Dynamique de l'holobionte corallien et plasticité transcriptomique : variabilité interindividuelle, interpopulationnelle et interspécifique

Kelly Brener-Raffalli

THÈSE

Pour obtenir le grade de

Docteur

Délivré par

UNIVERSITE DE PERPIGNAN VIA DOMITIA

Préparée au sein de l'école doctorale **ED 305**
Et de l'unité de recherche UMR5244-IHPE

Spécialité : BIOLOGIE

Présentée par BRENER-RAFFALLI Kelly

Dynamique de l'Holobionte Corallien et Plasticité Transcriptomique : Variabilité Interindividuelle, Interpopulationnelle et Interspécifique

Soutenue le 09/11/17 devant le jury composé de

Mme Christine COUSTAU, DR CNRS, UMR 1355-7254	Rapportrice
Mme Christine PAILLARD, DR CNRS, UMR 6539	Rapportrice
Mme Cécile SABOURAULT, MC UNICE, UMR 7138	Examinatrice
Mme Delphine DESTOUMIEUX, DR CNRS, UMR 5244	Examinatrice
Mme Eve TOULZA, MC UPVD, UMR5244	Co-directrice de thèse
M. Guillaume MITTA, PR UPVD, UMR 5244	Directeur de thèse

« Le dur et le rigide accompagnent la mort, le souple et le délicat accompagnent la vie. »

Tao Te Ching

RÉSUMÉ :

Dans le contexte du réchauffement climatique actuel, les récifs coralliens subissent des stress thermiques de plus en plus fréquents et intenses menant à des événements de blanchissement de masse. Ces événements de blanchissement sont dus à la rupture de la symbiose entre l'hôte corallien et son algue endosymbiotique. Cette algue, du genre *Symbiodinium,* fournit au corail plus de 90% de ses besoins nutritifs grâce auxquels il peut élaborer un squelette calcaire formant le support physique de l'écosystème corallien. Sous l'effet d'un stress thermique, l'algue produit un excès de molécules oxydantes que l'hôte corallien et son symbionte ne sont plus capables d'éliminer. L'algue devient alors toxique et est éliminée ou expulsée de l'endoderme du corail. Lors d'un stress thermique, on observe également un grand bouleversement dans la communauté bactérienne associée au corail, ce qui favorise l'émergence de maladies infectieuses. Malgré ces phénomènes responsables de mortalités de masse, certaines espèces/populations de corail montrent une thermotolérance supérieure à la moyenne et sont capables de résister ou résilier face à ces situations de stress thermique. D'après la théorie évolutive de l'hologénome, tous les compartiments de l'holobionte, c'est à dire l'hôte et les microorganismes symbiotiques associés, peuvent jouer un rôle sur le phénotype de l'hôte et donc sur la thermotolérance de ces populations particulièrement résistantes. Ceci a été démontré pour l'algue symbiotique dont on sait que certains clades ont une influence sur la thermotolérance, la photo-tolérance et la croissance du corail. D'après les théories évolutives et les nombreuses observations faites depuis l'accélération du réchauffement climatique, le mécanisme adaptatif le plus efficace face à des changements environnementaux brutaux serait une augmentation de la plasticité phénotypique, qui permettrait de générer un éventail de phénotypes plus large à partir d'un même génotype. En théorie, elle serait favorisée chez des populations expérimentant un environnement plus fluctuant. Dans le but de vérifier cette hypothèse, nous avons comparé la réponse au stress thermique de deux populations de *Pocillopora sp*. soumises soit à un régime thermique peu fluctuant (population thermo-sensible) soit à un régime beaucoup plus fluctuant (population thermo-tolérante). Tous les compartiments biologiques pouvant avoir une influence sur la thermotolérance de l'holobionte corallien ont été pris en compte dans cette analyse : la composition en *Symbiodinium*, la composition de la communauté bactérienne et le transcriptome de l'hôte corallien. Les résultats montrent une composition des micro-organismes symbiotiques stable pendant le stress et cohérente avec la littérature. Au travers de l'étude du transcriptome de l'hôte, nous avons pu montrer une plus grande plasticité transcriptomique chez la population thermo-tolérante ainsi qu'un mécanisme de « frontloading » (expression constitutive et plus forte d'un certain nombre de gènes de résistance au stress thermique dans la population thermo-tolérante). L'analyse de la fonction des gènes régulés dans la population thermo-tolérante a montré une induction des fonctions attendues pour la réponse au stress thermique (HSPs, réponse au stress oxydant ou apoptose), ainsi que la répression d'autres fonctions par un mécanisme de trade-off. De manière intéressante, on observe une forte induction des gènes impliqués dans la régulation épigénétique chez cette même population ; elle pourrait expliquer la plasticité transcriptomique observée. Au vu de ces résultats, nous proposons l'hypothèse selon laquelle le frontloading observé chez la population thermo-tolérante pourrait correspondre à une « assimilation génétique » de la plasticité, favorisée par un environnement fluctuant. Cette plasticité serait générée grâce aux mécanismes épigénétiques fortement actifs chez cette population.

Dans un second temps, nous avons cherché à identifier certains paramètres génétiques (haplotype de l'hôte) ou environnementaux (régime thermique) qui pourraient influencer la composition des communautés bactériennes et de *Symbiodinium* chez *P. damicornis*. Pour cela, nous avons comparé les communautés microbiennes (bactéries et *Symbiodinium*) en milieu naturel chez des colonies de *P. damicornis* provenant de 4 régions géographiques aux régimes thermiques très contrastés : la Polynésie française, la Nouvelle-Calédonie, Djibouti et Taïwan. Le séquençage de l'haplotype mitochondrial a révélé 2 clades d'hôtes : clade 1 pour Taïwan, Polynésie Française et Nouvelle-Calédonie et clade 2 pour Djibouti qui semblent avoir une influence aussi bien sur la communauté bactérienne que sur les *Symbiodinium*. La composition

du microbiote, analysée en métabarcoding 16S, s'est révélée très variable entre les échantillons mais un core bactérien émerge dans l'ensemble des populations (les genres *Endozoicomonas*, *Acinetobacter* et *Arcobacter*). La température influence également la composition de la communauté bactérienne, bien que l'effet soit plus faible que celui de l'haplotype de l'hôte. En ce qui concerne l'assemblage des *Symbiodinium*, on retrouve un seul clade majoritaire pour chacun des échantillons avec une grande diversité de clades très faiblement abondants (<1%). Les différences très marquées entre populations (clade D1 à Djibouti et Polynésie Française, clade C1 à Taïwan et Nouvelle-Calédonie) sont principalement influencées par les températures minimales des régimes thermiques subis. On observe ainsi par exemple une corrélation négative entre l'association avec le clade D, connu pour être thermo-tolérant, et les températures minimales. Ce nouveau résultat conforte que les communautés microbiennes, et notamment de *Symbiodinium,* sont une résultante de l'adaptation de *P. damicornis* à son environnement thermique.

A l'issue de ce travail, nous avons pu déterminer le rôle majeur de l'hôte dans la thermotolérance. En revanche, même si la structure du microbiote est stable en situation de stress, des études méta-transcriptomiques devront être menées pour mieux appréhender sa fonction dans ces situations. Il serait également intéressant de débrouiller le poids des mécanismes génétiques et épigénétiques dans la thermotolérance et la plasticité phénotypique. En effet, des études de génomique des populations permettraient d'identifier un certain nombre de loci candidats qu'il serait intéressant de comparer à ceux que nous avons identifiés. Il serait également pertinent d'approfondir le poids des mécanismes épigénétiques en étudiant l'impact de stress récurrents sur des populations clonales de coraux, afin d'évaluer les modifications épigénétiques induites et leur lien avec une éventuelle amélioration de la thermotolérance.

REMERCIEMENTS :

Difficile de résumer 4 années de travail, de complicité, de doutes et de victoires...
Ce qui est sûr c'est que cette thèse m'a permis de rencontrer toute sorte de personnes hautes en couleur, proches ou très éloignées de ma façon de voir les choses, la recherche, la vie, les gens. Ma vie n'en est que plus riche et rien que pour ça je vous en remercie.
Tout d'abord, merci à mes deux directeurs de thèse, merci de m'avoir guidé tant bien que mal dans les tumultes de la recherche. Merci *Guillaume Mitta* car je sais que ça n'a pas toujours été évident d'accorder nos violons tant nous sommes différents, mais tu as su prendre le taureau par les cornes dans mes moments d'égarement et ce n'était pas chose facile, je te l'accorde. Merci *Eve Toulza*, une vraie mère ! Tu as su être à l'écoute tout au long de ces quatre années tant sur le plan professionnel que personnel et c'est quelque chose de très apaisant dans cette aventure périlleuse qu'est la thèse. Merci pour toutes tes petites attentions, tes petits présents pour les petites ou grandes occasions. Merci de m'avoir enseigné tout ce que tu as pu sur les micro-organismes qui nous entourent et sur la bio-informatique. Et enfin, un gros MERCI de partager mon handicap, une absence totale du sens de l'orientation, ça me rassure d'avoir trouvé quelqu'un avec la même tare et qui a quand même réussi dans la vie ! Je pense que cette expérience aura été enrichissante pour tous les trois, je vous souhaite bonne continuation, j'espère qu'on restera en contact.
Pour essayer de n'oublier personne, je vais tenter de remonter le temps pour vous emmener au commencement, à la première étincelle. Pas trop loin non plus je vous rassure.
Ma première expérience dans ce labo, je l'ai obtenu grâce au secours d'un enseignant un peu potache, mais super sympa, j'ai nommé *Julien Portela* ! Désœuvrée de ne pas avoir trouvé de stage, je suis venue toquer à son mail ! Il m'a finalement confié à *Jérôme Boissier*, ce fou et passionné de science, *Gabriel Mouahid*, le chercheur le plus souriant de la planète et *Marion Picard*, aussi sensible que chaleureuse, à l'époque en 2ème année de thèse, tu m'impressionnais un peu, mais on allait vite se retrouver un an plus tard dans le même bateau. Merci à vous trois d'avoir supporté dans vos pattes la petite stagiaire de M1 que j'étais. J'ai vraiment beaucoup apprécié ce stage parmi vous, c'est à ce moment là que j'ai découvert ce qu'était le métier de chercheur et vous m'y avez donné goût.
C'est également durant ce stage de M1 que j'ai rencontré mon meilleur binôme !!!! Un canadien un peu louche au premier abord mais tellement unique !!! *David Roquis*, plus connu sous le nom d'étalon québécois au laboratoire. Moi je l'appelle mon champi, à l'image du lichens qui est composé d'une algue et d'un champignon symbiotique (et d'une levure... mais le 3ème symbionte est arrivé en cours de route on avait pas prévu ça !!). Heureusement que tu étais là durant mes premières années de

thèse, tu m'a fait me sentir à ma place, tu m'as rassuré et tu es une source de savoir inépuisable ! Je t'admire beaucoup pour ça, car je sais que tu t'es construit seul et tu peux être fier de ce que tu es devenu, tu n'as plus rien à prouver maintenant. Nos repas à l'estudiantine, nos balades dans Perpignan, mais aussi ta tendance à vouloir adopter la moindre bestiole (anémone, Triops, Daphnies), notre exploration des recoins les plus reculés du laboratoire grouillant de flasques remplies de spécimens exotiques et de vieilles encyclopédies vierges de tout étudiant, tes blagues que seul les scientifiques peuvent connaitre (la carboglace est la meilleur amie du scientifique blagueur), ton café qui sent bon et ta manie de tout réparer. Tout ça a égayé mes premières années et j'espère qu'on pourra un jour rebosser ensemble pour vivre de nouvelles péripéties!

C'est donc dans ce laboratoire que j'ai fait mes premières armes et que j'ai appris à aimer la science alors forcément j'ai voulu y retourner ! Bon je ne le savais pas encore mais bien sûr ces quelques années de recherche n'allaient pas être qu'une partie de plaisir. Loin de là même ! Il en aura fallu de la patience pour récupérer tous ces petits coraux égarés aux 4 coins de la planète. Cinq sessions d'échantillonnage pour pouvoir récupérer les deux populations de notre cher corail tropical *Pocillopora damicornis* (ou *acuta*, on en perd son latin). C'était sans compter ma patience à toute épreuve ! J'en aurai passé des mois à commander, préparer, réparer, acheter, chercher, bricoler... Heureusement je n'étais pas seule dans la tempête : il y a eu *Rémi Pillot*, ce perfectionniste dévoué avec qui on a pas arrêté de se creuser les méninges pour imaginer la source des problèmes et leur solutions, franchement un énorme merci à toi !!! Sans toi cette manip aurait été très bancale je crois. *Julien Loubet*, ou Huggy les bons tuyaux ! Lui c'est l'efficacité dans l'urgence, tu peux être sûr qu'il te trouvera une solution à n'importe quel problème pour éviter la catastrophe. Vous vous êtes bien trouvé tous les deux, vous êtes très complémentaires et l'aquarium de Banyuls a beaucoup de chance de vous avoir !! *Lionel Feuillassier*, mouahaha je ne raconterai pas toutes les anecdotes mais ton surnom de « bière » restera gravé dans les annales ! Merci à toi d'avoir participé à la mission d'échantillonnage omanaise (de graaaaannds moments) et d'avoir toujours été de très bon conseil une fois les coraux en aquarium. Travailler avec toi a été un plaisir et j'espère avoir l'occasion de te revoir un de ces 4.

Merci aussi à ceux qui avaient le nez dans la paperasse, *Pascal Romans*, *Mehdi Adjeroud*, *Didier Aurelle*. Merci également à *Jérémie Vidal-Dupiol*, qui n'a pas pu m'accompagner tout au long de l'aventure, mais dont les apparitions étaient toujours instigatrices de grands changements. Ton caractère tumultueux m'a souvent décontenancé mais je sais que tu as de grandes qualités de chercheurs et une passion dévorante qui te mèneront très loin.

Merci également à Cristian, notre Bio-informaticien chilien à qui je tente d'apprendre la langue française mais il ne fait pas beaucoup

d'effort... merci pour ton aide inépuisable, tes conseils, tes bavardages ou juste ta présence rassurante. Tu as accompagné mes derniers mois de thèse dans une ambiance sereine et pour ça je t'en suis très reconnaissante.
Bien sûr merci au laboratoire tout entier : *Jeff* et *Richard* toujours présents pour filer un coup de main, aider lors des manips ou juste lancer une petite blague au détour d'un couloir. *Anne* et *Diane,* sans qui travailler dans la recherche serait un vrai casse-tête. Merci à tous les enseignants-chercheurs du labo que j'ai d'abord eu en cours et qui m'ont ensuite accueilli chaleureusement, j'ai apprécié les plus ou moins longues discussions, sérieuses ou non, avec chacun d'entres vous, tantôt utiles, réconfortantes ou questionnantes mais toujours très enrichissantes.
Je remercie toutes les personnes du labo pour cette ambiance familiale grâce à laquelle il fait bon travailler parmi vous ! Mention spéciale à mes amis : *Cécile*, la pétillante, on aura vécu de très bons moments en très peu de temps, je te lâche pas ma poulette. *Nathalie*, la pas si sage, j'adore ta façon d'être et de penser, tu respires le positif et le courage. *Cindy*, notre normande qui s'est vite acclimatée au climat catalan, bonne continuation dans ta nouvelle vie. *Manon* ou manouche pour les intimes, mon padawan, tu es une belle personne pleine de surprise, crois en toi parce que moi j'y crois, t'es une guerrière !!

Merci à tous les doctorants et post-doctorants de Perpignan, Montpellier et Marseille avec qui on a partagé nos bons et mauvais moments une bière ou un coca à la main, je pense bien sûr à *Silvain*, *Anaïs*, *Guillaume* (*Tétreau*), *Camille* (fille et garçon), *Julien*, *Benoit*, *Sara*, *Rodolfo*, *Ronaldo*, *Tristan*, *Maxime*, *Aude*, *Marine*.
Et plein d'autres que je ne citerais pas parce que si je m'amuse à citer tout le monde j'en oublierai forcément !

En dernier, un Big Up à toute ma famille, mes parents, mes frères et sœur, mes amis de cœurs et mon chéri. Merci d'être ce que vous êtes, il est bon de revenir parmi vous le temps d'une semaine ou deux histoire de toucher à nouveau le sol et m'enraciner à nouveau. Pardon d'avoir été absente de vos vies pendant tout ce temps, promis, c'est bientôt fini !
Merci aussi à *Jérôme* qui m'a supporté durant toute ces années, tu es comme un frère pour moi et l'une des personne qui me connais le mieux. Merci d'être là.

Merci tout spécial à mon *Palinus*, j'ai conscience que sans toi ma vie aurait été bien différente et je ne serai jamais allé ni dans cette direction, ni jusque là, alors merci d'être là et de nous aimer comme tu le fais, cette thèse, c'est à toi que je la dédie.

