temperature, the main factor influencing coral associated bacteria? 126
III. Conclusions and perspectives 126
III. 1. Take home messages 126
III. 2. What is next? 127

Paper n° 2 – Interactive effects of ultraviolet radiation and thermal stress on two reef-building corals 131
Paper n° 3 – Effects of temperature and UVR on organic matter fluxes and the metabolic activity of *Acropora muricata* 151
Paper n° 4 - Seasonal variability in the physiology and composition of the coral *Acropora muricata* holobiont under different ultra-violet radiation levels 167
Paper n° 5 Effects of ultraviolet radiation and nutrient levels on the physiological response and mucus production of *Pocillopora damicornis* following thermal stress 203
Paper n° 6 – The c-Jun N-terminal kinase prevents oxidative stress induced by UV and thermal stresses in corals and human cells 237

References and Appendix 253

I. References 255
II. Appendix 277
Appendix 1. Translation of Introduction §III.2. 277
Appendix 2. English version of the popularization paper L. Ezzat and **L. Courtial.** OCEAN AND CLIMATE, 2016 – *Scientific Notes*, Second edition, Tome 2. www.ocean-climate.org, 92 pages. 282

Liste des figures | List of figures

Figure 1 | Formations des trois types de récifs coralliens. **17**

Figure 2 | Répartition géographique des récifs coralliens. Le zoom indique la localisation de la Nouvelle Calédonie; la zone 30 °N et 30°S est surlignée en rose et les points marquent la localisation des récifs. Source : adaptée d'oceanservice.noaa.gov **18**

Figure 3 | La Nouvelle Calédonie et ses récifs. Sources : adaptée de Google map. Photographies : ©L. Courtial. **19**

Figure 4 | Phylogénie des Cnidaires (simplifiée), d'après Veron et Stafford-Smith (2000). **21**

Figure 5 | Le corail à différentes échelles. Colonie de *Stylophora pistillata* (a, photographie ©E. Tambutté), polypes de *S. pistillata* (b, photographie ©E. Tambutté), *Symbiodinium* en culture (c). Schéma d'un polype (d, adapté de Tambutté et al. 2007) et du tissu oral dans lequel sont contenus les *Symbiodinium* (e, adapté de Kvennefors et al. 2010). **22**

Figure 6 | Illustration des différentes formes que peuvent arborer les coraux : branchu (A), digité (B), tabulaire (C), en bois de cerf (D), foliacé (E), encroûtant (F), sub-massif (G), massif (H), champignon (I). Photographies: © L. Courtial (C,E,F,I), ©J. Courtial (A,B,G), ©E. Matson (G). **30**

Figure 7 | Les coraux, une source de nutrition pour le récif. Poisson perroquet qui broute le corail (A, ©J. Courtial), trace laissée par les poissons perroquets sur une colonie de *Porites* sp. (B, ©J. Courtial), excrétion de mucus par une colonie d'*Acropora humilis* (C, source : www.casmbenvis.nic.in) et de *Porites astreoides* (D, source : www.sci.sdsu.edu). **30**

Figure 8 | Spectre et spectre d'absorption de la lumière dans l'eau de mer. **35**

Figure 9 | Carte de la température de surface des océans (janvier 2017). Les pointillés délimitent la zone autour de la ceinture équatoriale dans laquelle sont recensés la plupart des récifs coralliens. Source : http://www.ospo.noaa.gov/data/sst/contour/global_small.cf.gif **36**

Figure 10 | Effets des UVs sur les coraux. L'énergie issue de l'*absorption* des UVs peut être soit dissipée (*dissipation*) sous forme de fluorescence ou de chaleur soit provoquer des *dommages* à l'organisme. Les dommages peuvent être directement induits par la dégradation photochimique des *cellules* et des bio*molécules* (1) ou indirectement induits via la production de *dérivés actifs de l'oxygène* (2). L'organisme peut activer des mécanismes de *réparation* des dommages ou de *neutralisation* des ROS. Le stress net se traduit par une augmentation de la *demande énergétique*; par des changements à l'échelle cellulaires (*composition des cellules* ; ex: contenu lipidique); par un ralentissement de la *croissance* du aux dommages causés et à l'allocation de l'énergie à d'autres mécanismes; et par une augmentation de la *mortalité* si le stress est trop long. **42**

Figure 11| Aquarium facilities in Monaco (A), nubbins of *P. damicornis* under UVR (B). The neon lights on top of the aquariums are the artificial UVR lamps. **68**

Figure 12| Distribution spectra of the tested (A, B) and (C) used UV lamps. The first lamp tested is a lamp delivering mainly UVA (spectrum A, 20 W m^{-2} of UVA: 315-400 nm and 0.34 W m^{-2} of UVB: 280-315 nm) which was associated with a UVB lamp (spectrum B, 20 W of UVs: 290-320 nm). The UV lamp used for all experiments conducted in Monaco is a Q-Panel UVA 340 with a similar UVR spectrum than the sun (C, 290-400 nm). **69**

Figure 13 | Experimental set up in the Aquarium des lagons in Nouméa. The experimental tanks were settled in a big buffer tank to regulate the temperature and shade cloths were installed above them

to attenuate the solar radiation (A). UVR filters were installed on the top of some of the experimental tanks (BC). **72**

Figure 14 | A) Average daily incident shortwave solar energy (kWh) reaching the ground per square meter in Nouméa (orange line) with 25th to 75th and 10th to 90th percentile bands. This report illustrates the typical weather in Nouméa, based on a statistical analysis of historical hourly weather reports and model reconstructions from January 1, 1980 to December 31, 2016. © weatherspark.com; https://weatherspark.com/y/144754/Average-Weather-in-Noum%C3%A9a-New-Caledonia B) UV index at Nouméa airport between the 02/09/2015 and the 31/08/2016. The UV Index is a number linearly related to the intensity of sunburn-producing UV *i.e.* relative to the proportion UVB/UVA. © weatheronline.co.uk; https://weatherspark.com/y/144754/Average-Weather-in-Noum%C3%A9a-New-Caledonia). **73**

Figure 15 | Data recording during the thermal stress experiment (Paper n° 3). The red line is the temperature recorded in high temperature tanks while the blue line is the temperature in ambient temperature condition. ΔT is the week of temperature increase and T=30°C is the thermal stress period. **75**

Figure 16 | Coral species used in this thesis: *Pocillopora damicornis* (A), *Stylophora pistillata* (B), *Turbinaria reniformis* (C), *Acropora muricata* (D). The outlined pictures show the nubbins used in the experiments; black lines are 1 cm long. Photo credit: ©L. & J. Courtial. **76**

Figure 17 | Emission spectrum of a nubbin before (blue curve) and after (red curve) exposure for 30 minutes in Carboxy-H_2DCFDA probe under thermal stress (30°C). **78**

Figure 18 | Summary of the results obtained for an exposition of *S. pistillata* to a combined stress of UVR and high temperature. JNK signalling pathway is activated by the stress exposure and down regulates the increase in intracellular ROS (A). JNK inhibitor blocks JNK activation which impairs ROS scavenging capacities and results in coral bleaching (B). The western blot images show JNK activation in the control condition (C), under UVR alone (UV), at high temperature (T), under the combination of UVR and high temperature (UVT). **105**

Figure 19 | Summary of the effects of UVR and seasons on coral and mucus microbiomes. "Ø" indicates no effects. Exposure to today's levels of UVR has no effects coral microbiome but induces changes in the mucus microbiome. Seasons induce changes in both coral and mucus bacterial communities. **115**

Figure 20| Summary of the effects of UVR and thermal stress on coral physiology (metabolism and tissue parameters). "-" indicates negative effects, "Ø" indicates no effect. Large arrow indicates high effects. UVR has no effect on acclimated corals and/or harbour high protective pigment concentrations. UVR has negative effects when colonies harbour high symbiont density or under thermal stress. Thermal stress negatively affects coral physiology. High N+P supply decreases these negative effects but UVR and nutrient deficiency worsen them. **122**

Figure 21 | Summary of the effects of UVR and thermal stress on organic matter release. "-" indicates negative effects, "+" indicates positive effects and "Ø" indicates no effect. Today's UVR levels has no effect on POM and DOM release but increased UVR doses triggers a decrease in TN release. Thermal stress does not affect total organic matter fluxes but negatively impacts POM release and increases DOM release (*i.e.* thermal stress modifies the released matter quality). **123**

Les photographies utilisées pour illustrer la thèse sont soumises à un copyright.

Liste des tableaux | List of tables

Table 1| Les différents types de symbiose. **24**

Table 2 | Récapitulatif des différentes formes que peuvent arborer les coraux. **29**

Table 3 | Récapitulatif des connaissances sur les effets des UVs sur les coraux. **43**

Table 4 | Résumé des expériences réalisées au cours de cette thèse faisant l'objet de publications sous forme d'articles scientifiques. Dans la colonne « conditions environnementales », 0UV et UV désignent les conditions sans et avec UVs; T désigne une condition de forte température ; W et S désignent les conditions hiver et été ; D, C, E désignent une condition déplétée, ambiante et enrichie en nutriments. **61**

Table 5 | Summarize of the temperatures and UVR levels at the different time of the experiments. "+" give a comparison between UVR levels at different time of the year (deduced from Figure): "+++" is the highest level, "++" is a medium level and "+" is the lowest level. **74**

Table 6 | Validated antibodies. **81**

Table 7 | Physiological differences between *P. damicornis* and *T. reniformis* (paper n°2). The red numbers highlight differences between the two species by showing the greatest value between the two. **100**

Table 8 | Summary of the effects of temperature (T), ultraviolet radiations (UV) and the combination of both stressors (UV+T) *on P. damicornis* and *T. reniformis*. "+" means positive effect: an increase of the value compared to the control condition, "-" means negative effect: a decrease of the value compared to the control condition and "=" means no effect, the final value is not different from the control condition. Red indicates significant effects of temperature alone; violet indicates effects of UVR alone and yellow indicates an interaction between UVR and temperature. In brackets is indicated whether the effects are additive (no interaction of UV and T in the ANOVA's results), synergistic or antagonistic (significant interaction between UV and T in the ANOVA's results). **101**

Table 9 | Comparison of the tissue parameters of fed and unfed nubbins of *P. damicornis* (papers n°2 and 5). "Temp." is the temperature; "UVR cond." Is the UVR condition (0UV means no UVR, UV means exposed to UVR); "Symbionts", "Chl *a*" and *"Proteins are* the symbiont, chlorophyll *a* and protein contents in %. **102**

Table 10 | Effects of season and UVR on coral physiology. "+" indicates positive effects," -" indicates negative effects, "Ø" indicates no effects, "S" indicates Summer and "W" indicates winter values. Indicated values are means ± standard deviation. **103**

Table 11 | Summary of the 8 OTUs associated with *A. muricata* mucus. **112**

Table 12 |Summary of the effects of UVR and season on the 8 OTUs associated with *A. muricata* mucus. Blue highlights bacteria more abundant in winter; yellow indicates more abundant in summer; purple is bacteria more abundant under UVR in winter; grey is bacteria more abundant without UVR in winter. **113**

Table 13 | Major genera composing the coral associated bacterial community. Brown highlights bacteria with antibacterial or nutritional function; yellow indicates bacteria previously found to be associated with diseased or stressed corals; green is diazotrophic bacteria. **114**

Table 14 | Summary of the experiments carried out during the thesis. In the "environmental condition column", 0UV and UV designate the conditions without and with UVR respectively. T designates a high temperature condition; W and S designate winter and summer conditions; D, C, E designate depleted, control and enriched nutrient conditions respectively. **281**

Liste des encarts | List of boxes

Les encarts sont présents pour apporter des précisions et des informations supplémentaires non nécessaires à la compréhension du sujet.

Boxes are there to provide supplementary information non necessary for the thesis understanding.

Encart 1 | Les récifs de Nouvelle Calédonie **|** New Caledonian's reefs **19**

Encart 2 | Qu'est-ce qu'une symbiose ? **|** What is a symbiosis? **23**

Encart 3 | Les différentes formes du corail **|** Coral shapes **28**

Encart 4 | Blanchissement des coraux, un réservoir de biodiversité menacé **|** Coral bleaching, an imminent threat to marine biodiversity **51**

Encart 5 | Espèces de coraux étudiées **|** Studied species **75**

Liste des publications à comité de lecture international | Publications in international scientific journals

Subm. **| Courtial, L.**, J. van de Water, C. Rottier, R. Rodolfo-Metalpa F. Houlbrèque, and C. Ferrier-Pagès. Seasonal variability of the microbial communities associated with *A. muricata* exposed to or shaded from UVR.

Subm. **| Courtial, L.**, F. Houlbrèque, and C. Ferrier-Pagès. Effects of ultraviolet radiation and nutrient levels on the physiological response and mucus production of *Pocillopora damicornis* following thermal stress.

2017 | Courtial, L., C. Ferrier-Pagès, S. Jacquet, R. Rodolfo-Metalpa, S. Reynaud, C. Rottier, and F. Houlbrèque. 2017. Effects of temperature and UVR on organic matter fluxes and the metabolic activity of *Acropora muricata*. Biol. Open 6: 1190–1199. doi:10.1242/bio.026757

2017 | Courtial, L., V. Picco, G. Pagès, and C. Ferrier-Pagès. Validation of commercial ERK antibodies against the ERK orthologue of the scleractinian coral *Stylophora pistillata* [version 1; referees: awaiting peer review]. F1000Research 6. doi:10.12688/f1000research.11365.1

2017 | Courtial, L., V. Picco, R. Gorver, Y. Cormerais, C. Rottier, A. Labbe, G. Pagès, and C. Ferrier-Pagès. The c-Jun N-terminal kinase prevents oxidative stress induced by UV and thermal stresses in coral and human cells. Sci. Rep. 7: 45713. doi:10.1038/srep45713

2017 | Courtial, L., S. Roberty, J. M. Shick, F. Houlbrèque, and C. Ferrier-Pagès. Interactive effects of ultraviolet radiation and thermal stress on two reef-building corals. Limnol. Oceanogr. doi:10.1002/lno.10481

Communications orales à comité de lecture international et dans une émission de vulgarisation scientifique |Oral presentations in an international congress and in a popularization TV program

2016 | Courtial, L., V. Picco, R. Gorver, Y. Cormerais, C. Rottier, A. Labbe, G. Pagès, and C. Ferrier-Pagès. The c-Jun N-terminal kinase prevents oxidative stress induced by UV and thermal stresses in coral and human cells. 13th international Coral Reef Symposium, June 19-24, Honolulu, USA.

2016 | Vue sur Mer. Episode 3., Archipel Production. 17 Septembre 2016. http://la1ere.francetvinfo.fr/nouvellecaledonie/emissions/vues-sur-mer/episode-3-17-septembre-2016-421353.html

Publication de vulgarisation | Popularization publication

2016 | L. Ezzat et **L. Courtial.** OCÉAN ET CLIMAT, 2016 – *Fiches scientifiques*, Deuxième édition, Tome 2. www.ocean-climate.org, 100 pages. | L. Ezzat and **L. Courtial.** OCEAN AND CLIMATE, 2016 – *Scientific Notes*, Second edition, Tome 2. www.ocean-climate.org, 92 pages.

In prep. | **L. Courtial**, D. Allemand, P. Furla - Les Coraux : les Ingénieurs des Océans sont menacés – Encyclopédie de l'environnement mis en ligne par l'Université Grenoble Alpes.

Liste des abréviations majeures

UVs | Ultraviolets
UVR | Rayonnement ultraviolet
CO_2 | Dioxyde de carbone
A. muricata | *Acropora muricata*
P. damicornis| *Pocillopora damicornis*
T. reniformis | *Turbinaria reniformis*
S. pistillata | *Stylophora pistillata*
DOM | Matière organique dissoute
POM | Matière organique particulaire
MAAs| Acides aminés mycosporine- like
TOC | Carbone organique total
POC | Carbone organique particulaire
DOC | Carbone organique dissout
TN | Azote total
PN | Azote particulaire
DN | Azote dissout
PAR | Rayonnement photosynthétiquement actif
ROS | Espèces réactives de l'oxygène
MAPK | Mitogen activated protein kinase
N | Azote
P | Phosphore
NO_3 | Nitrate
PO_4 | Phosphate
EEA | Activité enzymatique extracellulaire
EEA_{max} | Activité enzymatique extracellulaire maximale
rETR | Taux de transport d'électron relatif
JNK | c-Jun N-terminal kinase
ERK | Extracellular signal-regulated kinase

List of the main abbreviations

UVs | Ultraviolets
UVR | Ultraviolet radiation
CO_2 | Carbon dioxide
A. muricata | *Acropora muricata*
P. damicornis| *Pocillopora damicornis*
T. reniformis | *Turbinaria reniformis*
S. pistillata | *Stylophora pistillata*
DOM | Dissolved organic matter
POM | Particulate organic matter
MAAs| Mycosporine like amino acids
TOC | Total organic carbon
POC | Particulate organic carbon
DOC | Dissolved organic carbon
TN | Total nitrogen
PN | Particulate nitrogen
DN | Dissolved nitrogen
PAR | Photosynthetic active radiation
ROS | Reactive oxygen species
MAPK | Mitogen activated protein kinase
N | Nitrogen
P | Phosphorous
NO_3 | Nitrate
PO_4 | Phosphate
EEA | Extracellular enzymatic activity
EEA_{max} | Maximal extracellular enzymatic activity
rETR | Relative electron transport rate
JNK | c-Jun N-terminal kinase
ERK | Extracellular signal-regulated kinase

Chapter 1

Introduction

Chapitre 1 | Introduction

I. Les récifs coralliens, un écosystème basé sur la symbiose corallienne

Un écosystème est un ensemble d'organismes vivants qui interagissent entre eux et avec le milieu dans lequel ils évoluent (environnement biologique, géologique, hydrologique, climatique…). Le récif corallien est défini comme l'écosystème marin le plus riche en terme de biodiversité et est souvent comparé à son équivalent terrestre : la forêt tropicale (Knowlton et al. 2010). Dans cette thèse, les récifs coralliens de Nouvelle Calédonie seront systématiquement pris pour exemple, puisqu'une partie du travail a été réalisée sur des coraux prélevés sur les récifs du phare Amédée et de l'îlot maître (lagon Sud-Ouest de la Nouvelle Calédonie).

I.1. Les récifs coralliens : formation, répartition et importance

Les récifs coralliens sont des bio-constructions formées principalement par les coraux Scléractiniaires qui synthétisent un squelette de carbonate de calcium ($CaCO_3$). Au cours des temps géologiques, les coraux se sont développés autour des îles volcaniques ou des plateaux continentaux en subduction et forment aujourd'hui les plus grandes structures solides construites par des organismes vivants.

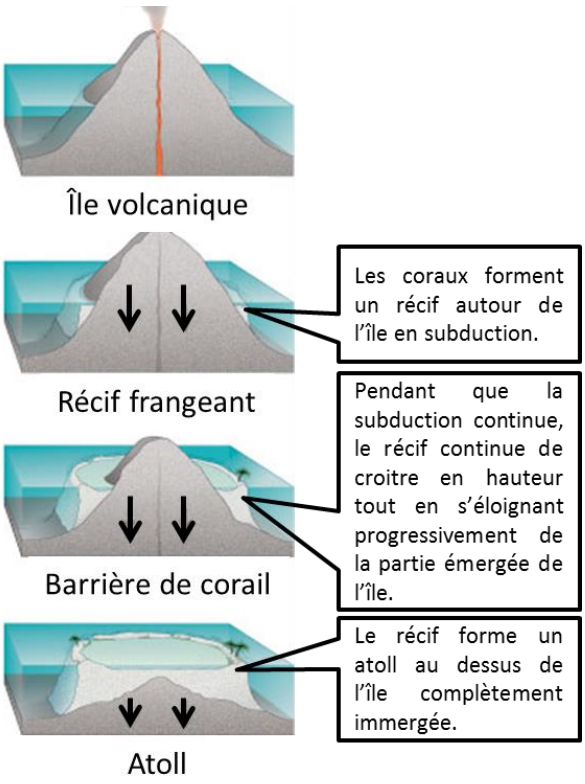


Figure 1. Formation des trois types de récifs coralliens.

En fonction de l'avancement de la subduction, on distingue trois types de récifs coralliens (Figure 1):

Les récifs frangeants qui se développent sur les bords du littoral d'une île en début de subduction. Ils sont constitués d'un court platier de faible profondeur recouvert de corail mort qui sépare la zone de croissance du corail de la plage (exemple : le récif frangeant de Yaté, Nouvelle Calédonie; Encart 1).

Les récifs-barrières situés à des kilomètres des côtes et séparés du littoral par le lagon (exemple : la Grande Barrière de Corail en Australie ou le récif barrière de Nouvelle Calédonie; Encart 1).

Les atolls qui sont d'anciens récifs-barrières dont l'île est partiellement ou complètement immergée (exemple : l'atoll d'Ouvéa en Nouvelle

Calédonie; Encart 1).

Les récifs coralliens sont principalement répartis entre 30 °N et 30 °S, dans les zones tropicales et subtropicales autour de la ceinture équatoriale (Figure 2). La majorité se développe entre 0 et 30 m de profondeur, mais ils peuvent atteindre plus de 100 m de profondeur dans certaines régions du globe comme la Mer Rouge. Leur répartition dépend principalement de la lumière (zone photique) et de la température de l'eau (Spalding et al. 2001). Les récifs coralliens représentent environ 0.16% (environ 600 000 km^2) des fonds marins et 10.8% des fonds côtiers (Spalding and Grenfell 1997). La plus grande formation corallienne au monde est la Grande Barrière de Corail en Australie qui s'étend sur plus de 2600 km de long et représente 17% des récifs mondiaux (Spalding et al. 2001). Malgré leurs faibles superficies, les écosystèmes récifaux sont des réserves écologiques majeures qui abritent plus de 25% de la biodiversité marine dont ¼ des espèces de poissons recensées (Allsopp et al. 2008).

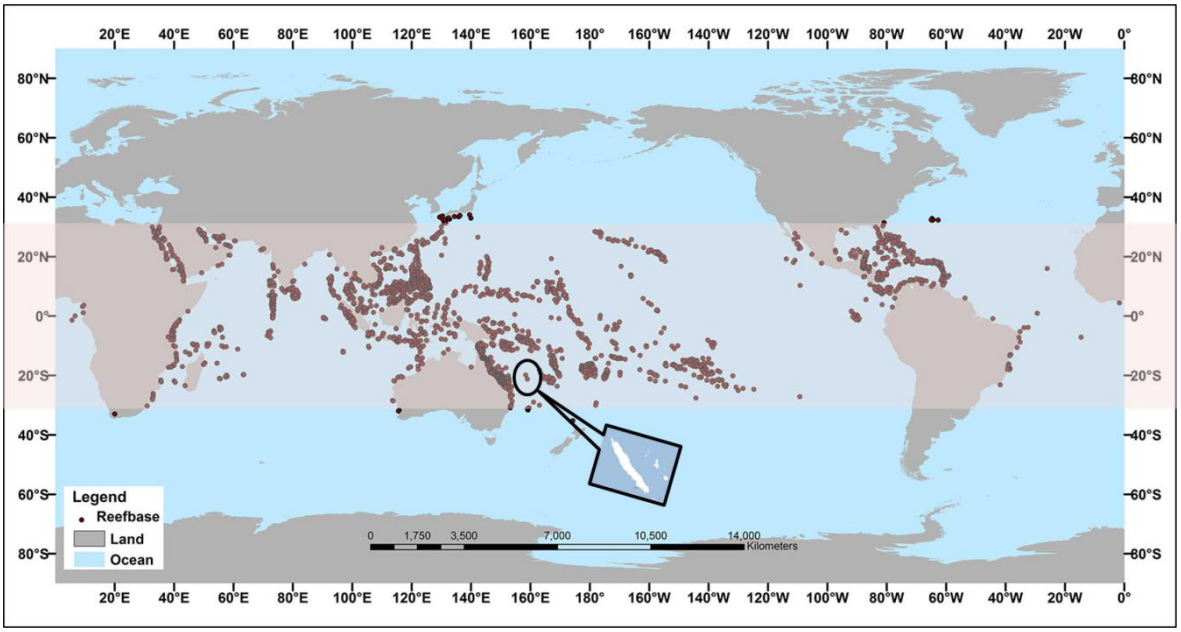


Figure 2. Répartition géographique des récifs coralliens. Le zoom indique la localisation de la Nouvelle Calédonie; la zone 30 °N et 30°S est surlignée en rose et les points marquent la localisation des récifs. Source : adaptée d'oceanservice.noaa.gov

Au-delà de leur importance écologique, les récifs coralliens rendent d'importants services écosystémiques à l'Homme (Moberg and Folke 1999). Ils bordent les côtes de plus d'une centaine de pays et jouent un rôle essentiel pour leurs populations. Leur extrême biodiversité contribue au développement économique de ces pays, grâce aux activités de pêche, au tourisme et à leurs apports en matériaux de construction. Ils assurent également un rôle de protection des côtes contre l'érosion, les tempêtes et les cyclones (Cesar 2002). Au total, plus de 500 millions de vie humaines sont directement liées aux services rendus par les récifs coralliens (Wilkinson and Souter 2008). Plus récemment, certains organismes des récifs se sont révélés d'intérêt pour les entreprises de

biotechnologie pour l'extraction de nouveaux gênes et molécules pour l'industrie alimentaire et pharmaceutique (UNEP-WCMC 2006). Les bénéfices nets apportés par les récifs coralliens (en prenant en compte la pêche, la protection des côtes et la biodiversité) varient selon les estimations de 30 milliards de dollars par an (Cesar 2002) à 375 milliards de dollars par an (Costanza et al. 1997). Le squelette des coraux est également utilisé comme archive paléoclimatique, pour reconstruire les conditions environnementales passées (Wyndham et al. 2004).

Encart 1. Les récifs de Nouvelle Calédonie

La Nouvelle Calédonie possède la première plus longue barrière récifale continue (1600 km) et la deuxième plus grande barrière au monde après la grande barrière de corail d'Australie. La barrière de corail longe les côtes calédoniennes à une distance variant entre 2 et 50 km, formant ainsi un lagon de 36 000 km^2 (Andréfouët et al. 2009), là aussi, le plus grand du monde. La Nouvelle Calédonie est caractérisée par une très grande diversité des formations récifales qu'elle abrite. On retrouve par exemple les trois grands types de récifs cités précédemment (Figure 3) ainsi qu'une configuration très rare sur certains endroits: une double barrière récifale.

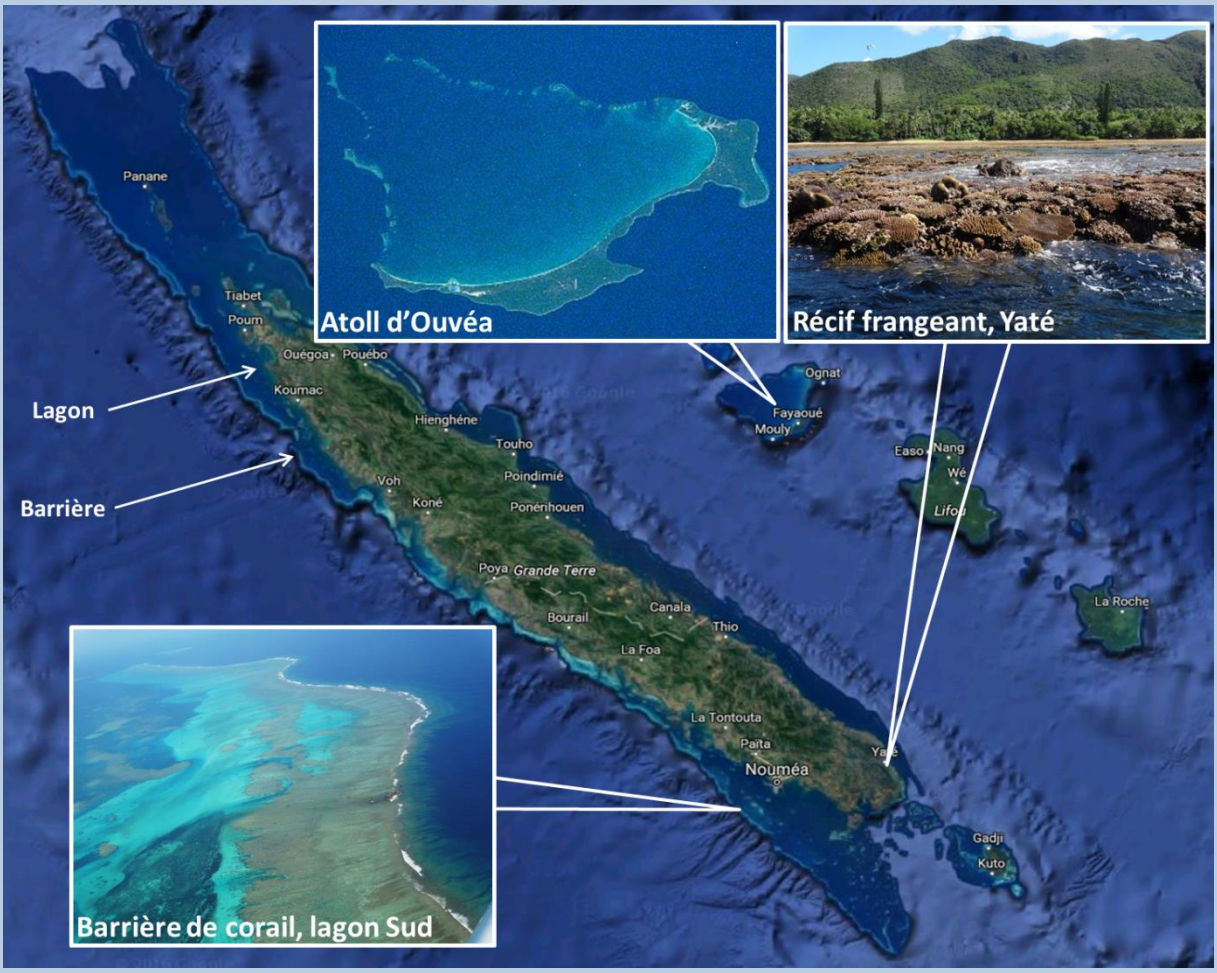


Figure 3. La Nouvelle Calédonie et ses récifs. Sources : adaptée de Google map. Photographies : ©L. Courtial.

Du point de vue de la biodiversité, les récifs calédoniens sont parmi les plus riches au monde avec 401 espèces de coraux (Lasne 2010) identifiées jusqu'ici, dont une espèce de fungiidae endémique *Cantharellus noumeae* (Gilbert et al. 2015). Cette **diversité exceptionnelle** est accrue par la présence d'une grande variété d'habitats (*i.e.* mangroves, algueraies, herbiers, estuaires, plaines lagonaires). Parmi les espèces avoisinant le récif, on retrouve des populations menacées de grands prédateurs, des tortues marines ainsi qu'un grand nombre de mammifères marins dont le dugong.

Après l'inscription de 15 000 km^2 de lagons et récifs de Nouvelle Calédonie au **patrimoine mondial de l'UNESCO en 2008**, le gouvernement de la Nouvelle Calédonie a créé, en avril 2014, le **Parc naturel de la mer de Corail**, la deuxième plus grande aire marine protégée au monde. Ce parc naturel couvre la totalité de l'Espace maritime de la Nouvelle Calédonie, soit 1 291 000 km^2 et a pour objectifs de protéger, valoriser et faire connaitre les ressources et la richesse exceptionnelle de ces environnements. Sur le long terme, la volonté des acteurs du parc est de réunir les conditions d'une économie bleue (*i.e.* respectueuse du milieu qu'elle exploite) et de participer à la dynamique de protection des océans au niveau international.

En Nouvelle-Calédonie, le premier blanchissement corallien massif a été observé durant l'été austral 95-96 (Richer de Forges and Garrigue 1997), à la suite d'un épisode durable de températures particulièrement élevées dans le lagon. Le phénomène ENSO (El Niño/Oscillation Australe) a souvent été évoqué pour expliquer les épisodes de blanchissement du Pacifique. Cependant en Nouvelle-Calédonie plusieurs phénomènes El Niño et la Niña se sont succédés depuis 1995 avec des épisodes de réchauffement de la surface sans qu'aucun phénomène de blanchissement n'y ait été rapporté. En Février 2016, un nouvel épisode durable de réchauffement a été observé et s'est accompagné d'un blanchissement massif en Nouvelle-Calédonie. Une surveillance a été mise en place par l'IRD et les observations collectées indiquent que le phénomène a touché la quasi-totalité des récifs frangeants côtiers de la Grande Terre et des récifs intermédiaires du lagon.

Pour en savoir plus :

https://nouvelle-caledonie.ird.fr

http://www.ifrecor.nc/spip.php?article5

http://www.aires-marines.fr/Les-aires-marines-protegees/Categories-d-aires-marines-protegees/Parc-naturel-de-la-mer-de-Corail-en-Nouvelle-Caledonie

http://whc.unesco.org/fr/list/1115

I. 2. Les coraux Scléractiniaires, des animaux symbiotiques à l'origine des récifs coralliens

I. 2. 1. Taxonomie des Cnidaires

Les coraux Scléractiniaires, principaux constructeurs de récifs, appartiennent à l'embranchement des Cnidaires, apparu au Précambrien (les plus anciens fossiles étant datés à -700 millions d'années, Stanley 2013). La phylogénie simplifiée des Cnidaires est présentée dans la Figure

4. Les organismes de cet embranchement sont caractérisés entre autres par la présence de cellules urticantes (les cnidocytes) et l'alternance de deux morphotypes au cours du cycle biologique : la forme sessile ou polype et la forme mobile ou méduse. L'embranchement des Cnidaires regroupe trois classes dont la classe des Anthozoaires divisée en deux sous-classes : les Hexacoralliaires et les Octocoralliaires. Les coraux Scléractiniaires (ordre des Scléractiniaires) appartiennent à la sous classe des Hexacoralliaires en opposition aux coraux mous et au corail rouge qui appartiennent aux Octocoralliaires. Cette sous-classe regroupe des organismes qui sécrètent un squelette calcaire à base d'aragonite et est divisée en deux groupes fonctionnels distinguant les coraux non constructeurs de récifs (ahermatypiques) et les coraux constructeurs de récifs (hermatypiques),

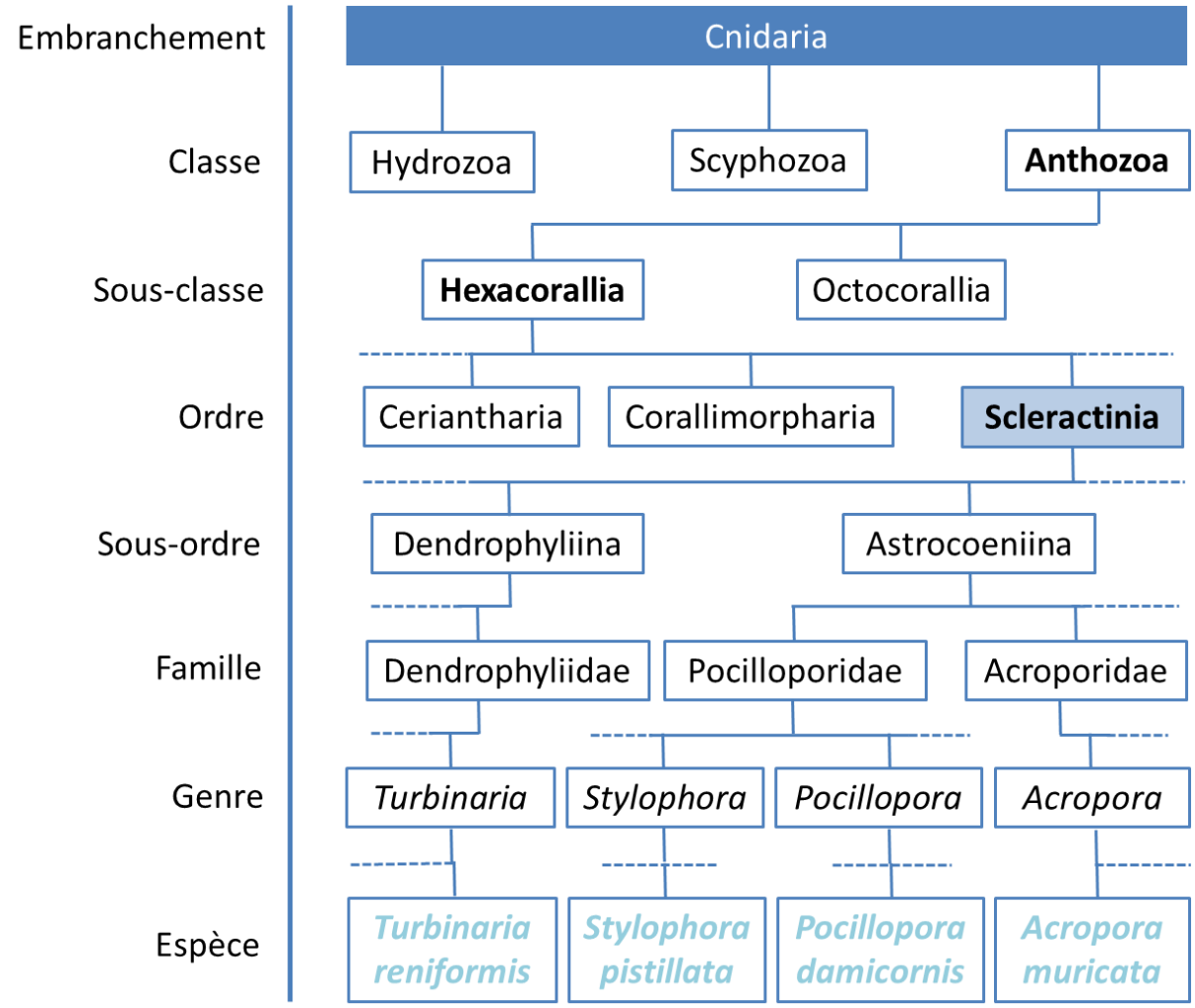


Figure 4. Phylogénie des Cnidaires (simplifiée), d'après Veron et Stafford-Smith (2000).

auxquels appartiennent toutes les espèces utilisées dans les expérimentations présentées ici (Figure 4). Dans la suite de ce travail, le terme « corail » désignera, sauf mention contraire, les coraux Scléractiniaires constructeurs de récifs.

I. 2. 2. Le corail, anatomie et histologie

Les coraux sont des animaux majoritairement coloniaux, c'est-à-dire qu'ils vivent en colonies d'individus appelés polypes (Figure 5AB) reliés les uns aux autres par une cavité commune (appelée cavité gastro-vasculaire ou cœlentéron) et par un réseau nerveux commun (Grimmelikhuijzen and Westfall 1995). Chaque polype est composé d'un orifice unique, appelé bouche, qui assure à la fois les fonctions de nutrition et d'excrétion. La bouche est entourée d'une couronne de 6 tentacules (Hexacoralliaires) servant à la fois à la nutrition et à la défense. A l'intérieur du polype, l'orifice se prolonge par un canal œsophagien (*stomodeum*) s'ouvrant sur la cavité gastrovasculaire (cœlentéron, Figure 5D). Le polype est délimité par deux tissus : le tissu oral, en contact direct avec l'eau de mer, et le tissu aboral, en contact avec le squelette de la colonie. Chacun des deux tissus est composé de deux couches cellulaires, l'épiderme sur la face externe du tissu et le gastroderme sur la face interne du tissu. Elles sont séparées par une couche acellulaire appelée mésoglée (Figure 5E). L'ensemble des couches cellulaires composant les tissus du polype ainsi que le cœlentéron se prolongent dans la colonie et forment le cœnosarque qui connecte les polypes entre eux.

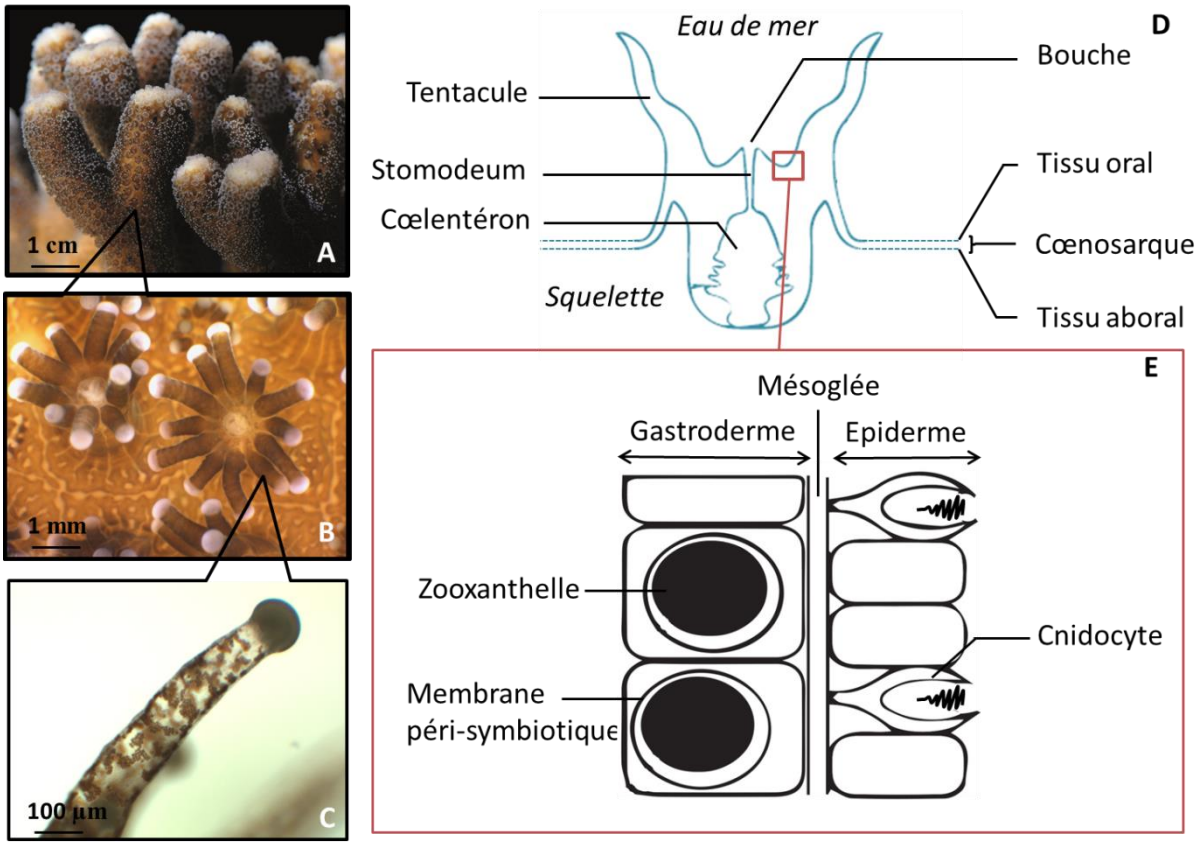


Figure 5. Le corail à différentes échelles. Colonie de *Stylophora pistillata* (A, photographie ©E. Tambutté), polypes de *S. pistillata* (B, photographie ©E. Tambutté), tentacule riche en symbiote

d'un polype de *S. pistillata* (C). Schéma d'un polype (D, adapté de Tambutté et al. 2007) et du tissu oral dans lequel sont contenus les *Symbiodinium* (E, adapté de Kvennefors et al. 2010).

I. 2. 3. Le corail, un animal en symbiose avec les *Symbiodinium*

La symbiose corallienne

Une grande partie des coraux constructeurs de récifs, et l'ensemble des espèces utilisées dans cette thèse, vivent en symbiose (association durable entre deux ou plusieurs organismes, voir Encart 2.) avec des micro-algues unicellulaires photosynthétiques du genre *Symbiodinium*, (Muscatine and Porter 1977). Cette symbiose est mutualiste car les deux partenaires, le corail (hôte) et les *Symbiodinium* (symbiotes) tirent des bénéfices de l'association (développés ci-après). Elle est également décrite comme endosymbiose intracellulaire car les *Symbiodinium* sont localisées à l'intérieur des cellules du gastroderme du tissu oral des polypes, séparés du cytosol par une membrane plasmique appelée péri-symbiotique, l'ensemble formant un symbiosome (Figure 5E). Enfin, l'association corail- *Symbiodinium* est considérée comme obligatoire pour le partenaire animal car il ne survit généralement pas longtemps à la perte de ses symbiontes (appelée blanchissement corallien, voir Introduction §II.2.). En revanche, les *Symbiodinium* peuvent vivre à l'état libre dans la colonne d'eau, rentrant alors dans la composition du phytoplancton. Pour la bonne lecture de cette thèse, le mot « corail » sera par la suite utilisé pour désigner l'ensemble hôte/symbiotes.

Encart 2. **Qu'est-ce qu'une symbiose ?**

Le mot symbiose vient du grec συμβίωσις "vivre ensemble". A l'origine il était communément utilisé pour décrire la vie en communauté. Ce n'est qu'en 1879 que le mot est accepté dans la communauté scientifique suite aux travaux d'Heinrich Anton de Bary qui définit la symbiose comme «**la vie en association de différentes espèces** » (De Bary 1879). Depuis, la définition du mot symbiose a été beaucoup débattue notamment concernant le type d'interaction biologique qu'elle décrit (mutualiste, commensaliste ou parasite, Table 1). Aujourd'hui, en français courant, le terme est réduit aux interactions mutualistes impliquant des avantages pour les deux organismes associés. Les organismes impliqués sont appelés **hôte** (organisme le plus gros) et **symbiote** (associé à l'hôte). Les scientifiques utilisent une définition plus large qui ne présuppose pas des modalités de l'interaction et ajoutent des distinctions selon la nature de l'interaction et la localisation du symbiote par rapport à l'hôte (Table 1). L'association symbiotique entre les coraux (hôte) et les *Symbiodinium* (symbiote) est caractérisée comme une endosymbiose intracellulaire mutualiste (Muscatine and Porter 1977).

Table 1. Les différents types de symbiose.

Type	Description
Distinction selon la nature de l'interaction hôte -symbiote	
Mutualisme	Association bénéfique pour les deux partenaires *Ex :* la symbiose corallienne
Commensalisme	Association bénéfique uniquement pour l'un des deux partenaires sans que l'autre soit lésé *Ex :* la symbiose entre poisson pilote et requin
Parasitisme	Association bénéfique pour l'un des partenaire s et nocive pour l'autre. *Ex :* le mildiou, un parasite de la vigne
Distinction selon la localisation du symbiote par rapport à l'hôte	
Endosymbiose intracellulaire	Le symbiote est localisé dans les cellules de l'hôte *Ex :* la symbiose corallienne
Endosymbiose extracellulaire	Le symbiote est localisé dans les tissus de l'hôte entre les cellules *Ex :* la symbiose entre Symbiodinium et bénitier
Ectosymbiose	Le symbiote est localisé en dehors de l'hôte *Ex :* la symbiose entre poisson clown et anémone

Les *Symbiodinium*, taxonomie et caractéristiques biologiques

Les algues symbiotiques du corail sont des dinoflagellés symbiotiques du genre *Symbiodinium* qui appartiennent au règne Alveolata et à l'embranchement des Dinophycea. Jusque dans les années 1980, elles étaient apparentées à une seule espèce, *Symbiodinium microadriaticum,* décrite pour la première fois en symbiose chez la méduse tropicale *Cassiopea xamachana* par Freudenthal en 1962. Grâce aux nouvelles techniques de génétique moléculaire, on distingue maintenant 9 groupes phylogénétiques distincts appelés clades (allant de A à I) (Rowan and Powers 1991; Rowan 1998). Les *Symbiodinium* associées au corail appartiennent majoritairement aux clades A, C et D (LaJeunesse 2001; Coffroth and Santos 2005). Au sein d'une même espèce animale, il est possible de trouver plusieurs clades de symbiotes différents en fonction des conditions environnementales. Le clade D par exemple, est connu pour être un clade plus résistant à la température. Des études ont montré qu'une même espèce de corail était capable de changer sa population de symbiotes pour avoir une dominance de clade D en réponse à une augmentation de la température (Baker et al. 2004; Jones et al. 2008). Les symbiotes sont des cellules de 8 à 12 µm de diamètre (Figure 5C) situées dans des vacuoles animales dans le cytoplasme endodermique de l'hôte (Figure 5E, Wakefield et al. 2000). La densité de symbiotes au sein d'une colonie est exprimée en nombre de cellules algales par cm^2 de surface de squelette. Cette densité dépend de multiples et complexes facteurs incluant l'état nutritionnel de l'hôte, la disponibilité des sels nutritifs ou de la lumière (Jones and Yellowlees 1997).

En condition de développement normal, elle est de l'ordre de 1 à 10 millions de cellules par cm^2 de squelette (Muscatine et al. 1998). Les *Symbiodinium* peuvent être directement transmises du parent à la descendance (on parle de transmission verticale) ou peuvent être acquises par l'endocytose de *Symbiodinium* libres dans l'eau de mer (on parle de transmission horizontale, Thompson et al. 2014). Les pigments photosynthétiques contenus dans les *Symbiodinium* (chlorophylle a et c, caroténoïdes…) sont responsables, en grand partie, de la couleur du corail.

Physiologie de la symbiose

L'association avec les *Symbiodinium* apporte des avantages importants pour le corail. Tout d'abord, le rôle du symbiote pour le corail est principalement d'ordre trophique. Le corail passe ainsi du statut d'organisme hétérotrophe (utilisation de sources de carbone organique via une nutrition hétérotrophe) à un statut d'organisme mixotrophe, c'est-à-dire qu'il peut utiliser à la fois le carbone organique et le carbone inorganique via l'autotrophie des *Symbiodiniu*m (Houlbrèque and Ferrier-Pagès 2009). Pour sa **nutrition hétérotrophe**, l'animal capture différents types de proies vivantes (allant des bactéries au macro-zooplancton) (Houlbrèque et al. 2004b) ou de la matière organique détritique dissoute (Grover et al. 2008) ou particulaire (Anthony 1999). Pour capturer et ingérer ces proies et cette matière organique, les coraux utilisent un éventail de dispositifs tels que du mucus, des structures ciliées, des filaments mésentériques, des tentacules et des nématocystes (Thorington and Hessinger 1996; Sorokin 2013). Les proies et le mucus englobant ces proies sont ensuite digérés dans le cœlentéron et constituent une source d'azote, de phosphore, de carbone et de vitamines (Houlbrèque and Ferrier-Pagès 2009) pour l'ensemble hôte-symbiote.

La photosynthèse réalisée par les symbiotes constitue une source majoritaire de carbone pour le corail puisqu'elle apporte des composés carbonés formés à partir de l'eau de mer et du CO_2 rejeté par l'hôte, pauvres en éléments essentiels comme l'azote et le phosphore. La plupart des photosynthétats (75-95%) sont transférés à l'hôte qui les utilise majoritairement pour sa propre respiration et son métabolisme basal (Muscatine and Porter 1977). Une partie des photosynthétats est également accumulée sous forme de lipides dans les réserves de l'hôte ou bien sert à la synthèse d'acides aminés essentiels et à la sécrétion du mucus. Les symbiotes peuvent ainsi contribuer jusqu'à 100% aux besoins énergétiques de l'hôte (Muscatine et al. 1981, 1984).

Les polypes des coraux réalisent **la calcification**, c'est-à-dire qu'ils synthétisent un squelette calcaire à partir des ions Ca^{2+} et HCO_3^- de l'eau de mer. Ce processus est appelé minéralisation biologiquement contrôlée ou biominéralisation. Le squelette des coraux est constitué de la superposition organisée de couches de cristaux d'aragonite (un polymorphe du carbonate de calcium $CaCO_3$) insérés dans une matrice organique constituée principalement de protéines riches en acides

aminés et en phospholipides (Allemand et al. 2011). La matrice organique permet au corail d'exercer un contrôle biologique sur la calcification, imposant la morphologie du squelette et le taux de croissance. Les symbiotes favorisent la calcification en fournissant à l'hôte l'énergie nécessaire à sa réalisation ainsi que des photosynthétats utilisés dans la synthèse de la matrice organique. De plus, la consommation du CO_2 pour la photosynthèse entraîne l'augmentation du pH dans les tissus de l'animal ce qui stimule la calcification (Gattuso et al. 1999; Allemand et al. 2004, 2011).

Bénéfices, coûts et rupture de la symbiose

Comme évoqué précédemment, la symbiose corallienne est une association qui bénéficie à la fois à l'hôte et aux symbiotes. L'hôte acquiert une nouvelle capacité métabolique qui lui apporte un avantage énergétique important via la photosynthèse. Les bénéfices se traduisent par une augmentation des réserves énergétiques, l'amélioration de la respiration et de la capacité de reproduction et la stimulation de la croissance cellulaire et squelettique (Muscatine and Cernichiari 1969; Shick et al. 1991; Allemand et al. 2004). En contrepartie, les symbiotes bénéficient d'un environnement protégé du broutage et de la stabilité du cytoplasme animal. Les symbiotes bénéficient en outre de l'azote et du phosphore provenant du recyclage des déchets métaboliques de l'hôte et du carbone inorganique (CO_2) produit directement par le métabolisme animal (Smith and Douglas 1987) évitant ainsi toute perte inutile dans un milieu globalement pauvre en nutriments.

Malgré ses avantages évolutifs importants, la symbiose impose également un coût aux deux partenaires (Muller-Parker and D'Elia 1997; Furla et al. 2011). Par exemple, en raison de ses besoins en lumière pour réaliser la photosynthèse, l'hôte est contraint de se développer uniquement dans la zone euphotique où l'exposition à de fortes intensités lumineuses peut provoquer un important stress oxydant (Dykens et al. 1992; Lesser and Farrell 2004; Richier et al. 2005). Les symbiotes eux sont contraints de céder à l'hôte la majeure partie des photosynthétats qu'ils produisent ce qui constitue une perte très supérieure aux 5% perdus dans le milieu par les algues libres (Battey 1992). De plus, l'accès au CO_2 et aux nutriments est soumis au contrôle de l'hôte qui peut limiter les apports.

Les conditions environnementales jouent un rôle primordial dans l'équilibre entre les coûts et les bénéfices de la symbiose. Cet équilibre fragile peut être rompu lorsque les conditions de lumière et de température ou la qualité de l'eau changent. La rupture se traduit par l'expulsion des symbiotes ou la perte des pigments photosynthétiques. Ce phénomène est communément appelé blanchissement (ou « bleaching » en anglais) et peut entraîner la mort du corail (Hoegh-Guldberg 1999; Hoegh-Guldberg et al. 2007). Le blanchissement ainsi que ses causes et conséquences seront discutés en détails ci-dessous (Introduction, §II.2.).

I. 2. 4. Le corail, un holobionte

Au-delà de la symbiose qu'il établit avec les *Symbiodinium*, le corail (animal) maintient une relation complexe avec divers micro-organismes indispensables à son bon développement. Le corail est ainsi décrit comme un **holobionte** (du grec "ὅλος", "tout") c'est-à-dire un assemblage de différentes espèces formant une unité écologique. Le terme a été utilisé pour la première fois pour décrire le corail en 2002 par Rowher et collaborateurs. Il désigne l'association entre l'animal (hôte), les *Symbiodinium* et de nombreux microorganismes tels que des bactéries, des ciliés, des eucaryotes etc. (synthèse et détails dans la revue de Thompson et al. 2014). Cette association semble être spécifique selon l'espèce de corail et dépendante du clade ou sous-clade de *Symbiodinium* impliqué dans la symbiose (Littman et al. 2009, 2010). Les microorganismes sont répartis en communautés qui se développent dans différents compartiments du corail présentant des conditions physicochimiques particulières (Sweet et al. 2011). On retrouve des communautés distinctes entre les **tissus** (Lesser et al. 2004), le **mucus** (Bourne and Munn 2005; Lema et al. 2012), le **squelette** (Frias-Lopez et al. 2002; Sweet et al. 2011) et la **cavité gastro-vasculaire** (Agostini et al. 2012). Leur rôle est varié et dépend là encore du compartiment dans lequel elles se situent. Par exemple, dans le mucus, qui nous intéresse plus particulièrement dans cette thèse, les ciliés empêchent la fixation d'organismes parasites en créant un courant de surface de l'ordre de 2 mm s^{-1} (Johnston and Rohwer 2007; Garren et al. 2014). Les bactéries associées au mucus ont quant à elles un rôle de protection contre d'autres bactéries pathogènes ou des virus (Bythell and Wild 2011). Ces bactéries associées au mucus dégradent également la matière organique excrétée par le corail et participent au recyclage des nutriments (voir Introduction §I.3.1). Dans les tissus et le squelette, des cyanobactéries, ou autres bactéries diazotrophes, réalisent la fixation de diazote, constituant une source potentielle d'azote pour l'hôte et les *Symbiodinium*. Dans des conditions de blanchissement, cette source d'azote ainsi que les sucres synthétisés par la photosynthèse des diazotrophes peuvent être de grande importance pour la survie de l'holobionte (Shashar et al. 1994; Fine and Loya 2002; Lesser et al. 2004; Bednarz et al. 2017). Dans la cavité gastrovasculaire, comme dans les intestins d'autres organismes, les microorganismes participent à la digestion et apportent à l'hôte des éléments essentiels tels que des vitamines et des acides aminés (Agostini et al. 2012). Certaines études ont également mis en évidence que l'activité antimicrobienne des micro-organismes associés aurait un rôle important i) pour la protection des œufs fécondés, ii) l'implantation de la larve dans l'environnement et iii) durant sa métamorphose (passage du stade larvaire, libre dans l'eau, au stade adulte, fixe sur un substrat, Dobretsov and Qian 2004; Marquis et al. 2005; Hadfield 2011). Tout comme l'association hôte-*Symbiodinium*, la relation entre l'hôte et ses micro-organismes associés est un équilibre fragile. La disponibilité des ressources organiques (accepteurs d'électrons, carbone…) et inorganiques (lumière,

nutriments…) régule la structure des populations qui coexistent. La modification de ces ressources, liée à des changements dans l'environnement du corail, peut fortement perturber la symbiose et parfois conduire à la mort de l'holobionte. Les perturbations se traduisent par l'attaque d'agents pathogènes qui ne sont plus contrés par les bactéries associées ou par la disparition des sources supplémentaires en sucres et nutriments fournies par les diazotrophes (Thurber et al. 2009; Meron et al. 2012; Thompson et al. 2014).

I.3. Le corail, son rôle dans le récif : protection et nutrition

I. 3. 1. Le corail, un refuge pour les autres organismes du récif

L'écosystème corallien se forme autour de la charpente corallienne qui forme des niches écologiques procurant un habitat à de nombreuses espèces. La structure physique de l'habitat, définie par l'ensemble des formes prises par le squelette calcaire des coraux (voir encart Encart **3**), influence profondément la biodiversité associée et le fonctionnement de l'écosystème (MacArthur and MacArthur 1961). Plus elle est complexe, plus elle facilite la coexistence et la division des espèces, la protection contre les prédateurs et les perturbations météorologiques (Bruno and Bertness 2001; Willis et al. 2005) ainsi que la reproduction et le développement des juvéniles. Par conséquent, la richesse, l'abondance et la biomasse des poissons et des invertébrés sont directement liées à la complexité architecturale du récif (Gratwicke and Speight 2005a; b; Idjadi and Edmunds 2006; Wilson et al. 2007).

Encart 3. **Les différentes formes du corail**

Les colonies de coraux constructeurs de récifs peuvent arborer plusieurs formes qui sont communément regroupées en dix ensembles (McManus et al. 1997, Table). La forme de la colonie est déterminée par son espèce mais est aussi conditionnée par des facteurs environnementaux tels que la lumière, les courants, l'action des vagues, la densité de coraux autour etc. (Barnes 1987; Lalli and Parsons 1997; Barnes and Hughes 1999). Ainsi, sur un même récif, la même espèce pourra arborer plusieurs formes en fonction de son emplacement sur le récif.

Table 2. Récapitulatif des différentes formes que peuvent arborer les coraux.

Type	Description	Exemple
Branchus	Possèdent des branches principales souvent des branches secondaires.	*Acropora muricata*,
Digités	Possèdent des courtes branches verticales sans ou avec peu de branches secondaires.	*Acropora sp.*
Tabulaires	Forment des plateaux horizontaux soutenus par un tronc central.	*Acropora sp.*
En bois de cerf (ou en spatule)	Possèdent des branches larges et aplaties en forme de spatules.	*Acropora palmata*
Foliacés	Forment des colonies en fines feuilles plus ou moins enroulées.	*Turbinaria reniformis*
Encroûtants	Se développent sur une surface et la recouvrent.	*Porites sp.*
Sub-massifs	Forment des colonnes massives, des nœuds qui s'élèvent d'une base encroûtante.	*Porites sp.*
Massifs	Forment des grosses masses compactes et robustes.	*Porites sp.*
Champignons	Constitués d'un unique polype souvent très gros et souvent non fixé à un substrat.	*Fungia sp.*
En coupe	Possèdent des formes torturées et aplaties.	*Turbinaria coccinea*

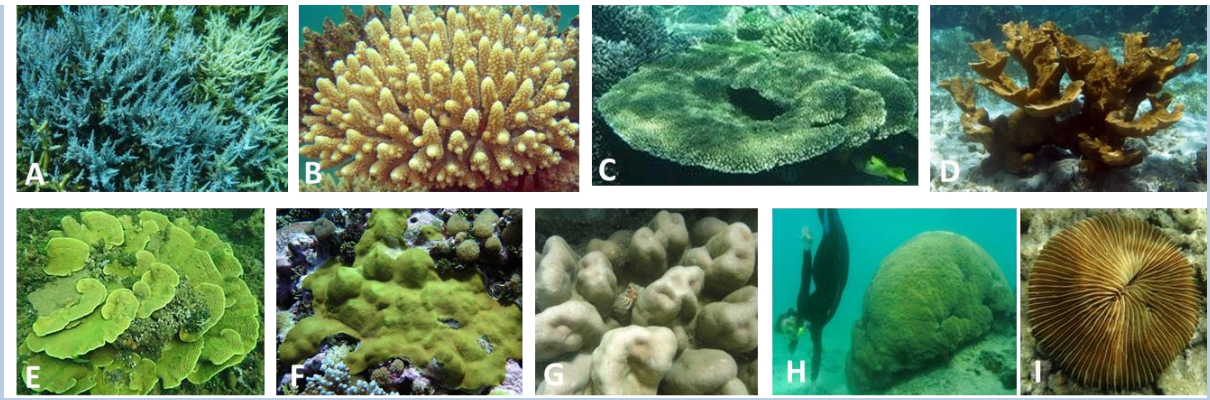


Figure 6. Illustration des différentes formes que peuvent arborer les coraux : branchu (A), digité (B), tabulaire (C), en bois de cerf (D), foliacé (E), encroûtant (F), sub-massif (G), massif (H), champignon (I). Photographies: © L. Courtial (C,E,F,I), ©J. Courtial (A,B,G), ©E. Matson (G).

I. 3. 2. Le corail, une source de nutrition

Au-delà de son rôle de protection, le corail est aussi une source de nutrition dans le récif. En effet, de nombreuses espèces de poissons broutent le corail et sont donc qualifiés de corallivores. C'est le cas par exemple de certains poissons demoiselles (Pomacentridae), de gobies (Gobiidae) de poissons papillons (Chaetodontidae) ou encore de poissons perroquets (Scaridae) (McIlwain and Jones 1997; Kokita and Nakazono 2001). La Figure 7 (A) montre un poisson perroquet en train de brouter le corail et les traces laissées par son passage sur un corail du genre Porites (B).

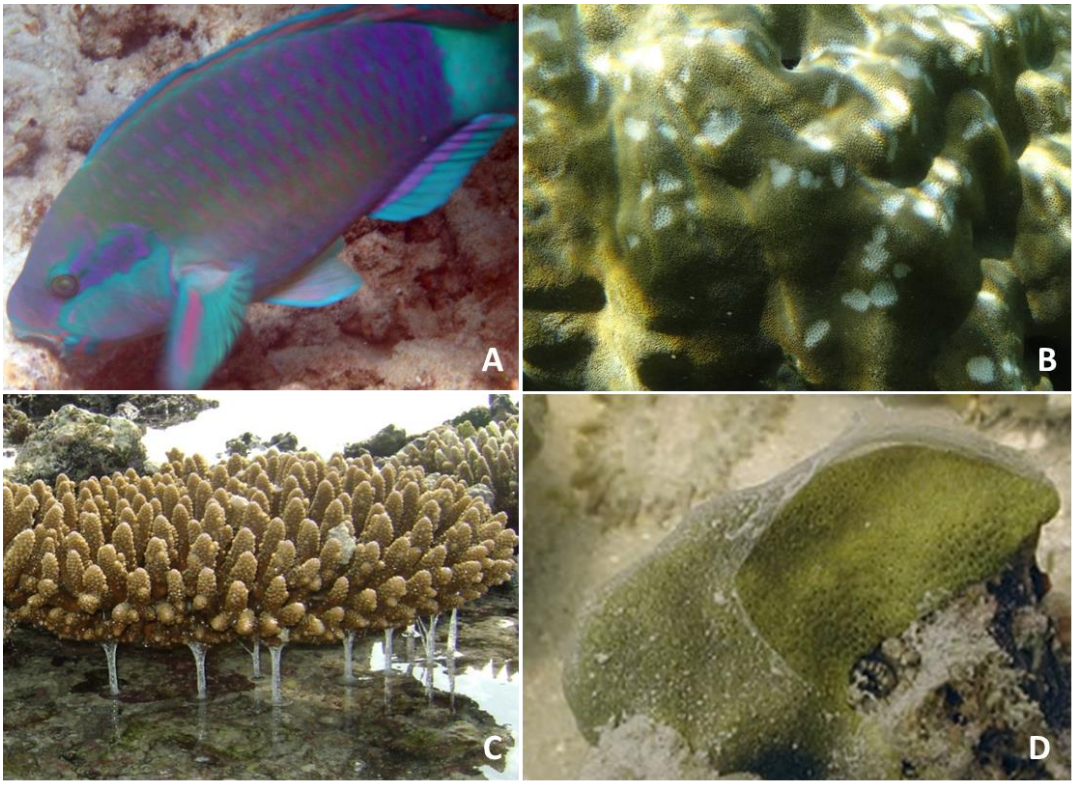


Figure 7. Les coraux, une source de nutrition pour le récif. Poisson perroquet qui broute le corail (A, ©J. Courtial), traces laissées par les poissons perroquets sur une colonie de *Porites* sp. (B, ©J.

Courtial), excrétion de mucus par une colonie d'*Acropora humilis* (C, source : www.casmbenvis.nic.in) et de *Porites astreoides* (D, source : www.sci.sdsu.edu).

Par ailleurs, le corail est également une **source majeure de matière organique** dans le récif via l'excrétion de mucus (Crossland et al. 1980; Davies 1984). Ce mucus est ensuite dispersé dans la colonne d'eau et utilisable par les autres organismes du récif (Figure 7Figure 7CD). Les paragraphes qui suivent résument les connaissances actuelles sur l'excrétion de la matière organique par le corail et son devenir dans le récif.

Synthèse du mucus, quoi, comment, pourquoi ?

Le corail excrète de la matière organique à la fois dissoute (**DOM**, de diamètre inférieur à 0.2 µm et directement assimilable par les organismes) et particulaire (**POM**, de diamètre supérieur à 0.2 µm, qui doit être dégradée par les micro-organismes du récif avant d'être consommée). Cette matière sous forme d'un complexe formé de lipides, de polysaccharides et de protéines appelé **mucus** (Bythell and Wild 2011, Figure 7), est donc riche en carbone et azote organique (Coffroth 1990). Le mucus est excrété par des cellules spécialisées nommées mucocytes, localisées dans l'ectoderme du corail (Marshall and Wright 1993). Il sert à la fois à la nutrition du corail en favorisant le transport des proies (voir Introduction §I.2.3. Physiologie de la symbiose) ainsi qu'à la protection contre les agents pathogènes (voir Introduction §I.2.4. Le corail, un holobiont) et contre les conditions extérieures (détaillées dans Brown and Bythell 2005). En effet, à l'intérieur du mucus, on trouve par exemple des molécules particulières appelées les acides aminés mycosporine-like (MAAs) qui jouent un rôle de « crème solaire » pour les coraux et les protègent contre le rayonnement ultraviolet du soleil (Drollet et al. 1993, 1997). Le mucus est également une protection contre la dessiccation lorsque le corail est exposé à l'air libre lors des marées basses (Daumas and Thomassin 1977; Krupp 1984). Il constitue également une barrière physique qui protège les tissus sous-jacents des dommages causés par les courants et les vagues, le dépôt intempestif de sédiments ou encore la pénétration de polluants (Jaap and Wheaton 1975; Mitchell and Chet 1975; Daumas and Thomassin 1977; Bastidas and Garcia 2004).

L'excrétion de matière dépend de l'espèce et des conditions environnementales

De nombreux travaux ont étudié l'excrétion du mucus chez les coraux Scléractiniaires. Ils ont montré que la quantité et la qualité de la matière excrétée par le corail dépendent à la fois de l'espèce étudiée et des conditions environnementales (Wild et al. 2004, 2008; Tanaka et al. 2008; Naumann et al. 2010; Levas et al. 2015). Naumann et ses collaborateurs (2010) ont, par exemple, étudié l'excrétion de matière de 6 genres dominants de coraux Scléractiniaires (*Acropora, Fungia, Goniastrea, Millepora, Pocillopora et Stylophora*) et ont montré que tous ces genres excrètent de la

POM avec des taux de carbone organique particulaire (POC) allant de 0.3 à 6.5 mg POC m^{-2} et d'azote particulaire (PN) entre 0.04 et 0.5 mg PN m^{-2}. A contrario, seulement la moitié d'entre eux (*Acropora*, *Goniastrea* and *Millepora*) excrètent de la DOM (entre 0.8 et 5.2 mg L^{-1}) tandis que les autres genres absorbent la DOM de l'eau de mer. La proportion et la quantité de POM/DOM qui sont transférées à la colonne d'eau (*i.e.* la matière excrétée sous forme de mucus moins la matière absorbée par le corail pour sa propre nutrition) dépend donc du genre et de l'espèce considérés. Les paramètres environnementaux tels que la lumière, la température, la turbidité, les courants *etc.* influencent également l'excrétion du mucus (Brown and Bythell 2005). Plus de détails concernant cette variabilité sont donnés dans la deuxième partie de l'introduction (voir Introduction §II.2.) qui traite des effets des paramètres environnementaux sur la physiologie du corail et les flux de matière organique.

Le devenir de la matière organique, son importance pour le récif

La matière excrétée sous forme de mucus est soit dégradée par les micro-organismes associés au mucus et utilisée pour leur propre développement, soit transférée à la colonne d'eau. Une fois décollée de la surface du corail, la matière organique forme une source de nutrition potentielle pour les autres organismes du récif. La partie dissoute intègre la réserve de DOM de la colonne d'eau, directement utilisable par les organismes benthopélagiques, tandis que la partie particulaire restante forme des agrégats en suspension dans la colonne d'eau qui finissent par couler vers le fond de l'océan et qui viennent alimenter la boucle microbienne des sédiments (Krupp 1984; Wild et al 2004, 2005). La matière organique excrétée par le corail est donc essentielle au fonctionnement du récif car elle favorise la croissance des micro-organismes associés au mucus, soutient la production benthopélagique et participe au recyclage des éléments essentiels (azote, phosphore…) dans l'eau très oligotrophe du récif (Wild et al. 2004; Bythell and Wild 2011).

II. Les coraux, des organismes dans un environnement changeant

Cette thèse portant sur les effets des ultraviolets (UVs) combinés aux changements de température et de sels nutritifs sur les coraux Scléractiniaires, les autres paramètres environnementaux tels que la salinité, le pH de l'eau ou la courantologie n'ont pas été développés dans la suite de ce manuscrit. Ces paramètres demeurent néanmoins importants pour la croissance et le développement du corail (Kleypas et al. 1999).

II.1. Conditions environnementales du récif et variabilité naturelle

II. 1. 1. La lumière, définition et conditions sur les récifs

Parce qu'ils vivent en symbiose avec des dinoflagellés autotrophes, la lumière est un élément indispensable au métabolisme et à la croissance des coraux Scléractiniaires. Sans lumière, les coraux ne pourraient pas réaliser la photosynthèse et soutenir leurs besoins énergétiques (Muscatine 1990). Les récifs coralliens se développent donc dans une **zone** dite **«photique»** dans laquelle la lumière est suffisante pour que la production d'oxygène, via la photosynthèse, soit supérieure à sa consommation par la respiration (Lee et al. 2007). Physiquement, cela correspond à la zone entre la surface et la profondeur à laquelle 99% de la lumière de surface a été atténuée dans la colonne d'eau. Environ 90% de la vie marine, dont les coraux Scléractiniaires symbiotiques, est située dans cette zone.

La lumière qui atteint la surface de la zone photique et pénètre dans l'océan peut être divisée en deux catégories (Figure 8) : le **rayonnement photosynthétiquement actif** (**PAR**) et le **rayonnement ultraviolet**. Le PAR désigne la partie du spectre lumineux que les coraux sont capables d'utiliser pour la photosynthèse. Cela correspond plus ou moins au spectre du visible c'est-à-dire aux longueurs d'ondes comprises entre 400 et 700 nm (Su et al. 2007). Les longueurs d'ondes plus longues (au-dessus de 700 nm) ne transportent pas suffisamment d'énergie pour permettre la photosynthèse et les longueurs d'ondes plus courtes (en dessous de 400 nm), très énergétiques, peuvent causer des dommages aux tissus et aux cellules des organismes (voir plus de détails Introduction §II.1.). Ces dernières sont appelées ultraviolets (UVs) et ne sont pas visibles par l'œil humain. Elles sont divisées en trois catégories: les UVA (400-315 nm), les UVB (315-280 nm) et les UVC (280-100 nm). Ces radiations sont fortement absorbées par l'ozone de l'atmosphère et l'absorption est d'autant plus forte que les longueurs d'onde sont courtes. Par conséquent, seuls les UVA et les UVB traversent la couche d'ozone et atteignent la surface de la terre.

L'énergie lumineuse disponible au niveau des récifs dépend principalement de trois variables : **l'intensité de la lumière à la surface de l'eau**, **l'atténuation dans la colonne d'eau** (fonction de la turbidité) et **la profondeur du récif** (Kleypas et al. 1999).

L'intensité de surface dépend du moment de la journée, du coefficient d'atténuation de l'atmosphère (météo, nuages, pollution *etc.*) et de l'angle d'inclinaison du soleil par rapport à la Terre (latitude et saison). Au cours de la journée, l'irradiance (éclairement) augmente du lever du soleil jusqu'à midi puis diminue jusqu'au coucher du soleil et varie en fonction du couvert nuageux. A plus grande échelle, l'irradiance est plus forte lorsque les rayons traversent la plus faible couche

atmosphérique, c'est-à-dire lorsque l'angle d'inclinaison du soleil par rapport à la Terre est proche de 90°. Cela se traduit par une irradiance plus forte, au niveau de l'équateur par rapport aux zones tempérées, et en été par rapport à l'hiver. Les récifs coralliens étant répartis sur une large bande de latitude, la quantité d'énergie lumineuse reçue en surface par les récifs est très variable. Par exemple, en été à Hawaii, l'irradiance peut atteindre 2000 µmol m^{-2} s^{-1} pour la lumière PAR, 40 W m^{-2} d'UVA et 2.5 W m^{-2} d'UVB (Torregiani and Lesser 2007) alors qu'à Eilat en mer rouge, l'irradiance maximale est autour de 700 µmol m^{-2} s^{-1} avec 20 W m^{-2} d'UVA et 1 W m^{-2} (Dishon et al. 2013).

L'**atténuation de la lumière dans la colonne d'eau** dépend de deux facteurs : la **diffusion et l'absorption** qui sont directement fonction des propriétés optiques de l'eau et de la **turbidité** (i.e. la présence de matière organique et inorganique qui occulte l'eau (Tedetti and Sempéré 2006; Su et al. 2007). Les différences de diffusion et d'absorption sont liées au fait que les molécules d'eau absorbent la lumière de manière inégale selon les longueurs d'ondes. Plus les longueurs d'ondes sont grandes (proche du rouge) et plus elles vont être absorbées (Wozniak and Dera 2007). Le rouge est donc atténué le plus rapidement dans la colonne d'eau tandis que le bleu pénètre plus loin (Figure 8). Les UVs, de plus courtes longueurs d'ondes que la lumière PAR, sont donc peu atténués par les molécules d'eau. En revanche, ils sont très efficacement absorbés par la matière organique colorée dissoute et particulaire présente dans la colonne d'eau, dont la concentration définit la turbidité de l'eau. Là aussi, l'absorption est inégale en fonction de la longueur d'onde incidente : les UVB sont atténués beaucoup plus rapidement que les UVA et que le PAR (Blough et al. 1993). Ainsi, les UVA représentent 95% du rayonnement UV reçu par les coraux tandis que les UVB n'en représentent que 5%. La turbidité de l'eau est souvent liée à l'éloignement à la côte : les zones proches du continent étant souvent plus riches en matière organique en raison de l'influence des apports terrigènes et anthropiques (Kleypas et al. 1999). La profondeur de pénétration de la lumière et en particulier des UVs varie énormément en fonction de la localisation géographique du récif. Ainsi, la pénétration des UVAs peut aller de 0.5 m dans les eaux côtières à plus de 50 m au large et celle des UVB peut atteindre 15 à 20 m de profondeur (Tedetti and Sempéré 2006).

Enfin, le dernier paramètre important qui définit la disponibilité de la lumière pour les coraux est **la profondeur du récif**. Plus le récif est profond, plus l'intensité lumineuse diminue et son spectre se restreint. Un récif profond recevra en majorité de la lumière bleue et des UVA tandis qu'un récif peu profond sera soumis à l'ensemble du spectre visible ainsi qu'aux deux types d'UVs (Figure 8). Sur un récif, les coraux peuvent croître à différentes profondeurs et être disposés de sorte que certaines colonies se trouvent davantage à l'ombre que d'autres (morphologie de la colonie, ombrage entre colonies, relief du récif *etc.*). Cela implique une grande variabilité de lumière même au sein d'un même récif.

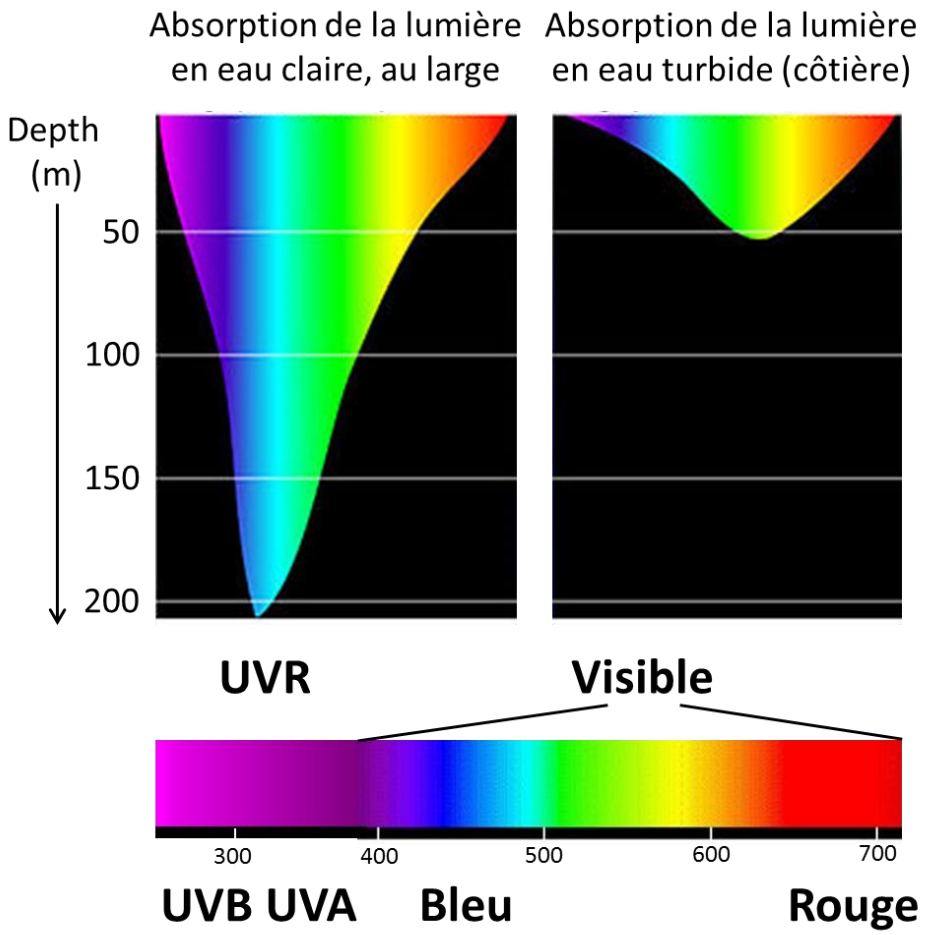


Figure 8. Spectre et spectre d'absorption de la lumière dans l'eau de mer.

En résumé, la quantité d'UVs reçu par les coraux dépend de la latitude du récif, de sa profondeur et de sa morphologie, de la turbidité de l'eau, de la saison, de la météo et du moment de la journée.

II. 1. 2. La température, conditions et variabilité

La température de surface (Figure 9) est un des facteurs déterminant expliquant la répartition géographique des récifs coralliens. En effet, bien que certains récifs grandissent dans des zones relativement extrêmes (températures descendant jusqu'à 16 °C au Nord du golfe Persique ou s'élevant à 34.4°C à l'intérieur du golfe), la majorité des récifs évoluent dans une gamme de températures relativement restreinte (entre 24.8 °C à 30.2°C, Kleypas et al. 1999), correspondant au pourtour de la ceinture équatoriale (Figure 9). La température de l'eau des récifs **varie au cours de la journée,** avec le cycle solaire et les marées, et en fonction de la saison. Les zones près des côtes, soumises à l'effet des marées et peu profondes (faible épaisseur de couche d'eau à refroidir ou à réchauffer), sont plus sensibles aux variations journalières de température (Soloviev and Lukas 2006).

Les **variations saisonnières** au large des côtes sont entre 2 et 8°C et peuvent aller jusqu'à 15°C près des côtes. De ce fait, dans une même région géographique, les coraux sont soumis à différents régimes thermiques en fonction de leur éloignement à la côte ; les récifs frangeants étant donc soumis à des variations journalières (cycle solaire et marées) et saisonnières plus grandes que les récifs barrières ou les atolls. En Nouvelle Calédonie par exemple, les températures varient entre 22°C l'hiver austral et 28-29°C l'été austral (Andréfouët et al. 2009).

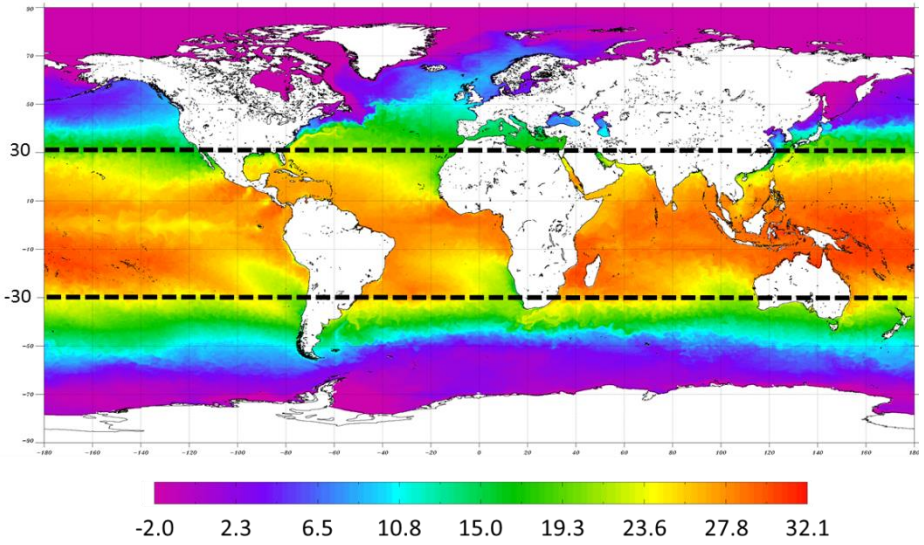


Figure 9. Carte de la température de surface des océans (janvier 2017). Les pointillés délimitent la zone autour de la ceinture équatoriale dans laquelle sont recensés la plupart des récifs coralliens. Source : http://www.ospo.noaa.gov/data/sst/contour/global_small.cf.gif

II. 1. 3. Les sels nutritifs

Les sels nutritifs sont des composés inorganiques ou organiques dissous dans l'eau qui sont nécessaires au fonctionnement des coraux (D'Elia 1977; Muscatine and D'elia 1978). Les nutriments majeurs sont l'azote et le phosphore. **L'azote** participe à la synthèse des acides aminés (protéines) et des acides nucléiques (ADN, ARN), il constitue donc l'unité de base des organismes. Les coraux utilisent majoritairement l'azote sous les formes de nitrate (NO_3^-), et d'ammonium (NH_4^+) pour les formes inorganiques (l'ammonium étant la forme la plus facilement ingérée, Grover et al. 2002), et sous les formes d'urée et d'acides aminés pour les formes organiques (Capone et al. 2008; Grover et al. 2008). **Le phosphore** est également un élément indispensable à la vie puisqu'il entre dans la composition de l'ADN et de l'ARN et qu'il est présent dans les phospholipides qui forment les membranes cellulaires. C'est aussi un constituant de l'ATP (l'adénosine-5'-triphosphate), la monnaie énergétique nécessaire à la réalisation des réactions chimiques et des mécanismes enzymatiques (Elser 2012; Ferrier-Pagès et al. 2016). Les coraux utilisent surtout le phosphore sous ses formes

inorganiques dissoutes ioniques (PO_4^{3-}, HPO_4^{2-}, H_2PO^{4-}, H_3PO_4 en fonction du pH) et phosphates condensés (Björkman and Karl 1994; Godinot et al. 2011b).

Les concentrations d'azote et de phosphore inorganiques dans l'eau des récifs sont faibles (conditions **oligotrophes**, Sorokin 2013). En effet, 90% des récifs ont moins de 0.6 µmol L^{-1} d'azote inorganique et moins de 0.20 µmol L^{-1} de phosphate inorganique (Kleypas et al. 1999). Aujourd'hui, avec l'augmentation de l'activité humaine (urbaine, agricole et industrielle) et le déversement des eaux usées, certains récifs sont exposés à des concentrations bien plus fortes en nutriments comme en Polynésie Française par exemple où la teneur en nitrate atteint 31 µM sur le site de Vaiare (Rouzé et al. 2015) ou encore à Guam (Tumon Bay), où la concentration excède 8.4 mg L^{-1} (Denton et al. 2005). L'enrichissement des eaux en nutriments, appelé **eutrophisation,** est souvent associé à un déclin des récifs coralliens (D'Angelo and Wiedenmann 2014). Ses effets sont développés dans la partie II.2.3 de l'introduction.

II.2. Les effets des paramètres environnementaux sur les coraux

II. 2. 1. La lumière

La qualité et la quantité de lumière sont parmi les paramètres environnementaux qui affectent le plus les coraux symbiotiques (Dubinsky et al. 1984; Falkowski et al. 1984, 1990; Iglesias-Prieto et al. 2004). La lumière est indispensable au fonctionnement de la symbiose puisqu'elle est l'élément principal de la photosynthèse, qui contribue (jusqu'à 100 %) aux besoins énergétiques de l'holobionte (Muscatine et al. 1981). Les UVs peuvent également se révéler très importants puisqu'ils sont responsables de l'activation des mécanismes de réparation de l'ADN et peuvent favoriser les défenses contre les infections bactériennes chez certaines espèces de coraux (Fine et al. 2002). Cependant, une trop forte dose de lumière ou d'UVs peut être une cause de stress important pouvant à terme conduire à la rupture de la symbiose (Hoegh-Guldberg 1999; Hoegh-Guldberg and Jones 1999). Les UVs peuvent causer des dommages moléculaires, cellulaires et physiologiques importants mais ils entraînent également la formation de dérivés réactifs de l'oxygène (ROS), des molécules instables qui contribuent à l'augmentation du stress oxydant dans les organismes (résumé des effets Figure 10). Seuls ces effets sur les coraux seront développés dans les paragraphes suivants. Ils sont résumés dans le Table 3.

Les effets directs des UVs

Chez les coraux, les UVs sont absorbés par les pigments de l'hôte et des symbiotes, les acides aminés aromatiques et les bases pyrimidines de l'ADN. Si les doses sont trop fortes, elles peuvent

causer des dommages aux molécules qui l'absorbent, et notamment à l'ADN. En effet, les photons provoquent la dimérisation de paires de bases (deux bases qui se lient entre elles) et une interruption de la chaine d'acides aminés qui rend la réplication de l'ADN impossible (Sinha and Häder 2002; Baruch et al. 2005). Les UVs peuvent également altérer les pigments photosynthétiques et diminuer l'efficacité de la photosynthèse des symbiotes (Halldal 1968; Fitt and Warner 1995; Ferrier-Pagès et al. 2007; Torres-Pérez and Armstrong 2012). Ce phénomène a particulièrement été observé lorsque des coraux de profondeur (>20 m), qui reçoivent peu ou pas de radiations UVs dans leur environnement naturel, sont transplantés à faibles profondeurs (Masuda et al. 1993; Shick et al. 1995; Cohen et al. 2013).

La dégradation des pigments et la baisse de la photosynthèse liée à l'exposition aux UVs peut entraîner la perte des symbiotes et favoriser le blanchissement (Lesser et al. 1990a; Gleason and Wellington 1993; Torres-Pérez and Armstrong 2012; Cohen et al. 2013). Cela a été observé en 1985 lors du blanchissement majeur sur la grande barrière de corail en Australie, les scientifiques avaient alors observé que les coraux blanchis étaient situés dans des récifs peu profonds ou dans des eaux très claires avec forte pénétration des radiations UVs (Fisk and Done 1985; Harriott 1985). Ces effets nocifs ont été nuancés par d'autres études montrant que des espèces telles que *Pocillopora damicornis*, *Acropora validata* ou *Porites lobata* n'étaient pas affectées par une augmentation des UVs seuls (Jokiel and York 1982; Glynn et al. 1993; D'Croz et al. 2001). Par contre, ces mêmes espèces ainsi que d'autres exposées à une augmentation simultanée de la température et du rayonnement UV avaient blanchi plus fortement que sous un stress de température seul (Glynn et al. 1993; Fitt and Warner 1995; Lesser 1996; D'Croz and Mate 2000; Lesser and Farrell 2004; Ferrier-Pagès et al. 2007), suggérant que le rayonnement UV est un facteur qui aggrave les effets de la température. D'Croz et Mate (2000) ont également observé une meilleure récupération des coraux après blanchissement lorsqu'ils sont protégés des radiations UVs. Avec le changement climatique et l'augmentation de la fréquence des phénomènes de blanchissement, les observations de D'Croz et Mate suggèrent que des récifs exposés aux UVs auront plus de difficultés à retrouver un équilibre entre deux périodes de blanchissement et seront donc plus fragiles face à un nouveau stress de température.

En plus d'affecter la symbiose et de provoquer le blanchissement, les UVs semblent également avoir des effets néfastes sur la croissance et la reproduction du corail. Cependant, là encore, les résultats des expériences menées en conditions contrôlées montrent des différences de sensibilité entre espèces. Alors que certaines espèces telles que *Pocillopora damicornis*, *Porites furcata* ou *Porites asteroides* ont présenté une croissance ralentie sous UVs (Jokiel and York 1982; Gleason 1993; Torres-Pérez and Armstrong 2012) d'autres espèces telles qu'*Acropora validata* ou *Porites compressa* n'ont-elles pas été affectées (Glynn et al 1993; Kuffner 2002). De même, les

résultats sont contradictoires en ce qui concerne l'effet des UVs sur la reproduction. En effet, alors que le nombre de larves pondues par *Pocillopora damicornis* est plus grand sous UVs (Jokiel and York 1982), la fécondité de *Porites furcata* et la survie des larves d'*Acropora palmata* et de *Montastrea annularis* se trouvent elles réduites (Wellington and Fitt 2003; Torres-Pérez and Armstrong 2012). Les observations ci-dessus suggèrent donc que des travaux complémentaires sont nécessaires pour confirmer ou infirmer ces résultats préliminaires.

A ma connaissance, aucune étude ne s'est intéressée aux effets des UVs sur les flux de matière organique et de nutriments entre le corail et son environnement. Pourtant, la lumière, qui est étroitement liée à l'activité photosynthétique des symbiotes, est un facteur connu pour influencer les taux d'absorption d'azote et de phosphore (Muscatine and D'elia 1978; Wilkerson and Trench 1986; Grover et al. 2002) ainsi que l'excrétion de matière organique des coraux (Crossland 1987; Naumann et al. 2010).

Les effets indirects des UVs : le stress oxydant

En plus de leurs effets directs, les UVs peuvent également endommager les organismes via la formation de dérivés réactifs de l'oxygène (**ROS,** voir la revue de Lesser, 2006). Ces espèces sont formées par la réduction du dioxygène lors de la respiration et de la photosynthèse, ainsi que lors de la formation d'ATP dans les mitochondries. Ces réactions entraînent la formation d'oxydes d'anion (O_2^-) très instables, qui réagissent ensuite pour former des espèces telles que le peroxyde d'hydrogène (H_2O_2) et les radicaux d'hydroxyde (HO^-). Lorsqu'ils sont formés naturellement, ces composés sont qualifiés d'endogènes et servent notamment de messagers secondaires dans les voies de signalisation moléculaires des cellules. Dans certaines conditions de stress telles qu'une forte exposition aux UVs ou lors d'un stress thermique, la quantité de ROS, alors qualifiés d'exogènes, peut fortement augmenter. L'accumulation de ces espèces instables est appelée **stress oxydant**. Si ce dernier n'est pas contenu par les mécanismes de défense de l'organisme (voir paragraphe suivant), il peut causer d'importants dommages cellulaires (effets toxiques sur l'ADN, les enzymes, les membranes protéiques et lipidiques, les photosystèmes des symbiotes *etc.*). En plus de sa réactivité propre, O_2^- peut également réagir avec l'oxyde de nitrite (NO) pour former des dérivés réactifs de l'azote (**NOx**). Ces composés sont eux aussi toxiques pour les cellules, car ils diffusent plus facilement que les ROS à travers les membranes, endommageant les lipides qui les constituent. Ils participent donc à l'augmentation du stress oxydant de l'organisme.

En raison de la difficulté à quantifier les ROS chez ces organismes, les preuves de l'induction du stress oxydant par les UVs chez les coraux sont majoritairement indirectes. Les études menées jusqu'alors ont montré une corrélation entre l'exposition aux UVs et l'augmentation de l'activité des

enzymes de défenses contre le stress oxydant (voir paragraphe suivant) dans les coraux et les *Symbiodinium* isolées (Lesser 1989, 1996b; Lesser and Shick 1989; Shick et al. 1991, 1995).

Les mécanismes de défense

L'ensemble des résultats présentés précédemment souligne la complexité et la diversité des réponses des coraux aux rayonnements UVs. Ils mettent en évidence que les effets des UVs sur le corail dépendent de l'espèce et des conditions de croissance des colonies mères utilisées pour réaliser les expériences. En effet au cours de l'évolution, mais aussi en fonction de leur environnement de croissance, certaines espèces se sont adaptées et acclimatées aux fortes irradiances, ce qui leur permet d'y faire face plus facilement. L'adaptation et l'acclimatation des coraux impliquent au moins deux types de mécanismes : les **mécanismes de photoprotection qui réduisent l'exposition aux UVs** et les **mécanismes de réparation et de défense qui limitent les effets des UVs** (Gleason 2001; Titlyanov and Titlyanova 2002; Banaszak and Lesser 2009).

La photoprotection passe par le contrôle du niveau d'énergie absorbé dans les cellules en 1) diminuant l'absorption par les pigments photosynthétiques, qui, si elle est trop importante, peut endommager les photosystèmes et augmenter le stress oxydant dans les tissus et 2) en augmentant l'absorption du surplus d'énergie par des molécules spécialisées capables de neutraliser ses effets. La quantité d'UVs absorbée par les pigments photosynthétiques peut être contrôlée par les symbiotes via la diminution de la concentration en pigments ou par l'hôte via le contrôle de la quantité de symbiotes dans ses tissus, le développement de clades de symbiotes plus résistants, et la modification de la morphologie de la colonie pour limiter l'exposition à la lumière (Falkowski and Dubinsky 1981; Berner et al. 1987; Muller-Parker et al. 1996). En parallèle, d'autres molécules spécialisées jouent le rôle de « crème solaire » en absorbant les UVs et dissipant leur énergie. Les principales sont des métabolites secondaires présents dans les tissus de l'hôte et des symbiotes connus sous le nom d'acides aminés mycosporine-like (**MAAs**) (Shick et al. 1995, 1996). Il existe une vingtaine de MAAs dont l'ensemble permet l'absorption de la lumière entre 300 et 306 nm (Shick and Dunlap 2002). Ils sont majoritairement synthétisés par les algues symbiotiques via la voie du shikimate (Shick and Dunlap 2002) et transférés ensuite presque en totalité à l'hôte. En effet, 95% des MAAs sont accumulés dans l'ectoderme de l'hôte qui forme ainsi la première barrière anti-UV de l'organisme. La concentration de ces composés dans les coraux est proportionnelle à la quantité d'UVs auxquels ils sont soumis (Shick and Dunlap 2002). Ainsi, des coraux vivant dans un environnement sans UVs sont dépourvus de MAAs. En revanche, s'ils sont de nouveau exposés, ils sont capables de synthétiser des MAAs en 7 jours (Shick 2004).

Si les mécanismes de photoprotection sont insuffisants, les UVs pénètrent dans les cellules et causent des dommages à l'ADN et une augmentation de l'état de stress oxydant des cellules. Les coraux ont alors plusieurs **mécanismes de réparation et de défense** qu'ils mettent en place pour réduire ces effets nocifs. Les organismes marins ont développés des **mécanismes de réparation** de l'ADN, impliquant une enzyme particulière appelée photolyase dont l'activation nécessite la présence d'UVA (Siebeck 1981, 1988). Cette enzyme n'a pas encore été étudiée chez les coraux mais est une hypothèse probable pour pallier aux dommages causés par les UVs. Le **système de défense contre le stress oxydant** est en revanche mieux connu. Il implique un grand nombre d'enzymes (superoxyde dismutase, catalase, ascorbate glutathione peroxydase etc.), et de molécules non enzymatiques telles que certains pigments de la famille des caroténoïdes et des xanthophylles, dont le rôle est de détoxifier les ROS (Demmig-Adams and Adams III 1992; Rozema et al. 2002; Lesser 2006). L'ensemble de ces mécanismes sont activés lorsque les coraux sont exposés aux UVs : l'activité des enzymes du stress oxydant et la concentration de diadinoxanthin et β-carotène dans les *Symbiodinium* augmentent (Lesser et al. 1990; Lesser and Shick 1989). Cela permet notamment aux coraux de faire face à la grande variabilité d'irradiance imposée par les variations journalières et saisonnières.

Si les mécanismes de défense des coraux contre les UVs commencent à être connus, il y a très peu d'information sur les voies de signalisation moléculaires qui sont impliquées dans le déclenchement de ces défenses. En d'autres termes, nous ne connaissons pas les intermédiaires mis en jeux entre la détection du stress et la réponse des animaux. Chez l'hydre et d'autres organismes marins, certaines voies de signalisation impliquant des protéines kinases nommées « mythogen-activated protein kinase » (MAPK) sont connues pour jouer des rôles important dans la réponse à un stress de température ou dans la gestion du stress oxydant (Anestis et al. 2008; Bridge et al. 2010; Yao and Somero 2012; Garcia-Gomez et al. 2012; Parages et al. 2013). Ces protéines et leurs fonctions sont très conservées dans tout le règne animal et végétal (Widmann et al. 1999) suggérant une possible conservation des mécanismes chez les coraux.

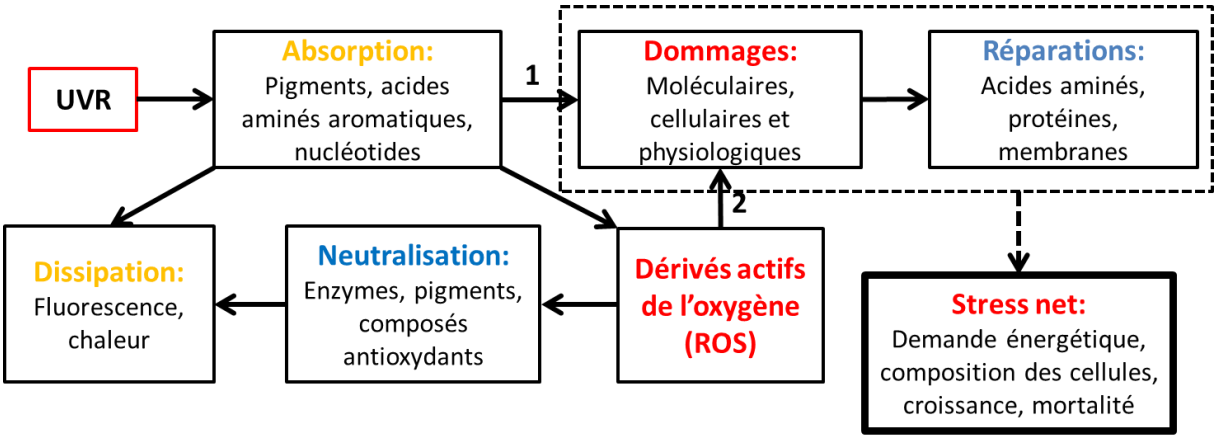


Figure 10. Effets des UVs sur les coraux. L'énergie issue de l'*absorption* des UVs peut être soit dissipée (*dissipation*) sous forme de fluorescence ou de chaleur soit provoquer des *dommages* à l'organisme. Les dommages peuvent être directement induits par la dégradation photochimique des *cellules* et des bio*molécules* (1) ou indirectement induits via la production de *dérivés actifs de l'oxygène* (2). L'organisme peut activer des mécanismes de *réparation* des dommages ou de *neutralisation* des ROS. Le stress net se traduit par une augmentation de la *demande énergétique*; par des changements à l'échelle cellulaires (*composition des cellules* ; ex: contenu lipidique); par un ralentissement de la *croissance* du aux dommages causés et à l'allocation de l'énergie à d'autres mécanismes; et par une augmentation de la *mortalité* si le stress est trop long.

Table 3. Récapitulatif des connaissances sur les effets des UVs sur les coraux.

Paramètres biologiques	Effets des UVs	Espèces/récifs	Références
Croissance et calcification	- Absorption de ^{45}Ca négativement corrélée à l'intensité en UVA	*Pocillopora damicornis*	Roth et al. 1982
	- Diminution de la croissance	*Pocillopora damicornis* *Porites furcata*	Jokiel and York 1982 Torres-Pérez and Armstrong 2012
	- Diminution de la calcification	*Porites asteroides*	Gleason 1993
	- Pas d'effet sur la croissance ou la calcification	*Acropora validata* *Porites compressa*	Glynn et al. 1993 Kuffner 2002
Blanchissement	- Observation in situ : le blanchissement n'a lieu qu'à très faible profondeur ou en profondeur dans des eaux très claires.	GBR Caraïbes	Fisk and Done 1985; Harriott 1985 Goenaga et al. 1989
	-Diminution de la quantité de symbiotes	*Palythoa caribaerum* *Montastrea annularis* *Stylophora pistillata*	Lesser et al. 1990 Gleason and Wellington 1993 Cohen et al. 2013
	-Diminution de la quantité de pigments photosynthétiques	*Porites furcata*	Torres-Pérez and Armstrong 2012
	- Pas de blanchissement suite à une exposition aux UVs seuls	*Pocillopora damicornis* *Acropora validata* *Porites Lobata*	Jokiel and York 1982; Glynn 1993 Glynn et al. 1993 D'Croz et al. 2001
	- Les UVs renforcent les effets négatifs de la température	*Pocillopora damicornis* *Acropora validata* *Montastrea cavernosa, annularis* *Agaricia agaricia, marcki*	Glynn et al. 1993 Glynn et al. 1993 Fitt and Warner 1995 Fitt and Warner 1995
	- Les coraux récupèrent moins bien d'un blanchissement sous UVR	*Pocillopora damicornis*	D'Croz and Mate 2000
Photosynthèse et respiration	- Diminution de l'efficacité photosynthétique Fv/Fm	Symbiote de *Montastrea annularis* *Stylophora pistillata*	Fitt and Warner 1995 Ferrier-Pagès et al. 2007
	-Diminution de la photosynthèse dans des coraux de profondeur (>20 m) exposés aux UVR	*Fungia spp.* *Acropora microphtalma* *Stylophora pistillata*	Masuda et al. 1993 Shick et al. 1995 Cohen et al. 2013
	- Inhibition de la photosynthèse	*Pocillopora damicornis*	Lesser and Lewis 1996
	- Photo-oxydation de la chlorophylle sous UVB et stimulation de la photosynthèse sous UVA	*Symbiotes de Favia pallida*	Halldal 1968

Paramètres biologiques	Effet des UVR	Espèce/récif	Références
Reproduction	- Augmentation du nombre de larve pondu	*Pocillopora damicornis*	Jokiel and York 1982
	- Diminution de la fécondité	*Porites furcata*	Torres-Pérez and Armstrong 2012
	-Diminution de la survie des larves	*Acropora palmata, Montastrea annularis,*	Wellington and Fitt 2003
ADN et biomolécules	- Augmentation du nombre de cassures dans l'ADN sous UVB	*Stylophora pistillata* *Porites Porites*	Baruch et al. 2005 Anderson et al. 2001
Micosporin like amino acids (MAAs)	- Augmentation de la concentration de MAAs	*Porites furcata* *Montipora verrucosa* *Porites compressa* *Pocillopora damicornis* *Montastraea annularis* *Stylophora pistillata*	Torres-Pérez and Armstrong 2012 Torregiani and Lesser 2007 Kuffner 2002 Jokiel et al. 1997 Muszynski et al. 1998 Shick et al. 1999
	-Sans UVs, la concentration de MAAs diminue	*Porites compressa*	Kuffner 2002
	-Synthèse de MAAs dans les 7 jours qui suivent l'exposition aux UVR et concentration maximal atteinte après 14 jours	*Stylophora pistillata*	Shick 2004
Stress oxydant	- Augmentation de l'activité des enzymes du stress oxydant	*Palythoa caribaeorum* *Symbiodinium bermudense*	Lesser et al. 1990 Lesser and Shick 1989
	- Augmentation de la production de ROS	*Symbiodinium bermudense* *Palythoa caribaeorum*	Lesser 1996 Lesser et al. 1990

II. 2. 2. La température

Effets sur la physiologie du corail et mécanismes de défense

La température est un paramètre primordial contrôlant le métabolisme et la croissance des coraux (Kleypas et al. 1999). En effet, la plupart des réactions chimiques de l'organisme sont catalysées par des enzymes dont l'activité dépend principalement de la température. L'activité enzymatique est minimale à faible température puis augmente jusqu'à un optimum de température au-dessus duquel elle est ensuite inhibée. Par conséquent, la photosynthèse, la respiration et la calcification augmentent de façon linéaire avec la température, jusqu'à un optimum qui dépend très souvent de l'espèce étudiée, avant de diminuer de nouveau (Coles and Jokiel 1977; Ip et al. 1991; Al-Horani 2005; Castillo and Helmuth 2005). L'optimum de température pour la calcification est d'ailleurs souvent le même que celui de la photosynthèse (light-enhanced calcification, Allemand et al. 2004). Comme statué précédemment (Introduction, §II.1.2.), la température sur un récif peut varier fortement au cours de l'année entraînant des variations annuelles du métabolisme avec un maximum en été et un minimum en hiver (Crossland 1984). Pour compenser ces changements, les coraux sont capables de diminuer la quantité de symbiotes lorsque le métabolisme est à son maximum pendant l'été (Stimson 1997; Brown et al. 1999; Fagoonee et al. 1999; Fitt et al. 2000) et ré-augmenter la quantité de symbiotes présents dans leur tissu pour rehausser leur métabolisme en hiver.

La frontière entre les effets bénéfiques et nocifs de la température est mince. En effet, une température trop élevée est responsable des phénomènes de blanchissement et de fortes mortalités sur les récifs (Hoegh-Guldberg 1999). En plus d'inhiber les enzymes du métabolisme, elle dénature les protéines et altère leur fonction, ce qui a pour conséquences de bloquer les réactions chimiques indispensables au fonctionnement de l'organisme et d'augmenter le stress oxydant dans les cellules (voir Introduction §II.2.1). En dégradant certains complexes protéiques, la température favorise la synthèse de ROS et empêche leur neutralisation en réduisant l'activité des enzymes du stress oxydant (Feierabend et al. 1992; Havaux 1992; Downs et al. 2002). Pour limiter les dommages causés par un stress thermique, les coraux synthétisent des protéines spécialisées appelées protéines de choc thermique qui permettent le maintien de la fonction d'autres protéines auxquelles elles s'associent. Certaines protègent par exemple la fonction des transporteurs d'électron de la photosynthèse et de la respiration, limitant ainsi la production de ROS (Downs and Heckathorn 1998; Downs et al. 1999; Ganea and Harding 2000). En dernier recours, lorsque le stress thermique est trop important et que les coraux ne sont plus capables de contenir le stress oxydant, ils expulsent de manière massive les symbiotes pour limiter la formation de ROS via la photosynthèse et la

respiration (Downs et al. 2002). Si le stress perdure, la quantité de symbiontes n'est plus suffisante pour que le corail maintienne ses fonctions primaires et la colonie meurt.

Conséquences sur les flux de matière organique et de nutriments

Comme détaillé précédemment (voir Introduction §I.3.2), le corail excrète de la matière organique sous forme de mucus qui joue un rôle primordial pour la nutrition et la protection de l'animal et qui est une composante majeure des flux de matière dans le récif. Les effets de la température sur l'excrétion du mucus ont été très peu étudiés bien que cet aspect puisse avoir des conséquences importantes sur le corail et l'écosystème associé. Les travaux réalisés jusque-là ont montré une grande hétérogénéité dans la quantité et la qualité (particulaire ou dissoute) du mucus excrété en fonction de l'espèce corallienne et de la localisation des récifs (Crossland 1987; Wild et al. 2010a; b; Houlbrèque et al. 2004a; Wild et al. 2004, 2005, 2008, Tanaka et al. 2008, 2009; Haas et al. 2010; Naumann et al. 2010; Levas et al. 2015). Levas et collaborateurs (2015) par exemple n'ont mesuré aucune variation dans la qualité ou la quantité de matière organique excrétée par *Turbinaria reniformis* et *Acropora sp.,* en fonction de la température. En revanche, plusieurs autres études ont observé une augmentation de l'excrétion de DOC et/ou de POC en été ou sous un stress thermique (Niggl et al. 2008; Haas et al. 2010; Naumann et al. 2010). Inversement, le corail peut absorber la matière organique dissoute ou particulaire de la colonne d'eau et l'utiliser comme source d'énergie pour son métabolisme. Cette nutrition hétérotrophe lui permet de compenser la diminution d'apports autotrophes à forte température (Houlbrèque et al. 2004b; Grottoli et al. 2006; Houlbrèque and Ferrier-Pagès 2009; Tremblay et al. 2012; Levas et al. 2016). Même si les conséquences de tels changements dans l'excrétion de matière organique par les coraux n'ont pas encore été étudiées, il est probable que les micro-organismes associés au mucus et ceux de la colonne d'eau en soient affectés. Dès lors, le recyclage de l'azote et du carbone sur l'ensemble du récif peut être modifié.

II. 2. 3. Effets de la disponibilité en sels nutritifs

Comme présenté précédemment**,** les coraux ont besoin d'incorporer des nutriments pour synthétiser les composés indispensables à leur fonctionnement. Une carence en nutriments, ou au contraire, une trop forte disponibilité peut perturber le fonctionnement de la symbiose.

Conséquences d'un manque de sels nutritifs

Peu d'études se sont intéressées à l'effet d'un manque en nutriments sur la physiologie du corail. Pourtant, certaines zones géographiques où se développent les coraux sont ultra-oligotrophes (Kleypas et al. 1999). Le métabolisme et la croissance des coraux y sont-ils limités? Ces coraux

répondent-ils de la même façon aux variations environnementales? Les quelques travaux réalisés sur le sujet suggèrent qu'une quantité minimale de sels nutritifs est requise pour que les coraux soient en bonne santé. De trop faibles concentrations en azote (<0.1 µM) et en phosphore (<0.01 µM) (conditions ultra-oligotrophes) entraînent une diminution de la densité en symbiotes, de la concentration en pigments chlorophylliens, de la photosynthèse et de la calcification par rapport à des conditions oligotrophes ou mésotrophes (Titlyanov et al. 2001; Wiedenmann et al. 2013; Ezzat et al. 2015; Rosset et al. 2015; Ezzat in *prep*). La loi du minimum qui stipule que le développement d'un organisme est limité par l'élément nutritif qui vient à manquer en premier (von Liebig 1855) implique que quelle que soit la concentration de l'ensemble des sels nutritifs, si un élément est limitant, le développement du corail sera diminué. Ceci a été observé dans plusieurs expériences où les coraux ont été placés dans des conditions limitées en phosphore, mais pas en azote. Ces conditions ont créé un déséquilibre nutritionnel au sein de l'holobionte entraînant 1) une diminution de la densité en symbiotes et de l'efficacité photosynthétique, 2) une modification de la composition des membranes lipidiques et 3) une susceptibilité au blanchissement plus importante (Marubini and Davies 1996; Ferrier-Pagès et al. 2001; Wiedenmann et al. 2013; D'Angelo and Wiedenmann 2014; Ezzat et al. 2016a). Jacobson et collaborateurs (2016) ont également montré que la limitation en nutriments pouvaient provoquer une dépression métabolique c'est-à-dire une réduction du taux de respiration et de la biomasse sans conséquence sur la production de squelette calcaire. Ceci pourrait être une stratégie de certaines espèces pour maintenir la compétitivité dans l'espace durant des périodes de limitation chronique en nutriments. Ces travaux apportent des premiers éléments de réponse aux questions que pose la déplétion en sels nutritifs. Cependant, d'autres études sont nécessaires pour compléter ces réponses et notamment pour mieux comprendre les effets combinés des changements environnementaux en conditions ultra-oligotrophes.

Conséquences d'un excès de sels nutritifs

L'usage excessif de fertilisants, le défrichement des terres agricoles et les exutoires d'eaux usées résultant des activités anthropiques, augmentent fortement les quantités d'azote et de phosphore dans les zones côtières. Ces enrichissements non naturels peuvent perturber les récifs coralliens de plusieurs manières: 1) en affectant la physiologie du corail, 2) en favorisant le développement bactérien induisant la propagation de maladies coralliennes (Ruiz-Morenol et al. 2012; Vega Thurber et al. 2014; Zaneveld et al. 2016), et 3) en créant un déséquilibre dans la compétition algue/corail qui favorise la prolifération de la biomasse algale (Pomeroy 1974; Lapointe et al. 2004; Smith and Schindler 2009). L'effet direct des sels nutritifs sur la physiologie corallienne est encore peu compris car les résultats obtenus jusqu'à présent dépendent de la forme azotée (nitrate ou ammonium), de la disponibilité en nutriments organiques, du contexte environnemental

et de l'état physiologique du corail (Fabricius et al. 2013; Ezzat 2016; Ezzat et al. 2016b). Dans l'ensemble, un double enrichissement en azote et en phosphate profite aux symbiotes (augmentation de la chlorophylle et/ou de la quantité de symbiotes) plus qu'à l'hôte (Muscatine et al. 1989; Tanaka et al. 2007; Wiedenmann et al. 2013; Wooldridge 2013; Ezzat et al. 2015, 2016b). En effet, pour satisfaire leurs besoins métaboliques, les symbiotes en croissance retiennent le carbone autotrophe et limitent la quantité transférée à l'hôte (Ezzat et al. 2015), ce qui entraîne souvent une diminution de la calcification (Renegar and Riegl 2005; Tanaka et al. 2007; Holcomb et al. 2010). En fonction de l'espèce corallienne concernée, la prolifération des symbiotes peut être bénéfique ou néfaste. Pour les espèces comme *Turbinaria reniformis* ayant, dans des conditions normales, une faible quantité de symbiotes, l'enrichissement permet de compenser les effets néfastes de la température et de maintenir des taux de photosynthèse et de calcification élevés même pendant un stress de température (Shantz and Burkepile 2014; Ezzat et al. 2016b). En revanche, pour d'autres espèces telles que *Pocillopora damicornis*, la densité trop forte de symbiotes peut entrainer un stress oxydant nocif pour l'organisme et ainsi accentuer les effets négatifs de la température ou des UVs (Cunning and Baker 2013). L'eutrophisation pourrait alors favoriser certaines espèces et induire des modifications dans la répartition et la composition des espèces au sein d'un récif.

L'enrichissement en nutriments peut également se faire de manière déséquilibrée en fonction de la source de pollution. Par exemple, les régions sous l'influence d'intenses activités agricoles ou urbaines sont souvent enrichies en azote (Howarth et al. 1996; Carpenter et al. 1998) tandis que les zones sous l'influence des eaux usées ou de l'activité bovine sont souvent enrichies en phosphore (Conley et al. 2009). Les récifs coralliens peuvent donc être soumis à des enrichissements partiels créant un déséquilibre dans le rapport azote/phosphore (N:P). Un enrichissement en ammonium seul a un effet neutre ou positif sur la physiologie du corail en augmentant la photosynthèse et la croissance des symbiotes et de l'hôte (Meyer et al. 1983; Meyer and Schultz 1985; Bongiorni et al. 2003; Béraud et al. 2013). En condition de stress, l'apport d'ammonium permet de préserver les capacités photosynthétiques de l'holobionte (Béraud et al. 2013, Ezzat *in prep*). A contrario, un enrichissement en nitrate seul est délétère pour la photosynthèse et la calcification des coraux (Marubini and Davies 1996; Ferrier-Pagès et al. 2001; Wiedenmann et al. 2013; Ezzat et al. 2015). En effet, l'absorption de nitrate, très coûteuse en électrons, entre en compétition directement avec la photosynthèse (Dagenais-Bellefeuille and Morse 2013). De plus, le nitrate est une espèce réactive de l'azote qui, lorsqu'elle est accumulée dans les tissus, peut devenir néfaste pour les cellules ce qui pourrait accentuer les effets de la lumière et de la température (Lesser 2006). Un enrichissement modéré en phosphate seul profite à la fois aux symbiotes et à l'hôte en favorisant leurs croissances (Godinot et al. 2011a; Ezzat et al. 2016a) et peut augmenter la résistance des

coraux aux stress environnementaux (Tchernov et al. 2004; Ezzat et al. 2016a). Les effets de l'enrichissement en nutriments sur la physiologie du corail sont donc contrastés et dépendent du type d'enrichissement et de l'espèce corallienne considérée. Cependant, l'augmentation de la prolifération de maladies coralliennes et la domination des algues suite à l'eutrophisation entraînent souvent le déclin des récifs (Garren 2016).

III. Devenir des récifs coralliens, prévision, incertitudes et enjeux

III.1. Les récifs coralliens, un écosystème en danger

Comme détaillé dans la partie précédente, les conditions environnementales jouent un rôle primordial sur le développement des coraux. A la variabilité naturelle de ces paramètres s'ajoutent des pressions induites par la présence de l'Homme et ses activités. Ces pressions sont locales : eutrophisation (Introduction, §II.2.3.), pollutions, sédimentation, développement côtier non durable, surpêche, sur-fréquentation *etc.* ; ou globales : le changement climatique et l'acidification des océans. Dans cette thèse, seuls les effets de l'eutrophisation et du réchauffement climatique ont été étudiés. Néanmoins, les autres pressions et notamment l'acidification des océans (*i.e.* la diminution du pH de l'océan due à l'absorption du CO_2 atmosphérique dans l'océan) influencent également la physiologie du corail et doivent être considérés lorsque l'on évalue les risques de mortalité d'un récif (Hoegh-Guldberg et al. 2007; Pandolfi et al. 2011).

III. 1. 1. Le changement climatique : définition et conséquences au niveau des zones récifales

Le « **réchauffement climatique** » désigne l'augmentation de la température de la basse atmosphère et de la surface de la terre liée à l'augmentation de l'**effet de serre.** Le terme « **Changement climatique** » inclue le réchauffement climatique et ses conséquences sur le climat et l'océan. L'effet de serre est un phénomène thermique où une couche de gaz de l'atmosphère (appelés gaz à effet de serre) joue un rôle de couverture en piégeant la chaleur que la Terre émet sous forme d'infrarouge et en la réémettant ensuite dans toutes les directions, y compris la Terre. Ce phénomène naturel réchauffe la surface de la Terre et la basse atmosphère et permet de maintenir une température de surface propice à la vie. Les gaz à effet de serre tels que la vapeur d'eau, le CO_2 et le méthane sont produits de manière naturelle par exemple via l'évaporation de l'eau de surface, le volcanisme. Depuis la révolution industrielle, l'activité humaine est devenue une source importante (et souvent majoritaire) de gaz. En effet, les dégagements industriels et domestiques, la déforestation, la culture des sols, l'utilisation des énergies fossiles *etc.* sont autant de sources qui

renforcent l'effet de serre (Santer et al. 1996). Un tel renforcement est la cause du réchauffement climatique qui a des conséquences nombreuses incluant l'augmentation des températures de l'atmosphère et de l'océan, des modifications de la circulation atmosphérique, le dérèglement des précipitations, l'augmentation de la fréquence des phénomènes météorologiques extrêmes tels que les tempêtes ou les cyclones, l'augmentation du niveau marin *etc.* (*i.e.* Collins et al. 2013; Vose et al. 2014).

Toutes les perturbations citées ci-dessus impactent les récifs coralliens, en particulier la température de l'eau dont la plus grande augmentation est attendue en surface dans les zones tropicales et subtropicales. Différents scénarios dépendant principalement de la quantité de gaz rejetée à l'avenir par l'activité humaine, sont envisagés pour prédire l'augmentation de température de l'océan. Le plus optimiste prévoit une augmentation de 0.6°C tandis que le scénario le plus pessimiste prévoit 2°C de plus d'ici 2050 (IPCC 2014). Outre l'augmentation de la température, les récifs coralliens sont inquiétés par l'accélération de la circulation de Brewer-Dobson, qui achemine l'ozone formé dans la stratosphère tropicale vers les zones subtropicales. Certains modèles prédisent une déplétion d'ozone dans la stratosphère induisant une augmentation de plus de 2% des UVB au niveau des récifs coralliens tropicaux (Butchart et al. 2010; Eyring et al. 2010; Watanabe et al. 2011). Cette pression sur les coraux est également augmentée par d'autres phénomènes climatologiques naturels dont la fréquence est amplifiée par le réchauffement climatique, tels que les cyclones ou le phénomène El Niño (Vose et al. 2014). L'accumulation des pressions environnementales sur les coraux favorise les épisodes de blanchissement dont l'ampleur et la fréquence augmentent (Nicholls et al. 2007; Collins et al. 2013). En 2016 par exemple, le phénomène El-Niño s'est renforcé et a entraîné des anomalies de température de surface allant jusqu'à +3°C dans certaines régions (https://coralreefwatch.noaa.gov) et provoqué de sévères épisodes de blanchissements coralliens (voir définition Introduction §II.2.2.) partout dans le monde (Hughes et al. 2017). Cette année encore (2017), un blanchissement massif a touché la grande barrière de corail (https://www.jcu.edu.au/news/releases/2017/april/two-thirds-of-great-barrier-reef-hit-by-back-to-back-mass-coral-bleaching).

Les quelques études qui ont suivi la croissance des récifs sur le long terme montrent une diminution nette de la couverture corallienne et un déclin des récifs. Par exemple, De'ath et collaborateur (2012) ont mis en évidence une perte de 50.7% du couvert corallien entre 1985 et 2012 sur la Grande Barrière de corail en Australie et le *Global Coral Reef Monitoring Network* (GCRMN) estime que 19 % des récifs mondiaux sont actuellement détruits et 15% sont sérieusement endommagés. L'encart 4 résume les conséquences du réchauffement climatique sur le blanchissement corallien et donne un aperçu des enjeux de la menace qui pèse sur les coraux.

Encart 4. Blanchissement des coraux, un réservoir de biodiversité menacé

L'article ci-dessous est paru dans le rapport « OCÉAN ET CLIMAT, 2016 – Fiches scientifiques, Deuxième édition, Tome 2 », p 42-46. Il a également été traduit en anglais (voir http://www.ocean-climate.org/wp-content/uploads/2016/10/internet-161024_ScientificNotes_Oct2016_BD_ppp.pdf ; ou en annexe 2).

ocean-climate.org

Blanchissement des coraux, un réservoir de biodiversité menacé

Leïla Ezzat
et Lucile Courtial

Depuis une trentaine d'années, la température moyenne des océans ne cesse d'augmenter, ce qui renforce l'intensité, la durée et l'extension du phénomène de blanchissement des coraux. En 2014-2016, suite à une hausse anormale de la température de l'eau de mer accentuée par un épisode *El Niño* très marqué, on constate un événement de blanchissement d'une intensité et d'une ampleur exceptionnelles qui pourrait s'étendre bien au-delà de l'année 2017. Les modèles climatiques prévoient une hausse de la température de surface des océans de 1 à 3 °C d'ici la fin du XXIe siècle, menaçant la survie des récifs du monde entier d'ici 2050. Le degré de résilience des récifs reste faible, voire ponctuel et le stress qu'ils subissent est accentué par d'autres facteurs d'origine anthropique (acidification, hausse du niveau des mers, surexploitation, pollutions…). Pour protéger ce patrimoine naturel dont dépendent plus de 500 millions de personnes dans le monde, il apparaît nécessaire, au-delà des actions locales, de prendre des décisions à l'échelle des gouvernements afin de diminuer l'impact des humains sur le climat.

Malgré leur importance écologique et économique, les récifs coralliens sont, de nos jours, affectés par de nombreux facteurs de stress aussi bien à une échelle locale (surexploitation, techniques de pêche destructrices, pression touristique, pollutions marines, développement côtier) que par des facteurs de stress globaux, telles que la hausse de la température de surface des océans ainsi que l'acidification du milieu marin. Les pressions anthropiques et le changement climatique menacent actuellement la plupart des écosystèmes récifaux dans le monde. À terme, ces facteurs de stress peuvent mener à la rupture de l'association formée entre l'hôte corallien et ses symbiotes photosynthétiques, un phénomène que l'on nomme « blanchissement » (*coral bleaching* en anglais) qui s'apparente, comme le nom l'indique, à une décoloration de l'organisme (perte des symbiotes et/ou des pigments photosynthétiques associés) s'échelonnant sur une période de temps plus ou moins longue. Une diminution modérée de la concentration en symbiotes et/ou en photopigments associés résulte toutefois d'un phénomène saisonnier et naturel, survenant dès que la température de surface de l'eau de mer excède les moyennes maximales saisonnières et ceci sur une courte période de temps qui varie selon les sites observés. Cependant, depuis une trentaine d'années, la température moyenne des océans ne cesse d'augmenter, dû au réchauffement global, renforçant l'intensité et la durée du phénomène que les scientifiques caractérisent alors de « blanchissement », et qui affecte aujourd'hui les zones récifales du monde entier, du Pacifique à l'océan Indien, en passant par les Caraïbes et la mer Rouge.

LE BLANCHISSEMENT : UN PHÉNOMÈNE RÉCURRENT DANS L'HISTOIRE

Le premier épisode de blanchissement corallien semble avoir été reporté par Yonge et Nicholls sur la Grande Barrière de corail en mars 1929 alors

ocean-climate.org

que la température des eaux de surface atteignait 35 °C, mais il faut attendre le début des années 1980 pour constater une augmentation de la fréquence, de l'intensité et de l'extension des épisodes de blanchissement à travers le monde. En cause, une hausse « record » de la température de surface des océans due au réchauffement climatique, combinée au renforcement du phénomène *El Niño*. Deux événements de blanchissement majeurs ont été reportés en 1998 et 2010. L'épisode de 1998 avait concerné 60 pays et nations insulaires à travers l'océan Pacifique, Indien, Atlantique (Caraïbes), le golfe Persique ainsi que la mer Rouge. Les zones couvrant l'océan Indien avaient été particulièrement touchées, avec plus de 70 % de mortalité observées sur un gradient de profondeur pouvant s'échelonner jusqu'à plus de 50 m. Les fortes anomalies de température de surface de l'océan ont entraîné la perte de plus de 16 % des récifs coralliens à travers le monde. L'année 1998 fut ainsi le premier « épisode de blanchissement global » déclaré par la National Oceanic and Atmospheric Administration (NOAA). En 2010, à nouveau, un intense phénomène *El Niño* a déclenché un blanchissement extrême des coraux touchant l'ensemble des récifs de la planète avec, pour certaines régions comme l'Asie du sud-est, des conséquences plus importantes en terme d'extension et de mortalité.

2014-2016 : UN ÉVÉNEMENT D'UNE INTENSITÉ ET AMPLEUR EXCEPTIONNELLES

L'événement de blanchissement actuel, qui s'étend sur les trois bassins océaniques principaux (Pacifique, Atlantique et Indien) résulte de l'effet combiné d'une hausse anormale de la température de l'eau de mer, accentuée par le réchauffement climatique, et d'un phénomène *El Niño* très marqué, décrit comme l'un des plus intenses jamais observé. Cet événement de blanchissement exceptionnel a débuté en juin 2014 dans la zone ouest du Pacifique, aux abords des Îles de Guam et Mariannes puis s'est étendu jusqu'aux côtes hawaïennes. Il s'est par la suite propagé dans le Pacifique sud, affectant les territoires britanniques de l'océan Indien et la zone des Caraïbes, des Florida Keys, englobant Cuba et la République dominicaine, les Îles Marshall.

En octobre 2015, la NOAA a officiellement annoncé qu'un 3e événement de blanchissement global était en cours dans les trois bassins océaniques principaux et menaçait 95 % des récifs coralliens américains. À l'issu de l'année 2015, 32 % des récifs à travers le monde avaient été exposés à une anomalie de température équivalente à 4 °C, entraînant des mortalités sur plus de 12000 km². Le début de l'année 2016 fut marqué par la propagation du phénomène dans le Pacifique sud et également sur la Grande Barrière de corail et la côte ouest australienne. Durant le mois de mars 2016, la température moyenne de l'eau de mer était supérieure d'1,5 à 2 °C aux valeurs enregistrées entre 1971 et 2000 dans la zone nord de la Grande Barrière, pour la même période de l'année. Les programmes de prospection aériens et sous-marins ont révélé que sur un total de 911 récifs individuels observés sur la Grande Barrière, 93 % avaient été affectés, et particulièrement les 1000 km de côtes longeant la zone nord de Port Douglas, pourtant considérées jusqu'ici comme parfaitement préservées, car à l'écart des activités humaines. « Nous n'avions jamais vu jusqu'à présent un phénomène de blanchissement à cette échelle. Dans le nord de la Grande Barrière de corail, c'est comme si 10 cyclones avaient eu lieu en même temps » a déclaré le professeur Terry Hughes[1], directeur du Centre d'excellence pour la recherche sur les coraux en Australie (AIMS). De larges colonies, d'une centaine d'années, se meurent, montrant le caractère exceptionnel du phénomène. Dans le Pacifique encore, des événements sévères de blanchissement ont été reportés en Nouvelle-Calédonie ainsi qu'aux Îles Fidji et aux Kiribati où le taux de mortalité a atteint 80 % (Eakin, 2016, person comm, 8th of April). L'événement s'étend au fur et à mesure à la zone ouest de l'océan Indien, aux Maldives, au Kenya, aux Seychelles avec des impacts particulièrement importants dans la zone couvrant le Triangle de corail, en Indonésie. De fortes anomalies de température sont également enregistrées au Moyen-Orient : le golfe Persique, le golfe d'Aqaba ainsi que le golfe de Suez sont encore classés en alerte de niveau 2 (associés à des événements de blanchissements et de mortalités significatifs et étendus), alors que le golfe d'Oman et la zone récifale de la mer Rouge rattachée à l'Égypte restent en état d'alerte niveau 1 (associé à des événements de blanchissements et de mortalités

1 https://www.coralcoe.org.au/media-releases/only-7-of-the-great-barrier-reef-has-avoided-coral-bleaching

ocean-climate.org

significatifs). D'autres événements de blanchissements sont attendus durant l'été 2016 au Japon ainsi que dans les Caraïbes. Les chercheurs de la NOAA suggèrent que le blanchissement global observé pourrait s'étendre bien au-delà de l'année 2017.

LE DEVENIR DES RÉCIFS CORALLIENS : ENTRE ESPOIR ET INQUIÉTUDE

En 2015, la température de surface moyenne de l'eau de mer était supérieure de 0,74 °C comparé aux moyennes du XX[e] siècle, excédant de 0,11 °C le record de l'année 2014. Les modèles climatiques prévoient une hausse de la température de surface des océans de 1 à 3 °C d'ici la fin du XXI[e] siècle. Ainsi, le dépassement du seuil limite de thermotolérance au-dessus duquel les coraux blanchissent, jusque-là exceptionnel et ponctuel, est prévu de devenir annuel ou biannuel et de menacer la survie des récifs du monde entier d'ici 2050. Les conséquences sont alarmantes puisque l'augmentation de la fréquence du blanchissement, comme celle observée dans les Caraïbes (1995, 1998, 2005 puis 2010) par exemple, limite le temps de résilience de l'écosystème et peut entraîner, sur le long terme, de plus forts taux de mortalité.

De récentes études ont toutefois démontré le potentiel d'acclimatation physiologique des coraux aux fortes anomalies de température. Par exemple, certaines espèces coralliennes sont à même de modifier leur population d'algues symbiotiques afin d'optimiser leur résistance au stress thermique ou encore de réguler l'expression de leurs gènes pour renforcer les mécanismes de défenses. Cependant, ce degré de résilience reste faible, voire ponctuel. Il est donc improbable qu'il joue un rôle significatif alors que la température de l'océan ne cesse d'augmenter de manière significative. De plus, la combinaison d'autres facteurs environnementaux tels que l'acidification des océans et la hausse du niveau marin couplés aux menaces locales (la surpêche, la pollution, les dommages physiques, l'érosion des sols, etc.) sont à prendre en considération dans la prédiction du devenir des récifs coralliens. L'effet synergique de ces facteurs de stress (pollutions marines et surpêche) peut altérer les relations trophiques entre organismes au sein de l'écosystème récifal, en favorisant la croissance excessive d'algues filamenteuses au détriment des coraux, devenant plus susceptibles aux maladies et infections.

LA NÉCESSITÉ D'UNE PRISE DE CONSCIENCE

Ces événements récents inquiètent la communauté scientifique et ont déclenché une prise de conscience sur la nécessité d'agir afin de protéger les récifs coralliens. De nouvelles techniques et de nombreuses ressources ont été mises en place afin de suivre l'extension géographique et l'évolution du blanchissement permettant de mieux comprendre et appréhender la réponse des coraux. Par exemple, l'expédition « Catlin Seaview survey », qui a été lancée en septembre 2012, établit un suivi de l'état des récifs à travers le monde. Dans la même lignée, la France vient, quant à elle, de fêter les 15 ans de l'initiative française pour les récifs coralliens (Ifrecor). Ces efforts peuvent aboutir à la mise en place de plans d'action locaux afin de réduire l'empreinte de l'homme sur les écosystèmes récifaux. Mumby & Harborne (2010) ont par exemple démontré l'efficacité des aires marines protégées pour la résilience des récifs dans les Caraïbes. De même, en 2014, la Nouvelle-Calédonie a annoncé la création du Parc naturel de la mer de corail, l'une des plus grandes aires marines protégées au monde (1,3 million de km2). Des solutions d'ingénierie biologique ont même été proposées, suggérant d'utiliser des colonies coralliennes « optimisées » en fonction des nouvelles conditions environnementales dans le but de restaurer les récifs dégradés. Certains scientifiques proposent en effet d'utiliser « l'évolution assistée » pour modifier le seuil de résilience des coraux en réalisant une sélection artificielle en laboratoire, consistant à exposer les coraux à divers stress ou en sélectionnant des souches de symbiotes thermotolérantes.

Le rôle des autorités locales et des organisations non gouvernementales (ONG) est ainsi primordial afin de

ocean-climate.org

préserver ce patrimoine, dont plus de 500 millions de personnes dépendent. Au-delà des actions locales, des décisions à l'échelle des gouvernements ont été prises afin de diminuer l'impact des humains sur le climat. En décembre 2015, un accord international fixant comme objectif une limitation du réchauffement mondial à 1,5 °C - 2 °C d'ici 2100 a été validé par 195 pays (dont la France) participant à la Conférence internationale sur le climat (COP21). Le 3 septembre 2016, la Chine et les USA, les deux plus grandes puissances mondiales et plus gros pollueurs de la planète, ont ratifié cet accord, joignant ainsi l'effort commun. Dans le but de préserver les récifs de notre planète pour les futures générations, il nous faudra alors proposer des solutions réalistes à travers des programmes fédérateurs, s'adressant à tous : aussi bien aux politiques et entreprises qu'au grand public, aux plus jeunes d'entre nous ainsi qu'aux communautés directement impliquées. Car une protection effective de la biodiversité repose, entre autre, sur l'amélioration des conditions de vie des populations locales, et sur leurs capacités à gérer durablement les ressources des écosystèmes dont elles dépendent[2].

2 Voir en particulier l'action d'ONG telle que Coral Guardian (www.coralguardian.org/association-coral-guardian).

RÉFÉRENCES

- AIMS, 2016 – Western Australian reefs feel the heat from global bleaching event, www.aims.gov.au/docs/media/featured-content.html/-/asset_publisher/Ydk18I5jDwF7/content/western-australian-reefs-feel-the-heat-fromglobal-bleaching-event.
- AINSWORTH T. D. *et al.*, 2016 – *Climate Change Disables Coral Bleaching Protection on the Great Barrier Reef.* Science 352, 338-342 (2016).
- BAHR K. D., JOKIEL P. L. and RODGERS K. S., 2015 – *The 2014 Coral Bleaching And Freshwater Flood Events In Kāne'ohe Bay*, Hawai'i. PeerJ 3, e1136.
- BROWN B., 1997 – *Coral Bleaching : Causes and Consequences.* Coral reefs 16, S129-S138.
- DONNER S. D., SKIRVING W. J., LITTLE C. M., OPPENHEIMER M. and HOEGH-GULDBERG O., 2005 – *Global Assessment of Coral Bleaching and Required Rates of Adaptation under Climate Change.* Global Change Biology 11, 2251-2265.
- EAKIN C. M. *et al.*, 2010 – *Caribbean Corals in Crisis : Record Thermal Stress, Bleaching, and Mortality in 2005.* PloS one 5, e13969.
- FABRICIUS K. E., CSÉKE S., HUMPHREY C. and DE'ATH G., 2013 – *Does Trophic Status Enhance or Reduce the Thermal Tolerance of Scleractinian Corals ? A Review, Experiment and Conceptual Framework.* PloS one 8, e54399 (2013).
- FISCHLIN A. *et al.*, 2007 – *Ecosystems, Their Properties, Goods and Services.*
- FITT W., MCFARLAND F., WARNER M. and CHILCOAT G. – *Seasonal Patterns of Tissue Biomass and Densities of Symbiotic Dinoflagellates in Reef Corals and Relation to Coral Bleaching.* Limnology and oceanography 45, 677-685 (2000).
- GLYNN P., 1993 – *Coral Reef Bleaching : Ecological Perspectives.* Coral reefs 12, 1-17.
- GLYNN P. W., PEREZ M. and GILCHRIST S. L., 1985 – *Lipid Decline in Stressed Corals and their Crustacean Symbionts.* The Biological Bulletin 168, 276-284.
- HERON S. F. *et al.*, 2016 – *Validation of Reef-Scale Thermal Stress Satellite Products for Coral Bleaching Monitoring.* Remote Sensing 8, 59.
- HOEGH-GULDBERG O. and RIDGWAY T., 2016 – *Coral Bleaching Hits Great Barrier Reef As Global Temperatures Soar.* Green Left Weekly, 10.
- HOEGH-GULDBERG O., 1999 – *Climate Change, Coral Bleaching and the Future of the World's Coral Reefs.* Marine and freshwater research 50, 839-866.

ocean-climate.org

- ISRS, 2016 – Reef Encounter, 31.
- JOKIEL P. and COLES S., 1990 – *Response of Hawaiian and Other Indo-Pacific Reef Corals to Elevated Temperature*. Coral reefs 8, 155-162. PALUMBI S. R., BARSHIS D. J., TRAYLOR-KNOWLES N. and BAY R. A., 2014 – *Mechanisms of Reef Coral Resistance to Future Climate Change*. Science 344, 895-898.
- MUMBY P. J. and HARBORNE A. R., 2010 – *Marine Reserves Enhance the Recovery of Corals on Caribbean Reefs*. PLoS ONE 5, e8657, doi : 10.1371/journal.pone.0008657.
- NICHOLLS R. J. *et al.*, 2007 – *Coastal Systems and Low-Lying Areas*.
- NOAA, 2015 – *Noaa Declares Third Ever Global Coral Bleaching Event : Bleaching Intensifies in Hawaii, High Ocean Temperatures Threaten Caribbean Corals*. www.noaanews.noaa.gov/stories2015/100815-noaa-declares-third-ever-global-coralbleaching-event.html.
- NOAA, 2015 – *NOAA Coral Reef Watch : 2015 Annual Summaries of Thermal Conditions Related to Coral Bleaching for NCRMP Jurisdictions*.
- NOAA., 2015 – *State of the Climate : Global Analysis for Annual 2015*. National Centers for Environmental Information, www.ncdc.noaa.gov/sotc/global/201513.
- NORMILE D., 2010 – *Restoration or devastation ?* Science 327, 1568-1570.
- PALUMBI S. R., BARSHIS D. J., TRAYLOR-KNOWLES N. and BAY R. A., 2014 – *Mechanisms of Reef Coral Resistance to Future Climate Change*. Science 344, 895-898.
- Studies, C.C.A.C.o.E.C.R., 2016 – *Only 7 % of the Great Barrier Reef Has Avoided Coral Bleaching*. www.coralcoe.org.au/media-releases/only-7-of-the-greatbarrier-reef-has-avoided-coral-bleaching.
- VAN OPPEN M. J. H., OLIVER J. K., PUTNAM H. M. and GATES R. D., 2015 – *Building Coral Reef Resilience Through Assisted Evolution*. Proceedings of the National Academy of Sciences 112, 2307-2313, doi : 10.1073/pnas.1422301112.
- VEGA THURBER R. L. *et al.*, 2014 – *Chronic Nutrient Enrichment Increases Prevalence and Severity of Coral Disease and Bleaching*. Global change biology 20, 544-554.
- WAKE B., 2016 – *Snapshot : Snow White Coral*. Nature Climate Change 6, 439-439.
- WILKINSON C. R., SOUTER D. N. and NETWORK G. C. R. M., 2008 – *Status of Caribbean Coral Reefs after Bleaching and Hurricanes in 2005*. Global Coral Reef Monitoring Network.
- YONGE C. M., NICHOLLS A. G. and YONGE M. J., 1931 – *Studies on the Physiology of Corals*. Vol. 1, British Museum.
- ZANEVELD J. R. *et al.*, 2016 – *Overfishing and Nutrient Pollution Interact with Temperature to Disrupt Coral Reefs Down to Microbial Scales*. Nat Commun 7, doi : 10.1038/ncomms11833.

III. 1. 2. Les enjeux de la situation actuelle

Si le réchauffement climatique a déjà entrainé la mort de 19% des récifs coralliens (source : GCRMN), les récentes observations et les modèles prédisent la perte de 90% des récifs d'ici le milieu du XXI^{ème} siècle dans le meilleur des cas, (Frieler et al. 2013), c'est à dire si les émissions de gaz à effet de serre sont réduites aux valeurs fixées par l'accord international sur le climat signé en 2015 par les 195 pays participants à la Conférence sur le Climat de Paris (COP21). L'objectif, ratifié ensuite par la Chine, est de réussir à stabiliser les émissions d'ici le milieu du siècle et de progressivement les diminuer pour limiter le réchauffement mondial à 1.5-2°C d'ici 2100. Pour optimiser les chances de survie des récifs, il est nécessaire 1) d'identifier les récifs les plus enclins à

faire face au réchauffement climatique, 2) de continuer à mettre en place des plans de protection pour diminuer les pressions locales sur les récifs, 3) de mettre au point et d'améliorer les techniques de restauration des récifs (réimplantation, bouturage…). **Les travaux de cette thèse participent à cet effort d'approfondissement des connaissances sur l'impact du changement climatique sur les coraux et notamment sur le rôle des UVs. Les paragraphes suivants développent les difficultés de la prédiction du devenir des récifs et montrent comment les objectifs de la thèse s'inscrivent dans l'effort commun de préservation des récifs coralliens.**

III.2. Les difficultés des prédictions : les enjeux de la thèse

Les expériences en aquarium sont les premiers outils utilisés pour mieux comprendre la réponse des coraux aux changements environnementaux et prédire le devenir des récifs. Bien que la complexité et la variabilité de l'environnement ne puissent pas être totalement reproduites en aquarium, ces études permettent de mettre en évidence la réponse et les mécanismes de défense à un stress particulier pour mieux comprendre ce que l'on observe *in situ* et mieux prédire le devenir des récifs. Les problèmes majeurs de ce type d'études, qui résument les difficultés rencontrées par la communauté scientifiques pour anticiper la réponse des récifs au changement climatique, sont 1) la complexité de la réponse du corail (*i.e.* la diversité des paramètres biologiques à prendre en considération), 2) la diversité des réponses entre espèces, 3) la prise en compte de plusieurs facteurs environnementaux qui varient et dont les effets peuvent être synergiques ou antagonistes , 4) la prise en compte du passé environnemental des coraux qui ne se développent pas dans les mêmes conditions et 5) la prise en compte des interactions entre les coraux et leur écosystème. **Les paragraphes qui suivent détaillent chacun de ces points en indiquant comment les travaux de la thèse apportent des éléments de réponse aux différentes difficultés rencontrées.**

III. 2. 1. Diversité des paramètres biologiques

Les pressions environnementales affectent l'ensemble des paramètres biologiques des coraux. La prise en compte d'un nombre limité de paramètres, imposé par les difficultés techniques de la réalisation des expériences, ne permet pas d'avoir une vue d'ensemble sur les conséquences des stress imposés. Par exemple, le suivi de la quantité de symbiotes et de pigments photosynthétiques donne une idée de la vitesse et de l'ampleur du blanchissement qui ne sont qu'une partie du puzzle. Les mesures de photosynthèse, respiration, nutrition hétérotrophe et des réserves énergétiques des coraux (protéines, lipides *etc.*) permettent le suivi du statut énergétique des coraux, élément essentiel de leur résistance aux stress (Lesser 2013). Les mesures de calcification sont également importantes puisque la réduction des taux peut signifier une diminution de la

compétitivité du corail pour l'espace et la lumière ou encore un squelette moins épais et donc moins résistant aux courants et à l'érosion (Allemand et al. 2011). D'autres changements physiologiques tels qu'une modification de l'excrétion de mucus ou de la diversité des micro-organismes associés peuvent avoir des conséquences sur les capacités du corail à se défendre face aux attaques des bactéries pathogènes (Brown and Bythell 2005; Thompson et al. 2014; Glasl et al. 2016). Chacun de ces paramètres biologiques (et d'autres encore) apporte une réponse partielle sur le futur d'une colonie et ce n'est qu'en combinant les paramètres que l'on pourra prédire de manière plus efficace la réponse des coraux au changement climatique.

Si la réponse des coraux à une élévation de température a été très étudiée, les effets des UVs sur l'ensemble des paramètres biologiques du corail sont moins connus. Les études réalisées par le passé se sont principalement focalisées sur la production de MAAs et le blanchissement des coraux. Moins de 10 études ont par contre mesuré le métabolisme des coraux (calcification, photosynthèse, respiration) et aucune n'a été faite sur les effets des UVs sur l'excrétion de matière organique et sa dégradation par les micro-organismes associés (voir Table 3). **Le premier objectif de cette thèse a donc été de renforcer nos connaissances sur les effets des UVs sur les coraux en incluant la mesure d'un maximum de paramètres biologiques : le blanchissement, le métabolisme, la nutrition hétérotrophe, l'excrétion de mucus, l'activité et la diversité des microorganismes associés au mucus ainsi que sur les voies moléculaires impliquées dans les mécanismes de défense des coraux.**

III. 2. 2. La variabilité inter-espèces

Un récif est constitué d'un grand nombre d'espèces ayant chacune des caractéristiques physiologiques différentes. L'une des difficultés à anticiper le devenir des récifs réside dans la diversité des réponses des différentes espèces face au changement climatique (Loya et al. 2001). Par exemple, l'ensemble des travaux réalisés sur les effets d'un stress de température sur les coraux montre que leur sensibilité diffère selon l'espèce étudiée (Hoegh-Guldberg 1999) : certains genres comme *Stylophora*, *Pocillopora* et *Acropora*, sont très sensibles au blanchissement tandis que *Cyphastrea*, *Goniopora*, *Galaxea* et *Pavona* sont plus résistants (McClanahan et al. 2004). Ces différences peuvent être attribuées à plusieurs facteurs. D'abord, le clade de symbiote a été identifié comme l'un des facteurs déterminant pour la sensibilité de l'holobionte; le clade D étant, plus thermo-tolérant que les autres clades (Glynn et al. 2001; Baker et al. 2004; Berkelmans and Van Oppen 2006; Jones et al. 2008; LaJeunesse et al. 2009). Ainsi, la capacité de certaines espèces de coraux à changer leur population de symbiotes pour que le clade dominant soit adapté aux conditions environnementales (Silverstein et al. 2012) leurs confère un mécanisme de défense supplémentaire pour répondre au changement climatique. Les bactéries associées semblent

également jouer un rôle dans la santé de l'hôte, comme il a été récemment suggéré pour le genre *Endozoicomonas* (Neave et al. 2016). L'hôte possède lui aussi des caractéristiques propres tels que la synthèse de pigments fluorescents (Salih et al. 1998, 2000, 2006), de protéines de choc thermique (Brown et al. 2002) ou la capacité à utiliser des sources hétérotrophes de carbone pour compenser la diminution de la photosynthèse (Grottoli et al. 2006). Cependant, toutes les espèces ne possèdent pas ces caractéristiques et n'ont de fait pas les mêmes capacités de réponse aux stress. Par exemple, les genres *Pocillopora* et *Acropora*, connus pour être sensibles au blanchissement ont une faible quantité de pigments fluorescents par rapport à d'autres tels que *Porites* et *Turbinaria* (Marshall and Baird 2000; Floros et al. 2004; Ulstrup et al. 2006; Schoepf et al. 2013a). La plasticité nutritionnelle (*i.e.* la capacité de passer d'un régime dominé par l'autotrophie à un régime hétérotrophe) n'est également pas observée chez toutes les espèces (Houlbrèque and Ferrier-Pagès 2009). Par exemple, pendant un blanchissement, *Montipora capitata* est capable de satisfaire totalement sa demande en énergie journalière via l'hétérotrophie (Grottoli et al. 2006) ce qui lui permet de se remettre plus rapidement d'un blanchissement par rapport à *Porites compressa* et *P. lobata*, incapables de changer leur régime nutritionnel (Rodrigues and Grottoli 2007).

Si la diversité des réponses des coraux à la température commence à être connue; le peu d'études réalisées sur les effets des UVs ne permet pas de conclure quant à la capacité des différentes espèces à contrer leurs effets. **C'est pourquoi, l'un des objectifs de cette thèse a été de comparer la réponse de plusieurs espèces de coraux ayant des morphologies et des physiologies différentes à une exposition aux UVs. La première expérience a permis de comparer *Turbinaria reniformis* et *Pocillopora damicornis*, deux espèces distantes génétiquement l'une de l'autre. Deux expériences conduites en Nouvelle Calédonie ont étudié *Acropora muricata*, une espèce très présente sur les récifs néo-calédoniens. Enfin, des travaux réalisés sur *Stylophora pistillata* ont permis de mettre en évidence une voie moléculaire de réponse au stress UVs.**

III. 2. 3. Approche multi-paramètres

Une autre difficulté dans la prédiction du devenir des récifs est liée au fait que les coraux doivent faire face aux effets combinés de plusieurs stress environnementaux. Par exemple, les récifs vont être soumis à la fois à l'augmentation de la température (Hoegh-Guldberg 1999) et à celle du rayonnement UVs (Watanabe et al. 2011), ou des sels nutritifs suite à l'eutrophisation des milieux côtiers (Fabricius 2011). L'analyse des effets de facteurs environnementaux isolés, bien que primordiale pour tester les prédictions de théories existantes ou pour en générer de nouvelles, ne suffit pas à anticiper le futur. Par exemple, de nombreuses expériences en laboratoire et de terrain ont mis en évidence le lien entre l'augmentation de température et le blanchissement des coraux

(Hoegh-Guldberg 1999), mais peu de travaux ont été menés sur la combinaison de l'augmentation de la température avec d'autres facteurs tels que les UVs. Pourtant, les quelques études portant sur le sujet tendent à montrer que les UVs peuvent accentuer le blanchissement de certaines espèces de coraux et le stress oxydant dans les *Symbiodinium* de culture (Fitt and Warner 1995; Lesser 1996; D'Croz and Mate 2000; Lesser and Farrell 2004; Ferrier-Pagès et al. 2007). Le facteur UVs pourrait donc modifier les prédictions de survie des récifs les plus exposés. Les UVs ne sont pas le seul exemple, d'autres paramètres du changement climatique tel que l'acidification des océans interagissent de manière positive ou négative avec la température et modifient la réponse des coraux (Reynaud et al. 2003; Anthony et al. 2008; Schoepf et al. 2013b; Wall et al. 2014; Kwiatkowski et al. 2015). C'est pourquoi, il est primordial d'adopter une approche multifactorielle dans notre tentative de prédiction du devenir des récifs coralliens. **Le troisième objectif de la thèse a été de compléter et d'approfondir nos connaissances sur ces interactions et d'identifier les mécanismes et les stratégies de défense des coraux. Nous avons donc systématiquement adopté une approche multifactorielle en combinant l'exposition aux UVs avec une augmentation de la température, différents niveaux de nutriments ou différentes saisons.**

III. 2. 4. Acclimatation : le rôle des conditions de croissance

Les conditions de croissance des coraux avant l'exposition au stress environnemental sont également à prendre en considération pour prédire le devenir des récifs. En Nouvelle Calédonie par exemple, les coraux sont acclimatés à des changements annuels de température de l'eau de l'ordre de 6°C (entre été et hiver, Andréfouët et al. 2009). Il est donc possible que ces coraux ne répondent pas de la même façon à un stress de température en fonction de la période de l'année où les expériences sont faites. De même, l'exposition au rayonnement UV n'aura certainement pas le même effet sur des coraux de faible profondeur déjà acclimatés aux UVs et ayant des mécanismes de défenses tels que les MAAs déjà développés (Shick and Dunlap 2002), que sur des colonies situées plus en profondeur ou dans des zones turbides, et qui sont dépourvus de MAAs (Masuda et al. 1993; Shick et al. 1995; Cohen et al. 2013). Ces considérations sont à prendre en compte dans l'interprétation et l'analyse d'expériences en laboratoire ou *in situ* et de fait dans les conclusions à tirer pour la prédiction du devenir des récifs. **Dans cette thèse, nous avons conduit des expériences sur des coraux maintenus sans UVs (pour mimer les effets du changement climatique sur des coraux « profonds ») ainsi que sur des coraux prélevés à faible profondeur, naturellement exposés aux UVs. De plus, deux expériences conduites sur *P. damicornis* ont permis de mettre en évidence l'effet de l'accès ou non à une source de nutrition hétérotrophe dans le milieu de culture sur la réponse des coraux aux stress combinés UV/température.**

III. 2. 5. Prise en compte des multi-échelles

Les expériences réalisées sur les coraux doivent également tenir compte des conséquences de l'altération de la physiologie du corail sur le reste de l'écosystème. Il existe en effet de nombreuses interactions/rétroactions entre les coraux et les autres organismes qui peuvent modifier la dynamique du récif et influencer leur futur. Par exemple, l'eutrophisation des récifs favorise la croissance des macroalgues directement en compétition avec les coraux pour le substrat, les sources nutritionnelles et/ou la lumière (Fabricius et al. 2005; Burkepile et al. 2013; Garren 2016). En parallèle, la croissance d'algues entraîne l'augmentation de l'excrétion de carbone organique dissous algal qui sert de substrat pour le développement de bactéries pathogènes pour les coraux (Ruiz-Morenol et al. 2012; Vega Thurber et al. 2014; Zaneveld et al. 2016). Ces effets indirects, souvent en défaveur des coraux (Mumby 2009; Cheal et al. 2010; Barott et al. 2012; Vieira et al. 2015), s'ajoutent aux effets négatifs de l'eutrophisation sur la physiologie du corail et peuvent contrebalancer les effets positifs parfois observés sur la résistance aux stress environnementaux (Tanaka et al. 2007; Holcomb et al. 2010; Béraud et al. 2013; Hoadley et al. 2016). Inversement, les changements physiologiques du corail peuvent affecter le reste du récif. En effet, comme évoqué précédemment (§I.3.2.), le corail est la principale source de production de matière organique dans le récif. Cette matière soutient la production benthopélagique et participe au recyclage des éléments essentiels (azote, phosphore…) dans l'eau très oligotrophe du récif (Wild et al. 2004; Bythell and Wild 2011). Ainsi, des changements dans l'excrétion de la matière organique peuvent modifier le reste de l'écosystème. **Si les expériences à l'échelle de l'écosystème sont difficiles à mettre en place en laboratoire, nous avons inclus des mesures de flux de carbone et d'azote organiques à la plupart de nos expériences. Les résultats ne sont qu'une première étape dans l'identification des interactions corail-récif mais les changements qualitatifs ou quantitatifs de l'excrétion de matière organique identifiés nous donnent des indications sur les effets potentiels sur les microorganismes du récif.**

III. 2. 6. Le déroulement de la thèse

Les objectifs et enjeux de la thèse ont mené à la formulation d'un certain nombre de questions auxquelles nous avons apporté des éléments de réponse par le biais de 5 expériences (résumées dans le Table 4) :

- L'exposition aux UVs naturels est-elle un facteur influençant le métabolisme, les paramètres tissulaires, l'excrétion de mucus du corail et son microbiome associé?
- Quelle est la réponse des coraux à l'augmentation du rayonnement UV ? Cette réponse est-elle généralisable où est-elle spécifique à l'espèce ?
- Comment les UVs affectent-ils l'excrétion et la dégradation de mucus ?

▪ Comment les UVs affectent-ils la réponse des coraux au réchauffement climatique et à l'eutrophication?

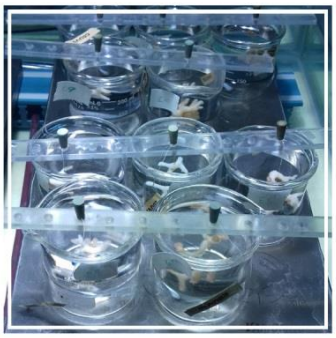
▪ Quels sont les mécanismes moléculaires impliqués dans la réponse des coraux aux UVs ?

Table 4. Résumé des expériences réalisées au cours de cette thèse faisant l'objet de publications sous forme d'articles scientifiques. Dans la colonne « conditions environnementales », 0UV et UV désignent les conditions sans et avec UVs; T désigne une condition de forte température ; W et S désignent les conditions hiver et été ; D, C, E désignent une condition déplétée, ambiante et enrichie en nutriments.

Article n°	Titre	Source d'UVs	Espèces	Statut hétérotrophique	Facteurs environ-nementaux testés	Conditions environ-nementales
1	Validation of commercial ERK antibodies against the ERK orthologue of the scleractinian coral *Stylophora pistillata*	Lampes	*S.pistillata*	Nourris	-	-
2	Interactive effects of ultraviolet radiation and thermal stress on two reef-building corals	Lampes	*P.damicornis* *T.reniformis*	Nourris	UVs et température	0UV; UV; 0UVT; UVT
3	Temperature and UVR effects on the organic matter fluxes and the metabolic activity of *Acropora muricata*	Radiations solaire	*A.muricata*	Nourris	UVs et température	0UV; UV; 0UVT; UVT
4	Seasonal variability of the microbial communities associated with *A. muricata* exposed to or shaded from UVR	Radiations solaire	*A.muricata*	Nourris	UVs et saison	W0UV; WUV; S0UV; SUV
5	Effects of ultraviolet radiation and nutrient levels on the physiological response and mucus production of *Pocillopora damicornis* following thermal stress	Lampes	*P.damicornis*	Non nourris	UVs, température, niveaux de nutriments	D0UV; DUV; DT0UV; DTUV; C0UV; CUV; CT0UV; CTUV; E0UV; EUV; ET0UV; ETUV
6	The c-Jun N-terminal kinase prevents oxidative stress induced by UV and thermal stresses in corals and human cells	Lampes	*S.pistillata*	Nourris	UVs et température	0UV; UV; 0UVT; UVT

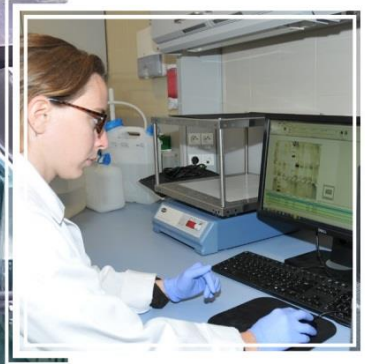
La démarche expérimentale et les protocoles expérimentaux utilisés pour répondre à ces questions sont décrits dans le deuxième chapitre de la thèse. L'ensemble des résultats sont ensuite décrits dans les chapitres 3 et 4. Le chapitre 3 décrit les effets des UVs combinés ou non avec un stress de température et différentes conditions de nutrition (niveau de nutriments et statut hétérotrophique) à différentes saisons sur la physiologie du corail (*i.e.* les paramètres tissulaires :

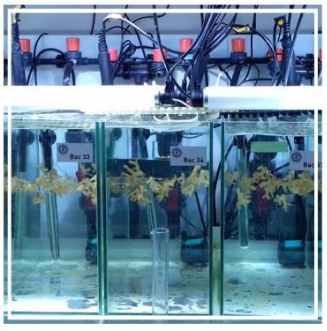
densité de symbiontes, concentration en pigments et protéines ; le métabolisme : photosynthèse, respiration, calcification et la nutrition hétérotrophe). Le chapitre 4 décrit les effets des mêmes paramètres environnementaux sur les flux de matière organique (qualité et quantité) ainsi que l'activité, le développement et la diversité des bactéries associées au mucus des coraux. Enfin, chacune des questions citées ci-dessus seront discutées à la lumière des nouveaux éléments apportés par nos résultats dans un cinquième chapitre.



Chapter 2

Materials and methods





Chapter 2 | Materials and methods

The following section describes the experimental procedures used and developed during the PhD and the technical problems encountered. Most of these techniques are common protocols in coral studies and are well described in the papers; they will therefore be briefly explained in this chapter. However, more details will be given for the protocols developed during the PhD. The different experiments (corresponding each to a different paper) carried out during the PhD will be referred as numbered in Table 4.

I. Experimental settings: two different but complementary approaches in Monaco and in New Caledonia

Because of technical constraints, the experimental setups in Monaco and New Caledonia were different, which led to different ecological relevance of the experiments. The following sections detail the differences in the two settings and highlight the difficulties we encountered during the thesis.

I.1. Experimental set up in Monaco

This experimental set up concerns the papers n° 2, 5 and 6.

I. 1. 1. UVR

The choice of UV lamps: the right quality and quantity of UVR

The facilities in Monaco consist in indoors aquaria, with an artificial source of UVR (see Figure 11). Two parameters are taken into account for the choice of the UVR source:

- The quality of the UVR (the wavelength spectrum), respecting the solar UVA/UVB ratio.
- The quantity of the UVR delivered.

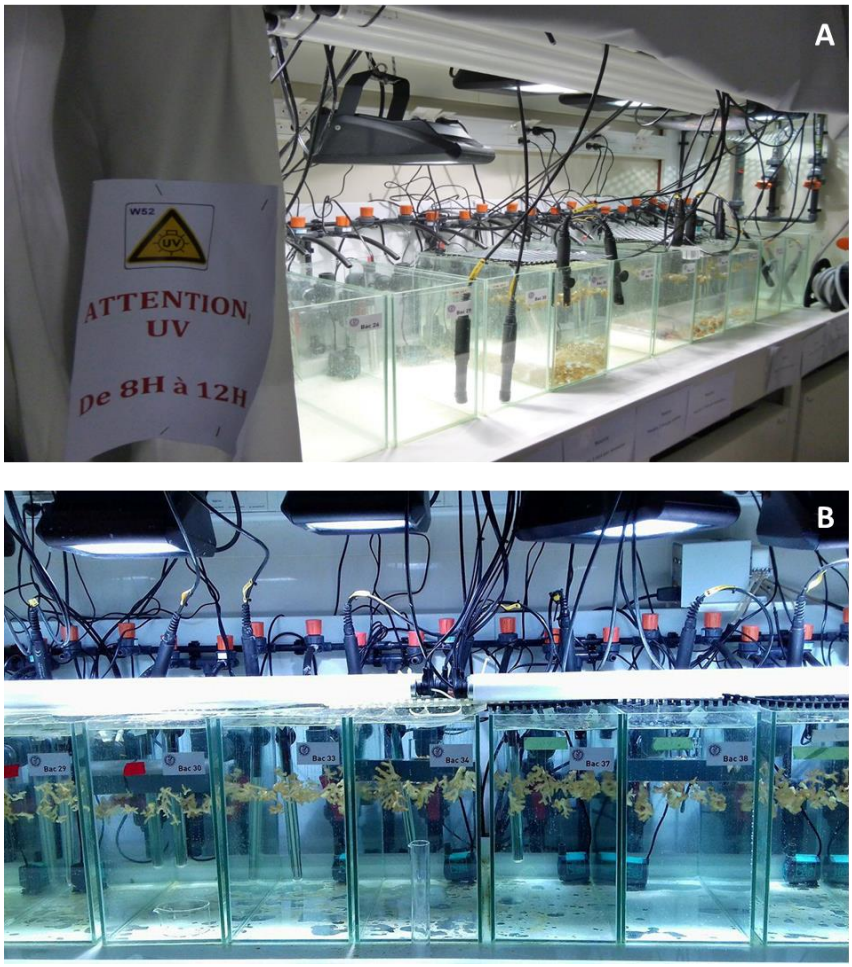


Figure 11. Aquarium facilities in Monaco (A), nubbins of *P. damicornis* under UVR (B). The neon lights on top of the aquariums are the artificial UVR lamps.

The quality of the light (*i.e.* the wavelength) is an important factor to consider because the shorter the wavelength is, the more damaging it is for living organisms. Yet, very little is known on the separate effects of UVA and UVB on corals. Therefore, we first tried to find UV lamps delivering ecologically relevant amounts of UVA (315-400 nm) and UVB (280-315 nm) separately to be able to isolate the effects of each type of UVR. We used a UVA lamp (CLEO Swift 100W-R) and a UVB lamp (G13 TL 20W /12 RS UVB) that we implemented at a different distance from the water surface to match the UVR conditions on a shallow reef (1.9 W m^{-2} of UVB and 20 W m^{-2} of UVA). The UVA/UVB intensities were controlled with an International Light ILT1400 portable radiometer and detectors (SEL240/UVB-1/TD and SEL033/UVA/TD).

Although these intensities of UVA and UVB were previously tested on *Stylophora pistillata* in several studies (Shick et al. 1999; Ferrier-Pagès et al. 2007) with a unique lamp delivering both UVA and UVB (Q-Panel UVA 340), nubbins in our test lost their tissue after only 2 days of exposure. We reduced the UVB intensities (down to 0.2 W m^{-2}) but could not prevent tissue loss after a few days.

The emission range (from 290-320 nm) of the UVB lamp tested was consistent with the solar radiation and with the lamp used in the previous cited studies. However, the difference between our test and the Q-Panel UVA 340 lamps was the distribution spectrum of UVB, that peaked at 302 nm for our lamp (Figure 12B,C). This is likely the reason why coral nubbins died in our experiment.

As we could not find artificial UVB lamps with similar spectrum than the solar spectrum, we opted for the Q-Panel UVA 340 (Figure 12C). These lamps are sold to be the best to reproduce solar radiation with a similar radiation spectrum. UV lamps were settled so that nubbins were exposed to 1.5 W m^{-2} UVB and 30 W m^{-2} UVA.

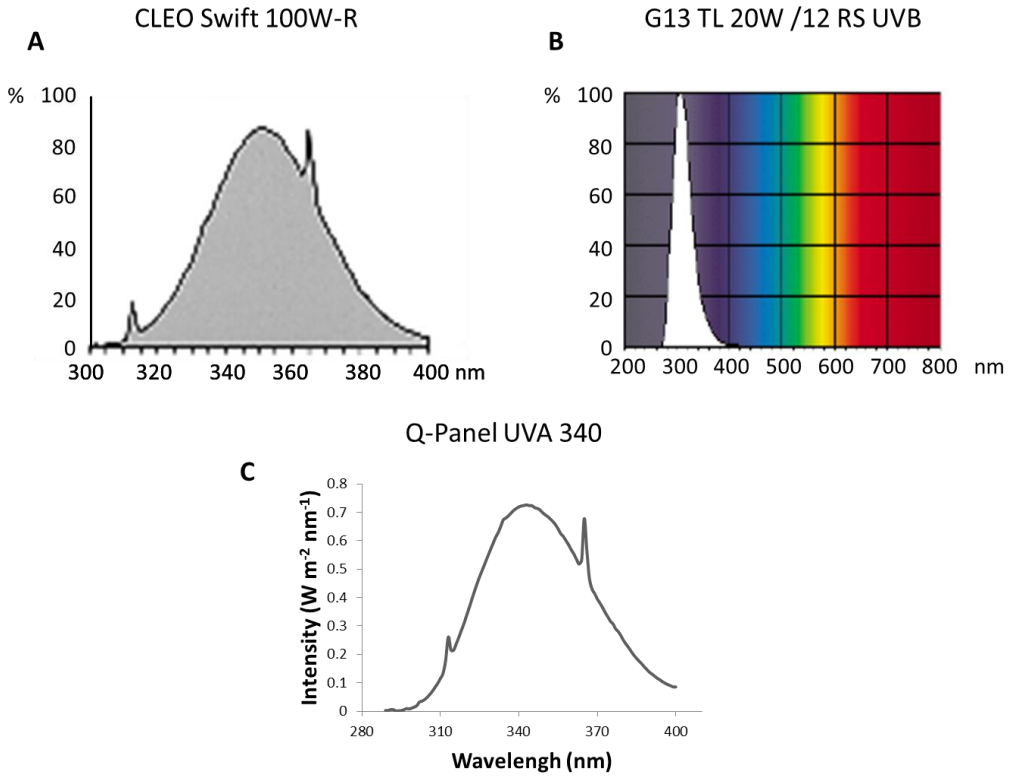


Figure 12. Distribution spectra of the tested (A, B) and (C) used UV lamps. The first lamp tested is a lamp delivering mainly UVA (spectrum A, 20 W m^{-2} of UVA: 315-400 nm and 0.34 W m^{-2} of UVB: 280-315 nm) which was associated with a UVB lamp (spectrum B, 20 W of UVs: 290-320 nm). The UV lamp used for all experiments conducted in Monaco is a Q-Panel UVA 340 with a similar UVR spectrum than the sun (C, 290-400 nm).

Limits of the experimental design: UV dose, UV intensity and duration of the exposure

One of the limitations in the use of artificial UVR lamps is the impossibility to reproduce the solar daily cycle (*i.e.* a progressive increase in intensity from dawn to midday and decrease from midday to sunset) as the lamps deliver a constant irradiation. However, it was possible to adjust the

daily dose of UVR (*i.e.* the amount of UVR received during the day) to the right amount. For example, in the case of this thesis, nubbins, which were exposed for 4 h to 1.5 W m^{-2} UVB and 30 W m^{-2} UVA received the same total UVR dose than reef corals exposed during 12 h to UVR (Torregiani and Lesser 2007). This technique is based on the assumption that the effects of UVR on corals are a matter of dose exposure and that the variability in intensity and time exposure has minor influence. However, this assumption was never tested before which needs to be kept in mind when interpreting results obtained with this experimental design. The most accurate way to test the difference between the two exposure length and intensity would have been to expose nubbins from the same parent colonies to the same dose of UVR provided either with a natural UVR exposure or with artificial lamps delivering constant intensity. This is very complex to implement as the natural UVR not only vary during the day but also vary between days as it depends on the cloud cover. An easier but less accurate way to test the difference in UVR exposure would have been to expose nubbins to the same dose with artificial lamps but with different intensities over shorter or longer exposure length. To validate the relevance of laboratory studies with UVR lamps, the latest experimental design would be interesting to test in the near future.

Ecological relevance of the experimental setting

In Monaco, the parent colonies were grown for several months without UVR prior the experiment and did not have MAAs in their tissue (Shick et al. 1999). In their work, Schick and collaborators showed that exposing them to UVR triggered the MAAs synthesis within 7 days with a maximal concentration reached after 14 days. Therefore, the response of such corals can be compared to deep-living or shaded corals facing an increase in UVR level. It is consistent with a UVR stress, and the aim of the experiment was to test the effects of this stress on the coral physiology and release of organic matter.

I. 1. 2. Thermal stress and nutrients levels

Thermal stress (in papers n°2 and 5) was induced by gradually increasing the temperature from 25°C (growth temperature for the parent colonies) to 30°C over a 10 days period. The temperature was controlled using heaters connected to Elli- Well PC 902/T controllers.

Three nutrient levels were also tested during the thesis (paper n°5): a control condition using ambient seawater levels (~0.5 µM nitrate (NO_3) and ~0.1 µM phosphate (PO_4)), a depleted condition (<0.1 µM NO_3 and <0.05 µM PO_4) and an enriched condition (3 µM NO_3 and 0.5 µM of phosphates). Nutrient concentrations were chosen in order to have a control condition matching the nutrient levels in oligotrophic reefs (Kleypas et al. 1999), an enriched condition corresponding to a moderate

input of nutrient by an upwelling or human activities (Nixon 1995; Smith 2003) and a depleted condition matching the concentration in stratified water (Polovina et al. 2008; Hoegh-Guldberg and Bruno 2010). Nutrient concentrations in each aquarium were monitored using an Autoanalyzer (Alliance Instrument, AMS, France) according to Treguer and Le Corre (1975). Nutrient depleted water was obtained with bio-chemical filters (Fluidize bed filter BF-700) installed in batch tank. Experimental tanks were continuously supplied with the depleted water from the batch tanks. To enrich the seawater, solutions of sodium nitrate ($NaNO_3$) and sodium dihydrogenophosphate (NaH_2PO_4) were added to batch tanks. This water was then continuously pumped with a peristaltic pump and delivered to the experimental tanks at a final concentration of 3 µM for $NaNO_3$ and 0.5 µM for NaH_2PO_4. The stock solutions were renewed three times a week.

I. 2. Experimental set up in New Caledonia

This experimental set up was used in papers n°3 and 4.

I. 2. 1. UVR

Light quality and quantity

The “Aquarium des lagons” of Nouméa, New Caledonia, has outdoor facilities, exposed to natural solar radiation. To match the underwater light conditions on a shallow reef, shade cloths were disposed above the aquaria (Figure 13A). In November, when the cloths were installed, the natural irradiances in aquaria, obtained at midday on a sunny day, were ~900 µmol photons m^{-2} s^{-1} PAR, ~20 W m^{-2} and ~1.2 W m^{-2} of UVA and UVB radiation, respectively. UV intensity was controlled with an International Light ILT1400 portable radiometer connected to detectors (SEL240/UVB-1/TD and SEL033/UVA/TD). The settings stayed identical over the 6 months of experiment. To shield nubbins from UVR, UV filters (226 Lee U.V. filters) were used (Figure 13BC).

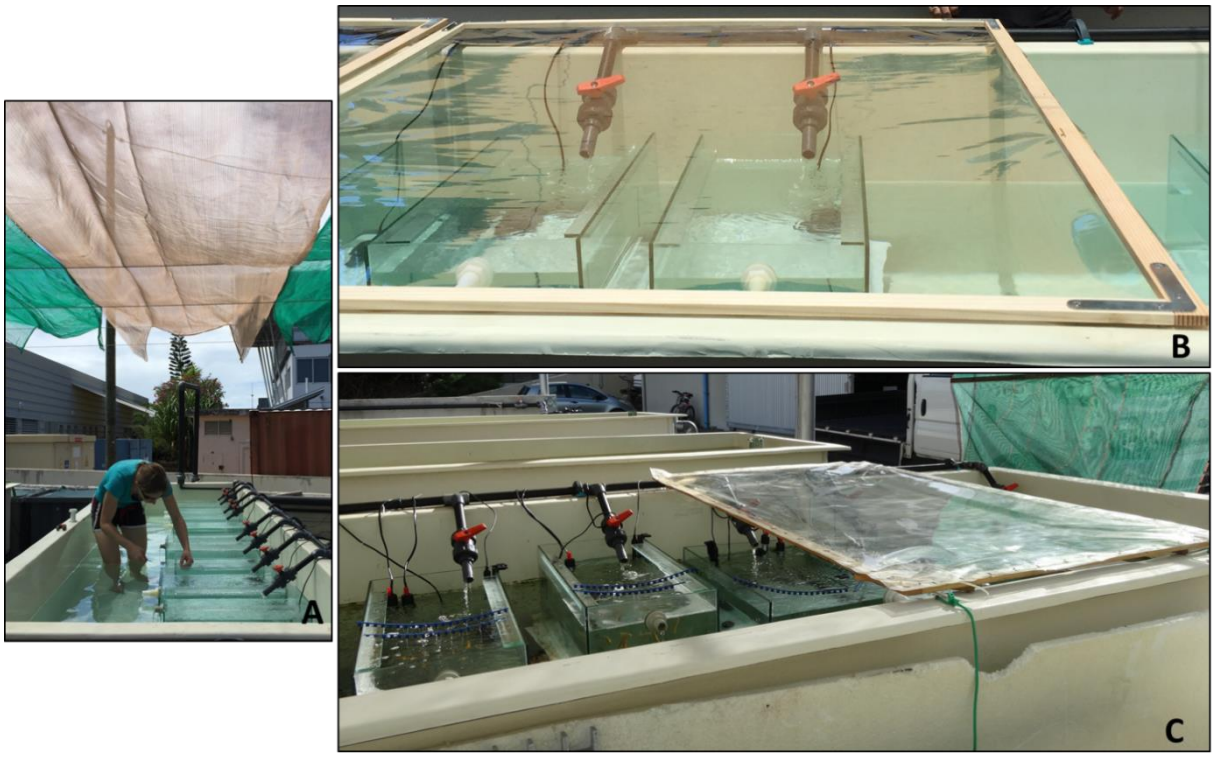


Figure 13. Experimental set up in the Aquarium des lagons in Nouméa. The experimental tanks were settled in a big buffer tank to regulate the temperature and shade cloths were installed above them to attenuate the solar radiation (A). UVR filters were installed on the top of some of the experimental tanks (BC).

As we were enabled to record UVR intensity variation over time, we used UV index (from Weather Online) recorded at the airport of Nouméa during the time period of the experiments and average annual variation reported by the NASA's MERRA-2 Modern-Era Retrospective Analysis, to give a general idea of the UVR seasonal variability (Figure 14). Table 5 summarizes the known environmental conditions at the time of the different experiments.

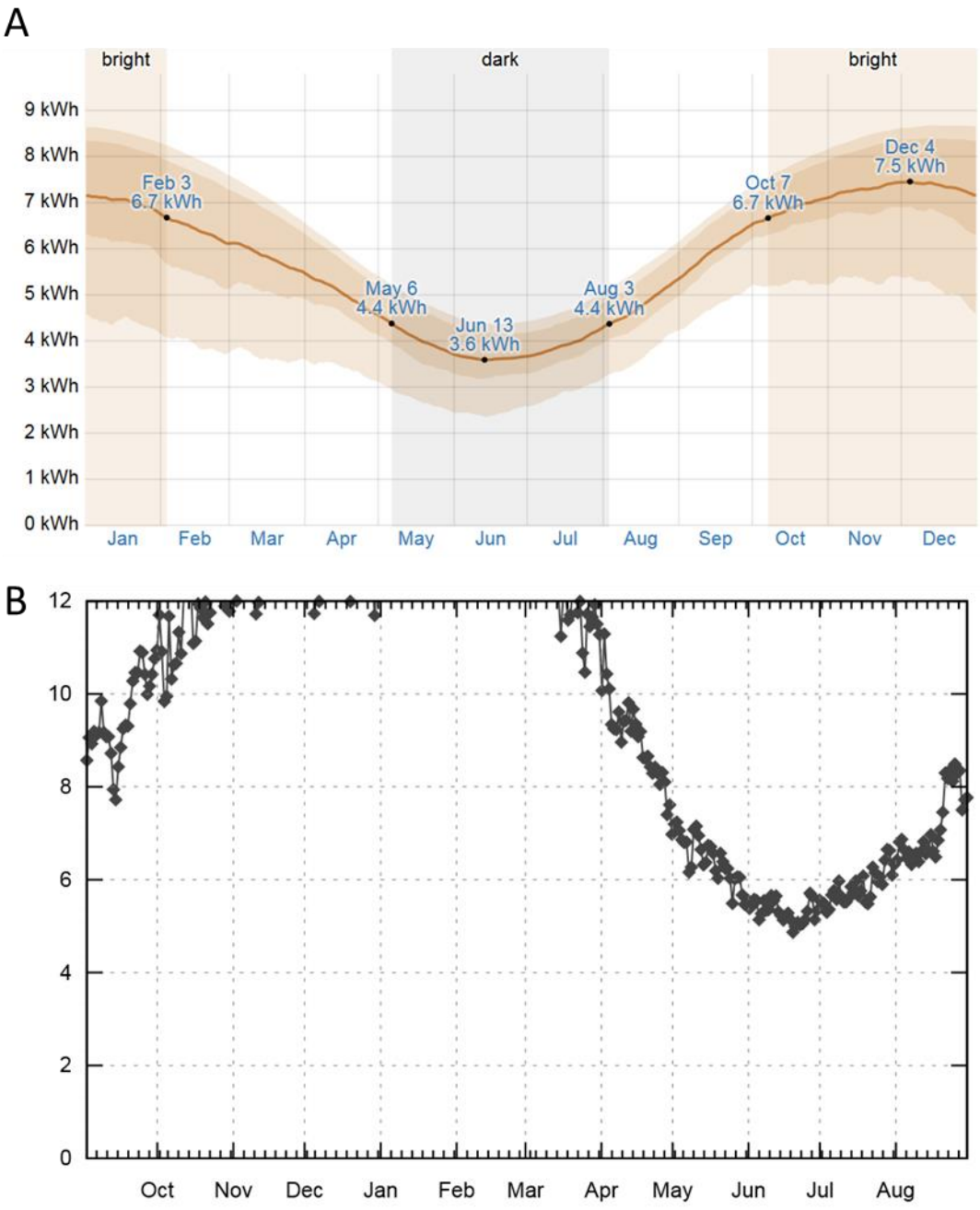


Figure 14. A) Average daily incident shortwave solar energy (kWh) reaching the ground per square meter in Nouméa (orange line) with 25th to 75th and 10th to 90th percentile bands. This report illustrates the typical weather in Nouméa, based on a statistical analysis of historical hourly weather reports and model reconstructions from January 1, 1980 to December 31, 2016. © weatherspark.com; https://weatherspark.com/y/144754/Average-Weather-in-Noum%C3%A9a-New-Caledonia B) UV index at Nouméa airport between the 02/09/2015 and the 31/08/2016. The UV Index is a number linearly related to the intensity of sunburn-producing UV *i.e.* relative to the proportion UVB/UVA. © weatheronline.co.uk; http://www.weatheronline.co.uk/weather/maps/city

Table 5. Summarize of the temperatures and UVR levels at the different time of the experiments. "+" give a comparison between UVR levels at different time of the year (deduced from Figure): "+++" is the highest level, "++" is a medium level and "+" is the lowest level.

Paper number	Condition	Month	Temperature	UVR level
4	Winter	July	23.1 ± 0.6°C	+
3	Autumn	May	26.3 ± 0.5°C	++
4	Summer	February	29.3 ± 0.9°C	+++
3	Thermal stress	May	30.1 ± 0.3°C	++

Ecological relevance of the experimental setting

Corals in the aquaria were exposed to natural and present time daily and seasonal variations in UVR, similar to the irradiance received in their original reef location. The aim of the experimental set up was to test the effects of natural UVR, or UVR shading (obtained by UV filters) on 1) the coral physiology, mucus quality/quantity and associated bacteria at different time of the year (in winter and summer) and on 2) the coral response to thermal stress.

I. 2. 2. Temperature: natural variability and thermal stress

The water in the experimental tanks was directly pumped from the nearest reef. The seasonal variability in temperature was therefore similar to the reef. To avoid excessive warming of the water due to the small size of the experimental tanks, these later were kept in a big buffer tank with a very high water renewal rate (Figure 3A). During austral summer months, this set up was not sufficient to compensate the effects of the sun heat during daytime. The water was therefore cooled down with a chiller before being distributed in the experimental tanks. The daily variability was kept to ~1°C difference between night and day.

The thermal stress experiment (paper n°3) was carried out in May, corresponding to the beginning of the austral winter, when the ambient temperature was around 26°C. The thermal stress was induced by gradually increasing the temperature from 26 to 30°C within a week. The temperature was controlled using heaters connected to a temperature-stat system (IKS, Karlsbad, accuracy ± 0.05°C) and recorded using HOBO data logger (Figure 15). Table 5 summarizes the average temperature during the time of the experiments.

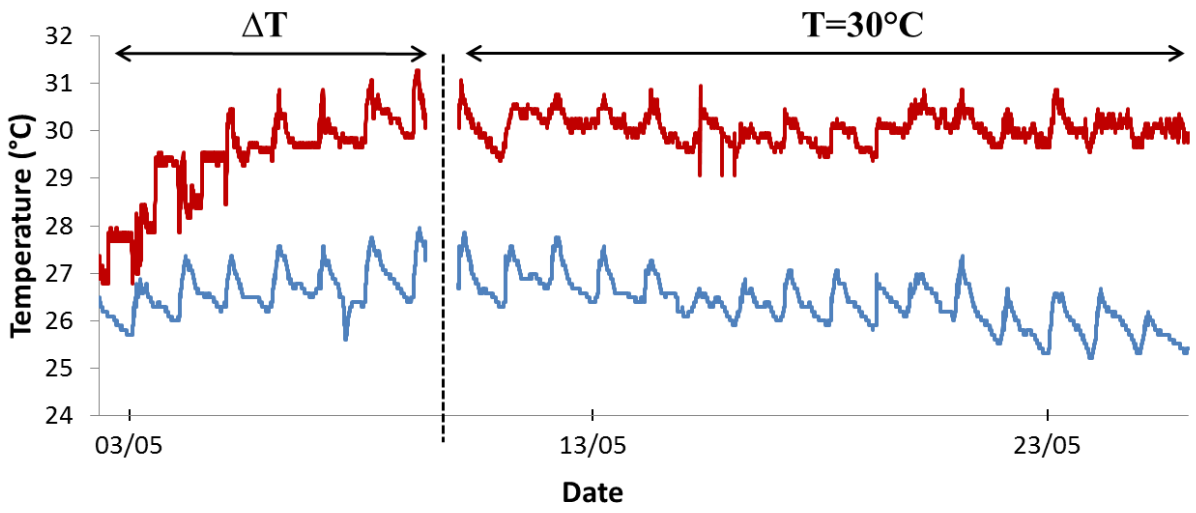


Figure 15. Data recording during the thermal stress experiment (Paper n° 3). The red line is the temperature recorded in high temperature tanks while the blue line is the temperature in ambient temperature condition. ΔT is the week of temperature increase and T=30°C is the thermal stress period.

II. Physiological and organic matter fluxes study

Several coral species were used during the thesis. Box 6 describes the different coral species studied in the thesis.

Box 5. Studied coral species

***Pocillopora damicornis* (Linnaeus, 1758) (Figure 16A)**

P. damicornis belongs to the Pocilloporidae family. It is a fast growing branching species widely distributed in the shallow waters of the Red Sea and the Indo-Pacific area (Veron and Stafford-Smith 2000). The nubbins used for the experiments in Monaco were collected in the Pacific Ocean and contained the clade C1 of *Symbiodinium* (Karako-Lampert et al. 2004).

***Stylophora pistillata* (Esper, 1797) (Figure 16B)**

Like *P. damicornis*, *S. pistillata* belongs to the Pocilloporidae family. This species is widely distributed in the Indo-Pacific Ocean and the Red Sea and is found in shallow and deep water (from 1 to 80 m depth, Veron and Stafford-Smith 2000). It is a fast growing branching species, whose branch thickness depends on the environmental conditions (light, sediment, wave and currents). The nubbins we used in Monaco were collected in the red Sea and contained the clade A of *Symbiodinium*.

***Turbinaria reniformis* (Bernard, 1986) (Figure 16C)**

Species of the *Turbinaria* genus and from the Dendrophylliidae family are described as the most diverse to date (Veron and Stafford-Smith 2000). *Turbinaria reniformis* is mainly distributed in the Indo-pacific area, in the Red Sea and along the coast of East Africa. It has a leaf shape and usually

grows in turbid water. The nubbins used in Monaco were collected in the Red Sea and contained clade C1 of *Symbiodinium*.

***Acropora muricata* (Linnaeus, 1758) (Figure 16D)**

A. muricata belongs to the Acroporidae family. It is a fast growing coral, which belongs to the branching species. It is one of the ten most abundant genera in New Caledonia (Fenner and Muir 2008) and worldwide (Veron and Stafford-Smith 2000). Nubbins used in New Caledonia were collected in shallow water (<5 m depth) on the New Caledonian reefs of "L'îlot maître" and of "Phare Amédée".

Figure 16. Coral species used in this thesis: *Pocillopora damicornis* (A), *Stylophora pistillata* (B), *Turbinaria reniformis* (C), *Acropora muricata* (D). The outlined pictures show the nubbins used in the experiments; black lines are 1 cm long. Photo credit: ©L. & J. Courtial.

II.1. Tissue parameters (symbiont, protein and pigment density)

Nubbin tissue was removed from the skeleton using an air pick and homogenized with a Potter tissue grinder. The surface, used to normalize the great majority of the data, was measured using the wax technique (Stimson and Kinzie 1991). The symbiont density was determined using either a microscope (in New Caledonia, Hoogenboom et al. 2010) or a coulter counter (in Monaco). Protein contents were measured using BCA assay (Smith et al. 1985) according to Hoogenboom et al.

(2010). The chlorophyll a and c2 contents were quantified by spectrophotometry (Hoogenboom et al. 2010) using the equations of Jeffrey and Humphrey's (1975).

II. 2. Coral metabolism (Photosynthesis, respiration, calcification and coral growth)

Respiration and photosynthesis rates were measured in closed chambers using either optodes (papers n° 3, 5) or O_2 electrodes (paper n°2). The photosynthetic efficiency of photosystem II of the symbiont was assessed by pulse amplitude modulated (PAM) fluorometry (Ralph and Gademann 2005). Coral calcification was assessed using the total alkalinity anomaly method (Smith and Key 1975). Coral growth was measured using the buoyant weight technique (Jokiel et al. 1978).

II. 3. Organic matter fluxes

The total and dissolved organic carbon/nitrogen (TOC/TON; DOC/DON respectively) fluxes were assessed by incubating nubbins for several hours in filtrated seawater and by subtracting the organic matter content of the seawater at the beginning of the incubation from the content at the end. Those values were then corrected by a blank obtained by incubating in parallel and in the same condition, filtrated seawater. Negative fluxes indicate a net uptake by coral nubbins, and positive fluxes indicate a net release from corals. To differentiate the dissolved from the total matter, seawater samples were filtered on 0.45 µm. Measurements were performed using a TOC-L analyzer (Shimadzu, Japan) according to Naumann and collaborators (2010). The particulate matter fluxes were calculated by subtracting the dissolved matter from the total matter.

II. 4. Reactive oxygen species concentration

To assess the intracellular ROS concentration in living coral tissue, a protocol based on fluorometric measurements was developed. Apical tips of *Stylophora pistillata* were incubated with Carboxy-H_2DCFDA, a cell-permeable probe that is non-fluorescent until it is converted to the fluorescent form, DCF (dichlorofluorescein) after being oxidized by ROS. The emitted fluorescence is directly proportional to the ROS concentration within the tissue. This probe was already used as an indicator for ROS in several species and namely in Human cells and free *Symbiodinium* (Wang and Joseph 1999; Roberty et al. 2015).

To optimize the signal measured in coral tissue, nubbins were first incubated for 30 minutes in the dark in 750 µL of 0.45 µm filtrated seawater containing 25 µM of Carboxy-H_2DCFDA dissolved

in 0.001% DMSO (hereafter called probe). Nubbins were then removed out of the probe, quickly rinsed in filtrated seawater and placed, out of the water, at a constant distance under the optical fiber. At this stage, the emission spectrum between 515 and 550 nm (495 nm excitation) of each coral was measured with a spectrofluorometer equipped with an optical fibre to establish a baseline signal. Nubbins were then incubated again for 30 more minutes in the probe and under the different environmental conditions to be tested. At the end of the incubation, the emission spectrum was measured again. The fluorescence was then calculated by removing the baseline from the final signal (example of the measurements Figure 17).

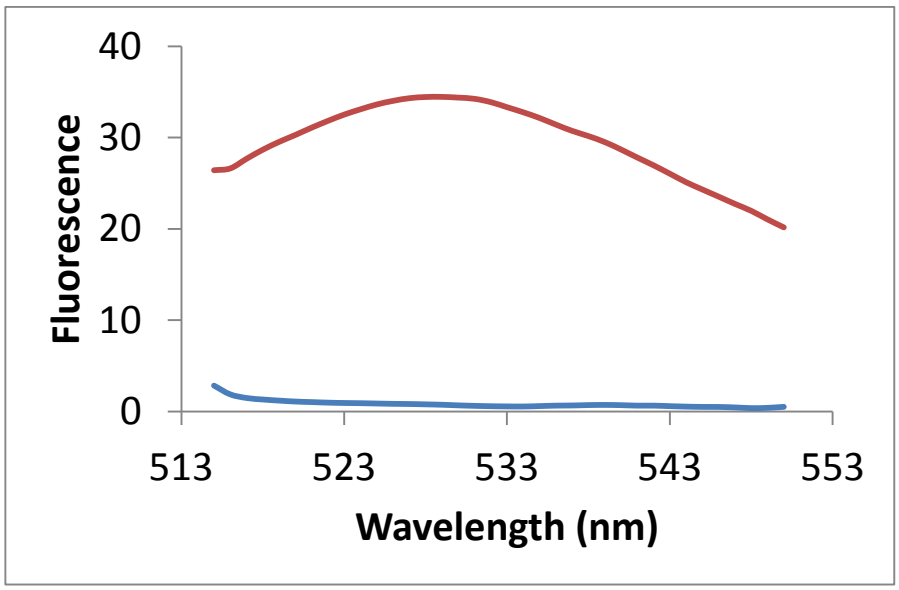


Figure 17. Emission spectrum of a nubbin before (blue curve) and after (red curve) exposure for 30 minutes in Carboxy-H_2DCFDA probe under thermal stress (30°C).

III. Micro-organisms associated with the mucus and coral tissue

III.1. Population identification

In paper n°4, we characterized the microbiome associated both with the mucus and the host of the scleractinian coral *Acropora muricata*, using the technique of 16S metabarcoding (Rohwer et al. 2001). Briefly, coral nubbins were incubated for 2 h in 1 L of 0.45 µm filtrated seawater for mucus production. At the end of the 2h, the incubation water was filtrated on 0.2 µm polycarbonate membrane filters. Nubbins and polycarbonate membranes were then separately frozen at -20°C in RNA later. Coral fragments were crushed in liquid nitrogen. The DNA of the coral powders and the polycarbonate membranes was extracted using the Genomic DNA Buffer Set and Genomic-tip 20/G columns (QIAGEN, Hilden, Germany) according to the manufacturer's protocol. For each sample, 16S

rDNA amplicon libraries were constructed using the 515F/806R primer pair (according to the Earth microbiome project protocol; http://www.earthmicrobiome.org/protocols-and-standards/16s/), which targets the V4 region of the 16S rRNA gene, and paired-end (2x 300bp) sequenced on the Illumina MiSeq platform. Samples were then sent to Molecular Research Laboratory (MR DNA, Shallowater, Texas, USA) for sequencing.

III.2. Growth and enzymatic activity

The population dynamics of mucus-associated microorganisms was determined by flow cytometry. For this purpose, coral nubbins were incubated for several hours in filtrated seawater for mucus production. Microorganism concentrations in seawater, were assessed by flow cytometry as described by Jacquet et al. (2013) at the beginning and end of the incubation. The population dynamic (growth or decline via coral predation) was calculated using the equations of Ribes et al. (1998) (see the equation in details in paper n°3). To only assess the changes in the mucus-associated microorganisms, the concentrations in the experimental beakers were corrected from a blank (filtrated seawater alone, no coral nubbin) incubated in parallel.

The activity of the mucus-associated bacteria was assessed by monitoring the extracellular activity (EEA) of the α-glucosidase and the aminopeptidase, the two main enzymes degrading carbon and nitrogen respectively. The EEA was measured by fluorometry following a protocol developed by Hoppe (1983) and adapted to corals by Fonvielle et al (2015). The principle of this technique is to incubate mucus (and associated bacteria) with fluorescent substrate analogues (4-methylumbeliferyl-α-D-glucopyranoside (αMUF) for the α-glucosidase and L-Leucine-7-amido-4-methyl-coumarin hydrochloride (Leu-MCA) for the aminopeptidase), which are then cleaved by the enzyme activity into two fluorescent compounds: the 4-methylumbeliferone (MUF) and the amino 4-methylcoumarin (MCA) respectively. The emitted fluorescence is directly proportional to the enzymatic activities and can be monitored using a spectrofluorometer. The relation between the fluorescence intensity and the quantity of hydrolyzed substrate is obtained by establishing a calibration curve using known concentrations of commercial MUF and MCA.

The matter degradation by the mucus-associated bacteria can be calculated considering the enzymatic activity and the concentration of total organic matter. The calculation was performed according to the following equation: **%X degraded = EEA_{max}x100/TOX** with X being either carbon (C) or nitrogen (N), EEA_{max} is the maximal enzymatic activity (nmol L^{-1} h^{-1}) of α-glucosidase (for C degradation) or aminopeptidase (for N degradation). TOX (nmol L^{-1} h^{-1}) is the quantity of organic X contained in the mucus. TOX was calculated as **$TOX=TOX_{coral}-TOX_{blk}$** with TOX_{coral} and TOX_{blk} (nmol L^{-}

[1] h^{-1}) the TOX concentrations in the seawater at the end of the incubation with or without nubbin respectively.

IV. Molecular approach: signalling pathways

IV.1. Aims and difficulties

In order to identify the molecular signalling pathways involved in the coral response to oxidative stress, we assessed the activity of the mitogen active protein kinases (MAPKs). MAPK activation occurs through its phosphorylation by an upstream kinase, the Mitogen-Activated protein Kinase Kinase (MAPKK). An appropriate readout for the activity of the pathway is therefore to monitor the phosphorylation of the MAPKs using western blot analysis.

The western blot is a widely used analytical technique that consists in separating proteins of an extract on a membrane and revealing the target protein signal with specific antibodies (in our case specific of the phosphorylated or the total form of the studied MAPKs). Without going into details, the main steps are (fully describe in Mahmood and Yang 2012) and are the following:

- Preparation of the protein extract
- Protein separation by size with a gel electrophoresis
- Transfer of the proteins from the gel to a membrane
- Staining of the membrane with specific antibodies
- Protein detection

The activity of the MAPK proteins has never been studied in corals before. The challenges of applying this technique to coral extracts were in the sample preparation step, to keep the protein phosphorylation intact and in the protein detection, to determine specific antibodies that target coral MAPKs.

IV.2. Description of the new steps of the experimental design

IV. 2. 1. Sample preparation

The preparation of coral protein extract needed 3 steps, as described in the following paragraph. The western blot was then performed according to the standard procedure (Mahmood and Yang 2012).

Tissue extraction in Laemmli buffer

Coral tissue was removed from the skeleton using an air-pick in 1 mL Laemmli buffer (1.5 X, Laemmli 1970). The uses of Laemmli buffer as well as the small extraction volume are essential to 1) preserve the protein phosphorylation and 2) concentrate the protein extract for a clear signal on the western blot.

Purification of the extract

Samples were then sonicated for 30 sec, and centrifuged (3 x 5 minutes at 15 000 g) to remove the lipid supernatant and debris. This step is important in order to obtain a clear extract suitable for western blot analysis.

Protein quantification and final preparation

To be able to load the same amount of proteins in each load of the gel, the protein concentration of each coral extract was quantified using a BCA protein assay (Thermo Fisher Scientific BCA kit), following the manufacturer's recommendation. 1,4 Dithiothreitol (1 mM) and bromophenol blue (0.1%) were then added to the samples before they were heated for 5 minutes at 95°C.

IV. 2. 2. Antibody validation

In the thesis, two different MAPKs were studied: the extracellular signal regulated kinase (ERK, paper n°1) and the c-Jun N-terminal kinase (JNK, paper n°6). For each MAPK; two antibodies were validated: an antibody targeting the total form of the protein and an antibody targeting the phosphorylated form of the protein (Table 6).

Table 6. Validated antibodies.

Antibody	Manufacturar	Catalog number	RRID
p44+42 MAPK (Erk1,2) Antibody (3F8)	Thermo Fisher Scientific	MA5-15605	AB_10983247
Phospho-p44 MAPK + p42 MAPK pTyr204 Antibody (B.742.5)	Thermo Fisher Scientific	MA5-15174	AB_10980347
pTPpY Antibody	Promega	V7931	AB_430864
pan-JNK Antibody	R&D Systems	AF1387	AB_2140743

The methodological paper (n°1) describes in details the steps of validation of the antibodies directed against the two forms of ERK. A similar approach was used to validate the JNK antibodies in the paper n°6.

Paper n°1 – F1000 Research

F1000Research F1000Research 2017, 6:577 Last updated: 21 JUL 2017

Check for updates

ANTIBODY VALIDATION ARTICLE

REVISED Validation of commercial ERK antibodies against the ERK orthologue of the scleractinian coral *Stylophora pistillata* [version 2; referees: 3 approved]

Lucile Courtial [1-3*], Vincent Picco [4*], Gilles Pagès[4,5], Christine Ferrier-Pagès[1]

[1]Marine Department, Centre Scientifique de Monaco, Monaco, MC-98000, Monaco
[2]Sorbonne Universités, Pierre and Marie Curie University, Paris, 75252, France
[3]Laboratoire d'Excellence, UMR ENTROPIE, Nouméa, 98848, New Caledonia
[4]Biomedical Department, Centre Scientifique de Monaco, Monaco, MC-98000, Monaco
[5]Institute for Research on Cancer and Aging of Nice (IRCAN), University Nice Sophia-Antipolis, CNRS UMR7284/INSERM U1081, Centre Antoine Lacassagne, Nice, 06189, France

* Equal contributors

v2 **First published:** 26 Apr 2017, **6**:577 (doi: 10.12688/f1000research.11365.1)
Latest published: 03 Jul 2017, **6**:577 (doi: 10.12688/f1000research.11365.2)

Abstract

The extracellular signal-regulated protein kinase (ERK) signalling pathway controls key cellular processes, such as cell cycle regulation, cell fate determination and the response to external stressors. Although ERK functions are well studied in a variety of living organisms ranging from yeast to mammals, its functions in corals are still poorly known. The present work aims to give practical tools to study the expression level of ERK protein and the activity of the ERK signalling pathway in corals. The antibody characterisation experiment was performed five times and identical results were obtained. The present study validated the immune-reactivity of commercially available antibodies directed against ERK and its phosphorylated/activated forms on protein extracts of the reef-building coral *Stylophora pistillata*.

This article is included in the SPONSORED GATEWAY
Antibody Validations
abcam
Organizations supporting this gateway have had no editorial control with respect to the content contained herein.

Open Peer Review

Referee Status: ✔ ✔ ✔

	Invited Referees 1	2	3
REVISED version 2 published 03 Jul 2017	✔ report		✔ report
version 1 published 26 Apr 2017	? report	✔ report	? report

1 **Andrea Pitzschke**, University of Salzburg, Austria

2 **Immacolata Castellano**, Stazione Zoologica Anton Dohrn, Italy

3 **María L. Parages**, University of Málaga, Spain

Discuss this article

Comments (0)

F1000Research F1000Research 2017, 6:577 Last updated: 03 JUL 2017

Corresponding authors: Vincent Picco (vpicco@centrescientifique.mc), Christine Ferrier-Pagès (ferrier@centrescientifique.mc)

Author roles: Courtial L: Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Writing – Original Draft Preparation, Writing – Review & Editing; **Picco V**: Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Project Administration, Supervision, Writing – Original Draft Preparation, Writing – Review & Editing; **Pagès G**: Conceptualization, Resources, Writing – Original Draft Preparation; **Ferrier-Pagès C**: Conceptualization, Resources, Supervision, Writing – Original Draft Preparation

Competing interests: No competing interests were disclosed.

How to cite this article: Courtial L, Picco V, Pagès G and Ferrier-Pagès C. **Validation of commercial ERK antibodies against the ERK orthologue of the scleractinian coral *Stylophora pistillata*** *F1000Research* , **6**:577 (doi: $version.doi)

Copyright: © 2017 Courtial L *et al*. This is an open access article distributed under the terms of the Creative Commons Attribution Licence, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. Data associated with the article are available under the terms of the Creative Commons Zero "No rights reserved" data waiver (CC0 1.0 Public domain dedication).

Grant information: Financial support to CFP, GP, LC and VP was provided by the Centre Scientifique de Monaco and Paris VI University.
The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

First published: 26 Apr 2017, **6**:577 (doi: 10.12688/f1000research.11365.1)

F1000Research 2017, 6:577 Last updated: 03 JUL 2017

REVISED **Amendments from Version 1**

In order to comply with the referees' requests, we added an experiment consisting of inducing ERK activity by exposing corals to UV radiation, thermal stress or a combination of both.

We added new figures as well as their corresponding text in the Methods and the Results sections. Specifically, in order to answer one of the referees' concerns, we have slightly modified Figure 2 and Supplementary Figure S1 by adding the amido black total protein stainings of the western blot membranes as a loading control. We have also added a new Figure 3 and the corresponding Supplementary Figure S2 (full length images of the western blot).