SOMMAIRE

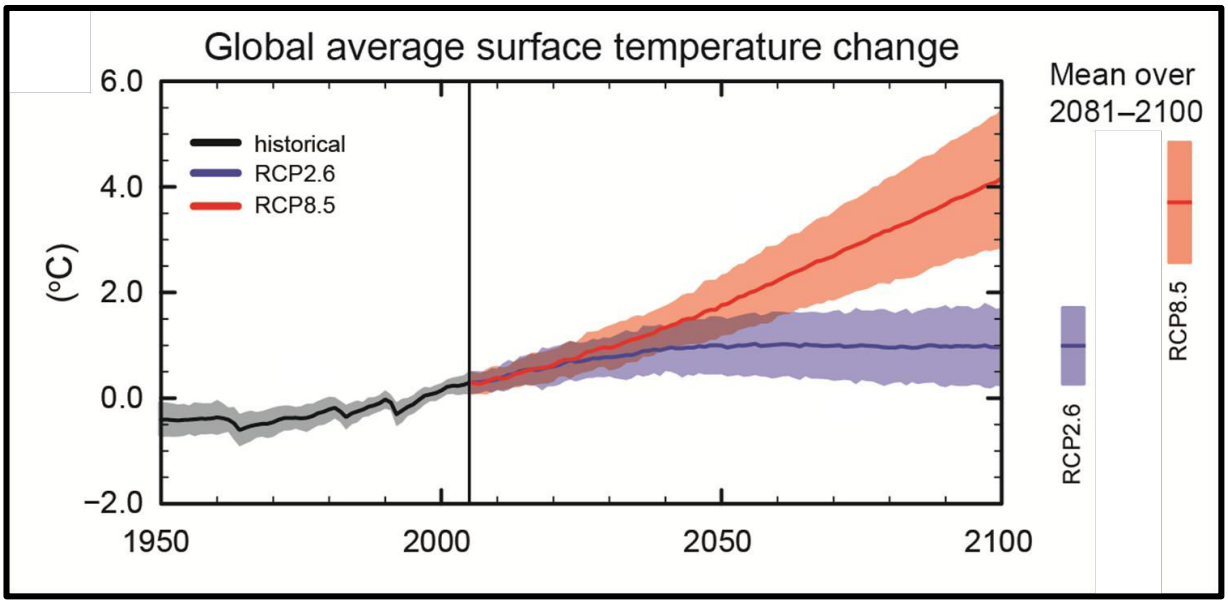


Figure 1 : Evolution de la température annuelle moyenne du globe en surface par rapport à la période 1986-2005. Les séries chronologiques des projections et une mesure de l'incertitude (parties ombrées) sont présentées pour les scénarios RCP2,6 (en bleu) et RCP8,5 (en rouge). Les RCP représentent « des profils représentatifs d'évolution de concentrations », c'est-à-dire des scénarios dans lesquels on prédit différentes valeurs pour les futures émissions ou concentrations de gaz à effet de serre, aérosols et autres facteurs anthropiques, selon les politiques climatiques mises en place d'ici 2100. Ici, les RCP 2.6 et 8.5 représentent le scénario le plus optimiste (CO2 atteignant 421 ppm en 2100, pour environ 390 ppm aujourd'hui) et le plus pessimiste (CO2 atteignant 936 ppm) respectivement. Le noir (couleur grise) représente l'évolution historique. Modifiée d'après (Stocker et al. 2013)

1. INTRODUCTION :

1.1. LE CHANGEMENT CLIMATIQUE : QUELLES CONSÉQUENCES SUR LA BIODIVERSITÉ ?

Le GIEC (Groupe d'experts Intergouvernemental sur l'Evolution du Climat ou IPCC, Intergovernmental Panel on Climate Change) est un organisme intergouvernemental chargé d'évaluer le changement climatique, aussi bien les causes que les conséquences passées, présentes et futures. Créé en 1988 par le Programme des Nations Unies pour l'environnement (PNUE) et par l'Organisation météorologique mondiale (OMM), il se réunit depuis chaque année et a déjà fourni cinq rapports scientifiques complets sur l'état des lieux, aussi bien d'un point de vue climatologique, qu'écologique et sociologique (IPCC 2013). Toutes les données relatives au changement climatique dans la présente introduction seront donc tirées du 5ème rapport du GIEC 2013.

Lorsque le GIEC parle de changement climatique, il englobe dans ce terme tout changement du climat persistant durant plus d'une décennie, qu'il soit attribuable aux processus naturels internes ou externes (cycle solaire, éruptions volcaniques, changement de la composition de l'atmosphère et de la nature des sols par l'activité anthropogénique), contrairement à l'UNFCCC (United Nations Framework Convention on Climate Change) qui définit le changement climatique comme un changement du climat attribuable directement ou indirectement aux activités humaines (Planton 2013). Le changement climatique est caractérisé par une augmentation de la concentration des gaz à effet de serre (+40% de dioxyde de carbone depuis 1850, dont 30% absorbés par les océans, menant à une acidification des eaux de -0.1 du pH), une fonte des neiges et de la glace, une augmentation du niveau de la mer et surtout un réchauffement de l'atmosphère et des océans.

La particularité de ce réchauffement réside dans la rapidité du processus comparé aux changements climatiques les plus récents de la planète. En effet, durant la dernière déglaciation, il y a 11650 ans, l'augmentation moyenne de la température était de 0,3°C à 0,8°C par millier d'années avec deux périodes plus rapides, entre 1°C et 1,5°C par millier d'années (Shakun et al. 2012). En comparaison, et comme le montre la Figure 1 ci-contre, la température de surface, des eaux et des océans a déjà augmenté de 0,85°C

durant la période 1880 à 2012. Selon les différents scénarios, 1°C à 4°C d'augmentation sont estimés pour les cents prochaines années (Stocker et al. 2013).

L'influence de l'être humain sur ce changement climatique est clairement établie. Il serait causé à hauteur de plus de 50% par l'augmentation de la concentration des gaz à effet de serre dans l'atmosphère liée notamment à l'utilisation des énergies fossiles (Stocker et al. 2013). Quand bien même un arrêt des émissions de CO_2 surviendrait, la plupart des facteurs engendrant le changement climatique persisteront pendant encore plusieurs siècles. C'est ce qu'on appelle l'inertie du changement climatique (Stocker et al. 2013).

Ces modifications rapides du climat, et en particulier le réchauffement climatique, ne sont pas sans conséquences sur l'équilibre écologique de la planète. En effet, de nombreuses études ont déjà montré des changements de comportement de nombreuses espèces depuis le début du changement climatique, il y a une centaine d'années (Merilä & Hendry 2014):

- La distribution spatiale de nombreuses espèces a été modifiée, avec un changement (Perry 2005; I.-C. Chen et al. 2011) ou une réduction (Parmesan et al. 1999) de leur aire de répartition afin de conserver un environnement optimal, soit vis-à-vis des températures soit vis-à-vis de leur source de nourriture. Ces changements de distribution mettent en contact des espèces jusque là séparées géographiquement, induisant une augmentation de la compétition pour les ressources, ainsi que l'introduction de nouvelles maladies (Capinha et al. 2013), mais aussi des phénomènes d'hybridations plus ou moins bénéfiques selon l'espèce concernée (Canestrelli et al. 2017; Muhlfeld et al. 2014; Becker et al. 2013; Hegarty 2012).
- On observe également des modifications au niveau de la phénologie de nombreuses espèces, c'est-à-dire une modification de la synchronisation des événements clés de la fitness des espèces, comme la floraison chez les plantes (Chmielewski & Rötzer 2002), ou la migration chez les oiseaux (MØller & Merilä 2004), les poissons (Crozier & Hutchings 2014) et les mammifères (Inouye et al. 2000). Certaines modifications sont clairement attribuables au réchauffement climatique induisant, par exemple, l'avancement du printemps de 2,8 jours par décennie (Parmesan 2007). Même si on pouvait voir dans certains phénomènes une adaptation des espèces au changement climatique, cela cause un dérèglement des réseaux trophiques par une

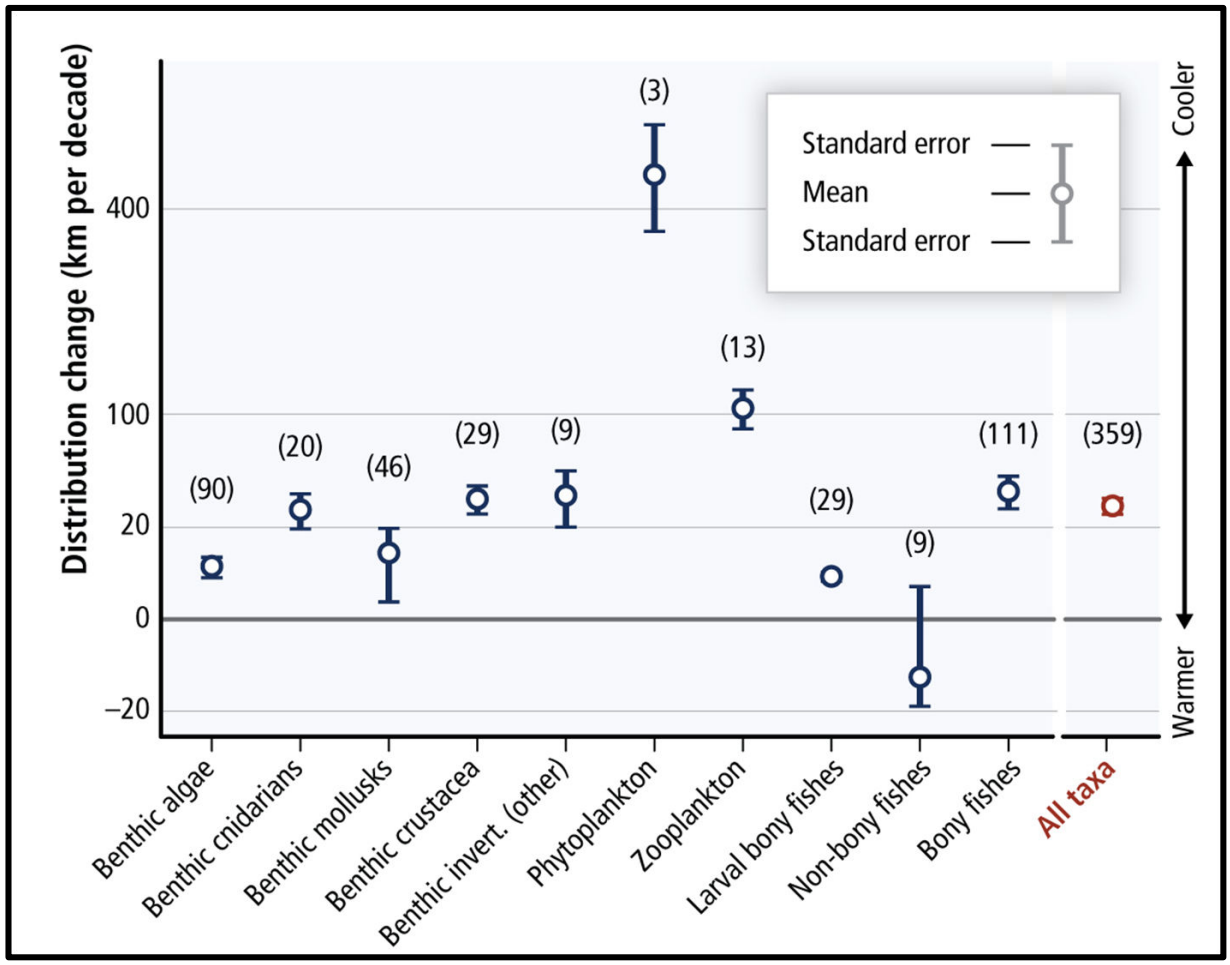


Figure 2 : Moyenne des changements de répartition par km et par décennie des différents groupes taxonomiques marins (observations de 1900 à 2010). Les changements positifs correspondent à des migrations dues au réchauffement (migration vers des eaux auparavant plus froides, généralement en direction des pôles). Les nombres entre parenthèses correspondent aux nombres de résultats analysés. Modifiée d'après (Field et al. 2014)

désynchronisation des espèces en interaction, notamment pour les relations proies/prédateurs (Thackeray et al. 2010).

- Le changement climatique entraîne également des modifications phénotypiques adaptatives comme la couleur brune du plumage chez la chouette hulotte (*Strix aluco*) en adaptation à la fonte des neiges (Karell et al. 2011), la réduction de la taille de nombreuses espèces suivant la règle de Bergman (tendance à la corrélation négative entre la masse corporelle et la température chez les animaux endothermes) (Teplitsky et al. 2008), mais aussi non-adaptatives comme le changement de sex-ratio de la plupart des reptiles qui produisent de moins en moins de mâles au fur et à mesure que les températures augmentent (Ewert et al. 2005; Rhen & W . Lang 1998; Janzen 1994).
- On assiste également à de nombreuses mortalités et une quantité notable d'extinctions ont été relevées durant les cent dernières années (Regan et al. 2001). En revanche, ces extinctions ne sont pas attribuables qu'au changement climatique car d'autres facteurs, comme la destruction directe des niches écologiques par l'être humain, sont fortement impactant (Settele et al. 2014). Dans une étude de Cahill *et al.* (2013), 136 extinctions sont analysées afin de déterminer leurs causes. Parmi elles, seulement 7 sont attribuables au changement climatique et aucune à l'augmentation de la température directement, mais plutôt aux nouvelles interactions interspécifiques et au manque de nourriture (Cahill et al. 2013). Le changement climatique et ses impacts sur la biodiversité affectent également la productivité primaire par la dégradation et le dérèglement des écosystèmes (Quéré & Raupach 2009).

Il est donc évident que la composition et le comportement des espèces changent depuis un siècle ; même si d'autres facteurs ont une influence (comme la dégradation directe des écosystèmes par l'être humain, la pollution des eaux, l'introduction de nouvelles espèces, etc.), les changements observés sur le fonctionnement des différents écosystèmes est largement attribuable à l'augmentation des températures. Or, c'est dans l'océan que 90% de cette énergie s'est emmagasiné, avec un réchauffement des 75 premiers mètres de 0.11°C par décennie depuis 1971 (Stocker et al. 2013). Ce réchauffement et, dans une moindre mesure la variation des autres paramètres physico-chimiques des océans, ont profondément perturbé les écosystèmes marins. On observe notamment un déplacement des organismes marins vers les latitudes plus élevées, comme le résume la Figure 2 tirée du GIEC 2013, ou vers des profondeurs plus importantes, ainsi que des modifications de leur phénologie.

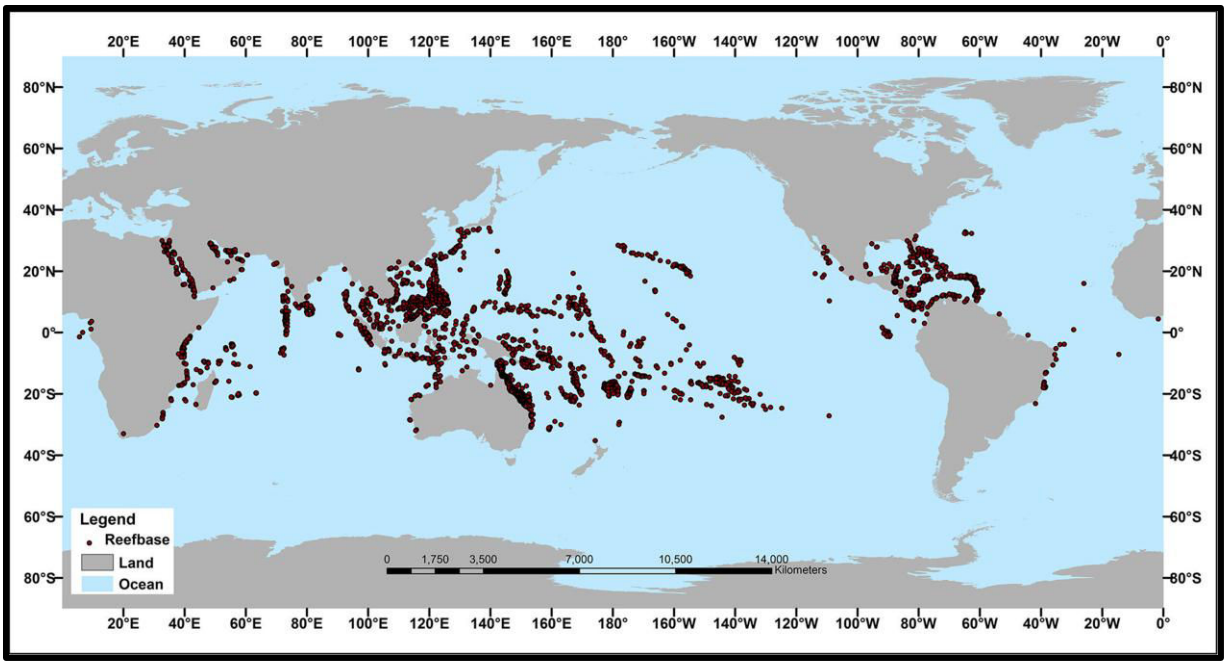


Figure 3 : carte représentant la répartition géographique des récifs coralliens mondiaux téléchargée à partir du site NOAA (National Oceanic and Atmospheric Administration) (NOAA Sites 2017)

Figure 4 : Photographie d'un récif frangeant de Moorea en Polynésie française, modifié d'après (runkle 2017).

Parmi les écosystèmes marins, l'écosystème des récifs coralliens est l'un des plus touchés par le réchauffement climatique. Il a subi de nombreux épisodes de blanchissement de masse et de mortalités (Cramer et al. 2014).

1.2. L'ÉCOSYSTÈME CORALLIEN

1.2.1. Le récif corallien

Les récifs coralliens sont des infrastructures bioconstruites dont les principaux producteurs sont des coraux durs (classe des anthozoaires, ordre des scléractiniaires). Formés de l'accumulation des squelettes de carbonate de calcium de ces animaux, les récifs finissent par constituer un rempart contre la houle autour de nombreuses îles situées essentiellement dans les océans tropicaux et subtropicaux (généralement dans les 70 premiers mètres des eaux chaudes et oligotrophes, Figure 3).

Ces barrières naturelles, en plus de protéger les côtes contre l'érosion (Figure 4), fournissent un abri pour d'innombrables espèces marines et constituent un réservoir de biodiversité. En effet, couvrant à peine 0,1% des océans, elles abritent pourtant plus d'un quart de toutes les espèces de poissons marins, ainsi que beaucoup d'autres organismes marins. On estime entre 1 et 3 millions le nombre d'espèces abritées par les récifs coralliens mondiaux (Spalding et al. 2001; Ormond & Roberts 1997).

Les récifs sont donc d'une grande importance écologique, mais également économique, pour plus d'un demi-milliard de personnes dans le monde via la pêche et le tourisme (Heron et al. 2017). On estime que les récifs coralliens rapportent plus de 352 $/ha/an en 2011(Costanza et al. 2014).

1.2.2. L'holobionte corallien

À la base de ces immenses structures que sont les récifs coralliens, il y a l'holobionte corallien, c'est-à-dire le corail et ses micro-organismes symbiotiques. Le terme d'holobionte, énoncé pour la première fois par Lynn Margulis en 1991, a été popularisé plus tardivement chez les coraux par Rohwer en 2002 (Rohwer et al. 2002; Margulis & Fester 1991). L'holobionte corallien désigne un méta-organisme constitué de l'hôte corallien, de l'algue endosymbiotique (la zooxanthelle), ainsi que de l'ensemble

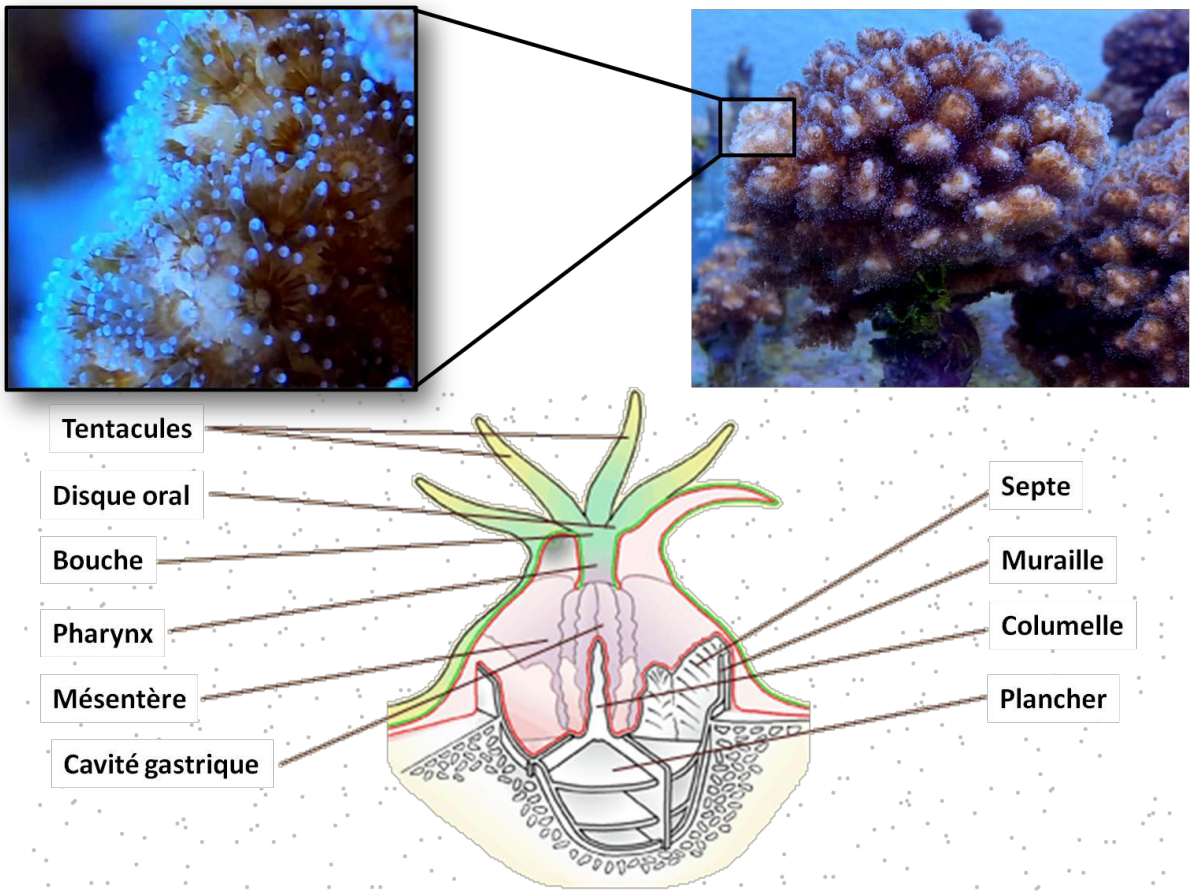


Figure 5 : anatomie d'un polype corallien, photographies d'une bouture de *Pocillopora damicornis* provenant d'Oman par Kelly Brener-Raffalli et dessin modifié d'après (Bay-Nouailhat & Bay-Nouailhat 2017).

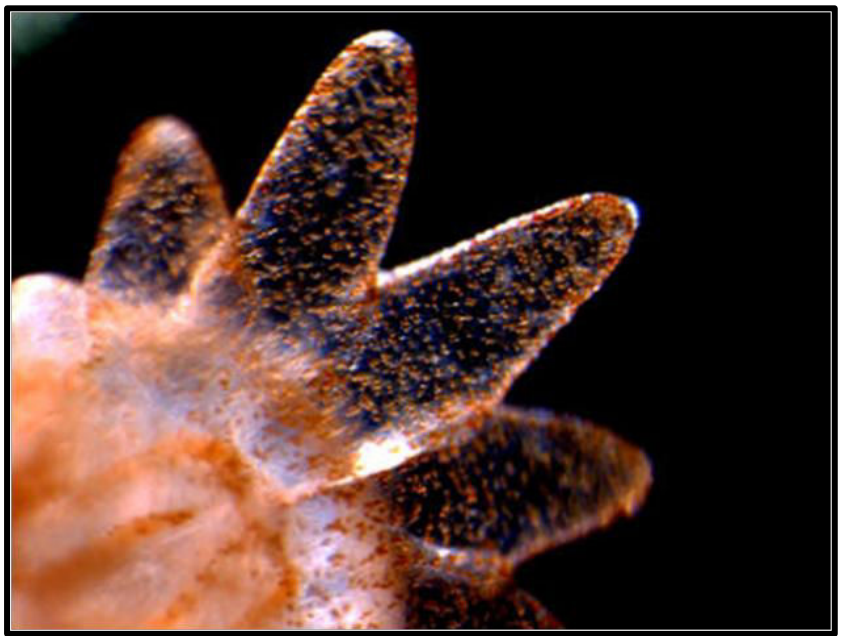


Figure 6 : présence de *Symbiodinium* dans les tissus d'un polype de corail (El-Awady 2015)

des micro-organismes associés. Ainsi, on englobe les protistes (W. W. Toller et al. 2001), les champignons (Bentis et al. 2000), les algues endolithiques (Odum & Odum 1955; Shashar et al. 1997; Bentis et al. 2000) et tout autre composante potentielle, y compris les virus (Rohwer et al. 2002). Tous ces micro-organismes pourraient contribuer à la fitness de leur hôte par la protection contre les pathogènes (Rohwer et al. 2002; Shnit-Orland & Kushmaro 2009), l'apport et le recyclage des nutriments (Lesser et al. 2004; Wegley et al. 2007).

1.2.2.1. Le cnidaire hôte

L'appellation corail désigne généralement des cnidaires de l'ordre des scléractiniaires. L'embranchement des cnidaires regroupent des animaux marins constitués de seulement deux couches de cellules monostratifiées : l'ectoderme et l'endoderme. Elles sont séparées par la mésoglée qui est une couche acellulaire riche en collagène. Sous forme de polype, ils possèdent des tentacules creux autour de la bouche et des cellules urticantes caractéristiques de l'embranchement: les cnidoblastes (Figure 5). Ces cellules sont formées d'une capsule (cnidocyste) contenant du venin et d'un filament urticant se terminant par un harpon. Elles permettent à l'animal de capturer des proies et de se défendre. Leurs systèmes nerveux et digestif sont rudimentaires et n'ayant pas de système respiratoire, les échanges gazeux se font au niveau de chaque cellule (l'oxygène dissous dans l'eau de mer diffuse selon le gradient de concentration à travers la membrane cellulaire et le CO_2 produit diffuse dans l'autre sens) (Vimal 2007). Les scléractiniaires sont capables de produire un exosquelette calcaire composé à 90% de carbonate de calcium sous forme d'aragonite.

Une colonie corallienne est composée d'une multitude de polypes issus de reproduction asexuée (et donc a priori clonaux) et reliés entre eux par le coenosarque (tissu de liaison à l'origine du squelette). C'est donc l'ensemble des polypes qui participent à générer le squelette calcaire dont la forme dépendra principalement de l'espèce.

Pour construire ces immenses et riches infrastructures dans des eaux pauvres en nutriments, les coraux ont besoin d'une grande quantité d'énergie. Cette énergie, ils la puisent de l'énergie solaire grâce à la photosynthèse. La photosynthèse est assurée par une algue symbiotique unicellulaire du genre *Symbiodinium* (aussi appelée zooxanthelle) au sein même des cellules de l'endoderme des coraux (Figure 6). C'est

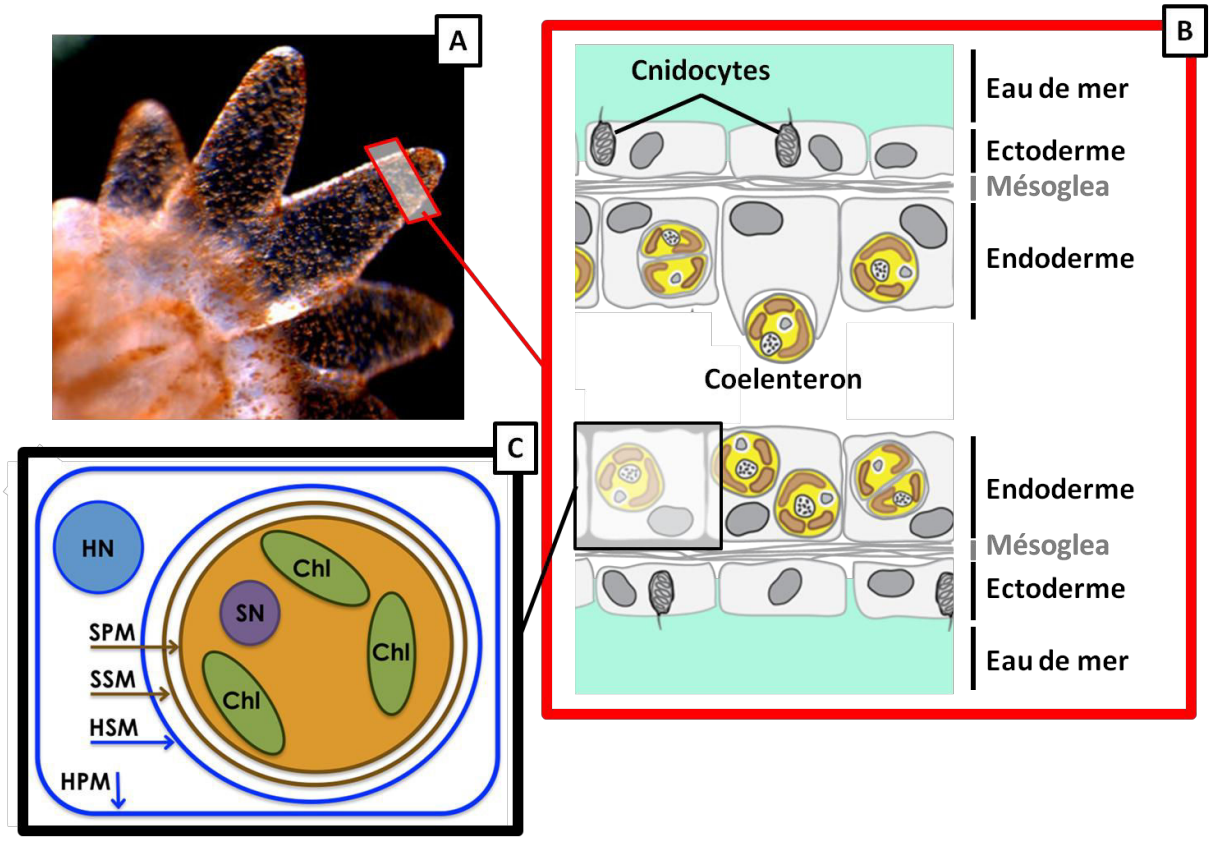


Figure 7 : schéma histologique et localisation des algues *Symbiodinium* dans les tissus du polype corallien : A) polype de corail dont les algues *Symbiodinium* sont visibles à l'œil nu, modifié d'après (El-Awady 2015); B) représentation schématique d'une coupe de tentacule, modifié d'après (LaJeunesse et al. 2012) ; C) représentation schématique d'une cellule de l'endoderme contenant un symbiosome. HN : Noyau de la cellule hôte, HPM : Membrane plasmique de la cellule hôte, HSM=Membrane du symbiosome issue de la membrane du phagosome produit lorsque l'hôte récupère le symbionte via la phagocytose, SSM=Membrane du symbiosome supposément issue de vésicules produites par le symbionte qui servent à la production des plaques de la thèque à l'état libre (Kazandjian et al. 2008), SPM : Membrane plasmique du *Symbiodinium*, SN : Noyau de l'algue symbiotique du genre *Symbiodinium*, Chl : Chloroplaste, histologie des coenosarques composés de tissu oral et aboral modifié d'après (Garrett et al. 2013)

d'ailleurs cette même algue, via ses pigments photosynthétiques, qui confère aux coraux leur couleur. Dans cette association symbiotique, le symbionte va fournir à son hôte jusqu'à 95% de sa production en sucres, acides aminés et peptides (Muscatine & Porter 1977) rendant ainsi l'holobionte corallien quasiment autotrophe. Le polype est également capable de récupérer des nutriments via une filtration passive et/ou la capture de proies (zooplancton) grâce notamment à ses tentacules contenant les cnidocystes.

1.2.2.2. L'algue symbiotique

Comme mentionné précédemment, l'algue du genre *Symbiodinium* apporte au corail scléractiniaire suffisamment d'énergie pour lui permettre d'élaborer son squelette calcaire (Barnes & Chalker 1990; Pearse & Muscatine 1971). Pour favoriser un échange de nutriment rapide et efficace, l'algue est contenue dans une vacuole à l'intérieur même des cellules de l'hôte (celles de l'endoderme) (Figure 7) (Wakefield & Kempf 2001; Colley & Trench 1983). Cette vacuole, appelée symbiosome, est issue de la phagocytose du symbionte et est caractérisée par un enchevêtrement de membranes bien particulier (Kazandjian et al. 2008).

Le genre *Symbiodinium* est subdivisé en neuf clades (de A à I), eux même subdivisés en différents sous-clades. Ces subdivisions ont été faites à partir du séquençage de différentes régions de l'ADN ribosomique nucléaire [18S (Rowan & Powers 1992), 28S (Pochon et al. 2001; Coffroth & Santos 2005), séquences inter-géniques transcrites 1 et 2, ITS1 et ITS2 (Pochon et al. 2001)] ou chloroplastique [cp23S-ADNr (Pochon et al. 2004; Rowan 2004; Pochon & Gates 2010)].

Chacun des clades possède une répartition géographique et/ou un spectre d'hôte assez bien défini. Chez les coraux scléractiniaires, on retrouve essentiellement les clades A, B, C et D (Rowan & Powers 1991; Pochon et al. 2001; Baker 2003; Coffroth & Santos 2005),mais les clades F et G peuvent également être présents (Pochon et al. 2006) ainsi que le clade H, récemment retrouvé chez *Pocillopora damicornis* (Schmidt-Roach et al. 2012). Les clades H et I sont quasiment exclusivement retrouvés chez les foraminifères (Pochon et al. 2006), le clade E est essentiellement retrouvé à l'état libre (Pochon et al. 2006; Pochon & Gates 2010).