See referee reports

Introduction

Mitogen activated protein kinases (MAPKs) are highly conserved proteins involved in signalling pathways and control key cellular processes such as proliferation, differentiation, migration, survival and apoptosis (Dhillon *et al.*, 2007). The MAPK gene family encompasses three major subfamilies: the extracellular signal-regulated kinase (ERK), p38/HOG and c-Jun N-terminal kinase (JNK) groups. The ERK family is the most studied in mammals (Boulton *et al.*, 1990; Dhillon *et al.*, 2007) because it is involved in meiosis, mitosis and post mitotic functions in differentiated cells, as well as in the oxidative stress response and wound healing (Castellano *et al.*, 2014; Johnson & Lapadat, 2002; Matsubayashi *et al.*, 2004; Runchel *et al.*, 2011). The ERK gene family is evolutionnarily conserved and is found in all eukaryotes, including yeasts, plants, vertebrates and invertebrates (Chen *et al.*, 2001; Widmann *et al.*, 1999). Although recent molecular studies have shown the existence of ERK genes in different coral species (Mayfield *et al.*, 2010; Siboni *et al.*, 2012; van de Water *et al.*, 2015), ERK activity and specific functions are not yet clearly defined. ERK activation occurs through phosphorylation of the Threonine and Tyrosine residues of an ERK-specific TEY motif by the upstream kinases of ERK, the mitogen-activated protein kinase kinase (MAPKK or MEK). ERK phosphorylation on these residues is classically considered the most appropriate readout for the activity of the ERK signalling pathway. However, it has never been monitored in corals. Overall, MAPK activities in corals have only been investigated once, in a study focusing on the JNK subfamily (Courtial *et al.*, 2017).

In this work, we used the scleractinian coral *Stylophora pistillata*, a very abundant species in most tropical reefs (Veron & Stafford-Smith, 2000). We applied the same protocol as in Courtial *et al.* (2017) to demonstrate the efficiency of antibodies directed against the mammalian phosphorylated forms of ERK (pERK) and total ERK to detect the ERK orthologs in *S. pistillata* (Table 1). According to the manufacturer's instructions, the antibody used in this study and directed against the Thr202/Tyr204 di-phosphorylated active ERK (Thermo Scientific Pierce; MA5-15174) showed reactivity with fruit fly, human, mink, mouse, non-human primate, pig, rat and zebrafish. The immunogen used to generate this rabbit IgG monoclonal antibody was a synthetic phosphopeptide corresponding to residues surrounding the phospho-Thr202/Tyr204 of the human p44/ERK1 MAP kinase. This antibody is not cross-reactive with the corresponding phosphorylated residues of either JNK/SAPK or p38. The ERK1/ERK2 antibody (Thermo Scientific Pierce; MA5-15605) used in the study previously showed reactivity with human and mouse samples. The immunogen used to generate this mouse IgG2b monoclonal antibody was a purified recombinant fragment of human MAPK.

Methods

Maintenance of *Stylophora pistillata* nubbins and human fibroblasts

Nubbins of *Stylophora pistillata* were collected from five mother colonies maintained in the aquaria facilities of the Centre Scientifique de Monaco. Two small nubbins (3–5 cm long) were

Table 1. Primary and secondary antibodies.

Antibody	Manufacturer	Catalogue number	RRID	Concentration
p44+42 MAPK (Erk1,2) Antibody (3F8)	Thermo Fisher Scientific	MA5-15605	AB_10983247	1µg/mL (1/1000)
Phospho-p44 MAPK + p42 MAPK pTyr204 Antibody (B.742.5)	Thermo Fisher Scientific	MA5-15174	AB_10980347	1µg/mL (1/1000)
Peroxidase-AffiniPure Goat Anti-Mouse IgG (H+L) antibody	Jackson ImmunoResearch Labs	115-035-003	AB_10015289	1µg/mL (1/10000)
Peroxidase-AffiniPure Goat Anti-Rabbit IgG (H+L) antibody	Jackson ImmunoResearch Labs	111-035-003	AB_2313567	1µg/mL (1/10000)
IRDye 800CW Goat Anti-Rabbit IgG (H+L)	LI-COR Biosciences	926-32211	AB_621843	0.1µg/mL (1/10000)
IRDye 680RD Goat anti-Mouse IgG (H + L)	LI-COR Biosciences	926-68070	AB_10956588	0.1µg/mL (1/10000)

cut off from each mother colony, and were allowed to heal for four weeks in 15 L open system tanks before the experiments. Corals were maintained in the same conditions as the mother colonies, *i.e.* at 25°C, under a photosynthetic active radiation of 200 µmol photon.m^{-2}.s^{-1} provided by 400 W metal halide lamps (HPIT, Philips) and were fed twice a week with *Artemia salina nauplii*. Seawater in the tanks was continuously renewed at a rate of 10 L.h^{-1}.

Immortalized skin fibroblasts (BJ-EHLT cells) were kindly provided by E. Gilson's lab (IRCAN) and cultured in Dulbecco's Modified Eagle's Medium (Invitrogen, Villebon-sur-Yvette, France) supplemented with 10% heat-inactivated fetal calf serum (Dutscher, Brumath, France) at 37°C in an atmosphere of 5% CO_2, as previously described (Biroccio *et al.*, 2013).

UO126 treatment of coral nubbins

Incubations were performed in 100 mL beakers containing one coral nubbin each, and filled with 40 mL of 0.45 µm filtered seawater. They were placed in the dark for one hour in either a control condition containing 0.005% DMSO (vehicle) or a condition with 5 µmol.L^{-1} UO126 (Selleck Chemicals), a MEK inhibitor (Tang *et al.*, 2003). The incubation medium was continuously stirred using magnetic stirrers at a constant temperature of 25°C. At the end of the incubation, nubbins were frozen and kept at – 80°C prior to western blot analysis.

UVR and temperature treatment of coral nubbins

Incubations were performed in 100 mL beakers containing one coral nubbin each and filled with 40 mL of 0.45 µm filtered seawater and continuously stirred using magnetic stirrers. High temperature or/and ultraviolet radiation (UVR) stresses (i.e. four environmental conditions: control (at 25°C and without UVR), thermal stress (30°C without UVR), UVR stress (25°C under UVR), thermal and UVR stresses (30°C and under UVR)) were applied to corals and ERK activation was monitored after 30 minutes of stress. Thermal stress corresponded to an increase in temperature from the normal culture condition of 25°C to 30°C. The UVR stress corresponded to an increase in UVR from 0 (HQI lamps in the culture conditions) to a radiation intensity of about 3 W.m^{-2} UVB and 30 W.m^{-2} UVA (Q-Panel UVA 340 lamps). At the end of the incubation, nubbins were frozen and kept at – 80°C prior to western blot analysis.

Western blot analysis

Immuno-detections were performed as in Courtial *et al.* (2017; Table 2 and Table 3). Briefly, nubbins were airbrushed in 1 mL Laemmli buffer (2% SDS, 10% glycerol, 50mM Tris HCL pH7), (Laemmli, 1970) using an air-pick (5 bars) to remove the totality of the tissues surrounding the skeleton was removed from coral. Samples were then sonicated for 30 seconds, and centrifuged (3 × 5 minutes at 15 000 g) to remove the lipid

Table 2. Tissue extraction and western blot protocol.

Process	Reagent	Manufacturer	Catalogue number	Concentration/Composition
Tissue extraction	Laemmli buffer 1.5X	Homemade		150 mM Tris-HCl pH 7, 25% glycerol, 2% SDS
Sample preparation	Laemmli - 1,4 Dithiothreitol - bromophenol blue solution	Homemade		1.5 X- 50 mM - 0.1%
Electrophoresis	ECL gradient gel 8–16%	GE Healthcare Lifesciences	29-9901-58	
	TG-SDS 10X running buffer	EUROMEDEX	EU0510	1X
Protein transfer	DUNN transfer buffer	Homemade		10 mM NaHCO3 - 3 mM Na2CO3 - 10% Ethanol (pH 9.9)
Blocking	Blocking reagent	Homemade		PBS + milk (3%)
Washes	Wash buffer 10X	Homemade		PBS 10X Tween 20 1N
Membrane coloration	Coloration buffer	Homemade		Isopropanol (25%) + acetic acid (10%) + amido black (0.1%)
Membrane destain	Destain buffer	Homemade		Isopropanol (25%) + acetic acid (10%)
Target detection	Immobilon Western HRP Substrate	Millipore	WBKLS0500	
Reagents	BCA QuantiPro BCA Assay Kit	Sigma-Aldrich	QPBCA-1KT	
	Milk	Itambe ®		
	Methanol	Sigma-Aldrich		

Table 3. Reagents for tissue extraction and western blots.

Protocol steps	Reagent	Time	Temperature
Tissue extraction	Laemmli 1.5 X (1mL)		RT
Sonication	Laemmli 1.5 X	30 sec	RT
Centrifugation (x3 15000 g)	Laemmli 1.5 X	3 × 5 min	RT
Addition of 1,4 Dithiothreitol - bromophenol blue solution	Laemmli 1.5 X		RT
Heat up		5 min	95°C
Electrophoresis (100 V)	Running buffer	variable	RT
Transfer (200 mA)	Transfer buffer	overnight	4°C
Coloration	Isopropanol (25%) + acetic acid (10%) + amido black (0.1%)	5 min	RT
Destain	Isopropanol (25%) + acetic acid (10%)	3 × 5 min	RT
Blocking	PBS + milk (3%)	30 min	RT
Primary antibodies	PBS + milk (1%) + ab (1/1000)	overnight	4°C
Washes (3 times)	Wash buffer 1X	3 × 15 min	RT
Secondary antibody	PBS + milk (1%) + ab (1/10000)	2h	RT
Washes (5 times)	Wash buffer 1X	5 × 15 min	RT
Detection	Immobilon Western HRP Substrate	30 sec – 10 min	RT

supernatant and debris. Fibroblasts were washed twice in phosphate buffered saline solution (PBS), lyzed in Laemmli buffer directly in the dishes and sonicated for 30 seconds. The total protein concentration of all samples was determined using a BCA protein Assay Kit (Thermo Fisher Scientific), according to the manufacturer's recommendation. 1,4 Dithiothreitol (1 mM) and bromophenol blue (0.1%) were added to the samples, which were then heated for 5 minutes at 95°C.

60 µg of proteins were separated on 10% polyacrylamide gels at 300 mA and 110 V at room temperature. Proteins were then transferred on a PVDF membrane at 4°C overnight in Dunn's transfer buffer at 200 mA. After a rinse in distilled water, membranes were saturated for 30 minutes in PBS - 3% low fat milk, rinsed in PBS and incubated with primary antibodies diluted in PBS - 1% low fat milk at 4°C overnight. The antibody directed against Thr202/Tyr204 di-phosphorylated active ERK was from Thermo Scientific Pierce (rabbit monoclonal; MA5-15174; batch no. OC1680806); the anti-ERK1/2 antibody was from Thermo Scientific Pierce (mouse monoclonal; MA5-15605; batch no. PH1895491). After extensive washing (4×30 minutes) in PBS – 0.1% Tween 20, membranes were incubated for 2 hours at room temperature in the simultaneous presence of IRDye 680RD goat anti-mouse (925-68070) and IRDye 800CW goat anti-rabbit (925-32211; Li-COR Biotechnology GmbH, Bad Homburg, Germany) secondary antibodies, or with anti-mouse and anti-rabbit HRP-conjugated antibody. Another set of extensive rinsing (4×30 minutes) in PBS – 0.1% Tween 20 was performed before membranes were imaged with an Odyssey device (LI-COR Biosciences, Lincoln, Nebraska) to detect fluorescence and HRP activity using Millipore ECL.

Densitometric analysis of the western blots was performed using Image Studio v2.1 software (Li-COR Biosciences). Intensity of the pERK signal was normalized to the intensity of ERK signal. The relative intensities between control and inhibitor conditions were compared using a t-test. Statistical analysis was done using the software Graphpad Prism v5.03.

Results and discussion

In order to confirm the presence of an ERK ortholog in corals, the human protein sequence of ERK1 (NP_001035145) was compared to the transcriptome database of *Stylophora pistillata* using the BLAST software (Altschul *et al.*, 1990; Karako-Lampert *et al.*, 2014). An open reading frame was retrieved from the best hit sequence with a predicted molecular weight of 42 kDa (Spi_isotig05348). This sequence (hereafter referred to as Spi-ERK for *S. pistillata* ERK) is the only one that shows an homology as high as 81%, 80% and 78% with the protein sequences of the cnidarians *Nematostella vectensis* ERK (Nv-ERK; XP_001629498.1), *Hydra vulgaris* ERK (Hv-ERK; XP_002154499.3) and the human MAPK3/ERK1 (Hs-ERK1), respectively (Figure 1)

F1000Research 2017, 6:577 Last updated: 03 JUL 2017

```
                                                                      D I
Spi_ERK      ----------------------MAAPPPPEVVRGQAFDVGPRYTNLSYIGEGAYGMVCSAL
Nv_ERK       ----------------------MAASTGPEVVRGQVFDVAPRYTNLNYIGEGAYGMVVAGV
Hv_ERK       -------------------------MASAEIVRGQVFDVGPRYKDLSYIGEGAYGMVCCAT
Hs_ERK1      MAAAAAQGGGGGEPRRTEGVGPGVPGEVEMVKGQPFDVGPRYTQLQYIGEGAYGMVSSAY
                                       *:*:** ***.***.:*.********** ..

                       D II            D III       D IV                D V
Spi_ERK      DNATGQRVAIKKISPFEHQTYCQRTLREIKILRRFKHENIIGIMDIIRAPNMDDMKDVYI
Nv_ERK       DNTTGERIAIKKISPFEHQTYCQRTLREIKILSRFKHENIIGILDIIRAPRAEDMKDIYI
Hv_ERK       DIYTGNKVAIKKISPFEHQTYCQRTLREIKILSRFNHENIINIQDIKRSPTIDDMKDVYI
Hs_ERK1      DHVRKTRVAIKKISPFEHQTYCQRTLREIQILLRFRHENVIGIRDILRASTLEAMRDVYI
             *     ::**********************:** **.***:*.* ** *:   : *:*:**

                                       D VIA           D VIB
Spi_ERK      VQSLMETDLYKLLKTQKLSNDHICYFLYQILRGLKYIHSANVLHRDLKPSNLLLNTTCDL
Nv_ERK       VQPLMETDMYKLLKTQRLSNDHICYFLYQILRGLKYIHSANVLHRDLKPSNLLLNTTCDL
Hv_ERK       VQTLMETDLYKLLKTQKLSNDHICYFLYQILRGLKYIHSANVLHRDLKPSNLLLNTTCDL
Hs_ERK1      VQDLMETDLYKLLKSQQLSNDHICYFLYQILRGLKYIHSANVLHRDLKPSNLLINTTCDL
             ** *****:*****:*:************************************:******

               D VII                          D VIII           D IX
Spi_ERK      KICDFGLARVADPEHDHTGFLTEYVATRWYRAPEIMLNSKGYSKAIDIWSVGCILAEMLS
Nv_ERK       KICDFGLARIADPDHDHTGFLTEYVATRWYRAPEIMLNSKGYSKAIDIWSVGCILAEMLA
Hv_ERK       KICDFGLARVADPEHDHTGMLTEYVATRWYRAPEIMLNSKGYTKSIDIWSVGCILAEMLS
Hs_ERK1      KICDFGLARIADPEHDHTGFLTEYVATRWYRAPEIMLNSKGYTKSIDIWSVGCILAEMLS
             *********:***:*****:**********************:*:**************:

                              D X
Spi_ERK      NRPLFPGKHYLDQLNRILEVLGSPSQDDLQCIMNDKARSYIQSLAYKPRVPWQRYFGGAD
Nv_ERK       NRPLFPGKHYLDQLNLILNVLGSPIADDLLCIKNEKARSYLQSLPYKPKTPFIKLFSNAD
Hv_ERK       NRPLFPGKHYLDQLNLILNVLGSPDKESLEFIRNLKAKSYLQGLPIKERTPWKTLFPEAD
Hs_ERK1      NRPIFPGKHYLDQLNHILGILGSPSQEDLNCIINMKARNYLQSLPSKTKVAWAKLFPKSD
             ***:*********** ** :****  :.*  * * **:.*:*.*  * :. :   *  :*

                                                                    D XI
Spi_ERK      NKAIELLDKMLTFNPDKRITIEEALAHPYLEQYYDPQDEPVAEEPFRFETELDDLPKERL
Nv_ERK       AKALDLIENLLAFNPNKRITVEEALAHPYLEQYYDPADEPVAETPFKFQMELDDLPKEKL
Hv_ERK       PKALDLLDKLLAFNPNKRVTVEEALAHEYLEQYYDPNDEPVAEEPFTFDTELDDLPKEKL
Hs_ERK1      SKALDLLDRMLTFNPNKRITVEEALAHPYLEQYYDPTDEPVAEEPFTFAMELDDLPKERL
              **::*::.:*:***:**:*:****** ******** ****** ** *  ********:*


Spi_ERK      KELIFKETASYPGSPYLGDFHLGQQ
Nv_ERK       KGKLNTKF-----------------
Hv_ERK       KEMIFESTHMYGSA-----------
Hs_ERK1      KELIFQETARFQPGVLEAP------
             *  :  .
```

Figure 1. Sequence alignment of MAPK orthologs. The ERK orthologs of *Stylphora pistillata* (Spi-ERK), *Nematostella vectensis* (Nv-ERK), *Hydra vulgaris* (Hv-ERK), and the human ERK1 (Hs-ERK1) protein sequences are shown. The ERK-specific TEY motif is highlighted in red. The eleven conserved kinase domains are underlined.

F1000Research 2017, 6:577 Last updated: 03 JUL 2017

(Krishna *et al.*, 2013; Putnam *et al.*, 2007). These sequences all contain both the conserved kinase domains (Hanks & Hunter, 1995) and the TEY motif of the catalytic domain, which is unique for ERK orthologs (Davis, 2000; Figure 1). An interesting point to note is that a unique sequence showing these features is present in *N. vectensis* and *H. vulgaris* genomes, as well as in the *S. pistillata* transcriptome database. This result suggests that a single ortholog of ERK is present in these cnidarians, consistently with previous work where only one ERK ortholog was found (Castellano *et al.*, 2014; Russo *et al.*, 2004) but as opposed to the two genes encoding ERKs in most mammalian genomes (Ip & Davis, 1998). Furthermore, based on the high level of sequence conservation between distant species (Hanks & Hunter, 1995), antibodies directed against portions of the ERK human proteins may recognize ERKs from other species. Accordingly, we detected a single immune-reactive band with the total-ERK antibody by western blot on *S. pistillata* extracts (Figure 2A and Supplementary Figure S1). Spi-ERK should retain the mechanism of activation by phosphorylation of the Threonine and the Tyrosine residues of the ERK-specific TEY motif. Hence, the MA5-15174 antibody directed against the phosphorylated Thr202 and the Tyr204 (*i.e.* the phosphorylated TEY motif) should detect a phosphorylated TEY motif of Spi-ERK (phospho-ERK). This is consistent with what we observed, as we detected a unique immune-reactive band of approximately 40 kDa with both antibodies (Figure 2A).

Interestingly, the fluorescent immunoblot technique showed that the bands detected with the phosphorylated- and the total-ERK antibodies mostly co-migrate, suggesting that the same protein is detected (Figure 2A). The slight electrophoretic migration shift of the band detected with the anti-phosphorylated ERK antibody would be consistent with the phosphorylation of the threonine and tyrosine residues of the TEY motif as previously described (Aoki *et al.*, 2011). These results suggest that ERK and its phosphorylated form are correctly recognized by the antibodies.

RNAi interference techniques are not available in coral, and the confirmation that the immune reactive bands observed here specifically correspond to ERK could not be obtained through this method. In order to test the specificity of the antibodies, we therefore used U0126, a very potent and selective inhibitor of MEK (Bain *et al.*, 2007). The limited thickness of the animal tissue covering the skeleton and the very large surface of contact of both ectoderm and endoderm with the seawater render *S. pistillata* suitable for treatment with drugs directly diluted in the seawater as we previously showed (Courtial *et al.*, 2017). U0126 was previously

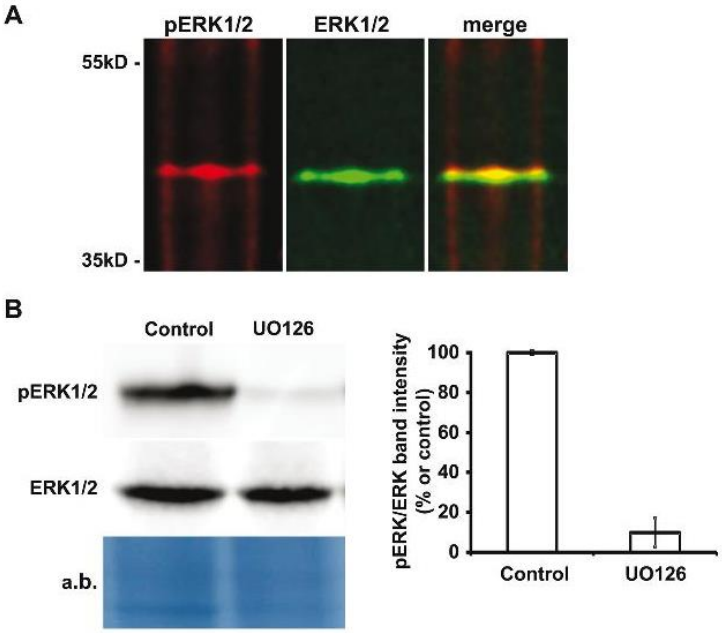


Figure 2. Detection of ERK activity in corals. (**A**) Fluorescent immunoblot revealing activated (pERK) and total forms of ERK (ERK) present in *Stylphora pistillata* nubbins. Molecular weight standards in kilo Daltons (kDa) are indicated on the left side of the figure. (**B**) Immunoblot performed with ERK and pERK antibodies on protein extracts from coral nubbins incubated in the absence (Control) or presence of the MEK inhibitor U0126. Densitometric analysis of activated ERK intensities is presented on the right of the figure. The amido black total protein staining of the western blot membrane is shown as a loading control. The medians and standard deviations of three independent experiments are presented (***, $p<0.01$, t-test).

shown to efficiently block MEK activity in a wide variety of organisms, including cnidarians (Hasse *et al.*, 2014; Picco *et al.*, 2007; Röttinger *et al.*, 2004). When the inhibitor was added to the seawater, the intensity of the band detected by the anti-total ERK did not vary, while the intensity of the band detected with the anti-phosphorylated ERK antibody was significantly reduced (Figure 2B and Supplementary Figure S1). Altogether, our results strongly suggest that the proteins detected with the two antibodies were ERK and pERK.

To confirm that Spi-ERK activity can dynamically respond to changes in experimental conditions, we performed an induction experiment by modifying culture conditions of the corals. Courtial *et al.* (2017) showed that thermal and UVR stresses induced the formation of reactive oxygen species which are known to trigger ERK phosphorylation (McCubrey *et al.*, 2006). ERK phosphorylation was enhanced in corals exposed to UVR, high temperature or a combination of both (Figure 3 and Supplementary Figure S2). These results confirm that the antibodies characterized herein can be used to monitor ERK activity in corals.

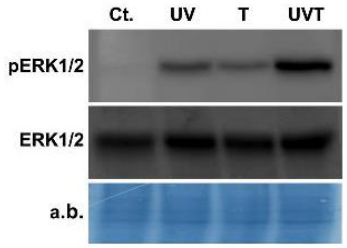


Figure 3. Induction of Spi-ERK phosphorylation by thermal and UV stresses. Immunoblot performed with ERK and pERK antibodies on protein extracts from coral nubbins incubated for 30 minutes in control (Cont.), thermal stress (T), UV stress (UV) or a combination of thermal and UV stresses (UV + T) conditions. The amido black total protein staining of the western blot membrane is shown as a loading control.

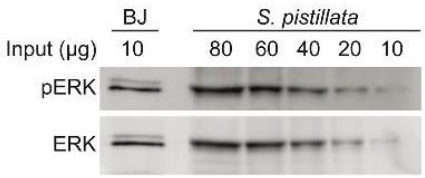


Figure 4. Relative sensitivities of ERK antibodies toward the human and coral proteins. Immunoblot performed with anti-ERK and anti-phospho-ERK on total protein extracts of human fibroblasts (BJ) and *Stylphora pistillata*. The amount of protein loaded in each lane is indicated on the Supplementary Figure S4.

Finally, to assess the performance of these antibodies, we compared the signal obtained on *S. pistillata* and human fibroblasts protein extracts (Figure 4 and Supplementary Figure S3). We loaded on the same gel 10µg of fibroblast total protein extract and different amounts of *S. pistillata* extracts (ranging from 80 to 10 µg). A signal comparable to the one obtained with the fibroblast extract was observed using 40 µg of coral proteins for both antibodies. This suggests that the affinity of the antibodies towards the coral proteins may be lower than for their human counterparts.

Dataset 1. Supplementary Figure S1. Uncropped blot images for Figure 2 and supplementary replicates

http://dx.doi.org/10.5256/f1000research.11365.d159188

(**A**) Biological replicates of fluorescent immunoblots performed in control conditions (Ct) are shown (Replicates 1 and 2). The portions of the images used in the main text are outlined. (**B**) Biological replicates of immunoblots performed on protein extracts from coral nubbins incubated in the absence (Control) or presence of the MEK inhibitor U0126 (UO) (Replicates 1 to 5). The amido black total protein staining of the western blot membrane is shown as a loading control. The portions of the images used in the main text are outlined.

Dataset 2. Supplementary Figure S2. Uncropped blot images for Figure 3 and supplementary replicates

http://dx.doi.org/10.5256/f1000research.11365.d166821

The portions of the images used in the main text are outlined.

Dataset 3. Supplementary Figure S3. Uncropped blot images for Figure 4 and supplementary replicates

http://dx.doi.org/10.5256/f1000research.11365.d166825

The portions of the images used in the main text are outlined.

Conclusion

This work showed that MA5-15174 and MA5-15605 are two specific antibodies that can be used to quantitatively assess *Stylophora pistillata* ERK phosphorylation/activity in different experimental or environmental conditions. We demonstrated the specificity of these antibodies and their good affinity towards their coral targets. It therefore provides the coral research community with a potent tool for the analysis of the activity of a signalling pathway involved in a wide variety of biological processes.

Data availability

Supplementary Figure S1. Uncropped blot images for Figure 2 and supplementary replicates. (**A**) Biological replicates of fluorescent immunoblots performed in control conditions (Ct) are shown (Replicates 1 and 2). The portions of the images used in the main text are outlined. (**B**) Biological replicates of

immunoblots performed on protein extracts from coral nubbins incubated in the absence (Control) or presence of the MEK inhibitor U0126 (UO) (Replicates 1 to 5). The portions of the images used in the main text are outlined. doi, 10.5256/f1000research.11365.d159188 (Courtial *et al.*, 2017a).

Supplementary Figure S2. Uncropped blot images for Figure 3 and supplementary replicates. The portions of the images used in the main text are outlined. doi, 10.5256/f1000research.11365.d166821 (Courtial *et al.*, 2017b).

Supplementary Figure S3. Uncropped blot images for Figure 4 and supplementary replicates. The portions of the images used in the main text are outlined. doi, 10.5256/f1000research.11365.d166825 (Courtial *et al.*, 2017c).

Author contributions

CFP, GP, LC and VP conceived and designed the experiments. LC and VP performed the experiments. CFP, GP, LC and VP analyzed the data. CFP and GP contributed reagents/materials/analysis tools. CFP, GP, LC and VP wrote the paper.

Competing interests

No competing interests were disclosed.

Grant information

Financial support to CFP, GP, LC and VP was provided by the Centre Scientifique de Monaco and Pierre and Marie Curie University.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgements

The authors thank Y. Cormerais for his help in the experimental setup, N. Caminiti-Seconds for the first coral extracts and assays as well as Pr. Denis Allemand, Director of the Centre Scientifique de Monaco for scientific support.

References

Altschul SF, Gish W, Miller W, *et al.*: **Basic local alignment search tool.** *J Mol Biol.* 1990; **215**(3): 403–410.
PubMed Abstract | Publisher Full Text

Aoki K, Yamada M, Kunida K, *et al.*: **Processive phosphorylation of ERK MAP kinase in mammalian cells.** *Proc Natl Acad Sci U S A.* 2011; **108**(31): 12675–12680.
PubMed Abstract | Publisher Full Text | Free Full Text

Bain J, Plater L, Elliott M, *et al.*: **The selectivity of protein kinase inhibitors: a further update.** *Biochem J.* 2007; **408**(3): 297–315.
PubMed Abstract | Publisher Full Text | Free Full Text

Biroccio A, Cherfils-Vicini J, Augereau A, *et al.*: **TRF2 inhibits a cell-extrinsic pathway through which natural killer cells eliminate cancer cells.** *Nat Cell Biol.* 2013; **15**(7): 818–828.
PubMed Abstract | Publisher Full Text

Boulton TG, Yancopoulos GD, Gregory JS, *et al.*: **An insulin-stimulated protein kinase similar to yeast kinases involved in cell cycle control.** *Science.* 1990; **249**(4964): 64–67.
PubMed Abstract | Publisher Full Text

Castellano I, Ercolesi E, Palumbo A: **Nitric oxide affects ERK signaling through down-regulation of MAP kinase phosphatase levels during larval development of the ascidian *Ciona intestinalis*.** *PLoS One.* 2014; **9**(7): e102907.
PubMed Abstract | Publisher Full Text | Free Full Text

Chen Z, Gibson TB, Robinson F, *et al.*: **MAP kinases.** *Chem Rev.* 2001; **101**(8): 2449–2476.
PubMed Abstract | Publisher Full Text

Courtial L, Picco V, Gorver R, *et al.*: **The c-Jun N-terminal kinase prevents oxidative stress induced by UV and thermal stresses in corals and human cells.** *Sci Rep.* 2017; **7**: 45713.
PubMed Abstract | Publisher Full Text | Free Full Text

Courtial L, Picco V, Pag s G, *et al.*: **Dataset 1 in: Validation of commercial ERK antibodies against the ERK orthologue of the scleractinian coral *Stylophora pistillata*.** *F1000Research.* 2017a.
Data Source

Courtial L, Picco V, Pag s G, *et al.*: **Dataset 2 in: Validation of commercial ERK antibodies against the ERK orthologue of the scleractinian coral *Stylophora pistillata*.** *F1000Research.* 2017b.
Data Source

Courtial L, Picco V, Pag s G, *et al.*: **Dataset 3 in: Validation of commercial ERK antibodies against the ERK orthologue of the scleractinian coral *Stylophora pistillata*.** *F1000Research.* 2017c.
Data Source

Davis RJ: **Signal transduction by the JNK group of MAP kinases.** *Cell.* 2000; **103**(2): 239–252.
PubMed Abstract | Publisher Full Text

Dhillon AS, Hagan S, Rath O, *et al.*: **MAP kinase signalling pathways in cancer.** *Oncogene.* 2007; **26**(22): 3279–90.
PubMed Abstract | Publisher Full Text

Hanks SK, Hunter T: **Protein kinases 6. The eukaryotic protein kinase superfamily: kinase (catalytic) domain structure and classification.** *FASEB J.* 1995; **9**(8): 576–596.
PubMed Abstract

Hasse C, Holz O, Lange E, *et al.*: **FGFR-ERK signaling is an essential component of tissue separation.** *Dev Biol.* 2014; **395**(1): 154–166.
PubMed Abstract | Publisher Full Text

Ip YT, Davis RJ: **Signal transduction by the c-Jun N-terminal kinase (JNK)--from inflammation to development.** *Curr Opin Cell Biol.* 1998; **10**(2): 205–219.
PubMed Abstract | Publisher Full Text

Johnson GL, Lapadat R: **Mitogen-activated protein kinase pathways mediated by ERK, JNK, and p38 protein kinases.** *Science.* 2002; **298**(5600): 1911–1912.
PubMed Abstract | Publisher Full Text

Karako-Lampert S, Zoccola D, Salmon-Divon M, *et al.*: **Transcriptome analysis of the scleractinian coral *Stylophora pistillata*.** *PLoS One.* 2014; **9**(2): e88615.
PubMed Abstract | Publisher Full Text | Free Full Text

Krishna S, Nair A, Cheedipudi S, *et al.*: **Deep sequencing reveals unique small RNA repertoire that is regulated during head regeneration in *Hydra magnipapillata*.** *Nucleic Acids Res.* 2013; **41**(1): 599–616.
PubMed Abstract | Publisher Full Text | Free Full Text

Laemmli UK: **Cleavage of structural proteins during the assembly of the head of bacteriophage T4.** *Nature.* 1970; **227**(5259): 680–685.
PubMed Abstract | Publisher Full Text

Matsubayashi Y, Ebisuya M, Honjoh S, *et al.*: **ERK activation propagates in epithelial cell sheets and regulates their migration during wound healing.** *Curr Biol.* 2004; **14**(8): 731–735.
PubMed Abstract | Publisher Full Text

Mayfield AB, Hsiao YY, Fan TY, *et al.*: **Evaluating the temporal stability of stress-activated protein kinase and cytoskeleton gene expression in the Pacific reef corals *Pocillopora damicornis* and *Seriatopora hystrix*.** *J Exp Mar Bio Ecol.* 2010; **395**(1–2): 215–222.
Publisher Full Text

McCubrey JA, LaHair MM, Franklin RA: **Reactive oxygen species-induced activation of the MAP kinase signaling pathways.** *Antioxid Redox Signal.* 2006; **8**(9–10): 1775–1789.
PubMed Abstract | Publisher Full Text

Picco V, Hudson C, Yasuo H: **Ephrin-Eph signalling drives the asymmetric division of notochord/neural precursors in *Ciona* embryos.** *Development.* 2007;

134(8): 1491–1497.
PubMed Abstract | Publisher Full Text

Putnam NH, Srivastava M, Hellsten U, *et al.*: **Sea Anemone Genome Reveals Ancestral Eumetazoan Gene Repertoire and Genomic Organization.** *Science.* 2007; **317**(5834): 86–94.
PubMed Abstract | Publisher Full Text

R ttinger E, Besnardeau L, Lepage T: **A Raf/MEK/ERK signaling pathway is required for development of the sea urchin embryo micromere lineage through phosphorylation of the transcription factor Ets.** *Development.* 2004; **131**(5): 1075–1087.
PubMed Abstract | Publisher Full Text

Runchel C, Matsuzawa A, Ichijo H: **Mitogen-activated protein kinases in mammalian oxidative stress responses.** *Antioxid Redox Signal.* 2011; **15**(1): 205–218.
PubMed Abstract | Publisher Full Text

Russo GL, Tosto M, Mupo A, *et al.*: **Biochemical and functional characterization of protein kinase CK2 in Ascidian *Ciona intestinalis* oocytes at fertilization. Cloning and sequence analysis of cDNA for alpha and beta subunits.** *J Biol Chem.* 2004; **279**(31): 33012–33023.
PubMed Abstract | Publisher Full Text

Siboni N, Abrego D, Seneca F, *et al.*: **Using bacterial extract along with differential gene expression in *Acropora millepora* Larvae to decouple the processes of attachment and metamorphosis.** *PLoS One.* 2012; **7**(5): e37774.
PubMed Abstract | Publisher Full Text | Free Full Text

Tang QQ, Otto TC, Lane MD: **Mitotic clonal expansion: A synchronous process required for adipogenesis.** *Proc Natl Acad Sci U S A.* 2003; **100**(1): 44–49.
PubMed Abstract | Publisher Full Text | Free Full Text

van de Water JA, Leggat W, Bourne DG, *et al.*: **Elevated seawater temperatures have a limited impact on the coral immune response following physical damage.** *Hydrobiologia.* 2015; **759**(1): 201–214.
Publisher Full Text

Veron JE, Stafford-Smith M: **Corals of the World. Volumes 1–3.** *Aust Inst Mar Sci.* Townsville, Aust, 2000.
Reference Source

Widmann C, Gibson S, Jarpe MB, *et al.*: **Mitogen-activated protein kinase: conservation of a three-kinase module from yeast to human.** *Physiol Rev.* 1999; **79**(1): 143–180.
PubMed Abstract

***Paper n°1 - Validation of commercial ERK antibodies against the ERK orthologue of the scleractinian coral* Stylophora pistillata**

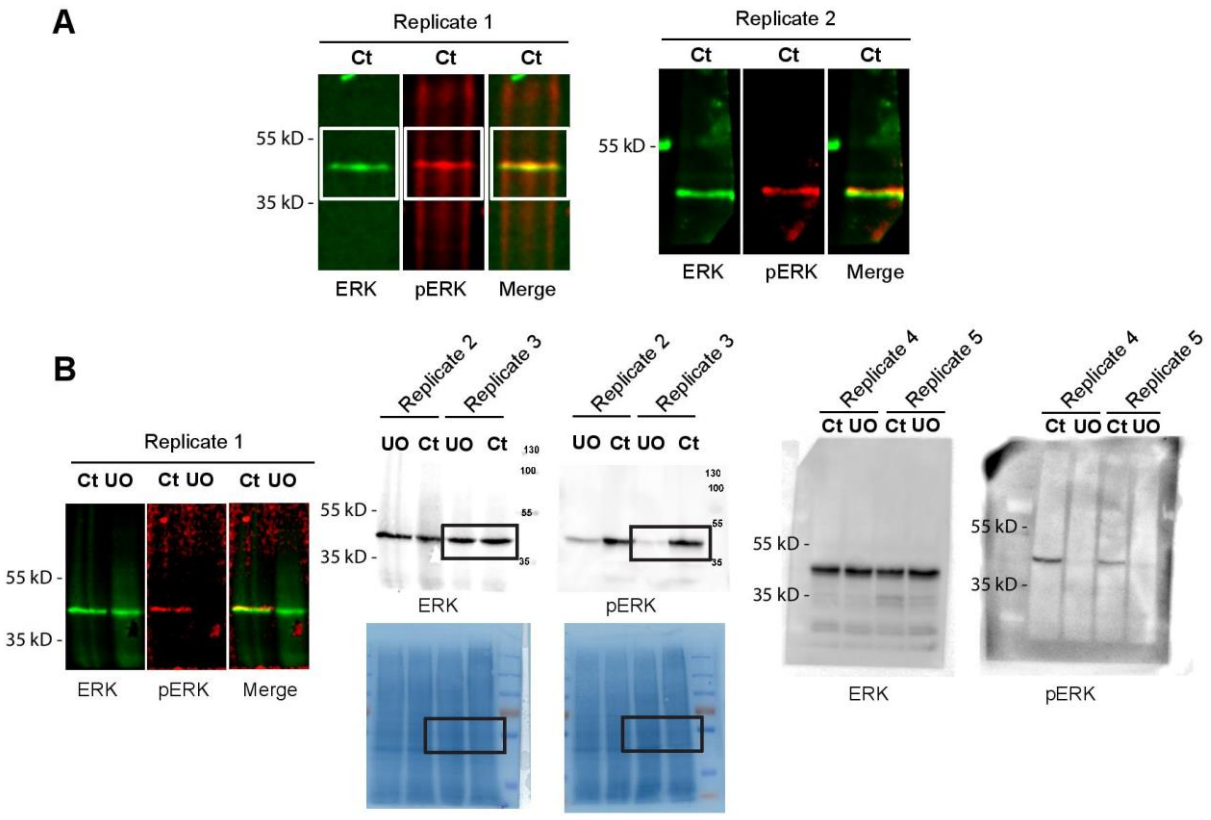


Figure S1. Uncropped blot images for Figure 2 and supplementary replicates.

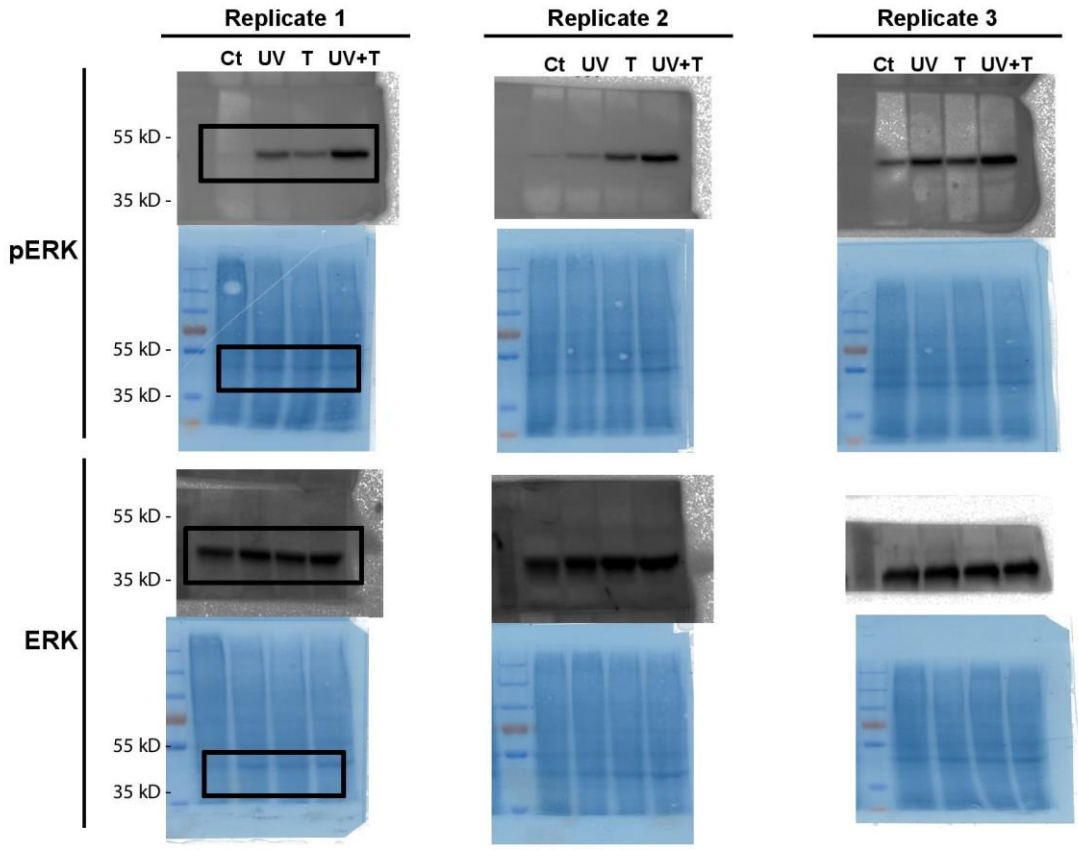


Figure S2. Uncropped blot images for Figure 3 and supplementary replicates.

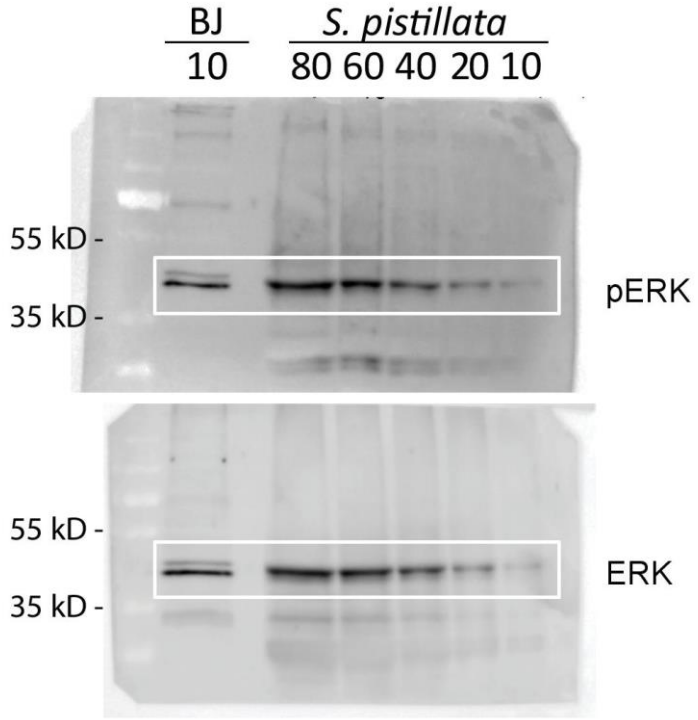
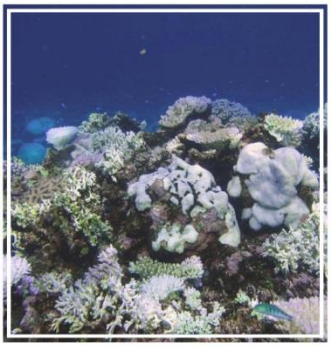
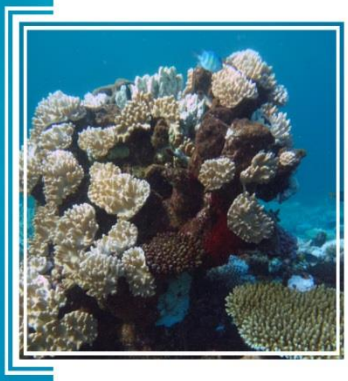


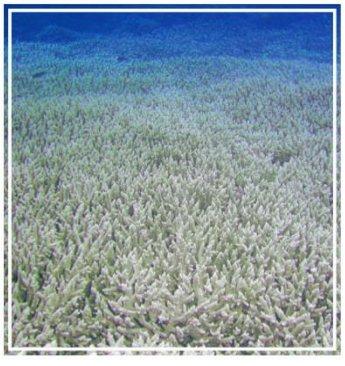
Figure S3. Uncropped blot images for Figure 4.





Chapter 3

Effects of UVR, temperature and nutrient condition on coral physiology - Summary of the results



Chapter 3 | Effects of UVR, temperature and nutrient condition on coral physiology - Summary of the results

As previously discussed, there is increasing evidence that sea surface temperature is the main factor driving physiological changes in corals (Hoegh-Guldberg 1999; Hughes et al. 2017). However, these changes are not always linearly correlated to temperature, which suggests that other environmental factors may mitigate coral response to temperature variations. UVR, for example, is an important factor to take into account when investigating coral health, because it may induce oxidative stress in cells and increase coral bleaching susceptibility (Shick et al. 1996). Despite the threat that represents the parallel climate change induced-increase in UVR and temperature (Watanabe et al. 2011; Collins et al. 2013), few studied investigated their combined effects on corals. In addition, these works mainly focused on few biological parameters, and the results obtained showed a great variability in the coral response (Table 3), suggesting species specificities and/or influences of the environmental context.

The first aim of this thesis was thus to improve our knowledge on the effects of UVR (natural exposure or increase in UVR, hereafter called UVR stress) on the physiology and response to thermal stress of several coral species. These coral species were maintained in different environmental conditions: (i) different feeding regimes (heterotrophic supplies or only autotrophy), (ii) different inorganic nutrient levels (depletion or enrichment), (iii) different seasons (summer and winter). The second aim was to identify one of the molecular pathways involved in the coral response to oxidative stress, in order to better understand what modulates coral response to environmental changes.

The results that will be presented in this chapter are a summary of those presented in five different studies summarized in Table 4 and referred to the number of the corresponding paper.

I. UVR and global warming

The following paragraphs summarize the main obtained results and show that: 1) the physiological response of corals to a UVR stress is species-dependent; 2) the physiology of acclimated coral is affected or not by natural UVR exposure; 3) UVR worsens the effect of thermal stress and 4) the environmental context can mitigate the effects of thermal stress and UVR.

I. 1. Coral response to a UVR stress is species specific

In the first experiment (paper n°2), we compared the effect of UVR stress (increased level from no UVR to a normal dose received at 5m depth) on the physiology of two genetically distant

coral species: *Pocillopora damicornis* and *Turbinaria reniformis* bearing the same symbiont clade (C1) in their tissue. Our results first showed several physiological differences between these two species (summary in Table 7). *T. reniformis,* presented 3 times less symbionts per surface area, twice higher chlorophyll per symbiont, and 2.6 times more proteins than *P. damicornis*. However, the two species had similar respiration and grazing rates even though *P. damicornis* had higher photosynthetic and calcification rates. The responses of the two coral species to UVR exposure were also very different (Table 8). UVR decreased the symbiont density, the protein content, the calcification and the grazing rates of *P. damicornis* while it had no effect on *T. reniformis*.

Table 7. Physiological differences between *P. damicornis* and *T. reniformis* (paper n°2). The red numbers highlight differences between the two species by showing the greatest value between the two.

Biological parameters	*P. damicornis*	*T. reniformis*
Symbiont density (x10^6 cm^{-2})	**2.27 ± 0.1**	0.746 ± 0.09
Protein content (mg cm^{-2})	0.115 ± 0.03	**0.295 ± 0.05**
Chlorophyll content (pg $cell^{-1}$)	2.15 ± 0.8	**5.14 ± 2**
Xanthophyll content (pg $cell^{-1}$)	0.123 ± 0.07	**0.451 ± 0.2**
Carotenoid content (pg $cell^{-1}$)	1.76 ± 0.5	**3.52 ± 0.5**
Dinoxanthin concentration (pg $cell^{-1}$)	0.0001 ± 0.0003	**0.006 ± 0.002**
Net photosynthesis (µmolO_2 cm^{-2} h^{-1})	**0.857 ± 0.09**	0.342 ± 0.1
Fv/Fm	**0.669 ± 0.019**	0.556 ± 0.044
Respiration rate (µmolO_2 cm^{-2} h^{-1})	-0.589 ± 0.1	-0.441 ± 0.2
Calcification (µmol$CaCO_3$ cm^{-2} d^{-1})	**14.8 ± 1.8**	9.22 ± 3
Grazing rate (prey cm^{-2} h^{-1})	42.3 ± 6.0	40.8 ± 20

Table 8. Summary of the effects of temperature (T), ultraviolet radiations (UV) and the combination of both stressors (UV+T) *on P. damicornis* and *T. reniformis*. "+" means positive effect: an increase of the value compared to the control condition, "-" means negative effect: a decrease of the value compared to the control condition and "=" means no effect, the final value is not different from the control condition. Red indicates significant effects of temperature alone; violet indicates effects of UVR alone and yellow indicates an interaction between UVR and temperature. In brackets is indicated whether the effects are additive (no interaction of UV and T in the ANOVA's results), synergistic or antagonistic (significant interaction between UV and T in the ANOVA's results).

Species	Envir. factors	Symbiont density	Protein content	Chlorophyll content	Pn	R	Calcification rate	Grazing rate
P. damicornis	T	-	-	+	=	=	-	=
	UV	-	-	=	=	=	-	-
	UV+T	- (additive)	- (synergistic)	+	- (additive)	- (additive)	- (synergistic)	= (antagonistic)
T. reniformis	T	=	=	=	=	=	=	-
	UV	=	=	=	=	=	=	=
	UV+T	=	=	=	=	=	- (synergistic)	-

I. 2. Coral response to UVR stress depends on the nutritional status

The different experiments carried out during the PhD allowed to assess the role of nutrition (both heterotrophic feeding (comparison papers n°2 and 5) and nutrient level (paper n°5)) on coral response to UVR.

Heterotrophic feeding

The comparison between fed (with *Artemia nauplii*) and unfed colonies of *P. damicornis* (papers n°2 and 5) showed that only fed colonies were impacted by UVR (significant decrease in symbiont density, protein content and calcification) (paper n°2; Chapter 3 §I.1), UVR had no effect on unfed colonies (paper n°5; Chapter 3 §I.2.2). The different responses were associated with differences in the tissue parameters: unfed nubbins had about 75% less symbionts than fed nubbins, 87% less chlorophyll *a* and 98% less proteins (Table 9).

Table 9. Comparison of the tissue parameters of fed and unfed nubbins of *P. damicornis* (papers n°2 and 5). "Temp." is the temperature; "UVR cond." Is the UVR condition (0UV means no UVR, UV means exposed to UVR); "Symbionts", "Chl *a*" and *"Proteins are* the symbiont, chlorophyll *a* and protein contents in %.

UNFED vs FED (% of change)				
Temp.	**UVR cond.**	**Symbionts**	**Chl*a***	**Proteins**
25°C	0UV	-74.1	-87.4	-97.7
25°C	UV	-48.7	-82.7	-92.6
30°C	0UV	-86.1	-93.8	-95.4
30°C	UV	-73.3	-91.3	-89.1

Nutrient level

Three nutrient levels were tested on unfed nubbins of *P. damicornis* (paper n°5): a depleted condition (<0.1 µM nitrate (NO_3) and <0.05 µM phosphate (PO_4)), a control condition (~0.5 µM NO_3 and ~0.1 µM PO_4) and an enriched condition (3 µM NO_3 and 0.5 µM PO_4). Nutrient limitation lowered gross photosynthetic rates while nutrient enrichment decreased coral growth. Despite these effects of nutrient levels on the coral physiology, nubbins were unaffected by UVR regardless of the nutrient condition.

I. 3. Effect of natural UVR exposure on the physiology of acclimated corals and seasonal variability

The access to shallow reef corals (*i.e*. acclimated to UVR) in New Caledonia allowed us to investigate how natural UVR exposure affects the physiology of acclimated corals and whether these effects change with the season (papers n°3 and 4). Our results showed that UVR status (exposed or shaded) had little effects on *A. muricata* regardless of the season (symbiont density chlorophyll *a* and protein contents, heterotrophic feeding and calcification rates, papers n°3 and 4), at the exception of the photosynthetic efficiency ($rETR_{max}$) which was increased by UVR in winter, and decreased in summer. However, nubbins physiology varied with the season itself: in summer, it had fewer proteins and a lower photosynthetic efficiency but higher calcification rates than in winter (paper n°4).

Table 10. Effects of season and UVR on coral physiology. "+" indicates positive effects," -" indicates negative effects, "Ø" indicates no effects, "S" indicates Summer and "W" indicates winter values. Indicated values are means ± standard deviation.

Biological parameters	Effects of season under ambiant UVR (S compared to W)	Effects of UVR in winter (UV compared to 0UV)	Effects of UVR in summer (UV compared to 0UV)
Symbiont density ($10^5 cm^{-2}$)	Ø	Ø	Ø
	3.43 ± 1.6 - 4.76 ± 1.0	4.76 ± 1.0 - 3.28 ± 0.9	3.43 ± 1.6 - 2.65 ± 0.9
Chla content (mg cm^{-2})	Ø	Ø	+
	0.25 ± 0.07 - 0.31 ± 0.06	0.31 ± 0.06 - 0.28 ± 0.02	0.25 ± 0.07 - 0.16 ± 0.05
Protein content (mg cm^{-2})	-	Ø	Ø
	0.45 ± 0.1 - 0.67 ± 0.1	0.67 ± 0.1 - 0.56 ± 0.08	0.45 ± 0.1 - 0.48 ± 0.08
rETR	-	+	-
	22.6 ± 3.2 - 41.7 ± 1.8	41.7 ± 1.8 - 29.6 ± 2.5	22.6 ± 3.2 - 33.5 ± 5.3
Calcification rate (µmol cm^{-2} d^{-1})	+	Ø	Ø
	19.7 ± 1.7 - 12.5 ± 2.5	12.5 ± 2.5 - 12.3 ± 2.2	19.7 ± 1.7 - 22.8 ± 2.7

I. 4. UVR worsens the thermal stress effects

I. 4. 1. Effect of thermal stress

Similarly to UVR exposure, coral responses to thermal stress were species-dependent. High temperature alone reduced the symbiont density, the protein content and the calcification rates of *P. damicornis* while only grazing rates were decreased in *T. reniformis* (paper n°2). *A. muricata* (paper n°3) was the most affected species as high temperature led to 1) a decrease in symbiont density, chlorophyll *a* concentration, protein content, photosynthetic efficiency (rETRmax) and net photosynthesis; 2) an increase in respiration rates and 3) an enhancement of prokaryotes consumption.

Our results showed that nutrient levels modulate the effects of high temperature on *P. damicornis* physiology (paper n° 5). Nutrient enrichment alleviated the negative effects of thermal stress on chlorophyll *a* content, net and gross photosynthesis as well as respiration rates while nutrient limitation led to a higher loss of chlorophyll *a* and lower growth rates.

I. 4. 2. UVR and thermal stress

Overall, UVR (both UVR stress and natural exposure) worsened the effects of high temperature on coral physiology, even in the most resistant species (*T. reniformis*). In *P. damicornis* (paper n°2), the combination of UVR with thermal stress led to higher loss of proteins and symbionts,

causing lower oxygen fluxes (net photosynthesis and respiration) and calcification rates. Although *T. reniformis* was unaffected by UVR alone, the combination of UVR with high temperature led to a decrease in the calcification rates. Similarly, despite the acclimation of *A. muricata* to UVR (paper n°3), the combination of natural UVR with high temperature led to higher decrease in symbiont density, chlorophyll *a* content and photosynthetic rates than under high temperature alone and triggered a consumption of small autotrophs in suspension in seawater.

The only exception was observed in unfed nubbins of *P. damicornis* (paper n°5) which remained unaffected by UVR even at high temperature.

II. JNK signalling pathway and oxidative stress defenses

The following paragraph summarizes the main results on the identification of one of the molecular pathways involved in the coral response to oxidative stress. The work was performed with *Stylophora pistillata* and based on the hypothesis that antioxidant defense are triggered by MAPK signalling pathways whose functions are conserved from bacteria to humans (paper n°6).

In this work, we first confirmed the presence of JNK signalling pathway in *S. pistillata* and showed that temperature and UVR triggered its activation similarly to human cells. In coral nubbins, JNK phosphorylation was maximal after 30 min exposure to high temperature or UVR. The combination of the two factors led to significantly higher JNK activation than single stressor exposure. We also showed that JNK pathway was involved in the oxidative stress response. While there was a slight increase in ROS concentrations under UVR, thermal stress, and the combination of the two stressors when JNK was activated, there was a significant increase when JNK activation was blocked with an inhibitor. These increases were proportional to the initial JNK activation; *i.e.* it was higher in nubbins exposed to a combined stress. After 24h in this latest condition (UVR + high temperature + JNK inhibitor), nubbins lost 30% on their symbiont while they did not bleach without the inhibitor (summary Figure 18).

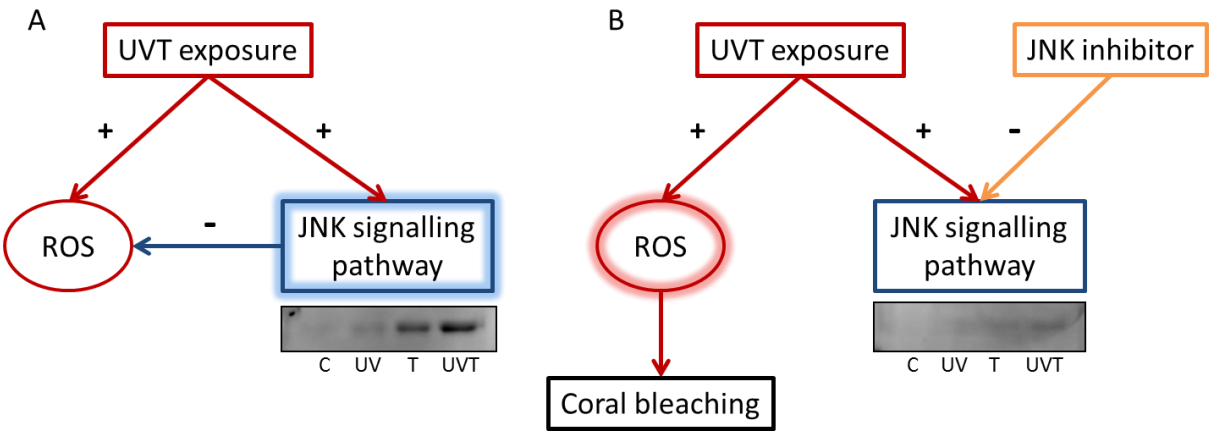
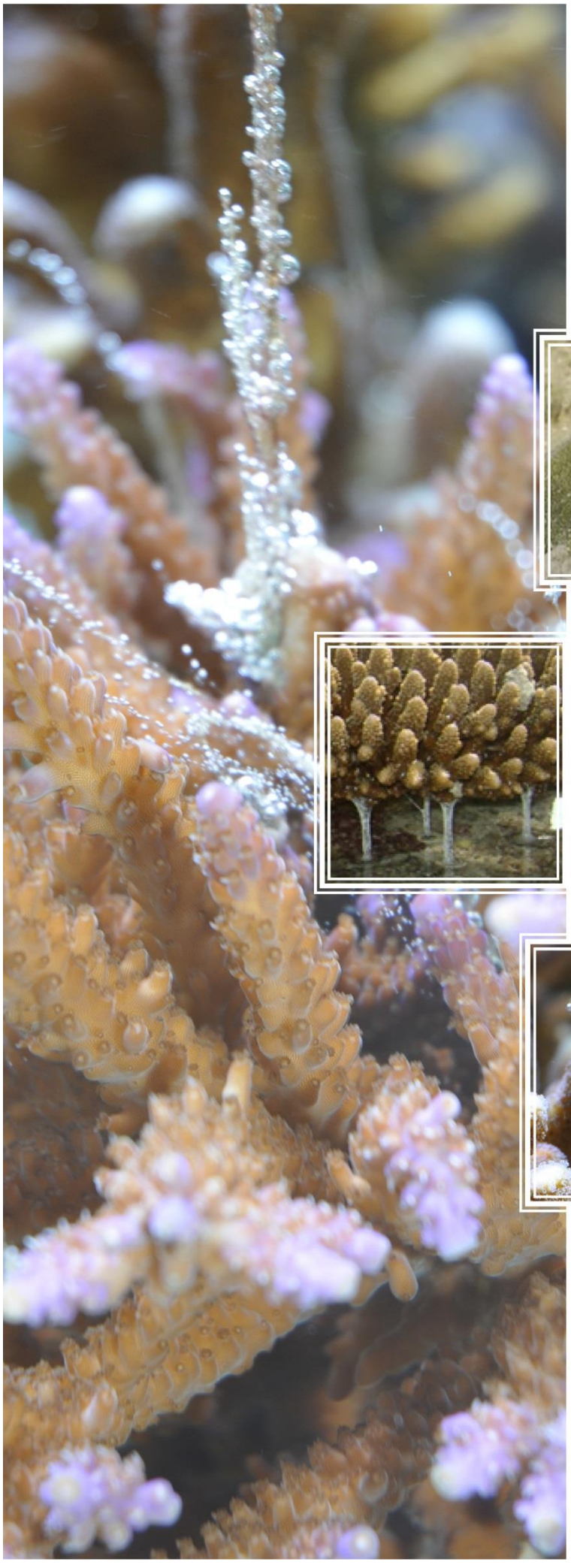


Figure 18. Summary of the results obtained for an exposition of *S. pistillata* to a combined stress of UVR and high temperature. JNK signalling pathway is activated by the stress exposure and down regulates the increase in intracellular ROS (A). JNK inhibitor blocks JNK activation which impairs ROS scavenging capacities and results in coral bleaching (B). The western blot images show JNK activation in the control condition (C), under UVR alone (UV), at high temperature (T), under the combination of UVR and high temperature (UVT).



Chapter 4

Combined effects of UVR, temperature, seasonality and nutrient level on mucus production and coral microbiome - Summary of the results

Chapter 4 | Combined effects of UVR, temperature, seasonality and nutrient level on mucus production and coral microbiome – Summary of the Results

As previously described in the introduction chapter, coral mucus plays a major role both for corals, as a first defense against environmental stresses (Brown and Bythell 2005), and for the mucus associated bacteria and the reef ecosystem as food source, energy carrier and particle trap (Wild et al. 2004; Levas et al. 2016). Despite its importance, very little is known about the direct and indirect (through changes in coral physiology) effects of environmental factors on mucus (organic matter) release, degradation by the associated microorganisms or transfer to the ecosystem. One of the aims of the thesis was to complement this poorly known aspect of the coral reef ecology by investigating the single and combined effects of UVR, temperature, season, and nutrient level on 1) coral derived organic matter fluxes, 2) organic matter degradation by mucus-associated bacteria; 3) mucus and/or coral associated bacterial community.

I. Organic matter fluxes, UVR, global warming, nutrient level and seasonal variability

In paper n°5, parent colonies of *Pocillopora damicornis* were grown without UVR and were therefore devoid of MAAs in their tissue at the start of the experiments. We thus tested the effect of an increased UVR exposure (*i.e.* UVR stress) during the experiment.

On the other hand, in papers n°3 and 4, *Acropora muricata* nubbins were sampled in the field and therefore acclimated to high levels of UVR. In these papers, we thus tested the effect of natural UVR exposure on corals.

For both species (*P. damicornis* and *A. muricata*), the fluxes of total organic matter (total organic carbon, TOC, and nitrogen, TN) were always positive, from the corals to the seawater, meaning that there was a net release of organic matter in all tested conditions.

- For *P. damicornis*, UVR stress was the main factor influencing organic matter release rates. It significantly decreased TN release, regardless of the inorganic nutrient enrichment performed while it increased TOC release when combined to a nutrient enrichment. High temperature alone did not affect the organic matter fluxes but it compensated the negative effects of UVR on TN release.
- *A. muricata* released the same amount of total organic matter (TN and TOC) regardless of the UVR or temperature condition (papers n°3 and 4). High temperature, which was the main environmental

factor influencing matter fluxes for this species, had a significant effect on the quality of the mucus released (*i.e.* particulate or dissolved matter). In the thermal stress experiment (paper n°3) a shift from POM to DOM release and DOM to POM uptake was observed from 26°C to 30°C (*i.e.* with temperature increasing to 30°C, more DOM is released compared to POM, while more POM is removed, compared to DOM). In the seasonal experiment (paper n°4), the mucus was only composed by POC in winter (23 ± 0.6°C) (nubbins took up DOC and released POC) and by a mix of POC and DOC in summer (29 ± 0.9°C).

II. Mucus associated bacterial activities: effect of UVR, global warming, nutrient level and seasonality

II. 1. Effects of UVR

In *P. damicornis* (paper n°5), UVR stress decreased α-glucosidase and aminopeptidase maximal extracellular enzymatic activity (EEA_{max}) while it had no effect on *A. muricata*'s microbiome enzymatic activities (paper n° 3) and little effects regardless of the season (paper n°4). The only exception was in summer, UVR exposure led to a decrease in α-glucosidase EEA_{max} in *A. muricata*'s mucus (paper n°4).

II. 2. Effects of high temperature

High temperature increased α-glucosidase and aminopeptidase EEA_{max} in both species (papers n°3 and 5) as well as summer compared to winter in *A. muricata* associated bacteria (paper n°4).

II. 3. Matter degradation

For both species, and regardless of the environmental conditions, the amount of mucus degraded by the α-glucosidases and aminopeptidases of the associated bacteria was only a small fraction of the total organic matter released by corals (between 0.6 and 8% for N and 0 and 1 % for C for *P. damicornis* and less than between 0.03% and 1% for C and 0 and 8% for N).

III. Coral and mucus associated bacteria: effect of seasonality and UVR exposure

III. 1. Mucus associated microbiome

The mucus associated bacterial community of *Acropora muricata* (paper n°4) was dominated by 8 different OTUs belonging to the genera *Oleibacter*, *Arenicella*, *Aestuariibacter*, Candidatus *Rhabdochlamydia*, *Coxiella*; the Rickettsiaceae family and the DB1-14 order (Table 11). Among them, four varied between summer and winter: *Oleibacter* and *Aestuariibacter* had higher abundance in summer while *Coxiella* and the α- proteobacterium from the DB1-14 class (hereafter called α-prot 1) had higher abundance in winter. In summer, UVR exposure had no effect on the bacterial community which was dominated by *Oleibacter* (about 75%) and *Coxiella* (about 15%). In winter however, UVR status impacted the bacterial community: the genus *Arenicella* and two OTUs from the DB1-14 class (α-prot 1 and 2) were more abundant under UVR while *Oleibacter*, *Coxiella* and a genus from the *Rickettsiaceae* family were more abundant without UVR. Under UVR, the bacterial community was dominated by the genus *Arenicella* (~43%), *Coxiella* (~20%), α-prot 1 (~16%) and *Oleibacter* (~11%). Without UVR, the community was dominated by *Coxiella* (43%), *Oleibacter* (~28%) a bacteria from the *Rickettsiales* family and α-prot 1 (~10 % each).

Table 11. Summary of the 8 OTUs associated with *A. muricata* mucus.

Phylum	Class	Order	Family	Genus	OTU	Information on the genus
Proteobacteria	Alphaproteobacteria	DB1-14	-	-	24581	-
Proteobacteria	Alphaproteobacteria	DB1-14	-	-	5726	-
Proteobacteria	Alphaproteobacteria	Rickettsiales	Rickettsiaceae	-	56046	Found in reef building corals (Casas et al 2004). Most human pathogens in this family are in genus Rickettsia. They spend part of their lifecycle in the bodies of arthropods.
Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	Aestuariibacter	5084	Found in corals of the red sea (Lee et al 2012); four known species (Yi et al. 2004)
Proteobacteria	Gammaproteobacteria	Arenicellales	Arenicellaceae	Arenicella	48357	Found in Platigyra carnosus (Ng et al 2015)
Proteobacteria	Gammaproteobacteria	Legionellales	Coxiellaceae	Coxiella	62614	Pathogens of mammals or symbiotic of Carios capensis (Piel 2004; Machado-Ferreira et al. 2016)
Proteobacteria	Gammaproteobacteria	Oceanospirillales	Oceanospirillaceae	Oleibacter	37807	Found in Acropora muricata (Sweet and Bulling 2017) ; degrades petroleum aliphatic hydrocarbons
Chlamydiae	Chlamydiae	Chlamydiales	Simkaniaceae	Candidatus Rhabdochlamydia	52824	Found in Aiptasia (Röthig et al 2016)

Table 12. Summary of the effects of UVR and season on the 8 OTUs associated with *A. muricata* mucus. Blue highlights bacteria more abundant in winter; yellow indicates more abundant in summer; purple is bacteria more abundant under UVR in winter; grey is bacteria more abundant without UVR in winter.

Phylum	Class	Order	Family	Genus	Variation of the abundance with season	Variation of the abundance with UVR in summer
Proteobacteria	Alphaproteobacteria	DB1-14	-	-	Higher in winter	Higher under UVR
Proteobacteria	Alphaproteobacteria	DB1-14	-	-	-	Higher under UVR
Proteobacteria	Alphaproteobacteria	Rickettsiales	Rickettsiaceae	-	-	Higher without UVR
Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*Aestuariibacter*	Higher in winter	-
Proteobacteria	Gammaproteobacteria	Arenicellales	Arenicellaceae	*Arenicella*	-	Higher under UVR
Proteobacteria	Gammaproteobacteria	Legionellales	Coxiellaceae	*Coxiella*	Higher in Summer	Higher without UVR
Proteobacteria	Gammaproteobacteria	Oceanospirillales	Oceanospirillaceae	*Oleibacter*	Higher in Summer	Higher without UVR
Chlamydiae	Chlamydiae	Chlamydiales	Simkaniaceae	Candidatus *Rhabdochlamydia*	-	-

III. 2. Coral associated microbiome

Within *A. muricata* tissue, 505 OTUs belonging to more than 100 different genera were found to be significantly different from the seawater. Among them, 19 different genera, which belonged to the core microbiome (*i.e.* the genera present in 90% of the samples), represented more than 0.5% of the total population (Table 13). Contrary to the mucus community, UVR exposure had no effect on coral associated bacteria. Temperature however led to significant changes in 7 genera: *Thalassospira* and 29 OTUs of *Endozoicomonas* were more abundant in winter while 22 other *Endozoicomonas* OTUs, Candidatus *Amoebaphilus*, *Leptilyngbya*, *Thalassotalea*, two genera from the Rhodobacteraceae and LWSR-14 families were more abundant in summer. The winter microbial community was dominated by *Endozoicomonas* (~60% of the community) and *Thalassospira* (~23%). In summer, a different population of *Endozoicomonas* represented about 77% of the community. Remarkably, summer was also characterized by a higher abundance of several minor Cyanobacteria (abundance <0.5% of the total population) as well as minor bacteria usually found in diseased, stressed or bleached corals (from the families of *Alteromonadaceae*, *Colwelliaceae*, *Rhodobacteriaceae*, *Planctomycetaceae* and the order *Rhizobiales*).

Table 13. Major genera composing the coral associated bacterial community. Brown highlights bacteria with antibacterial or nutritional function; yellow indicates bacteria previously found to be associated with diseased or stressed corals; green is diazotrophic bacteria.

Class	Order	Family	Genus	Genus or bacterial characteristics	Abundance variability with season
Actinobacteria	Corynebacteriales	Corynebacteriaceae	*Corynebacterium*	Found in different disease of *Stephanocoenia intersepta* coral (Sweet et al., 2013)	-
Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	*Thalassospira*	Found in healthy corals in winter (Chiu et al. 2011); some spepies can degrade crude oil (*Thalassospira xiamenensis*; Röthig et al., 2017)	Higher abundance in winter
Alphaproteobacteria	Rickettsiales	LWSR-14	-	Found in *Acropora sp.* No change between healthy and stressed corals (Casas et al., 2004)	Higher abundance in summer
Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	-	Oportunistic bacterial family more abundant in the most anthropogenic impacted sites (Ziegler et al 2016)	Higher abundance in summer
Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Sphingomonas*	Found to be a possible responsible for coral white plague (Rosenberg and Ben-Haim 2002; Miller et al., 2003)	-
Gammaproteobacteria	Alteromonadales	Colwelliaceae	*Thalassotalea*	Found in Montipora sp. (Chen et al 2016) and *Euphyllia glabrescens* (Tang et al 2016); some species were associated with white plague like disease of corals (Thompson et al 2006)	Higher abundance in summer
Gammaproteobacteria	Cellvibrionales	Spongiibacteraceae	*BD1-7 clade*	Found in *Corallium rubrum* (van de Water et al., 2016), in *Oculina patagonica* (Spring et al., 2015)	-
Gammaproteobacteria	Oceanospirillales	Hahellaceae	*Endozoicomonas*	See review Neave et al., 2016	Different community in summer or winter
Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	Found in *Acropora millepora* (Raina et al., 2009); antibacterial properties in soft coral (Radjasa et al., 2007)	-
Gammaproteobacteria	Thiotrichales	Thiotrichaceae	*Leucothrix*	-	-
Gammaproteobacteria	Thiotrichales	Thiotrichaceae	*Thiothrix*	Filamentus sulfur-oxidizing bacteria (Lakin et al., 1983)	-
Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	*Dyella*	Found in *Acropora eurystome* (Meron et al., 2011)	-
Bacilli	Bacillales	Staphylococcaceae	*Staphylococcus*	Pathogen agent associated with corals (Gowrishankar et al., 2006)	-
Cyanobacteria	SubsectionIII	FamilyI	*Leptolyngbya*	Diazotrophic bacteria found in *Acropora eurystoma* (Meron et al., 2011)	Higher abundance in summer
Cytophagia	Cytophagales	Flammeovirgaceae	Candidatus *Amoebophilus*	Found in coral tissue of *Orbicela faveolata*; *Montastrea cavernosa*, *Porites porites* (Apprill et al 2016)	Higher abundance in summer
Deltaproteobacteria	Myxococcales	P3OB-42	-	-	-
Flavobacteriia	Flavobacteriales	NS7 marine group	-	Found in bacterioplankton-dissolved organic matter (Lucas et al., 2016)	-
Planctomycetacia	Planctomycetales	Planctomycetaceae	*Rhodopirellula*	Found in deep corals (Yakimov et al., 2006) and in octocoral (Duque-Alarcon et al., 2012; Webster and Bourne 2007)	-
Verrucomicrobiae	Verrucomicrobiales	Rubritaleaceae	*Rubritalea*	Found in *Acropora muricata* (Lee et al., 2016), in *Lophelia pertusa* (Neulinger et al., 2008); found to degrade sugar and to have an antoxidant activity (Shindo et al., 2008)	-

The effects of UVR and seasons on coral and mucus microbiomes are summarized in Figure 19.

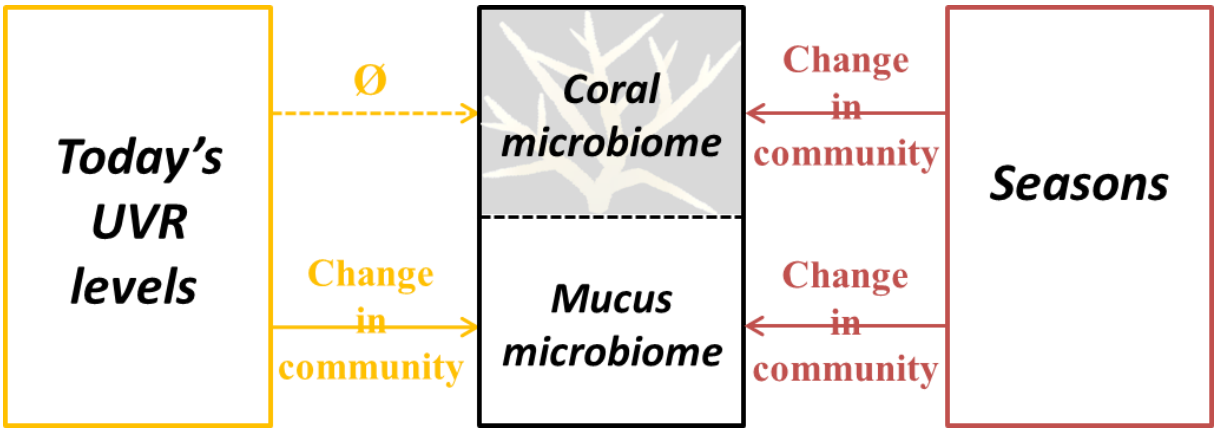
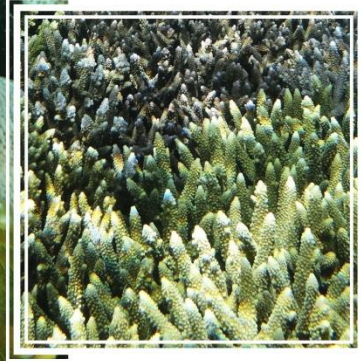


Figure 19. Summary of the effects of UVR and seasons on coral and mucus microbiomes. "Ø" indicates no effects. Exposure to today's levels of UVR has no effects coral microbiome but induces changes in the mucus microbiome. Seasons induce changes in both coral and mucus bacterial communities.



Chapter 5

General discussion and perspectives

Chapter 5 | General discussion and perspectives

I. UVR and corals

While most studies on the effects of UVR on corals mainly focused on MAAs and bleaching, and did not look at the interactions with other environmental parameters, the aim of this thesis was to assess the effect of increased or present time UVR level, alone or in combination with temperature and nutrients, on several biological parameters including symbiont density, protein and pigment contents, photosynthesis, respiration and calcification rates, heterotrophic feeding, mucus excretion and degradation by the associated bacteria as well as coral microbiome. The work brought several answers to the major questions presented in the introduction (§III.2.6) and which are listed below:

1) Which are the changes in coral physiology and in coral-associated microbes between corals exposed to natural and those UVR shaded from UVR?
2) How do corals respond to an increased UVR level? Is there a unique response or is the response species-specific?
3) How does increased UVR impact mucus release and degradation?
4) How does UVR affect the coral response to seawater warming and eutrophication?
5) Which are the molecular pathways involved in the coral's response to increased UVR?

Summary of the results are presented Figure 20 and Figure 21.

I. 1. Changes in coral physiology and in coral-associated microbes between corals shaded from UVR and those exposed to natural UVR

These experiments were performed with nubbins of *Acropora muricata*, from New Caledonia, at three time points corresponding to the austral winter, summer and intermediate season (February, May and July, papers n°3 and 4,Table 5). The results obtained first suggest that UVR has a different effect on *A. muricata* physiology depending on the season, *i.e.* on the temperature and the level of UVR and irradiance. In winter, when temperature and UVR levels were at their lowest range (23.1 ± 0.6°C), UVR enhanced the photosynthetic efficiency of the photosystem II and did not impact coral physiology. This observation, made for the first time in corals, is similar to what was previously observed in phytoplankton which was able to use UVA for photosynthesis (Nilawati et al. 1997; Barbieri et al. 2002; Helbling et al. 2003). In that case, UVR can be considered as beneficial to corals, as it is a source of supplementary energy for photosynthesis. However, in summer, the combined increase in temperature and UVR (29.3 ± 0.9°C) induced coral bleaching and the loss of photosynthetic efficiency due to enhanced oxidative stress (reviewed in Banaszak and

Lesser 2009). Despite these changes in coral physiology, bacterial communities associated with the tissue and skeleton of *A. muricata* were not affected by UVR, at both seasons suggesting that bacteria are protected from UVR likely because of the MAAs and absorbing pigments present inside the coral tissue (Shick and Dunlap 2002).

Concerning mucus production, UVR is known to photolyse organic matter (Herndl et al. 1997), and in that sense, should impact the quality of coral mucus released in seawater. However, we did not observe any change in the quality (dissolved versus particulate matter) of the mucus released by corals in presence or absence of UVR, likely because of the presence of protective MAAs (Drollet et al. 1997). Mucus composition (ratio POM/DOM) was rather affected by the heterotrophic activity of the coral holobiont which changed their uptake rates and release of dissolved and particulate organic matter.

The last observation concerned mucus-associated bacterial community, which shifted in winter from a community dominated by the genera *Coxiella* and *Oleibacter* without UVR to a community dominated by *Arenicella* under UV. These changes, never assessed before with mucus-associated bacteria, suggest a species-specific resistance to UVR, which may be attributed to differences in photoprotective mechanisms, as previously observed with marine biofilm communities (Elasri and Miller 1999; Joux et al. 1999; Arrieta et al. 2000; Winter et al. 2001). In summer, however, UVR had no effect on the community suggesting either changes in the bacterial sensitivity to UVR with temperature (Ravindran et al. 2013) or differences in mucus physical properties, leading to a higher protection. More experiments are needed to understand which factors govern the bacterial response to UVR.

I. 2. Coral response to an increased UVR level: is it species-specific? Is there an impact on mucus release and degradation?

The results obtained in this thesis clearly show that not all coral species were affected by an exposure to increased UVR level. For example, while *Pocillopora damicornis* bleached, slowed down its calcification and grazing rates and relied on its energetic reserves (*i.e.* proteins) to maintain photosynthesis and respiration rates at their maximal levels under UVR, *Turbinaria reniformis*, did not show any significant physiologic changes (paper n°2). Such difference in sensitivity could be attributed to: i) the tissue thickness and morphology, *T. reniformis* having more thicker tissue than *P. damicornis*; ii) pigment content of the symbiont cells and namely the carotenoid levels, also higher in *T. reniformis* and iii) symbiont density (lower in *T. reniformis*), and the balance between benefic energy and harmful ROS production. Previous work indeed showed that coral species having a high

symbiont density were more sensitive to bleaching (Cunning and Baker 2013). We tested this theory further by comparing *P. damicornis*, fed with *Artemia salina*, and containing a high symbiont density, with unfed *P. damicornis*, harboring 5 times less symbionts. *P. damicornis*, was negatively impacted by UVR, when fed, confirming that an excessive concentration of symbionts exacerbates UVR effects likely by inducing a parallel excessive production of harmful oxygen radicals (Wooldridge 2013; Cunning and Baker 2013). The results obtained in this thesis thus highlight a negative relationship between symbiont density and UVR impact on coral physiology, and may explain the different effects of UVR and thermal stress observed in the literature.

This thesis is also the first work to assess the effect of UVR stress on the release of organic matter by coral colonies. We found that UVR reduces total organic nitrogen (TN) release and therefore affected the composition of the coral-derived organic matter. This is likely due to a reduction in nitrogen uptake rates, as it was previously observed in phytoplankton (Serra et al. 1978; Butler et al. 1979; Olson 1980; Anderson and Roels 1981; Collos et al. 1996; Hessen et al. 1997). Modifications in mucus quality may change the reef water biochemistry and the reef productivity leading to potential modification of the food chain structure. For example, diazotrophic bacteria which are able to fix N_2 might be favored following an increase in UVR compared to other bacteria as the availability of coral-derived-nitrogen will decrease (Sandroni et al. 2007; Cardini et al. 2015). In the meantime, UVR also decreased the aminopeptidase activity of the mucus-associated bacteria likely because of photolysis (reviewed in Cunha et al., 2010). This suggests that despite the protective properties of the mucus, climatic conditions which will enhance UVR at the reef surface may lead to significant changes in the nitrogen cycle and food chain functioning of the whole reef.

I. 3. Effects of UVR on coral response to global warming, eutrophication and nutrient depletion

We found that corals exposed to UVR and thermal stress bleached more severely and faster than the same species protected from UVR either in UVR-acclimated species (paper n°3) or in resistant species (*i.e.* see *T. reniformis* in paper n°2). This is due to the fact that UVR induces ROS production (Lesser 2012) that comes in addition to the thermal-stress induced ROS production. To show this pattern, we developed a protocol to directly measure ROS production under different environmental conditions. Instead, previous studies evidenced increased ROS levels in corals only indirectly, through increasing antioxidant enzymatic activities for example (Lesser et al. 1990b; Yakovleva et al. 2004). These observations highlight the importance of considering UVR exposure for predicting coral bleaching and to take into account the depth at which colonies thrive and the position of the colony within the reef. Moreover, with climate change, reefs exposed to low UVR

today will deal with higher intensities in the next decades and might therefore become more sensitive to coral bleaching.

Our results also showed no effect of UVR on coral response to eutrophication or nutrient depletion (paper n°5). However, *P. damicornis* nubbins used in the experiment harbored a very low symbiont density that reduces the effects of UVR (see Discussion §I.2.). More studies are needed to see whether UVR can impact the response to different nutrient level of corals with higher symbiont density.

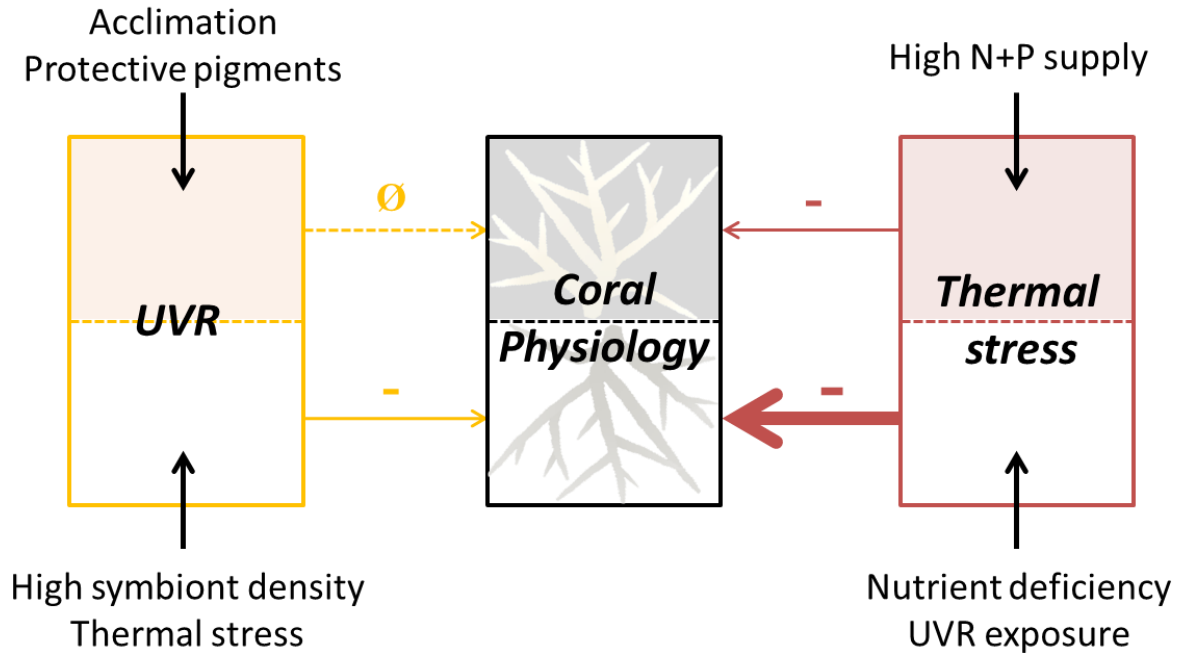


Figure 20. Summary of the effects of UVR and thermal stress on coral physiology (metabolism and tissue parameters). "-" indicates negative effects, "Ø" indicates no effect. Large arrow indicates high effects. UVR has no effect on acclimated corals and/or harbour high protective pigment concentrations. UVR has negative effects when colonies harbour high symbiont density or under thermal stress. Thermal stress negatively affects coral physiology. High N+P supply decreases these negative effects but UVR and nutrient deficiency worsen them.

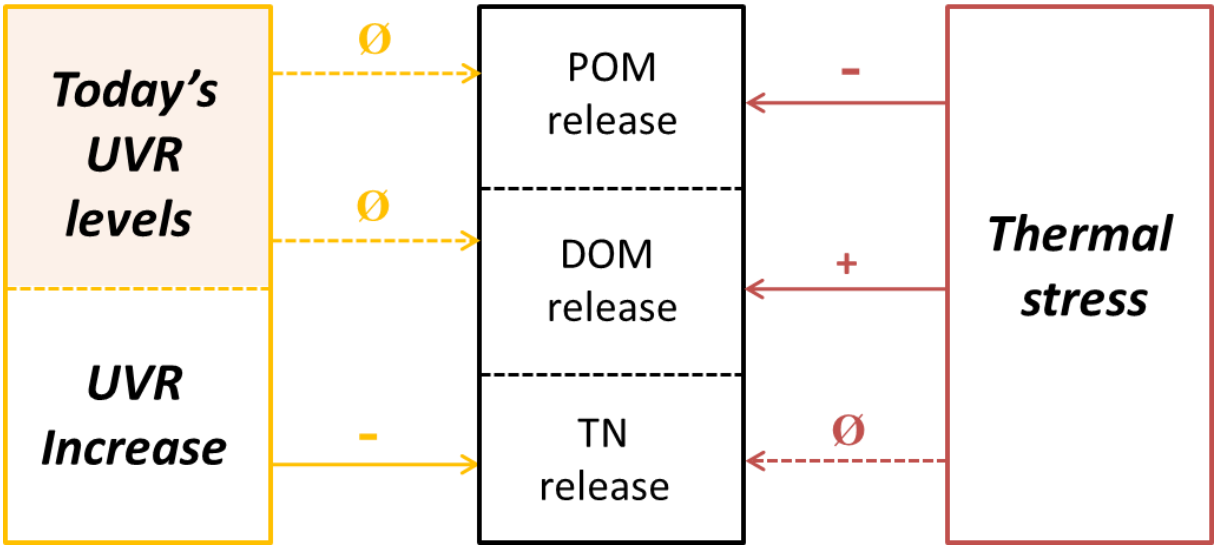


Figure 21. Summary of the effects of UVR and thermal stress on organic matter release. "-" indicates negative effects, "+" indicates positive effects and "Ø" indicates no effect. Today's UVR levels has no effect on POM and DOM release but increased UVR doses triggers a decrease in TN release. Thermal stress does not affect total organic matter fluxes but negatively impacts POM release and increases DOM release (*i.e.* thermal stress modifies the released matter quality).

I. 4. Molecular pathways involved in the coral response to increased UVR

During the PhD, we also reported for the first time one of the intracellular signalling that allows corals to rapidly respond to UVR and high temperature stresses. Our results show that the JNK protein activity is directly linked to coral capacities to cope with the ROS production induced by UVR or high temperature suggesting that the ancestral function of JNK is conserved (Runchel et al. 2011). In a context of rapid climate change where the environmental pressure on corals increases, the knowledge of cellular pathways involved in the response of corals to stressors is of particular importance as it defines coral capacities to overcome these changes. For example, we showed that under the combination of UVR and high temperature, the chemical inhibition of JNK pathway leads to a drastic increase in the cellular ROS concentration and in the long run, to coral bleaching. Any chemical compounds that would affect JNK activity might therefore also affect coral sensitivity to climate change. This information might therefore be of major importance for the water quality management.

II. Temperature effects on corals

In order to evaluate the combined effects of UVR and thermal or nutritional stress, we also studied the individual effects of temperature and/or nutrients on coral physiology, mucus production and degradation. Although thermal stress or nutrient level on coral physiology and symbiont-host

interactions have been very well studied in the past, our work is one of the few which has investigated the combined effects of these parameters, and to have taken into account the release of organic matter as well as its degradation by the associated bacteria. Overall, our results are consistent with recent field observations which found that temperature is the main environmental factor responsible for coral bleaching (Hughes et al. 2017) but also highlight the importance of considering a multifactorial approach to understand the coral response to environmental changes.

II. 1. Temperature, the main factor influencing coral physiology

The experiments conducted during the PhD, highlight some important factors, which determine the coral response to high temperature. First of all, we observed strong species-specific differences in energy allocation and stress response in two species (*P. damicornis* and *T. reniformis*) harbouring the same symbiont clade and experiencing the same recent growth history. While *P. damicornis* bleached rapidly following thermal stress, *T. reniformis* did not show any impact on its symbiosis and/or metabolism. These observations with those of previous work on the same species (Marshall and Baird 2000; Floros et al. 2004; Ulstrup et al. 2006; Schoepf et al. 2013b) suggest that the different resistances to thermal stress are likely linked to different thermal adaptations, due to the long genetic distance between the two species. Combined with different resistance to UVR (*T. reniformis* being also more resistant than *P. damicornis*), these adaptations are likely to provide great advantages for some species in the context of climate change. Another factor explaining thermal resistance might stand in the ability for corals to use heterotrophic feeding to compensate the loss of symbionts during coral bleaching. *A. muricata*, the most common species of New Caledonia, was indeed able to shift from autotrophy to heterotrophy during a thermal stress which allowed nubbins to increase their respiration rates and maintain their calcification rates. This capacity is believed to provide significant advantage over species that are unable to do so (Hughes and Grottoli 2013; Levas et al. 2016) and likely delays the disruption of the symbiosis. Such differences between species have to be taken into account when predicting the future evolution of a reef.

Another aspect that was addressed in this thesis is the importance of nutrient availability in seawater to understand the coral response to high temperature. First, we observed that nutrient depletion enhances the bleaching sensitivity, due to a general impairment of the coral metabolism, in agreement with few previous studies (Titlyanov et al. 2001; Wiedenmann et al. 2013; Rosset et al. 2015; Ezzat et al. 2015). Such observations, which suggest that corals living in very oligotrophic areas, or experiencing nutrient shortage due to water stratification will be particularly fragile during a thermal stress event, are also to take into account when predicting the future of the reefs. On the other hand, we found that high nutrient level in seawater mitigated the thermal stress effect.

Although this is in agreement with some studies showing that seawater supplementation with nutrients can alleviate the negative effects of high temperature or low pH (Holcomb et al. 2010; Béraud et al. 2013; Tanaka et al. 2014a; Hoadley et al. 2016), some other studies also showed higher bleaching sensitivity of corals maintained in eutrophicated waters (Schlöder and D'Croz 2004; Cunning and Baker 2013; Tanaka et al. 2014b; Vega Thurber et al. 2014). The variability in coral response to nutrient enrichment and thermal stress can be due to the source of nitrogen (nitrate versus ammonium) supplied to the corals (Ezzat et al. 2015), but we also hypothesized that it can be driven by differences in symbiont density. An excessive concentration of symbionts may indeed induce a parallel excessive production of harmful oxygen radicals (Wooldridge 2013; Cunning and Baker 2013) which exacerbates thermal stress negative effects (Schlöder and D'Croz 2004; Tanaka et al. 2014b), while species harboring low symbiont density might be able to use the additional nutrients to support the symbiosis during bleaching (Béraud et al. 2013; Hoadley et al. 2016, this thesis). These observations are congruent with the hypothesis developed above (Discussion, §I.2.) on the effect of symbiont density on *P. damicornis* susceptibility to UVR but more studies are needed to fully validate our hypothesis.

II. 2. Temperature variation and changes in the organic matter

The analysis of the dissolved and particulate fractions of the mucus showed that the quality of the OM changes with the environmental conditions under which corals are thriving. In *A. muricata*, temperature strongly affected the composition of the coral-derived organic matter, through changes in coral photosynthesis as well as in the heterotrophic/autotrophic balance. At ambient temperature, mucus produced by *A. muricata*, mainly composed of POM, is likely to rapidly sediment to the reef bottoms where it will contribute to the important nutrient recycling pathways observed in reefs (Muscatine and Porter 1977; Richter et al. 2001; Wild et al. 2004). At high temperature however, this mucus, mainly composed of labile DOM, will likely promote the development of free-living-bacteria (Ferrier-Pagès et al. 2000; Wild et al. 2004). Such variability might have consequences at the reef scale by promoting the growth of pathogenic bacterial communities at high temperature (Haas et al. 2013; Nelson et al. 2013) which might promote the invasion of corals by pathogens (Barott and Rohwer 2012). This aspect of the impacts of climate change on coral reefs is still poorly understood, although it might have great consequences on the biochemistry of the reef water and affect all organisms via the microbial loop.

II. 3. Temperature, the main factor influencing coral associated bacteria?

We also observed that temperature is the main factor responsible for the seasonal variability in the bacterial communities associated with the coral holobiont. Within the mucus, high temperature in summer enhanced the bacterial aminopeptidase and α-glucosidase activities and, together with the changes in the quality of the mucus, caused changes in the mucus-associated population. Within the coral microbiome, summer caused a shift in the population of *Endozoicomonas*, an increase in the abundance of bacteria previously associated with diseased and stressed corals as well as an increase in diazotrophic bacteria. These changes support the coral probiotic hypothesis which posits that a dynamic relationship exists between symbiotic microorganisms and environmental conditions which brings about the selection of the most advantageous coral holobiont (Reshef et al. 2006). This conclusion reinforces the interest that one should have toward the study of the associated microbial community as it likely is an early indicator of coral state of stress.

III. Conclusions and perspectives

III. 1. Take home messages

Exposure of UVR-acclimated corals to present time UVR dose has little effect on coral physiology and organic matter release likely because corals implement protective mechanisms to counteract the direct (photolysis) or indirect (ROS production) effects of UVR. The lack of effect on the mucus, the first layer to be exposed to solar radiation, even under high UVR intensities (summer radiations), suggests that the presence of UVR-absorbing compounds (MAAs like-compounds) within the mucus forms the first defense for corals against photolysis. Within the tissue, corals were able to limit the UVR-induced ROS production likely by implementing functional antioxidant mechanisms in their cells, as well as synthesizing MAAs like compounds. However, for some corals which are shaded from UVR, or which contain high densities of symbionts, these protective mechanisms are not sufficient to cope with an increase in UVR (*i.e.* UVR stress), which induces coral bleaching.

UVR worsens the effects of thermal stress on corals. This thesis largely complements the poor knowledge on the combined effects of UVR and high temperature and highlights the role of UVR in the severity of coral bleaching. It shows that corals exposed to UVR (shallow reefs), even at present time doses, will be more impacted by thermal stress and more susceptible to die during a long term event. We also showed that nutrient depletion or enrichment mitigates the effects of UVR-

thermal stress. In a time of common effort to protect coral reefs against climate change, these information are of major importance and highlight the need for multifactorial approaches.

UVR sensitivity is function of the symbiont density. One of the conclusions drawn by the comparison of different experiments performed in this thesis is that sensitivity to UVR can vary within a species depending on the symbiont density. Environmental conditions such as nutrient concentrations and heterotrophic prey availability vary between reefs and may locally influence the symbiont density of corals. This may partly explain why, two colonies of the same species growing in different close area can respond differently to climate change. More work on species at different locations or in different environmental conditions is needed to validate this hypothesis.

The signaling pathway responsible for the down regulation of the production of UVR-induced ROS in mammals is conserved in corals. JNK signaling pathway is known to be involved in the control of oxidative stress in mammalian cells. In corals, we showed that its basal activation was also necessary for limiting cellular ROS concentration. Moreover, we also demonstrated that JNK was activated by UVR and high temperature and that the inhibition of this activation led to a drastic increase in ROS concentration. This demonstrates that coral defenses against oxidative stress are at least partly activated by the JNK signaling pathway.

III. 2. What is next?

First of all, we raised a technical question on the use of artificial lamps that needs to be further addressed. As exposed in the materiel and method (§I.1.1.), UVR lamps deliver a constant irradiation and we only adjust the dose delivered over the day. A previous test done by M. Shick at CSM (unpublished) seems to indicate that the variability in intensity and time exposure has minor influence on corals compared to the received dose. More experiments would be interesting to confirm this preliminary result.

As we showed that coral response to UVR was function of different environmental and biological factors, there is an urgent need to study the cross-effects of UVR and other environmental factors such as ocean acidification, known to affect bleaching, productivity and calcification (Hoegh-Guldberg et al. 2007). Anthony et al. (2008) indeed proposed that CO_2 induces bleaching via its impact on photoprotective mechanisms of the photosystems; UVR could therefore be more harmful in acidified water. Moreover, this thesis indicates the need for exploring the sensitivity of different species namely to better understand whether resistance of some species, such as *T. reniformis*, is adaptive or acclimatory. To test the adaptive theory, the next step would be to compare the response to environmental stressors of other species from the same or a sister family as *T. reniformis*

that we found more resistant to UVR (Dendrophyllidae or Poritidae). Additionally, UVR acclimated corals seem to be unaffected by present time UVR dose. It would be interesting to compare the response of UVR acclimated nubbins to an increase in UVR. This could be done in New Caledonia by exposing nubbins collected at low depth to higher UVR using UV lamps.

According to our findings, coral sensitivity to UVR would be function of the symbiont density. Such hypothesis needs to be further investigated. Two aspects could be explored: 1) the comparison of more species harboring different symbiont density (as we did for *T. reniformis* and *P. damicornis*) to confirm that it is an important criteria to explain species specificity and 2) the comparison of individuals of the same species with different symbiont density. For example, the species Astrangia Danae, known to harbor different symbiont density within the same reef could be used. This would allow confirming that the different responses to UVR we saw are linked to the symbiont density rather than the changed environmental factor itself.

Our results also suggest that UVR stress might affect the biochemistry of the reef water and the microbial loop. More work is therefore needed to understand the consequences of coral response to stressors at the reef scale. The first step to test the consequences would be to monitor the variation of the diversity and the structure of the reef water microbial community following an increase in UVR to confirm that the microbial loop will be affected. Moreover, as a raise in UVR affects N fluxes between the coral and its environment, the endosymbiotic diazotrophic community might be also affected. More and more questions are raised about the role of endosymbiotic diazotrophic bacteria in N recycling within the coral, as they are thought to become an important source of N under stress conditions. Monitoring the changes in diazotroph density, community and N_2 fixation under UVR stress could therefore be relevant to better understand the role of these endosymbiont in the holobiont.

Finally, the work we did on JNK signalling pathway is a preliminary step in the understanding of molecular pathways involved in coral stress response. The next step of the work would be to identify JNK targets under UVR or thermal stress to understand what happens in between JNK activation and the control of ROS concentration. For example, previous work on mammals and hydra showed that JNK can activate the FOXO transcription factor responsible for activating antioxidant enzymes (Essers et al. 2004; Bridge et al. 2010). Such pathways could also be tested on corals. Other MAPk could also be tested in the context of climate change such as p38 also known to be involved in antioxidant defenses in mammalian cells (Runchel et al. 2011). Such work has already been initiated as p38 is one of the kinases tested on samples collected during Tara expedition.