Le clade A est le plus ancestral (Pochon et al. 2006), le clade C est la lignée dominante chez les coraux (toutes espèces confondues) du Pacifique (plus d'une centaine de sous-clades décrits), et co-dominante avec le clade B chez les coraux

d'Atlantique (LaJeunesse et al. 2003; Abrego et al. 2008). Le clade D (une dizaine de sous-clades décrits) est plus rare, mais retrouvé en association avec d'autres clades et souvent présent à moins de 10% (Lajeunesse 2005; Stat et al. 2013). Les clades A, B et F sont plus communs dans les hautes latitudes, tandis que le clade C est plus abondant dans les latitudes tropicales (Baker 2003).

En termes d'espèces, certains coraux montrent une spécificité de clade de *Symbiodinium* alors que d'autre abritent différents clades et sous-clades leur permettant de faire varier les proportions au gré des variations environnementales (Rowan & Knowlton 1995; Baker 2003; Ulstrup & Van Oppen 2003; Mieog et al. 2007; Berkelmans & van Oppen 2006).

Les symbiontes peuvent être acquis de deux manières : par transmission maternelle (transmission verticale dans l'œuf), ou bien par transmission horizontale, qui est l'acquisition du symbionte via la colonne d'eau au stade larvaire (planula) ou polype (Oppen 2001). La transmission verticale pourrait favoriser la spécificité et la coévolution entre les deux partenaires (Douglas 1998), tandis que la transmission horizontale, si la composition endosymbiotique diffère de celle de la colonne d'eau, peut révéler des mécanismes de sélection par l'hôte (Huang et al. 2013; Berkelmans & van Oppen 2006; Pochon & Gates 2010) ou favoriser la survie des coraux qui acquièrent le *Symbiodinium* local, adapté aux conditions environnementales (Cumbo et al. 2013; Blackall et al. 2015).

1.2.2.3. Le microbiote bactérien

Le microbiote des coraux, qui diffère de celui de la colonne d'eau aussi bien au niveau de sa composition que de son abondance, se répartit en plusieurs niches (Bourne & Munn 2005; Koren & Rosenberg 2006), chacune arborant une communauté bactérienne bien spécifique (Sweet et al. 2011):

- Le mucus, également nommé SML (Surface Mucus Layer), est essentiellement composé de glycoprotéines polymériques et de lipides qui favorisent la croissance des bactéries(Rohwer et al. 2002). Malgré un contact constant avec l'eau et les sédiments, il présente une communauté bactérienne très différentes de celle de son environnement, tant au niveau de sa diversité que de son abondance (Koren & Rosenberg 2006; Bourne & Munn 2005; Glasl et al. 2016; Kooperman et al. 2007); il constitue la première barrière physique contre les pathogènes et est renouvelé régulièrement par les mucocytes (Brown & Bythell 2005), ce qui permet une évacuation des déchets et/ou des éventuels

pathogènes ainsi que le renouvellement du microbiote (Glasl et al. 2016; Rohwer et al. 2002).

- Les tissus arborent également un microbiote abondant, mais différents de celui du mucus dans sa composition (Koren & Rosenberg 2006; Bourne & Munn 2005; Lesser et al. 2004).
- Le squelette contient des cyanobactéries pouvant assurer l'apport de matière organique via la photosynthèse. Cet apport pourrait compenser celui des zooxanthelles lorsque le corail blanchit (Schlichter et al. 1995).

Certaines bactéries du squelette et des tissus, telle que des cyanobactéries (Lesser et al. 2004) et des diazotrophes (bactéries et archéobactéries fixatrices de nitrogène) majoritairement du groupe rhizobia (Lema et al. 2012), sont essentielles à la physiologie de l'hôte corallien, fournissant en association avec les zooxanthelles plus de 50% des besoins en azote du corail (Ferrer & Szmant 1988; Lesser et al. 2004; Rädecker et al. 2015). D'autres, telles que des alphaprotéobactéries du genre *Roseobacteria* (Miller et al. 2004) ou de l'ordre de SAR11 (Tripp et al. 2008), permettent le recyclage des composés sulfurés produits par la zooxanthelle et dont les produits pourraient jouer le rôle d'antioxydants et d'antibactériens (Raina et al. 2010). L'apport d'antibiotiques produits par les bactéries du mucus serviraient à la défense contre les pathogènes (Ritchie 2006; Koh 1997).

On retrouve également parmi cette communauté de micro-organismes des archées dont la composition est similaire à la colonne d'eau environnante (Kellogg 2004), ce qui suggère une faible sélection de la part de l'hôte. Des virus ont également été retrouvés chez les coraux, dont une grande diversité dans le mucus (Nguyen-Kim et al. 2014), mais également dans les tissus (Wilson et al. 2005) et en association avec les zooxanthelles (Wilson et al. 2001). Lors de divers stress chez *Porites compressa*, il a été montré que l'abondance du virus *Herpesviridae* augmente significativement (Vega Thurber et al. 2008). (Bettarel et al. 2015) proposent d'ailleurs l'hypothèse selon laquelle les virus pourraient être en partie responsables du contrôle de la composition de la communauté bactérienne et, lors de stress environnementaux, du déclin de certains groupes bactériens (via des bactériophages) et même du blanchissement via des phytophages.

C'est la composition du microbiote bactérien qui a été la plus étudiée, notamment en fonction des différents facteurs environnementaux biotiques ou abiotiques :

- Les saisons (C.-P. Chen et al. 2011; Ceh et al. 2011; Kimes et al. 2013; Li et al. 2014) : bien qu'il soit difficile d'estimer lequel des paramètres environnementaux influe sur cette dynamique.
- Les précipitations affecteraient la composition du microbiote en apportant des bactéries retrouvées habituellement dans le sol (C.-P. Chen et al. 2011)
- Le régime thermique : une expérience de transplantation réciproque a montré que les communautés bactériennes de deux populations d'une même espèce, mais expérimentant des régimes thermiques différents (hautes variations contre faibles variations) présentaient des différences significatives dans la composition de leur microbiote bactérien (Ziegler et al. 2017) qui ont disparu après 17 mois de transplantation. En revanche, d'autres travaux n'ont pas mis en évidence de lien entre la composition du microbiote et le régime thermique (C.-P. Chen et al. 2011). Le régime thermique ne semble donc pas avoir une influence systématique sur la composition de la communauté bactérienne. Il serait intéressant de distinguer l'amplitude des variations et la température maximale et minimale dans les différentes études, afin de déterminer lequel ou lesquels de ces paramètres influencent le microbiote bactérien.
- L'hôte peut avoir un effet sur la composition du microbiote. On relève notamment une spécificité du microbiote pour l'espèce (même entre populations éloignées géographiquement), mais également, à l'intérieur d'une espèce, pour chaque colonie (C.-P. Chen et al. 2011; Rohwer et al. 2002; Morrow et al. 2012; Ritchie & Smith 1997).
- Il a également été montré que le clade de la zooxanthelle pouvait influencer la stabilité de la composition bactérienne lors d'un stress thermique (Littman et al. 2010). Il est toutefois difficile de distinguer l'influence directe du symbionte sur la communauté bactérienne de l'influence indirecte, via la physiologie de l'hôte perturbée par un changement de symbionte, qui peut entrainer une susceptibilité différente face au stress thermique (mécanisme décrit dans la partie 1.3.).

Différents paramètres biotiques et abiotiques vont donc influencer la composition du microbiote, ce qui va influencer l'état de santé du corail ainsi que sa capacité à réagir et résister aux variations environnementales.

1.3. LE BLANCHISSEMENT CORALLIEN

Les événements de blanchissement observés durant les cinquante dernières années se sont produits lorsque les températures locales ou régionales dépassaient les maximas typiques des mois d'été durant quelques semaines (Baker et al. 2008; Hoegh-Guldberg et al. 2007). Or, depuis 1980, les événements de blanchissement corallien de masse se produisent à un taux et une fréquence sans précédent (Heron et al. 2017; Burke, World Resources Institute., Mark Spalding, et al. 2011) et sont souvent suivis de mortalités de masse (Baker et al. 2008). Trois années ont été particulièrement critiques (1998, 2010 et 2014), avec de fortes températures concomitantes au phénomène El Niño, porteur de courants chauds induisant jusqu'à 70% de blanchissement des coraux à l'échelle du globe (année 2014). Le réchauffement climatique n'est pas le seul facteur induisant des dommages aux récifs coralliens : la baisse de la qualité des eaux, le développement côtier, l'augmentation de la pêche, et même le tourisme ont également été impliqués dans le déclin des communautés coralliennes dans les cinquante dernières années (Bruno et al. 2007). Étant donnée la synchronisation des derniers événements de blanchissement de masse avec les températures élevées et les connaissances sur l'impact des stress thermiques sur les coraux qui s'accumulent ces dernières années, on peut affirmer que le changement climatique joue un rôle majeur dans la dégradation des écosystèmes coralliens (Cramer et al. 2014).

Au cours du siècle à venir, selon le pire scénario du GIEC (RCP8,5), ce sont deux blanchissements de masse par décennie que devront subir plus de 86% des coraux du monde (Heron et al. 2017). Les récifs coralliens sont, et vont donc continuer à être, fragilisés par le réchauffement climatique et ceci, ajouté à d'autre facteurs naturels ou anthropiques, pourrait mener à terme à la destruction totale de ces écosystèmes écologiquement et économiquement précieux (Hoegh-Guldberg et al. 2007).

Cependant, les coraux ne sont pas tous sensibles au blanchissement de la même façon, et ce même au sein d'une espèce. Il existe, notamment dans le golfe persique, des populations capables de résister longtemps à des températures très importantes. Ces mêmes populations n'ont d'ailleurs pas ou peu subi les événements de blanchissement de masse les plus importants (Riegl et al. 2011). Comprendre les mécanismes de résistance au stress thermique de ces coraux pourrait permettre de prédire l'évolution future de ces organismes et d'envisager des mesures de sauvegarde efficaces pour les populations les plus fragiles. Pour cela, il est important de connaitre tous les éléments

qui peuvent influencer la thermo-tolérance des différentes espèces et populations de coraux.

1.3.1. Le symbionte et le stress thermique

Abriter et maintenir un organisme photosynthétique demande à l'hôte corallien un certain nombre de mécanismes de protection qui deviennent vite insuffisants lorsqu'un stress environnemental intervient. Lors d'un stress thermique ou lumineux, ce fragile équilibre est rapidement rompu et le corail, qui n'est plus apte à maintenir son symbionte devenu trop toxique, va alors expulser l'algue de ses cellules. C'est ce qu'on appelle le blanchissement corallien. En effet, la photosynthèse, en plus de nécessiter une forte exposition à la lumière, produit des molécules oxydantes appelées ROS (Reactive Oxygen Species), qui en excès, peuvent endommager les membranes plasmiques et l'ADN du corail (Muller-Parker et al. 2015; Weis 2008).

Ces ROS peuvent provenir de différents dérèglements de la machinerie de la photosynthèse. La dénaturation de la protéine D1 (Warner et al. 1999) et/ou le dysfonctionnement de la RUBISCO (Lesser 1996) induisent un maintien du photosystème II dans son état excité. L'endommagement de la membrane des thylakoides (Tchernov et al. 2004) va induire la rupture de la chaine de transport des électrons et entrainer un dysfonctionnement des deux photosystèmes. Le dérèglement de la machinerie de la photosynthèse va empêcher la production d'ATP et de NADPH, mais pas la production d'électrons, qui vont générer des ROS en se liant à diverses molécules. Par exemple, l'ion superoxyde O_2^- en se liant avec du dioxygène. Ce dernier peut être réduit par la superoxyde dismutase (SOD) en H_2O_2 (peroxyde d'hydrogène) moins réactif mais qui peut réagir avec l'ion ferreux (Fe^{2+}) et former l'ion hydroxyle (OH^-). Ces ROS vont continuer à endommager la machinerie et induire la production d'autres ROS. En addition de cette surproduction de ROS par l'algue symbiotique, des ROS sont également produites en excès par la mitochondrie de l'hôte lors de stress thermique ou lumineux (Dykens et al. 1992). En plus d'endommager la machinerie photosynthétique et de bloquer la production d'énergie, les ROS sont des molécules capables d'endommager protéines, lipides et membranes cellulaires, mais également l'ADN (Richier et al. 2005). Leur élimination est donc cruciale pour le symbionte et son hôte, mais les ROS étant alors en quantité trop importante pour être pris en charge par les différents systèmes de détoxification de l'hôte (ROS scavenger, SOD, peroxydase,

catalases, gluthation), le seul moyen de réduire leur production va être d'éliminer le symbionte (Downs et al. 2002).

Pour cela, le corail hôte peut procéder à la destruction directe de l'algue au sein de ses cellules (par nécrose, apoptose (Perez & Weis 2006; Richier et al. 2006) ou digestion (Downs et al. 2009; Brown et al. 1995)), à l'expulsion de l'algue par exocytose (Steen & Muscatine 1987; Brown et al. 1995) ou au détachement de la cellule hôte contenant son symbiote (Gates et al. 1992). Plusieurs facteurs biotiques et abiotiques peuvent mener à l'expulsion de l'algue symbiotique : la lumière (Gleason & Wellington 1993; Brown et al. 1995) ; la salinité (Goreau 1964; Van Woesik et al. 1995), l'hyper-sédimentation, la pollution aux métaux lourds (Mitchelmore et al. 2002; Hashimoto et al. 2004), mais le facteur le plus étudié reste la température (Glynn & D'Croz 1990; Rowan 2004; Baker et al. 2008; Obura 2009; Penin et al. 2013) pour lequel on connait maintenant assez bien le processus menant à l'expulsion du symbionte. La perte du symbionte n'est pas létale en soi si elle ne dure pas plus de quelques semaines. Le corail est en effet capable de récupérer des algues symbiotiques une fois le stress environnemental passé. C'est ce qu'on appelle la résilience (Rodrigues et al. 2008).

Par ailleurs, les différents clades de *Symbiodinium* peuvent avoir des performances photosynthétiques différentes et répondre à l'environnement de manière très spécifique influençant ainsi la valeur sélective de l'hôte (Knowlton & Rohwer 2003) :

- Les clades A, B et D sont retrouvés à de faibles profondeurs (0 à 6 m), où l'irradiation solaire est importante (Baker 2003; Rowan & Knowlton 1995; W. W. Toller et al. 2001). La photo-protection du clade A serait due à la production d'acides aminés de type mycosporines (T. Banaszak, et al. 2000). Le clade C est retrouvé à plus grande profondeur (3 à 14 m) et plus faible irradiation (Rowan et al. 1997).
- Le clade C serait plus productif à faible température que le clade D mais cette tendance s'inverse lors d'une augmentation de 24 à 30°C chez *Pocillopora damicornis* (Little et al. 2004; Jones & Berkelmans 2010) .
- Le clade D est généralement retrouvé chez des coraux soumis à des conditions non supportées par les autres clades de *Symbiodinium*. C'est le cas par exemple en profondeur à 35 m chez *Montastraea franski* (W. W. Toller et al. 2001), dans les sites exposés aux sédiments anthropiques (W W Toller et al. 2001; Ulstrup & Van Oppen 2003), ou bien chez des coraux résilients suite à un blanchissement (Baker 2001; W. W.

Toller et al. 2001). Le clade D est donc considéré comme généraliste ou opportuniste (Baker 2003). C'est également un clade thermorésistant (Stat & Gates 2011; Berkelmans & van Oppen 2006; Baker 2003), comme chez *Pocillopora*, où, lors de l'événement El Niño de 1998, les coraux de l'est du Pacifique associés majoritairement au clade D n'ont pas blanchi contrairement aux coraux associés à différents sous-clades du clade C (Glynn et al. 2001).

Les coraux peuvent arborer un mélange de plusieurs types de *Symbiodinium* (clades ou sous-clades) et montrer des variations de cette composition, notamment au niveau de leur abondance relative, selon les fluctuations environnementales au cours des saisons ou au cours des stress environnementaux (Jones et al. 2008; Hsu et al. 2012). Par exemple, *Isopora palifera* à Taïwan est associée aux clades D1a et C3 et semble pouvoir adapter l'abondance relative de ces deux clades en fonction des conditions environnementales. Le clade D1a est majoritaire lorsqu'il est soumis à de hautes températures ou suite à un événement de blanchissement mais on observe un retour au clade C3 lorsque les températures redeviennent stables (Hsu et al. 2012). De tels changements de symbiontes pourraient permettre une acclimatation des colonies aux variations des conditions environnementales (Jones et al. 2008; Berkelmans & van Oppen 2006). Cette forme de plasticité a donné lieu à l'émergence de la théorie du blanchissement adaptatif (Buddemeier & Fautin 1993) suite à différents constats :

- Les coraux provenant d'habitats plus variables ont tendance à être moins sensibles au blanchissement que les coraux d'habitats plus stables (Buddemeier & Fautin 1993).
- Dans un même habitat, on observe à la fois des espèces plus sensibles au blanchissement et d'autres plus résistantes et des individus d'une même espèce plus sensibles que d'autres (Buddemeier & Fautin 1993).
- Les zooxanthelles diffèrent biochimiquement selon l'hôte dans lequel elles sont abritées et différentes zooxanthelles auront différents effets sur un même hôte.
- Plusieurs espèces de zooxanthelles ainsi que de coraux hôtes sont présents dans les récifs, avec de nombreuses combinaisons possibles.
- Le blanchissement donne l'opportunité à l'hôte corallien d'être repeuplé par différents types de *Symbiodinium* ; des stress fréquents tendent à favoriser une combinaison résistante au stress.