Paper n°2 –Limnology and Oceanography

LIMNOLOGY
and
OCEANOGRAPHY

ASLO

Limnol. Oceanogr. 00, 2017, 00–00
© 2017 Association for the Sciences of Limnology and Oceanography
doi: 10.1002/lno.10481

Interactive effects of ultraviolet radiation and thermal stress on two reef-building corals

Lucile Courtial,[1,2,3]* Stéphane Roberty,[2,4] J.Malcolm Shick,[5] Fanny Houlbrèque,[3] Christine Ferrier-Pagès[2]*

[1]Sorbone Universités, UPMC Univ Paris 06 IFD-ED 129, Paris Cedex 05, France
[2]Centre Scientifique de Monaco, Equipe Ecophysiologie, Monaco, France
[3]UMR ENTROPIE (IRD), Laboratoire d'Excellence CORAIL, Nouméa Cedex, New Caledonia
[4]InBioS – Animal Physiology, Department of Biology, Ecology and Evolution, University of Liège, Liège, Belgium
[5]School of Marine Sciences, University of Maine, Orono, Maine

Abstract

In shallow tropical waters, ultraviolet radiation (UVR) occurs at high intensity simultaneously with high water temperatures, and both stressors are predicted to increase in the future and to have a major impact on reef coral survival. The poor knowledge of the interactive effects of those two factors, however, prevents a good estimation of the coral resistance to climate change pressure. The results obtained in this study on two genetically distant scleractinian coral species, *Pocillopora damicornis* and *Turbinaria reniformis*, highlight an interaction of temperature and UVR on the corals' physiology as well as a species-specific response. Overall, increase in UVR level exacerbated the negative impact of thermal stress. Whereas stressors did not reduce the autotrophic capacity (symbiont density, net photosynthetic rates) of *T. reniformis*, they significantly lowered it in *P. damicornis*, which initially contained twice more symbionts in its tissue. Only grazing rates (heterotrophy) were significantly decreased in *T. reniformis* under thermal stress. For both corals, calcification was slowed by the combination of thermal and UVR stress. Calcification was impaired likely due to a decrease in autotrophic energy supply in *P. damicornis*, and in heterotrophic energy supply in *T. reniformis*. This study confirms that the response of corals to global change needs to be studied using multifactorial approaches and a combination of different environmental factors.

Ultraviolet radiation (UVR) includes UVA wavelengths (315–400 nm) and high-energy UVB wavelengths (290–315 nm), the latter being mainly absorbed by ozone molecules in the atmosphere. Since the last century, human aerial gas discharge, in particular chlorofluorocarbon compounds and nitrous oxide produced by agriculture, has significantly reduced the ozone layer, increasing the amount of UVB reaching the earth surface (Madronich 1993). Although the Montreal protocol has legislated the amount of ozone-depleting substances that can be released to the atmosphere (Madronich et al. 1998), the ozone concentration is still low and is expected to be back to normal only by the middle of the century (Williamson et al. 2014). While ozone depletion is greater at high latitudes, lower latitudes, near the equator, generally receive more intense sunlight and UV levels (Madronich 1993). In the tropics, global warming is expected to even more increase UVR at the ocean surface and in shallow water through the enhancement of two physical processes: the Brewer–Dobson circulation, which decreases the ozone concentration above the tropics by re-distributing it in the extra-tropics (Butchart et al. 2010; Eyring et al. 2010a,b); and the stratification of the water column, which lowers the particulate organic matter concentrations, increasing water clarity and UV penetration (Häder et al. 2007). Meteorological models thus predict a 2% UVR increase at the ocean surface in tropical areas (Watanabe et al. 2011).

High levels of UVR have direct and indirect effects on living cells (Carrillo et al. 2015). UVR (especially UVB, Banaszak and Lesser 2009) is the most harmful and mutagenic component of the solar radiation that reaches the earth, photochemically degrading biomolecules (Leu et al. 2007) and indirectly damaging cells by forming highly energetic and

*Correspondence: lcourtial@centrescientifique.mc; ferrier@centrescientifique.mc

Authors Fanny Houlbrèque and Christine Ferrier-Pagès have equally contributed to the work.

Additional Supporting Information may be found in the online version of this article.

unstable reactive oxygen species (ROS) (Sharma et al. 2012). DNA bases, for example, are distorted directly by UVR and indirectly affected via ROS that exacerbate the conversion of coding bases to mutagenic or lethal non-coding lesions (Häder and Sinha 2005). Coral reef organisms are particularly exposed to UVR as they thrive in tropical shallow waters where UVR levels in the first meters can reach up to 2 W m^{-2} for UVB and 40 W m^{-2} for UVA (Torregiani and Lesser 2007).

Corals, which are the main reef builders, and create hotspots of biodiversity (Doney et al. 2012), have developed adaptations to high UV levels, including antioxidant and UV-absorbing compounds called mycosporine-like amino acids (MAAs) (Shick et al. 1996; Shick and Dunlap 2002; Rosic and Dove 2011). Although UVR is not lethal for corals that have acclimatized to it, it is however known to impact coral growth, reproduction, calcification, and viability (Shick et al. 1996; Gleason 2001; Banaszak and Lesser 2009; Torres-Pérez and Armstrong 2012). UV stress adds to other recurrent stresses linked to climate change such as elevated sea surface temperatures, increasing the environmental pressure on corals. Indeed, as corals live at or near their upper thermal tolerance limits (Jokiel 2004) they are thus increasingly affected by global warming, which induces bleaching (Hoegh-Guldberg 1999; Hoegh-Guldberg et al. 2007). Bleaching is the loss of the endosymbiotic phototrophic dinoflagellates living within the coral host tissue, which provide corals with up to 90% of their energy requirement (Muscatine 1990).

Even though elevated temperature and UV levels are simultaneously occurring on reefs, only few studies (fewer than ten) assessed their interactive effects on scleractinian corals, as compared with the impacts of each variable separately (more than a thousand). Most of the above studies focused on MAA production and oxidative stress either in isolated symbionts (Lesser 1996; Rogers et al. 2010) or in single coral species (Lesser et al. 1990*a*,*b*; Fitt and Warner 1995; D'Croz et al. 2001; Lesser and Farrell 2004). Due to disparate results in the several studies, their general conclusions concerning the corals' response are unclear. Indeed, D'Croz et al. (2001) found no significant effect of UV exposure when added to thermal stress on symbiont density and chlorophyll content of the scleractinian coral *Porites lobata*, while Fitt and Warner (1995) measured a decline in the photosynthetic efficiency of *Montastrea annularis* under UVA. Lesser et al. (1990*a*,*b*) as well as Lesser and Farrell (2004) observed enhanced production of ROS during exposure to high irradiances of solar radiation and elevated temperatures. The discrepancies observed in the above studies may be due to different combinations of temperature, PAR and UV levels, but also to a species-specific response to the stress. For example, Ferrier-Pagès et al. (2007) showed that different hosts and symbionts presented a holobiont-specific response to a coupled UV-and thermal stress. These species-specific responses to single-factor stress depend on pigment composition (Hoegh-Guldberg and Jones 1999), tissues thickness (Loya et al. 2001), colony shape (Glynn 1993) and oxidative stress level (Lesser 1996). Species-specific responses to two stressors might be much more complex as the effects of stress can be additive, synergistic, or antagonistic effects. It was indeed observed that UV counterbalanced thermal photoinhibition of diatoms (Halac et al. 2010) and vice-versa (Bouchard et al. 2006; Sobrino and Neale 2007); on the contrary, UVR was shown to exacerbate the harmful effect of thermal stress on the photosynthesis of brown algae (Cruces et al. 2013). Taken together, these observations highlight the complexity in the animal and algal response to combined stressors. In the context of climate change, it is important to understand how UVR and temperature impact growth, photosynthesis and energy metabolism of scleractinian corals, and which mechanisms each coral species implements for its protection.

The purpose of our study was to evaluate the responses of two coral species, *Pocillopora damicornis* and *Turbinaria reniformis*, to the individual and combined effects of increased temperature and UVR on photosynthesis, respiration, calcification, and grazing rates, as well as symbiont density, protein content, and pigment concentrations. These two species were chosen because they belong to two genetically distant scleractinian clades (robust vs. complex) that diverged at least 300 My ago (Romano and Palumbi 1996; Chen et al. 2002). *P. damicornis* belongs to the "robust" clade of taxa having relatively solid, heavily calcified skeletons while *T. reniformis* belongs to the "complex" clade of corals having relatively porous constructions due to a light, complex architecture (Romano and Palumbi 1996). The marked genetic distance between those two clades suggests important morphological and physiological differences and therefore possible differences in their response to environmental changes and bleaching susceptibilities (higher for *P. damicornis*, Ulstrup et al. 2006). In addition, *P. damicornis* is a key model species for Indo-Pacific reefs and is one of the most important species contributing to the reef framework (Sorokin 2013) while *T. reniformis* is well represented both in the Indo-Pacific and Red Sea reefs (Veron 2000). The data obtained will help predicting the response of key scleractinian coral species to global changes expected by the year 2100 (Collins et al. 2013).

Material and methods

The combined effects of UVR and temperature on coral physiology were assessed in two scleractinian coral species originating from the Red Sea and grown in aquaria at the Centre Scientifique de Monaco: a laminar species, *T. reniformis* (Dendrophyllidae) and a branching species *P. damicornis* (Pocilloporidae). Both species contained the clade C1 (Godinot et al. 2013; Ezzat et al. 2015). Five mother colonies of each species were grown for several months at 25°C, under a PAR (photosynthetically active radiation) of 200 μmol photons m^{-2} s^{-1} provided by 400 W metal halide lamps (HPIT,

Philips), shielded to eliminate UVR. A total of 80 nubbins per species were collected from each mother colony (16 nubbins per colony). After being cut, nubbins were allowed to heal for 3 weeks before experimental treatments, and were fed twice a week with *Artemia salina* nauplii. During this period, nubbins were maintained under the same conditions as the mother colonies.

Experimental settings

Nubbins were then evenly distributed in eight tanks of 25 L. Four conditions, representing two UVR levels and two temperatures, in a cross factor design, were tested in duplicated tanks, continuously supplied with seawater: 25°C (± 0.3°C) without UVR (control condition, hereafter called C); 25°C with UVR (CUV); 30°C (± 0.3°C) without UVR (CT); 30°C with UVR (UVT). PAR remained at 200 ± 10 μmol photons m^{-2} s^{-1} and controlled using a LI-COR data logger (LI-1000) connected to a spherical quantum sensor (LI-193). Temperature was controlled using heaters connected to Elli-Well PC 902/T controllers. UVR was provided by Q-Panel UVA 340 lamps and was adjusted to match the levels measured at 1–5 m depth on the reef (Ferrier-Pagès et al. 2007). The radiation intensity was ca. 1.5 W m^{-2} UVB and 30 W m^{-2} UVA, and was checked using an International Light ILT1400 portable radiometer and detectors (SEL240/UVB-1/TD and SEL033/UVA/TD). As corals were grown without UVR, their response can be compared with deep-living or shaded corals facing an increase in UVR level. All nubbins were maintained 5 weeks in the aquaria. Nubbins in the UVT and CUV treatments were first exposed to UVR for 1 week before temperature was gradually raised (within a week) from 25°C to 30°C in UVT. High temperature was maintained for three additional weeks and under UVR. The same thermal treatment was applied to CT corals but without UVR. At the end of the 5 weeks, 5 nubbins per condition were sampled to measure rates of photosynthesis, respiration, calcification, PSII fluorescence, symbiont density, and protein content. Three nubbins were used to assess grazing rates and pigment content the later was determined prior any treatment, and after 1, 2, and 5 weeks, see below. For all measurements, temperature and light were maintained constant and identical to the experimental conditions. Data were all normalized to the surface area of the corals, measured using the wax technique (Stimson and Kinzie 1991), or per symbiont cell (determined as described below).

Oxygen exchange measurements

Photosynthesis and respiration rates were measured using a Clark-type oxygen electrode (Si 928, Strathkelvin Instruments Ltd) fitted to a Plexiglas incubation chamber (Hoogenboom et al. 2010). The oxygen electrode was calibrated against oxygen-saturated water (100% air saturation) and a saturated aqueous solution of sodium dithionite (0% oxygen saturation). Actinic light was provided by LED light sources (white cold LEDS-5MP/WS, Monacor). Respiration and photosynthetic rates were measured during 10 min, after a 10-min dark or light acclimation period. Oxygen consumption or production was then deduced from the slope of the linear regression of oxygen concentration in the chamber vs. incubation time. At end of the measurements, coral nubbins were frozen at −20°C until the chlorophyll and protein concentrations, as well as symbiont density, were measured.

Chlorophyll fluorescence

The maximal photochemical quantum yield (F_V/F_m) was monitored in parallel with oxygen exchange measurements by using a DUAL-PAM 100 (Walz GmbH, Effeltrich, Germany). After a dark adaptation (DA) of 20 min, an induction curve was conducted with gain (G) and damping (D) set to 5 and 1 ms, respectively. The initial fluorescence level (F_0) was determined by applying weak modulated pulses of red measuring light (MI = 10). A 0.8 s saturating pulse of actinic light (SI = 10 corresponding to an intensity of ≈ 4000 μmol photons m^{-2} s^{-1}) was then applied after 1 min and every 2 min to measure the maximum fluorescence level (F_m), 5 min later the actinic light was provided. The maximal photochemical quantum yield (F_V/F_m) was calculated at the end of the dark incubation as $(F_m-F_0)/F_m$.

Chlorophyll, symbiont, and protein quantifications

Coral tissue was separated from the skeleton using compressed air and homogenized using a Potter tissue grinder. The symbiont density was quantified in a 100 μL sub-sample using a Z1 Coulter Particle Counter (Beckman Coulter). A 2 mL sub-sample was taken for protein extraction in sodium hydroxyde according to Hoogenboom et al. (2010) and measured using a BiCinchoninic acid Assay (BCA) assay kit according to Smith et al. (1985) on a Xenius spectrofluorometer (Safas). The remaining homogenate was then centrifuged at 2000 × g for 10 min at 4°C to separate the host tissue (supernatant) from the symbionts (pellet). *Symbiodinium* cells were then re-suspended in 5 mL of acetone and the samples were kept at 4°C for 24 h in darkness to insure total extraction. Samples were then centrifuged for 15 min at 11000 × g and the absorbance was measured at 630, 663, and 750 nm using a Xenius spectrofluorometer. Chlorophyll a and c_2 concentrations were calculated by using the equations of Jeffrey and Humphrey (1975).

Calcification and grazing rate

Calcification rates were measured using the alkalinity anomaly method (Smith and Key 1975). Coral nubbins were incubated for 6 h in individual beakers filled with 180 mL of 0.2 μm filtered seawater. The water was sampled at the beginning and the end of the incubation period and alkalinity was measured using a 888 Titrando titrator (Metrohm). Data were expressed as $\mu molCaCO_3$ cm^{-2} d^{-1}.

Feeding rates were assessed by incubating nubbins for 1.5 h in individual Plexiglas flow chambers filled with 0.45 μm filtered seawater containing 1500 *A. salina* nauplii

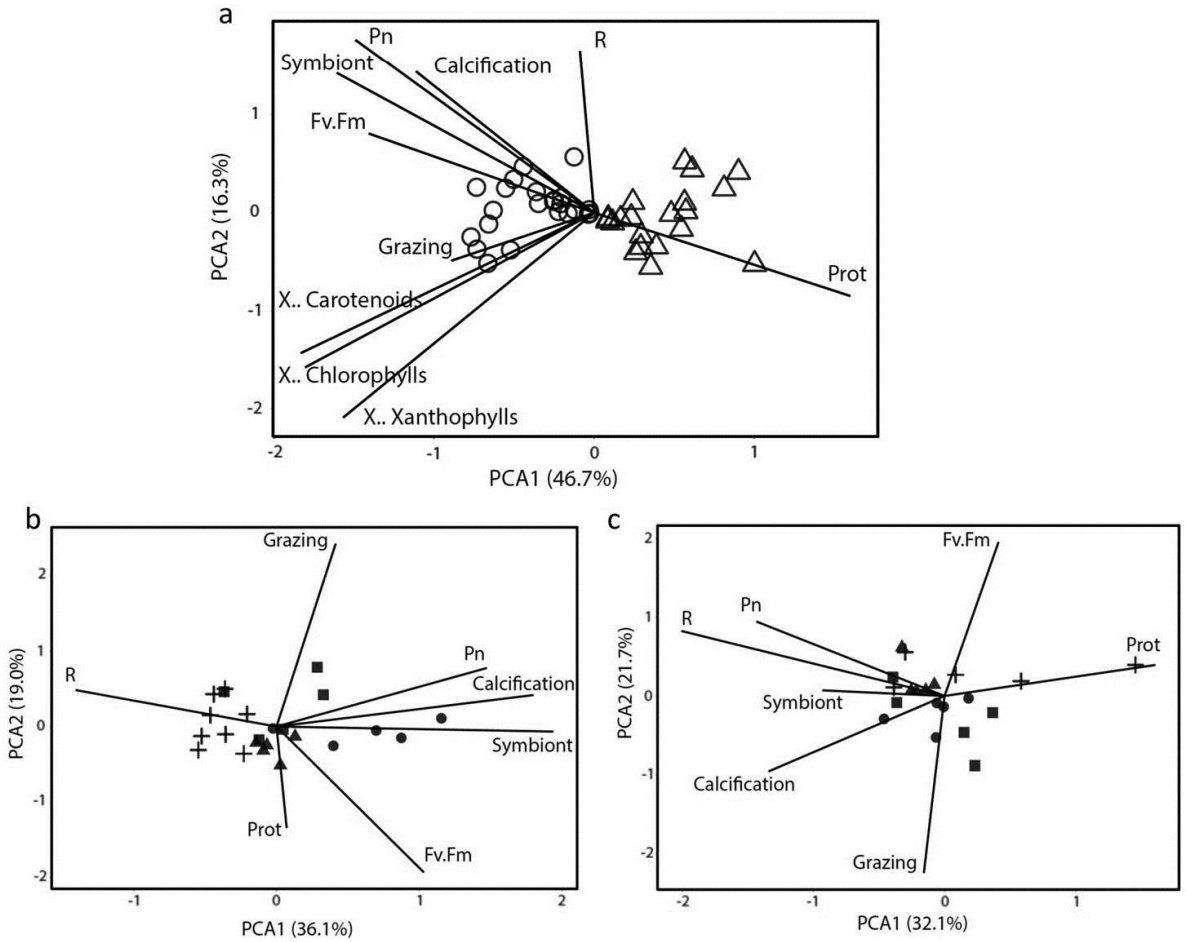


Fig. 1. Distance biplots resulting from the principal component analyses on all data (**a**), on *P. damicornis* (**b**) and on *T. reniformis* alone (**c**). Physiological traits (red lines) used as descriptors represented calcification rates (Calcification), respiration (R) and net photosynthetic rates (Pn), symbiont density (Symbiont), maximal photochemical quantum yield (F_V/F_m), grazing rates (Grazing), protein contents (Prot), and the sum of carotenoids, chlorophylls, and xanthophylls (X.Carotenoids, X.Chlorophylls, and X.Xanthophylls). (**a**) Open circles and triangles represent individuals of *Pocillopora damicornis* and *Turbinaria reniformis*, respectively. The first PCA axis accounted for 46.7% of the variability and the second PCA axis for 16.3%. (**b,c**) Plain circles, triangles, squares, and crosses represent nubbins in the control condition (C), at 30°C without UVR (CT), at 25°C with UVR (CUV), and at 30°C with UVR (UVT), respectively. The first PCA axis accounted for 36.1% of the variability and the second PCA axis for 19.0% (Fig. 1.b) and for 32.1% of the variability and the second PCA axis for 21.7%.

(Ferrier-Pagès et al. 2010). A control chamber with no coral inside was set up in parallel to the others. After 0, 15, 30, 60, and 90 min incubation, nauplii were counted 3 times in 10 mL water sampled in each chamber. Nauplii were then put back to the chamber to avoid artificial concentration decrease. Grazing rates were calculated as the difference between the final (after 1.5 h) and the initial concentration of nauplii in the chamber and normalized to the skeletal surface area. To calculate the total daily carbon acquisition via heterotrophy (CHAR), oxygen fluxes were converted to carbon equivalent using the respiratory quotient (RQ) equal to 0.8 (Muscatine et al. 1981; Gattuso and Jaubert 1990). It was calculated considering 2 h of grazing per day and a total content of 1.2 μmol carbon per *A. salina* (Tremblay et al. 2011) according to the equation: CHAR (%) = (grazing rates in μmolC cm^{-2} d^{-1})/(μmolO_2 consumed × 24 × RQ) × 100.

Pigment profile analysis

Coral nubbins were sampled at the same moment of the day in the different conditions, prior any treatment (T0),

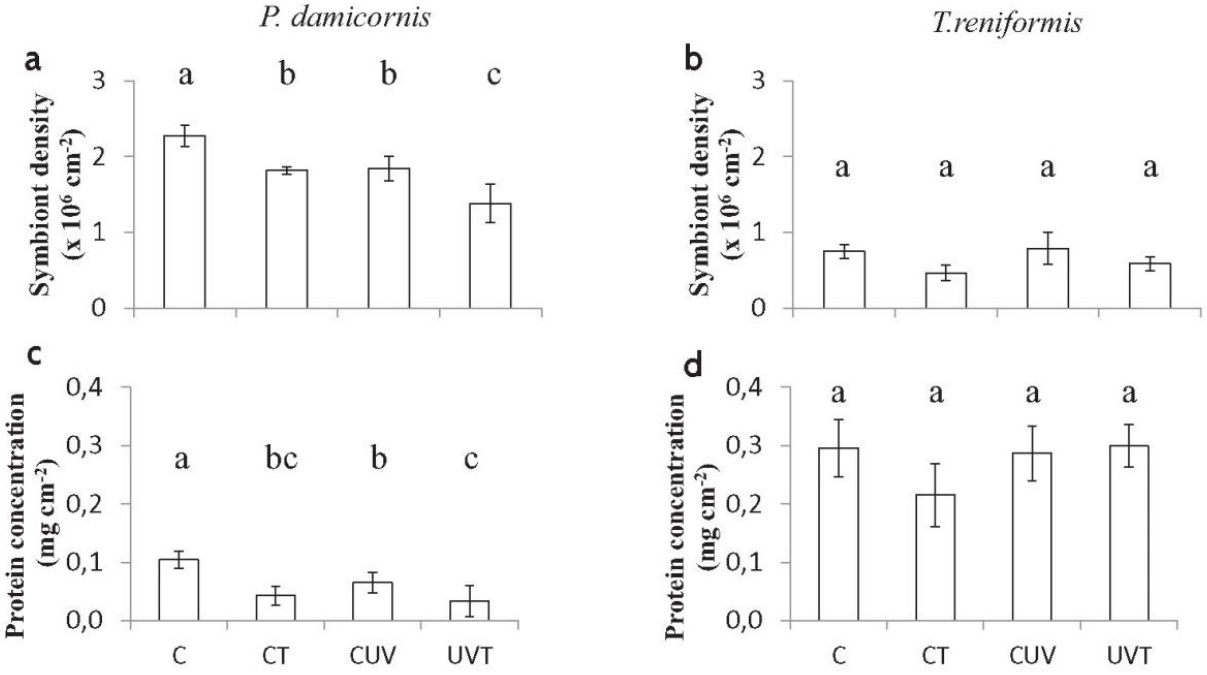


Fig. 2. Symbiont density ($\times 10^6$ cells cm^{-2}) and protein content (mg cm^{-2} of colony surface) of *Pocillopora damicornis* (**a, c**) and *Turbinaria reniformis* (**b, d**) in the control condition (C), at 30°C without UVR (CT), at 25°C with UVR (CUV) and at 30°C with UVR (UVT) after 5 weeks of experiment. Data are mean and standard deviations of five replicates. Values noted with the same letter are not significantly different (p value > 0.05).

and after 1 (T1), 2 (T2), and 5 (T5) weeks. They were placed for 15 min under 400 μmol photons m^{-2} s^{-1} PAR before being flash frozen in liquid nitrogen. They were then lyophilized, ground to powder, and pigments were extracted in 1.5 mL of MeOH (HPLC Grade, Merck). Samples were then vortexed for 3 × 15 s with 0.5 mL glass beads (Sigma Aldrich G1152) before being centrifuged at 16,000 × g for 20 min at 4°C. Pigments were then separated by reverse-phase HPLC, using Shimadzu Prominence HPLC system, comprising a DGU-20A5R Degassing Unit, a LC-20AT Liquid chromatograph, a SIL-20AC Autosampler, a CTO-10ASVP Column Oven and a SPD-M20A Diode Array Detector (Shimadzu). The HPLC column (Nova Pak C18, 60A column, 150-mm length and 4 μm pore size) was eluted with a mobile phase gradient (1 mL min^{-1}) set to the following program: 0.5 min with a gradient from 100% (v/v) solvent A (80% [v/v] methanol and 20% [v/v] 0.5 M ammonium acetate [pH 7]) to 100% (v/v) solvent B (90% [v/v] acetonitrile in water); then during 0.5 min with a gradient from 100% (v/v) solvent B to 90% (v/v) solvent B and 10% (v/v) solvent C (ethyl acetate); 10 min with a gradient from 90% (v/v) solvent B to 65% (v/v) solvent B and from 10% (v/v) solvent C to 35% (v/v) solvent C; 1 min with a gradient from 65% (v/v) solvent B to 55% (v/v) solvent B and from 35% (v/v) solvent C to 45% (v/v) solvent C; 3 min with a gradient from 55% (v/v) solvent B to 40% (v/v) solvent B and from 45% (v/v) solvent C to 60% (v/v) solvent C; 5 min with a gradient from 40% (v/v) solvent B to 0% (v/v) solvent B and from 60% (v/v) solvent C to 100% (v/v) solvent C; and finally during 2 min with a gradient from the latter solvent mixture to 100% (v/v) solvent A. Absorbance chromatograms were detected at 430 nm and quantified with pigments standards purchased from DHI Lab (Horstholm, Denmark). Acquisition and data treatment were performed using the Waters Empower software (Waters).

Statistical analysis

All data were expressed as mean ± standard deviation. When needed, data were transformed using the Box-Cox transformations to fulfill requirements of homescedasticity and normality of residuals. Data were compared using two-way ANOVA, with species and treatments as factors. When there were significant differences, a posteriori test (Tukey's test) was performed. Differences were considered statistically significant when $p < 0.05$. Principal component analysis (PCA) was performed on all nubbins and all conditions using 10 variables; the calcification rate (Calcification), the respiration rate (R), the net photosynthesis (Pn), the symbiont density (Symbiont), the maximal photochemical quantum yield

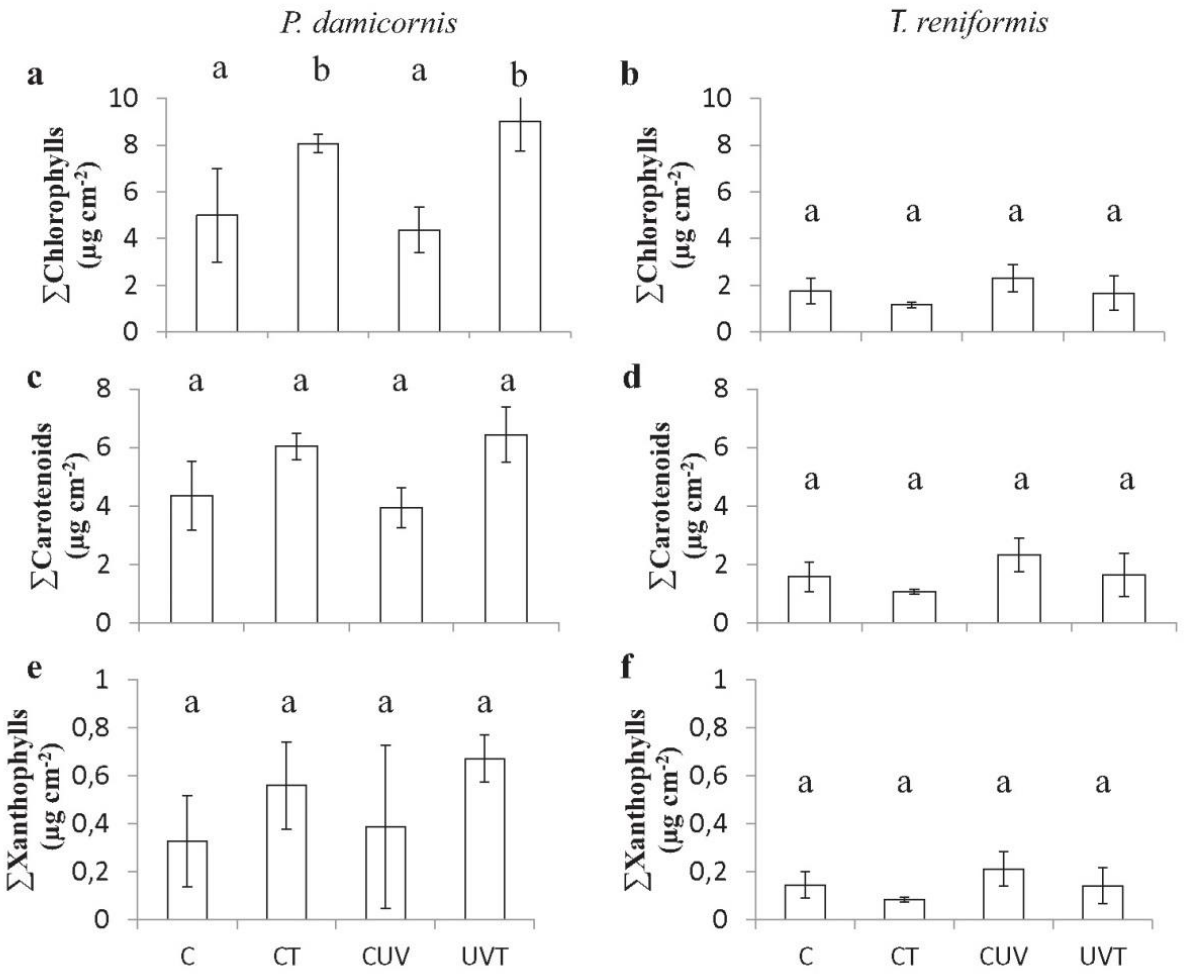


Fig. 3. Total concentration of chlorophylls (∑Chlorophylls), carotenoids (∑Carotenoids), and xanthophylls (∑Xanthophylls) (μg cm^{-2}) for *Pocillopora damicornis* (**a**, **c**, **e**) and *Turbinaria reniformis* (**b**, **d**, **f**) in the control condition (C), at 30°C without UVR (CT), at 25°C with UVR (CUV) and at 30°C with UVR (UVT) after 5 weeks of experiment. Data are mean and standard deviations of three replicates. Values noted with the same letter are not significantly different (p value > 0.05).

(Fv/F_m), the grazing rate (Grazing), the protein content (Prot), the sum of carotenoids, chlorophylls, and xanthophylls (X.Carotenoids, X.Chlorophylls, and X.Xanthophylls). PCAs were conducted as described above on the two species separately using seven variables: Calcification, R, Pn, Symbiont, Fv/F_m, Grazing, and Prot. One-way ANOVAs were performed to compare species in the control condition. Wilcoxon tests were used to compare pigment proportions between species. Temporal variations in pigment concentrations were assessed using Kruskal–Wallis tests with time as a predictor factor. In addition, when the combination of high temperature and UVR had a significant effect, we characterized it as antagonistic, synergistic, or additive depending on the ANOVA's results (Dunne 2010). When a significant interaction between temperature and UVR was found, the effects were characterized as synergistic if it was an amplified effect or antagonistic if it was a reduced effect.

Results

Exploratory statistics

PCA comparison of the physiological traits of both species maintained under the four UV-temperature conditions revealed clear interspecific physiological differences (Fig. 1a).

Table 1. Total concentration of chlorophylls (ΣChlorophylls), carotenoids (ΣCarotenoids), xanthophylls (ΣXanthophylls), and peridinin expressed per symbiont cell (pg cell^{-1}) and surface area (μg cm^{-2}) for *Turbinaria reniformis* at different incubation times (initial time, 1 week, 2 weeks, and 5 weeks) under UVR at 25°C (CUV). Values noted with the same letter are not significantly different (p value > 0.05). Values are mean ± standard deviation of three replicates.

	Pigment concentration (pg cell^{-1})				Pigment concentration (μg cm^{-2})			
	Initial time	1 Week	2 Weeks	5 Weeks	Initial time	1 Week	2 Weeks	5 Weeks
∑Chlorophylls	4.3 ± 0.6 (a)	5.6 ± 0.5 (a)	2.2 ± 1 (b)	5.4 ± 2 (a)	1.9 ± 0.7 (ab)	2.8 ± 0.4 (a)	0.95 ± 0.5 (b)	2.3 ± 0.6 (a)
∑Carotenoids	3.5 ± 0.5 (ab)	5.1 ± 0.1 (a)	2.1 ± 1 (b)	5.4 ± 2 (a)	1.6 ± 0.6 (ab)	2.6 ± 0.5 (a)	0.92 ± 0.5 (b)	2.3 ± 0.6 (a)
∑Xanthophylls	0.40 ± 0.1 (a)	0.56 ± 0.04 (a)	0.2 ± 0.08 (b)	0.51 ± 0.2 (a)	0.16 ± 0.1 (ab)	0.28 ± 0.05 (a)	0.081 ± 0.04 (b)	0.21 ± 0.07 (a)
Peridinin	3.2 ± 0.5 (ab)	4.5 ±0.1 (a)	1.9 ± 0.9 (b)	4.9 ±1.7 (a)	1.4 ± 0.5 (ab)	2.3 ± 0.5 (a)	0.84 ± 0.6 (b)	2.1 ± 0.5 (a)

The three first axes were significant and the first two axes explained more than 61% of the variance. All variables were well represented along the first axis, except the respiration and grazing rates, better described along the second and third PCA axes, respectively (Fig. 1). The PCA was clearly oriented by the physiological differences between species as the first axis, explaining the great majority of the variability, and separated two groups identified as the two species. The first group (circles), comprising only *P. damicornis* individuals, was characterized by lower protein content per surface area and higher values of pigment concentrations, symbiont density, Pn, F_V/F_m and calcification rate compared with the second group (triangles), which included all *T. reniformis* nubbins. In addition, each species responded differently to the thermal and UVR stress (Fig. 1b,c). For *P. damicornis*, the first PCA axis, representing 36.1% of the variance, clearly separated three groups composed of (1) UVT samples, (2) CT and CUV samples, and (3) control samples, the last of these presenting a higher symbiont density, as well as higher rates of calcification and Pn compared with the CT and CUV conditions, themselves presenting higher values than the UVT condition. On the contrary, no clear separation could be observed for *T. reniformis* among conditions (Fig. 2c). Those results suggest physiological differences between the two species under the control condition as well as differences in their stress responses. Those differences are detailed below.

Physiological differences between *P. damicornis* and *T. reniformis* under control conditions

Symbionts of both species belonged to *Symbiodinium* clade C1, and had a similar pigment composition with chlorophyll constituting 54% and carotenoids (including xanthophylls) 46% of total cellular pigments (data not shown). When expressed per skeletal surface area, and for the same culture condition, symbiont density was, however, three times higher in *P. damicornis* than in *T. reniformis* (Fig. 2a,b, and Supporting Information Table 1). As a consequence, total chlorophyll and carotenoid concentrations per surface area were 2.5 times higher (Fig. 3a–d) and in particular xanthophylls concentration was two times higher in *P. damicornis* than in *T. reniformis* (Fig. 3e,f, and Supporting Information Table 1). When expressed per *Symbiodinium* cell (Supporting Information Table 1), symbionts of *T. reniformis* had two times more pigments than *P. damicornis*, and much higher dinoxanthin concentration (0.006 ± 0.002 pg cell^{-1} against 0.0001 ± 0.0003 pg cell^{-1} for *P. damicornis*). The kinetics of the pigment response to UVR prior to the start of the experiment was different depending on the species. For *T. reniformis*, pigment concentrations per symbiont cell or per skeletal surface area (peridinin, total chlorophylls, carotenoids, and xanthophylls contents) dropped after 2 weeks of exposure to UVR (Table 1, and Supporting Information Table 4). The effect was transient since concentrations were back to their initial values at the end of the 5-week experiment. For *P. damicornis*, no significant decrease in the pigment concentration was recorded during the two first weeks (data not shown).

P. damicornis also presented significantly higher rates of Pn per surface area (Fig. 4a,b and Supporting Information Table 1), F_V/F_m (0.669 ± 0.019 against 0.556 ± 0.044) as well as calcification rates (Fig. 4e,f and Supporting Information Table 1) than *T. reniformis*. However, the protein content was 2.6 times greater in *T. reniformis* (Fig. 2c,d and Supporting Information Table 1). The respiration and grazing rates per surface area were similar for both species (Fig. 4c,d,g,h and Supporting Information Table 1).

Effect of thermal stress and UVR on tissue parameters

No significant effect of thermal or UVR stress could be observed in *T. reniformis* (Figs. 2, 3 and Supporting Information Table 2). On the contrary, symbiont density, protein and pigment concentrations per surface area were significantly affected by temperature and UVR in *P. damicornis* (Figs. 2, 3 and Supporting Information Table 2). In this species, after 5 weeks of the experiment, symbiont density significantly decreased by 25% in the CUV and CT treatments, and by 30% in the UVT treatment, showing an additive effect of the two stressors (Fig. 2A and Supporting Information Table 1). A synergistic effect was observed on the protein content, which was on average 38%, 60%, and 70% lower in CUV, CT, and UVT

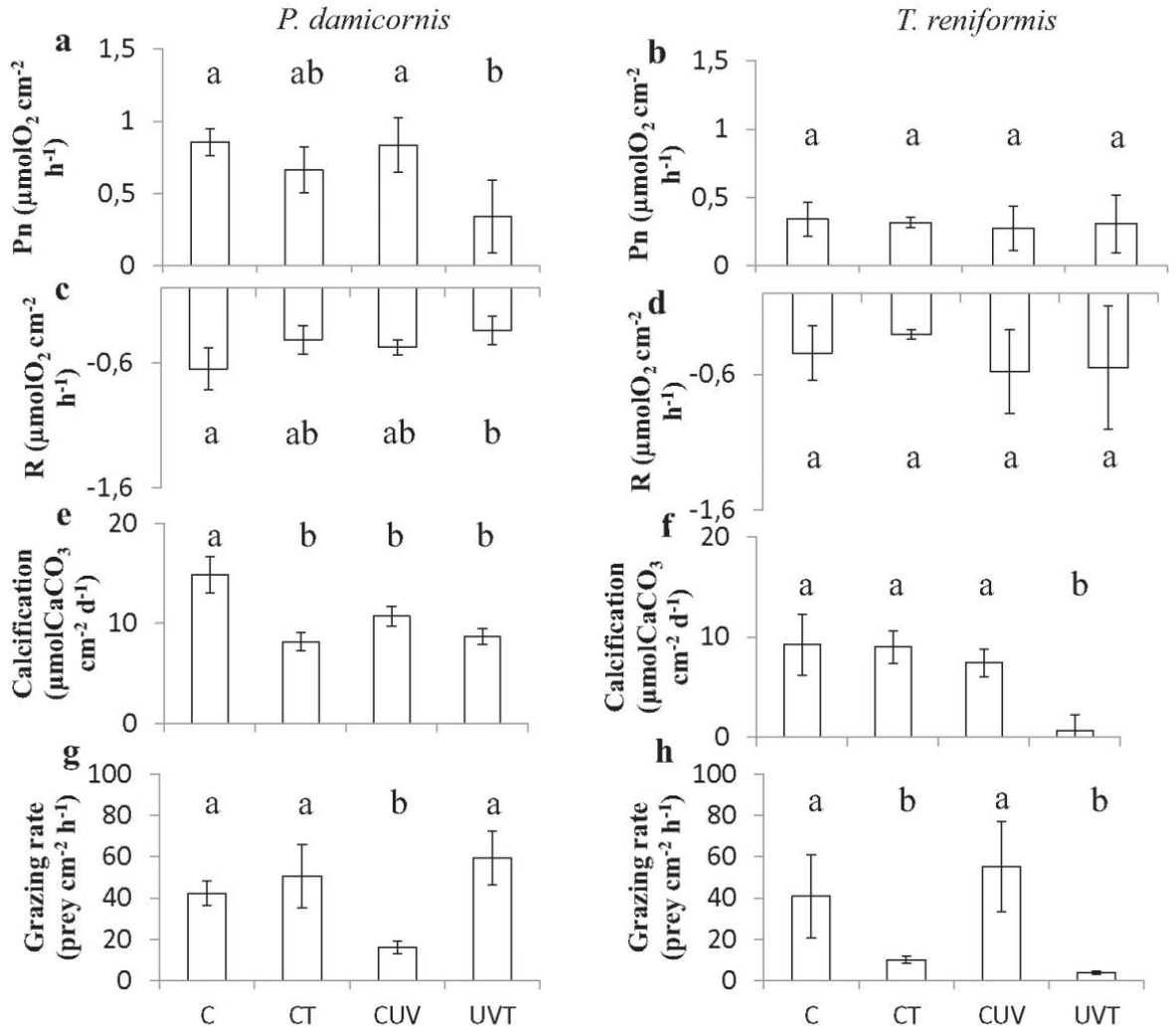


Fig. 4. Net photosynthesis Pn ($\mu molO_2\ cm^{-2}\ h^{-1}$), respiration rate R ($\mu molO_2\ cm^{-2}\ h^{-1}$), calcification rate ($\mu molO_2\ cm^{-2}\ d^{-1}$) and grazing rate (prey $cm^{-2}\ h^{-1}$) of *Pocillopora damicornis* (**a**, **c**, **e**, **g**) and *Turbinaria reniformis* (**b**, **d**, **f**, **h**) in the control condition (C), at 30°C without UVR (CT), at 25°C with UVR (CUV) and at 30°C with UVR (UVT) after 5 weeks of experiment. Error bars represent standard deviations calculated from five replicates except for the grazing rate for which calculations were done from three replicates. Values noted with the same letter are not significantly different (p value > 0.05).

conditions compared with the control (Fig. 2c and Supporting Information Table 2). Temperature (CT, UVT), however, significantly increased the total chlorophyll concentration per surface area (Fig. 3a and Supporting Information Table 3) and triggered a doubling in the diatoxanthin concentration (from 0.042 ± 0.01 to 0.090 ± 0.03 $\mu g\ cm^{-2}$). No effect of temperature or UVR was registered on the PSII maximal photochemical quantum yield of the symbionts that remained constant at 0.64 ± 0.05.

Effect of UVR and temperature on coral net photosynthesis, calcification and grazing rates

The effects of temperature and/or UVR alone were different according to the coral species. In *P. damicornis* there was an additive effect of temperature and UVR stress on the respiration rate per surface area as well as on the net photosynthesis rates per surface area or per symbiont cell, which significantly decreased in the UVT treatment, (Fig. 4a,c Table 2 and Supporting Information 2). Calcification rate, which

Table 2. Total concentrations of chlorophylls (ΣChlorophylls), carotenoids (ΣCarotenoids), and xanthophylls (ΣXanthophylls) expressed per symbiont cell (pg cell^{-1}), net photosynthesis Pn (pmolO_2 cell^{-1} h^{-1}) and respiration rate R (pmolO_2 cell^{-1} h^{-1}) per symbiont cell for the two coral species under the different treatments. Letters refer to values of one species in the different treatments. Values noted with the same letter are not significantly different (p value > 0.05). Values are mean ± standard deviation of three replicates for pigment concentrations and five replicates for Pn and R.

	P. damicornis				*T. reniformis*			
	C	CT	CUV	UVT	C	CT	CUV	UVT
∑Chlorophylls (pg cell^{-1})	2.1 ± 0.8 (a)	3.6 ± 0.4 (a)	3.8 ± 2.8 (a)	3.7 ± 0.5 (a)	4.2 ± 1.9 (a)	5.1 ± 2.2 (a)	6.4 ± 0.5 (a)	5.2 ± 0.2 (a)
∑Carotenoids (pg cell^{-1})	1.8 ± 0.5 (a)	2.08 ± 0.4 (a)	2.6 ± 1.4 (a)	2.6 ± 0.4 (a)	3.6 ± 0.5 (a)	4.5 ± 1.9 (a)	6.5 ± 1.1 (a)	5.2 ± 0.1 (a)
∑Xanthopylls (pg cell^{-1})	0.10 ± 0.07 (a)	0.25 ± 0.07 (a)	0.26 ± 0.2 (a)	0.27 ± 0.04 (a)	0.34 ± 0.1 (a)	0.37 ± 0.2 (a)	0.62 ± 0.1 (a)	0.47 ± 0.03 (a)
Pn (pmolO_2 cell^{-1} h^{-1})	0.39 ± 0.06 (a)	0.37 ± 0.09 (a)	0.44 ± 0.1 (a)	0.21 ± 0.06 (b)	0.36 ± 0.06 (a)	0.44 ± 0.1 (a)	0.47 ± 0.3 (a)	0.54 ± 0.3 (a)
R (nmolO_2 cell^{-1} h^{-1})	−0.29 ± 0.06 (a)	−0.23 ± 0.07 (a)	−0.24 ± 0.01 (a)	−0.16 ± 0.05 (a)	−0.50 ± 0.3 (a)	−0.38 ± 0.2 (a)	−1.0 ± 0.8 (a)	−1.1 ± 0.9 (a)

was positively correlated to the symbiont density ($R^2 = 0.84$), was negatively impacted by UVR, temperature, and both stressors (Fig. 4e and Table Supporting Information 2). However, this rate dropped by 30–40% in all conditions. Grazing rates were five times lower in the CUV condition (Fig. 4g and Supporting Information Table 2), leading to a collapse in the CHAR value in this treatment (from 75% in the control to 35% in the CUV); conversely, CHAR increased at high temperature in the CT and UVT treatments (127 and 181%, respectively), suggesting an antagonistic effect of UV and temperature on this parameter.

T. reniformis was less sensitive than *P. damicornis* to UVR and temperature change. Only two processes were significantly decreased: calcification rate dropped by 70% in the UVT treatment (Fig. 4f, Supporting Information Table 2), suggesting a synergistic effect of thermal and UVR stress, and grazing rates highly decreased at high temperature, with or without UVR (CT and UVT treatments) (Fig. 4h, Supporting Information Table 2). Consequently, CHAR values dropped from more than 96% in the C and CUV conditions to 34 and 6% in the CT and UVT conditions. For *T. reniformis*, no relationship was found between photosynthesis and calcification.

Discussion

The goal of this study was to evaluate simultaneously the effect of two main environmental stressors (UVR and temperature), on the physiological response of scleractinian coral species having two different bleaching sensitivities (Ulstrup et al. 2006). Not only global warming, but also high-energy UVR represents a threat for coral reef organisms (Banaszak and Lesser 2009), and both will increase and act in combination in the coming decades. Ocean warming indeed enhances bathymetric stratification and decreases the depth of the upper mixed layer, causing corals to be exposed to higher visible and UV radiations (Häder et al. 2007*a*,*b*). Results obtained in this study first reveal an overall interactive effect between increased temperature and UVR on the two coral species investigated, *T. reniformis* and *P. damicornis* (Table 3). The combined effect of the two stressors was indeed more deleterious than the individual effects on most physiological parameters. When both stressors were considered independently one from the other, temperature was more deleterious than UVR on coral physiology, especially on photosynthesis. Finally, the coral response to these stresses was species-specific, *P. damicornis* being much more affected than *T. reniformis*.

Long term exposure to UVR alone impacted only *P. damicornis*. Indeed, *T. reniformis* nubbins showed a transient decrease in their photosynthetic pigment content after 2 weeks of UV exposure but it returned to normal afterwards. Conversely, in *P. damicornis*, UVR induced significant bleaching, with both a loss in symbionts and pigments. Such a

Table 3. Summary of the effects of temperature (T), ultraviolet radiations (UV) and the combination of both stressors (UV+T) on *Pocillopora damicornis* and *Turbinaria reniformis*. + means positive effect: an increase of the value compared to the control condition, – means negative effect: a decrease of the value compared with the control condition and = means no effects, the final value is not different from the control condition. Pale grey indicates significant effects of temperature alone; dark grey indicates effects of UVR alone. In brackets is indicated whether the effects are additive (no interaction of UV and T in the ANOVA's results), synergistic or antagonistic (significant interaction between UV and T in the ANOVA's results).

Species	Environemental factor	Symbiont density	Protein concentration	Σ (Chlorophylls)	Σ (Carotenids)	Σ (Xanthophylls)	Pn	R	Calcification rate	Grazing rate
P. damicornis	T	–	–	+	=	=	=	=	–	=
	UV	–	–	=	=	=	=	=	–	–
	UV+T	–	–	+	=	=	–	–	–	=
		(additive)	(synergistic)				(additive)	(additive)	(synergistic)	(antagonistic)
T. reniformis	T	=	=	=	=	=	=	=	=	–
	UV	=	=	=	=	=	=	=	=	=
	UV+T	=	=	=	=	=	=	=	–	–
									(synergistic)	

different response between the two coral species might be due to differences in (1) tissue thickness and morphology, with, for example, twice more proteins in *T. reniformis*; (2) symbiont density, which was two to three times higher in *P. damicornis* than in *T. reniformis*, previous work indeed showed that coral species having a high symbiont density are more sensitive to bleaching (Cunning and Baker 2012), or (3) pigment content of the symbiont cells, with higher carotenoid levels (40 times more dinoxanthin) in *T. reniformis*. The antioxidant role of carotenoids was indeed demonstrated in higher plants, algae and marine animals (Frank et al. 1996; Lesser 2006). As both corals host the same symbiont genotype (Godinot et al. 2013; Ezzat et al. 2015), the different cellular pigment concentrations between *T. reniformis* and *P. damicornis* can either be due to different expressions of genes involved in pigment synthesis (Baumgarten et al. 2013) or to a modulation of pigment composition by the host (Ziegler et al. 2015). Symbionts indeed experience a different light environment depending on their location inside the host tissue (Wangpraseurt et al. 2014), or depending on the host tissue thickness and pigmentation (Dimond et al. 2012; Krämer et al. 2013). UVR also reduced by half zooplankton grazing rates by *P. damicornis*, lowering the CHAR from 75% under control conditions to 35%. This likely resulted in a 30% decrease in the protein content as well as in the calcification rates. This observation suggests that despite its higher symbiont density and higher photosynthetic rates compared to *T. reniformis*, *P. damicornis* might have strongly relied on heterotrophy, and its supply of essential molecules such as nitrogen and phosphorus (Houlbrèque and Ferrier-Pagès 2009) to maintain a maximal tissue and skeletal growth.

Thermal stress alone also induced significant species-specific changes in the corals' physiology, but differently compared with UVR stress. This is in line with previous studies showing different sensitivities of phytoplankton or corals to UVR and temperature (Halac et al. 2013). In *T. reniformis*, thermal stress alone decreased zooplankton grazing rates, most likely due to polyp retraction (L. C., personal observation), which is known to depend on environmental factors such as light, flow, and temperature (Pacherres et al. 2013). However, this significant reduction in heterotrophic nutrient acquisition (CHAR dropped from 96% to 34%) did not affect the short-term calcification and photosynthesis of *T. reniformis*, which remained equivalent to control condition. This suggests that nubbins had sufficient energetic reserves to overcome the thermal stress for some weeks, although there was a tendency (not significant) of declining protein content. Compared with *T. reniformis*, metabolism in *P. damicornis* was more impacted by thermal stress, which decreased symbiont density, as previously observed (Al-Sofyani and Floos 2013; Hill et al. 2014), and likely due to the same reasons as previously discussed for UVR. As a consequence of bleaching, Pn tended to be lowered compared to control

conditions, and this lack of autotrophic energy supply may be responsible for the reduction in respiration, calcification, and protein content, although protein decrease could have been due to heat-degradation of host tissue (Fransolet et al. 2014). Overall, *P. damicornis*'s calcification seems to be affected by both a decrease in autotrophic (under thermal stress) and heterotrophic (under UVR) supply, confirming that this process is energy-demanding.

For both coral species and most physiological factors, the combination of high UVR level and high temperature worsened the effects of individual stresses. *P. damicornis*'s bleaching was exacerbated and involved a larger decrease in photosynthesis and protein content of the tissue, indicating that this species was affected both in its energetic reserves and composition by the combined stressors. For *T. reniformis*, combined stresses led to a strong decrease in the calcification rate that was not observed under single-stress conditions. The double stress might have induced higher energetic costs for cellular repair, reducing the energy available for calcification. This drop in calcification occurred simultaneously with a low grazing rate and despite the fact that photosynthetic rates remained as high as in the control condition, suggesting that in stressed colonies of *T. reniformis* heterotrophy is needed to maintain a high calcification rate. In addition, the fact that respiration rates remained unchanged suggests that energy is used for other processes than calcification, such as defense mechanisms. Those results are consistent with previous studies demonstrating that when temperature increase is coupled to another stress such as high irradiance, the oxidative stress in cells is higher (Lesser et al. 1990*a*,*b*; Roberty et al. 2015), the corals' capacity to synthesize UV-protective MAAs is decreased (Fitt 1995) and therefore, the ability for corals to recover from a bleaching event decreases (D'Croz and Maté, 2002).

The results of this study also highlight strong species-specific differences in energy allocation and stress response despite the fact that both species harboured the same symbiont clade and experienced the same recent thermal history. In previous work, *P. damicornis* was already found to be more sensitive to thermal stress than *T. reniformis* (Ulstrup et al. 2006), and the genus *Turbinaria* is generally considered as one of the least susceptible taxa during natural bleaching events or environmental stress (Marshall and Baird 2000; Floros et al. 2004; Ulstrup et al. 2006; Schoepf et al. 2013). As the resistance of a specific coral to environmental stress can be due either to adaptation (genotypic traits that have evolved through natural selection) or to acclimation (environmentally inducible, and reversible phenotypic traits), our observations and previous ones suggest that the higher resistance of *T. reniformis* is likely linked to the long genetic distance between the two species and therefore due to an adaptation. However, to further investigate whether *T. reniformis* resistance is adaptive or acclimatory, the next step would be to compare the response to environmental stressors of other closely related species from the same or a sister family (Dendrophyllidae or Poritidae) also belonging to the "complex" clade.

The number of studies focusing on the UVR effects on reef corals has decreased in the last 10 yr because of stratospheric ozone restoration. However, recent studies tend to show that despite this restoration, UVB radiation will increase in tropics (Watanabe et al. 2011; Meul et al. 2016). Increasing our understanding of the interactive effects of UVR and elevated temperature is thus crucial to anticipate changes in the growth and productivity of reef corals, and species composition on coral reefs, by the end of this century. At the present time, published data are still insufficient to achieve these goals. Simplified experimental approaches such as the one developed in this study contribute to increase our knowledge on global change effects on marine systems and are essential to test and improve existing theories as well as to identify the causes of present bleaching events and reef deterioration.

References

Al-Sofyani, A. A., and Y. A. M. Floos. 2013. Effect of temperature on two reef-building corals *Pocillopora damicornis* and *P. verrucosa* in the Red Sea. Oceanologia **55**: 917–935. doi: 10.5697/oc.55-4.917

Banaszak, A. T., and M. P. Lesser. 2009. Effects of solar ultraviolet radiation on coral reef organisms. Photochem. Photobiol. Sci. **8**: 1276. doi:10.1039/b902763g

Baumgarten, S., T. Bayer, M. Aranda, Y. Liew, A. Carr, G. Micklem, and C. R. Voolstra. 2013. Integrating microRNA and mRNA expression profiling in *Symbiodinium microadriaticum*, a dinoflagellate symbiont of reef-building corals. BMC Genomics **14**: 704–721. doi:10.1186/1471-2164-14-704

Bouchard, R. W., M. A. Carrillo, S. A. Kells, and L. C. Ferrington. 2006. Freeze tolerance in larvae of the winter-active *Diamesa mendotae* Muttkowski (Diptera: Chironomidae): A contrast to adult strategy for survival at low temperatures. Hydrobiologia **568**: 403–416. doi:10.1007/s10750-006-0200-6

Butchart, N., and others. 2010. Chemistry-climate model simulations of twenty-first century stratospheric climate and circulation changes. J. Clim. **23**: 5349–5374. doi: 10.1175/2010JCLI3404.1

Carrillo, P., and others. 2015. Interactive effect of UVR and phosphorus on the coastal phytoplankton community of the Western Mediterranean Sea: Unravelling Eco-Physiological Mechanisms. PLoS One **10**: e0142987. doi: 10.1371/journal.pone.0142987

Chen, C. A., C. C. Wallace, and J. Wolstenholme. 2002. Analysis of the mitochondrial 12S rRNA gene supports a two-clade hypothesis of the evolutionary history of scleractinian corals. Mol. Phylogenet. Evol. **23**: 137–149. doi: 10.1016/S1055-7903(02)00008-8

Collins, M., and others. 2013. Long-term climate change: Projections, commitments and irreversibility pages 1029 to 1076, p. 1029–1136. *In* Intergovernmental Panel on Climate Change [ed.], Climate Change 2013 - The physical science basis. Cambridge Univ. Press.

Cruces, E., P. Huovinen, and I. Gómez. 2013. Interactive effects of UV radiation and enhanced temperature on photosynthesis, phlorotannin induction and antioxidant activities of two sub-Antarctic brown algae. Mar. Biol. **160**: 1–13. doi:10.1007/s00227-012-2049-8

Cunning, R., and A. C. Baker. 2012. Excess algal symbionts increase the susceptibility of reef corals to bleaching. Nat. Clim. Change **3**: 259–262. doi:10.1038/nclimate1711

D'Croz, L., J. L. Maté, and J. E. Oke. 2001. Responses to elevated sea water temperature and UV radiation in the coral *Porites lobata* from upwelling and non-upwelling environments on the Pacific coast of Panama. Bull. Mar. Sci. **69**: 203–214.

D'Croz, L., and J. L. Maté. 2002. The role of water temperature and UV radiation in the recovery of the experimentally bleached coral *Pocillopora damicornis* from the eastern Pacific Ocean (Panama), p. 1111–1116. Proceedings of the ninth international coral reef symposium, Bali, 23–27 October 2000.

Dimond, J. L., B. J. Holzman, and B. L. Bingham. 2012. Thicker host tissues moderate light stress in a cnidarian endosymbiont. J. Exp. Biol. **215**: 2247–2254. doi:10.1242/jeb.067991

Doney, S. C., and others. 2012. Climate change impacts on marine ecosystems. Ann. Rev. Mar. Sci. **4**: 11–37. doi:10.1146/annurev-marine-041911-111611

Dunne, R. P. 2010. Synergy or antagonism-interactions between stressors on coral reefs. Coral Reefs **29**: 145–152. doi:10.1007/s00338-009-0569-6

Eyring, V., and others. 2010*a*. Multi-model assessment of stratospheric ozone return dates and ozone recovery in CCMVal-2 models. Atmos. Chem. Phys. **10**: 9451–9472. doi:10.5194/acp-10-9451-2010

Eyring, V., and others. 2010*b*. Sensitivity of 21st century stratospheric ozone to greenhouse gas scenarios. Geophys. Res. Lett. **37**: 807–813. doi:10.1029/2010GL044443

Ezzat, L., J. F. Maguer, R. Grover, and C. Ferrier-Pagès. 2015. New insights into carbon acquisition and exchanges within the coral–dinoflagellate symbiosis under NH4+ and NO3− supply. Proc. R. Soc. B: Biol. Sci. **282**: 20150610. doi:10.1098/rspb.2015.0610

Ferrier-Pagès, C., C. Richard, D. Forcioli, D. Allemand, M. Pichon, and J. M. Shick. 2007. Effects of temperature and UV radiation increases on the photosynthetic efficiency in four scleractinian coral species. Biol. Bull. **213**: 76–87. doi:10.2307/25066620

Ferrier-Pagès, C., C. Rottier, E. Beraud, and O. Levy. 2010. Experimental assessment of the feeding effort of three scleractinian coral species during a thermal stress: Effect on the rates of photosynthesis. J. Exp. Mar. Bio. Ecol. **390**: 118–124. doi:10.1016/j.jembe.2010.05.007

Fitt, W. K., and M. E. Warner. 1995. Bleaching patterns of four species of Caribbean reef corals. Biol. Bull. **189**: 298–307. doi:10.2307/1542147

Floros, C. D., M. J. Samways, and B. Armstrong. 2004. Taxonomic patterns of bleaching within a South African coral assemblage. Biodivers. Conserv. **13**: 1175–1194. doi:10.1023/B:BIOC.0000018151.67412.c7

Frank, H. A., A. Cua, V. Chynwat, A. Young, D. Gosztola, and M. R. Wasielewski. 1996. The lifetimes and energies of the first excited singlet states of diadinoxanthin and diatoxanthin: The role of these molecules in excess energy dissipation in algae. Biochim. Biophys. Acta - Bioenerg. **1277**: 243–252. doi:10.1016/S0005-2728(96)00106-5

Fransolet, D., S. Roberty, and J. C. Plumier. 2014. Impairment of symbiont photosynthesis increases host cell proliferation in the epidermis of the sea anemone *Aiptasia pallida*. Mar. Biol. **161**: 1735–1743. doi:10.1007/s00227-014-2455-1

Gattuso, J. P., and J. Jaubert. 1990. Effect of light on oxygen and carbon dioxide fluxes and on metabolic quotients measured in situ in a zooxanthellate coral. Limnol. Oceanogr. **35**: 1796–1804. doi:10.4319/lo.1990.35.8.1796

Gleason, D. F. 2001. Ultraviolet radiation and coral communities, p. 118–149. *In* C. Cockell and A. Blaustein [eds.], Ecosystems, evolution, and ultraviolet radiation. Springer, New York.

Glynn, P. W. 1993. Coral reef bleaching: Ecological perspectives. Coral Reefs **12**: 1–17. doi:10.1007/BF00303779

Godinot, C., C. Ferrier-Pagès, S. Sikorski, and R. Grover. 2013. Alkaline phosphatase activity of reef-building corals. Limnol. Oceanogr. **58**: 227–234. doi:10.4319/lo.2013.58.1.0227

Häder, D. P., and R. P. Sinha. 2005. Solar ultraviolet radiation-induced DNA damage in aquatic organisms: Potential environmental impact. Mutat. Res. Mol. Mech. Mutagen. **571**: 221–233. doi:10.1016/j.mrfmmm.2004.11.017

Häder, D. P., H. D. Kumar, R. C. Smith, and R. C. Worrest. 2007. Effects of solar UV radiation on aquatic ecosystems and interactions with climate change. Photochem. Photobiol. Sci. **6**: 267–285. doi:10.1039/b700020k

Halac, S. R., V. E. Villafañe, and E. W. Helbling. 2010. Temperature benefits the photosynthetic performance of the diatoms *Chaetoceros gracilis* and *Thalassiosira weissflogii* when exposed to UVR. J. Photochem. Photobiol. B Biol. **101**: 196–205. doi:10.1016/j.jphotobiol.2010.07.003

Halac, S. R., S. D. Guendulain-García, V. E. Villafañe, E. W. Helbling, and A. T. Banaszak. 2013. Responses of tropical plankton communities from the Mexican Caribbean to solar ultraviolet radiation exposure and increased temperature. J. Exp. Mar. Bio. Ecol. **445**: 99–107. doi:10.1016/j.jembe.2013.04.011

Hill, R., M. Szabo, A. Ur Rehman, I. Vass, P. J. Ralph, and A. W. D. Larkum. 2014. Inhibition of photosynthetic CO2 fixation in the coral *Pocillopora damicornis* and its relationship to thermal bleaching. J. Exp. Biol. **217**: 2150–2162. doi:10.1242/jeb.100578

Hoegh-Guldberg, O. 1999. Climate change, coral bleaching and the future of the world's coral reefs. Mar. Freshw. Res. **50**: 839. doi:10.1071/MF99078

Hoegh-Guldberg, O., and R. Jones. 1999. Photoinhibition and photoprotection in symbiotic dinoflagellates from reef-building corals. Mar. Ecol. Prog. Ser. **183**: 73–86. doi:10.3354/meps183073

Hoegh-Guldberg, O., and others. 2007. Coral reefs under rapid climate change and ocean acidification. Science **318**: 1737–1742. doi:10.1126/science.1152509

Hoogenboom, M., E. Beraud, and C. Ferrier-Pagès. 2010. Relationship between symbiont density and photosynthetic carbon acquisition in the temperate coral *Cladocora caespitosa*. Coral Reefs **29**: 21–29. doi:10.1007/s00338-009-0558-9

Houlbrèque, F., and C. Ferrier-Pagès. 2009. Heterotrophy in tropical scleractinian corals. Biol. Rev. **84**: 1–17. doi:10.1111/j.1469-185X.2008.00058.x

Jeffrey, S. W., and G. F. Humphrey. 1975. New spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae and natural phytoplankton. Biochem. Physiol. Pflanz. **167**: 191–194. doi:10.1016/0022-2860(75)85046-0

Jokiel, P. L. 2004. Temperature stress and coral bleaching, p. 401–425. *In* E. Rosenberg and Y. Loya [eds.], Coral health and disease. Springer.

Krämer, W. E., V. Schrameyer, R. Hill, P. J. Ralph, and K. Bischof. 2013. PSII activity and pigment dynamics of *Symbiodinium* in two Indo-Pacific corals exposed to short-term high-light stress. Mar. Biol. **160**: 563–577. doi:10.1007/s00227-012-2113-4

Lesser, M. P. 1996. Elevated temperatures and ultraviolet radiation cause oxidative stress and inhibit photosynthesis in symbiotic dinoflagellates. Limnol. Oceanogr. **41**: 271–283. doi:10.4319/lo.1996.41.2.0271

Lesser, M. P. 2006. Oxidative stress in marine environments: Biochemistry and physiological ecology. Annu. Rev. Physiol. **68**: 253–278. doi:10.1146/annurev.physiol.68.040104.110001

Lesser, M. P., W. R. Stochaj, D. W. Tapley, and J. M. Shick. 1990*a*. Bleaching in coral reef anthozoans: Effects of irradiance, ultraviolet radiation, and temperature on the activities of protective enzymes against active oxygen. Coral Reefs **8**: 225–232. doi:10.1007/BF00265015

Lesser, M. P., W. R. Stochaj, D. W. Tapley, and J. M. Shick. 1990*b*. Coral Reefs effects of irradiance, ultraviolet radiation, and temperature. Coral Reefs **8**: 225–232. doi:10.1007/BF00265015

Lesser, M., and J. Farrell. 2004. Exposure to solar radiation increases damage to both host tissues and algal symbionts of corals during thermal stress. Coral Reefs **23**: 367–377. doi:10.1007/s00338-004-0392-z

Leu, E., S. Falk-Petersen, and D. O. Hessen. 2007. Ultraviolet radiation negatively affects growth but not food quality of arctic diatoms. Limnol. Oceanogr. **52**: 787–797. doi:10.4319/lo.2007.52.2.0787

Loya, Y., K. Sakai, K. Yamazato, Y. Nakano, H. Sambali, and R. van Woesik. 2001. Coral bleaching: the winners and the losers. Ecol. Lett. **4**: 122–131. doi:10.1046/j.1461-0248.2001.00203.x

Madronich, S. 1993. The atmosphere and UV-B radiation at ground level, p. 1–39. *In* Environmental UV photobiology. Springer.

Madronich, S., R. L. McKenzie, L. O. Björn, and M. M. Caldwell. 1998. Changes in biologically active ultraviolet radiation reaching the Earth's surface. J. Photochem. Photobiol. B Biol. **46**: 5–19. doi:10.1016/S1011-1344(98)00182-1

Marshall, P. A., and A. H. Baird. 2000. Bleaching of corals on the Great Barrier Reef: differential susceptibilities among taxa. Coral Reefs **19**: 155–163. doi:10.1007/s003380000086

Meul, S., M. Dameris, U. Langematz, J. Abalichin, and A. Kubin. 2016. Impact of rising greenhouse gas concentrations on future tropical ozone and UV exposure. Geophys. Res. Lett. **43**: 2–10. doi:10.1002/2016GL067997

Muscatine, L. 1990. The role of symbiotic algae in carbon and energy flux in reef corals. Ecosyst. World **25**: 75–87.

Muscatine, L., L. R. McCloskey, and R. E. Marian. 1981. Estimating the daily contribution of carbon from zooxanthellae to coral animal respiration. Limnol. Oceanogr. **26**: 601–611. doi:10.4319/lo.1981.26.4.0601

Pacherres, C. O., G. M. Schmidt, and C. Richter. 2013. Autotrophic and heterotrophic responses of the coral *Porites lutea* to large amplitude internal waves. J. Exp. Biol. **216**: 4365–4374. doi:10.1242/jeb.085548

Roberty, S., D. Fransolet, P. Cardol, J. C. Plumier, and F. Franck. 2015. Imbalance between oxygen photoreduction and antioxidant capacities in *Symbiodinium* cells exposed to combined heat and high light stress. Coral Reefs **34**: 1063–1073. doi:10.1007/s00338-015-1328-5

Rogers, J. E., D. T. Marcovich, S. J. Jordan, and S. N. Gallaher. 2010. Does temperature and UV exposure history modulate the effects of temperature and UV stress on *Symbiodinium* growth rates? Bull. Mar. Sci. **86**: 743–761.

Romano, S. L., and S. R. Palumbi. 1996. Evolution of scleractinian corals inferred from molecular systematics. Science **271**: 640–642. doi:10.1126/science.271.5249.640

Rosic, N. N., and S. Dove. 2011. Mycosporine-like amino acids from coral dinoflagellates. Appl. Environ. Microbiol. **77**: 8478–8486. doi:10.1128/AEM.05870-11

Schoepf, V., and others. 2013. Coral energy reserves and calcification in a high-CO_2 world at two temperatures. PLoS One **8**: e75049. doi:10.1371/journal.pone.0075049

Sharma, P., A. B. Jha, R. S. Dubey, and M. Pessarakli. 2012. Reactive oxygen species, oxidative damage, and antioxidative

defense mechanism in plants under stressful conditions. J. Bot. **2012**: 1–26. doi:10.1155/2012/217037

Shick, J., M. P. Lesser, and P. L. Jokiel. 1996. Effects of ultraviolet radiation on corals and other coral reef organisms. Glob. Chang. Biol. **2**: 527–545. doi:10.1111/j.1365-2486.1996.tb00065.x

Shick, J. M., and W. C. Dunlap. 2002. Mycosporin-like amino acids and related gadusols: Biosynthesis, accumulation, and UV-protective functions in aquatic organisms. Annu. Rev. Physiol. **64**: 223–262. doi:10.1146/annurev.physiol.64.081501.155802

Smith, P. K., and others. 1985. Measurement of protein using bicinchoninic acid. Anal. Biochem. **150**: 76–85. doi:10.1016/0003-2697(85)90442-7

Smith, S. V., and G. S. Key. 1975. Carbon dioxide and metabolism in marine environments. Limnol. Oceanogr. **20**: 493–495. doi:10.4319/lo.1975.20.3.0493

Sobrino, C., and P. J. Neale. 2007. Short-term and long-term effects of temperature on photosynthesis in the diatom *Thalassiosira pseudinana* under UVR exposures. J. Phycol. **43**: 426–436. doi:10.1111/j.1529-8817.2007.00344.x

Sorokin, Y. I. 2013. Coral reef ecology. Springer Science & Business Media.

Stimson, J., and R. A. Kinzie. 1991. The temporal pattern and rate of release of zooxanthellae from the reef coral *Pocillopora damicornis* (Linnaeus) under nitrogen-enrichment and control conditions. J. Exp. Mar. Bio. Ecol. **153**: 63–74. doi:10.1016/S0022-0981(05)80006-1

Torregiani, J. H., and M. P. Lesser. 2007. The effects of short-term exposures to ultraviolet radiation in the Hawaiian Coral *Montipora verrucosa*. J. Exp. Mar. Bio. Ecol. **340**: 194–203. doi:10.1016/j.jembe.2006.09.004

Torres-Pérez, J. L., and R. A. Armstrong. 2012. Effects of UV radiation on the growth, photosynthetic and photoprotective components, and reproduction of the Caribbean shallow-water coral *Porites furcata*. Coral Reefs **31**: 1077–1091. doi:10.1007/s00338-012-0927-7

Tremblay, P., A. Peirano, and C. Ferrier-Pagès. 2011. Heterotrophy in the Mediterranean symbiotic coral *Cladocora caespitosa*: Comparison with two other scleractinian species. Mar. Ecol. Prog. Ser. **422**: 165–177. doi:10.3354/meps08902

Ulstrup, K. E., R. Berkelmans, P. J. Ralph, and M. J. H. Van Oppen. 2006. Variation in bleaching sensitivity of two coral species across a latitudinal gradient on the Great Barrier Reef: The role of zooxanthellae. Mar. Ecol. Prog. Ser. **314**: 135–148. doi:10.3354/meps314135

Veron, J. V. 2000. Corals of the world. Australian Institute of Marine Science.

Wangpraseurt, D., L. Polerecky, A. W. D. Larkum, P. J. Ralph, D. A. Nielsen, M. Pernice, and M. Kühl. 2014. The in situ light microenvironment of corals. Limnol. Oceanogr. **59**: 917–926. doi:10.4319/lo.2014.59.3.0917

Watanabe, S., K. Sudo, T. Nagashima, T. Takemura, H. Kawase, and T. Nozawa. 2011. Future projections of surface UV-B in a changing climate. J. Geophys. Res. Atmos. **116**: D16118. doi:10.1029/2011JD015749

Williamson, C. E., and others. 2014. Solar ultraviolet radiation in a changing climate. Nat. Clim. Change **4**: 434–441. doi:10.1038/nclimate2225

Ziegler, M., C. M. Roder, C. Büchel, and C. R. Voolstra. 2015. Mesophotic coral depth acclimatization is a function of host-specific symbiont physiology. Front. Mar. Sci. **2**: 4. doi:10.3389/fmars.2015.00004

Conflict of Interest

None declared.

Submitted 04 May 2016
Revised 05 September 2016
Accepted 25 October 2016

Associate editor: Rebecca Vega Thurber

Table s1. Results of one-way ANOVA testing the difference between species in the control condition for all variables. * denotes a significant p-value lower than 0.05, ** denotes a significant p-value lower than 0.005, - denotes a non-significant p-value.

	Df	F-value	p
Symbiont density (cm^{-2})	1	209	**
Protein content ($mg\ cm^{-2}$)	1	30,45	**
F_V/F_M	1	16,79	**
Respiration rate ($\mu molO_2\ cm^{-2}\ h^{-1}$)	1	3,16	-
Net photosynthesis ($\mu molO_2\ cm^{-2}\ h^{-1}$)	1	61,71	**
Calcification rate ($\mu molCaCO_3\ cm^{-2}\ d^{-1}$)	1	7,824	**
Net photosynthesis per symbiont cell ($\mu molO_2\ cell^{-1}\ h^{-1}$)	1	0,764	-
Respiration rate per symbiont cell ($\mu molO_2\ cell^{-1}\ h^{-1}$)	1	2,834	-
Grazing rate (prey $cm^{-2}\ h^{-1}$)	1	0,245	-
∑Chlorophylls ($\mu g\ cm^{-2}$)	1	16,23	**
∑Carotenoids ($\mu g\ cm^{-2}$)	1	25,18	**
∑Xanthophylls ($\mu g\ cm^{-2}$)	1	5,378	*
Dinoxanthin ($\mu g\ cm^{-2}$)	1	42,45	**
∑Chlorophylls ($pg\ cell^{-1}$)	1	14,71	**
∑Carotenoids ($pg\ cell^{-1}$)	1	48,77	**
∑Xanthophylls ($pg\ cell^{-1}$)	1	26,64	**
Dinoxanthin ($pg\ cell^{-1}$)	1	47,92	**

Table s2. Results of two-factor ANOVA testing, for each species, the effect of UVR and temperature on all variables except pigment concentrations. * denotes a significant p-value lower than 0.05, ** denotes a significant p-value lower than 0.005, - denotes a non-significant p-value.

			P. damicornis		*T. reniformis*	
	Condition	Df	F-value	p	F-value	p
Symbiont density (cm^{-2})	UV	1	21,877	**	0,7	-
	T	1	25,219	**	10,011	-
	UV:T	1	1,296	-	0,017	-
Protein content ($mg\ cm^{-2}$)	UV	1	33,84	**	1,534	-
	T	1	57,53	**	0,593	-
	UV:T	1	10,74	**	2,16	-
F_V/F_M	UV	1	9,31	*	1	-
	T	1	0,287	-	1,005	-
	UV:T	1	0,79	-	0,998	-
Respiration rate ($\mu molO_2\ cm^{-2}\ h^{-1}$)	UV	1	3,415	-	3,087	-
	T	1	8,104	*	0,053	-
	UV:T	1	1,274	-	0,368	-
Net photosynthesis ($\mu molO_2\ cm^{-2}\ h^{-1}$)	UV	1	3,006	*	2,473	-
	T	1	10,043	**	0,024	-
	UV:T	1	3,351	-	0,015	-
Calcification rate ($\mu molCaCO_3\ cm^{-2}\ d^{-1}$)	UV	1	7,22	*	12,58	**
	T	1	24,073	**	11,18	*
	UV:T	1	9,385	*	11,03	*
Net photosynthesis per symbiont cell ($\mu molO_2\ cell^{-1}\ h^{-1}$)	UV	1	1,83	-	2,147	-
	T	1	7,212	*	0,482	-
	UV:T	1	5,436	*	0,011	-
Respiration rate per symbiont cell ($\mu molO_2\ cell^{-1}\ h^{-1}$)	UV	1	0,995	-	3,704	-
	T	1	0,501	-	3,707	-
	UV:T	1	2,033	-	3,704	-
Grazing rate ($prey\ cm^{-2}\ h^{-1}$)	UV	1	15,76	*	0,132	-
	T	1	10,73	*	39,642	**
	UV:T	1	13,87	*	0,379	-
CZAR	UV	1	0,813	-	15,307	*
	T	1	0,076	-	0,202	-
	UV:T	1	1,53	-	1,903	-
CHAR	UV	1	4,251	-	0,100	-
	T	1	2,331	-	12,323	*
	UV:T	1	0,914	-	1,703	-

Table s3. Results of two-factor ANOVA testing, for each species, the effect of UVR and temperature on pigment concentrations. * denotes a significant p-value inferior to 0.05, ** denotes a significant p-value inferior to 0.005, - denotes a non-significant p-value.

			P. damicornis		*T. reniformis*	
Variables	Conditions	Df	F-value	p	F-value	p
Diatoxanthin ($\mu g\ cm^{-2}$)	UV	1	2,574	-	0,005	-
	T	1	19,075	**	0,004	-
	UV:T	1	0,298	-	0,307	-
∑Chlorophylls ($\mu g\ cm^{-2}$)	UV	1	3,747	-	0,153	-
	T	1	27,401	**	3,71	-
	UV:T	1	3,337	-	0,004	-
∑Carotenoids ($\mu g\ cm^{-2}$)	UV	1	0,315	-	2,126	-
	T	1	4,625	-	3,177	-
	UV:T	1	0,025	-	0,06	-
∑Xanthophylls ($\mu g\ cm^{-2}$)	UV	1	1,963	-	0,629	-
	T	1	4,538	-	2,905	-
	UV:T	1	0,025	-	0,001	-
Diatoxanthin per symbiont cell ($pg\ cell^{-1}$)	UV	1	0,331	-	0,989	-
	T	1	0,006	-	0,183	-
	UV:T	1	0,067	-	3,98	-
∑Chlorophylls per symbiont cell ($pg\ cell^{-1}$)	UV	1	0,371	-	0,061	-
	T	1	1,406	-	0,565	-
	UV:T	1	0,197	-	0,004	-
∑Carotenoids per symbiont cell ($pg\ cell^{-1}$)	UV	1	0,398	-	1,23	-
	T	1	0,258	-	0,129	-
	UV:T	1	0,306	-	2,773	-
∑Xanthophylls per symbiont cell ($pg\ cell^{-1}$)	UV	1	0,322	-	0,11	-
	T	1	1,447	-	0,173	-
	UV:T	1	0,001	-	1,386	-

Table s4. Results of the Kruskal-Wallis test on pigment kinetics for *Turbinaria reniformis* under UVR at 25 °C. For all other conditions and for PO, there was no significant difference and results are not shown. * denotes a significant p-value inferior to 0.05, ** denotes a significant p-value inferior to 0.005, - denotes a non-significant p-value.

			P. damicornis		*T. reniformis*	
variables	condition	Df	F-value	p	F-value	p
Peridinin (µg cm^{-2})	CUV	1	2,315	-	5,002	*
∑Chlorophylls (µg cm^{-2})	CUV	1	1,112	-	3,738	*
∑Carotenoids (µg cm^{-2})	CUV	1	2,318	-	4,971	*
∑Xanthophylls (µg cm^{-2})	CUV	1	1,031	-	4,096	*
Peridinin per symbiont cell (pg $cell^{-1}$)	CUV	1	0,482	-	5,732	*
∑Chlorophylls per symbiont cell (pg $cell^{-1}$)	CUV	1	0,557	-	5,379	*
∑Carotenoids per symbiont cell (pg $cell^{-1}$)	CUV	1	0,568	-	5,758	*
∑Xanthophylls per symbiont cell (pg $cell^{-1}$)	CUV	1	0,544	-	3,799	*

Paper n°3 –Biology Open

© 2017. Published by The Company of Biologists Ltd | Biology Open (2017) 6, 1190-1199 doi:10.1242/bio.026757

RESEARCH ARTICLE

Effects of temperature and UVR on organic matter fluxes and the metabolic activity of *Acropora muricata*

Lucile Courtial[1,2,3,‡], Christine Ferrier-Pagès[2,*,‡], Stéphan Jacquet[4], Riccardo Rodolfo-Metalpa[3], Stéphanie Reynaud[2], Cécile Rottier[2] and Fanny Houlbrèque[3,*]

ABSTRACT

Coral bleaching events are predicted to occur more frequently in the coming decades with global warming. The susceptibility of corals to bleaching during thermal stress episodes depends on many factors, including the magnitude of thermal stress and irradiance. The interactions among these two factors, and in particular with ultra-violet radiation (UVR), the most harmful component of light, are more complex than assumed, and are not yet well understood. This paper explores the individual and combined effects of temperature and UVR on the metabolism of *Acropora muricata*, one of the most abundant coral species worldwide. Particulate and dissolved organic matter (POM/DOM) fluxes and organic matter (OM) degradation by the mucus-associated bacteria were also monitored in all conditions. The results show that UVR exposure exacerbated the temperature-induced bleaching, but did not affect OM fluxes, which were only altered by seawater warming. Temperature increase induced a shift from POM release and DOM uptake in healthy corals to POM uptake and DOM release in stressed ones. POM uptake was linked to a significant grazing of pico- and nanoplankton particles during the incubation, to fulfil the energetic requirements of *A. muricata* in the absence of autotrophy. Finally, OM degradation by mucus-associated bacterial activity was unaffected by UVR exposure, but significantly increased under high temperature. Altogether, our results demonstrate that seawater warming and UVR not only affect coral physiology, but also the way corals interact with the surrounding seawater, with potential consequences for coral reef biogeochemical cycles and food webs.

KEY WORDS: *Acropora muricata*, Thermal stress, UV radiation, Climate change, Organic matter fluxes, Microbial loop

INTRODUCTION

Tropical marine ecosystems, including coral reefs, harbor more than 30% of the marine biodiversity (Doney et al., 2012), and provide goods and services to almost one billion people every year (Moberg and Folke, 1999; Wilkinson et al., 1999). However, they are currently threatened by climate change-induced increase in sea surface temperature (Nicholls et al., 2007), and in the incident flux of ultra-violet radiation (UVR, 280-400 nm) (Häder et al., 2007). This later increase is due to the effects of global warming on the stratospheric circulation and to a greater water stratification (Watanabe et al., 2011), leading to a deeper penetration of UVR in the water column (Vodacek et al., 1997).

The effects of rising sea surface temperature on coral physiology have already been well studied. Since most corals live at or near their threshold of temperature tolerance (Hoegh-Guldberg, 1999), thermal stress induces coral bleaching (i.e. loss of photosynthetic symbionts and/or chlorophyll content) and reduces coral photosynthesis and calcification (Hoegh-Guldberg, 1999). The coral response is, however, species specific, depending on the symbiont clade associated to the coral species (Wham et al., 2017), or the energetic reserves of the host tissue (i.e. lipid and protein content) (Fitt et al., 2009). It is also influenced by a myriad of environmental factors, including the level of UVR received by corals. Although UVR is highly mutagenic and enhances cell oxidative state, especially under elevated temperatures (Häder et al., 2007; Sharma et al., 2012), the combined effects of UVR and temperature on coral physiology remain poorly understood because of the complexity of the interactions between these two factors (Courtial et al., 2017; D'Croz and Maté, 2002; D'Croz et al., 2001; Ferrier-Pagès et al., 2007; Fitt and Warner, 1995; Lesser and Farrell, 2004; Lesser et al., 1990). Indeed, while no change was observed on the photosynthetic/autotrophic capacities of *Porites lobata* or *Turbinaria reniformis* under the combined stressors (Courtial et al., 2017; D'Croz et al., 2001), these capacities were strongly affected in *Montastrea annularis* and *Pocillopora damicornis* (Courtial et al., 2017; D'Croz and Maté, 2002; Fitt and Warner, 1995). The scarcity of experimental studies in this field does not allow good predictions of the combined effects of UVR and temperature on coral physiology. More studies are thus needed to better understand the species-specific response to these factors, and the mechanisms underlying coral susceptibility to thermal stress.

Two other underestimated aspects of thermal and UVR stress on coral biology concern the changes in organic matter (OM) fluxes (uptake and/or release of OM by corals) and recycling by the associated bacteria. Under healthy conditions, corals can release half of the photosynthetically fixed carbon and nitrogen into the surrounding reef waters in the form of mucus, i.e. dissolved and particulate carbon (DOC and POC, respectively) and nitrogen (DON and PON, respectively) (Crossland et al., 1980; Davies, 1984). OM is then degraded by prokaryotes through their extracellular enzyme activity (EEA), and is used for bacterial growth (Cunha et al., 2010), or it enters into the recycling pathways of carbon and nitrogen (Wild et al., 2004). OM therefore supports pelagic and benthic production, and plays a major role in the

[1]Sorbone Universités, UPMC, 4 Place Jussieu, 75252 Paris Cedex 05, France. [2]Centre Scientifique de Monaco, Equipe Ecophysiologie, 8 Quai Antoine 1er, 98000, Monaco (Principality). [3]UMR ENTROPIE (IRD, Université de La Réunion, CNRS), Laboratoire d'Excellence CORAIL, BP A5, 98848, Nouméa Cedex, New Caledonia. [4]INRA, UMR CARRTEL, 75 avenue de Corzent, 74200 Thonon-les-Bains, France.
*These authors contributed equally to this work

‡Authors for correspondence (lcourtial@centrescientifique.mc, ferrier@centrescientifique.mc)

C.F.-P., 0000-0002-0357-4486

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution and reproduction in any medium provided that the original work is properly attributed.

Received 10 May 2017; Accepted 3 July 2017

RESEARCH ARTICLE Biology Open (2017) 6, 1190-1199 doi:10.1242/bio.026757

nutrient cycles and trophic structure of the whole reef ecosystem (Bythell and Wild, 2011). Elevated temperature, UVR and other stressors can, however, indirectly alter the quality and quantity of OM released by corals (Niggl et al., 2008; Tremblay et al., 2012; Wooldridge, 2009), and change the associated bacterial diversity (Ainsworth and Hoegh-Guldberg, 2009), likely affecting OM degradation rates. Although few studies have investigated OM fluxes in healthy and thermally stressed corals (Fonvielle et al., 2015; Grottoli et al., 2006; Levas et al., 2015; Niggl et al., 2008; Tremblay et al., 2012; Wooldridge, 2009), the effects of UVR on these fluxes remain unknown. As far as we know, the impact of elevated temperature and/or UVR on the enzymatic activities of mucus-associated bacteria has also never been investigated in tropical corals. The only knowledge on this subject comes from studies performed on water column bacteria from temperate and cold systems (reviewed in Cunha et al., 2010). They showed that bacterial enzymatic activities can be enhanced by temperature and repressed by UVR because of direct enzyme photolysis. Understanding how thermal and UVR stresses alter microbial degradation of coral OM and microbial growth will improve our understanding on future changes of the reef biogeochemical cycling, remineralization pathway and reef trophic structure.

The purpose of this study was to address some existing knowledge gaps regarding the effects of thermal stress, UVR and their combination on the quality, quantity and bacterial degradation of OM produced by a scleractinian coral, and to link these changes to coral metabolism. *A. muricata* was chosen because it belongs to one of the 10 most abundant genera in New Caledonia (Fenner and Muir, 2008) and worldwide (Veron, 2000), and is likely to be one of the major contributors affecting the reef biogeochemical processes. We hypothesize that UVR will exacerbate the effect of thermal stress on coral bleaching and overall metabolism. We also hypothesize that each stressor, alone or in combination, will alter organic carbon and nitrogen fluxes, both in terms of quantity and quality, which will likely change bacterial enzymatic activity in the released mucus. These changes will have a cascading effect on the whole pattern of reef nutrient recycling under global warming scenario.

RESULTS

Effects of temperature and UVR on coral physiology

Four conditions were tested: 26°C without UVR (LT0UV), 26°C with UVR (LTUV), 30°C without UVR (HT0UV) and 30°C with UVR (HTUV) (see Materials and Methods). After 2 weeks at 26°C, exposure to UVR (LTUV) had no significant effect on coral physiology, except for the protein concentration, which was significantly lower in UV treatment (Figs 1 and 2; Table S1). Conversely, exposure to elevated temperature alone (HT0UV) impaired the symbiont density, the chlorophyll *a* (chl *a*) and protein content, and the maximum relative electron transport rate ($rETR_{max}$) (Fig. 1; Table S1). The combination of both stressors had an interactive (protein and $rETR_{max}$) or synergistic (symbiont density and chl *a*) impact on coral physiology (HTUV; Table S1). Two weeks of thermal stress alone induced a 30% and 40% decrease in symbiont density and chl *a*, respectively, and this decline reached 70% and 60%, respectively, when thermal stress was combined with UVR exposure (Fig. 1A,B; Table S1). Net photosynthesis (Pn) normalized to the nubbins' surface area following the same trend as chl *a* (40% and 60% decrease in HT0UV and HTUV), while respiration rates increased by 40% in both treatments compared to 26°C (Fig. 1B and Fig. 2A; Tables S1 and S2). As photosynthesis slowed down and temperature increased under high temperature, the contribution of symbionts (Zooxanthellae) to the animal respiration (CZAR) significantly decreased from 137±33% in control, ambient temperature conditions (LT0UV or LTUV), to *ca.* 13±7% under both high temperature conditions (HT0UV or HTUV) (Fig. 2B; Table S2). However, after 2 weeks of thermal stress, no significant difference was observed between treatments in the $rETR_{max}$ (Fig. 1D, Tuckey's test), which was reached at 600 µmol photons m^{-2} s^{-1}. Photosynthetic apparatus was therefore not affected by the combination of stressors after 2 weeks. Despite significant changes in photosynthesis, calcification rates were not significantly different between conditions (Fig. 2C; Table S2).

After 4 weeks of thermal stress, symbiont density, chl *a* and protein content significantly decreased compared to measurements performed after 2 weeks (synergistic effects of time and temperature, Table S1). The $rETR_{max}$ also decreased showing that the photosynthetic apparatus is damaged by long-term exposure to stress. A synergistic effect between high temperature and UVR was also notable after 4 weeks of stress (Table S1). Therefore, corals lost 70% of their symbionts and chl *a* content in the HT0UV treatment, and almost 95% in the presence of UVR (HTUV) (Fig. 1A,B; Table S1). Their protein content declined by 30% and 50% in the HT0UV and HTUV treatments (Fig. 1C; Table S1), while the $rETR_{max}$ dropped by 50% and 77%, respectively (Fig. 1D; Table S1). Despite a significant bleaching per surface area, the chl *a* content and Pn normalized per symbiont cell stayed constant under stress and over time (12.3±1.5 pg $cell^{-1}$ and 17.7±7.6×10^{-7} µmol h^{-1} $cell^{-1}$, respectively) (Tables S1 and S2).

Heterotrophy and organic matter release

At 26°C (LTUV and LT0UV), autotrophs and prokaryotes presented positive and similar growth rates during the 4 h incubation. However, negative growth rates were observed for prokaryotes in HTUV and HT0UV conditions, and for autotrophs in the HTUV treatment (synergistic effect of UVR and temperature) (Fig. 3A,B; Table S2). These negative rates were linked to their ingestion by coral nubbins at a mean rate of 1.6±1×10^5 prokaryotes h^{-1} cm^{-2} and 4.6±1.7×10^3 autotrophs h^{-1} cm^{-2}, respectively.

Total organic matter (OM) fluxes were positive and not significantly different between treatments, showing a similar increase in total organic carbon (3.11±1.39 µg C cm^{-2} h^{-1}) and nitrogen (0.24±0.09 µg N cm^{-2} h^{-1}) concentrations in seawater. Particulate organic matter (POM) and dissolved organic matter (DOM) fluxes, however, showed inverse trends (Fig. 3C,D; Table S2), with positive fluxes for POM and negative fluxes for DOM at 26°C and the opposite trend at 30°C. There was, therefore, a significant temperature effect on the POM and DOM fluxes (Table S2), while no significant effect of UVR exposure was detected. Dissolved organic carbon (DOC) uptake at 26°C contributed to 39% of the daily respiratory needs ($CHAR_{DOC}$), while particulate organic carbon (POC) uptake at 30°C contributed to 80% of the respiratory needs ($CHAR_{POC}$).

Enzymatic activity and organic matter degradation

At 26°C, glucosidase maximum extracellular enzyme activity (EEA_{max}) was significantly higher in nubbins shaded from UVR (5.4±1.3 ng h^{-1} cm^{-2}, Fig. 4A). High temperatures significantly increased glucosidase EEA_{max}, which reached 15.2± 3.0 ng h^{-1} cm^{-2} (Fig. 4A; Table S2), without any effect of UVR (Table S2). Aminopeptidase EEA_{max} presented the same trends as the glucosidase EEA_{max}, with low rates at 26°C (Fig. 4B, 2.3± 2.3 ng h^{-1} cm^{-2}) and a significant increase at 30°C (16.1± 2.5 ng h^{-1} cm^{-2}) (Table S2). UVR exposure had no significant effect on this activity.

RESEARCH ARTICLE Biology Open (2017) 6, 1190-1199 doi:10.1242/bio.026757

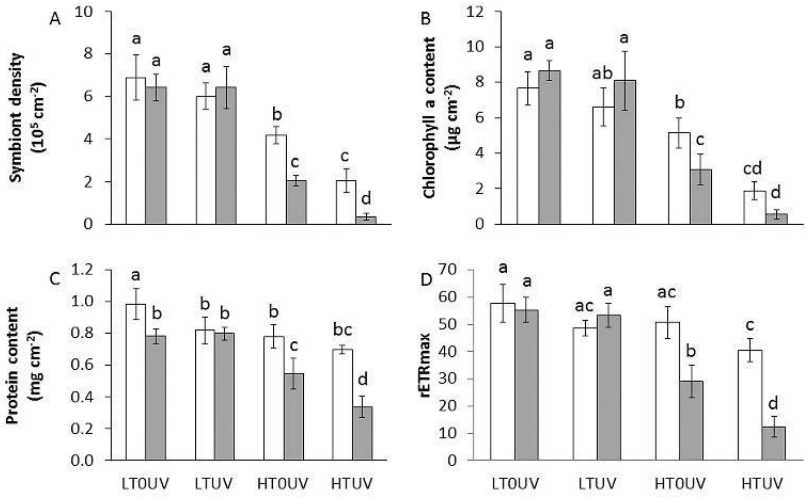


Fig. 1. Tissue parameters of *A. muricata* after 16 and 28 days of thermal stress with or without UVR. Symbiont density (A), chl *a* content (B), protein content (C) and $rETR_{max}$ (D) assessed at 26°C without UVR (LT0UV), 26°C with UVR (LTUV), 30°C without UVR (HT0UV) and 30°C with UVR (HTUV) after 16 (white columns) or 28 (grey columns) days of thermal stress. Data are mean±standard deviation (s.d.) of five replicates. Values with the same letter are not significantly different (P>0.05).

At low temperature, only a small fraction of the total OM released was degraded (0.03% and 1% of the total carbon and nitrogen released, respectively). However, at high temperature, 0.7% and 8% for the total carbon and nitrogen released were degraded, respectively (Fig. 4C,D; Table S2).

DISCUSSION

By simultaneously quantifying covariation in coral photosynthesis, calcification, tissue composition, OM fluxes and degradation by mucus-associated bacteria, this study allows deeper understanding of how two major environmental factors, elevated temperature and UVR, alone or in combination, impact the metabolism and close surrounding environment of *A. muricata*, a dominant coral reef species (Fenner and Muir, 2008). Specifically, our results demonstrate that elevated temperature was the main factor to affect the overall metabolism of *A. muricata*, as well as OM fluxes and bacterial activity. The results, however, highlight a significant combined effect of UVR and temperature on the bleaching susceptibility and photosynthetic efficiency of this coral species, as well as on the decrease in protein reserves over time. This study also reveals, for the first time, that the shift from auto- to heterotrophy that occurred in the short-term bleached *Acropora* nubbins led to a change in both the quality of the OM released and the population dynamics of the associated microorganisms. Finally, OM degradation by mucus-associated bacterial activity was unaffected by UVR exposure, but significantly increased under high temperature. Altogether, our results demonstrate that seawater warming not only affects coral physiology, but also the way corals interact with their nearest environment, with potential consequences for coral reef biogeochemical cycles and food webs.

A. muricata nubbins did not present any change in their physiology when they were experimentally shaded from UVR and maintained under their normal growth temperature. This lack of UVR effect was likely due to the acclimation to UVR of the colonies used in this experiment, which grew at 2-3 m depth and were therefore likely protected by mycosporine-like amino acids (MAAs), synthesized in most shallow water corals (Shick et al., 1995). This protection was, however, suppressed or reduced under

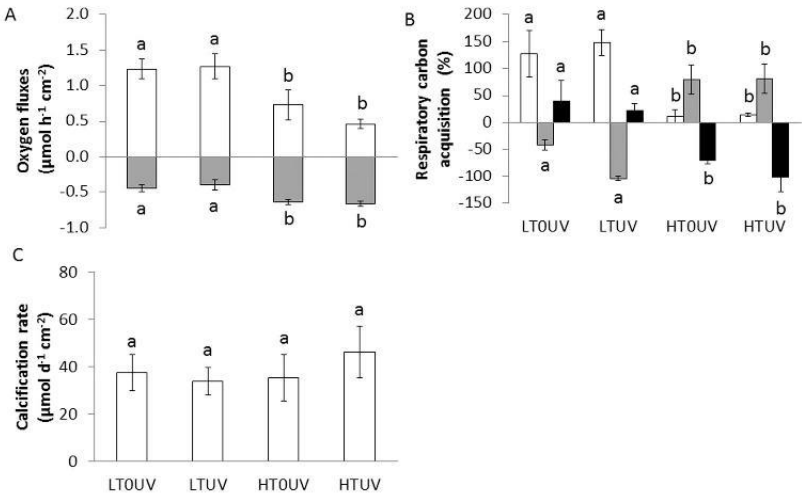


Fig. 2. Metabolism of *A. muricata* after 16 days of thermal stress with or without UVR. Oxygen fluxes (A), respiratory carbon acquisition (B), and calcification rate (C) after 2 weeks of thermal stress measured at 26°C without UVR (LT0UV), 26°C with UVR (LTUV), 30°C without UVR (HT0UV) and 30°C with UVR (HTUV). In A, white columns represent the net photosynthesis, grey columns represent respiration rates. Data are mean±s.d. of five replicates. In C, white columns are autotrophic carbon (CZAR), grey columns are $CHAR_{POC}$ and black columns are $CHAR_{DOC}$. Values with the same letter are not significantly different (P>0.05).

RESEARCH ARTICLE Biology Open (2017) 6, 1190-1199 doi:10.1242/bio.026757

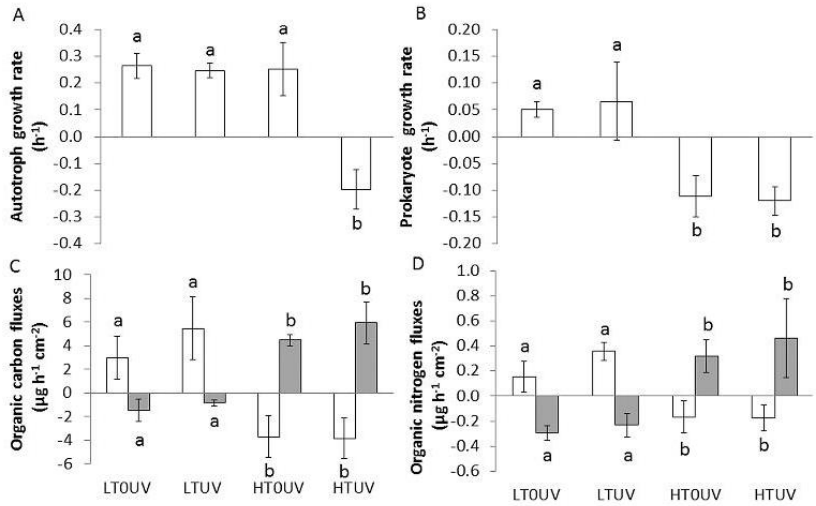


Fig. 3. Growth rates of the mucus-associated micro-organisms and organic matter fluxes. Autotroph growth rate (A), prokaryote growth rate (B), organic carbon fluxes (C) and organic nitrogen fluxes (D) at 26°C without UVR (LT0UV), 26°C with UVR (LTUV), 30°C without UVR (HT0UV) and 30°C with UVR (HTUV) after 2 weeks of thermal stress. In C and D, white columns are POM and grey columns are DOM. Data are mean±s.d. of five replicates. Values with the same letter are not significantly different (P>0.05).

thermal stress (Fitt and Warner, 1995), lowering the coral's capacity to cope with the accumulation of reactive oxygen species and oxidative stress (Lesser et al., 1990). Temperature presented an additive and synergistic effect with UVR on symbiont density and chl *a* content, respectively. Corals, therefore, bleached and lost 35% of their symbionts under thermal stress alone, and up to 68% under the combined stress, consistent with previous laboratory and field studies which showed greater effects of double than single stress in diverse coral species (Courtial et al., 2017; D'Croz and Maté, 2002; Ferrier-Pagès et al., 2007; Fitt and Warner, 1995). Bleaching was amplified with the stress duration as only 5% of the symbionts remained in nubbins kept for 4 weeks at high temperature under UVR. These results suggest that corals naturally exposed to low UVR could better resist long thermal stress events than UVR-exposed corals.