- Des conditions environnementales altérées (incluant des fréquences anormales d'événements stressants et des types de stress anormaux) favorisent l'établissement de combinaisons de symbiontes qui étaient moins adaptés aux conditions précédentes.
- Les combinaisons sensibles au stress peuvent avoir des avantages compétitifs en absence de stress (par mécanisme de trade-off), ce qui implique un retour progressif à des associations sensibles au stress, mais avantageuses dans des environnements stables et en l'absence de stress.

Selon cette théorie, même si le blanchissement représente une instabilité à court terme, il favorise une stabilité à long terme en augmentant les chances de survie du symbionte et de son hôte dans des conditions qui ne sont pas celles de l'environnement précédent le stress (Buddemeier & Fautin 1993). Tous les coraux n'ont pas la même capacité de plasticité symbiotique. Chez une population de *Pocillopora* en Californie, seulement 3% des colonies ont montré un changement de clade après un événement de blanchissement induit par un refroidissement de l'eau (McGinley et al. 2012). Dans une autre étude chez cette même espèce en Australie, on observe pourtant des changements de symbionte (avec intégration de nouveaux clades) à partir des *Symbiodinium* de sa biosphère « rare » (c'est à dire présents à moins de 1%) suite à deux événements de blanchissement (hausse des températures) jusqu'à finalement remplacer le clade dominant 18 mois plus tard par un de ces clades préalablement très faiblement abondants (Boulotte et al. 2016). Ces observations montrent que les interactions entre *Symbiodinium* et corail hôte peuvent être très différentes en fonction des espèces de corail et même des différentes populations (Blackall et al. 2015).

Les symbiontes ne sont pas les seuls micro-organismes abrités par l'hôte corallien. De nombreuses bactéries peuplent également les tissus, mais aussi le mucus et le squelette du corail (Bourne & Munn 2005; Koren & Rosenberg 2006). Un dérèglement de cet autre compartiment lors d'un stress thermique peut d'ailleurs être lui aussi responsable d'une rupture de l'homéostasie et entrainer un blanchissement de la colonie corallienne (Bourne et al. 2008).

1.3.2. Le microbiote bactérien et le stress thermique

Lors d'un stress thermique, il est connu que la communauté bactérienne connait un profond changement dans sa composition, pouvant mener à l'émergence de maladies (Bourne et al. 2008; Mao-Jones et al. 2010). Cela a déjà été montré pour de nombreuses

espèces de *Vibrio* (Littman et al. 2010; Munn 2015), pathogènes responsables de diverses maladies chez les coraux :

- *Vibrio shilonii* responsable du blanchissement saisonnier de *Oculina patagonica* (Rosenberg & Falkovitz 2004).
- *Vibrio coralliilyticus* responsable de blanchissements et nécroses chez *Pocillopora damicornis* (Ben-Haim et al. 2003).
- *Vibrio harveyi* impliqué dans le syndrome de la bande jaune et celui de la bande blanche chez plusieurs espèces de coraux scléractiniaires dont *Pocillopora damicornis* (Luna et al. 2009)
- La présence seule d'une espèce de *Vibrio* ne suffit pas toujours, et on retrouve notamment toute une population de *Vibrio* dans le cas du syndrome de la bande jaune chez les coraux de l'indopacifique : *Vibrio rotiferianus, Vibrio harveyi, Vibrio alginolyticus* et *Vibrio proteolyticus* (Cervino et al. 2008).

Cette liste non-exhaustive illustre l'importante implication des espèces de *Vibrio* dans de nombreuses maladies coralliennes, mais ce genre bactérien est également présent dans de moindres proportions dans le microbiote des coraux sains. *Vibrio* est alors présenté comme un pathogène opportuniste dont l'émergence serait induite dans certaines conditions environnementales comme un réchauffement des eaux (Munn 2015). Il a également été montré que *Vibrio shilonii* produisait des SOD (Super Oxide Dismutases) extracellulaires ce qui pourrait expliquer son émergence lorsque les températures augmentent puisque les zooxanthelles sont alors amenées à produire davantage de ROS qu'à la normale. Les bactéries capables de s'en protéger seraient donc favorisées par cet environnement (Banin et al. 2003).

Il existe de nombreuses autres bactéries causant les diverses maladies coralliennes qui accompagnent les stress thermiques, comme la maladie de la bande noire causée par un consortium bactérien dont des cyanobactéries (Cooney et al. 2002), la peste blanche causée par *Aurantimonas corallicida* (Denner et al. 2003), ou encore la variole blanche chez *Acropora palmata* causée par *Serratia marcescens* (Patterson et al. 2002).

Pour mieux comprendre l'implication possible du microbiote dans la thermo-tolérance et la résistance face aux pathogènes, il faut savoir que les coraux ont un système immunitaire inné. Leur système de défense consiste en une barrière physique

(l'épiderme et le mucus qui est renouvelé régulièrement), des phagocytes, des acides et des produits antibactériens solubles (Rosenberg & Loya 2004). Comme tous les invertébrés, ils n'ont pas de système immunitaire adaptatif et donc ne produisent pas d'anticorps, mais une grande partie des bactéries du mucus synthétisent des antibiotiques (Ritchie 2006; Koh 1997). De ce phénomène provient l'émergence de l'hypothèse probiotique, qui expliquerait le rôle du microbiote bactérien dans la résistance de certains coraux aux pathogènes et aux hautes températures. Quatre constatations supportent cette hypothèse : 1. les coraux hébergent une communauté bactérienne importante (mucus et tissus), 2. qui leur apporte plusieurs bénéfices (photosynthèse, fixation de l'azote et antibiotiques), 3. le microbiote change quand les facteurs environnementaux changent, 4. les coraux sont capables de développer une résistance contre les pathogènes. Ces éléments ont abouti à la formation de la théorie évolutive de l'hologénome (Reshef et al. 2006; Rosenberg et al. 2007) (voir plus bas).

Finalement, il a été montré de profondes modifications des communautés bactériennes au cours d'un stress thermique avec, comme le récapitule McDevitt-Irwin dans son article de 2017, l'augmentation de l'abondance de nombreux taxons, dont plusieurs ayant un pouvoir pathogène (Vibrionales (Tout et al. 2015; Bourne et al. 2008; Frydenborg et al. 2014; Lee et al. 2016), Flavobacteriales (Gignoux-Wolfsohn & Vollmer 2015) et Rhodobacterales (Welsh et al. 2015)) et la diminution d'autres connus pour avoir un rôle dans la physiologie de l'hôte corallien (Oceanosprillales (Pantos et al. 2015)) (McDevitt-Irwin et al. 2017). Cela suggère que lors d'un stress, le microbiote bactérien est fortement impacté (McDevitt-Irwin et al. 2017). La composition du microbiote bactérien peut être spécifique à une espèce, à une population voire même à une colonie corallienne (Rohwer et al. 2002; Morrow et al. 2012; Neave, Rachmawati, et al. 2016). Cet ensemble de données suggère que l'hôte pourrait être, au moins en partie, responsable de la sélection de son microbiote bactérien et que la capacité du corail à réguler son microbiote aurait tendance à diminuer lors d'un stress.

L'hôte semble donc jouer un rôle important dans le maintien et la stabilité de son microbiote, mais le microbiote lui-même semblerait jouer un rôle dans la résistance au stress. En effet, lors d'une transplantation réciproque de deux populations issues d'un environnement faiblement variable ou hautement variable, les communautés bactériennes initialement différentes entre les deux populations ne présentaient plus de différence significative avec celles des populations natives après 17 mois dans le nouvel

environnement. La population provenant de l'environnement le moins variable et transplantée dans un environnement variable présente un microbiote stable et peu ou pas de blanchissement, à l'inverse de la population provenant d'un environnement hautement variable transplantée dans un environnement faiblement variable (Ziegler et al. 2017). Une étude fonctionnelle des taxons bactériens des deux populations a également montré que la population de l'environnement variable présentait un enrichissement des processus cellulaires pouvant améliorer la résistance au stress du microbiote (carbohydrate metabolism, nitrogen-fixation protein, ferredoxin, bacterial chaperonin). Cette étude montre la possible implication du microbiote dans la thermotolérance de l'hôte, ainsi que la capacité d'adaptation des coraux à adapter leur microbiote à un environnement variable (microbiote plastique).

1.3.3. L'hôte cnidaire et le stress thermique

Lors du stress thermique, l'hôte doit assurer la « gestion » de ses micro-organismes symbiotiques, comme décrit précédemment, mais il est également capable de mettre en place un certain nombre de mécanismes de défenses macroscopiques et/ou moléculaires.

L'une des premières réactions physiologiques observable lors d'un stress (biotique ou abiotique) chez les coraux est la fermeture des polypes. Ce réflexe lui permet de diminuer sa surface d'exposition aux UV (en cas d'un stress thermique et/ou lumineux) ou à toute substances néfastes (en cas de pollution ou sédimentation) ou bien encore face à d'éventuels prédateurs (B. . Brown et al. 2002).

De très nombreuses études se sont intéressées à la réponse au stress thermique des coraux (Cikala et al. 1999; Townsend et al. 2002; Weis 2008; Granados-Cifuentes et al. 2013; Richier et al. 2006; Bellantuono et al. 2012; Barshis et al. 2013). Les principaux mécanismes moléculaires mis en avant par ces travaux sont les suivants :

- L'exocytose, l'apoptose et la nécrose, qui sont des mécanismes induits pouvant permettre l'élimination des symbiotes devenus toxiques ou tout simplement des cellules endommagées. Les molécules les plus caractéristiques de l'apoptose sont les TNF, TNRF, NFkB et les caspases (Ainsworth et al. 2011; Barshis et al. 2013; Maor-Landaw & Levy 2016; Chen 2005).
- La production de Heat Shock Proteins (protéines de choc thermique), dont les HSP 70, qui permettent de protéger la structure tridimensionnelle des protéines et

préservent ainsi les fonctions cellulaires les plus importantes (B. Brown et al. 2002; Carpenter et al. 2010; Maor-Landaw & Levy 2016).

- La production de molécules détoxifiantes qui permet d'éliminer les ROS produits par les symbiotes mais aussi par les mitochondries de la cellule corallienne (ROS scavenger, SOD, peroxydase...) (DeSalvo et al. 2010; Maor-Landaw et al. 2014; Yoda 2006).
- Des changements dans l'utilisation des ressources énergétiques avec, entres autres, la dégradation des acides gras servant de réserves d'énergie en cas de perte des symbiontes (induction de la dégradation des lipides, régulation du transport des lipides, répression de la synthèse des lipides) avec, en contrepartie, une diminution de la dégradation des sucres (Lehnert et al. 2014).
- Un changement de mode de nutrition avec le passage d'un mode autotrophe à un mode hétérotrophe (Anthony & Fabricius 2000; Grottoli et al. 2006; Houlbrèque & Ferrier-Pagès 2009).
- La fermeture des polypes et la rétractation des tissus, ainsi que le passage à un mode hétérotrophe (capture de proies), peuvent également induire une activité nerveuse et musculaire plus importante (Leclère & Röttinger 2017; Anctil 2009; Fisher et al. 2009; Vargas-Angel et al. 2006).
- On observe souvent un arrêt de la production du squelette calcaire, de la reproduction et de la croissance par un mécanisme de « trade-off » permettant de réallouer l'énergie aux fonctions métaboliques les plus utiles dans ce nouvel environnement stressant (Barshis et al. 2013; Kenkel et al. 2013; Debiasse & Kelly 2016; Vidal-dupiol et al. 2009).

1.4. LA THÉORIE DE L'HOLOGÉNOME

Face au réchauffement climatique, c'est donc un holobionte dans son ensemble qui est amené à s'adapter aux changements de conditions environnementales. L'adaptation peut se faire au niveau de l'hôte et/ou des communautés microbiennes symbiotiques. Il est donc important de prendre en compte toutes ces composantes lorsque l'on s'intéresse à l'évolution des coraux. C'est dans cette logique que la théorie de l'hologénome a vu le jour. Elle est énoncée pour la première fois par Rosenberg dans une revue s'intéressant au rôle des micro-organismes sur la santé et l'évolution du corail en 2007. C'est un an plus tard (Zilber-Rosenberg & Rosenberg 2008) que Rosenberg

formula sa théorie pour l'ensemble de la biosphère suite à quatre constats : l'ensemble de la faune et de la flore possèdent des microorganismes symbiotiques ; qui peuvent être transmis entre générations ; qui affectent la fitness de l'holobionte différemment selon les environnements, et l'ensemble des génomes de l'holobionte, forment un hologénome très variable puisque sa variabilité peut être induite par un changement génétique de l'hôte et des microorganismes (Zilber-Rosenberg & Rosenberg 2008).

Les variations génétiques de l'hôte passent par un brassage génétique, des mutations ou un réarrangement chromosomique qui doivent se produire sur les lignées germinales pour être transmis et intervenir sur plusieurs gènes pour impacter le phénotype de l'hôte. Selon le temps de génération de l'hôte, l'évolution du génome peut donc être assez lente, notamment dans le contexte des changements climatiques qui entrainent des variations rapides de l'environnement. Les microorganismes passent bien sur par ces mêmes mécanismes pour altérer leurs génomes, mais étant donné leur cycle de vie, la variation du génome est bien plus rapide. On a également tendance à considérer les différents génomes des microorganismes présents dans la communauté bactérienne comme un tout, un méta-génome. Ainsi, ce méta-génome peut varier par modifications individuelles des génomes présents, mais également par amplification d'un des microorganismes (changement dans la proportion des différents génomes), par acquisition d'un nouveau microorganisme (donc d'un nouveau génome) et par transferts horizontaux entre les différentes espèces (avec un microorganisme de passage, par exemple, ce qui permet l'inclusion d'un nouveau gène dans l'hologénome). Tout cela facilite l'acquisition très rapide de nouvelles fonctions.

Ces quatre observations, en plus des découvertes majeures du 20ème siècle [l'origine bactérienne des différents organelles comme la mitochondrie ou le chloroplaste (Margulis 1993); l'omniprésence des microorganismes (McFall-Ngai et al. 2013; McFall-Ngai et al. 2005)] ont donc mené Rosenberg à proposer la théorie de l'hologénome, selon laquelle l'holobionte et son hologénome (somme de l'ensemble des génomes des partenaires en interaction) doivent être considérés comme la véritable unité de la sélection naturelle (Webster & Reusch 2017; Zilber-Rosenberg & Rosenberg 2008).

Cette théorie met en avant qu'au cours de l'évolution, ce n'est pas un génome qui est sélectionné, mais le génome de l'hôte et de l'ensemble des génomes de son

microbiote (également modulés, en composition, abondance relative et activité, par l'environnement expérimenté).

1.5. LES STRATÉGIES ÉVOLUTIVES DANS UN ENVIRONNEMENT CHANGEANT

Face à des modifications profondes et durables de leur environnement, les organismes peuvent soit modifier leur aire de répartition afin de retrouver ailleurs les conditions environnementales initiales, soit faire varier leur phénotype (changement de phénologie, de taille, couleur, sex-ratio, etc.) (Chevin et al. 2013; Gienapp et al. 2008) afin de s'adapter au nouvel environnement.

1.5.1. Changement d'aire de répartition et dispersion larvaire

Face un environnement changeant, le corail étant est un animal sessile, il ne peut pas migrer vers des conditions environnementales plus favorables. A l'image des arbres qui disséminent leurs graines aux grés des vents, les coraux peuvent se « déplacer » via la dispersion larvaire. En effet, même si la reproduction sexuée n'est pas le mode de reproduction privilégié des coraux (McFadden 1997; Richmond & Hunter 1990), elle a ce double intérêt d'augmenter la diversité génétique grâce au mélange des gamètes et de permettre à l'espèce de conquérir de nouvelles aires géographiques.

Cependant, de nombreux obstacles peuvent entraver cette entreprise :

- La larve nageuse, la planula, est soumise à la prédation.
- Elle a une durée de vie limitée, variable selon l'espèce, durant laquelle elle doit survivre, trouver un support pour se fixer, et ce dans un environnement favorable.
- Une fois développée en colonie adulte, elle reste vulnérable plusieurs années et va mettre 3 ou 4 ans avant d'atteindre à son tour la maturité sexuelle.

La dispersion larvaire, peut donc être une solution pour la survie de l'espèce à long terme mais reste une entreprise risquée avec une forte probabilité d'échec. De plus, un récif corallien met des dizaines de milliers d'années à se former. Etant donné la rapidité du réchauffement climatique actuel, de nombreuses espèces abritées par les récifs coralliens mourront avant de pouvoir s'abriter dans des récifs coralliens nouvellement formés. De plus, ces récifs coralliens nouvellement formés seront également soumis au réchauffement climatique qui continue son ascension. Cette stratégie parait donc très incertaine en ce qui concerne les récifs coralliens.

1.5.2. Modifications du phénotype

Dans une population, les modifications phénotypiques peuvent être dues à différents mécanismes d'évolution adaptative : la sélection de gène ou génotypes déjà existant, la sélection de nouveaux gènes (mutation, hybridation) ou bien des changements d'expression des gènes (via les mécanismes épigénétiques par exemple).

1.5.2.1. L'adaptation et la microévolution

L'adaptation, c'est l'apparition chez un organisme d'un caractère nouveau et maintenu par la sélection naturelle (Antonovics 1987).

Si la sélection de gènes déjà existants et le changement d'expression des gènes sont deux mécanismes rapides pouvant se mettre en place en seulement quelques générations, la sélection de nouveaux gènes est un mécanisme beaucoup plus long et aléatoire et demande généralement plusieurs dizaines de générations. Or, dans le contexte du changement climatique, c'est bel et bien de temps qu'il est question puisque l'on assiste à des variations, notamment thermiques, dix fois plus rapides que celles rencontrées durant les derniers milliers d'années (Settele et al. 2014). La sélection de gènes ou génotypes déjà existants correspond à une échelle micro-évolutive et est basée sur une sélection directionnelle rapide (Hoffmann et al. 2011). On peut citer comme exemple la sélection de trait pour une performance thermique sous un environnement stressant thermiquement (Logan et al. 2014).

1.5.2.2. La plasticité phénotypique

Les changements d'expression des gènes représentent de la plasticité phénotypique, définie comme étant la capacité d'un génotype à générer des phénotypes différents en réponse à des changements environnementaux. Ceci ne doit pas être confondu avec le Bet-hedging, qui consiste à générer un phénotype différent à partir d'un seul génotype mais de manière complètement aléatoire afin de créer l'opportunité d'être adapté au futur environnement.

Il est difficile de prévoir l'apparition et l'évolution de la plasticité phénotypique car les mécanismes contrôlant cette capacité ne sont pas clairement définis. Ce que l'on sait sur la plasticité phénotypique, c'est que malgré sa capacité avantageuse à générer une réponse adaptée à un changement environnemental, son coût serait plus important que l'expression constitutive de ce même trait (Kaplan & Pigliucci 2001; DeJong 2005). La plasticité phénotypique ne devrait donc pas être favorisée ou conservée si la

population ne subit pas suffisamment de variations environnementales (Aubin-Horth & Renn 2009). La capacité d'un individu à être plastique devrait donc évoluer différemment chez les différentes espèces/populations selon les pressions de sélection auxquelles elles doivent faire face (Suzuki & Nijhout 2006).

D'après Bradshaw, la plasticité phénotypique serait favorisée par un environnement variable lorsqu'elle concerne des traits non directement liés à la fitness (morphologique ou physiologique) (Bradshaw 1965; Liefting et al. 2009). Les traits directement liés à la fitness comme la reproduction ou la survie sont fixés même dans un environnement variable (Félix & Wagner 2008).

La plasticité et la microévolution sont régulièrement mis en opposition dans les nombreuses études s'intéressant aux changements phénotypiques observés lors des cinquante dernières années (Merilä & Hendry 2014). Bien que ces deux mécanismes ne soient pas complètement opposables (la plasticité peut elle aussi être soumise à une micro-évolution) (Franks et al. 2014), il apparait que la plasticité phénotypique serait la stratégie évolutive qui expliquerait le plus grand nombre de ces variations phénotypiques (Gienapp et al. 2008). D'ailleurs, d'après Hofmann & Todgham (2010), à une échelle de temps de la génération ou de quelques générations, c'est cette stratégie évolutive qui devrait être favorisée par un environnement changeant (Hofmann & Todgham 2010). Étant donné le nombre d'extinctions d'espèces de ces dernières années (Regan et al. 2001), la question qui se pose alors est : quelles sont les espèces et/ou populations capables de générer de la plasticité phénotypique et donc d'augmenter leurs chances de survie dans un contexte d'environnement changeant très rapidement comme celui du changement climatique.

1.6. Mon modèle d'étude de la thermotolérance des coraux : *Pocillopora damicornis*

Pour répondre à cette problématique d'évolution dans un environnement changeant, le modèle corallien est optimal car :

- C'est un animal sessile. Vivant fixé il n'a pas la possibilité d'échapper aux perturbations environnementales. La mise en place d'un phénotype optimal est donc primordial pour sa survie.
- C'est un animal longévive. Certaines colonies peuvent atteindre plusieurs centaines d'années. Du fait de cette longévité, le corail expérimente forcément

une ou plusieurs perturbations environnementales au cours de sa vie, qui vont avoir une influence sur son phénotype à plus ou moins long terme.

- Il possède plusieurs modes de reproduction. On peut générer des individus clonaux à partir du bouturage.

L'espèce utilisée dans mes différentes études est *Pocillopora damicornis.* Cette espèce très abondante est retrouvée sur l'ensemble des récifs de l'Indo-Pacifique, y compris ceux de la Mer Rouge et du Golfe Persique (Veron 2000). En tant qu'espèce pionnière, c'est l'une des premières à coloniser de nouveaux milieux et à fournir, grâce à sa croissance rapide, un support pour la colonisation d'autres espèces coralliennes. Les colonies de *P. damicornis,* habituellement d'un diamètre d'une trentaine de centimètres, peuvent atteindre jusqu'à plusieurs mètres de diamètre dans certains récifs (Hoeksema 2017).

P. damicornis est un corail hermaphrodite « brooder », c'est-à-dire que lors de la reproduction sexuée le polype libère directement la larve nageuse (la planula), la fécondation est donc interne. Les planulas, qui sont émises à une périodicité lunaire (Richmond & Jokiel 1984), contiennent déjà leur algue symbiotique. On a donc une transmission verticale du symbionte. Les *Symbiodinium* généralement associés à *P. damicornis* appartiennent aux clades A, B, C et D, mais on retrouve également du clade G. La larve, grâce à l'énergie fournit par son symbionte, peut nager une centaine de jours pour aller se fixer sur un support et générer le premier polype. Ce laps de temps permet une dispersion des larves sur une longue distance (R. H. Richmond 1987). Il faut en moyenne trois à quatre ans pour qu'une colonie issue d'une planula arrive au stade adulte et soit mature sexuellement. Comme beaucoup de scléractiniaires, *P. damicornis* procède essentiellement à une reproduction asexuée par fragmentation, bourgeonnement ou émission de larves parthénogénétiques (Harrison & Wallace 1990; Yeoh & Dai 2010), beaucoup moins couteuse en énergie que la reproduction sexuée (Robert H. Richmond 1987). Parmi les scléractiniaires, *P. damicornis* est un modèle relativement bien étudié avec de nombreuses publications sur sa physiologie, ses modes de reproduction ou sa diversité génétique (Schmidt-roach et al. 2014; Magalon et al. 2005; Adjeroud & Tsuchiya 1999; Adjeroud et al. 2014). Au niveau moléculaire, quelques études sur son protéome (Mayfield et al. 2017) et son transcriptome (Vidal-dupiol 2011; Vidal-Dupiol et al. 2013; Vidal-Dupiol et al. 2014; Traylor-Knowles et al.

2011; Yuan et al. 2016; Mayfield et al. 2014) ont été publiées et le séquençage du génome est en cours de publication.

1.7. OBJECTIFS DE LA THÈSE : L'ÉVOLUTION DE LA PLASTICITÉ DE L'HOLOBIONTE CORALLIEN DANS LE CONTEXTE DU RÉCHAUFFEMENT CLIMATIQUE :

Comme décrit dans cette introduction, on assiste depuis plus d 'un siècle à un changement climatique sans précédent dans l'histoire de la planète. L'augmentation des gaz à effet de serre, principal moteur de ce bouleversement climatique, a notamment induit un réchauffement climatique de la surface terrestre et des eaux marines de surface de 1°C. Cette augmentation des températures a déjà eu de lourdes répercussions sur la biodiversité avec des extinctions de masse et la perturbation (voire dégradation) de nombreux écosystèmes. La dégradation des récifs coralliens par des événements de blanchissement de masse répétés des coraux scléractiniaires (support physique des écosystèmes coralliens) est probablement l'une des conséquences les plus alarmantes du réchauffement climatique. Malheureusement, la hausse des températures continue et devrait accélérer et atteindre une augmentation de 1°C d'ici l'année 2100 dans le meilleur des cas et 4°C si on en croit les prédictions les plus pessimistes.

Des mesures de sauvegarde des récifs coralliens sont donc plus que nécessaires pour prévenir les futurs événements de blanchissement de masse et les mortalités associées. L'un des espoirs de sauvegarde des récifs réside dans l'existence d'espèces, populations ou individus qui présentent des phénotypes très résistants au stress thermique.

La problématique principale de cette thèse est donc de comprendre comment, chez l'holobionte corallien, des phénotypes thermo-tolérants ont pu être sélectionnés et quels sont les mécanismes qui confèrent cette thermo-tolérance.

Pour ce faire, nous avons mené des approches comparatives de coraux issus de différentes populations soumises à des régimes thermiques différents. Dans un premier chapitre, nous présenterons une approche holistique comparative de la thermotolérance de deux populations de *P. damicornis* soumises à des régimes thermiques contrastés. Dans un deuxième chapitre, nous avons étudié le lien entre régime thermique et la composition du microbiote (bactérien et zooxanthelles) entre différentes populations de la même espèce.

A. Caractérisation de la thermo-tolérance d'holobiontes coralliens provenant d'environnements thermiquement contrastés chez l'espèce *Pocillopora sp.*

Dans ce premier chapitre, je pose l'hypothèse que les populations coralliennes thermo-tolérantes devraient présenter une plus grande plasticité phénotypique que les autres.

Cette hypothèse est formulée à partir du constat des nombreux changements phénotypiques déjà observés dans le milieu naturel dans la seconde moitié du siècle dernier; et des principales théories sur l'évolution de la plasticité phénotypique. Ces dernières prédisent que la plasticité phénotypique serait favorisée dans des environnements fluctuants, car malgré son coût énergétique présumé important, elle apporterait un avantage pour la survie des organismes en leur permettant la mise en place du phénotype le plus optimal dans chacun des environnements.

Pour vérifier cette hypothèse, nous avons choisi de procéder à une étude comparative (via la comparaison de deux populations issues d'environnements thermiquement contrastés) et intégrative (tous les compartiments de l'holobionte sont pris en compte) de la réponse au stress thermique chez l'holobionte corallien en utilisant des méthodes s'appuyant sur le séquençage à haut débit.

B. Analyse de l'influence du clade hôte, de la géographie et du régime thermique sur les communautés bactériennes et de *Symbiodinium* du corail scléractiniaire *Pocillopora damicornis sensu lato*.

L'holobionte corallien est un assemblage d'une multitude d'organismes : corail hôte, algue symbiotique et bactéries pour ne citer qu'eux. Selon la théorie de l'évolution de l'hologénome, ils peuvent tous avoir un impact sur le phénotype de l'hôte et donc sur sa valeur sélective. Bien que la nature des algues symbiotiques a déjà montré son influence sur la thermo-tolérance, la photo-tolérance et la croissance, on ne connait encore que peu de choses sur ce qui influence l'association entre une espèce corallienne et tel ou tel clade de *Symbiodinium*. Il en va de même pour la communauté bactérienne, pour laquelle on ne sait pas dans quelle mesure sa composition pourrait influencer la thermo-tolérance de l'hôte, mis à part le fait qu'une rupture de cette composition peut favoriser l'apparition et/ou l'augmentation des communautés bactériennes pathogènes et engendrer des maladies.

C’est donc toujours dans l’optique de mieux comprendre l’origine et la nature d’une meilleure thermo-tolérance chez l’holobionte corallien, que dans ce chapitre, nous avons cherché à connaitre les différents paramètres biotiques (haplotype de l’hôte) et abiotiques (géographie, température) qui pourraient influencer la distribution des micro-organismes symbiotiques du corail et dans quelle mesure ces communautés symbiotiques pourraient avoir une influence sur la plasticité phénotypique de l’holobionte corallien face à un environnement thermique variable.

2. CHAPITRE 1: CARACTÉRISATION DE LA THERMO-TOLÉRANCE D'HOLOBIONTES CORALLIENS PROVENANT D'ENVIRONNEMENTS THERMIQUEMENT CONTRASTÉS CHEZ L'ESPÈCE *POCILLOPORA SP.*

Ce chapitre correspond à un article soumis à BMC genomics (2017).

Dans le contexte du réchauffement climatique actuel, les récifs coralliens subissent des événements de blanchissement de masse de plus en plus fréquents et intenses. Malgré ces phénomènes entrainant des mortalités de masse, certaines espèces/populations ou individus, parmi les coraux scléractiniaires, sont capables de résister ou résilier face à ces situations de stress. D'après les théories de l'évolution et les nombreuses observations faites depuis l'accélération du réchauffement climatique, le mécanisme adaptatif le plus efficace face à ces changements brutaux serait la plasticité phénotypique. Cette capacité à générer différents phénotypes à partir d'un seul génotype et en fonction des variations environnementales pourrait être favorisée chez des populations expérimentant un environnement plus fluctuant. Dans le but de vérifier cette hypothèse et de comprendre les mécanismes sous-jacents qui permettent la mise en place d'une thermo-tolérance chez l'holobionte corallien, nous avons sélectionné deux populations de *Pocillopora sp*. provenant chacune d'un récif corallien soumis à un régime thermique peu fluctuant (population thermo-sensible) ou beaucoup plus fluctuant (population thermo-tolérante). Afin de comparer ces populations dans leur réponse maximale au stress thermique, elles ont été soumises à un stress thermique écologiquement réaliste en milieu contrôlé correspondant au régime thermique de leur milieu naturel. L'holobionte corallien a ensuite été analysé pour trois colonies de chaque population en condition contrôle (température moyenne des mois chauds) et en condition de stress (température maximale avant l'apparition du collapse physiologique). Tous les compartiments biologiques, pouvant avoir une influence sur la thermo-tolérance de l'holobionte, ont été pris en compte dans cette analyse : la composition en *Symbiodinium* (condition *In Situ*, contrôle et stress), la composition de la communauté bactérienne (condition *In Situ*, contrôle et stress) et le transcriptome de l'hôte corail (condition contrôle et stress). La composition du microbiote de chaque population est cohérente avec celles des autres études (dominance du genre

Endozoicomonas parmi les gamma-protéobactéries hautement majoritaires) et reste stable durant le stress thermique ; tout comme les populations de *Symbiodinium* dont les clades D1a et C1, qui sont respectivement retrouvés chez la population thermo-tolérante et thermo-sensible. Étant donné la stabilité des communautés symbiotiques au cours du stress et l'absence de blanchissement, la réponse au stress de l'hôte corallien ne sera pas due à une rupture de l'intégrité de l'holobionte, mais bien à une réponse au stress thermique. Au travers de l'étude du transcriptome de l'hôte, nous avons pu montrer une plus grande plasticité transcriptomique chez la population thermo-tolérante (plus de gènes régulés et des fluctuations d'expression plus importantes pour les gènes modules chez les deux populations) ainsi qu'un mécanisme de « frontloading » (une expression basale constitutive et plus forte chez la population thermo-tolérante pour la majorité des gènes qui sont induits chez la population thermo-sensible). L'analyse de la fonction de ces gènes régulés a montré qu'ils correspondaient à des fonctions attendues comme des protéines impliquées dans l'induction de l'apoptose (TNRF et TRAF) ou des protéines chaperonnes HSPs. Nous avons également mis en évidence un mécanisme de « trade-off » avec, par exemple, la répression de la biominéralisation. De manière intéressante, on observe une forte induction des gènes impliqués dans la régulation épigénétique et une augmentation de l'expression de gènes de rétrotransposons chez la population thermo-tolérante. Au vu de ces résultats, notre hypothèse est que le frontloading observé chez la population thermo-tolérante serait une assimilation de la plasticité favorisée par un environnement fluctuant. Nos résultats suggèrent que cette plasticité serait générée grâce aux mécanismes épigénétiques fortement actifs chez cette population. Il serait intéressant d'approfondir ces aspects en étudiant l'impact de stress récurrents sur des populations clonales de coraux afin d'évaluer les modifications épigénétiques induites et leur lien avec une éventuelle amélioration de la thermo-tolérance. De telles études, ainsi que les résultats obtenus dans le cadre de notre travail, pourrait permettre d'orienter des mesures futures de sauvegarde des récifs coralliens au travers de transplantations (de populations thermo-tolérantes) ou bien encore d'inductions artificielles de thermo-tolérance par des stress thermiques répétés.