In addition to studying the effects of thermal and UVR stress on coral physiology, we also expanded our measurements to assess the coral-induced changes in seawater biogeochemistry (via mucus release and degradation) with thermal and/or UVR stress. Coral mucus (i.e. dissolved and particulate organic carbon and nitrogen) has several functions, both for corals (defense against external stressors and food source (Brown and Bythell, 2005; Levas et al., 2016) and for the reef organisms (energy carrier and particle trap) (Wild et al., 2004); however, changes in mucus quality and quantity under different environmental conditions are still poorly understood (Niggl et al., 2008; Tremblay et al., 2012), because few studies have investigated both carbon and nitrogen fluxes, in dissolved or particulate forms, in healthy and stressed coral species (Bednarz et al., 2012; Naumann et al., 2010), preventing a comprehensive overview of OM fluxes in corals. Our results first show constant release rates of total organic carbon (TOC) and total organic nitrogen (TON) by *A. muricata*, irrespective of the stress state. The high TOC/TON ratio (13 to 15) of the released OM, already observed for several coral species of the Red Sea (Naumann et al., 2010), indicates a higher degree of nitrogen retention in coral tissue compared to carbon. Although *A. muricata* also presents 10 times higher TOC release rates than other species of the Red Sea or the Caribbean (Levas et al., 2016; Naumann et al., 2010), these rates are

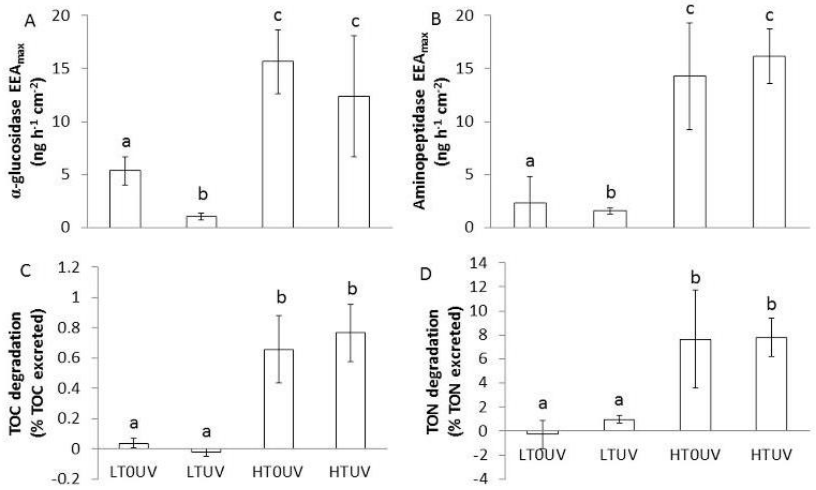


Fig. 4. Bacterial activity and organic matter degradation. Alpha-glucosidase EEA_{max} (A), aminopeptidase EEA_{max} (B), TOC degradation (C) and TON degradation (D), calculated at 26°C without UVR (LT0UV), 26°C with UVR (LTUV), 30°C without UVR (HT0UV) and 30°C with UVR (HTUV) after 2 weeks of thermal stress. Data are mean±s.d. of five replicates. Values with the same letter are not significantly different (P>0.05).

RESEARCH ARTICLE Biology Open (2017) 6, 1190-1199 doi:10.1242/bio.026757

in the range of previously reported values in several *Acropora* species from Malaysia and Jordan (Nakajima et al., 2009, 2010; Naumann et al., 2010). Overall, *Acropora* species tend to mainly be a source of energy-rich carbon compounds to the reef food chain. In New Caledonia, this production can partly explain the exceptionally high rates of N_2 fixation in the water column (Camps et al., 2016) compared to other reef systems (Bednarz et al., 2017), since diazotrophs need large amounts of energy-rich photosynthates to perform N_2 fixation (Bednarz et al., 2017).

The analysis of the DOM and POM forms shows that the quality of the OM (i.e. particulate or dissolved matter) changes with the environmental conditions under which corals are thriving. *A muricata* released POM and took up DOM under normal growth conditions, while the reverse was observed in bleached colonies. Although most previous studies show a release of DOC by healthy corals (Crossland, 1987; Wild et al., 2010a,b, 2004a,b, 2005, 2008; Houlbrèque et al., 2004; Tanaka et al., 2008, 2009; Haas et al., 2010; Naumann et al., 2010; Levas et al., 2015), some studies show the contrary (Houlbrèque et al., 2004; Naumann et al., 2010; Niggl et al., 2008). Observations of OM fluxes in bleached or thermally-stressed corals evidenced the same contrasted results: while *Porites divaricata*, *Porites astreoides* and *Orbicella faveolata*, were shown to take up DOC (Grottoli et al., 2006, 2014; Levas et al., 2013, 2016), *Acropora* sp., *Porites* spp. and *Stylophora pistillata* released it (Haas et al., 2010; Niggl et al., 2008; Tremblay et al., 2012). Overall, no common pattern can be deduced from these previous observations. Our measurements, which took into account the OM as well as the pico- and nanoplankton concentrations, however, suggest that the changes in POM and DOM fluxes in *A. muricata* are linked to its heterotrophic activity. POM includes both nonliving material and living particles such as bacteria and small autotrophs contained or grown in the mucus. Pico- and nanoplankton, which multiplied in the incubations with healthy coral colonies, were instead grazed by corals when bleached. A shift thus occurred between low-energy DOM uptake under healthy conditions (maximum of 1.5 µg C and N h^{-1} cm^{-2} when all DOM is consumed) to high energy POM uptake under bleached conditions, (maximum of 3.9 µg C and N h^{-1} cm^{-2} when all POM is consumed), suggesting a greater need for heterotrophic nutrients, likely to compensate for autotrophic loss and meet metabolic demand. POM uptake contributed 80% of the respiratory needs of the heat-stress colonies, compared to 40% for DOM under healthy conditions. In addition, POM consumption in bleached corals may have enhanced DOC release via sloppy feeding. Although this process was never studied in corals, it is well-known in copepods as a dominant mode of DOM production (Saba et al., 2011). Moreover, our results highlight a positive correlation between the stress level inflicted to the corals and their micro-heterotrophy level: corals shifted from total autotrophy under healthy conditions to partial heterotrophy on prokaryotes alone under thermal stress, and to predation on both prokaryotes and autotrophs when exposed to thermal and UVR stress. These results demonstrate the role of pico/nanoplankton food sources for *A. muricata* resilience to thermal stress, and for bleached corals in general (Houlbrèque and Ferrier-Pagès, 2009; Tremblay et al., 2012). They also clearly indicate that corals can feed on allochtonous aggregates (Coffroth, 1984), and on their own mucus and mucus associated particles, when needed. The ability to shift from autotrophy to heterotrophy is believed to provide significant advantage over species that are unable to do so (Hughes and Grottoli, 2013; Levas et al., 2016). In this study, high temperature induced a decrease in the rates of photosynthesis, without any impact on calcification, although the two processes are usually correlated (Gattuso et al. 1999). The shift to heterotrophy at high temperature indeed allowed *A. muricata* to increase its respiration rates, likely to keep up with energy costs associated with the reparation of damages caused by thermal stress (Coles and Jokiel, 1977; Fitt et al., 2001). This increased production of internal CO_2 likely sustained calcification rates, since more than 70% of the CO_2 used in calcification come from internal respiration (Furla et al., 2000).

Since bacteria are the first consumers of the carbon-rich compounds (i.e. wax esters, triglycerides, fatty acids) contained in the mucus, to convert them into bacterial biomass (Ferrier-Pagès et al., 1998; Herndl and Velimirov, 1986), we quantified in the different temperature and UV conditions, the activity of the two main bacterial enzymes responsible for carbon (α-glucosidase) and nitrogen (aminopeptidase) degradation. Shading nubbins from UVR had little effect on enzymatic activities, which contrasts with previous studies performed *in vitro* with isolated enzymes, showing a decrease in EEA under UVR because of photolysis (Espeland and Wetzel, 2001). MAAs release by corals in the mucus (Drollet et al., 1997) might have protected the associated bacteria and enzymes from UVR damage. On the other hand, and consistent with observations made on water column bacteria (Cunha et al., 2010; Price and Sowers, 2004), high temperature enhanced both aminopeptidase and α-glucosidase EEA, either directly or indirectly through increased bacterial concentration. As a consequence, carbon and nitrogen degradation rates were 20 and 10 times higher, respectively, than at normal temperature. Despite this large increase in OM degradation, the matter degraded by mucus-associated bacteria represented <1% of the carbon and 10% of the nitrogen contained in the excreted mucus. This shows that the recycling of the coral derived-matter is a long-term process, rather performed by bacteria free living in the water column or in reef sediment. Nitrogen was overall 10 times more degraded than carbon, likely because it is one of the major nutrients limiting bacterioplankton growth (Antia et al., 1991; Keil and Kirchman, 1991).

Overall, our study highlights the major changes in OM fluxes, composition and degradation following *A. muricata* bleaching. The potential organic carbon and nitrogen pathways expected with healthy (A) and bleached (B) *A. muricata* are represented in Fig. 5. Under healthy conditions, *A. muricata* releases POM, which is poorly degraded by prokaryotes. POM will thus rapidly sediment to the reef bottoms, where it will sustain bacterial growth, and will contribute to the important nutrient recycling pathways observed in reefs (Muscatine and Porter, 1977; Richter et al., 2001; Wild et al., 2004). During bleaching, *A. muricata* will release labile DOM, which is more likely to stay in the water column and therefore promote the development of free-living-bacteria (Ferrier-Pagès et al., 2000; Wild et al., 2004), including pathogenic communities (Haas et al., 2013; Nelson et al., 2013). Unless bacteria enter the microbial loop and higher trophic levels, such stimulation may lead to the 'microbialization' of the reef (Haas et al., 2016) with negative consequences for coral health such as the promotion of opportunistic pathogen invasion (Barott and Rohwer, 2012). Our study also emphasizes the importance of considering UVR exposure when predicting long-term coral bleaching. As UVR impact on coral physiology is increased with the stress duration, the effects measured during this short-term experiment could be underestimated on a longer term.

MATERIALS AND METHODS

Coral collection and experimental setup

Eighty 2-3 cm long *A. muricata* nubbins were collected between 2 and 3 m depth from 10 parent colonies on the reef of

RESEARCH ARTICLE

Biology Open (2017) 6, 1190-1199 doi:10.1242/bio.026757

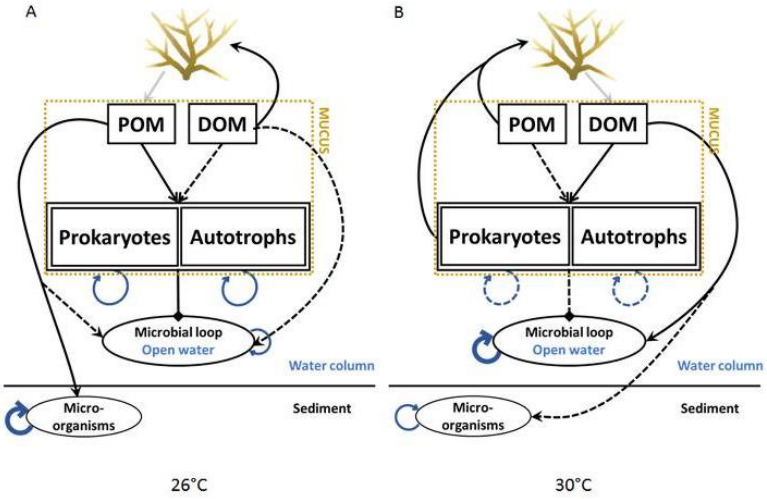


Fig. 5. Summary of organic matter exchange between coral microbial communities. Uptake (black arrows) and release (grey arrows) fluxes between coral nubbins-associated micro-organisms and near seawater at 26°C (A) and 30°C (B). Blue arrows indicate organisms' growth. Diamond-headed lines (from 'Prokaryotes/Autotrophs' to 'Microbial loop/Open water') indicate the contribution of mucus-associated microbiome to the open water microbial loop. Dashed lines indicate low fluxes, solid lines indicate enhanced processes. At normal temperature (26°C), coral releases POM and ingests DOM. Prokaryotes and autotrophs grow in the mucus and contribute to the open water microbial loop. The released POM sinks and is then available for the development of the microbial loop inside sediments and in open water. At high temperature (30°C), corals ingest POM and release DOM. They graze on associated microorganisms, which contribute less to the microbial loop of the water column. Overall, DOM is released to the seawater which enhances the development of microorganisms in the water column.

'phare Amédée' in the New Caledonian lagoon (22°28.845′S; 166°26.806′E) in April 2016 (APA-NCPS-2016-001). Nubbins were then transferred at the Aquarium des Lagons in Nouméa and left to recover for 1 month prior to the experiment. They were evenly distributed in eight 100 L outdoor tanks continuously supplied with the lagoon seawater at a rate of 72 L h^{-1}, and were fed once a week with *Artemia salina* nauplii. Shade cloths were disposed above aquaria to reach the underwater light conditions on the reef. Therefore, at the beginning of the experiment, the maximal natural irradiances in aquaria, obtained at midday, were ~900 µmol photons m^{-2} s^{-1} of photosynthetically active radiation (PAR), and ~20 W m^{-2} and ~1.2 W m^{-2} of ultra-violet A (UVA, 315-400 nm) and B (UVB, 280-315 nm) radiation, respectively. PAR was controlled using a LI-COR data logger (LI-1000, LI-COR, Lincoln, USA) connected to a spherical quantum sensor (LI-193, LI-COR); UVR was controlled using an ILT1400 portable radiometer (International Light Technologies, Peabody, USA) connected to detectors (SEL240/UVB-1/TD and SEL033/UVA/TD, International Light Technologies). In addition, during the 1 month acclimation period, four tanks were maintained under the above natural conditions (UV), while the other four tanks were shielded from UVA and UVB using UVR filters (226 Lee UV filters, La Boutique du Spectacle, Paris, France) (0UV).

After the acclimation period, for each treatment (0UV and UV), two temperature levels were set with duplicated tanks: 26°C (±0.5°C), corresponding to a natural condition, and 30°C (±0.5°C), corresponding to a heat-stress condition. Four conditions were thus reached in a cross factor design: 26°C without UVR (LT0UV); 26°C with UVR (LTUV); 30°C without UVR (HT0UV); 30°C with UVR (HTUV). For high-temperature (HT) conditions, temperature was gradually increased from 26°C to 30°C within 1 week, and was controlled using heaters connected to a temperature-stat system (IKS, Karlsbad, Germany; accuracy ± 0.05°C). Two samplings were performed after 16 days and 28 days incubation under the different conditions (for both 26°C and 30°C, with or without UVR). After 16 days, five nubbins per condition and for all conditions (LT0UV, LTUV, HT0UV, HTUV), randomly taken in the replicated tanks, were used to assess their photosynthetic performances (i.e. $rETR_{max}$, photosynthetic and respiration rates) and growth rates and then frozen at −20°C for symbiont, chl *a*, protein content and surface measurements. In the meantime, five additional nubbins per condition were used for OM fluxes and bacterial activity measurements (as described below) before being frozen at −20°C. Twelve days later (28 days of stress), the physiological parameters (chl *a*, symbiont density, protein and rETRmax) were re-assessed on the remaining nubbins (five per condition and for all conditions).

For all incubations, temperature and light were maintained constant and identical to the experimental conditions. All data were normalized to the surface area of the corals, measured using the wax technique (Stimson and Kinzie, 1991), or per symbiont cell (determined as described below).

Physiological measurements

Five nubbins per condition were incubated in the dark for 10 min before the relative electron transport rate (rETR) versus irradiance, or rapid light curves, were generated with a Pulse Amplitude Modulation (PAM) fluorometer (Diving-PAM, Walz, Effeltrich, Germany) according to Ralph and Gademann (2005). For this purpose, nubbins were illuminated for 10 s with seven different light intensities (from 0 to 900 µmol quanta m^{-2} s^{-1}) and the $rETR_{max}$ were deduced from the curves.

The same nubbins were then used to measure rates of photosynthesis, respiration and calcification. They were thus incubated in individual 100 ml beakers filled with 0.45 µm filtered seawater, continuously stirred with stirring bars and hermetically closed using transparent plastic film to avoid any oxygen exchange with the ambient air. Each beaker was equipped with an oxygen sensor spot (SP-PSt6-NAU, PreSens, Regensburg, Germany), and oxygen concentration was measured with a polymer optical fiber and Fibox 4 (PreSens) at the beginning and at the end of the incubations. Changes in oxygen production were measured during 1 h incubation in the dark for the determination of the respiration rate (R) and 30 min at the optimal photosynthetic light (600 µmol photons m^{-2} s^{-1}, Aquablue plus neon, Blue-white, 15,000 K, Giesemann, Nettetal, Germany) to assess net photosynthesis (Pn). This optimal irradiance was deduced from the rapid light curves and from preliminary Pn-irradiance curves (Fig. S1). Pn and R were estimated from the difference between the final and the initial oxygen concentrations. After the incubations,

RESEARCH ARTICLE Biology Open (2017) 6, 1190-1199 doi:10.1242/bio.026757

nubbins were frozen at –20°C for the subsequent determination of tissue parameters as described below. Data were expressed in µmol O_2 h^{-1} cm^{-2} d^{-1} and corrected against a blank (filtrated seawater incubated for the same period without nubbin).

The incubation seawater of each beaker was filtered through 0.2 µm filters and stored at 4°C for the subsequent determination of the calcification rates using the total alkalinity anomaly method (Smith and Key, 1975). The measurement was performed using a TIM865 titration manager (TitraLab, Hach, Loveland, USA). Titration error was verified using AT standards provided by A.G. Dickson (University of California, San Diego, USA; batch 142). Data were expressed in µmol $CaCO_3$ cm^{-2} d^{-1} and corrected against a blank (filtrated seawater incubated for the same period without nubbin).

To calculate the total daily CZAR, oxygen fluxes were converted to carbon equivalent using respiratory (RQ) and photosynthetic (PQ) quotients equal to 0.8 and 1.1, respectively (Gattuso and Jaubert, 1990; Muscatine et al., 1981). We considered that coral colonies photosynthesize during 12 h (light period) and respire during 24 h. CZAR (%) was calculated as

$$CZAR = ((\mu molO2\ produced \times 12 \times PQ)/(\mu molO2\ consumed \times 24 \times RQ)) \times 100 \quad (1)$$

Here, we considered the respiration of the whole coral holobiont (i.e. host, symbionts and microbiome), not only the animal, to take into account all respiratory needs.

Symbiont, chl *a* and protein content

Nubbin tissue was removed from the skeleton using an air pick and homogenized with a Potter tissue grinder. A sub-sample was taken for the determination of the symbiont density of each sample using a Neubauer cell, on five replicated counts. Another sub-sample was used to assess the protein content according to Hoogenboom et al. (2010), using a BCA assay kit (Smith et al., 1985). For chl *a* measurements, the last subsample was centrifuged at 5000×***g*** for 10 min at 4°C to separate the symbionts (in the pellet) from the host tissue. The pellet was then re-suspended in 10 ml acetone and kept in the dark at 4°C for 24 h prior measurements. Samples were then centrifuged for 15 min at 10,000×***g*** and the absorbance was measured at 630, 663 and 750 nm using an EVOLUTION 201 UV-Visible spectrophotometer (Thermo Fisher Scientific). Chlorophyll concentrations were computed using the equations of Jeffrey and Humphrey (1975).

POM and DOM fluxes

Five nubbins per condition were incubated for 4 h in 200 ml of 0.45 µm filtered seawater. Water samples were taken at the beginning (T_0) and at the end (T_f) of the incubation to assess (1) the organic carbon and nitrogen fluxes, (2) the abundance of heterotrophic prokaryotes and autotrophs in the organic matter released by the corals and (3) the EEA. All measurements were corrected against a blank (filtered seawater incubated without nubbin).

Organic carbon and nitrogen fluxes

For the determination of total organic carbon and nitrogen concentrations in the incubation medium at T_0 and T_f, 20 ml seawater were sampled at T_0 and T_f in each beaker with sterile syringes and transferred to glass vials, previously washed for 24 h in 10% HCl and burned at 500°C for 4 h (Naumann et al., 2010). Vials were amended with 42 µl H_3PO_4 to avoid any bacterial activity during storage, and they were then stored at −20°C until further analysis. The same procedure was applied to measure DOC and DON concentrations, except that the water was filtered through 0.45 µm filters prior to storage. Samples were analyzed using a TOC-L analyzer (Shimadzu, Kyoto, Japan).

To calculate the TOC/TON and DOC/DON fluxes, concentrations of each compound were first corrected from the blank (concentrations in beakers incubated without a coral nubbin), and the difference between T0 and Tf was then calculated to deduce a flux between corals and seawater. Negative fluxes indicate a net uptake by coral nubbins, and positive fluxes indicate a net release from corals. In addition, POC and PON were estimated by subtracting DOC or DON from TOC or TON. Data were expressed in µg h^{-1} cm^{-2}.

To calculate the total daily heterotrophic contribution of POC and DOC to the animal respiration ($CHAR_{POC}$ and $CHAR_{DOC}$, respectively), carbon fluxes were converted to µmol C h^{-1} cm^{-2} and 0.8 was used as a respiratory quotient (RQ) to convert oxygen fluxes to their carbon equivalent (Gattuso and Jaubert, 1990; Muscatine et al., 1981). CHAR (%) was calculated as

$$CHAR = (\mu molC\ taken\ up/(\mu molO2\ consumed \times RQ)) \times 100 \quad (2)$$

Abundance of pico-and nanoplankton

For the determination of the abundance of heterotrophic prokaryotes and autotrophs, 4.8 ml seawater was sampled at T_0 and T_f, fixed with 0.2 ml glutaraldehyde (25%) during 30 min in the dark, and stored at −80°C. Samples were then analyzed by flow cytometry as described by Jacquet et al. (2013). Particle growth rates or grazing rates were calculated according to the equations of Ribes et al. (1998). In brief, growth rates in the control beakers (k_c) and in the presence of coral nubbins (k_n) were computed as:

$$k = ln(Cf/C0)/(Tf - T0) \quad (3)$$

with C_0 and C_f representing the microorganism concentrations (cell ml^{-1}) at time T_0 and T_f, respectively, expressed per hour. These growth rates were then used to calculate g, the grazing coefficient (h^{-1}),

$$g = kc - kn \quad (4)$$

They were also used to calculate F, the filtration rate (ml h^{-1} cm^{-2})

$$F = V \times g/S \quad (5)$$

where V is the incubation volume (ml), S the coral surface (cm^2) and g (h^{-1}) the grazing coefficient. The ingestion rate I (ingested cell h^{-1} cm^{-2}) was finally calculated as

$$I = F \times C \quad (6)$$

where F (ml h^{-1} cm^{-2}) is the filtration rate and C (cell ml^{-1}) is the average prey concentration equal to

$$C = C0[e(k-g) \times (Tf - T0) - 1]/(k-g) \times (Tf - T0) \quad (7)$$

with C_0 the prey concentration at T_0. Because of the heterogeneity in the carbon content of the different autotrophs present in seawater, we could not quantify the equivalent carbon ingested by the coral using these data. However, this contribution was estimated using the $CHAR_{POC}$ above.

RESEARCH ARTICLE

Biology Open (2017) 6, 1190-1199 doi:10.1242/bio.026757

Extracellular enzymatic activities

EEAs were monitored using fluorescent substrate analogs according to standard protocols (Hoppe, 1983). L-Leucine-7-amido-4-methyl-coumarin hydrochloride (Leu-MCA) and 4-methylumbeliferyl-α-D-glucopyranoside (αMUF) were therefore used to assess aminopeptidase and alpha-glucosidase activities, respectively. These two compounds emit a fluorescent signal after cleavage by the enzymes. Correspondence between fluorescence value and the quantity of substrate hydrolyzed was obtained using amino 4-methylcoumarin (MCA) and 4-methylumbeliferone (MUF). Three replicates of the incubation water were sampled at the end of the 4 h incubation and added to the substrates to a final concentration of 250 µM. Samples were incubated in the dark for up to 168 h in a thermostated shaking bath at the experimental temperatures (26°C or 30°C). EEAs were measured every 24 h using a spectrofluorometer (Synergy-H1, BioTek, Bad Friedrichshall, Germany). For this purpose, 96-well plates (300 µl volume) were filled up with 240 µl sample and 60 µl of 10.8 Tris-HCl buffer was added to adjust pH to obtain maximal fluorescence intensity (Fonvielle et al., 2015).

Carbon degradation was calculated with the following equation:

$$\%Cdegraded = EEAmax \times 100/TOC \quad (8)$$

with EEA_{max} the α-glucosidase maximal enzymatic activity (nmol l^{-1} h^{-1}) of the mucus-associated bacteria and TOC (nmol l^{-1} h^{-1}) the quantity of organic carbon contained in the mucus, calculated as

$$TOC = TOCcoral - TOCblk \quad (9)$$

TOC_{coral} and TOC_{blk} (nmol l^{-1} h^{-1}) are the TOC concentrations in seawater after 4 h of incubation with or without nubbin, respectively.

Similarly, nitrogen degradation was calculated using the following equation:

$$\%Ndegraded = EEAmax \times 100/TON \quad (10)$$

with EEA_{max} the aminopeptidase maximal enzymatic activity (nmol l^{-1} h^{-1}) of the mucus-associated bacteria and TON (nmol l^{-1} h^{-1}) the quantity of total organic nitrogen contained in the mucus, calculated as

$$TON = TONcoral - TONblk \quad (11)$$

with TON_{coral} and TON_{blk} (nmol l^{-1} h^{-1}) the TON concentrations in seawater after 4 h of incubation with or without nubbin, respectively.

Statistical analysis

The effects of UVR and high temperature on the parameters measured after 16 days of thermal stress were assessed with two-way ANOVAs using UVR (presence, absence) and temperature (26°C and 30°C) as factors. To test the effects of high temperature and UVR over time, three-way ANOVAs using time (after 16 and 28 days), UVR and temperature as factors were performed on symbiont density, chl *a*, protein content and $rETR_{max}$. For all tests, normality of the residuals and variance homoscedasticity were tested using Shapiro and Bartlett tests, respectively. When needed, data were log transformed in order to fulfill those criteria. A Tukey's post hoc test was performed when results of the ANOVAs were significant.

Acknowledgements

We thank T. Biscéré, C. Bulteau, C. Peignon and J. Baly for laboratory assistance; C. Payri, director of the ENTROPIE UMR for scientific support; Mr. R. Farman, Director of the Aquarium des Lagons, Nouméa, for hosting the tanks and providing technical help during the experimental setup; and N. Wabete and M. Le Gall (Ifremer) for their collaboration in laboratory analysis.

Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: L.C., C.F.-P., F.H.; Methodology: L.C., C.F.-P., S.J., R.R.-M., S.R., C.R., F.H.; Formal analysis: L.C., C.F.-P., F.H.; Data curation: L.C.; Writing - original draft: L.C.; Writing - review & editing: L.C., C.F.-P., S.J., R.R.-M., S.R., F.H.; Supervision: C.F.-P., F.H.; Funding acquisition: C.F.-P., F.H.

Funding

This study was supported by Centre Scientifique de Monaco (C.F.-P., L.C., S.R., C.R.), Université Pierre et Marie Curie (Paris VI University) (L.C.), Institut National de la Recherche Agronomique (S.J.) and Département Soutien et Formation, Institut de Recherche pour le Développement (Nouméa, New Calédonia) (R.R.-M. and F.H.).

Supplementary information

Supplementary information available online at
http://bio.biologists.org/lookup/doi/10.1242/bio.026757.supplemental

References

Ainsworth, T. D. and Hoegh-Guldberg, O. (2009). Bacterial communities closely associated with coral tissues vary under experimental and natural reef conditions and thermal stress. *Aquat. Biol.* **4**, 289-296.

Antia, N. J., Harrison, P. J. and Oliveira, L. (1991). The role of dissolved organic nitrogen in phytoplankton nutrition, cell biology and ecology. *Phycologia* **30**, 1-89.

Barott, K. L. and Rohwer, F. L. (2012). Unseen players shape benthic competition on coral reefs. *Trends Microbiol.* **20**, 621-628.

Bednarz, V. N., Naumann, M. S., Niggl, W. and Wild, C. (2012). Inorganic nutrient availability affects organic matter fluxes and metabolic activity in the soft coral genus *Xenia*. *J. Exp. Biol.* **215**, 3672-3679.

Bednarz, V. N., Grover, R., Maguer, J.-F., Fine, M. Ferrier-Pagès, C. (2017). The assimilation of diazotroph-derived nitrogen by scleractinian corals depends on their metabolic status. *MBio* **8**, e02058-16.

Brown, B. E. and Bythell, J. C. (2005). Perspectives on mucus secretion in reef corals. *Mar. Ecol. Prog. Ser.* **296**, 291-309.

Bythell, J. C. and Wild, C. (2011). Biology and ecology of coral mucus release. *J. Exp. Mar. Bio. Ecol.* **408**, 88-93.

Camps, M., Benavides, M., Lema, K. A., Bourne, D. G., Grosso, O. and Bonnet, S. (2016). Released coral mucus does not enhance planktonic N_2 fixation rates. *Aquat. Microb. Ecol.* **77**, 51-63.

Coffroth, M. A. (1984). Ingestion and incorporation of coral mucus aggregates by a gorgonian soft coral. *Mar. Ecol. Prog. Ser. Oldend.* **17**, 193-199.

Coles, S. L. and Jokiel, P. L. (1977). Effects of temperature on photosynthesis and respiration in hermatypic corals. *Mar. Biol.* **43**, 209-216.

Courtial, L., Roberty, S., Shick, J. M., Houlbrèque, F. and Ferrier-Pagès, C. (2017). Interactive effects of ultraviolet radiation and thermal stress on two reef-building corals. *Limnol. Oceanogr.* **62**, 1000-1013.

Crossland, C. J. (1987). In situ release of mucus and DOC-lipid from the corals *Acropora variabilis* and *Stylophora pistillata* in different light regimes. *Coral Reefs* **6**, 35-42.

Crossland, C. J., Barnes, D. J. and Borowitzka, M. A. (1980). Diurnal lipid and mucus production in the staghorn coral *Acropora acuminata*. *Mar. Biol.* **60**, 81-90.

Cunha, A., Almeida, A., Coelho, F. J. R. C., Gomes, N. C. M., Oliveira, V. and Santos, A. L. (2010). Bacterial extracellular enzymatic activity in globally changing aquatic ecosystems. *Curr. Reseach, Technol. Educ. Top. Appl. Microbiol. Microb. Biotechnol.* **1**, 124-135.

Davies, P. S. (1984). The role of zooxanthellae in the nutritional energy requirements of *Pocillopora eydouxi*. *Coral Reefs* **2**, 181-186.

D'Croz, L. and Maté, J. L. (2002). The role of water temperature and UV radiation in the recovery of the experimentally bleached coral Pocillopora damicornis from the eastern Pacific Ocean (Panama). In Proceedings of the Ninth International Coral Reef Symposium, Bali, 23-27 October 2000, pp. 1111-1116.

D'Croz, L., Maté, J. L. and Oke, J. E. (2001). Responses to elevated sea water temperature and UV radiation in the coral Porites lobata from upwelling and non-upwelling environments on the Pacific coast of Panama. *Bull. Mar. Sci.* **69**, 203-214.

Doney, S. C., Ruckelshaus, M., Emmett Duffy, J., Barry, J. P., Chan, F., English, C. A., Galindo, H. M., Grebmeier, J. M., Hollowed, A. B., Knowlton, N. et al. (2012). Climate change impacts on marine ecosystems. *Ann. Rev. Mar. Sci.* **4**, 11-37.

Drollet, J. H., Teai, T., Faucon, M. and Martin, P. M. V. (1997). Field study of compensatory changes in UV-absorbing compounds in the mucus of the solitary coral *Fungia reparda* (Scleractinia: Fungiidae) in relation to solar UV radiation,

RESEARCH ARTICLE Biology Open (2017) 6, 1190-1199 doi:10.1242/bio.026757

sea-water temperature, and other coincident physico-chemical parameters. *Mar. Freshw. Res.* **48**, 329-333.

Espeland, E. M. and Wetzel, R. G. (2001). Complexation, stabilization, and UV photolysis of extracellular and surface-bound glucosidase and alkaline phosphatase: Implications for biofilm microbiota. *Microb. Ecol.* **42**, 572-585.

Fenner, D. and Muir, P. (2008). Reef corals of the northwestern lagoon of grande-terre, New Caledonia. In *A Rapid Marine Biodiversity Assessment of the Coral Reefs of the Northwest Lagoon, Between Koumac and Yandè, Province Nord, New Caledonia* (ed. S. A. McKenna, N. Baillon and J. Spaggiari), pp. 18-32. Washington: Conservation International.

Ferrier-Pagès, C., Gattuso, J. P., Cauwet, G., Jaubert, J. and Allemand, D. (1998). Release of dissolved organic carbon and nitrogen by the zooxanthellate coral *Galaxea fascicularis*. *Mar. Ecol. Prog. Ser.* **172**, 265-274.

Ferrier-Pagès, C., Leclercq, N., Jaubert, J. and Pelegrí, S. P. (2000). Enhancement of pico- and nanoplankton growth by coral exudates. *Aquat. Microb. Ecol.* **21**, 203-209.

Ferrier-Pagès, C., Richard, C., Forcioli, D., Allemand, D., Pichon, M. and Shick, J. M. (2007). Effects of temperature and UV radiation increases on the photosynthetic efficiency in four scleractinian coral species. *Biol. Bull.* **213**, 76-87.

Fitt, K. W. and Warner, M. E. (1995). Bleaching patterns of four species of Caribbean reef corals. *Biol. Bull.* **189**, 298-307.

Fitt, W. K., Brown, B. E., Warner, M. E. and Dunne, R. P. (2001). Coral bleaching: interpretation of thermal tolerance limits and thermal thresholds in tropical corals. *Coral Reefs* **20**, 51-65.

Fitt, W. K., Gates, R. D., Hoegh-Guldberg, O., Bythell, J. C., Jatkar, A., Grottoli, A. G., Gomez, M., Fisher, P., Lajuenesse, T. C., Pantos, O. et al. (2009). Response of two species of Indo-Pacific corals, *Porites cylindrica* and *Stylophora pistillata*, to short-term thermal stress: the host does matter in determining the tolerance of corals to bleaching. *J. Exp. Mar. Bio. Ecol.* **373**, 102-110.

Fonvielle, J. A., Reynaud, S., Jacquet, S., LeBerre, B. and Ferrier-Pages, C. (2015). First evidence of an important organic matter trophic pathway between temperate corals and pelagic microbial communities. *PLoS ONE* **10**, 1-22.

Furla, P., Galgani, I., Durand, I. and Allemand, D. (2000). Sources and mechanisms of inorganic carbon transport for coral calcification and photosynthesis. *J. Exp. Biol.* **203**, 3445-3457.

Gattuso, J.-P. and Jaubert, J. (1990). Effect of light on oxygen and carbon dioxide fluxes and on metabolic quotients measured in situ in a zooxanthellate coral. *Limnol. Oceanogr.* **35**, 1796-1804.

Gattuso, J.-P., Allemand, D. and Frankignoulle, M. (1999). Photosynthesis and calcification at cellular, organismal and community levels in coral reefs: a review on interactions and control by carbonate chemistry. *Am. Zool.* **39**, 160-183.

Grottoli, A. G., Warner, M. E., Levas, S. J., Aschaffenburg, M. D., Schoepf, V., McGinley, M., Baumann, J. and Matsui, Y. (2014). The cumulative impact of annual coral bleaching can turn some coral species winners into losers. *Glob. Chang. Biol.* **20**, 3823-3833.

Grottoli, A. G., Rodrigues, L. J. and Palardy, J. E. (2006). Heterotrophic plasticity and resilience in bleached corals. *Nature* **440**, 1186-1189.

Haas, A. F., Jantzen, C., Naumann, M. S., Iglesias-Prieto, R. and Wild, C. (2010). Organic matter release by the dominant primary producers in a Caribbean reef lagoon: implication for in situ O_2 availability. *Mar. Ecol. Prog. Ser.* **409**, 27-39.

Haas, A. F., Nelson, C. E., Rohwer, F., Wegley-Kelly, L., Quistad, S. D., Carlson, C. A., Leichter, J. J., Hatay, M. and Smith, J. E. (2013). Influence of coral and algal exudates on microbially mediated reef metabolism. *PeerJ* **1**, e108.

Haas, A. F., Fairoz, M. F. M., Kelly, L. W., Nelson, C. E., Dinsdale, E. A., Edwards, R. A., Giles, S., Hatay, M., Hisakawa, N. and Knowles, B. (2016). Global microbialization of coral reefs. *Nat. Microbiol.* **1**, 16042.

Häder, D. P., Helbling, E. W., Williamson, C. E. and Worrest, R. C. (2007). Effects of UV radiation on aquatic ecosystems and interactions with climate change. *Photochem. Photobiol. Sci.* **10**, 173.

Herndl, G. J. and Velimirov, B. (1986). Microheterotrophic utilization of mucus released by the Mediterranean coral *Cladocora cespitosa*. *Mar. Biol.* **90**, 363-369.

Hoegh-Guldberg, O. (1999). Climate change, coral bleaching and the future of the world's coral reefs. *Mar. Freshw. Res.* **50**, 839-866.

Hoogenboom, M., Beraud, E. and Ferrier-Pagès, C. (2010). Relationship between symbiont density and photosynthetic carbon acquisition in the temperate coral *Cladocora caespitosa*. *Coral Reefs* **29**, 21-29.

Hoppe, H.-G. (1983). Significance of exoenzymatic activities in the ecology of brackish water: measurements by means of methylumbelliferyl-substrates. *Mar. Ecol. Prog. Ser.* **11**, 299-308.

Houlbrèque, F. and Ferrier-Pagès, C. (2009). Heterotrophy in tropical scleractinian corals. *Biol. Rev.* **84**, 1-17.

Houlbrèque, F., Tambutté, E., Allemand, D. and Ferrier-Pagès, C. (2004). Interactions between zooplankton feeding, photosynthesis and skeletal growth in the scleractinian coral *Stylophora pistillata*. *J. Exp. Biol.* **207**, 1461-1469.

Hughes, A. D. and Grottoli, A. G. (2013). Heterotrophic compensation: A possible mechanism for resilience of coral reefs to global warming or a sign of prolonged stress? *PLoS ONE* **8**, 1-10.

Jacquet, S., Dorigo, U. and Personnic, S. (2013). A few tests prior to flow cytometry and epifluorescence analyses of freshwater bacterio-and virioplankton communities. *FLOW Cytom* **1**, 1-30.

Jeffrey, S. W. and Humphrey, G. F. (1975). New spectrophotometric equations for determining chlorophylls a, b, c_1 and c_2 in higher plants, algae and natural phytoplankton. *Biochemical Physiology Pflanz* **167**, 191-194.

Keil, R. G. and Kirchman, D. L. (1991). Contribution of dissolved free amino acids and ammonium to the nitrogen requirements of heterotrophic bacterioplankton. *Mar. Ecol. Prog. Ser* **73**, 1-10.

Lesser, M. P. and Farrell, J. H. (2004). Exposure to solar radiation increases damage to both host tissues and algal symbionts of corals during thermal stress. *Coral Reefs* **23**, 367-377.

Lesser, M. P., Stochaj, W. R., Tapley, D. W. and Shick, J. M. (1990). Bleaching in coral reef anthozoans: effects of irradiance, ultraviolet radiation, and temperature on the activities of protective enzymes against active oxygen. *Coral Reefs* **8**, 225-232.

Levas, S. J., Grottoli, A. G., Hughes, A., Osburn, C. L. and Matsui, Y. (2013). Physiological and biogeochemical traits of bleaching and recovery in the mounding species of coral *Porites lobata*: implications for resilience in mounding corals. *PLoS ONE* **8**, e63267.

Levas, S., Grottoli, A. G., Warner, M. E., Cai, W. J., Bauer, J., Schoepf, V., Baumann, J. H., Matsui, Y., Gearing, C., Melman, T. F. et al. (2015). Organic carbon fluxes mediated by corals at elevated pCO_2 and temperature. *Mar. Ecol. Prog. Ser.* **519**, 153-164.

Levas, S., Grottoli, A. G., Schoepf, V., Aschaffenburg, M., Baumann, J., Bauer, J. E. and Warner, M. E. (2016). Can heterotrophic uptake of dissolved organic carbon and zooplankton mitigate carbon budget deficits in annually bleached corals? *Coral Reefs* **35**, 495-506.

Moberg, F. and Folke, C. (1999). Ecological goods and services of coral reef ecosystems. *Ecol. Econ.* **29**, 215-233.

Muscatine, L. and Porter, J. W. (1977). Reef corals: mutualistic symbioses adapted to nutrient-poor environments. *Bioscience* **27**, 454-460.

Muscatine, L., McCloskey, L. R. and Marian, R. E. (1981). Estimating the daily contribution of carbon from zooxanthellae to coral animal respiration. *Limnol. Oceanogr.* **26**, 601-611.

Nakajima, R., Yoshida, T., Azman, B. A. R., Zaleha, K., Othman, B. H. R. and Toda, T. (2009). In situ release of coral mucus by *Acropora* and its influence on the heterotrophic bacteria. *Aquat. Ecol.* **43**, 815-823.

Nakajima, R., Yoshida, T., Fujita, K., Nakayama, A., Fuchinoue, Y., Othman, B. H. R. and Toda, T. (2010). Release of particulate and dissolved organic carbon by the scleractinian coral *Acropora formosa*. *Bull. Mar. Sci.* **86**, 861-870.

Naumann, M. S., Haas, A., Struck, U., Mayr, C., El-Zibdah, M. and Wild, C. (2010). Organic matter release by dominant hermatypic corals of the Northern Red Sea. *Coral Reefs* **29**, 649-659.

Nelson, C. E., Goldberg, S. J., Kelly, L. W., Haas, A. F., Smith, J. E., Rohwer, F. and Carlson, C. A. (2013). Coral and macroalgal exudates vary in neutral sugar composition and differentially enrich reef bacterioplankton lineages. *ISME J.* **7**, 962-979.

Nicholls, R. J., Wong, P. P., Burkett, V. R., Codignotto, J. O., Hay, J. E., McLean, R. F., Ragoonaden, S. and Woodroffe, D. C. (2007). Coastal systems and low-lying areas. *Assessment* 315-356.

Niggl, W., Glas, M., Laforsch, C., Mayr, C. and Wild, C. (2008). First evidence of coral bleaching stimulating organic matter release by reef corals. In Proceedings of the 11th International Coral Reef Symposium', pp. 905-910.

Price, P. B. and Sowers, T. (2004). Temperature dependence of metabolic rates for microbial growth, maintenance, and survival. *Proc. Natl. Acad. Sci. USA* **101**, 4631-4636.

Ralph, P. J. and Gademann, R. (2005). Rapid light curves: a powerful tool to assess photosynthetic activity. *Aquat. Bot.* **82**, 222-237.

Ribes, M., Coma, R. and Gili, J.-M. (1998). Heterotrophic feeding by gorgonian corals with symbiotic zooxanthella. *Limnol. Oceanogr.* **43**, 1170-1179.

Richter, C., Wunsch, M., Rasheed, M., Kötter, I. and Badran, M. I. (2001). Endoscopic exploration of Red Sea coral reefs reveals dense populations of cavity-dwelling sponges. *Nature* **413**, 726-730.

Saba, G. K., Steinberg, D. K. and Bronk, D. A. (2011). The relative importance of sloppy feeding, excretion, and fecal pellet leaching in the release of dissolved carbon and nitrogen by *Acartia tonsa* copepods. *J. Exp. Mar. Bio. Ecol.* **404**, 47-56.

Sharma, P., Jha, A. B., Dubey, R. S. and Pessarakli, M. (2012). Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *J. Bot.* **2012**, 1-26.

Shick, J. M., Lesser, M. P., Dunlap, W. C., Stochaj, W. R., Chalker, B. E. and Won, J. W. (1995). Depth-dependent responses to solar ultraviolet radiation and oxidative stress in the zooxanthellate coral *Acropora microphthalma*. *Mar. Biol.* **122**, 41-51.

Smith, S. V. and Key, G. S. (1975). Carbon dioxide and metabolism in marine environments. *Limnol. Oceanogr.* **20**, 493-495.

Smith, P. K., Krohn, R. I., Hermanson, G. T., Mallia, A. K., Gartner, F. H., Provenzano, M. D., Fujimoto, E. K., Goeke, N. M., Olson, B. J. and Klenk, D. C. (1985). Measurement of protein using bicinchoninic acid. *Anal. Biochem.* **150**, 76-85.

Stimson, J. and Kinzie, R. A. (1991). The temporal pattern and rate of release of zooxanthellae from the reef coral *Pocillopora damicornis* (Linnaeus) under nitrogen-enrichment and control conditions. *J. Exp. Mar. Bio. Ecol.* **153**, 63-74.

Tanaka, Y., Miyajima, T., Koike, I., Hayashibara, T. and Ogawa, H. (2008). Production of dissolved and particulate organic matter by the reef-building corals *Porites cylindrica* and *Acropora pulchra*. *Bull. Mar. Sci.* **82**, 237-245.

Tanaka, Y., Miyajima, T., Umezawa, Y., Hayashibara, T., Ogawa, H. and Koike, I. (2009). Net release of dissolved organic matter by the scleractinian coral *Acropora pulchra*. *J. Exp. Mar. Bio. Ecol.* **377**, 101-106.

Tremblay, P., Naumann, M. S., Sikorski, S., Grover, R. and Ferrier-Pagès, C. (2012). Experimental assessment of organic carbon fluxes in the scleractinian coral *Stylophora pistillata* during a thermal and photo stress event. *Mar. Ecol. Prog. Ser.* **453**, 63-77.

Veron, J. V. (2000). *Corals of the World*. (ed. Australian Institute of Marine Science). Odyssey Publishing: Townsville.

Vodacek, A., Blough, N. V., DeGrandpre, M. D., Peltzer, E. T. and Nelson, R. K. (1997). Seasonal variation of CDOM and DOC in the Middle Atlantic Bight: Terrestrial inputs and photooxidation. *Limnol. Oceanogr.* **42**, 674-686.

Watanabe, S., Sudo, K., Nagashima, T., Takemura, T., Kawase, H. and Nozawa, T. (2011). Future projections of surface UV-B in a changing climate. *J. Geophys. Res. Atmos.* **116**, D16118.

Wham, D. C., Ning, G. and LaJeunesse, T. C. (2017). Symbiodinium glynnii sp. nov., a species of stress-tolerant symbiotic dinoflagellates from pocilloporid and montiporid corals in the Pacific Ocean. *Phycologia* **56**, 396-409.

Wild, C., Huettel, M., Klueter, A., Kremb, S. G., Rasheed, M. Y. M. and Jørgensen, B. B. (2004a). Coral mucus functions as an energy carrier and particle trap in the reef ecosystem. *Nature* **428**, 66-70.

Wild, C., Rasheed, M., Werner, U., Franke, U., Johnsone, R. and Huettel, M. (2004b). Degradation and mineralization of coral mucus in reef environments. *Mar. Ecol. Prog. Ser.* **267**, 159-171.

Wild, C., Woyt, H. and Huettel, M. (2005). Influence of coral mucus on nutrient fluxes in carbonate sands. *Mar. Ecol. Prog. Ser.* **287**, 87-98.

Wild, C., Mayr, C., Wehrmann, L., Schöttner, S., Naumann, M., Hoffmann, F. and Rapp, H. (2008). Organic matter release by cold water corals and its implication for fauna–microbe interaction. *Mar. Ecol. Prog. Ser.* **372**, 67-75.

Wild, C., Niggl, W., Naumann, M. S. and Haas, A. F. (2010a). Organic matter release by Red Sea coral reef organisms-Potential effects on microbial activity and in situ O_2 availability. *Mar. Ecol. Prog. Ser.* **411**, 61-71.

Wild, C., Naumann, M., Niggl, W. and Haas, A. (2010b). Carbohydrate composition of mucus released by scleractinian warm-and cold-water reef corals. *Aquat. Biol.* **10**, 41-45.

Wilkinson, C., Lindén, O., Cesar, H., Hodgson, G., Rubens, J. and Strong, A. E. (1999). Ecological and socioeconomic impacts of 1998 coral mortality in the Indian Ocean: An ENSO impact and a warning of future change? *Ambio* **28**, 188-196.

Wooldridge, S. A. (2009). A new conceptual model for the enhanced release of mucus in symbiotic reef corals during "bleaching" conditions. *Mar. Ecol. Prog. Ser.* **396**, 145-152.

S1 Table. Results of tri-factor ANOVA testing the effect of time, UVR and temperature on variables. Significant values ($p<0.05$) are highlighted in red.

Variables	Conditions	Df	F values	p values
Symbiont density (cm^{-2})	Time	1	43	$2x10^{-7}$
	UV	1	55	$2x10^{-8}$
	T	1	386	$<2x10^{-16}$
	Time: UV	1	0.8	0.4
	Time:T	1	17	$2x10^{-4}$
	UV:T	1	12	$2x10^{-3}$
	Time:UV:T	1	0.2	0.6
Chlorophyll a content ($\mu g\ cm^{-2}$)	Time	1	15	$5x10^{-4}$
	UV	1	36	$1x10^{-6}$
	T	1	277	$<2x10^{-16}$
	Time: UV	1	0.3	0.5
	Time:T	1	23	$4x10^{-5}$
	UV:T	1	13	$1x10^{-3}$
	Time:UV:T	1	0.04	0.8
Protein content ($mg\ cm^{-2}$)	Time	1	99	$1x10^{-10}$
	UV	1	24	$4x10^{-5}$
	T	1	76	$2x10^{-9}$
	Time: UV	1	0.006	0.9
	Time:T	1	11	$3x10^{-3}$
	UV:T	1	0.5	0.5
	Time:UV:T	1	7.7	$1x10^{-2}$
$rETR_{max}$	Time	1	47	$7x10^{-7}$
	UV	1	48	$6x10^{-7}$
	T	1	118	$3x10^{-10}$
	Time: UV	1	0.6	0.4
	Time:T	1	40	$2x10^{-6}$
	UV:T	1	4	0.05
	Time:UV:T	1	3	0.09
Chlorophyll a content ($pg\ cell^{-1}$)	Time	1	14	0.4
	UV	1	3	0.1
	T	1	0.2	0.6
	Time: UV	1	0.6	0.4
	Time:T	1	1	0.3
	UV:T	1	1	0.3
	Time:UV:T	1	4	0.04

S2 Table. Results of two-factor ANOVA testing the effect of UVR and temperature on variables after two weeks of thermal stress. Significant values ($p<0.05$) are highlighted in red.

Variables	Conditions	Df	F values	p values
Pn ($\mu mol\ h^{-1}\ cm^{-2}$)	UV	1	5	0.05
	T	1	58	**$2x10^{-5}$**
	UV:T	1	3	0.1
R ($\mu mol\ h^{-1}\ cm^{-2}$)	UV	1	1	0.3
	T	1	71	**$4x10^{-6}$**
	UV:T	1	2	0.2
Calcification rate ($\mu mol\ d^{-1}\ cm^{-2}$)	UV	1	0.5	0.5
	T	1	2	0.2
	UV:T	1	3	0.1
TOC ($\mu g\ h^{-1}\ cm^{-2}$)	UV	1	0.4	0.5
	T	1	5	0.05
	UV:T	1	2	0.2
TON ($\mu g\ h^{-1}\ cm^{-2}$)	UV	1	0.5	0.5
	T	1	0.5	0.5
	UV:T	1	1	0.3
POC ($\mu g\ h^{-1}\ cm^{-2}$)	UV	1	4	0.07
	T	1	62	**$4x10^{-6}$**
	UV:T	1	2	0.2
PON ($\mu g\ h^{-1}\ cm^{-2}$)	UV	1	5	**0.04**
	T	1	55	**$4x10^{-5}$**
	UV:T	1	3	0.1
DOC ($\mu g\ h^{-1}\ cm^{-2}$)	UV	1	1	0.3
	T	1	128	**$1x10^{-6}$**
	UV:T	1	0.5	0.5
DON ($\mu g\ h^{-1}\ cm^{-2}$)	UV	1	1	0.3
	T	1	55	**$2x10^{-5}$**
	UV:T	1	0.2	0.6
Autotroph growth rate (h^{-1})	UV	1	0.2	**$2x10^{-4}$**
	T	1	0.1	**$5x10^{-4}$**
	UV:T	1	0.2	**$4x10^{-4}$**
Autotroph Ingestion rate ($prey\ h^{-1}\ cm^{-2}$)	UV	1	0.5	0.5
	T	1	28	**$3x10^{-4}$**
	UV:T	1	60	**$9x10^{-6}$**
Prokaryote growth rate (h^{-1})	UV	1	0.2	0.6
	T	1	69	**$5x10^{-6}$**

Prokaryote ingestion rate (prey h^{-1} cm^{-2})	UV:T	1	0.3	0.6
	UV	1	4	0.06
	T	1	62	**$4x10^{-6}$**
Glucosidase EEA_{max} (µg h^{-1} cm^{-2})	UV:T	1	0.4	0.5
	UV	1	8	**0.02**
	T	1	37	**$1x10^{-4}$**
Aminopeptidase EEA_{max} (µg h^{-1} cm^{-2})	UV:T	1	0.1	0.8
	UV	1	7	**0.02**
	T	1	78	**$1x10^{-6}$**
Pn per symbiont cell (µmol h^{-1} $cell^{-1}$)	UV:T	1	0.8	0.4
	UV	1	5	0.06
	T	1	0.02	0.9
R per symbiont cell (µmol h^{-1} $cell^{-1}$)	UV:T	1	4	0.07
	UV	1	25	**$2x10^{-4}$**
	T	1	139	**$3x10^{-9}$**
CZAR (%)	UV:T	1	23	**$2x10^{-4}$**
	UV	1	0.7	0.4
	T	1	45	**$1x10^{-4}$**
$CHAR_{POC}$ (%)	UV:T	1	0.2	0.6
	UV	1	3	0.1
	T	1	114	**$4x10^{-5}$**
$CHAR_{DOC}$ (%)	UV:T	1	5	0.06
	UV	1	0.3	0.6
	T	1	87	**$1x10^{-5}$**
$CHAR_{TOC}$ (%)	UV:T	1	0.3	0.6
	UV	1	0	1
	T	1	10	0.06
	UV:T	1	1	0.3

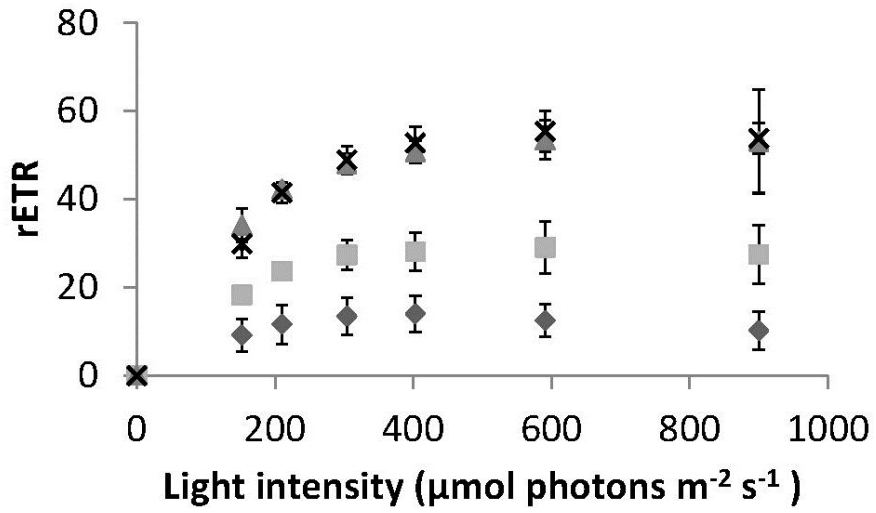


Figure S1. Rapid light curves. Rapid light curves at normal temperature (26°C) with (triangles) or without (crosses) UV radiation or at high temperature (30°C) with (diamonds) or without (squares) UV radiation. Data are mean and standard deviations of five replicates.

Paper n°4 – *in prep.*

Seasonal variability in the physiology and composition of the coral *Acropora muricata* holobiont under different ultra-violet radiation levels

Lucile Courtial[1,2,3], Jeroen A.J.M. van de Water[2], Fanny Houlbrèque[3¶*], Christine Ferrier-Pagès[2¶*]

1. Sorbonne Universités, UPMC Univ Paris 06 IFD-ED 129, 4 Place Jussieu, 75252 PARIS cedex 05, France
2. Centre Scientifique de Monaco, Equipe Ecophysiologie, 8 Quai Antoine 1er, 98000 Monaco (Principality)
3. UMR ENTROPIE (UMR250), Laboratoire d'Excellence « CORAIL», BP A5, 98848, Nouméa cedex, New Caledonia

This work was conducted in Nouméa, New Caledonia.

*Corresponding authors: Fanny Houlbrèque, IRD Nouméa, 101 Promenade Roger Laroque, Nouméa 98848, Nouvelle Calédonie ; (+687) 26 07 46 ; fanny.houlbreque@ird.fr- Christine Ferrier-Pagès, Centre Scientifique de Monaco, 8 Quai Antoine Ier, 98000 Monaco, +37797774480 ; ferrier@centrescientifique.mc

[¶]These authors contributed equally to the work

Running title: Seasonal and UVR effects on *A. muricata* holobiont

23 **Abstract**

24 Seasonal variations of the physico-chemical parameters of coral reef waters, as well as
25 exposure to ultraviolet radiation (UVR), affect the population dynamic and reproductive
26 cycles of microorganisms and scleractinian corals, the reef engineers. This study is one of the
27 first to investigate the seasonal variability, under different UVR levels, in the physiology and
28 holobiont composition of *Acropora muricata*. Our results highlight distinct communities
29 between mucus and coral microbiomes that varied with seasons and were differently affected
30 by UVR. The mucus-associated microbiome, affected by UVR level, was restricted to 8
31 ubiquitous bacteria, specialized in the degradation of complex carbon compounds (*Oleibacter,*
32 *Aestuariibacter*) or adapted to hypoxic environments (*Arenicella)*. The dominance of
33 *Oleibacter* in the mucus microbiome in summer and the enhanced enzymatic activities
34 observed were linked to the large availability in dissolved organic carbon in this mucus. The
35 microbiome associated with the coral host, unaffected by UVR level, contained many more
36 bacterial OTUs than the mucus microbiome. Although coral microbiome differed significantly
37 between seasons, the *Endozoicomonas-A. muricata* association persisted throughout the
38 seasons, with however a shift in the relative abundance of *Endozoicomonas*-related OTUs,
39 together with a change in coral physiology. The seasonal difference in *Endozoicomonas*-OTU
40 might be linked to differences in the nutritional host's requirements.

41 **Originality-Significance Statement.** This study is one of the first to provide an overview of
42 the seasonal and UVR-induced changes in both the coral physiology and the microbiome
43 associated with the tissue and mucus of the widely distributed scleractinian coral *Acropora*
44 *muricata*.

45

Introduction

Population dynamics and reproductive cycles of many species are influenced by seasonal pulses of nutrients and/or variations in environmental factors (Clarke, 1988; Gooday *et al.*, 1990; Coma *et al.*, 2000) leading to a long-standing interest in the way organisms react to the changing of seasons. Tropical corals live in a relatively stable environment, but still experience changes in sea surface temperature, photosynthetically active radiation (PAR), ultra-violet radiation (UVR), and eventually nutrients (brought by heavy rains) between winter and summer (Brown *et al.*, 1999). Seasonality is reflected in the coral physiology, such as calcification rates (Crossland, 1984), inorganic carbon acquisition (Warner *et al.*, 2002; Ulstrup *et al.*, 2008) and symbiont density within the host tissue (Brown *et al.*, 1999; Fitt *et al.*, 2000), as well as differences in disease prevalence (Gil-Agudelo and Garzón-Ferreira, 2001; Willis *et al.*, 2004). These physiological parameters are particularly affected in summer by elevated temperature, PAR and UVR levels, which may induce bleaching (loss of symbionts and/or photosynthetic pigments), as well as decreases in photosynthesis and calcification rates (Shick *et al.*, 1996; Hoegh-Guldberg, 1999).

While previous studies have focused on the seasonal changes in the physiology of the animal host and photosynthetic symbionts, seasonal patterns in the community composition of the other microbial partners within the coral holobiont (*sensu* Rohwer et al. 2001) have been poorly investigated. Corals are associated with a diverse array of microorganisms, (e.g. fungi, bacteria, archaea and viruses), which likely play an active role in maintaining coral nutrition and health (Rosenberg *et al.*, 2007; Bourne and Webster, 2013). Several microniches, including the mucus layer, the coral tissues, and the skeleton provide habitats for this diverse microbial life (Sweet *et al.*, 2011; Bourne and Webster, 2013). Experimental studies showed that several biotic and abiotic factors, such as temperature, pH, light, nutrient and dissolved organic carbon availablity cause shifts in coral-associated bacterial diversity (Chen *et al.*, 2011; Chiu *et al.*, 2012; Bourne and Webster, 2013; Sweet *et al.*, 2013; Li *et al.*, 2014). However, no clear trend can be deduced from these studies, as results were not performed over a seasonal cycle, not linked to the coral physiological state, and sometimes contradictory. For example, Gamma-proteobacteria have been shown to dominate coral microbiomes either in winter (Hong *et al.*, 2009; Ceh *et al.*, 2011), or summer during coral bleaching (Bourne *et al.*, 2008; Littman *et al.*, 2010; Kimes *et al.*, 2013). These discrepancies may be due to differences in host physiology and/or the photosynthetic activity of the algal symbionts, which may affect the coral-associated bacterial communities either directly, or indirectly through

79 modifications in the mucus composition (Brown and Bythell, 2005; Thompson *et al.*, 2014).
80 High UVR levels in summer may also disturb both tissue and mucus-associated bacteria as
81 UVR is one of the primary stressors for a variety of marine organisms (Lesser, 2006) and has
82 significant impacts on bacterial biofilms (Elasri and Miller, 1999; Arrieta *et al.*, 2000; Winter
83 *et al.*, 2001; Elifantz *et al.*, 2013). These observations highlight the importance to better
84 understand the seasonal variations in the coral microbiome, which may influence the health of
85 corals and their susceptibility to disease or bleaching (Ritchie, 2006).

86 To this end, we aimed to investigate changes in the bacterial communities associated
87 with tissue and mucus of the scleractinian coral *Acropora muricata* between two different
88 seasons (summer and winter). *A. muricata* is a dominant coral species in New Caledonia,
89 whose populations have severely suffered from mass bleaching events in summer 2016. To
90 better understand which factors contribute to the observed changes in bacterial communities,
91 we monitored the coral physiology, as well as the quantity and quality of the released mucus,
92 under normal growth conditions and under reduced UVR in parallel. Here, we describe that
93 the coral- and mucus-associated bacterial communities were highly distinct and varied with
94 seasons, but were differentially affected by UVR. Overall, our results provide novel insights
95 into how environmental conditions influence the interactions between corals and their resident
96 microbes.

97 **Results**

98 *Coral tissue parameters and mucus excretion*

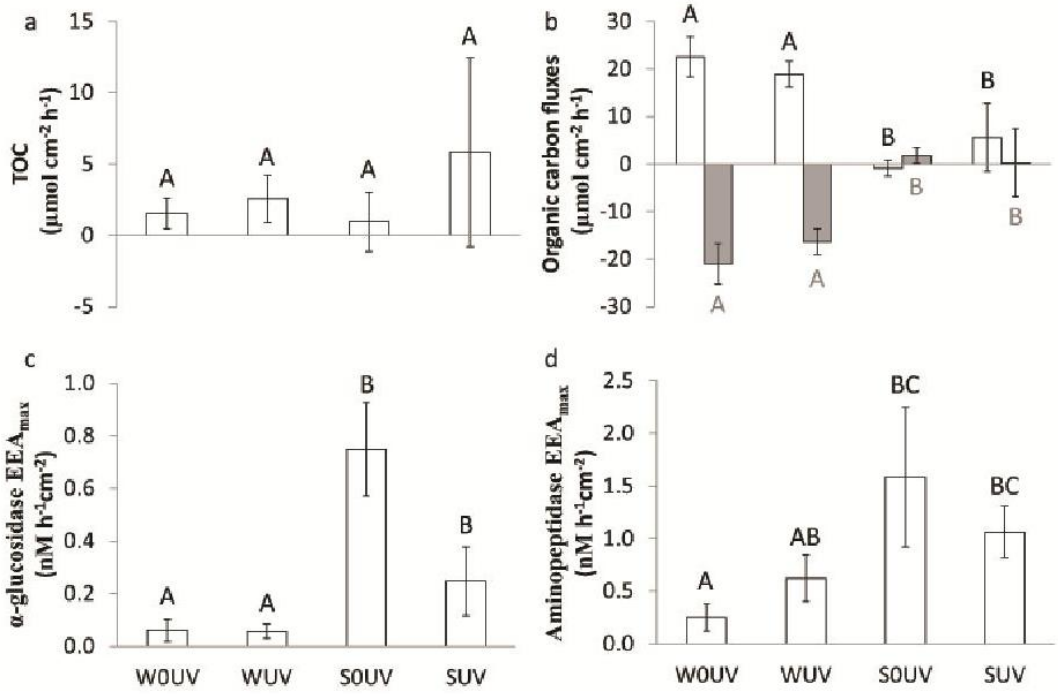
99 Coral tissue parameters and calcification rates were measured during both seasons,
100 under UV and reduced UV radiation conditions. In winter, natural UVR exposure had no
101 effect on coral physiology, except on the $rETR_{max,}$ which increased by 140% (Table 1). On the
102 contrary, UVR in summer exposure significantly decreased the rETR by 67% and increased
103 the chl *a* content. Under natural UVR exposure, the season had no effect on the symbiont
104 density or chl *a* content (Table S1). However, in summer the protein content and the $rETR_{max}$
105 were decreased by 67% and 54%, respectively compared to winter, while the calcification was
106 increased by 157% (Table 1, Table S1).

107 Positive fluxes of organic matter (OM) in seawater will be considered as OM release
108 by the corals while negative fluxes will be considered as OM uptake. Overall, corals released
109 total organic carbon at similar rates (2.8 ± 4.0 µg C cm^{-2} h^{-1}) in all conditions (Fig. 1a, Table

Table 1. Effects of season and UVR on coral physiology. "+" indicates higher values in summer than in winter or under UVR compared to no UVR, " –" indicates the opposite, "Ø" indicates no effects. Indicated values are means ± standard deviation, S indicates Summer and W indicates winter values.

Biological parameters	Effects of season under ambiant UVR (S compared to W)	Effects of UVR in winter (UV compared to 0UV)	Effects of UVR in summer (UV compared to 0UV)
Symbiont density ($10^5 cm^{-2}$)	Ø	Ø	Ø
	S: 3.43 ± 1.6 - W: 4.76 ± 1.0	UV: 4.76 ± 1.0 - 0UV: 3.28 ± 0.9	UV: 3.43 ± 1.6 - 0UV: 2.65 ± 0.9
Chl*a* content ($mg\ cm^{-2}$)	Ø	Ø	+
	S: 0.25 ± 0.07 - W: 0.31 ± 0.06	UV: 0.31 ± 0.06 - 0UV: 0.28 ± 0.02	UV: 0.25 ± 0.07 - 0UV: 0.16 ± 0.05
Protein content ($mg\ cm^{-2}$)	-	Ø	Ø
	S: 0.45 ± 0.1 - W: 0.67 ± 0.1	UV: 0.67 ± 0.1 - 0UV: 0.56 ± 0.08	UV: 0.45 ± 0.1 - 0UV: 0.48 ± 0.08
rETR	-	+	-
	S: 22.6 ± 3.2 - W: 41.7 ± 1.8	UV: 41.7 ± 1.8 - 0UV: 29.6 ± 2.5	UV: 22.6 ± 3.2 - 0UV: 33.5 ± 5.3
Calcification rate ($\mu mol\ cm^{-2}\ d^{-1}$)	+	Ø	Ø
	S: 19.7 ± 1.7 - W: 12.5 ± 2.5	UV: 12.5 ± 2.5 - 0UV: 12.3 ± 2.2	UV: 19.7 ± 1.7 - 0UV 22.8 ± 2.7

Figure 1. Organic matter fluxes and mucus associated bacterial activity. Total organic carbon (TOC) fluxes (a), particulate (white columns) and dissolved (grey columns) organic carbon fluxes (b), alpha-glucosidase maximal extracellular enzymatic activity (EEA_{max}, c), aminopeptidase EEA_{max} organic nitrogen fluxes (d) in winter without UVR (W0UV), winter under UVR (WUV), summer without UVR (S0UV) and summer under UVR (SUV). Data are mean and standard deviations of five replicates. Values noted with the same letter are not significantly different (*p-value* > 0.05).



S1). Season and UVR had however interactive significant effects on particulate (POC) organic matter and season on dissolved organic matter (DOC) fluxes. They both showed inverse trends in winter (Fig. 1b), with a net release of POC and uptake of DOC with and without UVR. In summer, fluxes were very low with no net release or uptake of DOC and POC. UVR effects on POC were low and tend to increase the release in summer. TN fluxes were ten to thirty times lower than TOC fluxes. Despite a trend for UVR to increase TN release, no clear information in TN fluxes was found under the different treatments because of the high variability among samples of a same condition (Fig. S1).

Identity and variability of A. muricata *mucus associated bacteria*

To assess the effects of seasonal changes of stress on the resident mucus-associated bacteria, we performed differential abundance analysis to select OTUs that were statistically significantly more abundant in the mucus compared with seawater. Only eight OTUs were found to match this criterion and were consistently present in all mucus samples (Table 2; Supplementary dataset). Four of these OTUs were assigned to the Gammaproteobacteria and belonged to the genera *Aestuariibacter* (OTU 5084), *Arenicella* (OTU 48357), *Coxiella* (OTU 62614) and *Oleibacter* (OTU 37807), while three OTUs were assigned to the Alphaproteobacteria orders DB1-14 (OTU 5726 and 24581) and Rickettsiales (OTU 56046) (Table 2). In addition, one OTU was identified as genus Candidatus *Rhabdochlamydia* (OTU 52824), from the Chlamydiae class (Table 2).

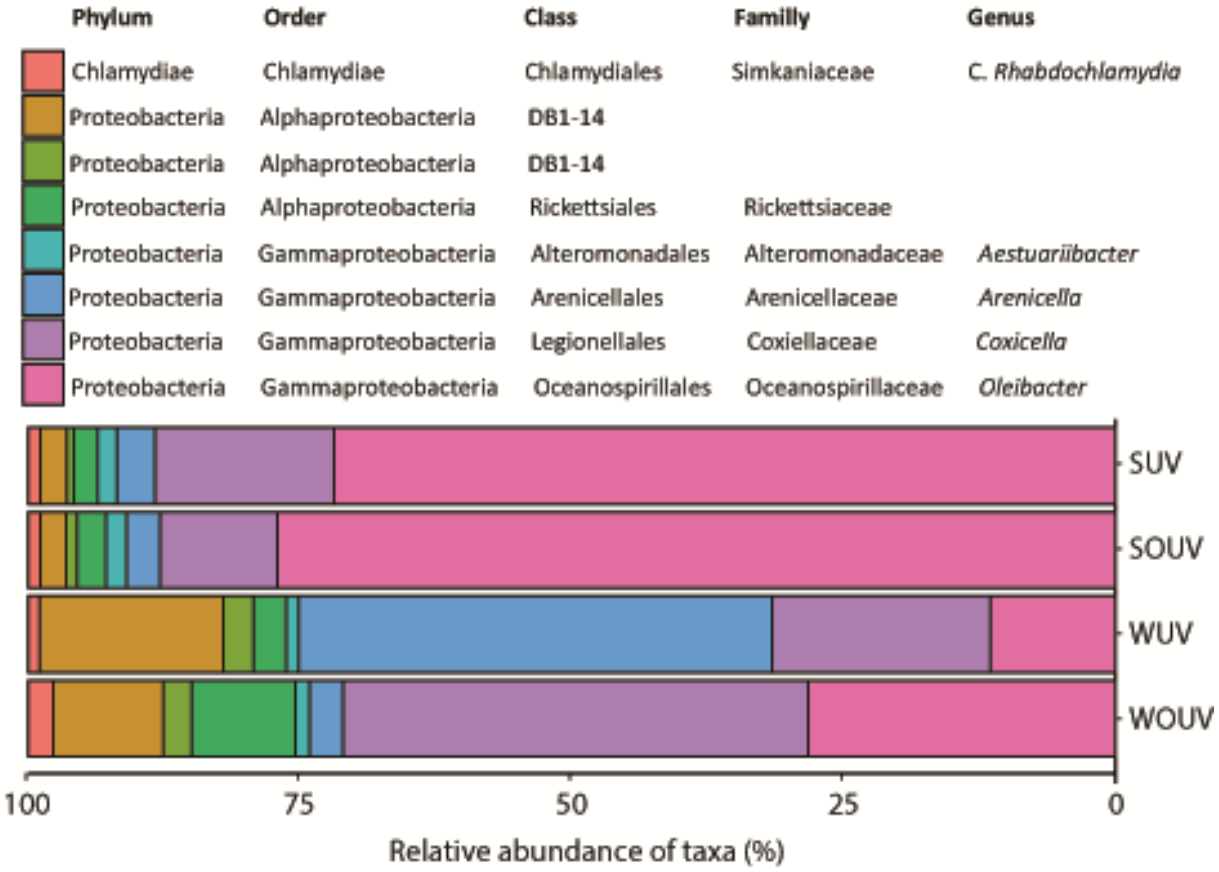
The bacterial communities varied with seasons (p=0.001, Table S2) and an interactive effect of UVR with season was also found (p=0.043). Seasonal differences were characterized by a higher abundance in summer of the *Oleibacter* and *Aestuariibacter* OTUs, but a lower abundance of *Arenicella*, *Coxiella* and DB1-14 (OTU 5726) (Fig. 2). In summer, the mucus-specific microbiome was dominated by *Oleibacter* and *Coxiella* and was unaffected by UVR exposure. In winter, however, it had a significant effect in winter (p=0.012). UVR exposure indeed induced an increase of *Arenicella,* both BD1-14 OTUs while it decreased the populations of *Oleibacter*, *Coxiella* and bacteria from the *Rickettsiales* family. The community under UVR was therefore dominated, in this order, by *Arenicella*, *Coxiella*, DB1-14 (OTU 5726) and *Oleibacter*, while the community shaded from UVR was dominated, in this order by *Coxiella*, *Oleibacter,* DB1-14 (OTU 5726) and bacteria from the *Rickettsiales* family.

Activity of the mucus associated bacteria

Table 2. Summary of OTUs found in *A. muricata* mucus.

Phylum	Class	Order	Family	Genus	OTU	Information on the genus
Proteobacteria	Alphaproteobacteria	DB1-14	-	-	24581	-
Proteobacteria	Alphaproteobacteria	DB1-14	-	-	5726	-
Proteobacteria	Alphaproteobacteria	Rickettsiales	Rickettsiaceae	-	56046	Found in reef building corals (Casas et al 2004). Most human pathogens in this family are in genus *Rickettsia* . They spend part of their lifecycle in the bodies of arthropods.
Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*Aestuariibacter*	5084	Found in corals of the red sea (Lee et al 2012); four known species (Yi et al. 2004)
Proteobacteria	Gammaproteobacteria	Arenicellales	Arenicellaceae	*Arenicella*	48357	Found in *Platigyra carnosus* (Ng et al 2015)
Proteobacteria	Gammaproteobacteria	Legionellales	Coxiellaceae	*Coxiella*	62614	Pathogens of mammals or symbiotic of *Carios capensis* (Piel 2004; Machado-Ferreira et al. 2016)
Proteobacteria	Gammaproteobacteria	Oceanospirillales	Oceanospirillaceae	*Oleibacter*	37807	Found in *Acropora muricata* (Sweet and Bulling 2017) ; degrades petroleum aliphatic hydrocarbons
Chlamydiae	Chlamydiae	Chlamydiales	Simkaniaceae	Candidatus *Rhabdochlamydia*	52824	Found in *Aiptasia* (Röthig et al 2016)

Figure 2. Relative abundance of the mucus associated bacteria in winter without UVR (W0UV), winter under UVR (WUV), summer without UVR (S0UV) and summer under UVR (SUV).



142 α-glucosidase and aminopeptidase EEAmax of the mucus-associated bacteria were
143 increased in summer regardless of the UVR level (Fig. 1cd, Table S1).

144 *Diversity of bacteria associated with the coral* A. muricata *and seasonal variability*

145 The bacterial community associated to *A. muricata* was distinct (Supplementary File)
146 from the mucus and seawater communities and was composed of 505 OTUs grouped in more
147 than 100 different genera (79 identified, Supplementary File). Nineteen genera represented
148 each more than 0.5% of the total bacterial community (Supplementary File, Table 3, Fig. 3):
149 *BD1-7*, Candidatus *Amoebophilus*, *Corynebacterium*, *Dyella*, *Endozoicomonas*, *Lecothrix*,
150 *Leptolyngbya*, *Pseudomonas*, *Rhodopirellula*, *Rubritelea*, *Sphingomonas*, *Staphylococcus*,
151 *Thalassospira*, *Thalassotalea*, *Thiotrix* and genera from the LWSR-14, NS7 marine group and
152 Rhodobacteraceae families.

153 The coral-associated bacterial community was significantly affected by season
154 (p=0.001, Table S3), but not by UVR. In summer the microbial population was largely
155 dominated by *Endozoicomonas*, while in winter the bacterial community was mainly
156 composed of *Endozoicomonas* and *Thalassospira* (Fig. 3). To assess which OTUs were
157 primarily responsible for the seasonal shift in microbiome composition, differential
158 abundance analysis was performed. Results showed that OTUs whose abundances varied
159 between summer and winter belonged to the proteobacteria (*Endozoicomonas*, *Sphingomonas*,
160 *Thalassotalea* and unknown bacterium from the LWSR-14 and the Rhodobacteraceae
161 families) with the exception of *Leptolyngbya*, a cyanobacterium, and Candidatus
162 *Amoebophilus*, belonging to the Bacteroidetes (Fig. 3 & 4). While *Endozoicomonas* was the
163 most dominant genus in both seasons, significant differences in the OTUs belonging to this
164 genus were observed (Fig. 4). As such, there appeared to be a major shift in *Endozoicomonas*
165 community between the two seasons. In addition, higher abundance of Candidatus
166 *Amoebophilus*, *Leptolyngbya*, *Thalassotalea* and of two unknown bacterium from the LWSR-
167 14 and Rhodobacteraceae families were observed in summer compared to winter. Ten minor
168 OTUs (<0.5% of the total abundance) from the cyanobacteria and six minor genera previously
169 found in diseased or stressed corals were also more abundant in summer than in winter (Fig.
170 4, Supplementary File).

171 **Discussion**

Table 3. Characteristics of major genera (>0.5 % of the total community) making up the coral associated bacterial community. Brown highlights bacteria with putative antibacterial or nutritional functions; yellow indicates bacteria previously found to be associated with diseased or stressed corals; diazotrophic bacteria are highlighted in green.

Class	Order	Family	Genus	Genus or bacterial characteristics	Variation with season
Actinobacteria	Corynebacteriales	Corynebacteriaceae	*Corynebacterium*	Found in different desease of *Stephanocoenia intersepta* coral (Sweet et al., 2013)	-
Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	*Thalassospira*	Found in healthy corals in winter (Chiu et al. 2011); some spepies can degrade crude oil (*Thalassospira xiamenensis*; Röthig et al., 2017)	Higher abundance in winter
Alphaproteobacteria	Rickettsiales	LWSR-14	-	Found in *Acropora sp.* No change between healthy and stressed corals (Casas et al., 2004)	Higher abundance in summer
Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	-	Oportunistic bacterial family more abundant in the most anthropogenic impacted sites (Ziegler et al 2016)	Higher abundance in summer
Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Sphingomonas*	Found to be a possible responsible for coral white plague (Rosenberg and Ben-Haim 2002; Miller et al., 2003)	-
Gammaproteobacteria	Alteromonadales	Colwelliaceae	*Thalassotalea*	Found in *Montipora sp.* (Chen et al 2016) and *Euphyllia glabrescens* (Sheu et al 2016); some species were associated with white plague like disease of corals (Thompson et al 2006)	Higher abundance in summer
Gammaproteobacteria	Cellvibrionales	Spongiibacteraceae	*BD1-7 clade*	Found in *Corallium rubrum* (van de Water et al., 2016), in *Oculina patagonica* (Spring et al., 2015)	-
Gammaproteobacteria	Oceanospirillales	Hahellaceae	*Endozoicomonas*	See review Neave et al., 2016	Different community in summer or winter
Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	Found in *Acropora millepora* (Raina et al., 2009); antibacterial properties in soft coral (Radjasa et al., 2007)	-
Gammaproteobacteria	Thiotrichales	Thiotrichaceae	*Leucothrix*	-	-
Gammaproteobacteria	Thiotrichales	Thiotrichaceae	*Thiothrix*	Filamentus sulfur-oxidizing bacteria (Larkin et al., 1983)	-
Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	*Dyella*	Found in *Acropora eurystome* (Meron et al., 2011)	-
Bacilli	Bacillales	Staphylococcaceae	*Staphylococcus*	Pathogen agent associated with corals (Gowrishankar et al., 2006)	-
Cyanobacteria	SubsectionIII	FamilyI	*Leptolyngbya*	Diazotrophic bacteria found in *Acropora eurystoma* (Meron et al., 2011)	Higher abundance in summer
Cytophagia	Cytophagales	Flammeovirgaceae	Candidatus *Amoebophilus*	Found in coral tissue of *Orbicela faveolata*; *Montastrea cavernosa*, *Porites porites* (Apprill et al 2016)	Higher abundance in summer
Deltaproteobacteria	Myxococcales	P3OB-42	-	-	-
Flavobacteriia	Flavobacteriales	NS7 marine group	-	Found in bacterioplankton-dissolved organic matter (Lucas et al., 2016)	-
Planctomycetacia	Planctomycetales	Planctomycetaceae	*Rhodopirellula*	Found in deep corals (Yakimov et al., 2006) and in octocoral (Duque-Alarcon et al., 2012; Webster and Bourne 2007)	-
Verrucomicrobiae	Verrucomicrobiales	Rubritaleaceae	*Rubritalea*	Found in *Acropora muricata* (Lee et al., 2016), in *Lophelia pertusa* (Neulinger et al., 2008); found to degrade sugar and to have an antoxidant activity (Shindo et al., 2008)	-

Figure 3. Relative abundance of the major coral associated bacterial genera (>0.5 % of the total community) in winter without UVR (W0UV), winter under UVR (WUV), summer without UVR (S0UV) and summer under UVR (SUV).

Phylum	Order	Class	Family	Genus
Actinobacteria	Actinobacteria	Corynebacteriales	Corynebacteriaceae	*Corynebacterium*
Bacteroidetes	Cytophagia	Cytophagales	Flammeovirgaceae	Candidatus *Amoebophilus*
Bacteroidetes	Flavobacteriia	Flavobacteriales	NS7 marine group	
Chlamydiae	Chlamydiae	Chlamydiales	Simkaniaceae	
Cyanobacteria	Cyanobacteria	Subsection III	Family I	*Leptolyngbya*
Firmicutes	Bacilli	Baciallales	Staphylococcaceae	*Staphylococcus*
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	*Thalassospira*
Proteobacteria	Alphaproteobacteria	Rickettsiales	LWSR-14	
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Sphingomonas*
Proteobacteria	Deltaproteobacteria	Myxococcales	P3OB-42	
Proteobacteria	Gammaproteobacteria	Alteromonadales	Colwelliaceae	*Thalassotalea*
Proteobacteria	Gammaproteobacteria	Cellvibrionales	Spongiibacteraceae	BD1-7 clade
Proteobacteria	Gammaproteobacteria	Oceanospirillales	Hahellaceae	*Endozoicomonas*
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*
Proteobacteria	Gammaproteobacteria	Thiotrichales	Thiotrichaceae	*Leucothrix*
Proteobacteria	Gammaproteobacteria	Thiotrichales	Thiotrichaceae	*Thiothrix*
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	*Dyella*
Proteobacteria	Gammaproteobacteria	Verrucomicrobiales	Ribritaleaceae	*Rubritalea*
Other				

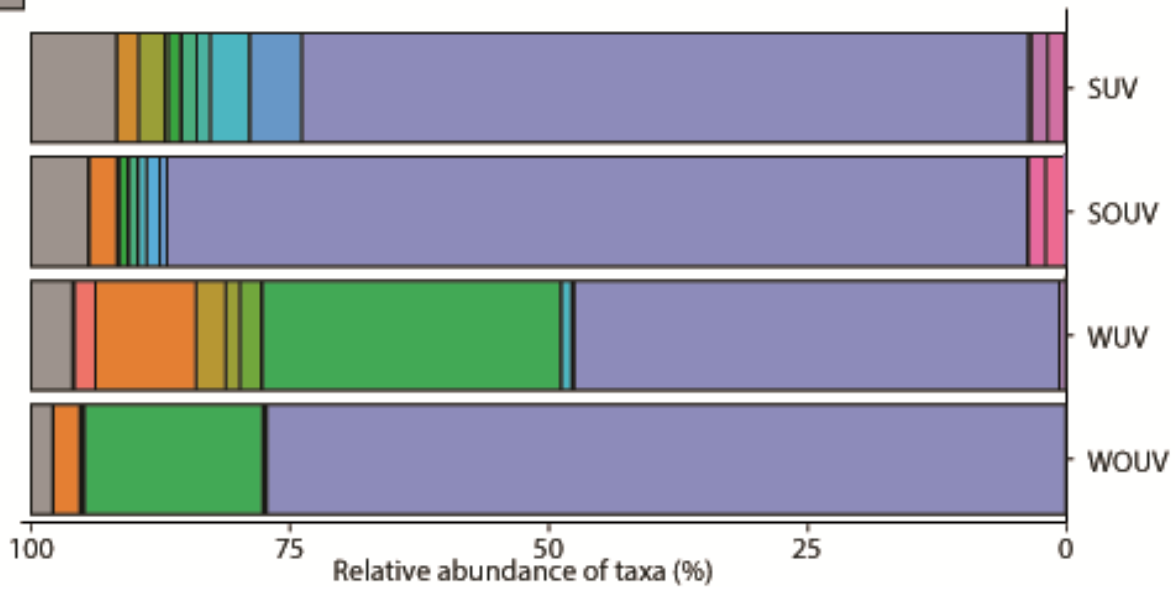
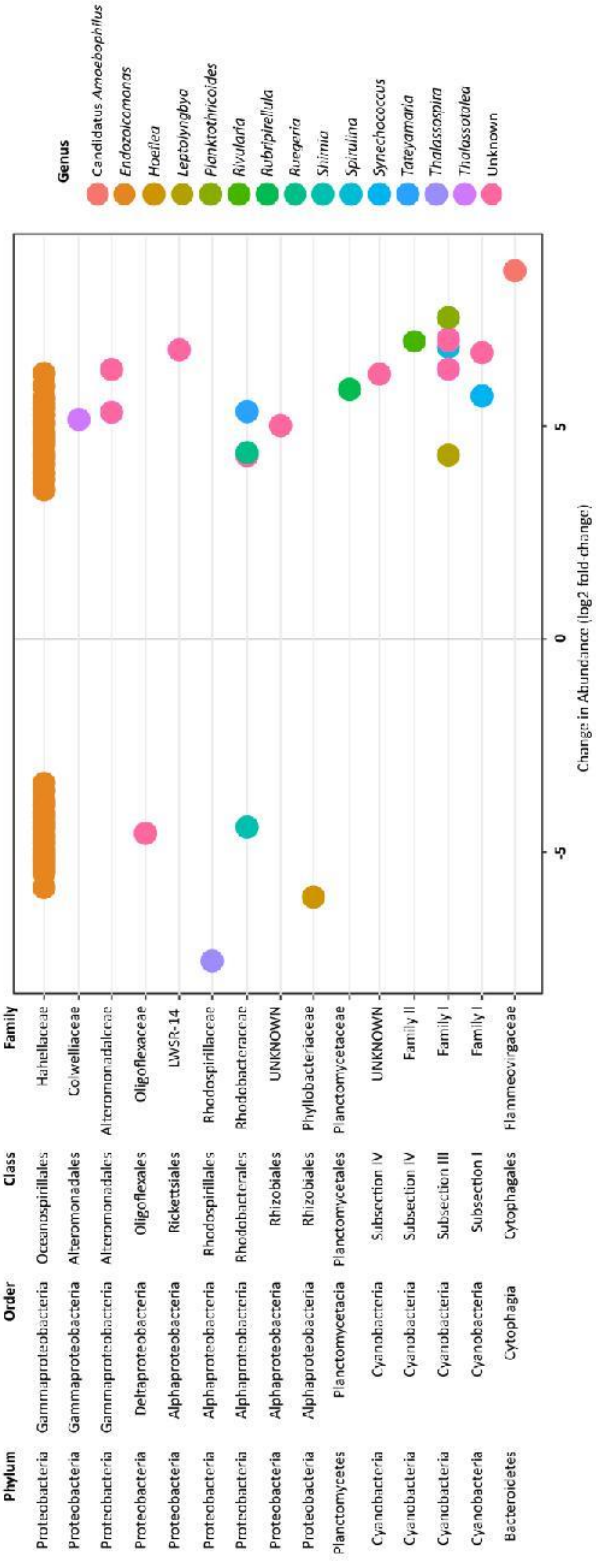


Figure 4. Differential abundance plot of coral-associated OTUs. Positive log2 fold-change values indicate OTUs with abundances higher in summer than in winter, while negative values indicate OTUs which were more abundant in winter compared with summer.



172 This study provides an overview of the seasonal and UVR-induced changes in the
173 bacterial communities associated with the tissue and mucus of the widely distributed
174 scleractinian coral *Acropora muricata*. While few bacterial OTUs were specifically present in
175 the mucus of *A. muricata*, a much higher microbial diversity was observed in the coral tissue.
176 The community composition of the mucus-associated bacteria varied between seasons, which
177 may have been related to changes in the quality and quantity of the mucus released by *A.*
178 *muricata*, and was also impacted by UVR in winter. The composition of the coral-associated
179 bacterial community also varied with the season, but UVR had no impact. Seasonal variability
180 in the coral was primarily driven by a shift within the *Endozoicomonas* community, which
181 was the dominant genus at both seasons. In the meantime, cyanobacteria and bacteria
182 previously found on diseased or bleached corals were more abundant in summer compared to
183 winter. These changes may be linked to the coral physiology, which presented significant
184 variations in calcification and photosynthetic efficiency with season.

185 We found only 8 bacterial OTUs specifically associated with the mucus of *A. muricata*.
186 Previous studies investigating the microbial diversity in the coral mucus have found large
187 numbers of OTUs (Bourne and Munn, 2005; Brown and Bythell, 2005; Sweet *et al.*, 2011);
188 however, this has recently been attributed to the sampling methods used to collect mucus,
189 which pick up both resident and transient bacteria from the surrounding environment
190 (Teplitski *et al.*, 2016). Here, we used the established beaker incubation technique (Herndl
191 and Velimirov, 1986; Wild *et al.*, 2008) to retrieve the mucus naturally released by the coral
192 colonies and statistically only isolated bacteria different from the seawater. Among the 8
193 OTUs identified, *Oleibacter* was previously found in the tissue/mucus and skeleton of
194 scleractinian corals (Ng *et al.*, 2015; Sweet and Bulling, 2017). Some species from this genera
195 are known to degrade complex hydrocarbons (Teramoto *et al.*, 2011) and this bacterium might
196 therefore be well adapted to use coral mucus as a food source. Another bacterium, *Arenicella*
197 has also been previously detected on corals (Ng *et al.*, 2015; Sweet and Bulling, 2017) and is
198 a common symbiont of the brown alga *Saccharina japonica* (Balakirev). *Arenicella* members
199 are commonly found in hydrothermal vents, or in hypoxic waters, overall suggesting that they
200 are able to grow in poor-oxygen media and may present a sulphate reduction or sulphide
201 oxidation activity (Parsons *et al.*, 2015; Bell *et al.*, 2016). The third most abundant bacterial
202 genus, *Coxiella* has been described as a pathogen for mammals (Seshadri *et al.*, 2003), but
203 also as a symbiotic bacterium in arthropods (Piel, 2004; Machado-Ferreira *et al.*, 2016), acting
204 as a vitamin provisioning endosymbiont (Smith *et al.*, 2015). Bacteria belonging to the other

genera, such as *Aestuariibacter*, a close neighbor of a agar hydrolyzing marine bacterium (Shi *et al.*, 2012), Candidatus *Rhabdochlamydia*, and those belonging to the Rickettsiaceae family have also previously been found in the tissue and mucus of scleractinian corals (Casas *et al.*, 2004; Lee *et al.*, 2012; Röthig *et al.*, 2016), although sequences related to the 2 OTUs from the DB1-14 class, have only been observed in intertidal outcrops and biofilms in Eastern Mediterranean coastal seawater (OTUs best match, Elifantz and others 2013; Couradeau and others 2017). Overall, our results suggest that the microbiome associated with the mucus of scleractinian corals is restricted to a few ubiquitous bacteria, and is dominated by bacteria specialized in the degradation of complex carbon compounds or adapted to hypoxic environments. By degrading the mucus, they could increase the availability of organic matter for the water column micro-organisms and therefore contribute to sustain the microbial loop.

We found that mucus composition may be involved in the changes observed within the mucus-associated microbiome, through the proportion of particulate and dissolved organic matter in the mucus. As such, the availability of dissolved organic carbon (20 µmol h^{-1} cm^2) and the enhanced enzymatic activities observed may be linked to the dominance of *Oleibacter* (more than 70% of the bacterial community) which may be capable of using mucus as carbon source. On the contrary, the abundance of this genus decreased to < 30% of the bacterial community in winter, in parallel with the reduction (to almost 0) in DOC. In winter, UVR also induced significant changes in the mucus-associated bacterial community. For example, we observed a switch from the dominance of *Coxiella* without UVR to the dominance of *Arenicella* under UVR, suggesting that *Arenicella* has a particular resistance to UVR. Such differences in sensitivity to UVR were previously observed in marine biofilm bacteria and were attributed to *Arenicella*'s higher levels of photoprotective mechanisms (Joux *et al.*, 1999). The lack of UVR effect in summer might be due to i) changes in the bacterial sensitivity to UVR with temperature (Ravindran *et al.*, 2013) or ii) differences in mucus physical properties leading to better protection against UVR (Drollet *et al.*, 1993).

Our results also show that the microbiome associated with the coral host contains many more specific bacterial OTUs than the mucus microbiome and is characterized by a high abundance of *Endozoicomonas*. This dominant genus is also commonly found in gorgonians and corals (Bayer, Arif, *et al.*, 2013; Bayer, Neave, *et al.*, 2013; van de Water *et al.*, 2016, 2017; Neave *et al.*, 2017) as well as in a wide range of other marine organisms (Forget and Kim Juniper, 2013; Fiore *et al.*, 2015; Katharios *et al.*, 2015). Although its exact functions are unknown, *Endozoicomoans* may be involved in host health through nutrient acquisition and

provision, and in structuring of the host microbial community function (reviewed in Neave et al. 2016). The 19 other most abundant genera found in our study (Table 3) have previously been observed in several coral species and may therefore play important roles in holobiont functioning (see table 2 for references) through nutrient cycling (Wild *et al.*, 2004; Naumann, 2009) and N-fixation (Lesser *et al.*, 2004), and coral health (Ritchie, 2006; Rosenberg *et al.*, 2007; Bourne *et al.*, 2009). The cyanobacterium *Leptolyngbya* and the proteobacteria from the Rhizobiales class are diazotrophs which are known to fix dinitrogen and to represent a potential significant nitrogen source for corals (Lesser *et al.*, 2004; Lema *et al.*, 2012, 2014). *Rubritalea,* and *Thalassospira* have been shown to degrade organic matter and thereby also potentially supply nitrogen to corals (Lesser *et al.*, 2007; Bednarz *et al.*, 2017). Other genus such as *Pseudomonas,* is known to have antibacterial properties (Radjasa *et al.*, 2007).

In contrast to mucus-associated bacteria, the coral-associated bacterial community compositions were unchanged by UVR, which could be related to the high concentrations of protective MAAs and fluorescent proteins in coral tissue (Shick and Dunlap, 2002). However, these communities differed significantly between seasons, which could be linked to seasonal patterns in other (non-UVR) environmental conditions and/or coral host physiology, which also significantly changed between the seasons. Our results are in accordance with several previous studies which reported highly different profiles between two sampling times/seasons, and the lack of long-term homeostasis between the coral host and its associated bacteria (Hong *et al.*, 2009; Ceh *et al.*, 2011; Kimes *et al.*, 2013). Although factors such as rain or nutrient input cannot be excluded, the large differences in seawater temperatures between the sampling time points (23.1 ± 0.6 in winter and 29.3 ± 0.9 in summer) suggest that temperature may drive the microbiome changes observed in this study, as it was previously suggested by Koren and Rosenberg (2006)

Although spatial and temporal factors are important in shaping microbial communities, distinct microbe-host associations can nevertheless be observed, such as the *Endozoicomonas - A. muricata* association, which persisted throughout the seasons. However we observed a shift in the relative abundance of *Endozoicomonas*-related OTUs between seasons reflecting a change in the community of this genus. A similar observation was made for *Endozoicomonas* species associated to Mediterranean gorgonians (van de Water *et al.*, 2017). Given the assumption that *Endozoicomonas* lives in beneficial relationships with its coral host (Neave *et al.*, 2016), it makes sense that corals harbour different *Endozoicomonas* community compositions at different times according to the benefit these bacteria can provide

to the host's requirements. This would be similar to 'symbiont shuffling' observed with the algal symbiont *Symbiodinium* in tropical scleractinian corals (Baker, 2001; Berkelmans and Van Oppen, 2006) and recently proposed for bacteria associated with temporal gorgonians (van de Water *et al.*, 2016): low-abundance genera become dominant during environmental disturbance and help the host maintain its physiological requirements. In summer, we also observed a significantly higher abundance of putative diazotrophic bacteria, such as *Leptolyngbya* and bacteria from the Rhizobiales class as well as several minor cyanobacterial OTUs, which are all known associates of scleractinian corals (Shashar *et al.*, 1994; Lesser *et al.*, 2004; Michael P. Lesser *et al.*, 2007; Meron *et al.*, 2011). In particular, Rhizobiales bacteria were identified in several coral species of the South-West Pacific such as *Acropora sp*. (Lema *et al.*, 2012, 2014; Krediet *et al.*, 2013). An increase in diazotroph abundance and diversity at elevated temperatures (Santos *et al.*, 2014; Cardini *et al.*, 2016) is an important functional process for corals thriving in oligotrophic tropical environments as fixation of dinitrogen by these bacteria can provide up to 15% of new nitrogen to nitrogen-limited corals (Bednarz *et al.*, 2017). The New Caledonian lagoon is considered as a hot-spot for diazotrophy as planktonic diazotrophs like Rhizobia are particularly abundant (Bonnet *et al.*, 2015; Turk-Kubo *et al.*, 2015). The presence of this family of bacteria in coral microbiome could partly be due to their ingestion by corals (Benavides *et al.*, 2016). As the abundance of these planktonic diazotrophs in the lagoon is greater in summer (Garcia *et al.*, 2007; Bonnet *et al.*, 2015), this might explain the difference in coral tissue concentrations between winter and summer. Taken together, our results converge to an acclimation of the holobiont to environmental changes.

Finally, the coral-associated microbial community in summer contained a higher abundance of bacteria that are generally found in increased numbers in diseased or stressed corals (Sekar *et al.*, 2008; Meron *et al.*, 2011; Apprill *et al.*, 2013; Diaz *et al.*, 2016; Ziegler *et al.*, 2016), such as *Thalassotalea, Rhodobacteraceae* family, as well as several bacteria belonging to the *Ruegeria* and *Rubripirellula* genera and the *Alteromonadaceae* and *Rhodobacteraceae* families. These shifts in the bacterial community composition in summer towards a more pathogenic microbiome may affect coral health and increase the susceptibility of the host to disease (Bourne and Munn, 2005; Ritchie, 2006; Bourne *et al.*, 2008). While the exact cause is unknown, high temperatures in summer compared to winter may directly affect bacterial growth and/or physiology, or indirectly alter bacterial antibiotic production and bacteria–bacteria interactions (Rypien *et al.*, 2010). Thermal stress may also impair the coral

304 immune system (van de Water *et al.*, 2015), that may lead to a reduced ability of the coral
305 host to regulate its microbiome. As the immunocompetence of an animal largely depends on
306 its energy reserves, the higher occurrence of potentially pathogenic OTUs in *A. muricata* in
307 summer may be linked to a reduced fitness of the host, as evidenced by the decrease in tissue
308 biomass (proteins) and photosynthetic efficiency.
309
310 Altogether, our results highlight important changes in the composition of the mucus- and
311 coral-associated bacteria and suggest a different control on these communities. While
312 temperature appears to be a main driver for changes in both communities, mucus associated
313 bacteria are also influenced by UVR and likely by the quality of the organic matter released
314 by corals. On the contrary, coral-associated community, more protected from the
315 environment, may be more affected by the physiological state of its coral host.

316 **Experimental procedures**

317 Coral collection and experimental setup

318 Twenty 2-3 cm long nubbins of *Acropora muricata* were collected between 2 and 3 m
319 depth at the beginning of January 2016 (Austral summer) and the beginning of June 2016
320 (Austral winter) (permit number: APA-NCPS-2016-001) from the same 10 parental colonies
321 (two fragments per colony) of the reef of "îlot Maître" in the New Caledonian lagoon.
322 Nubbins were evenly distributed over four 100 L outdoor experimental tanks continuously
323 supplied with lagoon water at a rate of 72 L h^{-1} and fed once a week with *Artemia salina*
324 nauplii. Using shade cloth, the irradiance level received by the corals in the experimental
325 tanks was maintained at *in situ* irradiance levels. PAR intensity data obtained from a LI-COR
326 data logger (LI-1000) connected to a spherical quantum sensor (LI-193) was used to inform
327 tank shading regimes. An International Light ILT1400 portable radiometer connected to UVA
328 and UVB detectors (SEL240/UVB-1/TD and SEL033/UVA/TD) was used to measure UVR.
329 In summer, the maximal irradiance obtained at midday in a sunny day was
330 ~900 µmole photons m^{-2} s^{-1} of photosynthetically active radiation (PAR), ~20 W m^{-2}
331 ultraviolet A (UVA, 315-400 nm) and ~1.2 W m^{-2} of ultraviolet B (UVB, 280-315 nm)
332 radiation. In winter, irradiance levels on a sunny day at mid-day were ~500 µmole photons m^{-2} s^{-1} of PAR, ~9 W m^2 of UVA, ~0.7 W m^2 of UVB. Two out of the four tanks were shielded
334 from UVR (condition hereafter called 0UV) using specific UVR filters (226 Lee U.V. filters),
335 while the two other tanks received the ambient dose of UVR (condition hereafter called UV).

Following collection, coral nubbins were maintained under experimental UVR conditions for 1.5 months prior to the assay incubations (described below) in summer (hereafter called S) and winter (hereafter called W). At both time points, five nubbins from each condition (UV and 0UV), were randomly sampled from the two tanks per condition, and used to assess tissue parameters (symbiont density, chlorophyll and protein concentrations), calcification rates, photosynthetic efficiency, organic matter fluxes, and mucus associated bacterial activity (described below). Following incubations, nubbins were frozen at -20 °C until further analyses. The remaining five nubbins per condition were used to assess coral and mucus-associated bacterial community (see details below).

All incubations were performed under the same temperature (29.3 ± 0.9°C in summer and 23.1 ± 0.6°C in winter) and light regime as the experimental tanks. All data were normalized to the nubbin surface area (cm^2) measured using the wax technique (Stimson and Kinzie, 1991).

Photochemical efficiency

Five nubbins per condition were incubated in the dark for 10 minutes before the relative electron transport rate (rETR) versus irradiance, or rapid light curves, were generated with a Pulse Amplitude Modulation (PAM) fluorometer (Diving-PAM, Walz, Germany) according to Ralph and Gademann (2005). For this purpose, nubbins were illuminated for 10 seconds with seven different light intensities (from 0 to 900 µmole quanta m^{-2} s^{-1}) and the $rETR_{max}$ was deduced from the curves.

Calcification rates

Nubbins were incubated 2 h in individual 100 mL beakers filled with 0.45 µm filtered seawater, continuously stirred with stirring bars and hermetically closed to avoid any oxygen exchange with the ambient air. The incubation seawater in each beaker was filtered through 0.2 µm and stored at 4°C for the subsequent determination of the calcification rates using the alkalinity anomaly method (Smith and Key, 1975). The measurement was performed using a TIM865 titration manager (Titralab). Data were expressed in µmol $CaCO_3$ cm^{-2} d^{-1} and corrected against a blank (filtrated seawater incubated for the same period without nubbin).

Organic matter (particulate and dissolved) release

Nubbins were also incubated for 2 h in 200 mL of 0.45 µm filtered seawater. Water samples were taken at the beginning (T_0) and at the end (T_f) for organic carbon and nitrogen measurements. The total organic carbon/nitrogen (TOC/TON) fluxes were assessed by sampling 20 mL seawater with sterile syringes at T_0 and T_f from each beaker. Samples were transferred to glass vials, previously washed for 24 h in 10 % HCl and burned at 500°C for 4 h and amended with 420 µL of H_3PO_4 to avoid any bacterial activity during storage at -20°C. Dissolved organic carbon (DOC) and nitrogen (DON) fluxes were assessed with a similar procedure with the exception that the water was filtered through 0.45 µm filters prior storage. Samples were analyzed using a TOC-L analyzer (Shimadzu, Japan) and data were expressed in µg h^{-1} cm^{-2}. All values were corrected against a blank (filtered seawater incubated without nubbin).

Aminopeptidase and α-glucosidase extracellular enzymatic activity assay

Aminopeptidase and α-glucosidase extracellular enzymatic activities (EEA) were monitored using fluorescent substrate analogues (L-Leucine-7-amido-4-methyl-coumarin hydrochloride (Leu-MCA, Sigma) and 4-methylumbeliferyl-α-D-glucopyranoside (αMUF, Sigma) respectively) according to the standard protocols (Hoppe, 1983). The two compounds become fluorescent after enzyme cleavage. The fluorescence intensity correlates with the quantity of hydrolysed substrates 7-amino 4-methylcoumarin (MCA, Sigma) and 4-methylumbeliferone (MUF, Sigma) and was measured using a spectrofluorometer (Synergy-H1, Biotek) with emission/excitation wavelengths of 448/360 nm for MUF and 442/356 MCA. At the end of 2 h incubations (described in the previous paragraph), 2000 µL of the incubation water (thereafter called mucus) were sampled three times per incubation and blank in 15 mL falcons. To the mucus, 3000 µL of 0.45 µm filtrated seawater and 1000 µL of 1.5 mM substrates (Lea-MCA or αMUF) were added for a final substrate concentration of 250 µM (Fonvielle *et al.*, 2015). Samples were incubated in the dark for up to 168 h in a shaking water bath at the temperature corresponding with the season (23 °C (winter) or 29 °C (summer)). EEAs were measured every 24 h. For this purpose, 96 white well plates of 300 µL were filled up with 240 µL of sample and 60 µL of 10.8 Tris-HCl buffer was added to adjust pH to obtain maximal fluorescence intensity.

Symbiont, chlorophyll *a* and protein content

After the incubations, nubbins were frozen at -20°C for the subsequent determination of tissue parameters. Nubbin tissue was removed from the skeleton using an air pick and

398 homogenized with a Potter-Elvehjem tissue grinder. A sub-sample was taken for the
399 determination of the symbiont density of each sample using a Neubauer cell, on five
400 replicated counts. A second sub-sample was used to assess the protein content according to
401 Hoogenboom et al. (2010), using BCA assay kit (Interchim Protein Quantification Kit). For
402 chl *a* measurements, another subsample was centrifuged at 5000 x g for 10 min at 4 °C to
403 separate the symbionts (in the pellet) from the host tissue. The pellet was then re-suspended in
404 10 mL acetone and kept in the dark at 4°C for 24 h prior measurements. Samples were then
405 centrifuged for 15 min at 10 000 x g and the absorbance was measured at 630, 663 and
406 750 nm using an EVOLUTION 201 UV-Visible spectrophotometer (Thermo Scientific).
407 Chlorophyll concentrations were computed using the equations of Jeffrey and Humphrey's
408 (1975).

409 Bacterial community analysis

410 Five nubbins of each condition (and a blank per condition) were incubated for 2 h in
411 1L of 0.45 µm filtered seawater continuously stirred with stirring bars. Following the
412 incubation, nubbins were stored at 4°C for 24 h in RNA*later* before being frozen at -20°C.
413 To isolate mucus associated bacteria, the incubation water was filtered sequentially through
414 10 µm, 3 µm and 0.2 µm Whatman Nuclepore Track-Etched filters (Sigma-Aldrich) and the
415 last filter was kept in RNA*later* at -20°C. Coral nubbins were then crushed on liquid nitrogen.
416 DNA was extracted from crushed coral and from the filter using the Genomic DNA Buffer
417 Set and Genomic-tip 20/G columns (QIAGEN, Hilden, Germany) according to the
418 manufacturer's protocol. DNA samples were sent to Molecular Research Laboratory (MR
419 DNA, Shallowater, Texas, USA) for 16S rDNA amplicon library generation using the
420 515F/806R primer pair, which targets the V4 region of the 16S rRNA gene, and paired-end
421 (2x 300bp) sequencing on the Illumina MiSeq platform.

422

423 16S rRNA gene amplicon data analysis

424 The quality (.qual) and reads (.fasta) files were obtained from MR DNA and the QIIME
425 pipeline (Caporaso *et al.*, 2010) was used to process the data as detailed in van de Water *et al.*
426 (2017). Briefly, low quality (Phred <20) sequences, reads <200 bp in length were removed
427 and each read were assigned to its respective sample using the split_libraries.py script.
428 Chimeric sequences were identified and removed using the UCHIME algorithm (Edgar *et al.*,
429 2011) and the SILVA v123 database (Quast *et al.*, 2013) as reference. Operational Taxonomic

Units (OTUs) were defined at the level of 97% similarity. Taxonomic identities were assigned using the UCLUST algorithm (Edgar, 2010) against the SILVA reference database (version 123). Singletons, unassigned OTUs, and OTUs classified as chloroplast or mitochondria were removed from the dataset. Raw sequences were deposited in the NCBI Sequence Read Archive.

Negative binomial modelling (likelihood ratio tests for the effect of experimental factors and Wald tests for pair-wise comparisons) implemented in the DESeq2 package in R (Love *et al.*, 2014), was used to test differential abundance. First, we identified OTUs which were 1) mucus-specific (i.e. significantly more abundant in mucus versus seawater samples) and 2) coral-specific (i.e. significant higher abundance in coral versus seawater samples). Eight OTUs were considered to be exclusively associated with the mucus, while 505 OTUs were found to compose the coral-associated bacterial community. Permutational Analysis of Variance (permANOVA) was performed on those selected communities, to test for the overall effects of UV radiation and season on the mucus-specific and coral-associated bacterial communities. Differential abundance analysis was also used to assess which OTUs were significantly impacted by UV radiation and/or season, and results were plotted using ggplot2 (REF). The phyloseq package (McMurdie and Holmes, 2013) integrated in R was used to generate statistically relevant graphical presentations of the microbiome data obtained, including 1) abundance plots of relevant taxa, and 2) a non-metric dimensional scaling (nMDS) on Bray-Curtis dissimilarity matrices, to visualise differences in coral-associated bacterial communities on seasonal scales (ellipses representing the 95% confidence intervals).

Statistical analysis

Effects of UVR and high temperature on tissue parameters, coral metabolism, organic matter fluxes and bacterial activity were assessed with two-way ANOVAs using UVR and temperature as factors. Normality of the residuals and variance homoscedasticity were tested using Shapiro and Bartlett tests respectively. When needed, data were log transformed in order to fulfill those criteria. A Tukey's post hoc test was performed when results of the ANOVAs were significant. The results of the pairwise comparisons (W0UV-WUV, S0UV-SUV and WUV-SUV conditions) were used to determine the effects of UVR in winter or in summer and the effects of the season in natural conditions (*i.e.* under UVR).

Acknowledgments

462 The authors would like to thank R. Rodolfo Metalpa and C. Rottier for laboratory assistance,
463 C. Payri, director of the ENTROPIE UMR, for scientific support and Mr. R. Farman, Director
464 of the "Aquarium des lagons", Nouméa, for hosting the tanks and providing technical help
465 during the experimental setup. We also would like to thank N. Wabete and M. Le Gall
466 (Ifremer) for their collaboration in lab analysis. CFP and JvW would like to thank the Paul
467 Hamel Foundation for financial support.

468

469

470

References

Apprill, A., Hughen, K., and Mincer, T. (2013) Major similarities in the bacterial communities associated with lesioned and healthy Fungiidae corals. *Environ. Microbiol.* **15**: 2063–2072.

Apprill, A., Weber, L.G., and Santoro, E. (2016) Distinguishing between Microbial Habitats Unravels Ecological Complexity in Coral Microbiomes. *mSystems* **1**:.

Arrieta, J.M., Weinbauer, M.G., Gerhard, J., and Mari, S. (2000) Interspecific Variability in Sensitivity to UV Radiation and Subsequent Recovery in Selected Isolates of Marine Bacteria Interspecific Variability in Sensitivity to UV Radiation and Subsequent Recovery in Selected Isolates of Marine Bacteria. *Appl. Environ. Microbiol.* **66**: 1468–1473.

Baker, A.C. (2001) Ecosystems: reef corals bleach to survive change. *Nature* **411**: 765–766.

Bayer, T., Arif, C., Ferrier-Pagès, C., Zoccola, D., Aranda, M., and Voolstra, C.R. (2013) Bacteria of the genus Endozoicomonas dominate the microbiome of the Mediterranean gorgonian coral Eunicella cavolini. *Mar. Ecol. Prog. Ser.* **479**: 75–84.

Bayer, T., Neave, M.J., Alsheikh-Hussain, A., Aranda, M., Yum, L.K., Mincer, T., et al. (2013) The microbiome of the Red Sea coral Stylophora pistillata is dominated by tissue-associated Endozoicomonas bacteria. *Appl. Environ. Microbiol.* **79**: 4759–4762.

Bednarz, V.N., Grover, R., Maguer, J.-F., Fine, M., and Ferrier-Pagès, C. (2017) The assimilation of diazotroph-derived nitrogen by scleractinian corals depends on their metabolic status. *MBio* **8**:.

Bell, J.B., Reid, W.D.K., Pearce, D.A., Glover, A.G., Sweeting, C.J., Newton, J., and Woulds, C. (2016) Hydrothermal activity lowers trophic diversity in Antarctic sedimented hydrothermal vents. *Biogeosciences Discuss.*

Benavides, M., Houlbrèque, F., Camps, M., Lorrain, A., Grosso, O., and Bonnet, S. (2016) Diazotrophs: a non-negligible source of nitrogen for the tropical coral Stylophora pistillata. *J. Exp. Biol.* **219**: 2608–2612.

Berkelmans, R. and Van Oppen, M.J.H. (2006) The role of zooxanthellae in the thermal tolerance of corals: a "nugget of hope"for coral reefs in an era of climate change. *Proc. R. Soc. London B Biol. Sci.* **273**: 2305–2312.

Bonnet, S., Rodier, M., Turk-Kubo, K.A., Germineaud, C., Menkes, C., Ganachaud, A., et al. (2015) Contrasted geographical distribution of N2 fixation rates and nifH phylotypes in the Coral and Solomon Seas (southwestern Pacific) during austral winter conditions. *Global Biogeochem. Cycles* **29**: 1874–1892.

Bourne, D., Iida, Y., Uthicke, S., and Smith-Keune, C. (2008) Changes in coral-associated microbial communities during a bleaching event. *ISME J.* **2**: 350–363.

Bourne, D.G., Garren, M., Work, T.M., Rosenberg, E., Smith, G.W., and Harvell, C.D. (2009) Microbial disease and the coral holobiont. *Trends Microbiol.* **17**: 554–562.

Bourne, D.G. and Munn, C.B. (2005) Diversity of bacteria associated with the coral

Pocillopora damicornis from the Great Barrier Reef. *Environ. Microbiol.* **7**: 1162–1174.

Bourne, D.G. and Webster, N.S. (2013) Coral reef bacterial communities. In, *The Prokaryotes*. Springer, pp. 163–187.

Brown, B.E. and Bythell, J.C. (2005) Perspectives on mucus secretion in reef corals. *Mar. Ecol. Prog. Ser.* **296**: 291–309.

Brown, B.E., Dunne, R.P., Ambarsari, I., Le Tissier, M.D.A., and Satapoomin, U. (1999) Seasonal fluctuations in environmental factors and variations in symbiotic algae and chlorophyll pigments in four Indo-Pacific coral species. *Mar. Ecol. Prog. Ser.* **191**: 53–69.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., et al. (2010) QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods* **7**: 335–336.

Cardini, U., van Hoytema, N., Bednarz, V.N., Rix, L., Foster, R.A., Al-Rshaidat, M.M.D., and Wild, C. (2016) Microbial dinitrogen fixation in coral holobionts exposed to thermal stress and bleaching. *Environ. Microbiol.* **18**:.

Casas, V., Kline, D.I., Wegley, L., Yu, Y., Breitbart, M., and Rohwer, F. (2004) Widespread association of a Rickettsiales-like bacterium with reef-building corals. *Environ. Microbiol.* **6**: 1137–1148.

Ceh, J., Van Keulen, M., and Bourne, D.G. (2011) Coral-associated bacterial communities on Ningaloo Reef, Western Australia. *FEMS Microbiol. Ecol.* **75**: 134–144.

Chen, C.-P., Tseng, C.-H., Chen, C.A., and Tang, S.-L. (2011) The dynamics of microbial partnerships in the coral Isopora palifera. *ISME J.* **5**: 728–740.

Chen, W.-M., Liu, L.-P., AChen, C.A., and Sheu, S.-Y. (2016) Thalassotalea montiporae sp. nov., isolated from the encrusting pore coral Montipora aequituberculata. *Int. J. Syst. Evol. Microbiol.* **66**: 4077–4084.

Chiu, J.M.Y., Li, S., Li, A., Po, B., Zhang, R., Shin, P.K.S., and Qiu, J.W. (2012) Bacteria associated with skeletal tissue growth anomalies in the coral Platygyra carnosus. *FEMS Microbiol. Ecol.* **79**: 380–391.

Clarke, A. (1988) Seasonality in the Antarctic marine environment. *Comp. Biochem. Physiol. Part B Comp. Biochem.* **90**: 461–473.

Coma, R., Ribes, M., Gili, J.-M., and Zabala, M. (2000) Seasonality in coastal benthic ecosystems. *Trends Ecol. Evol.* **15**: 448–453.

Couradeau, E., Roush, D., Guida, B.S., and Garcia-Pichel, F. (2017) Diversity and mineral substrate preference in endolithic microbial communities from marine intertidal outcrops (Isla de Mona, Puerto Rico). *Biogeosciences* **14**: 311–324.

Crossland, C.J. (1984) Seasonal variations in the rates of calcification and productivity in the coral Acropora formosa on a high-latitude reef. *Mar. Ecol. Prog. Ser. Oldend.* **15**: 135–140.

Diaz, J.M., Hansel, C.M., Apprill, A., Brighi, C., Zhang, T., Weber, L., et al. (2016) Species-

549 specific control of external superoxide levels by the coral holobiont during a natural
550 bleaching event. *Nat. Commun.* **7**: 13801.

551 Drollet, J.H., Glaziou, P., and Martin, P.M. V (1993) A study of mucus from the solitary coral
552 Fungia fungites (Scleractinia: Fungiidae) in relation to photobiological UV adaptation.
553 *Mar. Biol.* **115**: 263–266.

554 Duque-Alarcón, A., Santiago-Vázquez, L.Z., and Kerr, R.G. (2012) A microbial community
555 analysis of the octocoral Eunicea fusca. *Electron. J. Biotechnol.* **15**: 15.

556 Edgar, R.C. (2010) Search and clustering orders of magnitude faster than BLAST.
557 *Bioinformatics* **26**: 2460–2461.

558 Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., and Knight, R. (2011) UCHIME
559 improves sensitivity and speed of chimera detection. *Bioinformatics* **27**: 2194–2200.

560 Elasri, M.O. and Miller, R. V. (1999) Study of the response of a biofilm bacterial community
561 to UV radiation. *Appl. Environ. Microbiol.* **65**: 2025–2031.

562 Elifantz, H., Horn, G., Ayon, M., Cohen, Y., and Minz, D. (2013) Rhodobacteraceae are the
563 key members of the microbial community of the initial biofilm formed in Eastern
564 Mediterranean coastal seawater. *FEMS Microbiol. Ecol.* **85**: 348–357.

565 Fiore, C.L., Labrie, M., Jarett, J.K., and Lesser, M.P. (2015) Transcriptional activity of the
566 giant barrel sponge, Xestospongia muta Holobiont: molecular evidence for metabolic
567 interchange. *Front. Microbiol.* **6**: 364.

568 Fitt, W.K., McFarland, F.K., Warner, M.E., and Chilcoat, G.C. (2000) Seasonal patterns of
569 tissue biomass and densities of symbiotic dinoflagellates in reef corals and relation to
570 coral bleaching. *Limnol. Oceanogr.* **45**: 677–685.

571 Fonvielle, J.A., Reynaud, S., Jacquet, S., LeBerre, B., and Ferrier-Pages, C. (2015) First
572 evidence of an important organic matter trophic pathway between temperate corals and
573 pelagic microbial communities. *PLoS One* **10**: 1–22.

574 Forget, N.L. and Kim Juniper, S. (2013) Free-living bacterial communities associated with
575 tubeworm (Ridgeia piscesae) aggregations in contrasting diffuse flow hydrothermal vent
576 habitats at the Main Endeavour Field, Juan de Fuca Ridge. *Microbiologyopen* **2**: 259–
577 275.

578 Garcia, N., Raimbault, P., and Sandroni, V. (2007) Seasonal nitrogen fixation and primary
579 production in the Southwest Pacific: nanoplankton diazotrophy and transfer of nitrogen
580 to picoplankton organisms. *Mar. Ecol. Prog. Ser.* **343**: 25–33.

581 Gil-Agudelo, D.L. and Garzón-Ferreira, J. (2001) Spatial and seasonal variation of dark spots
582 disease in coral communities of the Santa Marta area (Colombian Caribbean). *Bull. Mar.*
583 *Sci.* **69**: 619–630.

584 Gooday, A.J., Turley, C.M., and Allen, J.A. (1990) Responses by benthic organisms to inputs
585 of organic material to the ocean floor: a review [and discussion. *Philos. Trans. R. Soc.*
586 *London A Math. Phys. Eng. Sci.* **331**: 119–138.

587 Herndl, G.J. and Velimirov, B. (1986) Microheterotrophic utilization of mucus released by the
588 Mediterranean coral Cladocora cespitosa. *Mar. Biol.* **90**: 363–369.

589 Hoegh-Guldberg, O. (1999) Climate change, coral bleaching and the future of the world's
590 coral reefs. *Mar. Freshw. Res.* **50**: 839–866.

591 Hong, M.-J., Yu, Y.-T., Chen, C.A., Chiang, P.-W., and Tang, S.-L. (2009) Influence of
592 species specificity and other factors on bacteria associated with the coral Stylophora
593 pistillata in Taiwan. *Appl. Environ. Microbiol.* **75**: 7797–7806.

594 Hoogenboom, M., Beraud, E., and Ferrier-Pagès, C. (2010) Relationship between symbiont
595 density and photosynthetic carbon acquisition in the temperate coral Cladocora
596 caespitosa. *Coral Reefs* **29**: 21–29.

597 Hoppe, H.-G. (1983) Significance of exoenzymatic activities in the ecology of brackish water:
598 measurements by means of methylumbelliferyl-substrates. *Mar. Ecol. Prog. Ser.* **11**:
599 299–308.

600 Jeffrey, S.W. and Humphrey, G.F. (1975) New spectrophotometric equations for determining
601 chlorophylls a, b, c1 and c2 in higher plants, algae and natural phytoplankton.
602 Biochemical Physiology Pflanz 167: 191–194. *Biochem. Physiol. Pflanz.* 191–194.

603 Joux, F., Jeffrey, W.H., Lebaron, P., and Mitchell, L. (1999) Marine Bacterial Isolates Display
604 Diverse Responses to UV-B Radiation Marine Bacterial Isolates Display Diverse
605 Responses to UV-B Radiation. **65**: 3820–3827.

606 Katharios, P., Seth-Smith, H.M.B., Fehr, A., Mateos, J.M., Qi, W., Richter, D., et al. (2015)
607 Environmental marine pathogen isolation using mesocosm culture of sharpsnout
608 seabream: striking genomic and morphological features of novel Endozoicomonas sp.
609 *Sci. Rep.* **5**: 17609.

610 Kimes, N.E., Johnson, W.R., Torralba, M., Nelson, K.E., Weil, E., and Morris, P.J. (2013)
611 The Montastraea faveolata microbiome: Ecological and temporal influences on a
612 Caribbean reef-building coral in decline. *Environ. Microbiol.* **15**: 2082–2094.

613 Koren, O. and Rosenberg, E. (2006) Bacteria associated with mucus and tissues of the coral
614 Oculina patagonica in summer and winter. *Appl. Environ. Microbiol.* **72**: 5254–5259.

615 Krediet, C.J., Ritchie, K.B., Alagely, A., and Teplitski, M. (2013) Members of native coral
616 microbiota inhibit glycosidases and thwart colonization of coral mucus by an
617 opportunistic pathogen. *ISME J.* **7**: 980–90.

618 Larkin, J.M. and Strohl, W.R. (1983) Beggiatoa, thiothrix, and thioploca. *Annu. Rev.*
619 *Microbiol.* **37**: 341–367.

620 Lee, O.O., Yang, J., Bougouffa, S., Wang, Y., Batang, Z., Tian, R., et al. (2012) Spatial and
621 species variations in bacterial communities associated with corals from the Red Sea as
622 revealed by pyrosequencing. *Appl. Environ. Microbiol.* **78**: 7173–7184.

623 Lee, S.T.M., Davy, S.K., Tang, S.-L., and Kench, P.S. (2016) Mucus Sugar Content Shapes
624 the Bacterial Community Structure in Thermally Stressed Acropora muricata. *Front.*
625 *Microbiol.* **7**: 371.

626 Lema, K.A., Willis, B.L., and Bourne, D.G. (2014) Amplicon pyrosequencing reveals spatial
627 and temporal consistency in diazotroph assemblages of the Acropora millepora
628 microbiome. *Environ. Microbiol.* **16**: 3345–3359.

629 Lema, K.A., Willis, B.L., and Bourne, D.G. (2012) Corals form characteristic associations
630 with symbiotic nitrogen-fixing bacteria. *Appl. Environ. Microbiol.* **78**: 3136–3144.

631 Lesser, M.P. (2006) Oxidative stress in marine environments: biochemistry and physiological
632 ecology. *Annu. Rev. Physiol.* **68**: 253–278.

633 Lesser, M.P., Bythell, J.C., Gates, R.D., Johnstone, R.W., and Hoegh-Guldberg, O. (2007)
634 Are infectious diseases really killing corals? Alternative interpretations of the
635 experimental and ecological data. *J. Exp. Mar. Bio. Ecol.* **346**: 36–44.

636 Lesser, M.P., Falcón, L.I., Rodríguez-Román, A., Enríquez, S., Hoegh-Guldberg, O., and
637 Iglesias-Prieto, R. (2007) Nitrogen fixation by symbiotic cyanobacteria provides a source
638 of nitrogen for the scleractinian coral Montastraea cavernosa. *Mar. Ecol. Prog. Ser.* **346**:
639 143–152.

640 Lesser, M.P., Mazel, C.H., Gorbunov, M.Y., and Falkowski, P.G. (2004) Discovery of
641 symbiotic nitrogen-fixing cyanobacteria in corals. *Science (80-.).* **305**: 997–1000.

642 Li, J., Chen, Q., Long, L.-J., Dong, J.-D., Yang, J., and Zhang, S. (2014) Bacterial dynamics
643 within the mucus, tissue and skeleton of the coral Porites lutea during different seasons.
644 *Sci. Rep.* **4**: 7320.

645 Littman, R.A., Bourne, D.G., and Willis, B.L. (2010) Responses of coral associated bacterial
646 communities to heat stress differ with Symbiodinium type on the same coral host. *Mol.*
647 *Ecol.* **19**: 1978–1990.

648 Love, M.I., Huber, W., and Anders, S. (2014) Moderated estimation of fold change and
649 dispersion for RNA-seq data with DESeq2. *Genome Biol.* **15**: 550.

650 Lucas, J., Koester, I., Wichels, A., Niggemann, J., Dittmar, T., Callies, U., et al. (2016) Short-
651 Term Dynamics of North Sea Bacterioplankton-Dissolved Organic Matter Coherence on
652 Molecular Level. *Front. Microbiol.* **7**: 321.

653 Machado-Ferreira, E., Vizzoni, V.F., Balsemão-Pires, E., Moerbeck, L., Gazeta, G.S.,
654 Piesman, J., et al. (2016) Coxiella symbionts are widespread into hard ticks. *Parasitol.*
655 *Res.* **115**: 4691–4699.

656 McMurdie, P.J. and Holmes, S. (2013) phyloseq: an R package for reproducible interactive
657 analysis and graphics of microbiome census data. *PLoS One* **8**: e61217.

658 Meron, D., Atias, E., Iasur Kruh, L., Elifantz, H., Minz, D., Fine, M., and Banin, E. (2011)
659 The impact of reduced pH on the microbial community of the coral Acropora eurystoma.
660 *ISME J.* **5**: 51–60.

661 Miller, J., Rogers, C., and Waara, R. (2003) Monitoring the coral disease, plague type II, on
662 coral reefs in St. John, US Virgin Islands. *Rev. Biol. Trop* **51**: 47–55.

663 Naumann, M. (2009) Biogeochemical consequences of organic matter release by reef-
664 building scleractinian corals. *Coral Reefs 28* 109–117.

665 Neave, M.J., Apprill, A., Ferrier-Pagès, C., and Voolstra, C.R. (2016) Diversity and function
666 of prevalent symbiotic marine bacteria in the genus Endozoicomonas. *Appl. Microbiol.*
667 *Biotechnol.* **100**: 8315–8324.

668 Neave, M.J., Rachmawati, R., Xun, L., Michell, C.T., Bourne, D.G., Apprill, A., and
669 Voolstra, C.R. (2017) Differential specificity between closely related corals and
670 abundant Endozoicomonas endosymbionts across global scales. *ISME J.* **11**: 186.

671 Neulinger, S.C., Jarnegren, J., Ludvigsen, M., Lochte, K., and Dullo, W.-C. (2008)
672 Phenotype-Specific Bacterial Communities in the Cold-Water Coral Lophelia pertusa
673 (Scleractinia) and Their Implications for the Coral's Nutrition, Health, and Distribution.
674 *Appl. Environ. Microbiol.* **74**: 7272–7285.

675 Ng, J.C.Y., Chan, Y., Tun, H.M., Leung, F.C.C., Shin, P.K.S., and Chiu, J.M.Y. (2015)
676 Pyrosequencing of the bacteria associated with Platygyra carnosus corals with skeletal
677 growth anomalies reveals differences in bacterial community composition in apparently
678 healthy and diseased tissues. *Front. Microbiol.* **6**:.

679 Parsons, R.J., Nelson, C.E., Carlson, C.A., Denman, C.C., Andersson, A.J., Kledzik, A.L., et
680 al. (2015) Marine bacterioplankton community turnover within seasonally hypoxic
681 waters of a subtropical sound: Devil's Hole, Bermuda. *Environ. Microbiol.* **17**: 3481–
682 3499.

683 Piel, J. (2004) Metabolites from symbiotic bacteria. *Nat. Prod. Rep.* **21**: 519–538.

684 Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., et al. (2013) The
685 SILVA ribosomal RNA gene database project: improved data processing and web-based
686 tools. *Nucleic Acids Res.* **41**: D590–D596.

687 Radjasa, O.K., Salasia, S.I.O., Sabdono, A., Weise, J., Imhoff, J.F., Lammler, C., and Risk,
688 M.J. (2007) Antibacterial Activity of Marine Bacterium Pseudomonas sp. Associated
689 with Soft Coral Sinularia polydactyla againstStreptococcus equi Subsp. zooepidemicus.
690 *Int. J. Pharmacol.* **3**: 170–174.

691 Raina, J.B., Tapiolas, D., Willis, B.L., and Bourne, D.G. (2009) Coral-associated bacteria and
692 their role in the biogeochemical cycling of sulfur. *Appl. Environ. Microbiol.* **75**: 3492–
693 3501.

694 Ralph, P.J. and Gademann, R. (2005) Rapid light curves: a powerful tool to assess
695 photosynthetic activity. *Aquat. Bot.* **82**: 222–237.

696 Ravindran, J., Kannapiran, E., Manikandan, B., Francis, K., Arora, S., Karunya, E., et al.
697 (2013) UV-absorbing bacteria in coral mucus and their response to simulated
698 temperature elevations. *Coral Reefs* **32**: 1043–1050.

699 Ritchie, K.B. (2006) 2006.Ritchie.Regulation of microbial populations by coral surface mucus
700 and mucus associated bacteria. **322**: 1–14.

701 Rohwer, F., Breitbart, M., Jara, J., Azam, F., and Knowlton, N. (2001) Diversity of bacteria
702 associated with the Caribbean coral Montastraea franksi. *Coral reefs* **20**: 85–91.

703 Rosenberg, E. and Ben-Haim, Y. (2002) Microbial diseases of corals and global warming.
704 *Environ. Microbiol.* **4**: 318–326.

705 Rosenberg, E., Koren, O., Reshef, L., Efrony, R., and Zilber-Rosenberg, I. (2007) The role of
706 microorganisms in coral health, disease and evolution. *Nat. Rev. Microbiol.* **5**: 355–362.

707 Röthig, T., Costa, R.M., Simona, F., Baumgarten, S., Torres, A.F., Radhakrishnan, A., et al.

(2016) Distinct Bacterial Communities Associated with the Coral Model Aiptasia in Aposymbiotic and Symbiotic States with Symbiodinium. *Front. Mar. Sci.* **3**: 234.

Rypien, K.L., Ward, J.R., and Azam, F. (2010) Antagonistic interactions among coral-associated bacteria. *Environ. Microbiol.* **12**: 28–39.

Santos, H.F., Carmo, F.L., Duarte, G., Dini-Andreote, F., Castro, C.B., Rosado, A.S., et al. (2014) Climate change affects key nitrogen-fixing bacterial populations on coral reefs. *ISME J.* **8**: 2272–2279.

Sekar, R., Kaczmarsky, L.T., and Richardson, L.L. (2008) Microbial community composition of black band disease on the coral host Siderastrea siderea from three regions of the wider Caribbean. *Mar. Ecol. Prog. Ser.* **362**: 85–98.

Seshadri, R., Paulsen, I.T., Eisen, J.A., Read, T.D., Nelson, K.E., Nelson, W.C., et al. (2003) Complete genome sequence of the Q-fever pathogen Coxiella burnetii. *Proc. Natl. Acad. Sci.* **100**: 5455–5460.

Shashar, N., Cohen, Y., Loya, Y., and Sar, N. (1994) Nitrogen fixation(acetylene reduction) in stony corals: Evidence for coral-bacteria interactions. *Mar. Ecol. Prog. Ser. Oldend.* **111**: 259–264.

Sheu, S.-Y., Liu, L.-P., Tangs, S.-L., and Chen, W.-M. (2016) Thalassotalea euphylliae sp. nov., isolated from the torch coral Euphyllia glabrescens. *Int. J. Syst. Evol. Microbiol.* **66**: 5039–5045.

Shi, X., Yu, M., Yan, S., Dong, S., and Zhang, X.-H. (2012) Genome sequence of the thermostable-agarase-producing marine bacterium Catenovulum agarivorans YM01T, which reveals the presence of a series of agarase-encoding genes. *J. Bacteriol.* **194**: 5484.

Shick, J., Lesser, M.P., and Jokiel, P.L. (1996) Effects of ultraviolet radiation on corals and other coral reef organisms. *Glob. Chang. Biol.* **2**: 527–545.

Shick, J.M. and Dunlap, W.C. (2002) Mycosporine-like amino acids and related gadusols: Biosynthesis, accumulation, and UV-protective functions in aquatic organisms. *Annu. Rev. Physiol.* **64**: 223–262.

Shindo, K., Asagi, E., Sano, A., Hotta, E., Minemura, N., Mikami, K., et al. (2008) Diapolycopenedioic acid xylosyl esters A, B, and C, novel antioxidative glyco-C30-carotenoic acids produced by a new marine bacterium Rubritalea squalenifaciens. *J. Antibiot. (Tokyo).* **61**: 185.

Smith, S. V. and Key, G.S. (1975) Carbon dioxide and metabolism in marine environments. *Limnol. Oceanogr.* **20**: 493–495.

Smith, T.A., Driscoll, T., Gillespie, J.J., and Raghavan, R. (2015) A Coxiella-like endosymbiont is a potential vitamin source for the Lone Star tick. *Genome Biol. Evol.* **7**: 831–838.

Spring, S., Scheuner, C., Göker, M., and Klenk, H.-P. (2015) A taxonomic framework for emerging groups of ecologically important marine gammaproteobacteria based on the reconstruction of evolutionary relationships using genome-scale data. *Front. Microbiol.* **6**:.

749 Stimson, J. and Kinzie, R.A. (1991) The temporal pattern and rate of release of zooxanthellae
750 from the reef coral Pocillopora damicornis (Linnaeus) under nitrogen-enrichment and
751 control conditions. *J. Exp. Mar. Bio. Ecol.* **153**: 63–74.

752 Sweet, M., Burn, D., Croquer, A., and Leary, P. (2013) Characterisation of the Bacterial and
753 Fungal Communities Associated with Different Lesion Sizes of Dark Spot Syndrome
754 Occurring in the Coral Stephanocoenia intersepta. *PLoS One* **8**: 1–9.

755 Sweet, M.J. and Bulling, M.T. (2017) On the Importance of the Microbiome and Pathobiome
756 in Coral Health and Disease. *Front. Mar. Sci.* **4**: 9.

757 Sweet, M.J., Croquer, A., and Bythell, J.C. (2011) Bacterial assemblages differ between
758 compartments within the coral holobiont. *Coral Reefs* **30**: 39–52.

759 Teplitski, M., Krediet, C.J., Meyer, J.L., and Ritchie, K.B. (2016) The Cnidaria, Past, Present
760 and Future Goffredo,S. and Dubinsky,Z. (eds) Springer International Publishing, Cham.

761 Teramoto, M., Ohuchi, M., Hatmanti, A., Darmayati, Y., Widyastuti, Y., Harayama, S., and
762 Fukunaga, Y. (2011) Oleibacter marinus gen. nov., sp. nov., a bacterium that degrades
763 petroleum aliphatic hydrocarbons in a tropical marine environment. *Int. J. Syst. Evol.*
764 *Microbiol.* **61**: 375–380.

765 Thompson, F.L. (2006) Thalassomonas loyana sp. nov., a causative agent of the white plague-
766 like disease of corals on the Eilat coral reef. *Int. J. Syst. Evol. Microbiol.* **56**: 365–368.

767 Thompson, J.R., Rivera, H.E., Closek, C.J., and Medina, M. (2014) Microbes in the coral
768 holobiont: partners through evolution, development, and ecological interactions. *Front.*
769 *Cell. Infect. Microbiol.* **4**: 176.

770 Turk-Kubo, K.A., Frank, I.E., Hogan, M.E., Desnues, A., Bonnet, S., and Zehr, J.P. (2015)
771 Diazotroph community succession during the VAHINE mesocosm experiment (New
772 Caledonia lagoon). *Biogeosciences* **12**: 7435–7452.

773 Ulstrup, K.E., Hill, R., Van Oppen, M.J.H., Larkum, A.W.D., and Ralph, P.J. (2008) Seasonal
774 variation in the photo-physiology of homogeneous and heterogeneous Symbiodinium
775 consortia in two scleractinian corals. *Mar. Ecol. Prog. Ser.* **361**: 139–150.

776 Warner, M., Chilcoat, G., McFarland, F., and Fitt, W. (2002) Seasonal fluctuations in the
777 photosynthetic capacity of photosystem II in symbiotic dinoflagellates in the Caribbean
778 reef-building coral Montastraea. *Mar. Biol.* **141**: 31–38.

779 van de Water, J.A.J.M., Melkonian, R., Voolstra, C.R., Junca, H., Beraud, E., Allemand, D.,
780 and Ferrier-Pagès, C. (2016) Comparative Assessment of Mediterranean Gorgonian-
781 Associated Microbial Communities Reveals Conserved Core and Locally Variant
782 Bacteria. *Microb. Ecol.* 1–13.

783 van de Water, J.A.J.M., Voolstra, C.R., Rottier, C., Cocito, S., Peirano, A., Allemand, D., and
784 Ferrier-Pagès, C. (2017) Seasonal stability in the microbiomes of temperate gorgonians
785 and the red coral Corallium rubrum across the Mediterranean Sea. *Microb. Ecol.*

786 van de Water, J. a. J.M., Lamb, J.B., van Oppen, M.J.H., Willis, B.L., and Bourne, D.G.
787 (2015) Comparative immune responses of corals to stressors associated with offshore
788 reef-based tourist platforms. *Conserv. Physiol.* **3**: cov032.

789 Webster, N.S. and Bourne, D. (2007) Bacterial community structure associated with the
790 Antarctic soft coral, Alcyonium antarcticum. *FEMS Microbiol. Ecol.* **59**: 81–94.

791 Wild, C., Huettel, M., Klueter, A., Kremb, S.G., Rasheed, M.Y.M., and Jørgensen, B.B.
792 (2004) Coral mucus functions as an energy carrier and particle trap in the reef ecosystem.
793 *Nature* **428**: 66–70.

794 Wild, C., Mayr, C., Wehrmann, L., Schöttner, S., Naumann, M., Hoffmann, F., and Rapp, H.
795 (2008) Organic matter release by cold water corals and its implication for fauna–microbe
796 interaction. *Mar. Ecol. Prog. Ser.* **372**: 67–75.

797 Willis, B.L., Page, C.A., and Dinsdale, E.A. (2004) Coral disease on the great barrier reef. In,
798 *Coral health and disease*. Springer, pp. 69–104.

799 Winter, C., Moeseneder, M.M., and Gerhard, J. (2001) Impact of UV radiation on
800 bacterioplankton community composition. **67**: 665–672.

801 Yakimov, M.M., Cappello, S., Crisafi, E., Tursi, A., Savini, A., Corselli, C., et al. (2006)
802 Phylogenetic survey of metabolically active microbial communities associated with the
803 deep-sea coral Lophelia pertusa from the Apulian plateau, Central Mediterranean Sea.
804 *Deep Sea Res. Part I Oceanogr. Res. Pap.* **53**: 62–75.

805 Yi, H., Bae, K.S., and Chun, J. (2004) Aestuariibacter salexigens gen. nov., sp. nov. and
806 Aestuariibacter halophilus sp. nov., isolated from tidal flat sediment, and emended
807 description of Alteromonas macleodii. *Int. J. Syst. Evol. Microbiol.* **54**: 571–576.

808 Ziegler, M., Roik, A., Porter, A., Zubier, K., Mudarris, M.S., Ormond, R., and Voolstra, C.R.
809 (2016) Coral microbial community dynamics in response to anthropogenic impacts near
810 a major city in the central Red Sea. *Mar. Pollut. Bull.* **105**: 629–640.

811

812

Table S1. **Results of two-factor ANOVA testing the effect of season and UVR on variables.** Significant values ($p<0.05$) are highlighted in bolt.

	Factor	F	*p*	significativity
Symbiont density	UVR	5.17	**0.0309**	*
	Season	4.27	**0.0481**	*
	UVRxSeason	0.519	0.4771	-
Chlorophyll a content	UVR	15.74	**0.0006**	**
	Season	13.32	**0.0013**	**
	UVRxSeason	1.55	0.2246	-
Protein content	UVR	0.16	0.6915	-
	Season	13.75	**0.0010**	**
	UVRxSeason	2.62	0.1171	-
Calcification rate	UVR	4.01	0.0684	-
	Season	54.05	**<0.0001**	**
	UVRxSeason	1.87	0.1963	-
TOC	UVR	3.12	0.1029	-
	Season	5.56	**0.0356**	*
	UVRxSeason	0.44	0.5191	-
DOC	UVR	0.03	0.865	-
	Season	172.7	**<0.0001**	**
	UVRxSeason	4.38	0.0537	-
POC	UVR	11.34	**0.0056**	*
	Season	43.44	**<0.0001**	**
	UVRxSeason	18.97	**0.0009**	**
TN	UVR	17.34	**0.0009**	**
	Season	2.95	0.1077	-
	UVRxSeason	4.57	0.0506	-
DN	UVR	1.20	0.2910	-
	Season	9.47	**0.00767**	*
	UVRxSeason	5.17	**0.0380**	*
PN	UVR	15.65	**0.0014**	**
	Season	8.38	**0.0118**	*
	UVRxSeason	0.87	0.3676	-
rETRmax	UVR	1.43	0.2484	-
	Season	20.86	**0.0003**	**
	UVRxSeason	39.28	**<0.0001**	**
Aminopeptidase EEAmax	UVR	0.006	0.9417	-
	Season	22.68	**0.0008**	**
	UVRxSeason	5.77	**0.0372**	*
α-glucosidase EEAmax	UVR	0.52	0.4852	-
	Season	37.21	**0.0001**	**
	UVRxSeason	1.45	0.0884	-
N degradation	UVR	13.55	**0.0051**	*
	Season	4.03	0.0755	-
	UVRxSeason	0.05	0.8344	-
C degradation	UVR	0.09	0.775	-
	Season	0.08	0.78	-
	UVRxSeason	0.63	0.443	-

Table S2. Results of permANOVA performed on the mucus associated bacteria.

	Df	F model	R^2	p
Season	1	10.2	0.33	0.001
UV	1	3.2	0.1	0.012
SeasonxUV	1	2.5	0.08	0.043
Residuals	15		0.49	

Table S3. Results of permANOVA performed on the coral associated bacteria.

	Df	**F model**	**R^2**	**p**
Season	1	3.4	0.18	0.001
UV	1	1.1	0.06	0.300
SeasonxUV	1	0.9	0.05	0.510
Residuals	14		0.72	

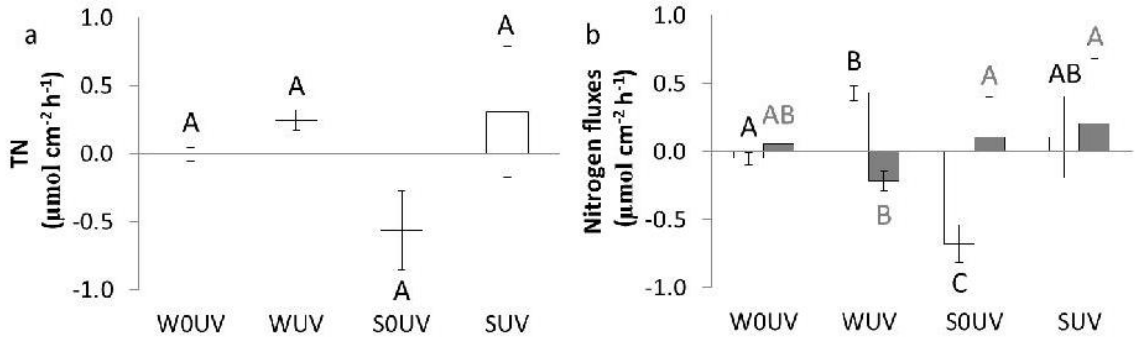


Figure S1. Nitrogen fluxes. Total nitrogen fluxes (a), dissolved (grey columns) and particulate (white columns) nitogen fluxes (b) in winter without UVR (W0UV), winter under UVR (WUV), summer without UVR (S0UV) and summer under UVR (SUV). Data are mean and standard deviations of five replicates. Values noted with the same letter are not significantly different (*p-value* > 0.05).

Paper n°5 – *In prep.*

Effects of ultraviolet radiation and nutrient levels on the physiological response and mucus production of *Pocillopora damicornis* following thermal stress

Stress of life in corals

Lucile Courtial[1,2,3*], Fanny Houlbrèque[3], Christine Ferrier-Pagès[2*]

1. Sorbone Universités, UPMC Univ Paris 06 IFD-ED 129, 4 Place Jussieu, 75252 PARIS cedex 05, France
2. Centre Scientifique de Monaco, Equipe Ecophysiologie, 8 Quai Antoine 1er, 98000 Monaco (Principality)
3. UMR ENTROPIE (IRD, Université de La Réunion, CNRS), Laboratoire d'Excellence « CORAIL», BP A5, 98848, Nouméa cedex, New Caledonia

*Corresponding authors: lcourtial@centrescientifique.mc (+37797774465), ferrier@centrescientifique.mc (+37797774480)

Keywords: Climate change, global warming, ultraviolet radiation, nutrient depletion, eutrophication, coral physiology, organic matter fluxes, *Pocillopora damicornis*

Primary Reasearch Article

Abstract

The increasing occurrence of coral bleaching worldwide has prompted scientists to understand which factors enhance or mitigate the impact of high temperatures on corals. On the one hand, global warming is usually associated with high ultraviolet radiation levels, and depletes surface waters from nutrients, due to the stratification of the water column. On the other hand, coastal reefs are subject to increased levels of inorganic nutrients (nitrate, phosphate) due to eutrophication. In this study, we thus assessed the individual and crossed effects of high temperature, ultraviolet radiation and nutrient level on the physiology of the reef building coral *Pocillopora damicornis*. We also investigated how these three factors affect the organic matter fluxes between seawater and *P. damicornis*, as well as the organic matter degradation by mucus-associated bacteria. Ultraviolet radiation exposure had minimal effect on *P. damicornis* thermally-induced bleaching but nutrient starvation increased the bleaching intensity and impaired coral growth. Contrary to the above observations, ultraviolet radiation significantly decreased nitrogen release and organic matter degradation by mucus associated bacteria whereas nutrient level had minimal effect on the release of organic carbon and nitrogen by *P. damicornis*. However, elevated seawater temperature moderated the ultraviolet radiation impact on organic matter degradation. Overall, our results show that each biological parameter of the coral will be differently affected by changes in the physico-chemical quality of the environment. In addition, the interactional effects of the three environmental factors studied, suggest that multifactorial approaches are required to understand the response of coral reefs to environmental changes.

1. Introduction

Global warming is a threat to the structure and function of tropical marine ecosystems, and thus to the services they provide to nature and humans (Doney et al., 2012; Eakin et al., 2016; Oliver et al., 2009; Spalding and Brown, 2015). In tropical areas, global warming manifests primarily in terms of increased seawater temperature and ultraviolet radiation (UVR) levels (because of changes in the stratospheric circulation, Watanabe et al. 2011), leading to enhanced stratification and nutrient depletion (Hoegh-Guldberg and Bruno, 2010; Polovina et al., 2008). In parallel, tropical coastal ecosystems increasingly suffer from chronic local pressures such as water eutrophication (*i.e.* enhanced nutrients, particles, and sedimentation levels) (Nixon, 1995; Smith, 2003) which come on top of the global changes. Discharges of inorganic nutrients from urbanization and human agricultural activities disturb the nutrient stoichiometry in seawater (i.e. Sterner and Elser 2002), which in turn impacts organisms' physiology and increase competition for nutrient, light and space (Lapointe et al., 2004; Pomeroy, 1974; Smith and Schindler, 2009). There is thus growing evidence that these shifting conditions, which significantly alter the surface water conditions, will cause changes in shallow benthic community composition and functions (Bruno and Bertness, 2001; Doney et al., 2012).

Coral reefs, which are one of the major tropical coastal ecosystems, and more specifically corals, the main reef builders, are rapidly declining because of these environmental changes (Baker et al., 2004; Beardall et al., 2014; D'Angelo and Wiedenmann, 2014; Doney et al., 2012; Hoegh-Guldberg et al., 2007). Over the last decades, corals indeed experienced more frequent and intense mass bleaching events (*i.e.* global loss of symbionts or photosynthetic pigments from the host tissue), responsible for high coral mortality (Glynn, 2012; Hoegh-Guldberg, 1999). Rising sea surface temperature is the main factor triggering coral bleaching (Hoegh-Guldberg, 1999; Hughes et al., 2017) as it increases the oxidative stress in cells (Lesser, 2012, 2006) and damages the structural and metabolic proteins (Warner et al., 1999). However, there is not always a cause-effect relationship between high temperature and coral bleaching, which suggests that other environmental factors, including UVR and nutrient level, which change in parallel to temperature, are important to consider (Hoegh-Guldberg, 1999). These environmental factors may enhance, reduce or even reverse the thermal stress effect on corals, so that our abilities to forecast the ecological consequences of global warming on reef corals are still limited. While no study combined UVR, nutrient level and temperature, those combining two of these factors led to contradicting results. For example, UVR worsened the

thermally-induced coral bleaching in some species (Courtial et al., 2017; D'Croz et al., 2001; Ferrier-Pagès et al., 2007; Fitt and Warner, 1995; Lesser and Farrell, 2004; Lesser et al., 1990) but not in others (Courtial et al., 2017; D'Croz et al., 2001; Ferrier-Pagès et al., 2007). Similarly, the few studies which combined high temperature and nutrient enrichment reached different conclusions depending on the nutrient species (Ezzat et al., 2016b; Holcomb et al., 2010; Schlöder and D'Croz, 2004; Shantz and Burkepile, 2014; Vega Thurber et al., 2014; Wiedenmann et al., 2013). These conflicting evidences lies in the poor mechanistic understanding of the interactive effects of physico-chemical factors on coral physiology and highlight the necessity of considering a multi-factorial approach to predict coral's responses to climate change.

In addition, the reduction in coral metabolism following bleaching may also affect the overall reef ecosystem biochemistry and productivity, for example through changes in mucus production. This is indeed an important process both for corals for prey capture and defense against environmental pressure (Brown and Bythell, 2005) and for reefs as a main organic matter (OM) source for microorganisms. Heterotrophic bacteria and cyanobacteria, including diazotrophic cells, indeed rely on OM supply for their growth, di-nitrogen fixation and recycling activities within the reef (Amon and Benner, 1996; Bednarz et al., 2017; Camps et al., 2016; Tranvik, 1992). Modifications in OM quality and/or quantity may therefore change the reef water biochemistry and the reef food chain structure. However, very few studies have assessed changes in OM fluxes under environmental stress, at the exception of thermal stress (Fitt et al., 2009; Levas et al., 2016; Niggl et al., 2008; Piggot et al., 2009; Tremblay et al., 2012). Overall, conflicting results were again observed (enhanced or reduced OM production with temperature increase). The addition of UVR, which is known to degrade OM in the water column (Tedetti and Sempéré, 2006), and nutrient enrichment, which may enhance OM production by corals (Bednarz et al., 2012) makes any future previsions even harder. Further work is therefore needed to better understand the single and cross effects of these changing environmental factors on OM fluxes in order to have an overall comprehension of the effects of climate change on coral reefs.

Here, we describe the individual and cross effects of thermal stress, UVR and nutrient (NO_3^-;PO_4^{3-}) enrichment on the physiology of the reef-building coral, *Pocillopora damicornis* as well as on its production of OM. We also monitored the degradation rate of this OM by mucus-associated bacteria, to predict the changes that may occur within the reef water column. We hypothesize that (1) UVR exposure worsens the temperature-induced bleaching;

(2) nutrient enrichment or depletion shape the metabolic response of *P. damicornis* under both normal and stressful conditions; (3) temperature, UVR and nutrient levels induce changes in quality of the released OM and the bacterial activity.

2. Material and methods

Mother colonies of *Pocillopora damicornis* (Pocilloporidae), originating from the Red Sea, were grown at 25°C in the aquaria set up of the Centre Scientifique de Monaco. They were maintained in oligotrophic seawater (~0.5 µM NO_3 and ~0.1 µM PO_4) filtrated on 5 µm without UVR, under a PAR (photosynthetically active radiation) of 200 µmol photons m^{-2} s^{-1} provided by 400 W metal halide lamps (HPIT, Philips) and were fed twice a week with *Artemia salina* nauplii. A total of 60 nubbins were collected from 10 mother colonies (6 nubbins per colony). After being cut, nubbins were allowed to heal under the same conditions as the mother colonies for four weeks and then kept unfed for three weeks before the experiment.

2.1. Experimental settings

Nubbins were then evenly distributed in twenty-four tanks of 25 L, continuously supplied with seawater. Twelve conditions, representing two UVR levels, two temperatures, and three nutrient conditions in a cross factor design were tested in duplicated tanks: depleted seawater (<0.1 µM nitrate (NO_3) and <0.05 µM phosphate (PO_4)) without UVR (hereafter called D0UV) and with UVR (DUV); ambient seawater (~0.5 µM NO_3 and ~0.1 µM PO_4) without UVR (i.e. control condition, C0UV) and with UVR (CUV); enriched seawater (3 µM NO_3 and 0.5 µM of phosphates) without UVR (E0UV) and with UVR (EUV). The six conditions were maintained at ambient temperature (AT, 25°C ± 0.3) or high temperature (HT, 30°C ± 0.3). PAR remained at 200 ± 10 µmol photons m^{-2} s^{-1} inside the tanks (12: 12 light: dark). UVR was provided 4 h a day by Q-Panel UVA 340 lamps and was adjusted to match the levels measured at 1-5 m depth on the reef (Ferrier-Pagès et al., 2007), ca. 1.5 W m^{-2} UVB and 30 W m^{-2} UVA. The radiation intensity was controlled using a LI-COR data logger (LI-1000) connected to a spherical quantum sensor (LI-193) for PAR and using an International Light ILT1400 portable radiometer and detectors (SEL240/UVB-1/TD and SEL033/UVA/TD) for UVR. As corals were grown without UVR prior the experiment, their response can be compared to deep-living or shaded corals facing an increase in UVR level. Temperature was controlled using heaters connected to ElliWell PC 902/T controllers. Seawater in the tanks was renewed at a flow rate of 20 L h^{-1}. Nutrient depleted tanks were

continuously supplied with water from batch tanks depleted in nutrients with a bio-chemical filter (Fluidize bed filter BF-700). The filters were installed following the manufacturer's recommendation several months prior the experiment. In nutrient enriched conditions, tanks were supplied with a solution of sodium nitrate ($NaNO_3$) in combination with sodium dihydrogenophosphate (NaH_2PO_4) continuously pumped from batch tanks with a peristaltic pump and delivered to the experimental tanks at a final concentration of 3 µM for $NaNO_3$ and 0.5 µM for NaH_2PO_4. The stock solutions were renewed every 3 d and added to the tanks at a constant flow rate of 0.3 L h^{-1}. Nutrient concentrations in each aquarium were monitored in triplicate once a week using an Autoanalyzer (Alliance Instrument, AMS, France) according to Tréguer and Le Corre (1975).

The experiment was run for a total of 9 weeks. During the first 4 weeks, all nubbins were acclimated to the UVR and nutrient conditions. The temperature was then gradually increased (within a week) from 25°C to 30°C in HT tanks. High temperature was maintained for 3 additional weeks before the measurements were conducted. At the end of the 9 weeks, 5 nubbins per condition, randomly taken in the replicated tanks, were used to measure photosynthesis, respiration and growth rates, total organic carbon (TOC) and total nitrogen (TN) fluxes and extracellular α-glucosidase and aminopeptidase activities. At the end of the measurements, nubbins were frozen at -20°C to assess the symbiont density, chlorophyll *a* (chl *a*) and protein contents. For all measurements, temperature, UVR, light and nutrient conditions were maintained constant and identical to the experimental tanks except for the short-term measurements of photosynthesis, which were made without UVR. Data were all normalized to the surface area of the corals, measured using the wax technique (Stimson and Kinzie 1991).

2.2. Physiological measurements

Nubbins were incubated in 50 mL glass chambers filled with water from the different nutrient conditions, filtered at 0.45 µm and continuously stirred with stirring bars. Rates of net photosynthesis (Pn) and respiration (R) were assessed at 200 and 0 µmol photons m^{-2}. Each chamber was equipped with oxygen-sensors (Unisense optode) connected to the Oxy-4 software (Chanel fiber-optic oxygen meter, Presens, Regensburg, Germany). Two calibrations of the optodes (100% and 0% oxygen) were done prior each measurement using air-saturated and nitrogen saturated seawater. Pn and R were deduced from the regressing oxygen production or consumption against time. Gross photosynthesis (Pg) was calculated by adding

the absolute values of R to Pn. Data were expressed in µmol O_2 h^{-1} cm^{-2} d^{-1} and corrected against a blank (filtrated seawater incubated for the same period without nubbin). Growth rates were assessed using the buoyant weight technique (Jokiel et al., 1978) on 5 nubbins per treatment the week before the beginning of the experiment and after the 9 weeks of treatments. The growth in % d^{-1} was then calculated using the equation: $(BW_f - BW_i)/(BW_i x Time) x100$ where BW_f and BW_i are the final and the initial buoyant weight respectively and Time is the number of days between the two measurements.

2.3. Symbiont, chlorophyll a and protein content

Coral tissue was extracted from the skeleton using an air pick and homogenized with a Potter tissue grinder. The symbiont density was then quantified with three sub-samples of 100 µL using a Z1 Coulter Particle Counter (Beckman Coulter, Godinot et al. 2013). Proteins were extracted according to Hoogenboom et al. (2010) in sodium hydroxide and quantified using a BCA essay kit according to Smith et al. (1985) on a Xenius spectrofluorometer (Safas, Monaco). To quantify chl *a*, the remaining homogenate was centrifuged at 5000 x *g* for 10 min at 4°C to separate the host tissue (supernatant) from the symbionts (pellet) and the pellet was re-suspended in 5 mL of acetone. Samples were then kept at 4°C for 24 h in darkness to insure total extraction. The next day, samples were centrifuged for 15 min at 11000 x *g* and the absorbance was measured at 630, 663 and 750 nm using a Xenius spectrofluorometer. Chl *a* concentrations were calculated using the equations of Jeffrey and Humphrey (1975).

2.4.Organic matter (particulate and dissolved) release and external enzymatic activity

To assess the total organic carbon (TOC) and the total nitrogen (TN) fluxes as well as the extracellular enzymatic activity (EEA), nubbins, were incubated for 6 h in 250 mL of 0.45 µm-filtered seawater, taken from the different nutrient conditions. Blanks (seawater without nubbin) were run in parallel.

For TOC and TN measurements, triplicates of 20 mL of seawater from each beaker were sampled with sterile syringes at the beginning and the end of the incubation. Samples were stored at -20°C in glass vials, previously washed for 24 h in 10% HCl and burned at 500°C for 4 h (Naumann et al., 2010), before being analyzed using a TOC-L analyzer (Shimadzu, Japan). Fluxes (TOC or TN, nM h^{-1} cm^{-2}) were calculated as: $TM=(TM_{coral}-TM_{blk})/$ (Surface x Time) where TM designates TOC or TN, TM_{coral} and TM_{blk} (nM h^{-1}) are the TM concentrations in the seawater after 6 h incubation respectively with or

without nubbin, Time (h) is the 6 h incubation time and Surface is the nubbin surface (cm^{-2}). Negative fluxes indicate a net uptake by coral nubbins, and positive fluxes indicate a net release from corals.

EEA were measured using two fluorescent analogue substrates of the α-glucosidase and aminopeptidase; the 4-methylumbeliferyl-α-D-glucopyranoside (αMUF) and the L-Leucine-7-amido-4-methyl-coumarin hydrochloride (Leu-MCA). The substrates emit a fluorescent signal after cleavage by the enzymes which can be transformed into quantity of substrate hydrolyzed using known concentrations of amino 4-methylcoumarin (MCA) and 4-methylumbeliferone (MUF). Three replicates of 2 mL of water in which corals were incubated during 6 h for the TOC and TN measurements were sampled from each beaker and added to the two fluorescent substrates diluted in filtrated water from the different nutrient condition at final volume and concentration of 6 mL and 250 µM (according to Hoppe 1983). The obtained solutions were then incubated in the dark for up to 168 h in thermostated shaking bath at the experimental temperatures (25°C or 30°C). EEAs were measured every 24 h using a spectrofluorometer (Synergy-H1, Biotek) in 96 white well plates. For this purpose, wells were filled with 240 µL of solution and to obtain maximal fluorescence intensity, the pH was adjusted by the addition of 60 µL of 10.8 Tris-HCl buffer (Fonvielle et al., 2015). The maximal EEAs (EEA_{max}) were assessed from the EEAs vs Time curves and used to calculate carbon and nitrogen degradation rates.

Carbon degradation rate was calculated following the equation: %C degraded = EEA_{max}x100/TOC with EEA_{max} the α-glucosidase maximal enzymatic activity (nmol L^{-1} h^{-1}) of the mucus-associated-bacteria and TOC (nmol L^{-1} h^{-1}) the quantity of organic carbon contained in the mucus (TOC_{coral}-TOC_{blk}). Similarly, nitrogen degradation was calculated as %N degraded = EEA_{max}x100/TON with EEA_{max} the aminopeptidase maximal enzymatic activity (nmol L^{-1} h^{-1}) of the mucus-associated-bacteria and TON (nmol L^{-1} h^{-1}) the quantity of total organic nitrogen contained in the mucus.

2.5.Statistical analysis

All data were expressed as mean ± standard deviation. Principal component analysis (PCA) was performed on all nubbins and all conditions using 8 variables: the growth rate (Growth), the respiration rate (R), the net photosynthesis (Pn), the symbiont density (Symbiont), the protein content (Protein), the chlorophyll *a* content (Chla), TOC release (TOC) and TN release (TN). The effects of UVR, high temperature and nutrient enrichment on the biological parameters were assessed with three-way ANOVAs using UVR (presence,

absence), temperature (25°C and 30°C) and nutrient level (depleted, control and enriched) as factors. Because of the lack of significant effects of UVR on coral physiology, two-way ANOVAs using only high temperature and nutrient level were performed on Pn, R, growth, chl *a*, symbiont and protein contents. For all ANOVAs, data were log transformed when needed in order to fulfill the normality of the residuals and variance homoscedasticity criteria which were tested using Shapiro and Bartlett tests respectively. A Tukey's post hoc test was performed when results of the ANOVAs were significant ($p < 0.05$). In addition, when the combination of two environmental factors had a significant effect, we characterized it as "synergistic" when the interaction factor between them was significant or as "additive" when it was not significant (Dunne, 2010).

3. Results

3.1. Global analysis of the environmental effects on coral physiology, and organic matter fluxes

PCA conducted with all data related to corals maintained under the different conditions is represented in Figure 1. The two first axes are significant and explain 52% of the variance. All variables are well represented along the first axis except for the protein content (Protein), and the total organic carbon fluxes (TOC), better described along the second axis (Fig. 1). The first axis of the PCA is clearly oriented by the physiological differences driven by the two experimental temperatures and divides the corals into two groups (Fig. 1a). The first group (blue symbols) contains only nubbins growing at 25°C, characterized by a higher symbiont and chl *a* content, a higher metabolism (Pn, R and growth) and lower TN release rates compared to the second group (red symbols) representing nubbins undergoing a thermal stress (30°C). Overall, no clear effect of UVR or nutrient level is detected by the PCA (Fig. 1b).

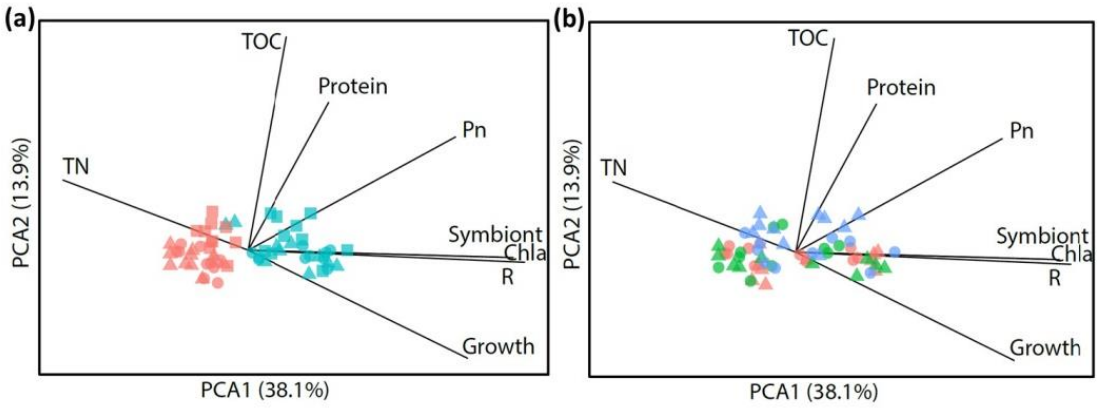


Figure 1. Distance biplots resulting from the Principal component analyses on all coral data. Physiological traits (black) used as descriptors represented symbiont density (Symbiont), protein (Protein) and chlorophyll a (Chla) contents, growth (Growth), respiration (R) and net photosynthetic rates (Pn), total organic carbon (TOC) and total nitrogen (TN) fluxes. The first PCA axis accounted for 38.1% of the variability and the second PCA axis for 13.9%. Figure 1.a: blue and red symbols represent nubbins at 25°C and 30°C respectively; triangles, circles and squares represent nubbins in depleted, control and enriched nutrient conditions. Figure 1.b: green, red and blue symbols are depleted, control and enriched nutrient condition respectively; triangles and circles represent nubbins with or without UVR respectively.

3.2.Coral physiology

The above PCA results suggest that among the different factors tested (temperature, UVR and nutrient level), temperature was the most important driver of changes in coral physiology. Three-way-ANOVAs and the Tuckey tests however showed numerous significant effects of the nutrient level whereas it confirmed the lack of UVR effect on the coral physiology (Table 1; Table S1). Therefore, for clarity in the presentation of the following results, data from nubbins exposed or not to UVR were pooled together for each temperature and nutrient condition.

Source of Variation	df	Sum of quares	F	*p*
Symbiont density (cell cm^{-2})				
T	1	296.33	216.769	<0.0001
NUT	2	55.75	20.024	<0.0001
UV	**1**	**1.51**	**1.108**	**0.2988**
T x NUT	2	12.72	4.653	0.0151
T x UV	1	2.97	2.175	0.1479
NUT x UV	2	4.34	1.587	0.2168
T x NUT x UV	2	6.08	2.225	0.1210
Chlorophyll a (mg cm^{-2})				
T	1	6.967	327.110	<0.0001
NUT	2	0.482	11.325	0.0002
UV	**1**	**0.004**	**0.193**	**0.6636**
T x NUT	2	0.435	10.216	0.0004
T x UV	1	0.006	0.285	0.5973
NUT x UV	2	0.587	13.778	<0.0001
T x NUT x UV	2	0.090	2.111	0.1382
Protein content (µg cm^{-2})				
T	1	0.901	2.276	0.1403
NUT	2	11.126	14.057	<0.0001
UV	**1**	**1.233**	**3.116**	**0.0862**
T x NUT	2	5.495	6.943	0.0030
T x UV	1	2.566	6.485	0.0154
NUT x UV	2	1.077	1.361	0.2696
T x NUT x UV	2	0.845	1.067	0.3548
Growth rate (% d^{-1})				
T	1	0.2719	55.411	<0.0001
NUT	2	0.0182	1.858	0.1741
UV	**1**	**0.0104**	**2.130**	**0.1552**
T x NUT	2	0.0055	0.557	0.5792
T x UV	1	0.0068	1.378	0.2500
NUT x UV	2	0.0478	4.874	0.0150
T x NUT x UV	2	0.0923	9.404	0.0007

Source of Variation	df	Sum of quares	F	*p*
Pn (µmol cm^{-2} h^{-1})				
T	1	14.407	982.389	<0.0001
NUT	2	1.719	58.607	<0.0001
UV	**1**	**0.040**	**2.708**	**0.1110**
T x NUT	2	0.265	9.040	0.0009
T x UV	1	0.002	0.169	0.6845
NUT x UV	2	0.015	0.505	0.6086
T x NUT x UV	2	0.105	3.596	0.0407
R (µmol cm^{-2} h^{-1})				
T	1	157.77	884.385	<0.0001
NUT	2	8.44	23.668	<0.0001
UV	**1**	**0.05**	**0.289**	**0.5940**
T x NUT	2	7.62	21.363	<0.0001
T x UV	1	0.00	0.013	0.9100
NUT x UV	2	0.16	0.437	0.6490
T x NUT x UV	2	0.55	1.537	0.2290
Pg (µmol cm^{-2} h^{-1})				
T	1	103.65	2082.721	<0.0001
NUT	2	5.51	55.116	<0.0001
UV	**1**	**0.16**	**3.116**	**0.0848**
T x NUT	2	0.61	6.153	0.0045
T x UV	1	0.32	6.530	0.0143
NUT x UV	2	0.35	3.470	0.0403
T x NUT x UV	2	0.00	0.015	0.9849

Table 1. Three way ANOVA results for physiological parameters. Results were considered significant when $p< 0.05$. Bolted figures highlight the non-significant effects of UVR.

At 25°C, there was no significant effect of the nutrient level on the tissue parameters (symbiont density, chl *a* and protein concentrations, Table 2, Fig. S1). However, nutrient depletion lowered Pg (Fig. 2b), while nutrient enrichment slowed down coral growth (Fig. 2d). Moreover, Pg:R ratios were higher in the enriched nutrient condition than in control or depleted conditions (Fig. 2b). Exposure to elevated temperature significantly reduced the

symbiont density, the chl *a* content (Table 2) as well as Pg, Pn and R in all nutrient condition (Fig. 2a,b,c). In addition, the combination of thermal stress and nutrient depletion had an additive and negative effect on coral growth as well as a synergistic and negative effect on chl *a* (Table S2 avec ANOVAs). Nutrient-depleted nubbins therefore experienced a higher decrease in chl *a* and in growth rate following thermal stress than in any other condition. On the other hand, the combination of thermal stress and nutrient enrichment had synergistic positive effect on chl *a*, Pg, Pn and R, which limited the negative impact of temperature on nubbins (Table S2 avec ANOVAs). Nutrient-enriched nubbins therefore presented the lowest decrease in chl *a*, Pg, Pn and R rates following thermal stress than in any other seawater condition.

Comparison	Parameters	Dep.	Cont.	Enr.
At 25°C	Symbiont	- (A)	- (A)	- (A)
	Chlorophyll *a*	- (A)	- (A)	- (A)
	Protein	- (A)	- (A)	- (A)
At 30°C	Symbiont	- (A)	- (A)	- (A)
	Chlorophyll a	- (A)	- (A)	+ 67.4 % (B)
	Protein	- (A)	- (A)	+ 56.8 % (B)
Between 25 and 30°C	Symbiont	-64.9 %	-58.7 %	-57.9 %
	Chlorophyll *a*	-78.5 %	-54.8 %	-40.0 %
	Protein	-	-	-

Table 2. Changes observed (%) in tissue parameters at and between 25 and 30°C in depleted (Dep.), control (Cont.) and enriched (Enr.) nutrient conditions. For each comparison (at 25°C, at 30°C and between 25 and 30°C), percentages were calculated using the control condition at 25°C, 30°C and 25°C respectively. Letters indicate significant differences after performing Tukey's post hoc tests.

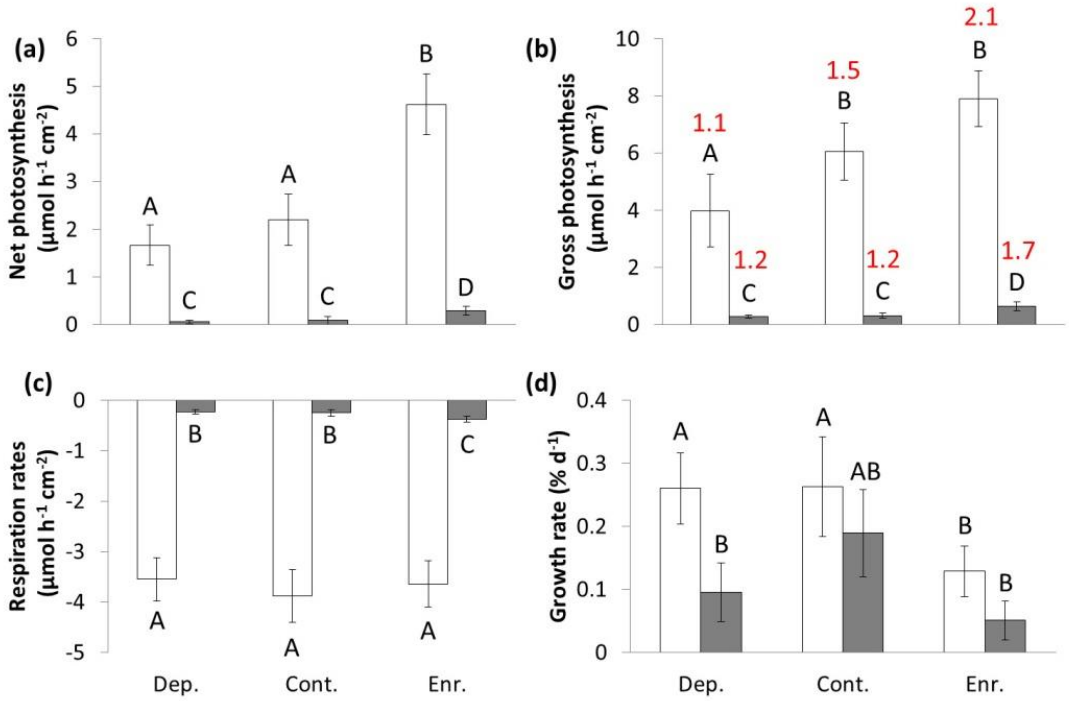


Figure 2. Net photosynthesis (µmol O_2 cm^{-2} h^{-1}, a), gross photosynthesis (µmol O_2 cm^{-2} h^{-1}, b), respiration rates (µmol O_2 cm^{-2} h^{-1}, c) and growth rates (% d^{-1}) in depleted (Depl.), control (Cont.) and enriched (Enr.) nutrient condition at 25°C (white columns) and 30°C (grey columns). Red numbers in figure b are Pg/R ratio values. Error bars represent standard deviations calculated from five replicates. Values noted with the same letter are not significantly different (*p-value* > 0.05).

3.3.Organic matter fluxes

In the following chapters, positive fluxes (from corals to seawater) and negative fluxes (from seawater to corals) will be translated into "release rates" and "uptake rates" respectively. The OM and nutrient fluxes were significantly affected by temperature, UVR and nutrient level, with cross effects between these parameters (Table S3).

Overall, TOC fluxes did not follow any logic trend, due to the numerous interactions between temperature, UVR and nutrients (Fig. 3a,b). For example, UVR respectively reduced (at 25°C) and increased (at both 25°C and 30°C) TOC release rates in the depleted and enriched nutrient conditions compared to the control conditions. TN fluxes were more consistent, with a decrease in release rates at 25°C under UVR and in all nutrient conditions

(Table S3, Fig. 3c). However, this negative UVR effect was counteracted by elevated temperature in the nutrient enriched and depleted conditions (Fig. 3d).

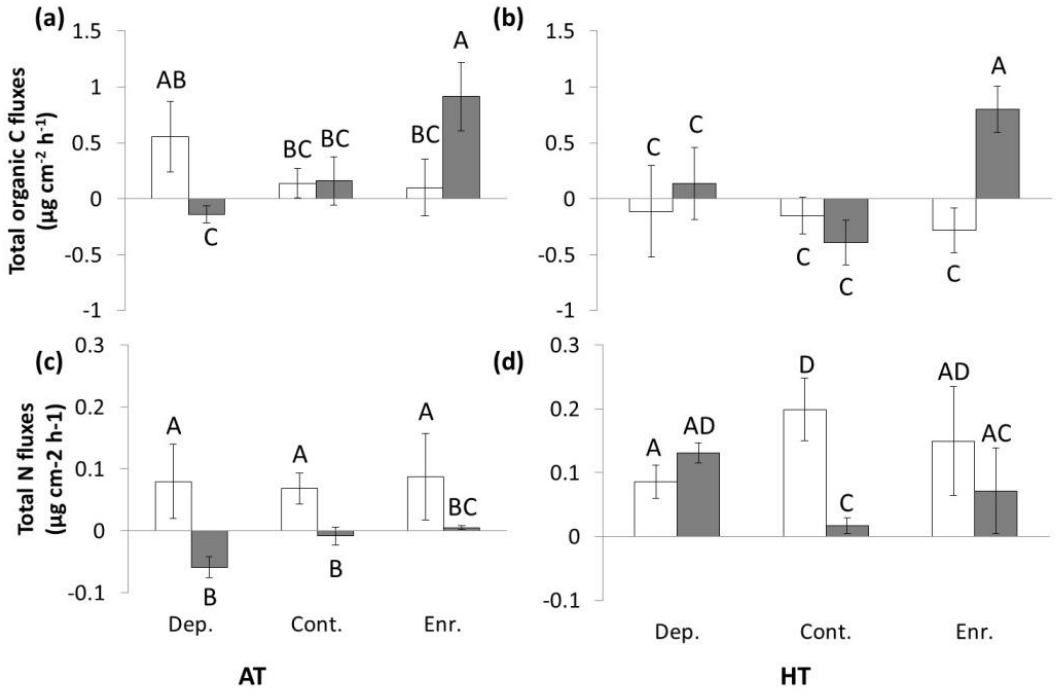


Figure 3. Total organic carbon (TOC) fluxes (µg cm^{-2} h^{-1}) and Total nitrogen (TN) fluxes (µg cm^{-2} h^{-1}) in depleted (Dep.), control (Cont.) and enriched (Enr.) nutrient condition at 25°C (AT, a,c) and 30°C (HT, b,d). White columns are nubbins growing without UVR; dark grey columns are nubbins growing under UVR. Positive values represent release and negative values represent uptakes. Error bars represent standard deviations calculated from five replicates. Values noted with the same letter (in ab or in cd) are not significantly different (*p-value* > 0.05).

3.4.Organic matter degradation by associated micro-organisms

Both enzymes (aminopeptidase and α-glucosidase) responded similarly to changes in environmental condition. At 25°C, their activities were not affected by the nutrient level but were decreased by UVR (Table S3, Fig. 4a,c). High temperature increased the kinetics of the enzymatic activities which reached their maxima after 24 h at 30°C against 48 h at 25°C (in all treatments, data not shown). High temperature also enhanced the EEAmax values in control and enriched water, regardless of the UV levels (Table S3, Fig. 4b,d). Overall, UVR's effects were inhibited by high temperature so that HT0UV and HTUV conditions were not

significantly different at the exception of the control condition where the temperature does not fully compensate the negative effects of UVR for MUF EEAmax. N and C degraded were a low part of the released matter (between 0.6 and 8% for N and 0 and 1% for C, data not shown).

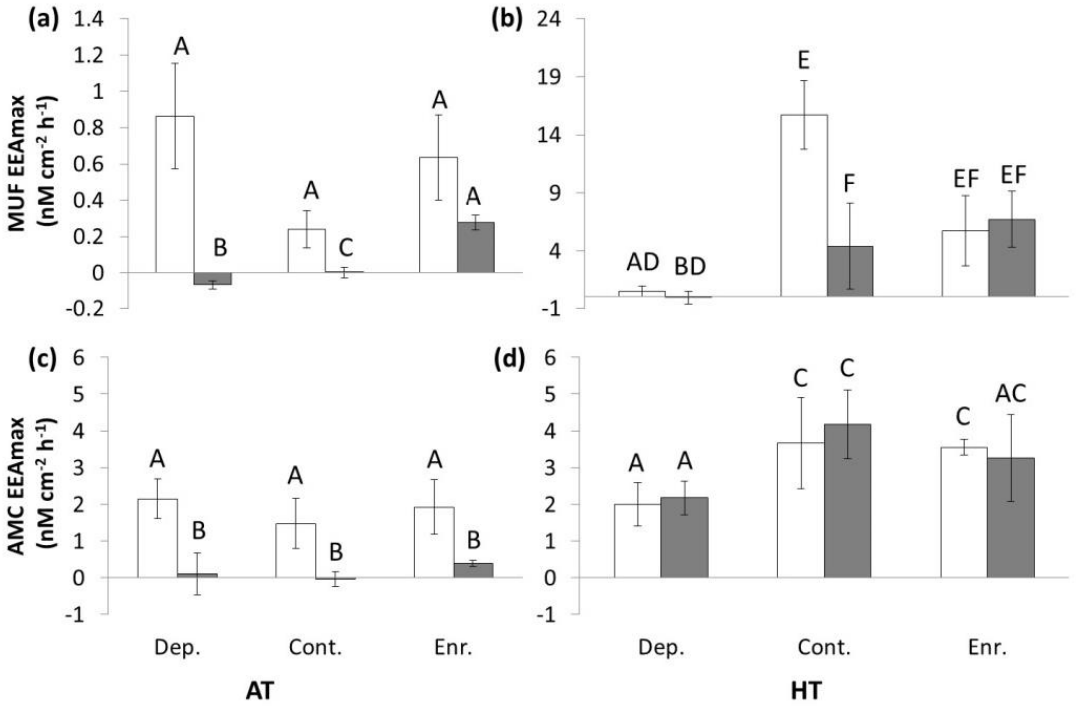


Figure 4. α-glucosidase (MUF, nM cm^{-2} h^{-1}) and aminopeptidase (nM cm^{-2} h^{-1}) maximal enzymatic activities in depleted (Dep.), control (Cont.) and enriched (Enr.) nutrient condition at 25°C (AT, a,c) and 30°C (HT, b,d). White columns are nubbins growing without UVR; dark grey columns are nubbins growing under UVR. Error bars represent standard deviations calculated from five replicates. Values noted with the same letter (in ab or in cd) are not significantly different (*p-value* > 0.05).

4. Discussion

By simultaneously quantifying co-variation in coral photosynthesis, growth, tissue composition, OM fluxes and degradation by mucus-associated bacteria, this study provides a better understanding of how the combination of global and local stressors (temperature, nutrient level and UVR exposure) impacts the widespread species *Pocillopora damicornis*. Specifically our results show that seawater temperature was the main factor altering the coral physiology although inorganic nutrient availability modulated this thermal effect (Summary

Fig. 5). While the bleaching susceptibility of *P. damicornis* under thermal stress was reduced with inorganic nutrient supplementation, it was on the contrary enhanced under nutrient depletion. UVR exposure, in turn, significantly impacted both mucus composition and degradation, as it reduced the release of organic nitrogen and the enzymatic activity of mucus-associated bacteria. In addition, enzymatic activities were limited by phosphorus availability in depleted conditions and by temperature when nutrients were available. Altogether, our results suggest that the coral physiology, as well as the reef biogeochemistry, will be differently affected by thermal stress in UVR exposed or UVR shaded reefs (surface versus deep reefs, clear versus cloudy sky) and in oligotrophic, or nutrient-enriched conditions. Our results also highlight the importance of applying a multifactorial approach to understand the response of corals to anthropogenic changes.

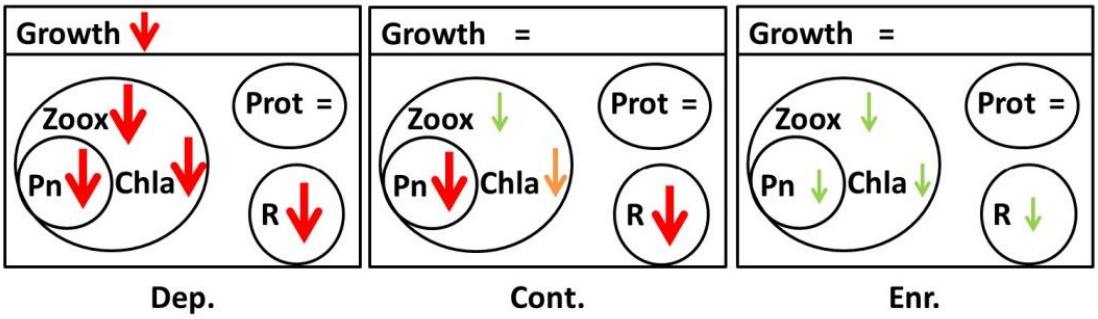


Figure 5. Change (compared to the control condition at 25°C) in coral physiology between 25°C and 30°C in depleted (Depl), control (Cont), and enriched (Enr.) = means no change, the arrows indicate a reduction. Large red arrows indicate the highest change, thinner and orange arrows indicate intermediate values and thin green arrows indicate the lowest change.

Temperature was the most harmful factor for *P. damicornis* physiology among the three factors tested in this study. Consistently with previous observations (Al-Sofyani and Floos, 2013; Courtial et al., 2017; D'Croz and Maté, 2004, 2002; Mayfield et al., 2013), thermal stress indeed led to a significant bleaching and impairment of the photosynthesis and respiration rates of *P. damicornis* independently of the nutrient and UV conditions (Hughes et al., 2017). This thermal effect was however modulated by the availability in dissolved inorganic nutrients. Nutrient depletion resulted in an enhancement of the bleaching sensitivity of *P. damicornis*, due to a general impairment of the coral metabolism (lower growth rate and chl *a* content compared to ambient nutrient conditions). Such metabolic depression was

observed in previous studies (Ezzat et al., 2015; Rosset et al., 2015; Titlyanov et al., 2001; Wiedenmann et al., 2013), and was explained by the need for nitrogen in CO_2 fixation and protein renewal (Evans, 1989, 1983), and for phosphorus in phospholipid membranes and ATP reserves (Tchernov et al., 2004; Wiedenmann et al., 2013). Our results thus suggest that corals living in very oligotrophic areas, or experiencing nutrient shortage due to water stratification will be particularly fragile during a thermal stress. On the contrary, high nutrient level in seawater mitigated the thermal stress effect on *P. damicornis*, as colonies supplemented with NO_3 and PO_4 maintained higher chl *a* and protein content as well as higher photosynthetic and respiratory rates compared to bleached colonies in control water. These results are in agreement with previous studies showing that supplementation of corals with nutrients can alleviate the negative effects of high temperature or low pH on the corals' photosynthetic capacities and on their resistance to bleaching (Béraud et al., 2013; Hoadley et al., 2016; Holcomb et al., 2010; Tanaka et al., 2014a). Nitrate supplementation indeed enhances the nitrate reductase activity, which uses electrons within the photosynthetic apparatus and therefore reduces oxidative pressure and damages on the PSII reaction center (Lomas and Glibert, 1999; Rodríguez-Román and Iglesias-Prieto, 2005). In addition, greater nitrogen availability increases repair rates of the D1 protein of PSII, particularly susceptible to thermal stress (Steglich et al., 2001; Warner et al., 1999) and high phosphorus concentration increases the production of ATP for repair mechanisms (Benitez-Nelson, 2000; Elser et al., 2007). Overall, these observations suggest that corals exposed to only pulses (short-term exposure) of inorganic nutrients, such as those located in upwelling areas (Bayraktarov et al., 2013, 2012) may take advantage of this nutrient source during the warming season.

Our observations however differ from studies showing higher bleaching sensitivity of corals maintained under nutrient enrichment or thriving in eutrophicated areas (Cunning and Baker, 2013; Schlöder and D'Croz, 2004; Tanaka et al., 2014b; Vega Thurber et al., 2014). Such variability in coral response to nutrient enrichment and thermal stress may either depend on the exposure time to nutrient enrichment or may likely be driven by differences in symbiont density in the coral tissue. According to the results obtained in the few studies which have investigated the bleaching susceptibility of corals under different nutrient enrichment and monitored symbiont density, two trends are observed: a positive effect of nutrients on the maintenance of the symbiosis when symbiont density remained below 10^6 symbionts cm^{-2} (Béraud et al. 2013; Hoadley et al. 2016, this study) and a negative effect for coral species bearing more than 10^6 symbionts cm^{-2} in their tissue (Schlöder and D'Croz, 2004; Tanaka et al., 2014b). An excessive concentration of symbionts may induce a parallel

excessive production of harmful oxygen radicals which exacerbates thermal stress negative effects (Cunning and Baker, 2013; Wooldridge, 2013).

Despite the positive effect of nutrient enrichment on the photosynthetic capacities of *P. damicornis* under thermal stress, NO_3 and PO_4 supplementation led to a drop in coral growth, consistently with previous observations (Wiedenmann et al. 2013; Kinsey and Davies 1979; Renegar and Riegl 2005; Holcomb et al. 2010). This was interpreted as a competition i) for inorganic carbon between algal photosynthesis and host skeletogenesis (Marubini and Davies, 1996; Stambler et al., 1991); and/or ii) for energy between the assimilation of nitrate and coral growth (Dagenais-Bellefeuille and Morse, 2013; Grover et al., 2008, 2002). In our study, the carbon-limitation hypothesis does not fit as the growth was reduced without any change in photosynthesis or symbiont density, leaving the second explanation as the most likely hypothesis.

Finally, our results showed no effect of UVR on *P. damicornis* physiology even when combined to high temperature, which differs from a previous finding that thermal and UVR stress enhanced *P. damicornis* bleaching (Courtial et al. 2017). The only difference between these two studies relies in the symbiont density inside the host tissue, since fed colonies in Courtial et al. (2017) hosted 70% higher symbiont density than unfed colonies of this study. These two experiments thus highlight a negative relationship between symbiont density and UVR impact on coral physiology, and may explain the different effects of UVR and thermal stress observed in the literature. These observations are congruent with the hypothesis developed above on the effect of symbiont density on *P. damicornis* bleaching susceptibility in nutrient enriched water.

Concerning the OM fluxes, our results first highlight a negative effect of UVR on TN release under control conditions, likely due to an impairment of nitrogen uptake by the coral colonies. Although this has never been investigated in corals, and will need more in depth experiments, several studies on phytoplankton observed a positive correlation between TN release and nitrogen uptake (Anderson and Roels, 1981; Butler et al., 1979; Collos et al., 1996; Olson, 1980; Serra et al., 1978), as well as a reduction in nitrogen uptake under UVR (Hessen et al., 1997). Also in phytoplankton and macroalgae, such reduction was attributed to membrane damage and to the inhibition of the enzymes involved in nitrogen metabolism (Behrenfeld et al., 1995; Döhler, 1985; Döhler et al., 1995; Hessen et al., 1997). In addition to reducing the amount of organic nitrogen released by the corals, UVR also decreased the aminopeptidase activity of the mucus-associated bacteria. This is likely due to photolysis as previously observed in *in vitro* experiments on enzymes (Espeland and Wetzel, 2001), as well

as on the enzymatic activity of the water-column bacteria (Cunha et al., 2010). The chemical structure of the mucus, as well as the presence of potential protecting molecules such as MAAs, did not preserve bacterial activity from external stress. Climatic conditions which will enhance UVR at the reef surface may therefore lead to significant changes in the nitrogen recycling and food chain functioning of the whole reef. We however observed that high temperature counteracted the negative effects of UVR on TN release and degradation. For TN release, several assumptions could be made: (i) high temperature may either have enhanced the uptake of nitrogen (which is unlikely considering previous studies, Godinot et al. 2011; Ezzat et al. 2016a), or (ii) have triggered defense mechanisms which have protected cell membranes and enzymes involved in nitrogen uptake and catabolism from UVR effects. High temperature also enhanced the aminopeptidase activity under phosphorus availability. This is consistent with previous observations made with free marine bacteria, where a positive correlation was found between temperature and enzyme kinetics (Cunha et al., 2010; Price and Sowers, 2004) or between phosphorus availability and OM consumption by bacteria (Elser et al., 1995; Kjelleberg, 1993; Zweifel et al., 1993). Reduction in the EEAmax by very low phosphorus concentration or UVR is likely to lead to a reduction in the OM consumption already known to be associated with a limitation of the bacterial growth (Wambeke et al., 2002; Zweifel et al., 1993).

Organic carbon fluxes presented a complete different response to environmental changes compared to N fluxes. This suggests that TN and TOC fluxes are not correlated, likely because they depend on different processes the photosynthetic activity for TOC, and the uptake of inorganic N for TN. This observation is consistent with the high variability observed in C:N ratios of the coral-derived OM, both between species (Bednarz et al., 2012; Haas et al., 2010; Nakajima et al., 2009; Naumann et al., 2010; Wild et al., 2008, 2005) and environnemental conditions (Bednarz et al., 2012; Haas et al., 2010; Naumann et al., 2010). TOC release was enhanced by UVR exposure only when nutrient concentrations were high suggesting that this combination of factors (stress and nutrient energy) was necessary to induce changes in OM production. As nutrients enhanced the Pg/R ratio in corals, more photosynthates might have been available for mucus production, which was increased to protect corals from UV stress (Brown and Bythell, 2005). Although the effect of temperature and UVR on TOC release was different from the effect on the TN release, the activity of the α-glucosidase was affected the same way than the aminopeptidase by the three environmental factors suggesting that the quality of the mucus does not affect the bacterial response to

environmental changes. This is coherent with our results on the OM degradation that showed that less than 1% and 8% for the total C and N released is degraded by mucus-associated bacteria. Therefore, even under UVR when less TN is released, the amount of mucus available for bacteria is in excess. This also suggests that most of the mucus degradation is performed by bacteria free living in the water column or sediment.

One of the most significant challenges now for the scientific community is to identify the factors that promote or worsen coral's resilience to global warming. Interactions among several stressors, where the effect of one is dependent on the magnitude of another, are very common in nature (Jackson et al., 2016). The results obtained in this study show that temperature is the main driver for coral bleaching, as it has been suggested from a recent meta-analysis on coral bleaching (Hughes et al., 2017). However, they also emphasize the importance of considering other abiotic and biotic factors, such as nutrient levels, or symbiont density within the coral tissue, when predicting the resistance of corals to bleaching. Overall, our study highlights the need for a better mechanistic understanding of the physiological response of corals to multiple stressors, and the necessity to improve our knowledge on changes occurring at the reef level following modifications in the OM fluxes and degradation.

Acknowledgements: The authors would like to thank C. Rottier and A. Labbe for laboratory assistance and technical help during the experimental setup as well as Pr. Denis Allemand, Director of the Centre Scientifique de Monaco for scientific support. Financial support to L.C. was provided by the Centre Scientifique de Monaco and Paris VI University.

References

Al-Sofyani, A.A., Floos, Y.A.M., 2013. Effect of temperature on two reef-building corals *Pocillopora damicornis* and *P. verrucosa* in the Red Sea. Oceanologia 55, 917–935. doi:10.5697/oc.55-4.917

Amon, R.M.W., Benner, R., 1996. Bacterial utilization of different size classes of dissolved organic matter. Limnol. Oceanogr. 41, 41–51. doi:10.4319/lo.1996.41.1.0041

Anderson, S.M., Roels, O.A., 1981. Effects of light intensity on nitrate and nitrite uptake and excretion by *Chaetoceros curvisetus*. Mar. Biol. 62, 257–261. doi:10.1007/BF00397692

Baker, A.C., Starger, C.J., McClanahan, T.R., Glynn, P.W., 2004. Coral reefs: Corals' adaptive response to climate change. Nature 430, 741–741. doi:10.1038/430741a

Bayraktarov, E., Pizarro, V., Eidens, C., Wilke, T., Wild, C., 2013. Bleaching susceptibility and recovery of Colombian Caribbean corals in response to water current exposure and seasonal upwelling. PLoS One 8, e80536. doi:10.1371/journal.pone.0080536

Bayraktarov, E., Pizarro, V., Eidens, C., Wilke, T., Wild, C., 2012. Upwelling mitigates coral bleaching in the Colombian Caribbean, in: Proc. 12th Int. Coral Reef Symp., Australia: 9A-2.

Beardall, J., Stojkovic, S., Gao, K., 2014. Interactive effects of nutrient supply and other environmental factors on the sensitivity of marine primary producers to ultraviolet radiation: Implications for the impacts of global change. Aquat. Biol. 22, 5–23. doi:10.3354/ab00582

Bednarz, V.N., Grover, R., Maguer, J.-F., Fine, M., Ferrier-Pagès, C., 2017. The assimilation of diazotroph-derived nitrogen by scleractinian corals depends on their metabolic status. MBio 8. doi:10.1128/mBio.02058-16

Bednarz, V.N., Naumann, M.S., Niggl, W., Wild, C., 2012. Inorganic nutrient availability affects organic matter fluxes and metabolic activity in the soft coral genus *Xenia*. J. Exp. Biol. 215, 3672–3679. doi:10.1242/jeb.072884

Behrenfeld, M.J., Lean, D.R.S., Lee, H., 1995. Ultra violet-B radiation effects on inorganic nitrogen uptake by natural assemblages of oceanic plankton. J. Phycol. 31, 25–36. doi:10.1111/j.0022-3646.1995.00025.x

Benitez-Nelson, C.R., 2000. The biogeochemical cycling of phosphorus in marine systems. Earth-Science Rev. 51, 109–135. doi:10.1016/S0012-8252(00)00018-0

Béraud, E., Gevaert, F., Rottier, C., Ferrier-Pagès, C., 2013. The response of the scleractinian coral Turbinaria reniformis to thermal stress depends on the nitrogen status of the coral holobiont. J. Exp. Biol. 216, 2665–2674. doi:10.1242/jeb.085183

Brown, B.E., Bythell, J.C., 2005. Perspectives on mucus secretion in reef corals. Mar. Ecol.

Prog. Ser. 296, 291–309. doi:10.3354/meps296291

Bruno, J.F., Bertness, M.D., 2001. Habitat modification and facilitation in benthic marine communities. Mar. community Ecol. 413, 201–218.

Butler, E.I., Knox, S., Liddicoat, M.I., 1979. The relationship between inorganic and organic nutrients in sea water. J. Mar. Biol. Assoc. United Kingdom 59, 239. doi:10.1017/S0025315400046312

Camps, M., Benavides, M., Lema, K.A., Bourne, D.G., Grosso, O., Bonnet, S., 2016. Released coral mucus does not enhance planktonic N_2 fixation rates. Aquat. Microb. Ecol. 77, 51–63.

Collos, Y., Descolas-Gros, C., Mornet, F., 1996. Diel variations in dissolved organic nitrogen in a coastal pond: Relationships with carbon and nitrogen metabolism. J. Exp. Mar. Bio. Ecol. 202, 177–189.

Courtial, L., Roberty, S., Shick, J.M., Houlbrèque, F., Ferrier-Pagès, C., 2017. Interactive effects of ultraviolet radiation and thermal stress on two reef-building corals. Limnol. Oceanogr. doi:10.1002/lno.10481

Cunha, A., Almeida, A., Coelho, F.J.R.C., Gomes, N.C.M., Oliveira, V., Santos, A.L., 2010. Bacterial extracellular enzymatic activity in globally changing aquatic ecosystems. Curr. Reseach, Technol. Educ. Top. Appl. Microbiol. Microb. Biotechnol. 124–135.

Cunning, R., Baker, A.C., 2013. Excess algal symbionts increase the susceptibility of reef corals to bleaching. Nat. Clim. Chang. 3, 259–262. doi:10.1038/nclimate1711

D'Angelo, C., Wiedenmann, J., 2014. Impacts of nutrient enrichment on coral reefs: New perspectives and implications for coastal management and reef survival. Curr. Opin. Environ. Sustain. 7, 82–93. doi:10.1016/j.cosust.2013.11.029

D'Croz, L., Maté, J.L., 2004. Experimental responses to elevated water temperature in genotypes of the reef coral *Pocillopora damicornis* from upwelling and non-upwelling environments in Panama. Coral Reefs 23, 473–483. doi:10.1007/s00338-004-0397-7

D'Croz, L., Maté, J.L., 2002. The role of water temperature and UV radiation in the recovery of the experimentally bleached coral *Pocillopora damicornis* from the eastern Pacific Ocean (Panama), in: Proceedings of the Ninth International Coral Reef Symposium, Bali, 23-27 October 2000,. pp. 1111–1116.

D'Croz, L., Maté, J.L., Oke, J.E., 2001. Responses to elevated sea water temperature and UV radiation in the coral *Porites lobata* from upwelling and non-upwelling environments on the Pacific coast of Panama. Bull. Mar. Sci. 69, 203–214.

Dagenais-Bellefeuille, S., Morse, D., 2013. Putting the N in dinoflagellates. Front. Microbiol. 4, 369. doi:10.3389/fmicb.2013.00369

Döhler, G., 1985. Effect of UV-B Radiation (290–320nm) on the Nitrogen Metabolism of

Several Marine Diatoms. J. Plant Physiol. 118, 391–400. doi:10.1016/S0176-1617(85)80199-1

Döhler, G., Hagmeier, E., David, C., 1995. Effects of solar and artificial UV irradiation on pigments and assimilation of 15N ammonium and 15N nitrate by macroalgae. J. Photochem. Photobiol. B Biol. 30, 179–187. doi:10.1016/1011-1344(95)07189-9

Doney, S.C., Ruckelshaus, M., Emmett Duffy, J., Barry, J.P., Chan, F., English, C.A., Galindo, H.M., Grebmeier, J.M., Hollowed, A.B., Knowlton, N., Polovina, J., Rabalais, N.N., Sydeman, W.J., Talley, L.D., 2012. Climate change impacts on marine ecosystems. Ann. Rev. Mar. Sci. 4, 11–37. doi:10.1146/annurev-marine-041911-111611

Dunne, R.P., 2010. Synergy or antagonism-interactions between stressors on coral reefs. Coral Reefs 29, 145–152. doi:10.1007/s00338-009-0569-6

Eakin, C.M., Liu, G., Gomez, A., De la Cour, J., Heron, S., Skirving, W., Geiger, E., Tirak, K., Strong, A., 2016. Global coral bleaching 2014–2017: status and an appeal for observations. Reef Encount. 31, 20–26. doi:10.3390/rs8010012

Elser, J., Stabler, L., Hassett, R., 1995. Nutrient limitation of bacterial growth and rates of bacterivory in lakes and oceans: a comparative study. Aquat. Microb. Ecol. 9, 105–110. doi:10.3354/ame009105

Elser, J.J., Bracken, M.E.S., Cleland, E.E., Gruner, D.S., Harpole, W.S., Hillebrand, H., Ngai, J.T., Seabloom, E.W., Shurin, J.B., Smith, J.E., 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecol. Lett. 10, 1135–1142. doi:10.1111/j.1461-0248.2007.01113.x

Espeland, E.M., Wetzel, R.G., 2001. Complexation, stabilization, and UV photolysis of extracellular and surface-bound glucosidase and alkaline phosphatase: Implications for biofilm microbiota. Microb. Ecol. 42, 572–585. doi:10.1007/s00248-001-1023-7

Evans, J.R., 1989. Photosynthesis and nitrogen relationships in leaves of C3 plants. Oecologia 78, 9–19. doi:10.1007/BF00377192

Evans, J.R., 1983. Nitrogen and photosynthesis in the flag leaf of wheat (*Triticum aestivum L.*). Plant Physiol. 72, 297–302. doi:10.1104/pp.72.2.297

Ezzat, L., Maguer, J.-F., Grover, R., Ferrier-Pagès, C., 2016a. Limited phosphorus availability is the Achilles heel of tropical reef corals in a warming ocean. Sci. Rep. 6, 31768. doi:10.1038/srep31768

Ezzat, L., Maguer, J.-F., Grover, R., Ferrier-Pagès, C., 2015. New insights into carbon acquisition and exchanges within the coral–dinoflagellate symbiosis under NH^{4+} and NO^{3-} supply. Proc. R. Soc. B Biol. Sci. 282, 20150610. doi:10.1098/rspb.2015.0610

Ezzat, L., Towle, E., Irisson, J., Langdon, C., Ferrier-Pagès, C., 2016b. The relationship between heterotrophic feeding and inorganic nutrient availability in the scleractinian

coral *T. reniformis* under a short-term temperature increase. Limnol. Oceanogr. 61, 89–102. doi:10.1002/lno.10200

Ferrier-Pagès, C., Richard, C., Forcioli, D., Allemand, D., Pichon, M., Shick, J.M., 2007. Effects of temperature and UV radiation increases on the photosynthetic efficiency in four scleractinian coral species. Biol. Bull. 213, 76–87. doi:10.2307/25066620

Fitt, K.W., Warner, M.E., 1995. Bleaching patterns of four species of Caribbean reef corals. Biol Bull 189, 298–307. doi:10.2307/1542147

Fitt, W.K., Gates, R.D., Hoegh-Guldberg, O., Bythell, J.C., Jatkar, A., Grottoli, A.G., Gomez, M., Fisher, P., Lajuenesse, T.C., Pantos, O., Iglesias-Prieto, R., Franklin, D.J., Rodrigues, L.J., Torregiani, J.M., van Woesik, R., Lesser, M.P., 2009. Response of two species of Indo-Pacific corals, *Porites cylindrica* and *Stylophora pistillata*, to short-term thermal stress: The host does matter in determining the tolerance of corals to bleaching. J. Exp. Mar. Bio. Ecol. 373, 102–110. doi:10.1016/j.jembe.2009.03.011

Fonvielle, J.A., Reynaud, S., Jacquet, S., LeBerre, B., Ferrier-Pages, C., 2015. First evidence of an important organic matter trophic pathway between temperate corals and pelagic microbial communities. PLoS One 10, 1–22. doi:10.1371/journal.pone.0139175

Glynn, P.W., 2012. Global warming and widespread coral mortality: evidence of first coral reef extinctions, in: Saving a Million Species. Springer, pp. 103–119.