CHARACTERIZATION OF THE THERMOTOLERANCE OF THE HOLOBIONT POCILLOPORA SP. FROM CONTRASTING TEMPERATURE ENVIRONMENTS

AUTHORS

Brener-Raffalli K.[1] (kelly.brener@univ-perp.fr), Vidal-Dupiol J.[2] (Jeremie.Vidal.Dupiol@ifremer.fr), Adjeroud M.[3] (mehdi.adjeroud@ird.fr), Romans P.[4] (pascal.romans@obs-banyuls.fr), Rey O.[1] (olivier.rey@univ-perp.fr), Pratlong M.[5,6] (marine.pratlong@gmail.com), Haguenauer A.[5] (anne.haguenauer@imbe.fr), Pillot R.[4] (remi.pillot@obs-banyuls.fr), Feuillassier L.[4] (lionel.feuillassier@mnhn.fr), Claereboudt M.[7] (michelc@squ.edu.om), Aurelle D.[5] (didier.aurelle@imbe.fr), Pontarotti P.[6] (pierre.pontarotti@imbe.fr), Mitta G.[1] (mitta@univ-perp.fr) and Toulza E.[1]* (eve.toulza@univ-perp.fr)

AFFILIATIONS

1 Université de Perpignan Via Domitia, CNRS UMR 5244, Interactions Hôtes-Pathogènes-Environnements (IHPE), 66860 Perpignan France.

2 IFREMER, IHPE UMR 5244, Univ. Perpignan Via Domitia, CNRS, Univ. Montpellier, F-34095 Montpellier, France.

3 Institut de Recherche pour le Développement, UMR 9220 ENTROPIE, Laboratoire d'Excellence "CORAIL", UPVD 52 Avenue Paul Alduy, 66860 Perpignan, France.

4 Sorbonne Universités, UPMC Univ Paris 06, UMS 2348, Centre de Ressources Biologiques Marines, Observatoire Océanologique, F-66650 Banyuls/Mer, France.

5 Aix-Marseille Université, CNRS, IRD, Avignon Université, IMBE UMR 7263, 13007, Marseille, France.

6 Aix-Marseille Université, CNRS UMR 7353, Équipe Evolution Biologique et Modélisation, Campus Saint-Charles, 3 place Victor Hugo, 13331 Marseille France.

7 Department of Marine Science and Fisheries, College of Agricultural and Marine Sciences, Sultan Qaboos University, Box 34, Al-Khod, 123, Sultanate of Oman.

* Corresponding author

ABSTRACT

Background: Ecosystems worldwide are being affected by climate change. For example, the diverse ecosystems of coral reefs are globally threatened by increasing sea surface temperatures. However, phenotypic plasticity provides the potential for organisms to respond rapidly and effectively to environmental change, and is predicted to be favored in variable environments. In this study we investigated the thermal stress response of *Pocillopora* sp. corals from contrasting thermal environments (more stable in New Caledonia, more variable in Oman) by exposing them to an ecologically realistic thermal stress. We compared the physiological state, bacterial and *Symbiodinium* communities (using 16S and ITS2 metabarcoding), and gene expression levels (using RNAseq) of control corals (held at the mean temperature of the warmer months in their respective natural environments) with corals exposed to thermal stress conditions (the temperature just below the first signs of physiological collapse).

Results: Corals of the two populations remained apparently normal and had open and colored polyps during exposure to the experimental thermal stress, and no change in bacterial and *Symbiodinium* community composition was found. In contrast, the populations differed in their transcriptome responses. The corals from the thermotolerant population had a more plastic transcriptome, having more differentially expressed genes, greater changes in expression levels, and frontloaded genes (genes having a higher and constant expression level) compared with those from the thermosensitive population. In terms of biological function we observed a trade-off between stress response (including induction of tumor necrosis factor receptor, heat shock proteins, and detoxification of reactive oxygen species) and morpho-anatomical functions. Gene regulation (transcription factors, chromatin modeling, mobile elements) appeared to be highly enhanced, indicating possible epigenetic regulation.

Conclusion: These results provide new insights into the balance between plasticity and frontloading, and provides insights into the origin and evolution of these strategies.

Keywords: phenotypic plasticity, frontloading, coral holobiont, *Pocillopora* sp., *Symbiodinium*, RNAseq, metabarcoding, thermal stress

2.1. BACKGROUND

In the context of global warming, biodiversity has been subject to environmental changes that have never occurred previously. For example, since the last deglaciation (11,650 years ago) the temperature has increased on average by 0.3–0.8°C per thousand years, with two extreme values of 1°C and 1.5°C per thousand years (Shakun et al. 2012). In comparison, the land and ocean surface temperatures have increased by 0.85°C since 1880, and a 1–4°C increase is expected over the next 100 years (Stocker & T.F., D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia 2015). The recent increase in temperature has already led to a changes in biocenosis, and therefore in ecosystems (Parmesan 2006).

These temperature increases have had major impacts on species of great ecological importance, including reef-building corals, which are the biological and physical support of the coral reef ecosystem. These corals support a large diversity of marine organisms (~600 coral species, ~2000 fish species, and ~5000 mollusk species (Veron & Stafford-Smith 2000; Reaka-Kudla 1997)), but are exceptionally vulnerable to climate change (Wilkinson & Network 2008; Burke, World Resources Institute., M. Spalding, et al. 2011). In recent decades the increased frequency and severity of abnormally high temperatures have been responsible for mass bleaching events (expulsion of the symbiotic alga *Symbiodinium*), and have resulted in the permanent degradation of > 20% of coral reefs worldwide (Burke, World Resources Institute., M. Spalding, et al. 2011; Bellwood et al. 2004; Hoegh-Guldberg et al. 2007).

However, some closely related coral populations have been found to be more tolerant of heat stress than others, providing some hope for coral survival based on the capacity of these organisms to adapt (Palumbi et al. 2014; Dixon 2015). The mechanisms underlying coral thermotolerance have been investigated at small spatial scales using transplantation experiments in which the responses of corals in a highly fluctuating and/or high temperature environment were compared with corals in a lesser fluctuating and/or lower temperature environment. These studies have highlighted the fundamental role of phenotypic plasticity in coral adaptation and acclimation (Palumbi et al. 2014; Dixon 2015). However, the “universality” of these mechanisms and the extent of adaptation possible in a population needs to be defined. Populations of some species subject to extreme temperatures and temperature variations can be found at the limits of their geographical range. The mechanisms underlying thermotolerance and the

extent of adaptability in such coral populations can be investigated by comparisons with populations inhabiting areas having optimal temperature conditions (Riegl et al. 2011).

All partners (bionts) involved in a stable symbiosis effectively form the entire organism, and constitute what is referred to as the holobiont (Margulis & Fester 1991). A decade after this term was defined its use has been particularly popularized in reference to corals (Rohwer et al. 2002), and subsequent research has led to the hologenome theory of evolution (Rosenberg et al. 2007; Zilber-Rosenberg & Rosenberg 2008). In this context the hologenome is defined as the sum of the genetic information of the host and its symbiotic microorganisms. Phenotypes are thus the product of the collective genomes of the holobiont partners, which constitute the unit of biological organization and thus the object of natural selection (McFall-Ngai et al. 2013; Bordenstein et al. 2015; Guerrero et al. 2013), although the role of the coral-associated microbes and their potential to modify holobiont adaptability remain unclear. It is clear that the genotype, or association of genotypes, of the photosynthetic mutualist *Symbiodinium* sp. plays a key role in the thermotolerance of the holobiont (Hume et al. 2015). There is less certainty about the involvement of the coral bacterial community, although some studies revealing differences in function of the disease states(Cróquer et al. 2013; Meyer et al. 2016; Sato et al. 2010) or the environmental conditions (Webster & Bourne 2007; Pantos et al. 2015; Hernandez-Agreda et al. 2017) highlights the need for further investigations. In this context, it is necessary to consider the coral microbial community in studies of the adaptability and thermotolerance of the coral holobiont.

The aim of the present study was to investigate the molecular mechanisms underlying the thermotolerance of coral holobionts adapted to different temperature regimes. We undertook an integrative analysis of the response of two populations from environments having contrasting temperature conditions. We used the scleractinian coral *Pocillopora damicornis sensu lato* as a model species because it has a broad spatial distribution throughout the Indo-Pacific (Veron & Stafford-Smith 2000), enabling access to populations subject to high and fluctuating temperatures and populations having optimal temperatures for coral reef development (lower temperatures and smaller fluctuations). In addition, this species is considered to be one of the most environmentally sensitive corals (van Woesik et al. 2011) but its widespread distribution suggests exceptional potential for adaptation. To avoid biases inherent in transplantation-based field experiments resulting from environmental factors other

Table 1 : Temperature regime in the natural environment of the two coral populations

	New Caledonia	Oman
Mean SST (°C)	24.8	27.9
Variance (°C)	2.7	9.5
Min SST (°C)	22.6	22.1
Max SST (°C)	27.1	33.2
Mean SST of warmer months (°C)	26.8	31.3
Mean SST of cooler months (°C)	22.8	23.8

than temperature, we undertook our comparative study in a controlled environment in which we imposed ecologically realistic conditions to study the thermal stress response. This approach included holding both populations of corals under the same environmental conditions for a long period (six months) prior to initiating the experiments, to enable acclimation of the corals following collection from the field. To characterize the holobiont response we used an RNA-seq approach to follow the host response at the population level, and used a metabarcoding approach to study changes in the holobiont microbial community (*Symbiodinium* sp. and bacterial populations).

2.2. METHODS

2.2.1. Coral sampling and maintenance

The sampling was designed to enable the study of populations derived from two contrasting temperature environments. To distinguish population and individual impacts on thermotolerance, we sampled 3 colonies from each population.

For each population, *Pocillopora* sp. colonies were collected in June 2014 from three sampling sites in Oman (Om), representing the population subject to a variable thermal environment, and in December 2014 from three sampling sites in New Caledonia (NC), representing the population subject to a more stable temperature environment (see Table 1 for the temperature regime of each population).

To decrease the probability of collecting colony clones (Adjeroud et al. 2014), the colonies (20 cm in diameter) chosen for sampling at each site were separated by at least 10 m. Immediately following collection a 1 cm tip of each colony was excised, rinsed three times in filtered seawater (0.22 µm), and placed in RNAlater solution (Sigma Aldrich) for the *in situ* microbiota analysis. The remainder of the colony was fragmented into 20 branches each of 10 cm length, and these were individually placed in plastic bags containing oxygenated seawater, and transported by aircraft to the research aquarium of the Banyuls-sur-Mer oceanographic observatory (France). The coral branches were maintained in artificial seawater (Seachem Reef Salt) at 26°C, and supplied daily with *Artemia* nauplii to satisfy their heterotrophic demand. The conditions in the maintenance tank were controlled to reproduced the physicochemical parameters of coral reefs (pH: 8.2; salinity: 36 g/L; light Intensity: 150 to 250 photons/m^2/s; photoperiod: 14h night/10h day; kH: 6–7.5 dkH; calcium concentration: 410–450 mg/L;

inorganic phosphate concentration: < 0.1 mg/L; magnesium concentration: 1300–1400 mg/L; nitrate concentration: < 5mg/L).

Following acclimation of the coral branches for at least two months under these maintenance conditions the branches were cut to produce several clones from each colony. This involved cutting nubbins from the mother colony using a stainless steel clamp to provide fragments ≤ 3 cm in length. These were individually fixed to a support (here a p1000 tip) using an epoxy adhesive. To facilitate healing (evident as tissue extending to cover the epoxy adhesive) prior to use in the experiment, the nubbins were maintained under the above maintenance conditions for at least one month following cutting.

2.2.2. Host haplotype

To assess the extent to which the two populations were phylogenetically related we analyzed the mitochondrial variable open reading frame (ORF) sequence of each colony, and aligned these against known sequences.

The ORF was amplified using FATP6.1 and RORF primers (Flot & Tillier 2007). The product was sequenced using Sanger sequencing, and the corresponding protein coding sequences were analyzed using MEGA version 6 (Tamura et al. 2013). Sequence alignment was performed using Muscle. The best model (Hasegawa-Kishino-Yano) was selected for the lowest BIC (Bayesian Information Criterion). A maximum likelihood tree was generated from the best model using 1000 bootstrap replicates.

2.2.3. Ecologically realistic thermal stress

The aim of this experiment was to compare the response to thermal stress of two coral populations having the same physiological state, to investigate the molecular mechanisms involved during the stress exposure.

The experimental design comprised eight tanks of 53 L volume (four for each population) in which the seawater was continuously recycled. The water was sterilized using UV (rate 3200 l/H) and renewed twice per hour in each tank (recirculation rate: 100 L/h in each tank). The eight tanks shared the same seawater but were individually controlled (HOBBY Biotherm Pro, model 10892; 500W AquaMedic titanium heater), and the temperature was monitored using a HOBO TidbiT v2 logger. At least two nubbins (depending on the number of clones available) from three colonies per population were randomly placed in each tank (four per population) and acclimated for 2 weeks at 26°C.

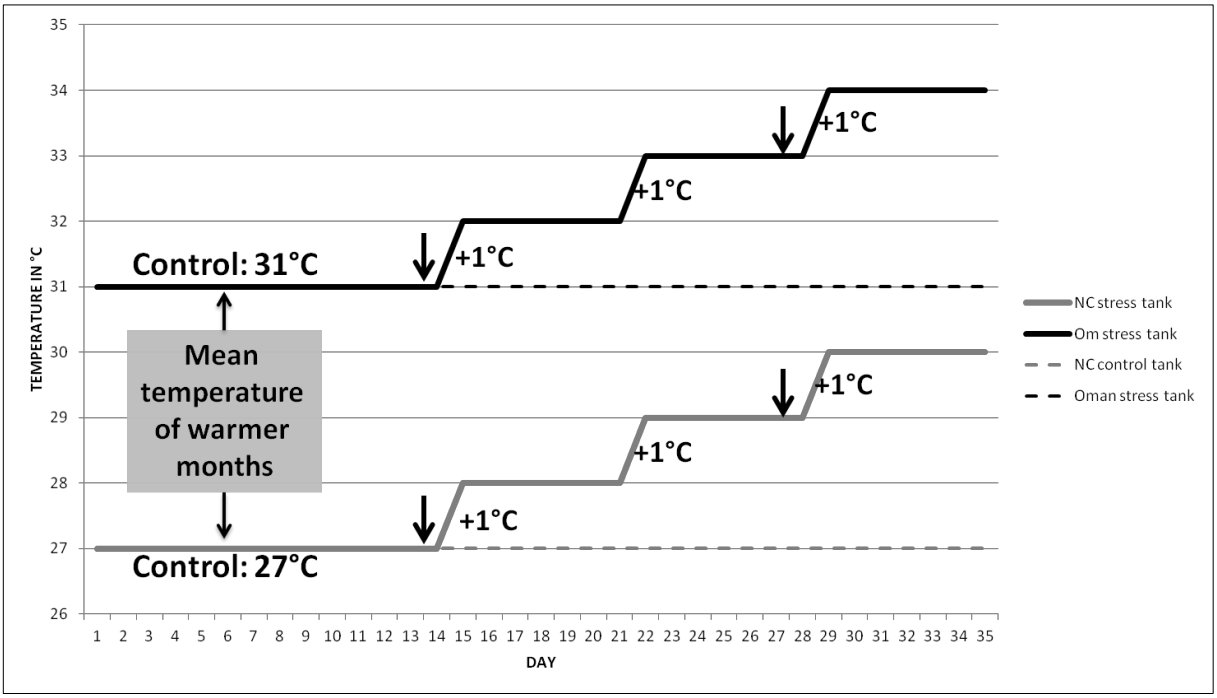


Figure 1 : Experimental design for exposure of corals from two different populations to an ecologically realistic thermal stress. The arrows represent points at which nubbins were used for analysis.

In the subsequent stress experiment, three tanks (stress condition) per population were subjected to a gradual temperature increase during the experiment, while the fourth tank (control) was maintained at the control temperature (Fig. 1). The control temperature was the mean water temperature for the three warmer months at the coral source location (Table 2): 31°C for the population from Om, and 27°C for the population from NC. In the stress treatment tanks the temperature was gradually increased by 1°C over 5 h each week until physiological collapse of the corals became evident (polyps closure, bleaching or necrosis), as described by Vidal-Dupiol et al. 2009 (Vidal-dupiol et al. 2009). Samples that were used to perform analyses were those samples in each tank just before the first increase of temperature from the control temperature, and just before the temperature that produced the first signs of physiological collapse (stress temperature). Thus, for each condition (control and stress) we obtained three replicates of each colony (three colonies per population) to give a total of 36 samples (2 populations × 3 colonies × 2 conditions × 3 replicates).

During the experiment a photographic record was kept to evaluate the general health of the nubbins at the various temperatures. This involved photographing one nubbin of each colony in each tank at noon each day (just prior to feeding) throughout the period of the experiment.