Godinot, C., Ferrier-Pagès, C., Sikorski, S., Grover, R., 2013. Alkaline phosphatase activity of reef-building corals. Limnol. Oceanogr. 58, 227–234. doi:10.4319/lo.2013.58.1.0227

Godinot, C., Houlbrèque, F., Grover, R., Ferrier-Pagès, C., 2011. Coral uptake of inorganic phosphorus and nitrogen negatively affected by simultaneous changes in temperature and pH. PLoS One 6, 1–10. doi:10.1371/journal.pone.0025024

Grover, R., Maguer, J.-F., Allemand, D., Ferrier-Pagès, C., 2008. Uptake of dissolved free amino acids by the scleractinian coral *Stylophora pistillata*. J. Exp. Biol. 211, 860–865. doi:10.4319/lo.2003.48.6.2266

Grover, R., Maguer, J.-F., Reynaud-Vaganay, S., Ferrier-Pagès, C., 2002. Uptake of ammonium by the scleractinian coral *Stylophora pistillata* : Effect of feeding, light, and ammonium concentrations. Limnol. Oceanogr. 47, 782–790. doi:10.4319/lo.2002.47.3.0782

Haas, A.F., Jantzen, C., Naumann, M.S., Iglesias-Prieto, R., Wild, C., 2010. Organic matter release by the dominant primary producers in a Caribbean reef lagoon: Implication for in situ O_2 availability. Mar. Ecol. Prog. Ser. 409, 27–39. doi:10.3354/meps08631

Hessen, D.A.G., De Lange, H., van Donk, E., 1997. UV-induced changes in phytoplankton cells and its effects on grazers. Freshw. Biol. 38, 513–524. doi:10.1046/j.1365-2427.1997.00223.x

Hoadley, K.D., Pettay, D.T., Grottoli, A.G., Cai, W.-J., Melman, T.F., Levas, S., Schoepf, V., Ding, Q., Yuan, X., Wang, Y., Matsui, Y., Baumann, J.H., Warner, M.E., 2016. High-temperature acclimation strategies within the thermally tolerant endosymbiont *Symbiodinium trenchii* and its coral host, Turbinaria reniformis, differ with changing pCO 2 and nutrients. Mar. Biol. 163, 134. doi:10.1007/s00227-016-2909-8

Hoegh-Guldberg, O., 1999. Climate change, coral bleaching and the future of the world's coral reefs. Mar. Freshw. Res. 50, 839–866. doi:10.1071/MF99078

Hoegh-Guldberg, O., Bruno, J.F., 2010. The impact of climate change on the world's marine ecosystems. Science. 328, 1523–1528.

Hoegh-Guldberg, O., Mumby, P.J., Hooten, A.J., Steneck, R.S., Greenfield, P., Gomez, E., Harvell, C.D., Sale, P.F., Edwards, A.J., Caldeira, K., Knowlton, N., Eakin, C.M., Iglesias-Prieto, R., Muthiga, N., Bradbury, R.H., Dubi, A., Hatziolos, M.E., 2007. Coral reefs under rapid climate change and ocean acidification. Science. 318, 1737–1742. doi:10.1126/science.1152509

Holcomb, M., McCorkle, D.C., Cohen, A.L., 2010. Long-term effects of nutrient and CO_2 enrichment on the temperate coral *Astrangia poculata* (Ellis and Solander, 1786). J. Exp. Mar. Bio. Ecol. 386, 27–33. doi:10.1016/j.jembe.2010.02.007

Hoogenboom, M., Beraud, E., Ferrier-Pagès, C., 2010. Relationship between symbiont density and photosynthetic carbon acquisition in the temperate coral *Cladocora caespitosa*. Coral Reefs 29, 21–29. doi:10.1007/s00338-009-0558-9

Hoppe, H.-G., 1983. Significance of exoenzymatic activities in the ecology of brackish water: measurements by means of methylumbelliferyl-substrates. Mar. Ecol. Prog. Ser. 11, 299–308. doi:10.3354/meps011299

Hughes, T.P., Kerry, J., Álvarez-Noriega, M., Álvarez-Romero, J., Anderson, K., Baird, A., Babcock, R., Beger, M., Bellwood, D., Berkelmans, R., Bridge, T., Butler, I., Byrne, M., Cantin, N., Comeau, S., Connolly, S., Cumming, G., Dalton, S., Diaz-Pulido, G., Eakin, C.M., Figueira, W., Gilmour, J., Harrison, H., Heron, S., Hoey, A.S., Hobbs, J.-P., Hoogenboom, M., Kennedy, E., Kuo, C.-Y., Lough, J., Lowe, R., Liu, G., Malcolm McCulloch, H.M., McWilliam, M., Pandolfi, J., Pears, R., Pratchett, M., Schoepf, V., Simpson, T., Skirving, W., Sommer, B., Torda, G., Wachenfeld, D., Willis, B., Wilson, S., 2017. Global warming and recurrent mass bleaching of corals. Nature. doi:10.1038/nature21707

Jackson, M.C., Loewen, C.J.G., Vinebrooke, R.D., Chimimba, C.T., 2016. Net effects of multiple stressors in freshwater ecosystems: a meta-analysis. Glob. Chang. Biol. 22, 180–189. doi:10.1111/ gcb.13028

Jeffrey, S.W., Humphrey, G.F., 1975. New spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae and natural phytoplankton. Biochemical Physiology Pflanz 167: 191–194. Biochem. Physiol. Pflanz.

doi:10.1016/0022-2860(75)85046-0

Jokiel, P.L., Maragos, J.E., Franzisket, L., 1978. Coral growth: buoyant weight technique, in: Stoddart, R., Johannes, D. (Eds.), Coral Reef: Research Methods. Unesco, pp. 379–396.

Kinsey, D.W., Davies, P.J., 1979. Carbon turnover, calcification and growth in coral reefs. Stud. Environ. Sci. 3, 131–162.

Kjelleberg, S., 1993. Starvation in bacteria. Springer US, Boston, MA. doi:10.1007/978-1-4899-2439-1

Lapointe, B.E., Barile, P.J., Matzie, W.R., 2004. Anthropogenic nutrient enrichment of seagrass and coral reef communities in the Lower Florida Keys: discrimination of local versus regional nitrogen sources. J. Exp. Mar. Bio. Ecol. 308, 23–58. doi:0.1029/2001GLO14198

Lesser, M., Farrell, J., 2004. Exposure to solar radiation increases damage to both host tissues and algal symbionts of corals during thermal stress. Coral Reefs 23, 367–377. doi:10.1007/s00338-004-0392-z

Lesser, M.P., 2012. Oxidative stress in tropical marine ecosystems, in: Oxidative stress in aquatic ecosystems. John Wiley & Sons, Ltd, Chichester, UK, pp. 7–19. doi:10.1002/9781444345988.ch1

Lesser, M.P., 2006. Oxidative stress in marine environments: biochemistry and physiological ecology. Annu. Rev. Physiol. 68, 253–278. doi:10.1146/annurev.physiol.68.040104.110001

Lesser, M.P., Stochaj, W.R., Tapley, D.W., Shick, J.M., 1990. Bleaching in coral reef anthozoans: effects of irradiance, ultraviolet radiation, and temperature on the activities of protective enzymes against active oxygen. Coral Reefs 8, 225–232. doi:10.1007/BF00265015

Levas, S., Grottoli, A.G., Schoepf, V., Aschaffenburg, M., Baumann, J., Bauer, J.E., Warner, M.E., 2016. Can heterotrophic uptake of dissolved organic carbon and zooplankton mitigate carbon budget deficits in annually bleached corals? Coral Reefs 35, 495–506. doi:10.1007/s00338-015-1390-z

Lomas, M.W., Glibert, P.M., 1999. Temperature regulation of nitrate uptake: A novel hypothesis about nitrate uptake and reduction in cool-water diatoms. Limnol. Oceanogr. 44, 556–572. doi:10.4319/lo.1999.44.3.0556

Marubini, F., Davies, P.S., 1996. Nitrate increases zooxanthellae population density and reduces skeletogenesis in corals. Mar. Biol. 127, 319–328. doi:10.1007/BF00942117

Mayfield, A.B., Chen, M.N., Meng, P.J., Lin, H.J., Chen, C.S., Liu, P.J., 2013. The physiological response of the reef coral *Pocillopora damicornis* to elevated temperature: Results from coral reef mesocosm experiments in Southern Taiwan. Mar. Environ. Res.

86, 1–11. doi:10.1016/j.marenvres.2013.01.004

Nakajima, R., Yoshida, T., Azman, B.A.R., Zaleha, K., Othman, B.H.R., Toda, T., 2009. In situ release of coral mucus by *Acropora* and its influence on the heterotrophic bacteria. Aquat. Ecol. 43, 815–823. doi:10.1007/s10452-008-9210-y

Naumann, M.S., Haas, A., Struck, U., Mayr, C., El-Zibdah, M., Wild, C., 2010. Organic matter release by dominant hermatypic corals of the Northern Red Sea. Coral Reefs 29, 649–659.

Niggl, W., Glas, M., Laforsch, C., Mayr, C., Wild, C., 2008. First evidence of coral bleaching stimulating organic matter release by reef corals, in: Proceedings of the 11th International Coral Reef Symposium'. pp. 905–910.

Nixon, S.W., 1995. Coastal marine eutrophication: A definition, social causes, and future concerns. Ophelia 41, 199–219. doi:10.1080/00785236.1995.10422044

Oliver, J.K., Berkelmans, R., Eakin, C.M., 2009. Coral bleaching in space and time, in: Coral Bleaching. Springer, pp. 21–39. doi:10.1007/978-3-540-69775-6_3

Olson, R.J., 1980. Nitrate and ammonium uptake in Antarctic waters. Limnol. Oceanogr. 25, 1064–1074. doi:10.4319/lo.1980.25.6.1064

Piggot, A.M., Fouke, B.W., Sivaguru, M., Sanford, R.A., Gaskins, H.R., 2009. Change in zooxanthellae and mucocyte tissue density as an adaptive response to environmental stress by the coral, *Montastraea annularis*. Mar. Biol. 156, 2379–2389. doi:10.1007/s00227-009-1267-1

Polovina, J.J., Howell, E.A., Abecassis, M., 2008. Ocean's least productive waters are expanding. Geophys. Res. Lett. 35, L03618. doi:10.1029/2007GL031745

Pomeroy, L.R., 1974. The Ocean's Food Web, A Changing Paradigm. Bioscience 24, 499–504. doi:10.2307/1296885

Price, P.B., Sowers, T., 2004. Temperature dependence of metabolic rates for microbial growth, maintenance, and survival. Proc. Natl. Acad. Sci. 101, 4631–4636. doi:10.1073/pnas.0400522101

Renegar, D., Riegl, B., 2005. Effect of nutrient enrichment and elevated CO2 partial pressure on growth rate of Atlantic scleractinian coral *Acropora cervicornis*. Mar. Ecol. Prog. Ser. 293, 69–76. doi:10.3354/meps293069

Rodríguez-Román, A., Iglesias-Prieto, R., 2005. Regulation of photochemical activity in cultured symbiotic dinoflagellates under nitrate limitation and deprivation. Mar. Biol. 146, 1063–1073. doi:10.1007/s00227-004-1529-x

Rosset, S., D'Angelo, C., Wiedenmann, J., 2015. Ultrastructural biomarkers in symbiotic algae reflect the availability of dissolved inorganic nutrients and particulate food to the reef coral holobiont. Front. Mar. Sci. 2, 103. doi:10.3389/fmars.2015.00103 Reef

Schlöder, C., D'Croz, L., 2004. Responses of massive and branching coral species to the combined effects of water temperature and nitrate enrichment. J. Exp. Mar. Bio. Ecol. 313, 255–268. doi:10.1016/j.jembe.2004.08.012

Serra, J.L., Llama, M.J., Cadenas, E., 1978. Nitrate utilization by the diatom Skeletonema costatum II. Regulation of nitrate uptake. Plant Physiol. 62, 991–994. doi:10.1104/pp.62.6.991

Shantz, A.A., Burkepile, D.E., 2014. Context-dependent effects of nutrient loading on the coral–algal mutualism. Ecology 95, 1995–2005. doi:10.1890/13-1407.1

Smith, P.K., Krohn, R.I., Hermanson, G.T., Mallia, A.K., Gartner, F.H., Provenzano, Md., Fujimoto, E.K., Goeke, N.M., Olson, B.J., Klenk, D.C., 1985. Measurement of protein using bicinchoninic acid. Anal. Biochem. 150, 76–85. doi:10.1016/0003-2697(85)90442-7

Smith, V.H., 2003. Eutrophication of freshwater and coastal marine ecosystems a global problem. Environ. Sci. Pollut. Res. 10, 126–139. doi:10.1065/espr2002.12.142

Smith, V.H., Schindler, D.W., 2009. Eutrophication science: where do we go from here? Trends Ecol. Evol. 24, 201–207. doi:10.1016/j.tree.2008.11.009

Spalding, M.D., Brown, B.E., 2015. Warm-water coral reefs and climate change. Science. 350, 769–771. doi:10.1126/science.aad0349

Stambler, N., Popper, N., Dubinsky, Z., Stimson, J., 1991. Effects of nutrient enrichment and water motion on the coral *Pocillopora damicornis*. Pacific Sci. doi:10125/1396

Steglich, C., Behrenfeld, M., Koblizek, M., Claustre, H., Penno, S., Prasil, O., Partensky, F., Hess, W.R., 2001. Nitrogen deprivation strongly affects photosystem II but not phycoerythrin level in the divinyl-chlorophyll b-containing cyanobacterium *Prochlorococcus marinus*. Biochim. Biophys. Acta (BBA)-Bioenergetics 1503, 341–349.

Sterner, R.W., Elser, J.J., 2002. Ecological stoichiometry: the biology of elements from molecules to the biosphere. Princeton University Press.

Stimson, J., Kinzie, R.A., 1991. The temporal pattern and rate of release of zooxanthellae from the reef coral Pocillopora damicornis (Linnaeus) under nitrogen-enrichment and control conditions. J. Exp. Mar. Bio. Ecol. 153, 63–74. doi:10.1016/S0022-0981(05)80006-1

Tanaka, Y., Iguchi, A., Nishida, K., Inoue, M., Nakamura, T., Suzuki, A., Sakai, K., 2014a. Nutrient availability affects the response of juvenile corals and the endosymbionts to ocean acidification. Limnol. Oceanogr. 59, 1468–1476. doi:10.4319/lo.2014.59.5.1468

Tanaka, Y., Inoue, M., Nakamura, T., Suzuki, A., Sakai, K., 2014b. Loss of zooxanthellae in a coral under high seawater temperature and nutrient enrichment. J. Exp. Mar. Bio. Ecol.

457, 220–225. doi:10.1016/j.jembe.2014.04.019

Tchernov, D., Gorbunov, M.Y., de Vargas, C., Narayan Yadav, S., Milligan, A.J., Haggblom, M., Falkowski, P.G., 2004. Membrane lipids of symbiotic algae are diagnostic of sensitivity to thermal bleaching in corals. Proc. Natl. Acad. Sci. 101, 13531–13535. doi:10.1073/pnas.0402907101

Tedetti, M., Sempéré, R., 2006. Penetration of ultraviolet radiation in the marine environment. A review. Photochem. Photobiol. 82, 389–397. doi:10.1562/2005-11-09-lR-733

Titlyanov, E.A., Titlyanova, T.V., Yamazato, K., van Woesik, R., 2001. Photo-acclimation of the hermatypic coral Stylophora pistillata while subjected to either starvation or food provisioning. J. Exp. Mar. Bio. Ecol. 257, 163–181. doi:10.1016/S0022-0981(00)00308-7

Tranvik, L.J., 1992. Allochthonous dissolved organic matter as an energy source for pelagic bacteria and the concept of the microbial loop. Hydrobiologia 229, 107–114. doi:10.1007/BF00006994

Tréguer, P., Le Corre, P., 1975. Manuel d'analyse des sels nutrifs dans l'eau de mer: utilisation de l'Autoanalyzer II Technicon (R). Université de Bretagne occidentale.

Tremblay, P., Naumann, M.S., Sikorski, S., Grover, R., Ferrier-Pagès, C., 2012. Experimental assessment of organic carbon fluxes in the scleractinian coral *Stylophora pistillata* during a thermal and photo stress event. Mar. Ecol. Prog. Ser. 453, 63–77. doi:10.3354/meps09640

Vega Thurber, R.L., Burkepile, D.E., Fuchs, C., Shantz, A.A., Mcminds, R., Zaneveld, J.R., 2014. Chronic nutrient enrichment increases prevalence and severity of coral disease and bleaching. Glob. Chang. Biol. 20, 544–554. doi:10.1111/gcb.12450

Wambeke, F., Christaki, U., Giannakourou, A., Moutin, T., Souvemerzoglou, K., 2002. Longitudinal and vertical trends of bacterial limitation by phosphorus and carbon in the Mediterranean Sea. Microb. Ecol. 43, 119–133. doi:10.1007/s00248-001-0038-4

Warner, M.E., Fitt, W.K., Schmidt, G.W., 1999. Damage to photosystem II in symbiotic dinoflagellates: A determinant of coral bleaching. Proc. Natl. Acad. Sci. 96, 8007–8012. doi:10.1073/pnas.96.14.8007

Watanabe, S., Sudo, K., Nagashima, T., Takemura, T., Kawase, H., Nozawa, T., 2011. Future projections of surface UV-B in a changing climate. J. Geophys. Res. Atmos. 116, D16118. doi:10.1029/2011JD015749

Wiedenmann, J., Angelo, C.D., Smith, E.G., Hunt, A.N., Legiret, F.-E., Postle, A.D., Achterberg, E.P., D'Angelo, C., Smith, E.G., Hunt, A.N., Legiret, F.-E., Postle, A.D., Achterberg, E.P., 2013. Nutrient enrichment can increase the susceptibility of reef corals to bleaching. Nat. Clim. Chang. 2, 1–5. doi:10.1038/nclimate1661

Wild, C., Mayr, C., Wehrmann, L., Schöttner, S., Naumann, M., Hoffmann, F., Rapp, H., 2008. Organic matter release by cold water corals and its implication for fauna–microbe interaction. Mar. Ecol. Prog. Ser. 372, 67–75. doi:10.3354/meps07724

Wild, C., Woyt, H., Huettel, M., 2005. Influence of coral mucus on nutrient fluxes in carbonate sands. Mar. Ecol. Prog. Ser. 287, 87–98. doi:10.3354/meps287087

Wooldridge, S.A., 2013. Breakdown of the coral-algae symbiosis: towards formalising a linkage between warm-water bleaching thresholds and the growth rate of the intracellular zooxanthellae. Biogeosciences 10, 1647–1658. doi:10.5194/bg-10-1647-2013

Zweifel, U., Norrman, B., Hagstrom, A., 1993. Consumption of dissolved organic carbon by marine bacteria and demand for inorganic nutrients. Mar. Ecol. Prog. Ser. 101, 23–32. doi:10.3354/meps101023

Additional information:

Figure S1. Symbiont density (a), protein content (b), chlorophyll *a* content per surface area (c) and chlorophyll *a* per symbiont cell (d) in depleted (D), control (C) and enriched (E) conditions at 25°C (white columns) or 30°C (grey columns).

Table S1. Partial Tukey test results for physiological parameters at normal temperature (LT), high temperature (HT), under UVR (UV) or without UVR (0UV) in control (C), depleted (D) or enriched (E) nutrient conditions.

Table S2. Two way ANOVA results for coral physiological parameters. Results were considered significant when $p < 0.05$.

Table S3. Three way ANOVA results for organic matter fluxes, associated bacterial enzymatic activities and matter degradation. Results were considered significant when $p < 0.05$.

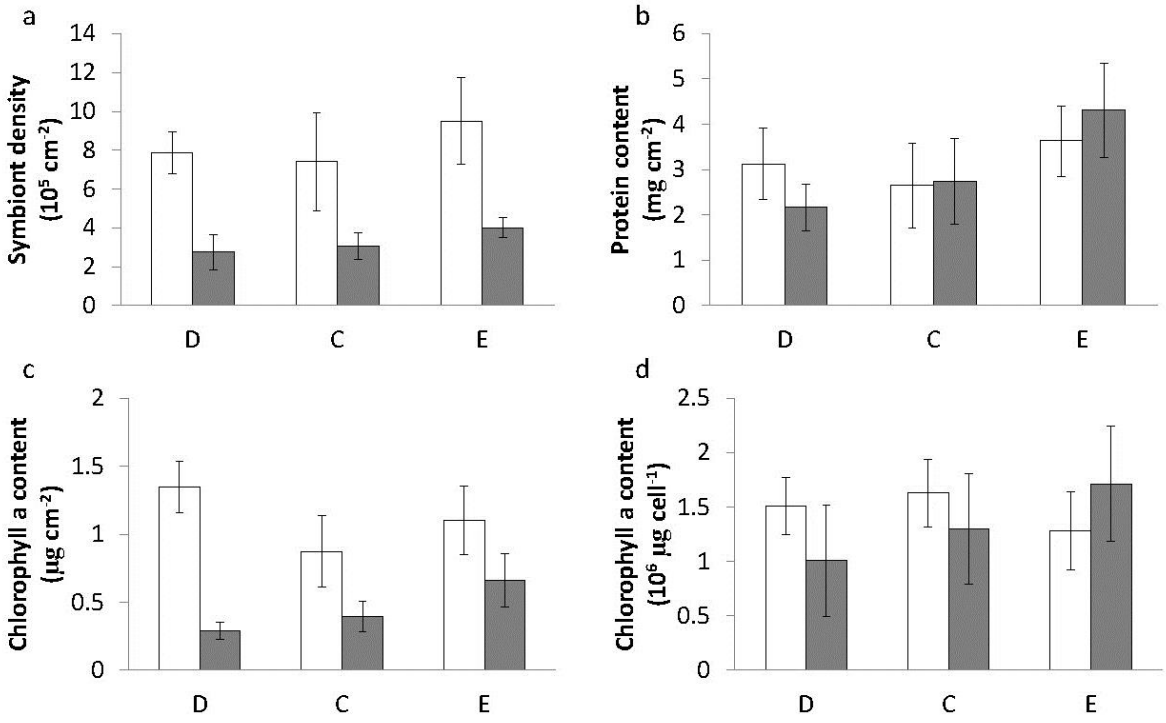


Figure S1. Symbiont density (a), protein content (b), chlorophyll *a* content per surface area (c) and chlorophyll *a* per symbiont cell (d) in depleted (D), control (C) and enriched (E) conditions at 25°C (white columns) or 30°C (grey columns).

Table S1. Partial Tukey test results for physiological parameters at normal temperature (LT), high temperature (HT), under UVR (UV) or without UVR (0UV) in control (C), depleted (D) or enriched (E) nutrient conditions.

	Symbiont	**Chl *a***	**Protein**	**Pn**	**R**	**Pg**	**Growth**
	p value	*p value*	*p value*	*p value*	*p value*	*p value*	*p value*
HTCUV-HTC0UV	1.0000	0.9952	1.0000	1.0000	1.0000	0.2078	0.9981
LTCUV-LTC0UV	0.9493	0.0754	0.9830	0.7507	1.0000	1.0000	0.8655
HTDUV-HTD0UV	1.0000	1.0000	0.9975	1.0000	0.9413	1.0000	0.9766
LTDUV-LTD0UV	0.2965	1.0000	1.0000	0.7345	1.0000	0.7929	0.0897
HTEUV-HTE0UV	1.0000	0.3446	1.0000	0.9069	0.9959	0.4765	1.0000
LTEUV-LTE0UV	0.9638	0.0323	0.0945	0.9754	1.0000	1.0000	0.1881

Table S2. Two way ANOVA results for coral physiological parameters. Results were considered significant when $p < 0.05$.

Source of Variation	df	Sum of quares	F	*p*
Symbiont density (cell cm^{-2})				
T	1	317.1	132.21	<0.0001
NUT	2	26.0	5.41	0.0077
T x NUT	2	3.1	0.64	0.5319
Chlarophyll a (mg cm^{-2})				
T	1	5.83	168.72	<0.0001
NUT	2	0.29	8.49	0.0007
T x NUT	2	1.00	14.47	<0.0001
Protein content (µg cm^{-2})				
T	1	0.31	0.42	0.5200
NUT	2	19.89	13.54	0.0001
T x NUT	2	5.73	3.90	0.0265
Growth rate (% d^{-1})				
T	1	0.11	31.96	<0.0001
NUT	2	0.12	17.78	<0.0001
T x NUT	2	0.01	2.12	0.1370

Source of Variation	df	Sum of quares	F	*p*
Pn (µmol cm^{-2} h^{-1})				
T	1	19.60	984.31	<0.0001
NUT	2	1.50	75.25	<0.0001
T x NUT	2	0.32	16.09	<0.0001
R (µmol cm^{-2} h^{-1})				
T	1	24.99	2931.81	<0.0001
NUT	2	0.06	3.41	0.0415
T x NUT	2	0.07	3.93	0.0264
Pg (µmol cm^{-2} h^{-1})				
T	1	103.65	1689.27	<0.0001
NUT	2	5.51	44.93	<0.0001
T x NUT	2	0.58	4.76	0.0130

Table S3. Three way ANOVA results for organic matter fluxes, associated bacterial enzymatic activities and matter degradation. Results were considered significant when $p < 0.05$.

Source of Variation	df	Sum of quares	F	*p*
TOC ($\mu g\ cm^{-2}\ h^{-1}$)				
T	1	0.85	13.96	0.0007
NUT	2	1.51	12.40	<0.0001
UV	1	0.37	6.09	0.0189
T x NUT	2	0.08	0.70	0.5017
T x UV	1	0.43	7.02	0.0123
NUT x UV	2	3.04	25.03	<0.0001
T x NUT x UV	2	0.68	5.64	0.0078
α-glucaosidase EEA_{max} ($nM\ cm^{-2}\ h^{-1}$)				
T	1	51.17	117.05	<0.0001
NUT	2	13.82	15.81	<0.0001
UV	1	10.11	23.13	<0.0001
T x NUT	2	30.69	35.10	<0.0001
T x UV	1	1.62	3.71	0.0646
NUT x UV	2	6.55	7.49	0.0026
T x NUT x UV	2	0.01	0.03	0.8632
TN ($\mu g\ cm^{-2}\ h^{-1}$)				
T	1	0.072	37.74	<0.0001
NUT	2	0.003	0.85	0.4386
UV	1	0.082	42.84	<0.0001
T x NUT	2	0.001	0.32	0.7249
T x UV	1	0.001	0.68	0.4150
NUT x UV	2	0.014	3.66	0.0378
T x NUT x UV	2	0.038	9.75	0.0005
Aminopeptidase EEA_{max} ($nM\ cm^{-2}\ h^{-1}$)				
T	1	65.92	120.35	<0.0001
NUT	2	4.79	4.37	0.0204
UV	1	5.28	9.63	0.0038
T x NUT	2	9.97	9.10	0.0007
T x UV	1	7.29	13.32	0.0009
NUT x UV	2	1.18	1.07	0.3533
T x NUT x UV	2	0.43	0.40	0.6756

Paper n°6 – *Scientific Reports*

www.nature.com/scientificreports

SCIENTIFIC REPORTS

OPEN

Received: 01 September 2016
Accepted: 03 March 2017
Published: 04 April 2017

The c-Jun N-terminal kinase prevents oxidative stress induced by UV and thermal stresses in corals and human cells

Lucile Courtial[1,2,3,*], Vincent Picco[4,*], Renaud Grover[1], Yann Cormerais[4], Cécile Rottier[1], Antoine Labbe[1], Gilles Pagès[4,5] & Christine Ferrier-Pagès[1]

Coral reefs are of major ecological and socio-economic interest. They are threatened by global warming and natural pressures such as solar ultraviolet radiation. While great efforts have been made to understand the physiological response of corals to these stresses, the signalling pathways involved in the immediate cellular response exhibited by corals remain largely unknown. Here, we demonstrate that c-Jun N-terminal kinase (JNK) activation is involved in the early response of corals to thermal and UV stress. Furthermore, we found that JNK activity is required to repress stress-induced reactive oxygen species (ROS) accumulation in both the coral *Stylophora pistillata* and human skin cells. We also show that inhibiting JNK activation under stress conditions leads to ROS accumulation, subsequent coral bleaching and cell death. Taken together, our results suggest that an ancestral response, involving the JNK pathway, is remarkably conserved from corals to human, protecting cells from the adverse environmental effects.

Living organisms have developed adaptive mechanisms allowing them to survive in a constantly changing environment[1,2]. Organisms that can rapidly adjust to these changes are likely to exhibit a greater degree of adaptation to a variety of stresses[3]. At the molecular level, cells have thus implemented complex signalling pathways to sense danger, and trigger a sequence of events that will mitigate any adverse effects. Among these pathways, mitogen-activated protein kinases (MAPKs) stimulate the expression of genes involved in the response to stress[4]. MAPKs also control key cellular processes such as proliferation, differentiation, migration, survival and apoptosis[5]. The MAPK gene family encompasses three major subgroups: the extracellular signal regulated kinase (ERK), the p38/HOG and the c-Jun N-terminal kinase (JNK) groups. JNKs are evolutionarily conserved and are found in all eukaryotes, including plants, vertebrates and anthozoans[6,7]. When activated by dual phosphorylation of a threonine-proline-tyrosine (TPY) motif, this family of kinases plays a key role in the response to thermal and ultra-violet radiation (UVR) stresses in animals as well as in several terrestrial and marine plants[8–13].

In human skin cells, which are often exposed to high solar radiation, UVR-induced reactive oxygen species (ROS) have been suggested to induce JNK activation[14], leading in turn to upregulation of anti-oxidant enzymes to overcome ROS accumulation[4,13,15]. Moreover, in cells, JNK has been proposed to have both pro-survival and pro-apoptotic activities, depending on the duration and intensity of the stress as well as the cellular context being examined[16,17]. Due to these different outcomes following activation of the JNK pathway, the primary function of JNK in the early response to UVR and thermal stress is still unclear[16,18–20].

Tropical corals are also exposed to high solar UVR and high temperature levels, especially in the face of climate change, which have already led to more than 20% destruction of coral reefs[21,22]. Exposure to these stresses

[1]Centre Scientifique de Monaco, Marine Department, 8 Quai Antoine Ier, MC-98000 Monaco, Principality of Monaco. [2]Sorbonne Universités, UPMC Univ Paris 06 IFD-ED 129, 4 Place Jussieu, 75252 PARIS cedex 05, France. [3]UMR ENTROPIE (IRD, Université de La Réunion, CNRS), Laboratoire d'Excellence « CORAIL», BP A5, 98848, Nouméa cedex, New Caledonia. [4]Centre Scientifique de Monaco, Biomedical Department, 8 Quai Antoine Ier, MC-98000 Monaco, Principality of Monaco. [5]University Nice Sophia-Antipolis, Institute for Research on Cancer and Aging of Nice (IRCAN), CNRS UMR7284/INSERM U1081, Centre Antoine Lacassagne, Nice 06189, France. *These authors contributed equally to this work. Correspondence and requests for materials should be addressed to G.P. (email: gpages@centrescientifique.mc) or C.F.-P. (email: ferrier@centrescientifique.mc)

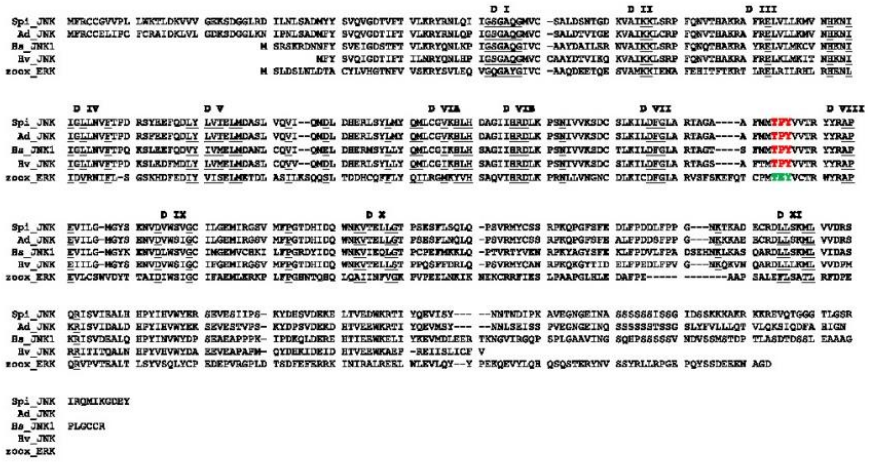


Figure 1. Sequence alignment of MAPK orthologs. The JNK orthologs of *Stylphora pistillata* (Spi-JNK), *Acropora digitifera* (Ad-JNK), *Hydra vulgaris* (Hv-JNK), the human MAPK8/JNK1 (Hs-JNK1) and the *Symbiodinium sp.* ERK ortholog (zoox_ERK) protein sequences are shown. The JNK-specific TPY activation motif and the ERK-specific TEY motif are highlighted in red and green respectively. The eleven conserved kinase domains are underlined.

leads to coral bleaching, i.e. the loss of coral algal symbionts and/or photosynthetic pigments[23,24], resulting in a drop in photosynthesis and calcification[25], and possibly leading to coral death. At the cellular level, bleaching has been proposed to be the consequence of an elevated production of ROS which damages cell components[26]. To prevent or to fight oxidative stress, corals have implemented defence strategies such as the synthesis of fluorescent pigments, heat-shock proteins, mycosporin-like amino acids[27,28] and the production of anti-oxidant enzymes[26]. An increasing number of studies indicate that corals express genes encoding MAPKs, including those encoding JNK[29–34]. This suggests the presence of a conserved stress response between coral and human cells at the molecular level, although the phylum cnidaria diverged from bilateria 550 million years ago[35]. An increase of the expression of genes coding for canonical substrates of JNK (c-Jun and c-Fos) was indeed observed in the coral *Acropora sp.* under thermal stress and following wounding[34,36,37]. Such induction may be specific to corals since post-translational and not transcriptional regulation of JNK signalling is most often observed in mammalian cells[38]. Although the above studies suggest a conservation of the stress response involving MAPK signalling cascades in corals, their specific functions have not been clearly defined. In particular, MAPK signalling pathway activity, which is classically considered the most appropriate readout, has never been monitored in corals.

Taking advantage of the amino acid sequence conservation of coral and human JNK orthologs, in particular the phosphorylatable TPY motif contained in the kinase domain, we used commercially available antibodies to analyse JNK activation upon thermal and UVR stress in both corals and human skin fibroblasts. Our aims were to confirm the presence of the JNK protein in corals, to study its phosphorylation (i.e. activation) under thermal and UVR stress, and to compare JNK activation in corals and human fibroblasts. In addition, we also endeavoured to link the JNK pathway activation to the defence against stress-induced ROS production in both models.

Results

Analysis of anthozoan transcriptomes. The human protein sequence for MAPK8/JNK1 (NCBI reference sequence: NM_001323322.1) was blasted against sequence databases including *Stylophora pistillata*[39] (GENBANK: GARY01004588), *Acropora digitifera*[40] (NCBI reference sequence: XM_015907276), *Hydra vulgaris*[41] (NCBI reference sequence: NM_001309762) and the coral symbiotic unicellular algae *Symbiodinium* clade B1[42] (accession number symbB.v1.2.036439). A sequence showing 89%, 74% and 64% homology with the protein sequences of *A. digitifera* (Ad-JNK), *H. vulgaris* (Hv-JNK) and the human MAPK8/JNK1 (Hs-JNK1), respectively, was identified in *S. pistillata*. This sequence (hereafter referred to as Spi-JNK for *S. pistillata* JNK) contains both the conserved kinase domains[43] and the TPY motif unique for JNK orthologs[15], like Ad-JNK, Hv-JNK and Hs-JNK sequences (Fig. 1). These results suggest that a single ortholog of JNK is present in *S. pistillata*, as opposed to the three genes encoding several JNK isoforms in the human genome[44]. Note that the unique and closest ortholog present in the *Symbiodinium* sequence database showed 35% homology with Spi-JNK (Fig. 1) and contained a TEY motif characteristic of ERK, instead of a TPY motif.

The TPY domain of JNK is phosphorylated by kinases upstream in its signal transduction pathway and as such its phosphorylation is an established readout of JNK activation. We therefore hypothesized that Spi-JNK, like its human counterpart, is activated by phosphorylation of the threonine and tyrosine residues contained in the TPY motif. To investigate this, we used commercially available anti-human JNK antibodies to test whether phosphorylated JNK (pJNK) could be detected in *S. pistillata* extracts. We used human fibroblasts to validate our approach and took advantage of the absence of a JNK ortholog in the *Symbiodinium* genome (Fig. 1) to prevent any misinterpretation due to potential cross contamination of animal and algae proteins in total protein extracts.

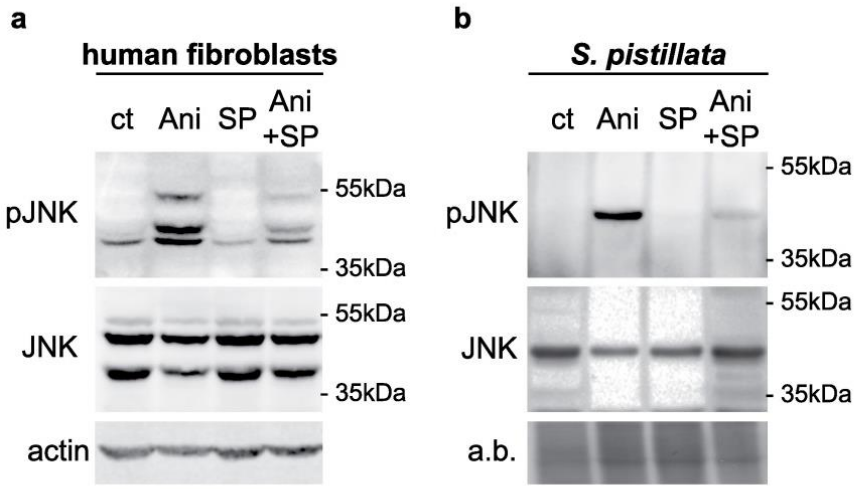


Figure 2. Detection of JNK activation in human fibroblasts and corals. Immunoblot revealing activated (pJNK) and total forms of JNK (JNK) present in (**a**) human fibroblasts and (**b**) *S. pistillata* nubbins incubated in the absence (control; ct) or presence of SP600125 (SP), anisomycin (Ani), or combination of both (Ani + SP). Molecular weight standards in kilodaltons (kDa) are indicated on the right side of the blots. (**a**) Total JNK and actin served as loading controls for human fibroblasts. (**b**) Total JNK and amido black staining (**a**,**b**) served as loading controls for *S. pistillata*. Data are representative of at least three independent experiments.

Characterization of JNK activation in corals and human cells. In corals, in agreement with the transcriptome analysis, only one JNK protein was present and therefore expressed in the western blots (Fig. 2a), while, in human fibroblasts, only JNK1 and JNK2 proteins were expressed (demonstrated by siRNA knockdown experiments, Fig. 2b and Fig. S1). In control culture conditions, phosphorylated forms of JNK (pJNK) were barely detectable in fibroblasts and undetectable in coral extracts (Fig. 2a and b). However, anisomycin, an activator of JNK[45], induced significant phosphorylation of JNK in both fibroblasts and *S. pistillata* (Fig. 2a and b). In fibroblasts, anisomycin induced the phosphorylation of isoforms that are not detected in the control condition. The anti-human pJNK antibody detected a unique immuno-reactive band in coral extracts, suggesting that this antibody recognized the phosphorylated TPY motif present in Spi-JNK (Fig. 2b). Consistent with our results indicating the absence of a JNK gene in the symbiont genome, isolated symbiont extracts did not present any immuno-reactive band when probed with the pJNK antibody (Supplementary Fig. S2). To further determine the specificity of the anti-human pJNK, we used the pharmacological inhibitor SP600125, commonly used as a JNK inhibitor[46,47]. It prevents autophosphorylation of JNK through competition with ATP, and the subsequent phosphorylation of JNK targets[48]. Treatment with SP600125 reduced the basal pJNK level in fibroblasts (Fig. 2a) and strongly reduced anisomycin induced of JNK phosphorylation in both *S. pistillata* and fibroblasts (Fig. 2a and b).

Both Spi- and Hs-JNK are activated by a combined thermal and UVR stress. To monitor the kinetics of JNK activation upon modifications of culture conditions, we subjected fibroblasts and coral nubbins to a combination of heat shock and UVR stress. In human cells, JNK activation was detectable after 10 minutes and reached a plateau after 30 minutes exposure in this experimental condition (Fig. 3a). In corals, JNK activation was at its maximum within 30 minutes and these levels were maintained at least for up to 4 hours under stress condition (Fig. 3b). Hereby, we show that, like in human cells, JNK activation is involved in the early response of corals to stress.

Respective contributions of UV and thermal stress to JNK activation. The effects of UV and temperature on early JNK activation were tested by subjecting fibroblasts and corals to the individual and combined stresses for 10 and 30 minutes, respectively. Ten minutes exposure to UVR alone had no significant effect on JNK activation in fibroblasts (Fig. 4a,c and Supplementary Table 1), although a longer exposure induced a strong activation (Supplementary Fig. S3). On the contrary, exposure to UVR triggered a detectable JNK phosphorylation in corals ($p = 1.4 \times 10^{-3}$) (Fig. 4b,d and Supplementary Table 1). Thermal stress strongly induced JNK activation in both models ($p = 1.25 \times 10^{-3}$ and $p = 5.38 \times 10^{-3}$ respectively). The combination of UV and thermal stress resulted in slightly higher but not statistically significant activation of JNK as compared to thermal stress alone (Fig. 4b,d and Supplementary Table 1). Treatment of cells and corals with the JNK inhibitor strongly reduced stress-induced JNK activation ($p = 6.62 \times 10^{-5}$ and $p = 6.51 \times 10^{-5}$ respectively). Notably, combining JNK inhibition to UVR stress had a dramatic effect on JNK protein stability in fibroblasts (Fig. 4a). We performed experiments to rule out the possibility that cell membrane disruption led to a leakage of the proteins out of the cells. First, we showed that the ROS carboxy-DCFDA probe used for ROS quantification did not leak out of the cells even after more than 30 minutes of exposure to a combination of UVR and SP600125 (Supplementary Fig. S4a).

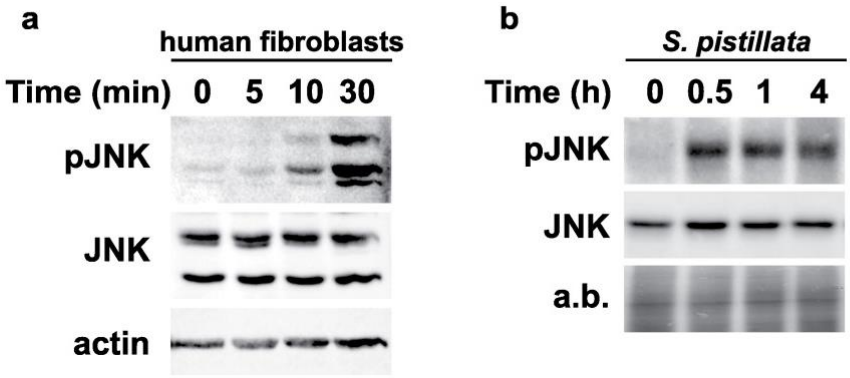


Figure 3. Time course of JNK activation by combined UVR and thermal stresses in human fibroblasts and coral. Immunoblot revealing activated (pJNK) and total JNK (JNK) in (**a**) human fibroblasts or (**b**) *S. pistillata* nubbins exposed to UVR and temperature rise for the indicated times. (**a**) Total JNK and actin served as loading controls for human fibroblasts. (**b**) Total JNK and amido black staining (**a**,**b**) served as loading controls for *S. pistillata*. Data are representative of three independent experiments.

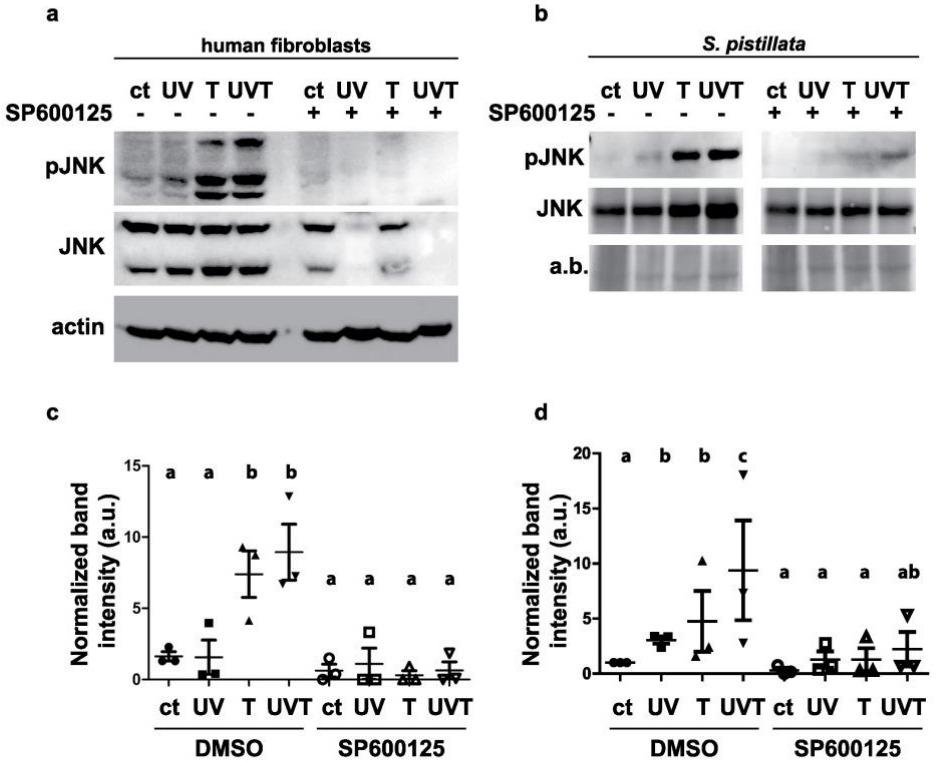


Figure 4. JNK activation by UVR, temperature rise and combined UVR and temperature rise in human fibroblasts and coral. Immunoblot revealing activated (pJNK) and total JNK (JNK) present in (**a**) human fibroblasts and (**b**) *S. pistillata* exposed to UVR (UV), increased temperature (T) or the combination of both stresses (UVT) in the absence (−) or presence (+) of SP600125. Total JNK and actin served as loading controls for human fibroblasts; total JNK and amido black staining (**a**,**b**) served as loading controls for *S. pistillata*. Data are representative of three independent experiments. Densitometric analysis of activated JNK intensities was performed on three independent blots for (**c**) human fibroblasts and (**d**) *S. pistillata*. The medians and standard deviations of three independent experiments are represented. Statistically significant differences, from the Tukey's post hoc test are indicated with letters. Similar letters indicates no significant differences between conditions.

Moreover, the quantification of the staining of the cells with the non-permeant fluorescent dye propidium iodide (PI) showed no difference between the control and UV + SP conditions (Supplementary Fig. S4b). These results suggest that the disappearance of the JNK bands was not associated to cell membrane disruption. We then hypothesized that JNK degradation could be due to a cleavage by proteases. We first showed that JNK degradation is not caused by caspase cleavage as caspase inhibitor Z-VAD-FMK did not prevent the degradation of JNK protein in cells exposed to a combination of UV and SP600125 (Fig. S5). Moreover, western blot analysis of the PARP protein showed a cleavage of PARP in the UV + SP condition (Fig. S5). Interestingly, this cleavage was not inhibited by Z-VAD-FMK and resulted in a band of different size than the staurosporin-induced caspase-dependent one, suggesting that non-caspase proteases are activated in these conditions. To test a potential autophagy-driven degradation of JNK, we assessed the expression level of the SQSTM1 protein. We observed no increase in SQSTM1 expression level, suggesting that the UV + SP treatment did not activate autophagy (Fig. S5). Finally, treatment with the proteasome pharmacological inhibitor MG132 did not result in stabilization of JNK in the UV + SP condition, suggesting that JNK is not degraded by the proteasome either (Fig. S6).

Inhibition of JNK in stress conditions impairs ROS scavenging capacities and results in strong adverse effects in corals and human cells. To gain more insight into the role of JNK activation in the early stress response, we assessed ROS levels in the experimental conditions described above. In both fibroblasts and corals, UVR induced a significant increase in ROS concentration ($p < 2 \times 10^{-16}$ and $p = 1.78 \times 10^{-10}$ respectively) (Fig. 5a,b and Supplementary Table 2). However, in both systems, thermal stress alone had no or very low effect on ROS content in the absence of JNK inhibitor (Fig. 5a,b and Supplementary Table 2). In corals, the inhibition of JNK activity leaded to an increase in ROS concentration in all conditions ($p = 4.60 \times 10^{-13}$) (Fig. 5b and Supplementary Table 2). A significant interaction between UVR and temperature led to a higher ROS concentration compared to each stress alone ($p = 2.53 \times 10^{-3}$) (Fig. 5b and Supplementary Table 2). In fibroblasts treated with JNK inhibitor, the ROS concentration did not change under high temperature but strongly increased under UVR with or without high temperature ($p < 2 \times 10–16$) (Fig. 5a and Supplementary Table 2).

Next we studied the physiological consequences of increased ROS cellular concentrations in human fibroblasts and corals. Hence, we determined the fibroblast viability and the loss of symbiont (bleaching) in coral as read-outs of ROS-mediated stress. As combined thermal stress and JNK inhibition had no effect on ROS content in fibroblasts, we only assessed the UVR response. Exposure to UVR or to SP600125 alone did not affect the fibroblasts viability, while the mean cell survival upon exposure to UVR plus the JNK inhibitor was less than 10% ($p = 1.07 \times 10^{-3}$) (Fig. 5c and Supplementary Table 3). In corals, exposure to SP600125 or UVT alone did not induce bleaching. However, exposure to the combination of UVR and high temperature in the presence of the JNK inhibitor for 24 hours led to a loss of more than 30% of the symbionts ($p = 1.21 \times 10^{-2}$) (Fig. 5d and Supplementary Table 3). These results suggest that JNK activity is necessary for the organisms to control the advert effects triggered by the stress-induced ROS burst.

Discussion

While the physiological response of corals to UVR or thermal stress has been well studied[27,28], the intracellular signalling that allows corals to rapidly respond to these stresses is still unknown. Our results strongly suggest the presence of a unique JNK protein in corals. This is in agreement with what was found in other cnidarians such as *Nematostela vectensis* or *Hydra vulgaris*[49,50]. For the first time, we showed a 100% conservation of the TPY motif in the catalytic domain of Spi and Hs-JNK. Phosphorylation of this domain by upstream kinases is essential for JNK activation. Hence, specific antibodies directed against the phosphorylated forms of Hs-JNK, which is an established player in the mammalian stress response[15], have been generated to monitor Hs-JNK activation. The specificity of these antibodies was further demonstrated for corals, since, as in human cells, Spi-JNK was activated and/or inhibited by the same mammalian activator/inhibitor. In addition, a significant increase in JNK phosphorylation was observed under exposure to high temperature and/or UVR, suggesting that activation of this pathway is a common response of *S. pistillata* to various environmental disturbances. Although previous studies suggested that mRNA encoding members of the JNK signalling pathway could be up-regulated in the corals' response to chronic stress conditions[34,36,37], JNK protein activity, which is classically considered the most appropriate readout, especially in an acute response setting, has never been monitored in corals. Our study thus demonstrates that JNK activity in corals is stimulated by environmental conditions in a way comparable to mammalian cells[4,51].

In *S. pistillata*, the phosphorylation of JNK was higher under thermal stress than UVR, and even seemed to be higher under the combination of both stressors, suggesting a cumulative effect of these two variables. This cellular response is also in agreement with the corals' physiological response to the same conditions[25,52,53] (Courtial *et al. in press*). Corals are usually well protected from UVR by a panel of natural sunscreens, such as mycosporine like amino acids[54] whereas they are more sensitive to thermal stress alone or in combination with other stressors[55]. Phosphorylation of JNK under the same stresses was previously observed in two kelp algae, but their response was mostly driven by UVR[11]. Nonetheless, a cumulative effect of temperature and UVR on JNK activation was observed in algae, suggesting that JNK is a good proxy for stress in marine organisms. Its activation is also proportional to the intensity of the stress. Similar to corals and algae, increased phosphorylation of JNK was observed in human fibroblasts under thermal stress. Instead, the UV stress inflicted to human cells was below the threshold value necessary to enhance the basal JNK activity (Fig. 4 and Supplementary Fig. S3). Nonetheless, the combination of UVR and JNK inhibition significantly decreased fibroblast viability, suggesting that the basal JNK activity is necessary and sufficient to overcome a moderate UV stress. Moreover, we observed a decrease in total JNK amount in fibroblasts by combining UVR and JNK inhibition. Such rapid disappearance of JNK has never been described and was not associated to cell membrane disruption, to caspase cleavage, to autophagy processes or to proteasomal degradation. Although we don't have a definitive explanation for such disappearance, the caspase-independent cleavage of PARP suggests that other proteases are activated in these conditions

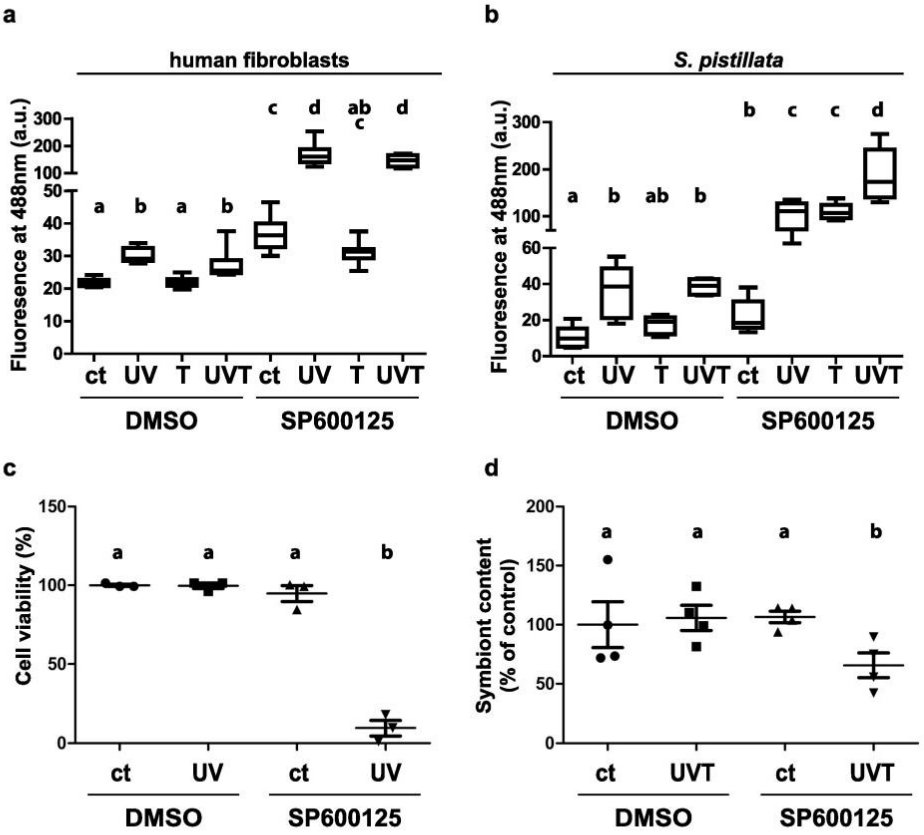


Figure 5. JNK activity protects corals and human cells from UV and temperature-induced oxidative stress and their adverse effects. Staining intensity of carboxy-H_2DCFDA (fluorescence at 488 nm), indicative of the presence of ROS, evaluated after 10 and 30 minutes respectively for (**a**) human fibroblasts and (**b**) *S. pistillata* with or without pre-incubation with SP 600125 in the following conditions: control (ct), UVR (UV), high temperature (T) and a combination of UVR and high temperature (UVT). The median, standard deviation and 95% confidence interval of at least three independent experiments are represented as boxplots. (**c**) Cell viability of human fibroblasts treated or not with SP600125 for 30 minutes prior to exposure to 10 minutes control (ct) or UVR (UV) measured 24 hours after the beginning of the experiment. (**d**) Symbiont content of *S. pistillata* nubbins treated or not with SP600125 for 1 hour prior to exposure to 4 hours control (ct) or combined UVR and thermal stresses (UVT) measured 24 hours after the beginning of the experiment. Medians and standard deviations are represented. Statistically significant differences, from the Tukey's post hoc test are indicated with letters. Similar letters indicates no significant differences between conditions.

and may also cleave JNK[56]. In both corals and fibroblasts, JNK inhibition by SP600125 indeed resulted in ROS accumulation. This "binary result" strongly suggests that JNK plays a pivotal role in protecting the cells against ROS damages; however, based on our results, it is difficult to conclude whether JNK activation results from ROS production or from another mechanism. In fibroblasts, the inactivation of JNK by SP600125 during exposure to fairly low dose of UVR[57,58] (3 W.m^{-2} UVB and 30 W.m^{-2} UVA for 10 minutes) led to the death of human cells. This observation supports the concept that, in the absence of active JNK, the fibroblasts cannot tolerate and neutralized the excessive production of UVR-induced ROS. This result contrasts with some studies showing that JNK activity is necessary for UVR-induced apoptosis[14,59,60]. However, our results are consistent with studies suggesting that JNK is also a key modulator of oxidative stress-mediated cell survival[14,60–62]. Discrepancies between studies may be explained by the use of different UV sources: whereas a UVA/B source that reproduces the UVR solar spectrum was used in our study, cells were exposed to UVC in the aforementioned studies[14,59,60]. In corals, the inactivation of the JNK pathway led to a slight bleaching (loss of symbiont cells) under the combined UVR/thermal stress, supporting the antioxidant function of the JNK signal transduction pathway. Consistent with our results, a protective role of JNK towards oxidative stress has also been reported in *C. elegans* and *D. melanogaster*[63–66], suggesting that this function is one of the primary functions of JNK throughout the animal kingdom. In humans, JNK activation has been linked to the upregulation of Manganese dependent-Super Oxide Dismutase (Mn-SOD) and catalases depending on the deacetylase SIRT1 and the FOXO transcription factor[13,64]. In a more

recent study, the early JNK response has been proposed to inhibit apoptosis[67]. Collectively, the results obtained in this study and other studies on various organisms suggest that the primary role of JNK signalling in thermal and solar spectrum UVR-induced oxidative stress consists of triggering a pro-survival anti-oxidant response. However, in case of a longer or more potent stress, e.g. exposure to UVC radiation[59], JNK might play a role in the induction of apoptosis.

Overall, our results suggest that JNK activation may play an evolutionarily-conserved role from cnidarians to mammals in protecting cells from oxidative stress. As the intensity of JNK phosphorylation is directly related to the intensity of oxidative stress in corals, JNK activation may represent a good and rapid proxy and an easily detectable marker in field studies to predict the effects of environmental perturbations on corals. In a time of rapid climate change, the knowledge of cellular pathways involved in the response of corals to stress is particularly important. Our findings bring new insight into the mechanisms that corals employ to face environmental pressures and raise several questions about the functions of JNK in both coral and human cells.

Methods

Maintenance of coral nubbins and human fibroblasts. The scleractinian coral *Stylophora pistillata* (*Pocilloporidae* family) was chosen as a model because it has a broad geographic range (from the Red Sea to the western central Pacific[68] and is widely used in laboratory experiments involving environmental stress effects, including short term thermal stress and UV exposure[25]. Colonies collected in the Gulf of Aqaba (Red Sea, Jordan) were cultured as previously described in Ferrier pages *et al.*[25].

Skin fibroblasts (BJ-EHLT cells) were kindly provided by E. Gilson's lab (IRCAN) and cultured in Dulbecco's Modified Eagle's Medium (DMEM) (Invitrogen, Villebon-sur-Yvette, France) supplemented with 10% heat-inactivated fetal calf serum (FCS, Dutscher, Brumath, France) at 37 °C in an atmosphere of 5% CO_2 as previously described[69].

Antibody validation. The antibody directed against the activated JNK (pTPpY) was from Promega, Madison, Wisconsin (V7931); the pan-JNK antibody was from R&D Systems, Minneapolis, Minnesota (AF1387).

Coral nubbins were incubated in individual beakers filled with 40 mL of 0.45 µm filtered seawater and placed during one hour under the following conditions: 1) 0.005% DMSO (drug solvent), 2) 100 µmol.L^{-1} anisomycin, which is a JNK activator[70] (Euromedex, Souffelweyersheim, France), 3) 40 µmol.L^{-1} of SP600125, which is a JNK inhibitor[48] (Euromedex); 4) a condition in which nubbins were pre-incubated with SP600125 during one hour, then stimulated with anisomycin while kept in the presence of SP600125 for another hour. The incubation medium was continuously stirred using magnetic stirrers at a constant temperature of 25 °C and a photosynthetically active radiation of 200 µmoles photons m^{-2} s^{-1}. At the end of the incubation, nubbins were frozen and kept at −80 °C prior to western blot analysis.

Skin fibroblasts (BJ-EHLT cells) were seeded at a density of 2×10^6 cells in 60 mm diameter dishes, and were subjected to the same treatments as the coral nubbins 24 hours later.

A *Symbiodinium* sp. culture (clade A1 isolated from *S. pistillata*,) in ASP8 medium[71] at 25 °C and 150 µmoles photons m^{-2} s^{-1} was also used to check for the presence of the JNK protein. Samples were exposed to the same 4 conditions as the coral nubbins, pelleted by centrifugation at 14 g for 10 minutes, frozen and kept at −80 °C prior to western blot analysis.

Assessment of JNK activity in response to combined thermal and UV stress. High temperature and UVR stresses were applied to corals and human cells. For corals, the thermal stress corresponded to an increase in temperature from the normal culture condition of 25 °C to 30 °C. The UVR stress corresponded to an increase in UVR from 0 (HQI lamps in the culture conditions) to a radiation intensity of about 3 W.m^{-2} UVB and 30 W.m^{-2} UVA (Q-Panel UVA 340 lamps). These intensities correspond to those reaching the sea surface in tropical areas[25]. Similar dose were used in previous work on corals[72–74] and in dermatological studies[57,58]. The UVR intensity was measured using ILT1400 portable radiometer and detectors (SEL240/UVB-1/TD and SEL033/UVA/TD) according to Shick *et al.*[75]. For human fibroblasts, thermal stress was induced by an increase in temperature from 37 °C to 42 °C, and UVR stress was the same as for coral nubbins.

JNK induction kinetics were evaluated on twelve coral nubbins, which were collected from the culture tanks and incubated during 4 h with constant agitation at 30 °C under UVR in beakers containing 40 mL of 0.45 µm filtered seawater. Three nubbins were frozen for each time point (0, 0.5, 1 and 4 hours). Fibroblasts were lyzed in Laemmli buffer prior to stress and after 5, 10 and 30 minutes incubations in the culture medium at 42 °C under UVR.

To assess JNK activity in response to individual and combined stress, coral nubbins were incubated in individual beakers, in the dark at 25 °C for 30 minutes in 40 mL of 0.45 µm filtered seawater containing 0.004% DMSO or 40 µmol.L^{-1} of JNK inhibitor (SP600125) prior to a 30 minutes exposure to the following conditions: 25 °C without UVR, 25 °C with UVR, 30 °C without UVR and 30 °C with UVR. Nubbins were flash frozen in liquid nitrogen and conserved at −80 °C prior to western blot analysis.

Similarly, BJ-EHLT fibroblasts were seeded at a density of 2×10^6 cells in 60 mm diameter dishes 24 h before the experiment. The cells were then incubated with 0.001% DMSO or 10 µmol.L^{-1} of SP600125 for 30 minutes at 37 °C prior to a 10 minutes exposure to the following conditions: 37 °C without UVR, 37 °C with UVR, 42 °C without UVR and 42 °C with UVR.

Western blot analysis. For coral samples, the tissue of each nubbin was removed from the skeleton using an air-pick in 1 mL of Laemmli buffer[76]. Samples were then sonicated for 30 seconds and centrifuged three times (5 minutes at 15 000 g) to remove the debris. Isolated *Symbiodinium* extracts were prepared from pellets re-suspended in 50 µL Laemmli buffer and then treated as the coral samples. Fibroblasts were washed twice in

phosphate buffered saline (PBS), lyzed in Laemmli buffer directly in the dishes and sonicated for 30 seconds. For all samples, the total protein concentration was determined using a BCA protein assay kit (Thermo Fisher Scientific) according to the manufacturer's recommendation. 1,4 Dithiothreitol (1 mM)-bromophenol blue solution (0.1% bromophenol blue) was added to all samples and they were then heated for 5 minutes at 95 °C and 60 µg of total protein were separated on 10% polyacrylamide gels and transferred onto a PVDF membrane at an intensity of 200 mA at 4 °C overnight in DUNN buffer. Membranes were stained in a 20% 2-propanol - 10% acetic acid - 0.1% amido black solution, destained in 2 washes with a 20% 2-propanol - 10% acetic acid solution, rinsed in distilled water, blocked for 30 minutes in PBS - 3% low fat milk, rinsed in PBS and incubated with primary antibodies (anti-activated JNK: Promega, V7931; anti-pan-JNK: R&D Systems, AF1387; anti-beta Actin: Pierce MA5- 15739; anti-GAPDH: Pierce, MA5-15738; anti-SQSTM1/p62 Antibody: Cell Signaling Technology 5114) diluted in PBS - 1% low fat milk at 4 °C overnight. After extensive washing with PBS, membranes were incubated for 2 hours in the presence of the HRP-conjugated secondary antibody, rinsed in PBS and HRP activity was detected using Millipore ECL according to the manufacturer recommendations. An Odyssey device (LI-COR Biosciences, Lincoln, Nebraska) was used to image the membranes.

Densitometric analysis of the western blots was performed using the Image Studio software (Li-COR Biosciences). Intensity of the pJNK signal was normalised to the intensity of the loading controls.

ROS quantification. ROS detection was performed using the carboxy-H_2DCFDA probe (Image-iT TM LIVE Green Reactive Oxygen Species Detection Kit (I36007), Molecular Probes Invitrogen. The apical tips of five coral branches were incubated in 750 µL of 0.45 µm filtered seawater containing 25 µmol.L^{-1} of carboxy-H_2DCFDA probe in the presence of 0.001% DMSO or 10 µmol.L^{-1} SP600125 per condition. The apical tips were incubated for 30 minutes in the dark at 25 °C prior to exposure to the following conditions: 25 °C without UVR, 25 °C with UVR, 30 °C without UVR and 30 °C with UVR. Samples were then rinsed in filtered seawater and fluorescence was directly measured in the coral tissue with a Xenius SOF spectrofluorometer (SAFAS, Monaco) equipped with an optical fiber. The emission spectrum was measured between 515 and 550 nm with an excitation wavelength of 495 nm.

Human fibroblasts (0.5×10^6 in 35 mm dishes) were incubated for 30 minutes in culture medium containing 25 µmol.L^{-1} of carboxy-H_2DCFDA probe in the presence of 0.001% DMSO or 10 µmol.L^{-1} SP600125 prior to exposure to the following conditions25 °C without UVR, 25 °C with UVR, 30 °C without UVR and 30 °C with UVR for 10 minutes. Cells were imaged using a LEICA DM4000 microscope with a 40x objective and a Zyla5.5 sCMOS camera (Andor Technology Ltd, Belfast, Northern Ireland). The software ImageJ[77] was used to quantify the fluorescence of at least five fields from three independent experiments per condition.

Measurement of bleaching and cellular viability. Coral nubbins were treated for 1 hour with 5 µmol.L^{-1} SP600125 or 0.005% DMSO in standard culture conditions prior to exposure to UVR at 30 °C for 4 hours. Nubbins were then, rinsed, and placed back into control culture conditions for 24 hours. The symbiont content was measured as previously described[78].

BJ-EHLT cells were seeded at a density of 10^6 cells in 60 mm diameter dishes the day before the experiment. The cells were then treated for 30 minutes with 1 µmol.L^{-0}f SP600125 or with 0.001% DMSO in standard culture conditions, exposed to UVR for 10 minutes and washed 3 times with culture medium before being placed back in normal culture conditions. The cell viability was assessed 24 hours after the initial treatment using an ADAM automatic fluorescence cell counter (AlphaMetrix Biotech, Rödermark, Germany) following the manufacturer's recommendations.

Statistical analysis. For ROS and western blot quantifications, conditions were compared using a three-way Analysis of Variance (ANOVA) with drugs (±SP 6000125), UVR (±UVR) and temperature (high or low temperature) as factors. The normality of the residuals and the homoscedasticity of the variances were tested using the Shapiro test and the Bartlett test, respectively. A Tukey's Honest Significant Difference post hoc test was performed when significant differences were found. Fibroblast viability and the relative amounts of symbionts left at the end of the experiment were compared using a t-test. Normality of data was checked prior to running tests. For each test, we used 0.05 as alpha level. For western blot quantifications, the ANOVAs were ran on n = 3 replicates; and on n = 15 and n = 5 replicates per condition for ROS concentration in fibroblast and coral respectively. T-tests were performed on n = 3 and n = 4 replicates respectively.

References

1. Meyers, L. A. & Bull, J. J. Fighting change with change: Adaptive variation in an uncertain world. *Trends Ecol. Evol.* **17,** 551–557 (2002).
2. Hofmann, G. E. & Todgham, A. E. Living in the now: physiological mechanisms to tolerate a rapidly changing environment. *Annu. Rev. Physiol.* **72,** 127–145 (2010).
3. Gallopin, G. C. Linkages between vulnerability, resilience, and adaptive capacity. *Glob. Environ. Chang.* **16,** 293–303 (2006).
4. Runchel, C., Matsuzawa, A. & Ichijo, H. Mitogen-activated protein kinases in mammalian oxidative stress responses. *Antioxid. Redox Signal.* **15,** 205–218 (2011).
5. Dhillon, A. S., Hagan, S., Rath, O. & Kolch, W. MAP kinase signalling pathways in cancer. *Oncogene* **26,** 3279–90 (2007).
6. Widmann, C., Gibson, S., Jarpe, M. B. & Johnson, G. L. Mitogen-activated protein kinase: conservation of a three-kinase module from yeast to human. *Physiol. Rev.* **79,** 143–180 (1999).
7. Chen, Z. *et al.* MAP Kinases. *Chem. Rev.* **101,** 2449–2476 (2001).
8. Dérijard, B. *et al.* JNK1: A protein kinase stimulated by UV light and Ha-Ras that binds and phosphorylates the c-Jun activation domain. *Cell* **76,** 1025–1037 (1994).
9. Bridge, D. *et al.* FoxO and stress responses in the cnidarian Hydra vulgaris. *PLoS One* **5** (2010).
10. Garcia-Gomez, C. *et al.* Cell survival after UV radiation stress in the unicellular chlorophyte Dunaliella tertiolecta is mediated by DNA repair and MAPK phosphorylation. *J. Exp. Bot.* **63,** 5259–5274 (2012).

11. Parages, M. L., Heinrich, S., Wiencke, C. & Jiménez, C. Rapid phosphorylation of MAP kinase-like proteins in two species of Arctic kelps in response to temperature and UV radiation stress. *Environ. Exp. Bot.* **91,** 30–37 (2013).
12. Yao, C.-L. & Somero, G. N. The impact of acute temperature stress on hemocytes of invasive and native mussels (Mytilus galloprovincialis and Mytilus californianus): DNA damage, membrane integrity, apoptosis and signaling pathways. *J. Exp. Biol.* **215,** 4267–77 (2012).
13. Brunet, A., *et al.* Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase. *Science* **303,** 2011–2015 (2004).
14. Shen, H. M. & Liu, Z. G. JNK signaling pathway is a key modulator in cell death mediated by reactive oxygen and nitrogen species. *Free Radic. Biol. Med.* **40,** 928–939 (2006).
15. Davis, R. J. Signal transduction by the JNK group of MAP kinases. *Cell* **103,** 239–252 (2000).
16. Liu, J. & Lin, A. Role of JNK activation in apoptosis: a double-edged sword. *Cell Res.* **15,** 36–42 (2005).
17. Tournier, C. The 2 faces of JNK signaling in cancer. *Genes Cancer* **4,** 397–400 (2013).
18. Bachelor, M. A. & Bowden, G. T. UVA-mediated activation of signaling pathways involved in skin tumor promotion and progression. *Semin. Cancer Biol.* **14,** 131–138 (2004).
19. Kim, E. K. & Choi, E.-J. Pathological roles of MAPK signaling pathways in human diseases. *Biochim. Biophys. Acta - Mol. Basis Dis.* **1802,** 396–405 (2010).
20. Dent, P., Yacoub, A., Fisher, P. B., Hagan, M. P. & Grant, S. MAPK pathways in radiation responses. *Oncogene* **22,** 5885–96 (2003).
21. Hoegh-Guldberg, O. *et al.* Coral reefs under rapid climate change and ocean acidification. *Science* **318,** 1737–1742 (2007).
22. Doney, S. C. *et al.* Climate change impacts on marine ecosystems. *Ann. Rev. Mar. Sci.* **4,** 11–37 (2012).
23. D'Croz, L & Maté, J. L. The role of water temperature and UV radiation in the recovery of the experimentally bleached coral Pocillopora damicornis from the eastern Pacific Ocean (Panama). In *Proceedings of the Ninth International Coral Reef Symposium, Bali, 23-27 October 2000* **2,** 1111–1116 (2002).
24. Drohan, A. F., Thoney, D. A. & Baker, A. C. Synergistic effect of high temperature and ultraviolet-B radiation on the gorgonian *Eunicea tourneforti* (Octocorallia: Alcyonacea: Plexauridae). *Bull. Mar. Sci.* **77,** 257–266 (2005).
25. Ferrier-Pagès, C. *et al.* Effects of temperature and UV radiation increases on the photosynthetic efficiency in four scleractinian coral species. *Biol. Bull.* **213,** 76–87 (2007).
26. Yakovleva, I., Bhagooli, R., Takemura, A. & Hidaka, M. Differential susceptibility to oxidative stress of two scleractinian corals: Antioxidant functioning of mycosporine-glycine. *Comp. Biochem. Physiol. - B Biochem. Mol. Biol.* **139,** 721–730 (2004).
27. Banaszak, A. T. & Lesser, M. P. Effects of solar ultraviolet radiation on coral reef organisms. *Photochem. Photobiol. Sci.* **8,** 1276 (2009).
28. Lesser, M. P. Oxidative stress in marine environments: biochemistry and physiological ecology. *Annu. Rev. Physiol.* **68,** 253–278 (2006).
29. Siboni, N. *et al.* Using bacterial extract along with differential gene expression in Acropora millepora Larvae to decouple the processes of attachment and metamorphosis. *PLoS One* **7** (2012).
30. Sun, J., Chen, Q., Lun, J. C. Y., Xu, J. & Qiu, J. W. PcarnBase: Development of a transcriptomic database for the brain coral Platygyra carnosus. *Mar. Biotechnol.* **15,** 244–251 (2013).
31. Mayfield, A. B., Hsiao, Y. Y., Fan, T. Y., Chen, C. S. & Gates, R. D. Evaluating the temporal stability of stress-activated protein kinase and cytoskeleton gene expression in the Pacific reef corals Pocillopora damicornis and Seriatopora hystrix. *J. Exp. Mar. Bio. Ecol.* **395,** 215–222 (2010).
32. Miller, D. J. *et al.* The innate immune repertoire in cnidaria-ancestral complexity and stochastic gene loss. *Genome Biol.* **8,** R59 (2007).
33. Shinzato, C. *et al.* Using the Acropora digitifera genome to understand coral responses to environmental change. *Nature* **476,** 320–323 (2011).
34. van de Water, J. A. J. M., Lamb, J. B., van Oppen, M. J. H., Willis, B. L. & Bourne, D. G. Comparative immune responses of corals to stressors associated with offshore reef-based tourist platforms. *Conserv. Physiol.* **3,** cov032 (2015).
35. Daly, M. *et al.* The phylum Cnidaria: A review of phylogenetic patterns and diversity 300 years after Linnaeus. *Zootaxa* **182,** 127–182 (2007).
36. van de Water, J. A. J. M. *et al.* Elevated seawater temperatures have a limited impact on the coral immune response following physical damage. *Hydrobiologia* **759,** 201–214 (2015).
37. van De Water, J. A. J. M. *et al.* The coral immune response facilitates protection against microbes during tissue regeneration. *Mol. Ecol.* **24,** 3390–3404 (2015).
38. Kaminska, B. MAPK signalling pathways as molecular targets for anti-inflammatory therapy - From molecular mechanisms to therapeutic benefits. *Biochim. Biophys. Acta - Proteins Proteomics* **1754,** 253–262 (2005).
39. Liew, Y. J. *et al.* Identification of microRNAs in the coral Stylophora pistillata. *PLoS One* **9,** 1–11 (2014).
40. Shinzato, C., Mungpakdee, S., Satoh, N. & Shoguchi, E. A genomic approach to coral-dinoflagellate symbiosis: Studies of Acropora digitifera and Symbiodinium minutum. *Front. Microbiol.* **5,** 1–17 (2014).
41. Krishna, S. *et al.* Deep sequencing reveals unique small RNA repertoire that is regulated during head regeneration in Hydra magnipapillata. *Nucleic Acids Res.* **41,** 599–616 (2013).
42. Barshis, D. J., Ladner, J. T., Oliver, T. A. & Palumbi, S. R. Lineage-specific transcriptional profiles of Symbiodinium spp. unaltered by heat stress in a coral host. *Mol. Biol. Evol.* **31,** 1343–1352 (2014).
43. Hanks, S. K. & Hunter, T. Protein kinases 6. The eukaryotic protein kinase superfamily: kinase (catalytic) domain structure and classification. *FASEB J.* **9,** 576–596 (1995).
44. Ip, Y. T. & Davis, R. J. Signal transduction by the c-Jun N-terminal kinase (JNK)—from inflammation to development. *Curr. Opin. Cell Biol.* **10,** 205–219 (1998).
45. Philipp, I., Holstein, T. W. & Hobmayer, B. HvJNK, a Hydra member of the c-Jun NH2-terminal kinase gene family, is expressed during nematocyte differentiation. *Gene Expr. Patterns* **5,** 397–402 (2005).
46. Manole, S., Richards, E. J & Meyer, A. S. JNK pathway activation modulates acquired resistance to EGFR/HER2 targeted therapies. *Cancer Res* (2016).
47. Sayeed, A. *et al.* β1 integrin- and JNK-dependent tumor growth upon hypofractionated radiation. *Oncotarget* (2016).
48. Bennett, B. L *et al.* SP600125, an anthrapyrazolone inhibitor of Jun N-terminal kinase. *Proc. Natl. Acad. Sci. USA* **98,** 13681–13686 (2001).
49. Putnam, N. H. *et al.* Sea anemone genome reveals ancestral eumetazoan gene repertoire and genomic organization. *Science* **317,** 86–94 (2007).
50. Philipp, I., Holstein, T. W. & Hobmayer, B. HvJNK, a Hydra member of the c-Jun NH 2-terminal kinase gene family, is expressed during nematocyte differentiation. *Gene Expr. patterns* **5,** 397–402 (2005).
51. Davis, R. In *Inflammatory Processes* (eds Letts, L. G. & Morgan, D.) 13–21 (Birkhäuser Basel, 2000).
52. Lesser, M. P., Stochaj, W. R., Tapley, D. W. & Shick, J. M. Coral Reefs effects of irradiance, ultraviolet radiation, and temperature. *Coral Reefs* **8,** 225–232 (1990).
53. D'Croz, L., Maté, J. L. & Oke, J. E. Responses to elevated sea water temperature and UV radiation in the coral Porites lobata from upwelling and non-upwelling environments on the Pacific coast of Panama. *Bull. Mar. Sci.* **69,** 203–214 (2001).
54. Shick, J., Lesser, M. P. & Jokiel, P. L. Effects of ultraviolet radiation on corals and other coral reef organisms. *Glob. Chang. Biol.* **2,** 527–545 (1996).

55. Hoegh-Guldberg, O. Climate change, coral bleaching and the future of the world's coral reefs. *Mar. Freshw. Res.* **50,** 839 (1999).
56. Chaitanya, G. V., Steven, A. J. & Babu, P. P. PARP-1 cleavage fragments: signatures of cell-death proteases in neurodegeneration. *Cell Commun Signal* **8,** 31 (2010).
57. Bernerd, F. & Asselineau, D. An organotypic model of skin to study photodamage and photoprotection *in vitro*. *J. Am. Acad. Dermatol.* **58,** S155–S159 (2008).
58. Marionnet, C. *et al.* Different oxidative stress response in keratinocytes and fibroblasts of reconstructed skin exposed to non extreme daily-ultraviolet radiation. *PLoS One* **5,** e12059 (2010).
59. Tournier C. *et al.* Requirement of JNK for Stress- Induced Activation of the Cytochrome c-Mediated Death Pathway. *Science* **288,** 870–874 (2000).
60. Yabu, T. *et al.* Stress-induced ceramide generation and apoptosis via the phosphorylation and activation of nSMase1 by JNK signaling. *Cell Death Differ.* **22,** 258–73 (2015).
61. Cao, G. *et al.* EGCG protects against UVB-induced apoptosis via oxidative stress and the JNK1/c-Jun pathway in ARPE19 cells. *Mol. Med. Rep.* **5,** 54–59 (2012).
62. Khoshnood, B., Dacklin, I. & Grabbe, C. Urm1: an essential regulator of JNK signaling and oxidative stress in Drosophila melanogaster. *Cell. Mol. Life Sci.* **73,** 1939–1954 (2016).
63. Wang, M. C., Bohmann, D. & Jasper, H. JNK signaling confers tolerance to oxidative stress and extends lifespan in Drosophila. *Dev. Cell* **5,** 811–816 (2003).
64. Essers, M. A. G. *et al.* FOXO transcription factor activation by oxidative stress mediated by the small GTPase Ral and JNK. *EMBO J.* **23,** 4802–12 (2004).
65. Venugopal, R. & Jaiswal, a. K. Nrf2 and Nrf1 in association with Jun proteins regulate antioxidant response element-mediated expression and coordinated induction of genes encoding detoxifying enzymes. *Oncogene* **17,** 3145–3156 (1998).
66. Kops, G. J. P. L. *et al.* Forkhead transcription factor FOXO3a protects quiescent cells from oxidative stress. *Nature* **419,** 316–321 (2002).
67. Brown, M. *et al.* An initial phase of JNK activation inhibits cell death early in the endoplasmic reticulum stress response. *J. Cell Sci.* **2,** jcs.179127 (2016).
68. Flot, J.-F. *et al.* Incongruence between morphotypes and genetically delimited species in the coral genus Stylophora: phenotypic plasticity, morphological convergence, morphological stasis or interspecific hybridization? *BMC Ecol.* **11,** 1 (2011).
69. Biroccio, A. *et al.* TRF2 inhibits a cell-extrinsic pathway through which natural killer cells eliminate cancer cells. *Nat. Cell Biol.* **15,** 818–828 (2013).
70. Iordanov, M. S. *et al.* Ribotoxic stress response: activation of the stress-activated protein kinase JNK1 by inhibitors of the peptidyl transferase reaction and by sequence-specific RNA damage to the alpha-sarcin/ricin loop in the 28S rRNA. *Mol. Cell. Biol.* **17,** 3373–3381 (1997).
71. Al-Moghrabi, S., Goiran, C., Allemand, D., Speziale, N. & Jaubert, J. Inorganic carbon uptake for photosynthesis by the symbiotic coral-dinoflagellate association.2. Mechanisms for bicarbonate uptake. *J. Exp. Mar. Bio. Ecol.* **199,** 227–248 (1996).
72. Courtial, L., Roberty, S., Shick, J. M., Houlbrèque, F. & Ferrier-Pagès, C. Interactive effects of ultraviolet radiation and thermal stress on two reef-building corals. *Limnol Oceanogr* (2017).
73. Shick, J. M. The continuity and intensity of ultraviolet irradiation affect the kinetics of biosynthesis, accumulation, and conversion of mycosporine-like amino acids (MAAs) in the coral Stylophora pistillata. *Limnol. Oceanogr.* **49,** 442–458 (2004).
74. Torres-Pérez, J. L. & Armstrong, R. A. Effects of UV radiation on the growth, photosynthetic and photoprotective components, and reproduction of the Caribbean shallow-water coral Porites furcata. *Coral Reefs* **31,** 1077–1091 (2012).
75. Shick, J. M., Lioud, S., Reynaud, S., Ferrier-Pagès, C. & Gattuso, J.-P. Ultraviolet-B radiation stimulates shikimate pathway-dependent accumulation of mycosporine-like amino acids in the coral Stylophora pistillata despite decreases in its population of symbiotic dinoflagellates. *Limnol. Oceanogr.* **44,** 1667–1682 (1999).
76. Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227,** 680–685 (1970).
77. Schneider, C., Rasband, W. S. & Eliceiri, K. W. NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* **9,** 671–675 (2012).
78. Godinot, C., Ferrier-Pagès, C., Sikorski, S. & Grover, R. Alkaline phosphatase activity of reef-building corals. *Limnol. Oceanogr.* **58,** 227–234 (2013).

Acknowledgements

The authors thank D. Desgré for technical help during the experimental setup, Dr. Jeroen van de Water and Didier Zoccola for editorial assistance, Mrs Natacha Caminiti-Segonds for the first coral extracts and assays as well as Pr. Denis Allemand, Director of the Centre Scientifique de Monaco for scientific support. Financial support to L.C. was provided by the Centre Scientifique de Monaco and Paris VI University, GP was supported by the Fondation de France, the Association for Cancer Research, the National Institute of Cancer (InCA) and "Cordon de vie, Monaco".

Author Contributions

Conceived and designed the experiments: C.F.P., G.P., L.C., V.P., Y.C. Performed the experiments: L.A., L.C., R.C., R.G., V.P., Y.C. Analysed the data: C.F.P., G.P., L.C., V.P. Contributed reagents/materials/analysis tools: C.F.P., G.P. Wrote the paper: C.F.P., G.P., L.C., V.P.

Additional Information

Supplementary information accompanies this paper at http://www.nature.com/srep

Competing Interests: The authors declare no competing financial interests.

How to cite this article: Courtial, L. *et al.* The c-Jun N-terminal kinase prevents oxidative stress induced by UV and thermal stresses in corals and human cells. *Sci. Rep.* **7,** 45713; doi: 10.1038/srep45713 (2017).

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

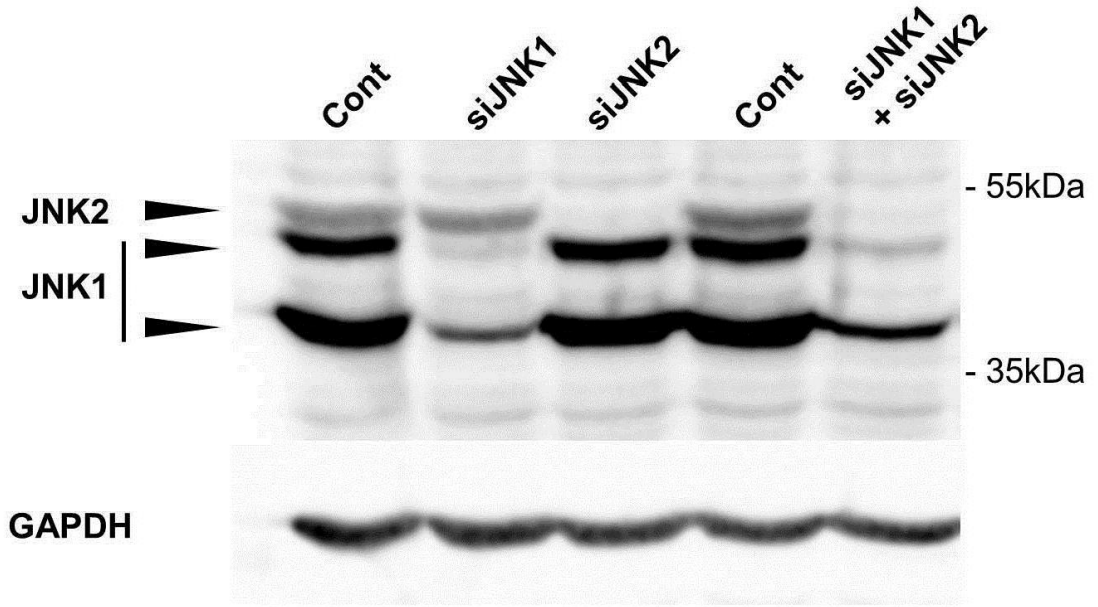
This work is licensed under a Creative Commons Attribution 4.0 International License. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder to reproduce the material. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/

© The Author(s) 2017

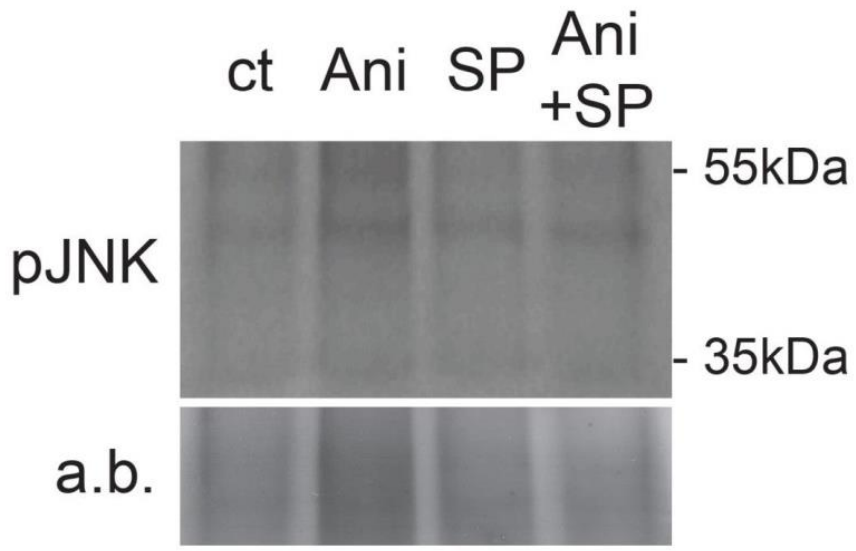
Supplementary information

The c-Jun N-terminal kinase prevents oxidative stress induced by UV and thermal stresses in corals and human cells

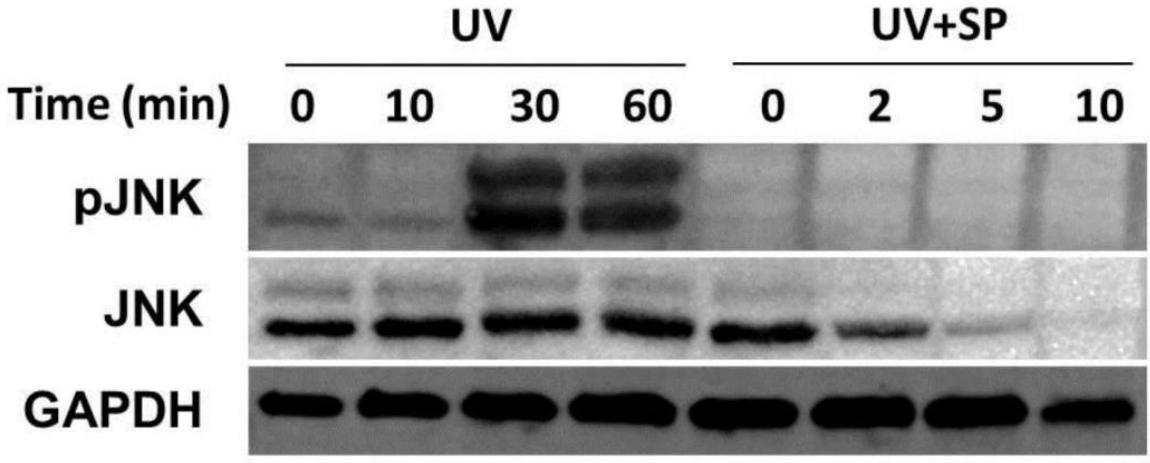
L.Courtial, V. Picco, R. Grover, Y.Cormerais, C. Rottier, A. Labbe, G. Pagès and C. Ferrier-Pagès



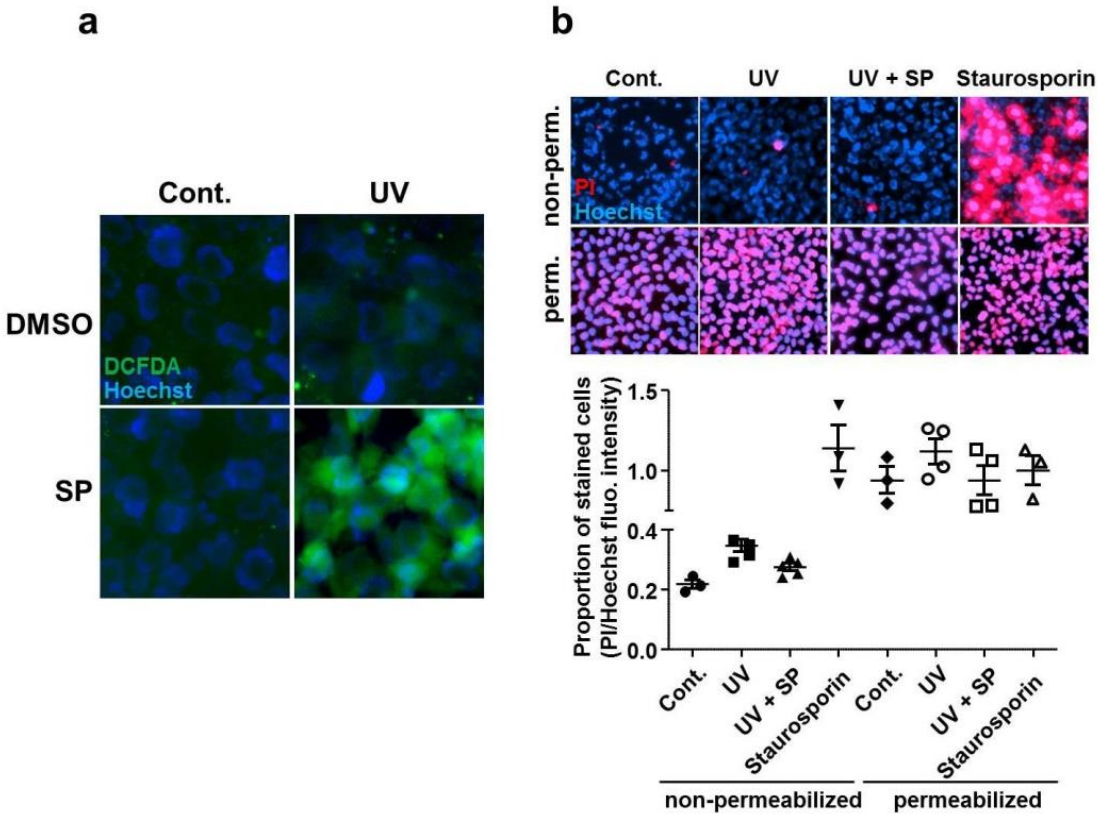
Supplementary Figure S1: JNK genes and isoforms expression in human BJ fibroblasts. The determination of the genes and isoforms expression was conducted by a siRNA-based approach. Briefly, siRNA pools directed against the human JNK1 and JNK2 genes (respectively ON-TARGETplus Human MAPK8 (5599) and MAPK9 (5601) SMARTpools) and the non-target control siRNA pool (the ON-TARGETplus Non-targeting Pool) were transfected with Dharmafect 1 transfection reagent (T-2001-02) as recommended by the manufacturer (GE Healthcare Europe, Velizy-Villacoublay, France). Cell lysates prepared for western blotting as described in the manuscript and were analyzed by immunoblot using a pan-JNK antibody. In the figure, the Cont, siJNK1, siJNK2 and siJNK1+2 columns represent cells transfected with non-target siRNAs, with siRNAs directed against JNK1 (siJNK1), JNK2 (siJNK2) or a combination of both siRNAs (siJNK1+siJNK2) respectively. GAPDH served as loading control. Molecular weight standards are indicated on the right part of the figure.



Supplementary Figure S2: Absence of JNK activity in Symbiodinium clade B1.
Immunoblot confirming the absence of activated JNK (pJNK) in Symbiodinium clade B1 cultures treated as described for coral nubbins, i.e. in the absence or presence of SP600125 (SP) and anisomycin (Ani and Ani+SP). Amido black staining (a.b.) served as loading control.



Supplementary Figure S3: Time course assessment of JNK activation and degradation.
Immunoblots revealing activated JNK (pJNK) in human fibroblasts exposed to UV radiation for the indicated times in the absence (UV) or presence (UV+SP) of JNK inhibitor SP600125. JNK and GAPDH served as loading control.



Supplementary Figure S4: Assessment of cell membrane permeability by dual Hoechst 33342 and propidium iodide staining after exposure to UVR and JNK pharmacological inhibitor.

Cells were treated with UVR and/or SP600125 as previously described and incubated for 20 minutes with 30 µM propidium iodide and 1µg.mL^{-1} Hoechst 33342. Hoechst 33342 is a cell membrane-permeant DNA dye with excitation/emission maxima of 361/497 nm whereas propidium iodide is a cell membrane-impermeable DNA dye with excitation/emission maxima of 535/617 nm. The difference in cell-permeability between the two dyes allows a discrimination of the cells with damaged membranes from intact cells. Cells were imaged using a Leica DMI4000 microscope as previously described. In the figure, (a) Cells stained with the ROS carboxy-DCFDA probe (DCFDA, green) still retained fluorescence 30 minutes after exposure to a combination of UVR and SP600125. (b) Intracellular staining of the cells with the cell impermeable propidium iodide dye (PI, red) did not increase under UVR and SP600125 single or combined treatments, as opposed to treatment with the apoptosis inducer staurosporin or the permeabilizing agent Triton X100 (perm.)

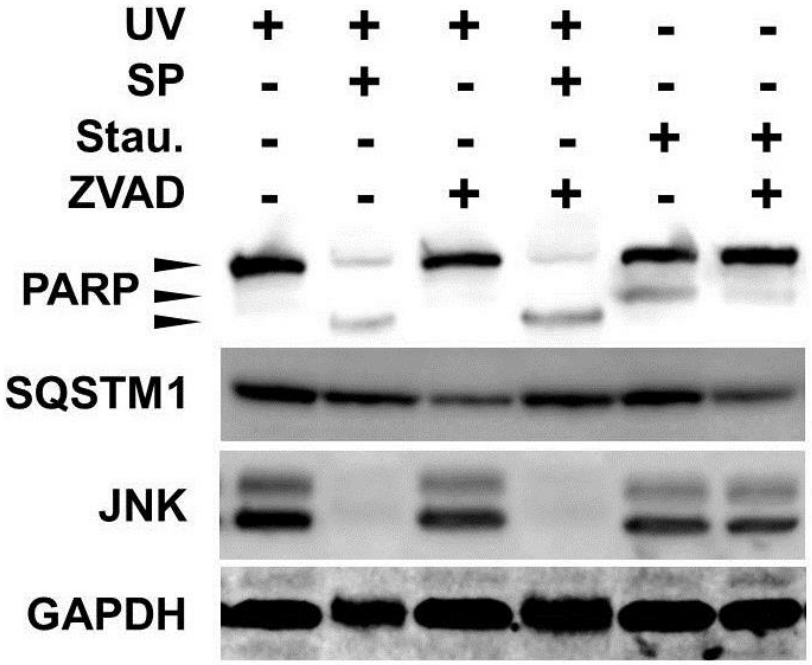


Figure S5: Activation of cell death pathways in various experimental conditions. Immunoblots revealing PARP, SQSTM1 and JNK in human fibroblasts in the absence (-) or presence (+) of UV radiation (UV), the JNK inhibitor SP600125 (SP), the pharmacological activator of apoptosis staurosporin (Stau.) and the caspase inhibitor Z-VAD-FMK (ZVAD). GAPDH served as loading control.

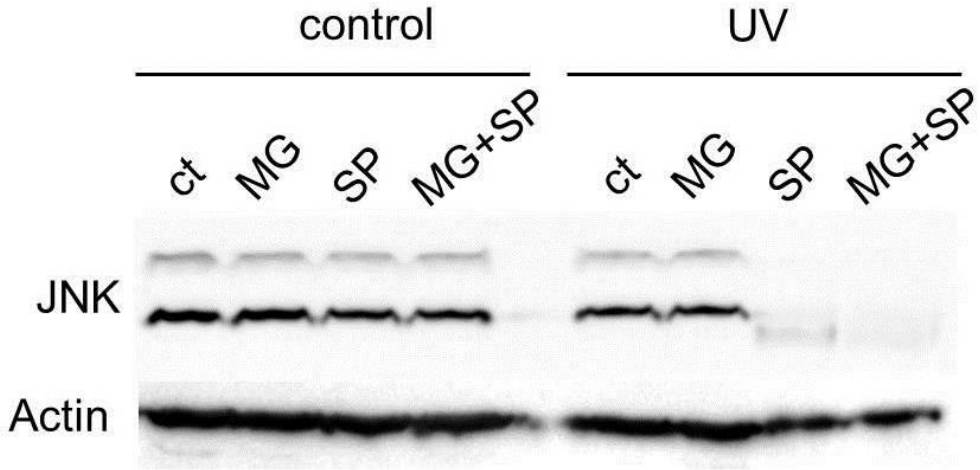
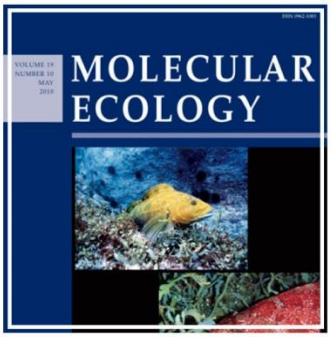


Figure S6: Pharmacological inhibition of the proteasome does not prevent JNK degradation. Immunoblot revealing total JNK form (JNK) in human fibroblasts in the absence (ct) or presence of MG132 (MG), JNK inhibitor SP600125 (SP) or a combination of both (MG+SP) and exposed (UV) or not (control) to UVR. Actin served as loading control.



References and Appendix

References

A

Agostini, S., Y. Suzuki, T. Higuchi, B. E. Casareto, K. Yoshinaga, Y. Nakano, and H. Fujimura. 2012. Biological and chemical characteristics of the coral gastric cavity. Coral Reefs **31**: 147–156.

Al-Horani, F. A. 2005. Effects of changing seawater temperature on photosynthesis and calcification in the scleractinian coral *Galaxea fascicularis*, measured with O_2, Ca^{2+} and pH microsensors. Sci. Mar. **69**: 347–354.

Allemand, D., C. Ferrier-Pagès, P. Furla, and others. 2004. Biomineralisation in reef-building corals: from molecular mechanisms to environmental control. Comptes Rendus Palevol **3**: 453–467.

Allemand, D., É. Tambutté, D. Zoccola, and S. Tambutté. 2011. Coral calcification, cells to reefs, p. 119–150. *In* Coral reefs: an ecosystem in transition. Springer.

Allsopp, M., S. E. Pambuccian, P. Johnston, and D. Santillo. 2008. State of the World's Oceans, Springer Science & Business Media.

Anderson, S. M., and O. A. Roels. 1981. Effects of light intensity on nitrate and nitrite uptake and excretion by *Chaetoceros curvisetus*. Mar. Biol. **62**: 257–261.

Anderson, S., R. Zepp, J. Machula, D. Santavy, L. Hansen, and E. Mueller. 2001. Indicators of UV exposure in corals and their relevance to global climate change and coral bleaching. Hum. Ecol. Risk Assess. An Int. J. **7**: 1271–1282.

Andréfouët, S., G. Cabioch, B. Flamand, and B. Pelletier. 2009. A reappraisal of the diversity of geomorphological and genetic processes of New Caledonian coral reefs: a synthesis from optical remote sensing, coring and acoustic multibeam observations. Coral Reefs **28**: 691–707.

Anestis, A., H. O. Pörtner, A. Lazou, and B. Michaelidis. 2008. Metabolic and molecular stress responses of sublittoral bearded horse mussel *Modiolus barbatus* to warming sea water: implications for vertical zonation. J. Exp. Biol. **211**: 2889–98.

Anthony, K. R. N. 1999. Coral suspension feeding on fine particulate matter. J. Exp. Mar. Bio. Ecol. **232**: 85–106.

Anthony, K. R. N., D. I. Kline, G. Diaz-Pulido, S. Dove, and O. Hoegh-Guldberg. 2008. Ocean acidification causes bleaching and productivity loss in coral reef builders. Proc. Natl. Acad. Sci. **105**: 17442–17446.

Arrieta, J. M. J. M., M. G. Weinbauer, J. Gerhard, and S. Mari. 2000. Interspecific variability in sensitivity to UV radiation and subsequent recovery in selected isolates of marine bacteria. Appl. Environ. Microbiol. **66**: 1468–1473.

B

Baker, A. C., C. J. Starger, T. R. McClanahan, and P. W. Glynn. 2004. Coral reefs: Corals' adaptive response to climate change. Nature **430**: 741–741.

Banaszak, A. T., and M. P. Lesser. 2009. Effects of solar ultraviolet radiation on coral reef organisms.

Photochem. Photobiol. Sci. **8**: 1276.

Barbieri, E. S., V. E. Villafañ, and E. W. Helbling. 2002. Experimental assessment of UV effects on temperate marine phytoplankton when exposed to variable radiation regimes. Limnol. Oceanogr. **47**: 1648–1655.

Barnes, R. D. 1987. Invertebrate zoology., WB Saunders company.

Barnes, R. S. K., and R. N. Hughes. 1999. An introduction to marine ecology, John Wiley & Sons.

Barott, K. L., B. Rodriguez-Mueller, M. Youle, K. L. Marhaver, M. J. A. Vermeij, J. E. Smith, and F. L. Rohwer. 2012. Microbial to reef scale interactions between the reef-building coral *Montastraea annularis* and benthic algae. Proc. R. Soc. London B Biol. Sci. **279**: 1655–1664.

Barott, K. L., and F. L. Rohwer. 2012. Unseen players shape benthic competition on coral reefs. Trends Microbiol. **20**: 621–628.

Baruch, R., N. Avishai, and C. Rabinowitz. 2005. UV incites diverse levels of DNA breaks in different cellular compartments of a branching coral species. J. Exp. Biol. **208**: 843–848.

De Bary, A. 1879. Die erscheinung der symbiose, Verlag von Karl J. Trübner.

Bastidas, C., and E. M. Garcia. 2004. Sublethal effects of mercury and its distribution in the coral *Porites astreoides*. Mar. Ecol. Prog. Ser. **267**: 133–143.

Battey, J. F. 1992. Carbon metabolism in zooxanthellae-coelenterate symbiosis. Algae and symbioses. Biopress 153–187.

Bednarz, V. N., R. Grover, J.-F. Maguer, M. Fine, and C. Ferrier-Pagès. 2017. The assimilation of diazotroph-derived nitrogen by scleractinian corals depends on their metabolic status. MBio **8**.

Béraud, E., F. Gevaert, C. Rottier, and C. Ferrier-Pagès. 2013. The response of the scleractinian coral *Turbinaria reniformis* to thermal stress depends on the nitrogen status of the coral holobiont. J. Exp. Biol. **216**: 2665–2674.

Berkelmans, R., and M. J. H. Van Oppen. 2006. The role of zooxanthellae in the thermal tolerance of corals: a “nugget of hope”for coral reefs in an era of climate change. Proc. R. Soc. London B Biol. Sci. **273**: 2305–2312.

Berner, T., Y. Achituv, Z. Dubinsky, and Y. Benayahu. 1987. Pattern of distribution and adaptation to different irradiance levels of zooxanthellae in the soft coral *Litophyton arboreum* (Octocorallia, Alcyonacea). Symbiosis **3**: 23–39.

Björkman, K., and D. M. Karl. 1994. Bioavailability of inorganic and organic. Mar. Ecol. Prog. Ser. **111**: 265–273.

Blough, N. V, O. C. Zafiriou, and J. Bonilla. 1993. Optical absorption spectra of waters from the Orinoco River outflow: Terrestrial input of colored organic matter to the Caribbean. J. Geophys. Res. Ocean. **98**: 2271–2278.

Bongiorni, L., S. Shafir, D. Angel, and B. Rinkevich. 2003. Survival, growth and gonad development of two hermatypic corals subjected to in situ fish-farm nutrient enrichment. Mar. Ecol. Prog. Ser. **253**: 137–144.

Bourne, D. G., and C. B. Munn. 2005. Diversity of bacteria associated with the coral *Pocillopora damicornis* from the Great Barrier Reef. Environ. Microbiol. **7**: 1162–1174.

Bridge, D., A. G. Theofiles, R. L. Holler, E. Marcinkevicius, R. E. Steele, and D. E. Martínez. 2010. FoxO and stress responses in the cnidarian *Hydra vulgaris*. PLoS One **5**.

Brown, B. E., and J. C. Bythell. 2005. Perspectives on mucus secretion in reef corals. Mar. Ecol. Prog. Ser. **296**: 291–309.

Brown, B. E., C. A. Downs, R. P. Dunne, and S. W. Gibb. 2002. Exploring the basis of thermotolerance in the reef coral *Goniastrea aspera*. Mar. Ecol. Prog. Ser. **242**: 119–129.

Brown, B. E., R. P. Dunne, I. Ambarsari, M. D. A. Le Tissier, and U. Satapoomin. 1999. Seasonal fluctuations in environmental factors and variations in symbiotic algae and chlorophyll pigments in four Indo-Pacific coral species. Mar. Ecol. Prog. Ser. **191**: 53–69.

Bruno, J. F., and M. D. Bertness. 2001. Habitat modification and facilitation in benthic marine communities. Mar. community Ecol. **413**: 201–218.

Burkepile, D. E., J. E. Allgeier, A. A. Shantz, C. E. Pritchard, N. P. Lemoine, L. H. Bhatti, and C. A. Layman. 2013. Nutrient supply from fishes facilitates macroalgae and suppresses corals in a Caribbean coral reef ecosystem. Sci. Rep. **3**: 1493.

Butchart, N., I. Cionni, V. Eyring, and others. 2010. Chemistry-climate model simulations of twenty-first century stratospheric climate and circulation changes. J. Clim. **23**: 5349–5374.

Butler, E. I., S. Knox, and M. I. Liddicoat. 1979. The relationship between inorganic and organic nutrients in sea water. J. Mar. Biol. Assoc. United Kingdom **59**: 239.

Bythell, J. C., and C. Wild. 2011. Biology and ecology of coral mucus release. J. Exp. Mar. Bio. Ecol. **408**: 88–93.

C

Capone, D. G., D. A. Bronk, M. R. Mulholland, and E. J. Carpenter. 2008. Nitrogen in the marine environment, Academic Press.

Cardini, U., V. N. Bednarz, M. S. Naumann, N. van Hoytema, L. Rix, R. A. Foster, M. M. D. Al-Rshaidat, and C. Wild. 2015. Functional significance of dinitrogen fixation in sustaining coral productivity under oligotrophic conditions. *Proc. R. Soc. B*. The Royal Society. 20152257.

Carpenter, S. R., N. F. Caraco, D. L. Correll, R. W. Howarth, A. N. Sharpley, and V. H. Smith. 1998. Nonpoint pollution of surface waters with phosphorus and nitrogen. Ecol. Appl. **8**: 559–568.

Castillo, K. D., and B. S. T. Helmuth. 2005. Influence of thermal history on the response of *Montastraea annularis* to short-term temperature exposure. Mar. Biol. **148**: 261–270.

Cesar, H. S. J. 2002. Coral reefs: their functions, threats and economic value.

Cheal, A. J., M. A. MacNeil, E. Cripps, M. J. Emslie, M. Jonker, B. Schaffelke, and H. Sweatman. 2010. Coral–macroalgal phase shifts or reef resilience: links with diversity and functional roles of herbivorous fishes on the Great Barrier Reef. Coral reefs **29**: 1005–1015.

Coffroth, M. A. 1990. Mucous sheet formation on poritid corals: an evaluation of coral mucus as a nutrient source on reefs. Mar. Biol. **105**: 39–49.

Coffroth, M. A., and S. R. Santos. 2005. Genetic diversity of symbiotic dinoflagellates in the genus *Symbiodinium*. Protist **156**: 19–34.

Cohen, I., G. Dishon, D. Iluz, and Z. Dubinsky. 2013. UV-B as a photoacclimatory enhancer of the hermatypic coral *Stylophora pistillata*. Open J. Mar. Sci. **3**: 15–27.

Coles, S. L., and P. L. Jokiel. 1977. Effects of temperature on photosynthesis and respiration in hermatypic corals. Mar. Biol. **43**: 209–216.

Collins, M., R. Knutti, J. Arblaster, and others. 2013. Long-term Climate Change: Projections, Commitments and Irreversibility Pages 1029 to 1076, p. 1029–1136. *In* Intergovernmental Panel on Climate Change [ed.], Climate Change 2013 - The Physical Science Basis. Cambridge University Press.

Collos, Y., C. Descolas-Gros, and F. Mornet. 1996. Diel variations in dissolved organic nitrogen in a coastal pond: Relationships with carbon and nitrogen metabolism. J. Exp. Mar. Bio. Ecol. **202**: 177–189.

Conley, D. J., H. W. Paerl, R. W. Howarth, D. F. Boesch, S. P. Seitzinger, K. E. Havens, C. Lancelot, and G. E. Likens. 2009. Controlling eutrophication: nitrogen and phosphorus. Science. **323**: 1014–1015.

Crossland, C., D. Barnes, and M. Borowitzka. 1980. Diurnal lipid and mucus production in the staghorn coral *Acropora acuminata*. Mar. Biol. **60**: 81–90.

Crossland, C. J. 1984. Seasonal variations in the rates of calcification and productivity in the coral *Acropora formosa* on a high-latitude reef. Mar. Ecol. Prog. Ser. Oldend. **15**: 135–140.

Crossland, C. J. 1987. In situ release of mucus and DOC-lipid from the corals *Acropora variabilis* and *Stylophora pistillata* in different light regimes. Coral reefs **6**: 35–42.

Crossland, C., D. Barnes, and M. Borowitzka. 1980. Diurnal lipid and mucus production in the staghorn coral Acropora acuminata. Mar. Biol. **60**: 81–90.

Cunha, A., A. Almeida, F. J. R. C. Coelho, N. C. M. Gomes, V. Oliveira, and A. L. Santos. 2010. Bacterial extracellular enzymatic activity in globally changing aquatic ecosystems. Curr. Reseach, Technol. Educ. Top. Appl. Microbiol. Microb. Biotechnol. 124–135.

Cunning, R., and A. C. Baker. 2013. Excess algal symbionts increase the susceptibility of reef corals to bleaching. Nat. Clim. Chang. **3**: 259–262.

D

D'Angelo, C., and J. Wiedenmann. 2014. Impacts of nutrient enrichment on coral reefs: New perspectives and implications for coastal management and reef survival. Curr. Opin. Environ. Sustain. **7**: 82–93.

D'Croz, J. L., and J. L. Mate. 2000. The role of water temperature and UV radiation in the recovery of the experimentally bleached coral *Pocillopora damicornis* from the eastern Pacific Ocean (Panama). Proc. 9th Int. Coral Reef Symp. Bali, Indones. **2**: 1111–1116.

D'Croz, L., J. L. Maté, and J. E. Oke. 2001. Responses to elevated sea water temperature and UV radiation in the coral *Porites lobata* from upwelling and non-upwelling environments on the Pacific coast of Panama. Bull. Mar. Sci. **69**: 203–214.

D'Elia, C. F. 1977. The uptake and release of dissolved phosphorus by reef corals1, 2. Limnol. Ocean. **22**: 301–315.

Dagenais-Bellefeuille, S., and D. Morse. 2013. Putting the N in dinoflagellates. Front. Microbiol. **4**: 369.

Daumas, R., and B. A. Thomassin. 1977. Protein fractions in coral and zoantharian mucus: possible evolution in coral reef environments. *Proc 3rd Int Coral Reef Symp*. 517–523.

Davies, P. S. 1984. The role of zooxanthellae in the nutritional energy requirements of *Pocillopora eydouxi*. Coral Reefs **2**: 181–186.

De'ath, G., K. E. Fabricius, H. Sweatman, and M. Puotinen. 2012. The 27-year decline of coral cover on the Great Barrier Reef and its causes. Proc. Natl. Acad. Sci. **109**: 17995–17999.

Demmig-Adams, B., and W. W. Adams Iii. 1992. Photoprotection and other responses of plants to high light stress. Annu. Rev. Plant Biol. **43**: 599–626.

Denton, G. R. W., L. P. Concepcion, H. R. Wood, and R. J. Morrison. 2005. Trace metals in sediments of four harbours in Guam. Mar. Pollut. Bull. **50**: 1133–1141.

Dishon, G., Z. Dubinsky, M. Fine, and D. Iluz. 2013. Underwater light field patterns in subtropical coastal waters: A case study from the Gulf of Eilat (Aqaba). Isr. J. Plant Sci. **60**: 265–275.

Dobretsov, S., and P.-Y. Qian. 2004. The role of epibotic bacteria from the surface of the soft coral Dendronephthya sp. in the inhibition of larval settlement. J. Exp. Mar. Bio. Ecol. **299**: 35–50.

Downs, C. A., and S. A. Heckathorn. 1998. The mitochondrial small heat-shock protein protects NADH: ubiquinone oxidoreductase of the electron transport chain during heat stress in plants. FEBS Lett. **430**: 246–250.

Downs, C. A., S. L. Ryan, and S. A. Heckathorn. 1999. The chloroplast small heat-shock protein: evidence for a general role in protecting photosystem II against oxidative stress and photoinhibition. J. Plant Physiol. **155**: 488–496.

Downs, C., J. Fauth, J. Halas, P. Dustan, J. Bemiss, and C. Woodley. 2002. Oxidative stress and seasonl coral bleaching. Free Radic. Biol. Med. **33**: 533–543.

Drollet, J. H., P. Glaziou, and P. M. V Martin. 1993. A study of mucus from the solitary coral *Fungia fungites* (Scleractinia: Fungiidae) in relation to photobiological UV adaptation. Mar. Biol. **115**: 263–266.

Drollet, J. H., T. Teai, M. Faucon, and P. M. V Martin. 1997. Field study of compensatory changes in UV-absorbing compounds in the mucus of the solitary coral *Fungia repanda* (Scleractinia: Fungiidae) in relation to solar UV radiation, sea-water temperature, and other coincident physico-chemical parameters. Mar. Freshw. Res. **48**: 329–333.

Dubinsky, Z., P. G. Falkowski, J. W. Porter, and L. Muscatine. 1984. Absorption and utilization of radiant energy by light-and shade-adapted colonies of the hermatypic coral *Stylophora pistillata*. Proc. R. Soc. London B Biol. Sci. **222**: 203–214.

Dykens, J. A., J. M. Shick, C. Benoit, G. R. Buettner, and G. W. Winston. 1992. Oxygen radical production in the sea anemone A*nthopleura elegantissima* and its endosymbiotic algae. J. Exp. Biol. **168**: 219–241.

E

Elasri, M. O., and R. V. Miller. 1999. Study of the response of a biofilm bacterial community to UV

radiation. Appl. Environ. Microbiol. **65**: 2025–2031. doi:0099-2240

Elser, J. J. 2012. Phosphorus: a limiting nutrient for humanity? Curr. Opin. Biotechnol. **23**: 833–838.

Essers, M. A. G., S. Weijzen, A. M. M. de Vries-Smits, I. Saarloos, N. D. de Ruiter, J. L. Bos, and B. M. T. Burgering. 2004. FOXO transcription factor activation by oxidative stress mediated by the small GTPase Ral and JNK. EMBO J. **23**: 4802–12. doi:10.1038/sj.emboj.7600476

Eyring, V., I. Cionni, J. F. Lamarque, and others. 2010. Sensitivity of 21st century stratospheric ozone to greenhouse gas scenarios. Geophys. Res. Lett. **37**: 807–813.

Ezzat, L., J.-F. Maguer, R. Grover, and C. Ferrier-Pagès. 2015. New insights into carbon acquisition and exchanges within the coral–dinoflagellate symbiosis under NH^{4+} and NO^{3-} supply. Proc. R. Soc. B Biol. Sci. **282**: 20150610.

Ezzat, L. 2016. Effets de la disponibilité en sels nutritifs sur la réponse physiologique des coraux tropicaux dans le contexte du changement climatique. Université Pierre et Marie Curie.

Ezzat, L., J.-F. Maguer, R. Grover, and C. Ferrier-Pagès. 2016a. Limited phosphorus availability is the Achilles heel of tropical reef corals in a warming ocean. Sci. Rep. **6**: 31768.

Ezzat, L., E. Towle, J. Irisson, C. Langdon, and C. Ferrier-Pagès. 2016b. The relationship between heterotrophic feeding and inorganic nutrient availability in the scleractinian coral *T . reniformis* under a short-term temperature increase. Limnol. Oceanogr. **61**: 89–102.

F

Fabricius, K., G. De'ath, L. McCook, E. Turak, and D. M. Williams. 2005. Changes in algal, coral and fish assemblages along water quality gradients on the inshore Great Barrier Reef. Mar. Pollut. Bull. **51**: 384–398.

Fabricius, K. E. 2011. Factors determining the resilience of coral reefs to eutrophication: a review and conceptual model, p. 493–505. *In* Coral Reefs: An ecosystem in transition. Springer.

Fabricius, K. E., G. De'ath, C. Humphrey, I. Zagorskis, and B. Schaffelke. 2013. Intra-annual variation in turbidity in response to terrestrial runoff on near-shore coral reefs of the Great Barrier Reef. Estuar. Coast. Shelf Sci. **116**: 57–65.

Fagoonee, I., H. B. Wilson, M. P. Hassell, and J. R. Turner. 1999. The dynamics of zooxanthellae populations: a long-term study in the field. Science. **283**: 843–845.

Falkowski, P. G., and Z. Dubinsky. 1981. Light-shade adaptation of *Stylophora pistillata*, a hermatypic coral from the Gulf of Eilat. Nature **289**: 172–174.

Falkowski, P. G., Z. Dubinsky, L. Muscatine, and J. W. Porter. 1984. Light and the bioenergetics of a symbiotic coral. Bioscience **34**: 705–709.

Falkowski, P. G., P. L. Jokiel, and R. A. Kinzie. 1990. Irradiance and corals. Ecosyst. world **25**: 89–107.

Feierabend, J., C. Schaan, and B. Hertwig. 1992. Photoinactivation of catalase occurs under both high- and low-temperature stress conditions and accompanies photoinhibition of photosystem II. Plant Physiol. **100**: 1554–1561.

Fenner, D., and P. Muir. 2008. Reef corals of the northwestern lagoon of grande-terre, New Caledonia, p. 18–32. *In* A rapid marine biodiversity assessment of the coral reefs of the

northwest lagoon, between Koumac and Yandé, province Nord, New Caledonia. Conservation International.

Ferrier-Pagès, C., C. Godinot, C. D'Angelo, J. Wiedenmann, and R. Grover. 2016. Phosphorus metabolism of reef organisms with algal symbionts. Ecol. Monogr. **86**: 262–277.

Ferrier-Pages, C., N. Leclercq, J. Jaubert, and S. P. Pelegri. 2000. Enhancement of pico- and nanoplankton growth by coral exudates. Aquat. Microb. Ecol. **21**: 203–209.

Ferrier-Pagès, C., V. Schoelzke, J. Jaubert, L. Muscatine, and O. Hoegh-Guldberg. 2001. Response of a scleractinian coral, *Stylophora pistillata*, to iron and nitrate enrichment. J. Exp. Mar. Bio. Ecol. **259**: 249–261.

Ferrier-Pagès, C., C. Richard, D. Forcioli, D. Allemand, M. Pichon, and J. M. Shick. 2007. Effects of temperature and UV radiation increases on the photosynthetic efficiency in four scleractinian coral species. Biol. Bull. **213**: 76–87.

Fine, M., and Y. Loya. 2002. Endolithic algae: an alternative source of photoassimilates during coral bleaching. Proc. R. Soc. London B Biol. Sci. **269**: 1205–1210.

Fine, M., U. Oren, and Y. Loya. 2002. Bleaching effect on regeneration and resource translocation in the coral *Oculina patagonica*. Mar. Ecol. Prog. Ser. **234**: 119–125.

Fisk, D. D. A., and T. T. J. Done. 1985. Taxonomic and bathymetric patterns of bleaching in corals, Myrmidon Reef. *Proceedings of the 5th International Coral Reef Congress, Tahiti, 27 May-1 June 1985-pages: 6: 149-154*.

Fitt, K. W., and M. E. Warner. 1995. Bleaching patterns of four species of Caribbean reef corals. Biol Bull **189**: 298–307.

Fitt, W. K., F. K. McFarland, M. E. Warner, and G. C. Chilcoat. 2000. Seasonal patterns of tissue biomass and densities of symbiotic dinoflagellates in reef corals and relation to coral bleaching. Limnol. Oceanogr. **45**: 677–685.

Floros, C. D., M. J. Samways, and B. Armstrong. 2004. Taxonomic patterns of bleaching within a South African coral assemblage. Biodivers. {&} Conserv. **13**: 1175–1194.

Fonvielle, J. A., S. Reynaud, S. Jacquet, B. LeBerre, and C. Ferrier-Pagès. 2015. First evidence of an important organic matter trophic pathway between temperate corals and pelagic microbial communities. PLoS One **10**: 1–22.

Freudenthal, U. D. 1962. *Symbiodinium gen. nov.* and *Symbiodinium microadriaticum sp. nov.*, a Zooxanthella: Taxonomy, Life Cycle, and Morphology. J. Protozool. **9**: 45–52.

Frias-Lopez, J., A. L. Zerkle, G. T. Bonheyo, and B. W. Fouke. 2002. Partitioning of bacterial communities between seawater and healthy, black band diseased, and dead coral surfaces. Appl. Environ. Microbiol. **68**: 2214–2228.

Frieler, K., M. Meinshausen, A. Golly, M. Mengel, K. Lebek, S. D. Donner, and O. Hoegh-Guldberg. 2013. Limiting global warming to 2°C is unlikely to save most coral reefs. Nat. Clim. Chang. **3**: 165–170.

Furla, P., S. Richier, and D. Allemand. 2011. Physiological adaptation to symbiosis in cnidarians, p. 187–195. *In* Coral Reefs: an Ecosystem in Transition. Springer.

G

Ganea, E., and J. J. Harding. 2000. α-Crystallin protects glucose 6-phosphate dehydrogenase against inactivation by malondialdehyde. Biochim. Biophys. Acta (BBA)-Molecular Basis Dis. **1500**: 49–58.

Garcia-Gomez, C., M. L. Parages, C. Jimenez, A. Palma, M. T. Mata, and M. Segovia. 2012. Cell survival after UV radiation stress in the unicellular chlorophyte *Dunaliella tertiolecta* is mediated by DNA repair and MAPK phosphorylation. J. Exp. Bot. **63**: 5259–5274. doi:10.1093/jxb/ers185

Garren, M., K. Son, J.-B. Raina, and others. 2014. A bacterial pathogen uses dimethylsulfoniopropionate as a cue to target heat-stressed corals. ISME J. **8**: 999–1007.

Garren, M. 2016. Microbial ecology: Algae feed a shift on coral reefs. Nat. Microbiol. **1**: 16061.

Gattuso, J.-P., D. Allemand, and M. Frankignoulle. 1999. Photosynthesis and calcification at cellular, organismal and community levels in coral reefs: a review on interactions and control by carbonate chemistry. Am. Zool. **39**: 160–183.

Gilbert, A., T. Heintz, B. W. Hoeksema, F. Benzoni, J. M. Fernandez, C. Fauvelot, and S. Andréfouët. 2015. Endangered New Caledonian endemic mushroom coral *Cantharellus noumeae* in turbid, metal-rich, natural and artificial environments. Mar. Pollut. Bull. **100**: 359–369.

Glasl, B., G. J. Herndl, and P. R. Frade. 2016. The microbiome of coral surface mucus has a key role in mediating holobiont health and survival upon disturbance. Isme J 1–13.

Gleason, D. F. 1993. Differential effects of ultraviolet radiation on green and brown morphs of the Caribbean coral Porites astreoides. Limnol. Oceanogr. **38**: 1452–1463.

Gleason, D. F., and G. M. Wellington. 1993. Ultraviolet radiation and coral bleaching. Nature **365**: 836–838.

Gleason, D. F. 2001. Ultraviolet radiation and coral communities, p. 118–149. *In* Ecosystems, Evolution, and Ultraviolet Radiation. Springer New York.

Glynn, PW, Imai R, Sakai K, Nakano Y, Y. K. 1993. Experimental responses of Okinawan (Ryukyu Island, Japan) reef corals to high seawater temperature and UV radiation. *Proceedings of Seventh International Coral Reef Symposium, Guam*. University of Guam Press. Vol. 1 27-37.

Glynn, P. W., J. L. Maté, A. C. Baker, and M. O. Calderón. 2001. Coral bleaching and mortality in Panama and Ecuador during the 1997–1998 El Niño–Southern Oscillation event: spatial/temporal patterns and comparisons with the 1982–1983 event. Bull. Mar. Sci. **69**: 79–109.

Godinot, C., R. Grover, D. Allemand, and C. Ferrier-Pagès. 2011a. High phosphate uptake requirements of the scleractinian coral *Stylophora pistillata*. J. Exp. Biol. **214**: 2749–54.

Godinot, C., F. Houlbrèque, R. Grover, and C. Ferrier-Pagès. 2011b. Coral uptake of inorganic phosphorus and nitrogen negatively affected by simultaneous changes in temperature and pH. PLoS One **6**: 1–10.

Goenaga, C., V. P. Vicente, and R. A. Armstrong. 1989. Bleaching induced mortalities in reef corals from La Parguera, Puerto Rico: A precursor of change in the community structure of coral reefs. Caribb. J. Sci. **25**: 59–65.

Gratwicke, B., and M. R. Speight. 2005a. Effects of habitat complexity on Caribbean marine fish assemblages. Mar. Ecol. Prog. Ser. **292**: 301–310.

Gratwicke, B., and M. R. Speight. 2005b. The relationship between fish species richness, abundance and habitat complexity in a range of shallow tropical marine habitats. J. Fish Biol. **66**: 650–667.

Grimmelikhuijzen, C. J. P., and J. A. Westfall. 1995. The nervous systems of cnidarians, p. 7–24. *In* The nervous systems of invertebrates: an evolutionary and comparative approach. Springer.

Grottoli, A. G., L. J. Rodrigues, and J. E. Palardy. 2006. Heterotrophic plasticity and resilience in bleached corals. Nature **440**: 1186–1189.

Grover, R., J.-F. Maguer, S. Reynaud-Vaganay, and C. Ferrier-Pagès. 2002. Uptake of ammonium by the scleractinian coral *Stylophora pistillata*: Effect of feeding, light, and ammonium concentrations. Limnol. Oceanogr. **47**: 782–790.

Grover, R., J.-F. Maguer, D. Allemand, and C. Ferrier-Pagès. 2008. Uptake of dissolved free amino acids by the scleractinian coral *Stylophora pistillata*. J. Exp. Biol. **211**: 860–865.

H

Haas, A. F., C. Jantzen, M. S. Naumann, R. Iglesias-Prieto, and C. Wild. 2010. Organic matter release by the dominant primary producers in a Caribbean reef lagoon: Implication for in situ O_2 availability. Mar. Ecol. Prog. Ser. **409**: 27–39.

Haas, A. F., C. E. Nelson, F. Rohwer, and others. 2013. Influence of coral and algal exudates on microbially mediated reef metabolism. PeerJ **1**: e108.

Hadfield, M. G. 2011. Biofilms and marine invertebrate larvae: what bacteria produce that larvae use to choose settlement sites. Ann. Rev. Mar. Sci. **3**: 453–470.

Halldal, P. 1968. Photosynthetic capacities and photosynthetic action spectra of endozoic algae of the massive coral Favia. Biol. Bull. **134**: 411–424.

Harriott, V. J. 1985. Mortality rates of scleractinian corals before and during a mass bleaching event. Mar. Ecol. Prog. Ser. Oldend. **21**: 81–88.

Havaux, M. 1992. Stress tolerance of photosystem II in vivo antagonistic effects of water, heat, and photoinhibition stresses. Plant Physiol. **100**: 424–432.

Helbling, E. W., K. Gao, R. J. Gonçalves, H. Wu, and V. E. Villafañe. 2003. Utilization of solar UV radiation by coastal phytoplankton assemblages off SE China when exposed to fast mixing. Mar. Ecol. Prog. Ser. **259**: 59–66.

Herndl, G. J., A. Brugger, S. Hager, E. Kaiser, I. Obernosterer, B. Reitner, and D. Slezak. 1997. Role of ultraviolet-B radiation on bacterioplankton and the availability of dissolved organic matter, p. 42–51. *In* UV-B and biosphere. Springer.

Hessen, D. A. G., H. De Lange, and E. van Donk. 1997. UV-induced changes in phytoplankton cells and its effects on grazers. Freshw. Biol. **38**: 513–524.

Hoadley, K. D., D. T. Pettay, A. G. Grottoli, and others. 2016. High-temperature acclimation strategies within the thermally tolerant endosymbiont *Symbiodinium trenchii* and its coral host, *Turbinaria reniformis*, differ with changing pCO_2 and nutrients. Mar. Biol. **163**: 134.

Hoegh-Guldberg, O. 1999. Climate change, coral bleaching and the future of the world's coral reefs. Mar. Freshw. Res. **50**: 839–866.

Hoegh-Guldberg, O., and R. Jones. 1999. Photoinhibition and photoprotection in symbiotic dinoflagellates from reef-building corals. Mar. Ecol. Prog. Ser. **183**: 73–86.

Hoegh-Guldberg, O., P. J. Mumby, A. J. Hooten, and others. 2007. Coral Reefs Under Rapid Climate Change and Ocean Acidification. Science. **318**: 1737–1742.

Hoegh-Guldberg, O., and J. F. Bruno. 2010. The impact of climate change on the world's marine ecosystems. Science. **328**: 1523–1528.

Holcomb, M., D. C. McCorkle, and A. L. Cohen. 2010. Long-term effects of nutrient and CO_2 enrichment on the temperate coral *Astrangia poculata* (Ellis and Solander, 1786). J. Exp. Mar. Bio. Ecol. **386**: 27–33.

Hoogenboom, M., E. Beraud, and C. Ferrier-Pagès. 2010. Relationship between symbiont density and photosynthetic carbon acquisition in the temperate coral *Cladocora caespitosa*. Coral Reefs **29**: 21–29.

Hoppe, H.-G. 1983. Significance of exoenzymatic activities in the ecology of brackish water: measurements by means of methylumbelliferyl-substrates. Mar. Ecol. Prog. Ser. **11**: 299–308.

Houlbrèque, F., E. Tambutté, D. Allemand, and C. Ferrier-Pagès. 2004a. Interactions between zooplankton feeding, photosynthesis and skeletal growth in the scleractinian coral *Stylophora pistillata*. J. Exp. Biol. **207**: 1461–1469.

Houlbrèque, F., E. Tambutté, C. Richard, and C. Ferrier-Pagès. 2004b. Importance of a micro-diet for scleractinian corals. Mar. Ecol. Prog. Ser. **282**: 151–160.

Houlbrèque, F., and C. Ferrier-Pagès. 2009. Heterotrophy in tropical scleractinian corals. Biol. Rev. **84**: 1–17.

Howarth, R. W., G. Billen, D. Swaney, and others. 1996. Regional nitrogen budgets and riverine N & P fluxes for the drainages to the North Atlantic Ocean: Natural and human influences, p. 75–139. *In* Nitrogen cycling in the North Atlantic Ocean and its watersheds. Springer.

Hughes, A. D., and A. G. Grottoli. 2013. Heterotrophic compensation: A possible mechanism for resilience of coral reefs to global warming or a sign of prolonged stress? PLoS One **8**: 1–10.

Hughes, T. P., J. Kerry, M. Álvarez-Noriega, and others. 2017. Global warming and recurrent mass bleaching of corals. Nature.

I

Idjadi, J. A., and P. J. Edmunds. 2006. Scleractinian corals as facilitators for other invertebrates on a Caribbean reef. Mar. Ecol. Prog. Ser. **319**: 117–127.

Iglesias-Prieto, R., V. H. Beltran, T. C. LaJeunesse, H. Reyes-Bonilla, and P. E. Thome. 2004. Different algal symbionts explain the vertical distribution of dominant reef corals in the eastern Pacific. Proc. R. Soc. London, Ser. B Biol. Sci. **271**: 1757–1763.

Ip, Y. K., A. L. L. Lim, and R. W. L. Lim. 1991. Some properties of calcium-activated adenosine triphosphatase from the hermatypic coralGalaxea fascicularis. Mar. Biol. **111**: 191–197.

IPCC, 2014: Climate change 2014: Synthesis Report. contribution of working groups I, II and III to the fifth assessment report of the intergovernmental panel on climate change [Core Writing Team, R. K. P. and L. A. M. (eds.)]. IPCC, Geneva, Switzerland, 151 pp.

J

Jaap, W. C., and J. Wheaton. 1975. Observations on Florida reef corals treated with fish-collecting chemicals.

Jacobson, L. M., P. J. Edmunds, E. B. Muller, and R. M. Nisbet. 2016. The implications of reduced metabolic rate in resource-limited corals. J. Exp. Biol. **219**: 870–877.

Jacquet, S., U. Dorigo, and S. Personnic. 2013. A few tests prior to flow cytometry and epifluorescence analyses of freshwater bacterio-and virioplankton communities. FLOW Cytom. 1.

Jeffrey, S. W., and G. F. Humphrey. 1975. New spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae and natural phytoplankton. Biochemical Physiology Pflanz 167: 191–194. Biochem. Physiol. Pflanz. 191–194.

Johnston, I. S., and F. Rohwer. 2007. Microbial landscapes on the outer tissue surfaces of the reef-building coral Porites compressa. Coral Reefs **26**: 375–383.

Jokiel, P. L., J. E. Maragos, and L. Franzisket. 1978. Coral growth: buoyant weight technique, p. 379–396. *In* R. Stoddart and D. Johannes, Coral reef: Research methods. Unesco.

Jokiel, P. L., and J. R. H. York. 1982. Solar ultraviolet photobiology of the reef coral *Pocillopora damicornis* and symbiotic zooxanthellae. Bull. Mar. Sci. **32**: 301–315.

Jones, A. M., R. Berkelmans, M. J. H. van Oppen, J. C. Mieog, and W. Sinclair. 2008. A community change in the algal endosymbionts of a scleractinian coral following a natural bleaching event: field evidence of acclimatization. Proc. R. Soc. London B Biol. Sci. **275**: 1359–1365.

Jones, R. J., and D. Yellowlees. 1997. Regulation and control of intracellular algae (= zooxanthellae) in hard corals. Philos. Trans. R. Soc. London B Biol. Sci. **352**: 457–468.

Joux, F., W. H. Jeffrey, P. Lebaron, and D. L. Mitchell. 1999. Marine bacterial isolates display diverse reponses to UV-B radiation. Appl. Environ. Microbiol. **65**: 3820–3827.

K

Karako-Lampert, S., D. J. Katcoff, Y. Achituv, Z. Dubinsky, and N. Stambler. 2004. Do clades of symbiotic dinoflagellates in scleractinian corals of the Gulf of Eilat (Red Sea) differ from those of other coral reefs? J. Exp. Mar. Bio. Ecol. **311**: 301–314.

Kleypas, J. A., J. W. McManus, and L. A. B. Meñez. 1999. Environmental limits to coral reef development: where do we draw the line? Am. Zool. 146–159.

Knowlton, N., R. E. Brainard, R. Fisher, M. Moews, L. Plaisance, and M. J. Caley. 2010. Coral reef biodiversity. Life World's Ocean. Divers. Distrib. Abundance 65–74.

Kokita, T., and A. Nakazono. 2001. Rapid response of an obligately corallivorous filefish *Oxymonacanthus longirostris* (Monacanthidae) to a mass coral bleaching event. Coral Reefs **20**: 155–158.

Krupp, D. A. 1984. Mucus production by corals exposed during an extreme low tide. Pacif. Sci. **38**: 1–11.

Kuffner, I. B. 2002. Effects of ultraviolet radiation and water motion on the reef coral, *Porites compressa* Dana: a transplantation experiment. J. Exp. Mar. Bio. Ecol. **270**: 147–169.

Kvennefors, E. C. E., W. Leggat, C. C. Kerr, T. D. Ainsworth, O. Hoegh-Guldberg, and A. C. Barnes. 2010. Analysis of evolutionarily conserved innate immune components in coral links immunity and symbiosis. Dev. Comp. Immunol. **34**: 1219–1229.

Kwiatkowski, L., P. Cox, P. R. Halloran, P. J. Mumby, and A. J. Wiltshire. 2015. Coral bleaching under unconventional scenarios of climate warming and ocean acidification. Nat. Clim. Chang. **5**: 777–781.

L

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227**: 680–685.

LaJeunesse, T. C. 2001. Investigating the biodiversity, ecology, and phylogeny of endosymbiotic dinoflagellates in the genus Symbiodinium using the ITS region: in search of a “species” level marker. J. Phycol. **37**: 866–880.

LaJeunesse, T. C., R. T. Smith, J. Finney, and H. Oxenford. 2009. Outbreak and persistence of opportunistic symbiotic dinoflagellates during the 2005 Caribbean mass coral “bleaching”event. Proc. R. Soc. London B Biol. Sci. **276**: 4139–4148.

Lalli, C., and T. R. Parsons. 1997. Biological oceanography: an introduction, Butterworth-Heinemann.

Lapointe, B. E., P. J. Barile, and W. R. Matzie. 2004. Anthropogenic nutrient enrichment of seagrass and coral reef communities in the Lower Florida Keys: discrimination of local versus regional nitrogen sources. J. Exp. Mar. Bio. Ecol. **308**: 23–58.

Lasne, G. 2010. Inventaire des coraux scléractiniaires du grand lagon de Nouvelle-Calédonie, CRISP.

Lee, Z., A. Weidemann, J. Kindle, R. Arnone, K. L. Carder, and C. Davis. 2007. Euphotic zone depth: Its derivation and implication to ocean-color remote sensing. J. Geophys. Res. **112**: C03009.

Lema, K. A., B. L. Willis, and D. G. Bourne. 2012. Corals form characteristic associations with symbiotic nitrogen-fixing bacteria. Appl. Environ. Microbiol. **78**: 3136–3144.

Lesser, M. P. 1989. Photobiology of natural populations of zooxanthellae from the sea anemone *Aiptasia pallida*: assessment of the host’s role in protection against ultraviolet radiation. Cytometry **10**: 653–658.

Lesser, M. P., and J. M. Shick. 1989. Effects of irradiance and ultraviolet radiation on photoadaptation in the zooxanthellae of *Aiptasia pallida*: primary production, photoinhibition, and enzymic defenses against oxygen toxicity. Mar. Biol. **102**: 243–255.

Lesser, M. P., W. R. Stochaj, D. W. Tapley, and J. M. Shick. 1990a. Bleaching in coral reef anthozoans: effects of irradiance, ultraviolet radiation, and temperature on the activities of protective enzymes against active oxygen. Coral Reefs **8**: 225–232.

Lesser, M. P., W. R. Stochaj, D. W. Tapley, and J. M. Shick. 1990b. Coral Reefs effects of irradiance , ultraviolet radiation , and temperature. Coral Reefs **8**: 225–232.

Lesser, M. P. 1996. Elevated temperatures and ultraviolet radiation cause oxidative stress and inhibit photosynthesis in symbiotic dinoflagellates. Limnol. Oceanogr. **41**: 271–283.

Lesser, M. P., and S. Lewis. 1996. Action spectrum for the effects of UV radiation on photosynthesis in the hermatypic coral *Pocillopora damicornis*. Mar. Ecol. Prog. Ser. **134**: 171–177.

Lesser, M., and J. Farrell. 2004. Exposure to solar radiation increases damage to both host tissues and algal symbionts of corals during thermal stress. Coral Reefs **23**: 367–377.

Lesser, M. P., C. H. Mazel, M. Y. Gorbunov, and P. G. Falkowski. 2004. Discovery of symbiotic nitrogen-fixing cyanobacteria in corals. Science. **305**: 997–1000.

Lesser, M. P. 2006. Oxidative stress in marine environments: biochemistry and physiological ecology. Annu. Rev. Physiol. **68**: 253–278.

Lesser, M. P. 2012. Oxidative stress in tropical marine ecosystems, p. 7–19. *In* Oxidative Stress in Aquatic Ecosystems. John Wiley & Sons, Ltd.

Lesser, M. P. 2013. Using energetic budgets to assess the effects of environmental stress on corals: are we measuring the right things? Coral Reefs **32**: 25–33.

Levas, S., A. G. Grottoli, M. E. Warner, and others. 2015. Organic carbon fluxes mediated by corals at elevated pCO_2 and temperature. Mar. Ecol. Prog. Ser. **519**: 153–164.

Levas, S., A. G. Grottoli, V. Schoepf, M. Aschaffenburg, J. Baumann, J. E. Bauer, and M. E. Warner. 2016. Can heterotrophic uptake of dissolved organic carbon and zooplankton mitigate carbon budget deficits in annually bleached corals? Coral Reefs **35**: 495–506.

von Liebig, J. F. 1855. Principles of agricultural chemistry: With special reference to the late researches made in England, Walton & Maberly.

Littman, R. A., B. L. Willis, and D. G. Bourne. 2009. Bacterial communities of juvenile corals infected with different *Symbiodinium* (dinoflagellate) clades. Mar. Ecol. Prog. Ser. **389**: 45–59.

Littman, R. A., D. G. Bourne, and B. L. Willis. 2010. Responses of coral associated bacterial communities to heat stress differ with Symbiodinium type on the same coral host. Mol. Ecol. **19**: 1978–1990.

Loya, Y., K. Sakai, K. Yamazato, Y. Nakano, H. Sambali, and R. van Woesik. 2001. Coral bleaching: the winners and the losers. Ecol. Lett. **4**: 122–131.

M

MacArthur, R. H., and J. W. MacArthur. 1961. On bird species diversity. Ecology **42**: 594–598.

Mahmood, T., and P.-C. Yang. 2012. Western blot: technique, theory, and trouble shooting. N. Am. J. Med. Sci. **4**: 429.

Marquis, C. P., A. H. Baird, R. De Nys, C. Holmström, and N. Koziumi. 2005. An evaluation of the antimicrobial properties of the eggs of 11 species of scleractinian corals. Coral Reefs **24**: 248–253.

Marshall, A. T., and O. P. Wright. 1993. Confocal laser scanning light microscopy of the extra-thecal epithelia of undecalcified scleractinian corals. Cell Tissue Res. **272**: 533–543.

Marshall, P. A., and A. H. Baird. 2000. Bleaching of corals on the Great Barrier Reef: Differential susceptibilities among taxa. Coral Reefs **19**: 155–163.

Marubini, F., and P. S. Davies. 1996. Nitrate increases zooxanthellae population density and reduces skeletogenesis in corals. Mar. Biol. **127**: 319–328.

Masuda, K., M. Goto, T. Maruyama, and S. Miyachi. 1993. Adaptation of solitary corals and their zooxanthellae to low light and UV radiation. Mar. Biol. **117**: 685–691.

McClanahan, T. R., A. H. Baird, P. A. Marshall, and M. A. Toscano. 2004. Comparing bleaching and mortality responses of hard corals between southern Kenya and the Great Barrier Reef, Australia. Mar. Pollut. Bull. **48**: 327–335.

McIlwain, J. L., and G. P. Jones. 1997. Prey selection by an obligate coral-feeding wrasse and its response to small-scale disturbance. Mar. Ecol. Prog. Ser. **155**: 189–198.

McManus, J. W., M. C. Ablan, S. G. Vergara, L. A. Menez, K. P. Reyes, M. L. Gorospe, and L. Halmarick. 1997. Reefbase aquanaut survey manual. ICLARM Educ. Ser. **18**.

Meron, D., R. Rodolfo-Metalpa, R. Cunning, A. C. Baker, M. Fine, and E. Banin. 2012. Changes in coral microbial communities in response to a natural pH gradient. ISME J. **6**: 1775–1785.

Meyer, J. L., E. T. Schultz, and G. S. Helfman. 1983. Fish schools: an asset to corals. Science. **220**: 1047–1049.

Meyer, J. L., and E. T. Schultz. 1985. Tissue condition and growth rate of corals associated with schooling fish. Limnol. Oceanogr. **30**: 157–166.

Mitchell, R., and I. Chet. 1975. Bacterial attack of corals in polluted seawater. Microb. Ecol. **2**: 227–233.

Moberg, F., and C. Folke. 1999. Ecological goods and services of coral reef ecosystems. Ecol. Econ. **29**: 215–233.

Muller-Parker, G., K. W. Lee, and C. B. Cook. 1996. Changes in the ultrastructure of symbiotic zooxanthellae (*Symbiodinium sp.*, Diniphyceae) in fed and starved sea anemones maintained under high and low light. J. Phycol. **32**: 987–994.

Muller-Parker, G., and C. F. D'Elia. 1997. Interactions between corals and their symbiotic algae. Life Death Coral Reefs **5**: 96–113.

Mumby, P. J. 2009. Phase shifts and the stability of macroalgal communities on Caribbean coral reefs. Coral Reefs **28**: 761–773.

Muscatine, L., and E. Cernichiari. 1969. Assimilation of photosynthetic products of zooxanthellae by a reef coral. Biol. Bull. **137**: 506–523.

Muscatine, L., and J. W. Porter. 1977. Reef corals: mutualistic symbioses adapted to nutrient-poor environments. Bioscience **27**: 454–460.

Muscatine, L., and C. F. D'elia. 1978. The uptake, retention, and release of ammonium by reef corals. Limnol. Ocean. **23**: 725–734.

Muscatine, L., L. R. McCloskey, and R. E. Marian. 1981. Estimating the daily contribution of carbon from zooxanthellae to coral animal respiration. Limnol. Oceanogr. **26**: 601–611. doi:10.4319/lo.1981.26.4.0601

Muscatine, L., P. G. Falkowski, J. W. Porter, and Z. Dubinsky. 1984. Fate of photosynthetic fixed carbon in light-and shade-adapted colonies of the symbiotic coral *Stylophora pistillata*. Proc. R. Soc. London B Biol. Sci. **222**: 181–202.

Muscatine, L., P. G. Falkowski, Z. Dubinsky, P. A. Cook, and L. R. McCloskey. 1989. The effect of external nutrient resources on the population dynamics of zooxanthellae in a reef coral. Proc. R. Soc. London B Biol. Sci. **236**: 311–324.

Muscatine, L. 1990. The role of symbiotic algae in carbon and energy flux in reef corals. Ecosyst. world **25**: 75–87.

Muscatine, L., C. Ferrier-Pagès, A. Blackburn, R. D. Gates, G. Baghdasarian, and D. Allemand. 1998. Cell-specific density of symbiotic dinoflagellates in tropical anthozoans. Coral Reefs **17**: 329–337.

Muszynski, F. Z., A. Bruckner, R. A. Armstrong, J. M. Morell, and J. E. Corredor. 1998. Within-colony variations of UV absorption in a reef building coral. Bull. Mar. Sci. **63**: 589–594.

N

Naumann, M. S., A. Haas, U. Struck, C. Mayr, M. El-Zibdah, and C. Wild. 2010. Organic matter release by dominant hermatypic corals of the Northern Red Sea. Coral Reefs **29**: 649–659.

Neave, M. J., A. Apprill, C. Ferrier-Pagès, and C. R. Voolstra. 2016. Diversity and function of prevalent symbiotic marine bacteria in the genus *Endozoicomonas*. Appl. Microbiol. Biotechnol. **100**: 8315–8324.

Nelson, C. E., S. J. Goldberg, L. W. Kelly, A. F. Haas, J. E. Smith, F. Rohwer, and C. A. Carlson. 2013. Coral and macroalgal exudates vary in neutral sugar composition and differentially enrich reef bacterioplankton lineages. ISME J. **7**: 962–979.

Nicholls, R. J., P. P. Wong, V. R. Burkett, J. O. Codignotto, J. E. Hay, R. F. McLean, S. Ragoonaden, and C. D. 2007. Coastal systems and low-lying areas. Assessment 315–356.

Niggl, W., M. Glas, C. Laforsch, C. Mayr, and C. Wild. 2008. First evidence of coral bleaching stimulating organic matter release by reef corals. *Proceedings of the 11th International Coral Reef Symposium'*. 905–910.

Nilawati, J., B. M. Greenberg, and R. E. H. Smith. 1997. Influence of ultraviolet radiation on growth and photosynthesis of two cold ocean diatoms. J. Phycol. **33**: 215–224.

Nixon, S. W. 1995. Coastal marine eutrophication: A definition, social causes, and future concerns. Ophelia **41**: 199–219.

O

Olson, R. J. 1980. Nitrate and ammonium uptake in Antarctic waters. Limnol. Oceanogr. **25**: 1064–1074.

P

Pandolfi, J. M., S. R. Connolly, D. J. Marshall, and A. L. Cohen. 2011. Projecting coral reef futures under global warming and ocean acidification. Science. **333**: 418–422.

Parages, M. L., S. Heinrich, C. Wiencke, and C. Jiménez. 2013. Rapid phosphorylation of MAP kinase-like proteins in two species of Arctic kelps in response to temperature and UV radiation stress. Environ. Exp. Bot. **91**: 30–37.

Polovina, J. J., E. A. Howell, and M. Abecassis. 2008. Ocean's least productive waters are expanding. Geophys. Res. Lett. **35**: L03618.

Pomeroy, L. R. 1974. The ocean's food web, a changing paradigm. Bioscience **24**: 499–504.

R

Ralph, P. J., and R. Gademann. 2005. Rapid light curves: a powerful tool to assess photosynthetic activity. Aquat. Bot. **82**: 222–237.

Ravindran, J., E. Kannapiran, B. Manikandan, and others. 2013. UV-absorbing bacteria in coral mucus and their response to simulated temperature elevations. Coral Reefs **32**: 1043–1050.

Renegar, D., and B. Riegl. 2005. Effect of nutrient enrichment and elevated CO_2 partial pressure on growth rate of Atlantic scleractinian coral *Acropora cervicornis*. Mar. Ecol. Prog. Ser. **293**: 69–76.

Reshef, L., O. Koren, Y. Loya, I. Zilber-Rosenberg, and E. Rosenberg. 2006. The coral probiotic hypothesis. Environ. Microbiol. **8**: 2068–2073.

Reynaud, S., N. Leclercq, S. Romaine-Liuod, C. Ferrier-Pagès, J. Jaubert, and J.-P. Gattuso. 2003. Interacting effects of CO_2 partial pressure and temperature on photosynthesis and calcification in a scleractinian coral. Glob. Chang. Biol. **9**: 1660–1668.

Ribes, M., R. Coma, and J.-M. Gili. 1998. Heterotrophic feeding by gorgonian corals with symbiotic zooxanthella. Limnol. Oceanogr. **43**: 1170–1179.

Richer de Forges, B., and C. Garrigue. 1997. First observations of a major coral bleaching in New Caledonia. Proc. Mar. Benthic Habitat Their Living Resour. Monit. Manag. Appl. to Pacific Isl. Nations, Noumea, New Caledonia.

Richier, S., P. Furla, A. Plantivaux, P.-L. Merle, and D. Allemand. 2005. Symbiosis-induced adaptation to oxidative stress. J. Exp. Biol. **208**: 277–285.

Richter, C., M. Wunsch, M. Rasheed, I. Kotter, and M. I. Badran. 2001. Endoscopic exploration of Red Sea coral reefs reveals dense populations of cavity-dwelling sponges. Nature **413**: 726–730.

Roberty, S., D. Fransolet, P. Cardol, J.-C. C. Plumier, and F. Franck. 2015. Imbalance between oxygen photoreduction and antioxidant capacities in Symbiodinium cells exposed to combined heat and high light stress. Coral Reefs **34**: 1063–1073.

Rodrigues, L. J., and A. G. Grottoli. 2007. Energy reserves and metabolism as indicators of coral recovery from bleaching. Limnol. Oceanogr. **52**: 1874–1882.

Rohwer, F., M. Breitbart, J. Jara, F. Azam, and N. Knowlton. 2001. Diversity of bacteria associated with the Caribbean coral *Montastraea franksi*. Coral reefs **20**: 85–91.

Rohwer, F., V. Seguritan, F. Azam, and N. Knowlton. 2002. Diversity and distribution of coral-associated bacteria. Mar. Ecol. Prog. Ser. **243**: 1–10.

Rosset, S., C. D'Angelo, and J. Wiedenmann. 2015. Ultrastructural biomarkers in symbiotic algae reflect the availability of dissolved inorganic nutrients and particulate food to the reef coral

holobiont. Front. Mar. Sci. **2**: 103.

Roth, A. A., C. D. Clausen, P. Y. Yahiku, V. E. Clausen, and W. W. Cox. 1982. Some effects of light on coral growth.

Rouzé, H., G. Lecellier, M.-J. Langlade, S. Planes, and V. Berteaux-Lecellier. 2015. Fringing reefs exposed to different levels of eutrophication and sedimentation can support similar benthic communities. Mar. Pollut. Bull. **92**: 212–221.

Rowan, R. O. B., and D. A. Powers. 1991. A molecular genetic classification of zooxanthellae and the evolution of animal-algal symbioses. Science. **251**: 1348–1351.

Rowan, R. 1998. Review—diversity and ecology of zooxanthellae on coral reefs. J. Phycol. **34**: 407–417.

Rozema, J., L. O. Björn, J. F. Bornman, and others. 2002. The role of UV-B radiation in aquatic and terrestrial ecosystems—an experimental and functional analysis of the evolution of UV-absorbing compounds. J. Photochem. Photobiol. B Biol. **66**: 2–12.

Ruiz-Morenol, D., B. L. Willis, A. C. Page, and others. 2012. Global coral disease prevalence associated with sea temperature anomalies and local factors. Dis. Aquat. Organ. **100**: 249–261.

Runchel, C., A. Matsuzawa, and H. Ichijo. 2011. Mitogen-activated protein kinases in mammalian oxidative stress responses. Antioxid. Redox Signal. **15**: 205–218.

S

Salih, A., O. Hoegh-Guldberg, and G. Cox. 1998. Photoprotection of symbiotic dinoflagellates by fluorescent pigments in reef corals. *Proceedings of the Australian Coral Reef Society 75th Anniversary Conference*. School of Marine Science, The University of Queensland Brisbane, Australia. 217–230.

Salih, A., G. Cox, R. Szymczak, and others. 2006. The role of host-based color and fluorescent pigments in photoprotection and in reducing bleaching stress in corals. *Proc 10th Int Coral Reef Symp*. 746–756.

Salih, A., A. Larkum, G. Cox, M. Kühl, and O. Hoegh-Guldberg. 2000. Fluorescent pigments in corals are photoprotective. Nature **408**: 850–853.

Sandroni, V., P. Raimbault, C. Migon, N. Garcia, and E. Gouze. 2007. Dry atmospheric deposition and diazotrophy as sources of new nitrogen to northwestern Mediterranean oligotrophic surface waters. Deep Sea Res. Part I Oceanogr. Res. Pap. **54**: 1859–1870.

Santer, B. D., K. E. Taylor, T. M. L. Wigley, and T. C. Johns. 1996. A search for human influences on the thermal structure of the atmosphere. Nature **382**: 39.

Schlöder, C., and L. D'Croz. 2004. Responses of massive and branching coral species to the combined effects of water temperature and nitrate enrichment. J. Exp. Mar. Bio. Ecol. **313**: 255–268.

Schoepf, V., A. G. Grottoli, M. E. Warner, and others. 2013a. Coral energy reserves and calcification in a high-CO2 world at two temperatures. PLoS One **8**: e75049. doi:10.1371/journal.pone.0075049

Schoepf, V., A. G. Grottoli, M. E. Warner, and others. 2013b. Coral Energy Reserves and Calcification in a High-CO_2 World at Two Temperatures. PLoS One **8**: e75049.

Serra, J. L., M. J. Llama, and E. Cadenas. 1978. Nitrate utilization by the diatom *Skeletonema costatum II*. Regulation of nitrate uptake. Plant Physiol. **62**: 991–994. doi:10.1104/pp.62.6.991

Shantz, A. A., and D. E. Burkepile. 2014. Context-dependent effects of nutrient loading on the coral–algal mutualism. Ecology **95**: 1995–2005.

Shashar, N., Y. Cohen, Y. Loya, and N. Sar. 1994. Nitrogen fixation(acetylene reduction) in stony corals: Evidence for coral-bacteria interactions. Mar. Ecol. Prog. Ser. Oldend. **111**: 259–264.

Shick, J. M., M. P. Lesser, and W. R. Stochaj. 1991. Ultraviolet radiation and photooxidative stress in zooxanthellae Anthozoa: the sea anemone *Phyllodiscus semoni* and the octocoral *Clavularia sp.* Symbiosis **10**: 145–173.

Shick, J. M., M. P. Lesser, W. C. Dunlap, W. R. Stochaj, B. E. Chalker, and J. W. Won. 1995. Depth-dependent responses to solar ultraviolet radiation and oxidative stress in the zooxanthellate coral *Acropora microphthalma*. Mar. Biol. **122**: 41–51.

Shick, J. M., S. R.- Lioud, C. F.-P. S, and J.-P. Gattuso. 1999. Ultraviolet-B radiation stimulates shikimate pathway-dependent accumulation of mycosporine-like amino acids in the coral *Stylophora pistillata* despite decreases in its population of symbiotic dinoflagellates. Limnol. Oceanogr. **44**: 1667–1682. Shick, J., M. P. Lesser, and P. L. Jokiel. 1996. Effects of ultraviolet radiation on corals and other coral reef organisms. Glob. Chang. Biol. **2**: 527–545.

Shick, J. M., and W. C. Dunlap. 2002. Mycosporine-like amino acids and related gadusols: Biosynthesis, accumulation, and UV-protective functions in aquatic organisms. Annu. Rev.

Physiol. **64**: 223–262.

Shick, J. M. 2004. The continuity and intensity of ultraviolet irradiation affect the kinetics of biosynthesis, accumulation, and conversion of mycosporine-like amino acids (MAAs) in the coral *Stylophora pistillata*. Limnol. Oceanogr. **49**: 442–458.

Siebeck, O. 1981. Photoreactivation and depth-dependent UV tolerance in reef coral in the Great Barrier Reef/Australia. Naturwissenschaften **68**: 426–428.

Siebeck, O. 1988. Experimental investigation of UV tolerance in hermatypic corals(Scleractinia). Mar. Ecol. Prog. Ser. Oldend. **43**: 95–103.

Silverstein, R. N., A. M. S. Correa, and A. C. Baker. 2012. Specificity is rarely absolute in coral–algal symbiosis: implications for coral response to climate change. Proc. R. Soc. London B Biol. Sci. **279**: 2609–2618.

Sinha, R. P., and D.-P. Häder. 2002. UV-induced DNA damage and repair: a review. Photochem. Photobiol. Sci. **1**: 225–236.

Smith, S. V., and G. S. Key. 1975. Carbon dioxide and metabolism in marine environments. Limnol. Oceanogr. **20**: 493–495.

Smith, P. K., R. I. Krohn, G. T. Hermanson, and others. 1985. Measurement of protein using bicinchoninic acid. Anal. Biochem. **150**: 76–85.

Smith, D. C., and A. E. Douglas. 1987. The biology of symbiosis., Edward Arnold (Publishers) Ltd.

Smith, V. H. 2003. Eutrophication of freshwater and coastal marine ecosystems a global problem. Environ. Sci. Pollut. Res. **10**: 126–139.

Smith, V. H., and D. W. Schindler. 2009. Eutrophication science: where do we go from here? Trends Ecol. Evol. **24**: 201–207.

Soloviev, A., and R. Lukas. 2006. The near-surface layer of the ocean structure, dynamics and applications Vol. 31, Atmospheric and Oceanographic Science Library.

Sorokin, Y. I. 2013. Coral reef ecology, Springer Science & Business Media.

Spalding, M. D., and A. M. Grenfell. 1997. New estimates of global and regional coral reef areas. Coral Reefs **16**: 225–230.

Spalding, M., C. Ravilious, and E. P. Green. 2001. World atlas of coral reefs, Univ of California Press.

Stanley Jr, G. D. 2013. The history and sedimentology of ancient reef systems, Springer Science & Business Media.

Stimson, J., and R. A. Kinzie. 1991. The temporal pattern and rate of release of zooxanthellae from the reef coral *Pocillopora damicornis* (Linnaeus) under nitrogen-enrichment and control conditions. J. Exp. Mar. Bio. Ecol. **153**: 63–74.

Stimson, J. 1997. The annual cycle of density of zooxanthellae in the tissues of field and laboratory-held *Pocillopora damicornis* (Linnaeus). J. Exp. Mar. Bio. Ecol. **214**: 35–48.

Su, W., T. P. Charlock, F. G. Rose, and D. Rutan. 2007. Photosynthetically active radiation from Clouds and the Earth's Radiant Energy System (CERES) products. J. Geophys. Res. **112**: G02022.

Sweet, M. J., A. Croquer, and J. C. Bythell. 2011. Bacterial assemblages differ between compartments within the coral holobiont. Coral Reefs **30**: 39–52.

T

Tambutté, E., D. Allemand, D. Zoccola, A. Meibom, S. Lotto, N. Caminiti, and S. Tambutté. 2007. Observations of the tissue-skeleton interface in the scleractinian coral *Stylophora pistillata*. Coral Reefs **26**: 517–529.

Tanaka, Y., T. Miyajima, I. Koike, T. Hayashibara, and H. Ogawa. 2007. Imbalanced coral growth between organic tissue and carbonate skeleton caused by nutrient enrichment. Limnol. Oceanogr. **52**: 1139–1146.

Tanaka, Y., T. Miyajima, I. Koike, T. Hayashibara, and H. Ogawa. 2008. Production of dissolved and particulate organic matter by the reef-building corals *Porites cylindrica* and *Acropora pulchra*. Bull. Mar. Sci. **82**: 237–245.

Tanaka, Y., T. Miyajima, Y. Umezawa, T. Hayashibara, H. Ogawa, and I. Koike. 2009. Net release of dissolved organic matter by the scleractinian coral *Acropora pulchra*. J. Exp. Mar. Bio. Ecol. **377**: 101–106.

Tanaka, Y., A. Iguchi, K. Nishida, M. Inoue, T. Nakamura, A. Suzuki, and K. Sakai. 2014a. Nutrient availability affects the response of juvenile corals and the endosymbionts to ocean acidification. Limnol. Oceanogr. **59**: 1468–1476.

Tanaka, Y., M. Inoue, T. Nakamura, A. Suzuki, and K. Sakai. 2014b. Loss of zooxanthellae in a coral under high seawater temperature and nutrient enrichment. J. Exp. Mar. Bio. Ecol. **457**: 220–

Tchernov, D., M. Y. Gorbunov, C. de Vargas, S. Narayan Yadav, A. J. Milligan, M. Haggblom, and P. G.

Falkowski. 2004. Membrane lipids of symbiotic algae are diagnostic of sensitivity to thermal bleaching in corals. Proc. Natl. Acad. Sci. **101**: 13531–13535.

Tedetti, M., and R. Sempéré. 2006. Penetration of ultraviolet radiation in the marine environment. A review. Photochem. Photobiol. **82**: 389–397.

Thompson, J. R., H. E. Rivera, C. J. Closek, and M. Medina. 2014. Microbes in the coral holobiont: partners through evolution, development, and ecological interactions. Front. Cell. Infect. Microbiol. **4**: 176.

Thorington, G. U., and D. A. Hessinger. 1996. Efferent mechanisms of discharging cnidae: I. Measurements of intrinsic adherence of cnidae discharged from tentacles of the sea anemone, Aiptasia pallida. Biol. Bull. **190**: 125–138.

Thurber, R. V., D. Willner-Hall, B. Rodriguez-Mueller, and others. 2009. Metagenomic analysis of stressed coral holobionts. Environ. Microbiol. **11**: 2148–2163.

Titlyanov, E. A., T. V. Titlyanova, K. Yamazato, and R. van Woesik. 2001. Photo-acclimation of the hermatypic coral *Stylophora pistillata* while subjected to either starvation or food provisioning. J. Exp. Mar. Bio. Ecol. **257**: 163–181.

Titlyanov, E. a, and T. V Titlyanova. 2002. Reef-building corals: symbiotic autotrophs. 2. Ways and mechanisms of adaptation to light. Russ. J. Mar. Biol. **28**: 16–31.

Torregiani, J. H., and M. P. Lesser. 2007. The effects of short-term exposures to ultraviolet radiation in the Hawaiian Coral *Montipora verrucosa*. J. Exp. Mar. Bio. Ecol. **340**: 194–203.

Torres-Pérez, J. L., and R. A. Armstrong. 2012. Effects of UV radiation on the growth, photosynthetic and photoprotective components, and reproduction of the Caribbean shallow-water coral *Porites furcata*. Coral Reefs **31**: 1077–1091.

Tréguer, P., and P. Le Corre. 1975. Manuel d'analyse des sels nutrifs dans l'eau de mer: utilisation de l'Autoanalyzer II Technicon (R), Université de Bretagne occidentale.

Tremblay, P., M. S. Naumann, S. Sikorski, R. Grover, and C. Ferrier-Pagès. 2012. Experimental assessment of organic carbon fluxes in the scleractinian coral *Stylophora pistillata* during a thermal and photo stress event. Mar. Ecol. Prog. Ser. **453**: 63–77.

U

Ulstrup, K. E., R. Berkelmans, P. J. Ralph, and M. J. H. Van Oppen. 2006. Variation in bleaching sensitivity of two coral species across a latitudinal gradient on the Great Barrier Reef: The role of zooxanthellae. Mar. Ecol. Prog. Ser. **314**: 135–148.

V

Vega Thurber, R. L., D. E. Burkepile, C. Fuchs, A. A. Shantz, R. Mcminds, and J. R. Zaneveld. 2014. Chronic nutrient enrichment increases prevalence and severity of coral disease and bleaching. Glob. Chang. Biol. **20**: 544–554.

Veron, J. E. N., and M. Stafford-Smith. 2000. Corals of the World. Volumes 1-3. Aust. Inst. Mar. Sci. Townsville, Aust.

Vieira, C., C. Payri, and O. De Clerck. 2015. Overgrowth and killing of corals by the brown alga

Lobophora hederacea (Dictyotales, Phaeophyceae) on healthy reefs in New Caledonia: a new case of the epizoism syndrome. Phycol. Res. **63**: 152–153.

Vose, R. S., S. Applequist, M. A. Bourassa, and others. 2014. Monitoring and understanding changes in extremes: Extratropical storms, winds, and waves. Bull. Am. Meteorol. Soc. **95**: 377–386.

W

Wakefield, T. S., M. A. Farmer, and S. C. Kempf. 2000. Revised description of the fine structure of in situ "Zooxanthellae" genus *Symbiodinium*. Biol. Bull. **199**: 76–84.

Wall, C. B., T.-Y. Fan, and P. J. Edmunds. 2014. Ocean acidification has no effect on thermal bleaching in the coral *Seriatopora caliendrum*. Coral Reefs **33**: 119–130.

Wang, H., and J. A. Joseph. 1999. Quantifying cellular oxidative stress by dichlorofluorescein assay using microplate reader. Free Radic. Biol. Med. **27**: 612–616.

Watanabe, S., K. Sudo, T. Nagashima, T. Takemura, H. Kawase, and T. Nozawa. 2011. Future projections of surface UV-B in a changing climate. J. Geophys. Res. Atmos. **116**: D16118.

Wellington, G. M., and W. K. Fitt. 2003. Influence of UV radiation on the survival of larvae from broadcast-spawning reef corals. Mar. Biol. **143**: 1185–1192.

Widmann, C., S. Gibson, M. B. Jarpe, and G. L. Johnson. 1999. Mitogen-activated protein kinase: conservation of a three-kinase module from yeast to human. Physiol. Rev. **79**: 143–180.

Wiedenmann, J., C. D. Angelo, E. G. Smith, and others. 2013. Nutrient enrichment can increase the susceptibility of reef corals to bleaching. Nat. Clim. Chang. **2**: 1–5.

Wild, C; Rasheed, M; Werner, U; Franke, U; Johnsone, R; Huettel, M. 2004. Degradation and mineralization of coral mucus in reef environments. Mar. Ecol. Prog. Ser. **267**: 159–171.

Wild, C., M. Huettel, A. Klueter, S. G. Kremb, M. Y. M. Rasheed, and B. B. Jørgensen. 2004. Coral mucus functions as an energy carrier and particle trap in the reef ecosystem. Nature **428**: 66–70.

Wild, C., H. Woyt, and M. Huettel. 2005. Influence of coral mucus on nutrient fluxes in carbonate sands. Mar. Ecol. Prog. Ser. **287**: 87–98.

Wild, C., C. Mayr, L. Wehrmann, S. Schöttner, M. Naumann, F. Hoffmann, and H. Rapp. 2008. Organic matter release by cold water corals and its implication for fauna–microbe interaction. Mar. Ecol. Prog. Ser. **372**: 67–75.

Wild, C., M. Naumann, W. Niggl, and A. Haas. 2010a. Carbohydrate composition of mucus released by scleractinian warm-and cold-water reef corals. Aquat. Biol. **10**: 41–45.

Wild, C., W. Niggl, M. S. Naumann, and A. F. Haas. 2010b. Organic matter release by Red Sea coral reef organisms-Potential effects on microbial activity and in situ O_2 availability. Mar. Ecol. Prog. Ser. **411**: 61–71.

Wilkerson, F. P., and R. K. Trench. 1986. Uptake of dissolved inorganic nitrogen by the symbiotic clam *Tridacna gigas* and the coral *Acropora sp.* Mar. Biol. **93**: 237–246.

Wilkinson, C. R., and D. N. Souter. 2008. Status of Caribbean coral reefs after bleaching and hurricanes in 2005, Global Coral Reef Monitoring Network.

Willis, S. C., K. O. Winemiller, and H. Lopez-Fernandez. 2005. Habitat structural complexity and morphological diversity of fish assemblages in a Neotropical floodplain river. Oecologia **142**: 284–295.

Wilson, S. K., N. A. J. Graham, and N. V. C. Polunin. 2007. Appraisal of visual assessments of habitat complexity and benthic composition on coral reefs. Mar. Biol. **151**: 1069–1076.

Winter, C., M. M. Moeseneder, and J. Gerhard. 2001. Impact of UV Radiation on Bacterioplankton Community Composition Impact of UV Radiation on Bacterioplankton Community Composition †. **67**: 665–672.

Wooldridge, S. A. 2013. Breakdown of the coral-algae symbiosis: towards formalising a linkage between warm-water bleaching thresholds and the growth rate of the intracellular zooxanthellae. Biogeosciences **10**: 1647–1658.

Wozniak, B., and J. Dera. 2007. Light absorption in sea water, Springer.

Wyndham, T., M. McCulloch, S. Fallon, and C. Alibert. 2004. High-resolution coral records of rare earth elements in coastal seawater: biogeochemical cycling and a new environmental proxy. Geochim. Cosmochim. Acta **68**: 2067–2080.

Y

Yakovleva, I., R. Bhagooli, A. Takemura, and M. Hidaka. 2004. Differential susceptibility to oxidative stress of two scleractinian corals: Antioxidant functioning of mycosporine-glycine. Comp. Biochem. Physiol. - B Biochem. Mol. Biol. **139**: 721–730.

Yao, C.-L., and G. N. Somero. 2012. The impact of acute temperature stress on hemocytes of invasive and native mussels (*Mytilus galloprovincialis* and *Mytilus californianus*): DNA damage, membrane integrity, apoptosis and signaling pathways. J. Exp. Biol. **215**: 4267–77.

Z

Zaneveld, J. R., D. E. Burkepile, A. A. Shantz, and others. 2016. Overfishing and nutrient pollution interact with temperature to disrupt coral reefs down to microbial scales. Nat. Commun. **7**.

Appendix

Appendix 1. Translation of Introduction §III.2.

III.2. Organization and objectives of the thesis

Experiments in aquaria are meant to better understand the coral response to environmental changes and predict the future of coral reefs. Although the complexity and variability of the natural environment can hardly be reproduced in tanks, those experiments are useful because they highlight the responses and defense mechanisms of corals against a particular stressor. Taken together, they help to better understand how corals will face the future. The main problems of those studies are the difficulties in capturing 1) the complexity to assess the coral response to stress (*i.e.* many biological parameters to take into account), 2) the diversity in species-specific responses 3) the interactive effects of several stress factors that corals have to face simultaneously and 4) the complex relationship between corals and the reef ecosystem. **The following paragraphs tackle each of these difficulties and show how the studies conducted during the thesis highlight some aspects of the coral response to UV and thermal stress.**

III. 2. 1. Complexity to assess the coral response to stressors

We cannot assess coral response to stress, *i.e.* its resistance and resilience to a particular stressor, without taking into account all physiological parameters. Each stressor will have a different effect depending on the physiological parameter considered (photosynthesis, calcification, tissue parameters, organic matter excretion...). For example, thermally-induced bleaching is well characterized by measuring the concentration of symbionts and photosynthetic pigments, but to determine the coral resistance and resilience to thermal stress, we need to assess its energetic status, by measuring the rates of photosynthesis, respiration, as well as the heterotrophic supply of energy and the level of energetic reserves (lipid, proteins…) (Lesser 2013). Calcification rates are also of importance because the coral competitiveness for space and light, and the skeletal thickness will determine its resistance to erosion (Allemand et al. 2011). Other physiological changes such as variation in organic matter release or in coral-associated microbiome might affect coral health too, by impacting the coral capacity to face pathogen infection, for example (Brown and Bythell 2005; Thompson et al. 2014; Glasl et al. 2016). Each of these biological factors gives a different clue on the coral response to change in the environment and only the combination of these responses enable accurate prediction of coral faith under climate change.

Although a bunch of studies already investigated the response of different coral species, even different holobionts (coral host, symbionts and associated micro-organisms) to thermal stress, the effects of UVR on coral physiology are poorly known. Previous studies mainly focused on MAAs production and UVR-induced bleaching. For example, less than 10 studies measured changes in coral metabolism (calcification, photosynthesis and respiration) under UVR and none monitored organic matter excretion and degradation by associated-micro-organisms (see Table Table 3). **The first aim of this thesis was therefore to improve our knowledge on the effects of UVR on coral physiology by including as many biological factors as possible: symbiont density, calcification, photosynthesis and respiration, heterotrophic feeding, mucus excretion, coral associated-bacterial activity and diversity as well as the molecular pathways involved in the coral defense against oxidative stress.**

III. 2. 2. Species-specific response to stressors

One of the difficulties in predicting the future of coral reefs under climate change is due to the species specificity of the coral response to stress (Loya et al. 2001). For example, some genera such as *Stylophora*, *Pocillopora* and *Acropora*, are very sensitive to bleaching whereas *Cyphastrea*, *Goniopora*, *Galaxea* and *Pavona* are more resistant (McClanahan et al. 2004). Such differences can be attributed to several factors. The *Symbiodinium* clade, for example, is critical to define the holobiont sensitivity to thermal stress, with clade D being the most thermo-tolerant one (Glynn et al. 2001; Baker et al. 2004; Berkelmans and Van Oppen 2006; Jones et al. 2008; LaJeunesse et al. 2009). Therefore, corals which will be able to switch from a non-thermo-tolerant symbiont population to a more tolerant one will have an advantage in the face of global warming (Silverstein et al. 2012). The coral microbiome was also shown to change in order to fit environmental conditions (Ziegler et al. 2017). Finally, thermal resistance may depend on the coral host, which has its own defenses against thermal stress such as fluorescent pigments (Salih et al. 1998, 2000, 2006), heat shock proteins (Brown et al. 2002) or heterotrophic capacities (Grottoli et al. 2006). For example, *Pocillopora* and *Acropora* genera are known to be very sensitive to bleaching and harbor less fluorescent pigments than more resistant one such as *Porites* and *Turbinaria* (Marshall and Baird 2000; Floros et al. 2004; Ulstrup et al. 2006; Schoepf et al. 2013a). Heterotrophic plasticity (*i.e.* the ability to switch form autotrophy to heterotrophy as a main source of carbon for respiration) has also been observed in some species only (Houlbrèque and Ferrier-Pagès 2009). For example, during a bleaching event, *Montipora capitata* is able to support its daily energetic needs through heterotrophic feeding (Grottoli et al. 2006) leading to faster recovery than *Porites compressa* or *P. lobata*, unable to do so (Rodrigues and Grottoli 2007).

Although the diversity of the coral response to thermal stress has been well studied, it is not the case for the effects of UVR. **One of the goals of this thesis was therefore to compare the response to UVR of several distant-related species, such as *Turbinaria reniformis* and *Pocillopora damicornis*, (paper n°2), or *Acropora muricata* (papers n°3 and 4) Finally, one study performed with *Stylophora pistillata* evidenced a molecular pathway involved in the coral response to oxidative stress (paper n°6).**

III. 2. 3. Multi-factorial approach

The interactive effects (synergistic, additive or antagonistic) of different stressors on coral physiology represent another difficulty to predict the outcome of corals under climate change. For example, corals can simultaneously face a raise in temperature and in UVR level (Watanabe et al. 2011), combined with a high nutrient concentrations in eutrophicated areas (Fabricius 2011). Until now, the few existing work showed that UVR worsens coral bleaching in different species (Fitt and Warner 1995; Lesser 1996; D'Croz and Mate 2000; Lesser and Farrell 2004; Ferrier-Pagès et al. 2007) and thus decreases the survival chances of the most exposed reefs. Other climate-induced changes, such as ocean acidification, were also shown to modulate temperature effects on corals (Reynaud et al. 2003; Anthony et al. 2008; Schoepf et al. 2013b; Wall et al. 2014; Kwiatkowski et al. 2015). It's therefore mandatory to consider a multifactorial approach when trying to assess the coral response to future changes. **The third aim of this thesis was to improve our knowledge on these interactions and to identify the defense mechanisms used by corals to face them. We therefore systematically considered a multifactorial approach, by combining UVR exposure with temperature, nutrient levels or seasons.**

III. 2. 4. Acclimation to UVR: the role of growth conditions

Coral response to stressors depends on its growth conditions. In New Caledonia for example, corals experience in average 6°C variations in water temperature over the year (Andréfouët et al. 2009). Therefore, their response to thermal stress will likely be different from corals growing in environment harboring smaller variations. Similarly, UVR is likely to affect differently shallow water corals, which are already acclimated to UVR through protection mechanisms such as MAAs , than deeper reefs, deprived from MAAs (Masuda et al. 1993; Shick et al. 1995; Cohen et al. 2013). These observations are to take into account in the interpretation of laboratory or *in situ* experiments and in the conclusions drawn. **In this thesis, experiments were done on corals maintained without UVR (to mimic the effects of a UVR increase) as well as on corals sampled on shallow reefs and naturally**

exposed to UVR. Moreover, two experiments carried on *P. damicornis* enabled to show the effect of heterotrophy on coral response to a UV/temperature combined stress.

III. 2. 5. Multi-level investigation

Most studies investigating the effects of stressors on corals did not consider the interactions between corals and the other reef organisms, nor how the induced changes in coral physiology may impact the whole ecosystem. For example, water eutrophication not only affect coral physiology, but also favors macroalgae growth over coral's (Fabricius et al. 2005; Burkepile et al. 2013; Garren 2016). In turn, increased algae growth causes higher algal-derived-organic carbon release which enhances bacterial growth, including coral pathogens (Ruiz-Morenol et al. 2012; Vega Thurber et al. 2014; Zaneveld et al. 2016). These indirect effects, often at the disadvantage of corals (Mumby 2009; Cheal et al. 2010; Barott et al. 2012; Vieira et al. 2015), come in addition to the direct effects of eutrophication on coral physiology and might counteract the beneficial effects of nutrients sometimes observed on coral resistance to thermal stress (Tanaka et al. 2007; Holcomb et al. 2010; Béraud et al. 2013; Hoadley et al. 2016). Conversely, coral physiological changes may affect the rest of the reef. As previously discussed (Introduction §I.3.2.), corals are the main source of organic matter (OM) for reef bacteria. OM sustains the bentho-pelagic production and participates to the recycling of major elements (nitrogen, phosphorous...) in very oligotrophic reef waters (Wild et al. 2004; Bythell and Wild 2011). Changes in OM excretion might therefore modify the rest of the ecosystem. **Although it is difficult to perform reef scale experiments, we included measurements of organic carbon and nitrogen fluxes in most of our experiments. Results are however only a preliminary step in addressing the effects of qualitative and quantitative changes in OM on reef microorganisms.**

III. 2. 6. Thesis preview

The objectives and the stakes of the thesis raised several questions that we assessed in five experiments (summary Table 14):

- What are the changes in coral physiology and microbial activity and diversity between corals exposed to natural UVR levels and those shaded from UVR?
- How do corals respond to an increased UVR level? Is there a unique response or is the response species-specific?
- How does increased UVR impact mucus release and degradation?
- How does UVR affect the coral response to seawater warming and eutrophication?
- Which are the molecular pathways involved in the coral's response to increased UVR?

Table 14. Summary of the experiments carried out during the thesis. In the "environmental condition column", 0UV and UV designate the conditions without and with UVR respectively. T designates a high temperature condition; W and S designate winter and summer conditions; D, C, E designate depleted, control and enriched nutrient conditions respectively.

Paper n°	Title	UVR source	Species	Heterotrophic status	Environmental factors tested	Environmental conditions
1	Validation of commercial ERK antibodies against the ERK orthologue of the scleractinian coral Stylophora pistillata	Lamps	*S.pistillata*	Fed	-	-
2	Interactive effects of ultraviolet radiation and thermal stress on two reef-building corals	Lamps	*P.damicornis* *T.reniformis*	Fed	UVR and temperature	0UV; UV; 0UVT; UVT
3	Temperature and UVR effects on the organic matter fluxes and the metabolic activity of *Acropora muricata*	Solar radiation	*A.muricata*	Fed	UVR and temperature	0UV; UV; 0UVT; UVT
4	Seasonal variability of the microbial communities associated with *A. muricata* exposed to or shaded from UVR	Solar radiation	*A.muricata*	Fed	UVR and season	W0UV; WUV; S0UV; SUV
5	Effects of ultraviolet radiation and nutrient levels on the physiological response and mucus production of *Pocillopora damicornis* following thermal stress	Lamps	*P.damicornis*	Unfed	UVR, temperature, nutrient level	D0UV; DUV; DT0UV; DTUV; C0UV; CUV; CT0UV; CTUV; E0UV; EUV; ET0UV; ETUV
6	The c-Jun N-terminal kinase prevents oxidative stress induced by UV and thermal stresses in corals and human cells	Lamps	*S.pistillata*	Fed	UVR and temperature	0UV; UV; 0UVT; UVT

The experimental designs used to answer these questions are described in the second chapter of the thesis. Results are presented in chapters 3 and 4. Chapter 3 describes the effects of UVR combined or not with thermal stress and different nutrient conditions at different time of the year, on coral physiology (*i.e.* symbiont density, pigments and proteins, photosynthesis, respiration, calcification and heterotrophic feeding). Chapter 4 describes the effects of the same environmental factors on organic matter fluxes (quality and quantity) as well as on the activity, growth and diversity of coral associated bacteria. Finally, a fifth chapter discusses the main outcomes of the thesis.

Appendix 2. Popularization paper: L. Ezzat and **L. Courtial.** OCEAN AND CLIMATE, 2016 – *Scientific Notes*, Second edition, Tome 2. www.ocean-climate.org, 92 pages.

ocean-climate.org

Coral Bleaching, an Imminent Threat to Marine Biodiversity

Leïla Ezzat
and Lucile Courtial

For thirty years, the ocean mean temperature has been incessantly increasing, which reinforces the intensity and length of coral bleaching. From 2014 to 2016, following an unusual increase in ocean water temperature notably reinforced by a rather marked El Niño phenomenon, scientists observed an exceptionally intense and major bleaching event which could extend beyond 2017. Climate models expect a temperature increase of surface waters from 1 to 3 °C by the end of the 21st century, which threatens the survival of coral reefs around the world beyond the year 2050. The resilience level remains low and limited; the stress coral reefs endure is emphasized by other anthropogenic factors (acidification, sea-level rise, overexploitation, pollution…). In order to protect this natural heritage, which over 500 million people depend upon around the world, it is necessary for governments to take action, beyond local measures, towards reducing human impacts on climate.

Despite their ecological and economic importance, coral reefs are affected by many stress factors at both a local level (overexploitation, destructive fishery techniques, tourism pressure, marine pollution, and coastal development) and at a global level with, for instance, increasing temperature of surface waters and ocean acidification. Anthropogenic pressure and climate change currently threaten most reef ecosystems around the world. Over time, these stress factors can lead to a rupture between the coral host and its photosynthetic symbiotes, a phenomenon called "bleaching" which is, as the name suggests, a lack of color or bleaching of the organism (loss of symbiotes and/or associate photosynthetic pigments) over a more less long period of time.

A moderate decrease in symbiotes and/or associate photo pigment concentration is due to a seasonal and natural phenomenon. This occurs when surface water temperature exceeds seasonal mean maximum temperature over a short period of time which varies according to observed sites. However, for thirty years, the ocean mean temperature has been steadily increasing, due to global warming, reinforcing the intensity and length of the phenomenon scientists define as "bleaching", which now affects reef areas around the world, from the Pacific to the Indian Ocean, including the Caribbean and Red Sea.

BLEACHING: A RECURRENT PHENOMENON IN HISTORY

The first coral bleaching episode was seemingly reported by Yonge and Nicholls regarding the Great Barrier Reef in March 1929, when surface water temperature had reached 35 °C, but it is not until the 1980s that the frequency, intensity and expansion of bleaching episodes increased around the world. This is caused by a "record" increase of ocean surface water temperature due to climate change, combined with the reinforcement of the El Niño phenomenon. Two major bleaching events were reported in 1998 and 2010. The 1998 episode involved 60 island states and nations across the Pacific Ocean, the Indian Ocean , the Atlantic Ocean (Caribbean), the Persian Gulf and the Red Sea.

ocean-climate.org

The areas covering the Indian Ocean were particularly affected, with over 70% of mortality observed over a gradient depth up to 50 m. Significant ocean surface water temperature irregularity caused a loss of over 16% of coral reefs around the world. In fact, 1998 was the first "global bleaching episode" declared by the National Oceanic and Atmospheric Administration (NOAA). Again, in 2010, an intense El Niño phenomenon triggered extreme coral bleaching, affecting all reefs throughout the world with, in some regions such as South East Asia, greater consequences in terms of expansion and mortality.

2014-2016: AN EXCEPTIONALLY INTENSE AND MAJOR EVENT

The current bleaching event, spreading over three major ocean basins (Pacific, Atlantic and Indian) is caused by the combined effect of an unusual rise of ocean water temperature, emphasized by global warming, and a particularly marked El Niño phenomenon, described as one of the most intense ever observed. This exceptional bleaching event began in June 2014 in the West Pacific, near Guam and the Mariana Islands and spread to the Hawaiian coasts. It then propagated to the South Pacific, affecting British territories in the Indian Ocean, the Caribbean, and the Florida Keys, covering Cuba, the Dominican Republic and the Marshall Islands.

In October 2015, NOAA officially announced an ongoing 3rd global bleaching event in the three principal ocean basins, threatening 95% of American coral reefs. By the end of 2015, 32% of world reefs had been exposed to a temperature irregularity of 4°C, causing mortality on more than 12 000 km². The beginning of 2016 was marked by the propagation of the phenomenon in the South Pacific as well as along the Great Barrier Reef and the Australian West Coast.

During March 2016, the ocean water mean temperature was over 1.5 to 2°C compared to measurements registered between 1971 and 2000 in the Northern area of the Great Barrier, at the same time of the year. Aerial and underwater survey programs showed that out of a total of 911 individual reefs observed on the Great Barrier, 93% were affected and in particular, the 1000km coastal area along the North of Port Douglas, yet considered until then as perfectly protected because the area is isolated from human activity.

"We had never seen such a large scale bleaching phenomenon until now. The Northern area of the Great Barrier Reef looks as if 10 hurricanes had occurred at the same time" stated Professor Terry Hughes , Director of the Australian Institute of Marine Science (AIMS). Large hundred-year-old colonies are dying, showing the exceptional nature of this phenomenon. Again, in the Pacific, severe bleaching event were reported in New Caledonia, Fiji Islands and Kiribati, where the mortality rate has reached 80% (Eakin, 2016, person comm, 8th of April). The event is gradually spreading to the West Indian Ocean, the Maldives, Kenya and the Seychelles, with particularly severe consequences in the Coral Triangle area, in Indonesia. Significant temperature irregularities were also registered in the Middle East: the Persian Gulf, the Gulf of Aqaba, and the Gulf of Suez are ranked at a level 2 alert (associated with bleaching and significant and widespread mortality events), while the Gulf of Oman and the Red Sea Reef area attached to Egypt remain at a level 1 alert (associated with bleaching and significant mortality events). Other bleaching events are expected in Japan and the Caribbean during the summer of 2016. NOAA scientists have suggested that global bleaching observed could extend beyond 2017.

THE FUTURE OF CORAL REEFS: BETWEEN HOPE AND CONCERN

In 2015, the mean temperature of ocean surface waters was 0.74°C above 20th Century average temperatures, exceeding by 0.11°C the record for 2014. Climate models expect a temperature increase of surface waters of 1 to 3°C between now and the end of the 21st century. In fact, until recently temperatures only rarely and punctually exceeded the thermo tolerance limit, which causes coral to bleach. However scientists expect this phenomenon to occur on an annual or bi annual basis, thus threatening the survival of coral reefs around the world by 2050. Consequences are alarming because an increase in bleaching frequency, such as that observed in the Caribbean (1995, 1998, 2005 and 2010) for instance, limits ecosystem resilience periods

and can cause, in the long term, higher mortality rates. Recent studies have shown coral acclimatization potential to severe temperature anomalies[1]. For instance, certain coral species can modify their symbiotic algae population in order to optimize their resistance to thermal stress or even regulate their genes to reinforce their defense mechanisms. However, this resilience level is rather low and punctual. It is in fact unlikely that it might have a significant role while the ocean temperature steadily rises. Furthermore, the combination with other environmental factors, such as ocean acidification and sea-level rise paired with local threats (overfishing, pollution, physical damage, land erosion, etc.), have to be taken into consideration when predicting the future of coral reefs. The synergetic effect of these stress factors (marine pollution and overfishing) can deteriorate trophic relationships between organisms within the coral reef ecosystem, by stimulating excessive growth of filamentous algae to the detriment of coral which becomes more vulnerable to diseases and infections.

THE NEED FOR AWARENESS

These recent events have raised concern among the scientific community and have sparked awareness of the necessity to act in order to protect coral reefs. New technologies and many resources have been set up in order to follow the geographical spread and evolution of bleaching for a better understanding and apprehension of coral response. For instance, the "Catlin Seaview Survey" Expedition, launched in 2012, created a coral reef monitoring around the world. As a similar action, France is celebrating the 15 year anniversary of the French initiative for coral reefs (Ifrecor). These efforts can lead to the implementation of local actions to reduce human print on reef ecosystems. For instance, Mumby & Harborne (2010) have proved the efficiency of marine protected areas for reef resilience in the Caribbean. Likewise, in 2014, New Caledonia announced the establishment of a "Coral sea natural park", one of the biggest marine protected areas in the world (1.3 million km²). Biological engineering solutions have been proposed, suggesting the use of "optimized" coral colonies according to new environmental conditions in order to restore deteriorated reefs. Some scientists suggest using "assisted evolution" to modify the coral's resilience limit by performing an in lab artificial selection, consisting in exposing coral to various stress or by selecting thermo tolerant symbiote stem cells.

Local authorities and non-governmental organizations (NGOs) have a crucial role in protecting this heritage, which over 500 million people depend upon. Beyond local actions, governmental decisions have been made to reduce human impact on climate. In December 2015, an international agreement, setting a goal for a global warming limit of 1,5 °C - 2 °C by 2100 was validated by 195 countries (including France) participating in the international climate conference (COP21). In September 2016, China and the USA, two superpowers and major world polluters, ratified this agreement, joining the worldwide common effort. In order to preserve reefs for future generations, we need to propose realistic solutions through unifying programs, addressed to all: decision-makers, industry, general public, youth and directly concerned communities. An efficient protection of biodiversity relies on improving local communities living conditions and their ability to sustainably manage the ecosystem resources which they depend upon[2].

1 https://www.coralcoe.org.au/media-releases/only-7-of-the-great-barrier-reef-has-avoided-coral-bleaching

2 See NGO actions such as Coral Guardian (www.coralguardian.org/association-corel-guardian/).

ocean-climate.org

REFERENCES

- AIMS, 2016 – Western Australian reefs feel the heat from global bleaching event, www.aims.gov.au/docs/media/featured-content.html/-/asset_publisher/Ydk18I5jDwF7/content/western-australian-reefs-feel-the-heat-fromglobal-bleaching-event.
- AINSWORTH T. D. *et al.*, 2016 – *Climate Change Disables Coral Bleaching Protection on the Great Barrier Reef.* Science 352, 338-342 (2016).
- BAHR K. D., JOKIEL P. L. and RODGERS K. S., 2015 – *The 2014 Coral Bleaching And Freshwater Flood Events In Kāne'ohe Bay*, Hawai'i. PeerJ 3, e1136.
- BROWN B., 1997 – *Coral Bleaching: Causes and Consequences.* Coral reefs 16, S129-S138.
- DONNER S. D., SKIRVING W. J., LITTLE C. M., OPPENHEIMER M. and HOEGH-GULDBERG O., 2005 – *Global Assessment of Coral Bleaching and Required Rates of Adaptation under Climate Change.* Global Change Biology 11, 2251-2265.
- EAKIN C. M. *et al.*, 2010 – *Caribbean Corals in Crisis: Record Thermal Stress, Bleaching, and Mortality in 2005.* PloS one 5, e13969.
- FABRICIUS K. E., CSÉKE S., HUMPHREY C. and DE'ATH G., 2013 – *Does Trophic Status Enhance or Reduce the Thermal Tolerance of Scleractinian Corals? A Review, Experiment and Conceptual Framework.* PloS one 8, e54399 (2013).
- FISCHLIN A. *et al.*, 2007 – *Ecosystems, Their Properties, Goods and Services.*
- FITT W., MCFARLAND F., WARNER M. and CHILCOAT G. – *Seasonal Patterns of Tissue Biomass and Densities of Symbiotic Dinoflagellates in Reef Corals and Relation to Coral Bleaching.* Limnology and oceanography 45, 677-685 (2000).
- GLYNN P., 1993 – *Coral Reef Bleaching: Ecological Perspectives.* Coral reefs 12, 1-17.
- GLYNN P. W., PEREZ M. and GILCHRIST S. L., 1985 – *Lipid Decline in Stressed Corals and their Crustacean Symbionts.* The Biological Bulletin 168, 276-284.
- HERON S. F. *et al.*, 2016 – *Validation of Reef-Scale Thermal Stress Satellite Products for Coral Bleaching Monitoring.* Remote Sensing 8, 59.
- HOEGH-GULDBERG O. and RIDGWAY T., 2016 – *Coral Bleaching Hits Great Barrier Reef As Global Temperatures Soar.* Green Left Weekly, 10.
- HOEGH-GULDBERG O., 1999 – *Climate Change, Coral Bleaching and the Future of the World's Coral Reefs.* Marine and freshwater research 50, 839-866.
- ISRS, 2016 – Reef Encounter, 31.
- JOKIEL P. and COLES S., 1990 – *Response of Hawaiian and Other Indo-Pacific Reef Corals to Elevated Temperature.* Coral reefs 8, 155-162. PALUMBI S. R., BARSHIS D. J., TRAYLOR-KNOWLES N. and BAY R. A., 2014 – *Mechanisms of Reef Coral Resistance to Future Climate Change.* Science 344, 895-898.
- MUMBY P. J. and HARBORNE A. R., 2010 – *Marine Reserves Enhance the Recovery of Corals on Caribbean Reefs.* PLoS ONE 5, e8657, doi: 10.1371/journal.pone.0008657.
- NICHOLLS R. J. *et al.*, 2007 – *Coastal Systems and Low-Lying Areas.*
- NOAA, 2015 – *Noaa Declares Third Ever Global Coral Bleaching Event: Bleaching Intensifies in Hawaii, High Ocean Temperatures Threaten Caribbean Corals.* www.noaanews.noaa.gov/stories2015/100815-noaa-declares-third-ever-global-coralbleaching-event.html.
- NOAA, 2015 – *NOAA Coral Reef Watch: 2015 Annual Summaries of Thermal Conditions Related to Coral Bleaching for NCRMP Jurisdictions.*
- NOAA., 2015 – *State of the Climate: Global Analysis for Annual 2015.* National Centers for Environmental Information, www.ncdc.noaa.gov/sotc/global/201513.
- NORMILE D., 2010 – *Restoration or devastation?* Science 327, 1568-1570.
- PALUMBI S. R., BARSHIS D. J., TRAYLOR-KNOWLES N. and BAY R. A., 2014 – *Mechanisms of Reef Coral Resistance to Future Climate Change.* Science 344, 895-898.

ocean-climate.org

- Studies, C.C.A.C.o.E.C.R., 2016 – *Only 7 % of the Great Barrier Reef Has Avoided Coral Bleaching*. www.coralcoe.org.au/media-releases/only-7-of-the-greatbarrier-reef-has-avoided-coral-bleaching.
- VAN OPPEN M. J. H., OLIVER J. K., PUTNAM H. M. and GATES R. D., 2015 – *Building Coral Reef Resilience Through Assisted Evolution*. Proceedings of the National Academy of Sciences 112, 2307-2313, doi : 10.1073/pnas.1422301112.
- VEGA THURBER R. L. *et al.*, 2014 – *Chronic Nutrient Enrichment Increases Prevalence and Severity of Coral Disease and Bleaching*. Global change biology 20, 544-554.
- WAKE B., 2016 – *Snapshot : Snow White Coral*. Nature Climate Change 6, 439-439.
- WILKINSON C. R., SOUTER D. N. and NETWORK G. C. R. M., 2008 – *Status of Caribbean Coral Reefs after Bleaching and Hurricanes in 2005*. Global Coral Reef Monitoring Network.
- YONGE C. M., NICHOLLS A. G. and YONGE M. J., 1931 – *Studies on the Physiology of Corals*. Vol. 1, British Museum.
- ZANEVELD J. R. *et al.*, 2016 – *Overfishing and Nutrient Pollution Interact with Temperature to Disrupt Coral Reefs Down to Microbial Scales*. Nat Commun 7, doi : 10.1038/ncomms11833.

Lucile COURTIAL

Effets combinés du rayonnement ultraviolet et du réchauffement climatique sur les coraux Scléractiniaires

Les coraux Scléractiniaires se développent généralement dans la zone photique peu profonde, exposée au rayonnement ultraviolet (UVs), la composante la plus dangereuse du rayonnement solaire. Le rayonnement UVs augmente avec le réchauffement climatique et s'ajoute à l'ensemble des pressions auxquelles sont soumis les coraux. Les enjeux de cette thèse ont été 1) de mieux comprendre les effets des UVs sur la réponse physiologique des coraux, les flux de matière organique et les bactéries associées au mucus et au corail; et 2) de caractériser l'effet combiné des UVs et d'une augmentation de température, et/ou d'un changement de disponibilité en sels nutritifs. Les résultats obtenus montrent tout d'abord que l'exposition des coraux aux UVs amplifie l'effet négatif de la température sur leur physiologie. Il en est de même pour l'absence en sels nutritifs, essentiels pour la physiologie corallienne. Nos résultats indiquent également que la sensibilité des coraux à un stress UV dépend de l'espèce étudiée et de la densité de symbiontes présents dans les tissus. L'effet négatif des UVs augmente avec la densité de symbiontes, vraisemblablement dû à la formation d'espèces réactives de l'oxygène (ROS) qui provoquent des dommages à l'organisme. Dans cette thèse, nous avons montré que la voie de signalisation JNK (c-Jun N-terminal kinase), hautement conservée au sein des êtres vivants, est impliquée dans la gestion de ces espèces réactives et que son inhibition entraine un blanchissement très rapide des coraux sous UVs et forte température. Finalement, l'excrétion de matière organique ainsi que les bactéries associées sont également impactés par les UVs ce qui pourrait contribuer à d'importants changements biochimiques dans l'eau des récifs coralliens. Les travaux de cette thèse apportent de nouvelles connaissances sur les effets des UVs sur les coraux et soulignent l'importance de les prendre en considération lors de nos prédictions sur le devenir des récifs coralliens face au réchauffement climatique.

Mots clés : UVs | coraux Scléractiniaires | changement climatique | stress oxydant

Scleractinian corals mainly grow in the shallow euphotic zone, exposed to ultraviolet radiation (UVR), the most harmful part of the solar radiation. UVR increases with climate change and adds to the different environmental pressures that corals are facing. The aims of this thesis were to 1) better understand the effects of UVR on coral physiology, organic matter fluxes and associated bacteria; 2) assess the combined effects of UVR and thermal stress and/or nutrient level. Results show that UVR worsens the negative effect of temperature on coral physiology, similarly to nutrient depletion. Our results also indicate that the sensitivity to UVR stress (*i.e.* an increase in UVR) is species dependent and function of the symbiont density. The negative effects of UVR increase with the number of symbionts, likely due to the formation of reactive oxygen species (ROS) which cause cellular damages. In the thesis, we showed that the JNK signalling pathway (c-Jun N-terminal kinase), highly conserved in living organisms, is involved in the early response of corals to UVR and its activation is required to repress stress-induced ROS accumulation. Finally, organic matter release and mucus and coral-associated bacteria are also significantly impacted by UVR, which could contribute to important biochemical changes in reef waters. The work conducted in this thesis brings new insights into the effects of UVR on corals and highlights the importance of taking this environmental factor into account when predicting the future of coral reefs under climate change.

Key words: UVR | scleractinian corals | climate change | oxidative stress