2.2.4. Microbial community analysis using MiSeq 16S and ITS2 metabarcoding

The aim of this analysis was to investigate the composition and dynamics of the two principal symbiotic coral communities (bacterial and *Symbiodinium*) during thermal stress.

2.2.4.1. DNA extraction

At each sampling occasion a coral tip (1 cm) was excised for metabarcoding analysis (bacterial and *Symbiodinium* assemblages). The fragment was washed three times in filtered seawater, placed in RNAlater solution (Sigma Aldrich) at 4°C for 24 h, then stored at −20°C until DNA extraction. Extractions were performed using the DNeasy blood and tissue kit (Qiagen) following the manufacturer's instructions. DNA was quantified by spectrophotometry (NanoDrop).

2.2.4.2. Amplicon Sequencing

A bacterial 16S rDNA amplicon library was generated for each of the 42 samples (one *in situ* condition, three control conditions and three stress conditions per colony,

three colonies per population, two populations), using the 341F (CCTACGGGNGGCWGCAG) and 805R (GACTACHVGGGTATCTAATCC) primers, which target the variable V3/V4 loops (Klindworth et al. 2013). *Symbiodinium* assemblages were analyzed using ITS2 (internal transcribed spacer of the ribosomal RNA gene) amplicon libraries and specific primers targeting a sequence of approximately 350 bp (ITS2-F GTGAATTGCAGAACTCCGTG; ITS2-R CCTCCGCTTACTTATATGCTT) (Lajeunesse & Trench 2000; Quigley et al. 2014). For both markers, paired-end sequencing using a 250 bp read length was performed on the MiSeq system (Illumina) using the v2 chemistry, according to the manufacturer's protocol. A paired-end fragment library (2 × 250 bp) was constructed and sequenced on the Illumina MiSeq platform at the Centre d'innovation Génome Québec and McGill University, Montreal, Canada.

2.2.4.3. Bioinformatic analysis

The FROGS pipeline (Find Rapidly OTU with Galaxy Solution) implemented on a Galaxy platform (http://sigenae-workbench.toulouse.inra.fr/galaxy/) was used for data processing (Escudié et al. 2015). In brief, paired reads were merged using FLASH (Magoc & Salzberg 2011). After de-noising and removal of primer/adapters using cutadapt (Martin 2011), *de novo* clustering was performed using SWARM. This uses a local clustering threshold with an aggregation distance (d) of 3. Chimeras were removed using VSEARCH (Rognes et al. 2016). We filtered the dataset for singletons and performed affiliation using Blast+ against the Silva database (release 128, September 2016) for 16S amplicons (Altschul et al. 1990). For ITS2 metabarcoding, the *Symbiodinium* type was assessed using Blast+ against an in-house database of *Symbiodinium* reference sequences. An OTU table in standard BIOM format with taxonomic affiliation was produced for subsequent analysis.

For community composition analysis we used the phyloseq R package (McMurdie et al. 2013) to infer alpha diversity metrics at the OTU level, and beta diversity (between sample similarity) from the OTU table. Community similarity was assessed by Principal Coordinate Analysis (PCoA) using the Bray-Curtis distance matrices.

We performed one-way ANOVAs to compare alpha or beta diversity metrics among the groups of samples. Corrections based on multiple testing were performed using the Benjamini-Hochberg false discovery rate procedure (Benjamini & Hochberg 1995). For all analyses, the threshold significance level was set at 0.05.

2.2.5. Transcriptome analysis

The aim of this analysis was to compare the transcriptomes of the two populations under the two different temperature conditions (control and stress), and to identify specific molecular markers for each population based on its temperature history.

2.2.5.1. RNA extraction

Total RNA was extracted from each coral sample using TRIzol reagent (Invitrogen), according to the manufacturer's protocol. The quantity and integrity of the total RNA extracted was checked using an Agilent 2100 Bioanalyzer (Agilent Technologies) (mean RIN = 7.5). The paired-end fragment library (2 × 100 bp) was constructed and sequenced on the Illumina HiSeq 2000 platform at the Centre d'Innovation Génome Québec and McGill University, Montreal, Canada.

2.2.5.2. Bioinformatic analysis

Fastq read files were processed on a local Galaxy platform (Afgan et al. 2016). Quality control and initial cleaning of the reads was performed using the filter by quality program (version 1.0.0) based on the FASTX-toolkit (Blankenberg et al. 2010). Reads having fewer than 90% of bases having a Phred quality score ≤ 26 were discarded (probability of 2.5 incorrect base call out of 1000, and a base call accuracy of 99.75%). Adaptors used for sequencing were removed using the cutadapt program version 1.6 (Martin 2011). All paired-end reads were aligned using RNAstar software under default parameters, with at least 66% of the bases being required to align to the reference, and a maximum of ten mismatches per read (Dobin et al. 2013). The *Pocillopora* reference genome used in this study (manuscript in preparation) consisted of a draft assembly of 25,553 contigs (352 Mb total) and N50 = 171,375 bp. The resulting transcriptome served as the reference for reads mapping, and a GTF annotation file was constructed using cufflink/cuffmerge (Trapnell et al. 2010). HTseq was used to produce count files for genes (Anders et al. 2015). The DESeq2 package was used to estimate the normalized abundances of the transcripts, and to calculate differential gene expression for samples between the control temperature and the stress temperature for each population (Love et al. 2014), using default parameters. To analyze these genes by expression pattern, the genes were grouped manually according to their differential expression levels in one or both populations. Six groups were generated: common

overexpressed genes, NC-specific overexpressed genes, Om-specific overexpressed genes, common underexpressed genes, NC-specific underexpressed genes, and Om-specific underexpressed genes. Cluster 3.0 (de Hoon et al. 2004) and Treeview (Saldanha 2004) were used to build the heatmap.

2.2.5.3. Discriminant analysis of principal components (DAPC)

Our aim was to quantify differences associated with thermal stress in genome-wide transcriptome plasticity between coral populations from Om and NC. To achieve this we performed a discriminant analysis of principal components (DAPC) approach based on a log-transformed transcript abundance matrix (containing 26,600 genes) obtained from the 36 individuals (i.e. 9 control and 9 stressed replicates per population), as described previously (Kenkel & Matz 2016b). Specifically, we ran a DAPC analysis using the resulting log2 transformed dataset for the coral populations from NC and Om reared in controlled conditions as predefined groups in the adegenet package implemented in R (Jombart et al. 2010). Only 2 principal components and a single discriminant function were retained. We then predicted the position of stressed individuals from both populations (Om and NC) onto the unique discriminant function of the DAPC.

To compare the genome-wide gene expression plasticity of the populations in response to heat stress we compared the absolute mean DAPC scores between populations for each experimental group (control versus thermal stress) using the non-parametric Wilcoxon test. We also ran a general linear model (GLM) using the DAPC scores as the dependent variable, and accounted for the population of origin (NC and Om), the conditions (control and thermal stress), and the interaction between populations and conditions as explanatory variables. In particular, as an indicator of significant differences in the genome-wide gene expression reaction norms between populations we tested for significant effects of the interaction between the population and the condition effects.

2.2.5.4. GO enrichment of differentially expressed genes

The transcriptome was annotated *de novo* using a translated nucleotide query (blastx; (Altschul et al. 1990)) against the non-redundant protein sequence database (nr). The 25 best hits were then used to search for gene ontology terms using the Blast2Go program (Conesa et al. 2005). Lists of significantly up-regulated and down-

regulated genes were subjected to GO enrichment analysis by comparison with all expressed gene using a Fischer exact test and a FDR value of 0.05.

2.3. RESULTS

2.3.1. Host haplotype

To evaluate the phylogenetic relatedness of the two *Pocillopora* sp. populations used in the study we analyzed the mitochondrial variable open reading frame (ORF) for each individual analyzed (3 colonies for each population). Sequence alignment with referenced haplotypes (Schmidt-roach et al. 2014) showed that the colonies from the NC population belonged to clade 1, whereas colonies from the Om population belonged to clade 2.

2.3.2. Ecologically realistic thermal stress

Following collection from the field the corals from the different populations were acclimated in the same controlled conditions prior to exposure to an ecologically realistic thermal stress. Firstly, the individuals from NC and Om were maintained at 27°C and 31°C, respectively, which correspond to the mean temperature of the warmer months (Fig. 1). In the thermal stress treatments the temperature was then increased by 1°C every week until the first signs of physiological collapse occurred. Visual and photographic monitoring was used to assess coral health. The first sign of coral stress was the closure of polyps at day 30 for both populations, which occurred at 30°C and 34°C for the NC and Om populations, respectively. These temperatures corresponded to the warmest temperature experienced by these populations in the field (Table 1). No signs of stress were observed in control corals throughout the experiment.

To measure the response of the holobiont of each population to a temperature just less than that causing physiological stress we sampled NC and Om colonies at 29°C and 33°C, respectively. NC and Om control coral colonies were collected from the tanks maintained at 27°C and 31°C, respectively.

2.3.3. Microbiota composition

To assess the effect of thermal stress on the microbial assemblages of the coral holobionts we characterized the structure and dynamics of the bacterial and *Symbiodinium* communities during stress exposure using 16S and ITS2 metabarcoding, respectively, and compared these with barcoding for the appropriate controls. *In situ*

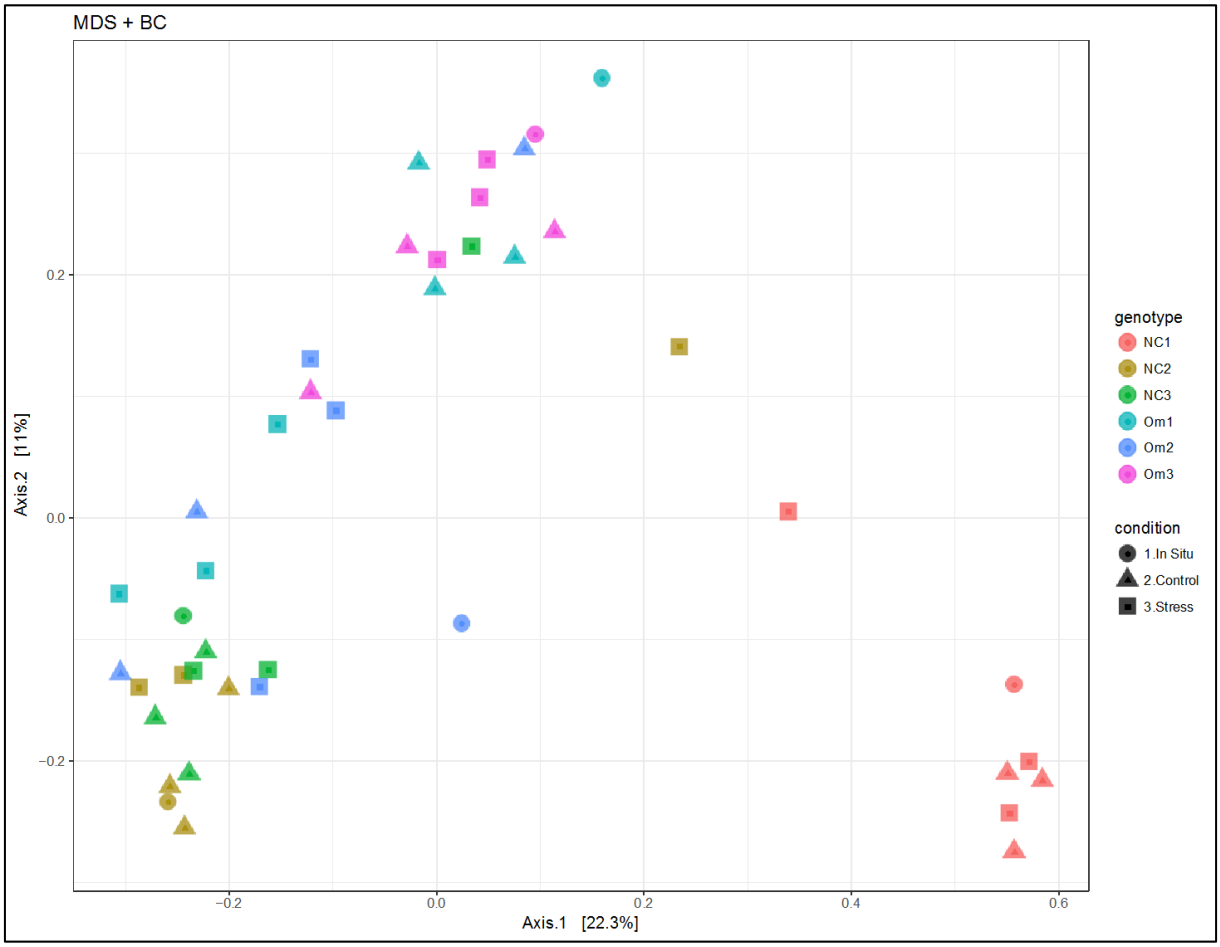


Figure 2 : Principal coordinate analysis plot for Bray-Curtis distances of the bacterial composition of each colony in each condition. Different colors represent different colonies, the squares represent the in situ conditions, the circles represent the control conditions, and the stars representing the stress conditions.

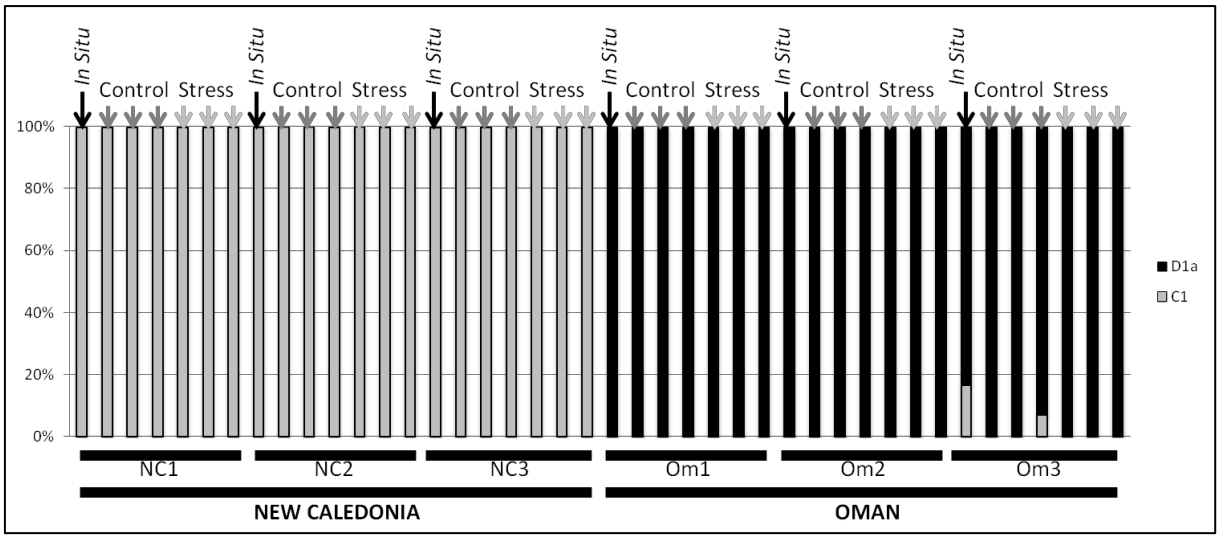


Figure 3 : Sub-clade composition of the *Symbiodinium* community in each population.

sampling was performed for each individual and each population to verify that the microbiota were not altered by the transfer of corals from their natural environment to the test tanks.

2.3.3.1. Bacterial communities

Analysis of the bacterial community structure and dynamics during exposure to stress was performed using 16S V3-V4 metabarcoding on 42 samples (one *in situ*, three control and three stress samples per colony).

Following cleaning and singleton filtering, we obtained a total of 5,308,761 sequences corresponding to 15,211 OTUs. The class composition is shown in Figure S1. In all samples the class Gammaproteobacteria was dominant (77.7%), particularly the genus *Endozoicomonas* (44.7% of the sequences); this genus is known to be an endosymbiont of numerous scleractinians (Neave, Rachmawati, et al. 2016). The PCoA of Bray-Curtis distances for all colonies (Fig. 2) showed no grouping based on conditions. We observed a loose grouping based on population and colony, especially for colony NC1, which appeared to have a more specific microbiota composition, as it had a different grouping associated with the first axis, which explained 22.3% of the variance. The one-way ANOVA for alpha diversity (Shannon index) revealed significant differences in the microbiota diversity between populations ($p = 0.00258$) and colonies ($p = 0.00129$), but no differences among the *in situ*, control, and stress conditions ($p = 0.885$). Similar results were obtained for the ANOVA for beta diversity (Bray-Curtis distance) (between populations: $p = 0.001$; between colonies: $p = 0.001$; between conditions: $p = 0.554$); the ANOVA results are summarized in Additional File 2. Thus, the bacterial composition appeared to be relatively specific to each colony within each population, but remained stable during the transition from the natural environment to artificial seawater, and during thermal stress exposure.

2.3.3.2. *Symbiodinium* assemblages

Analysis of the *Symbiodinium* composition was performed using ITS2 metabarcoding, which facilitated intra-clade resolution.

Removal of those OTUs having an abundance of < 1% left only 4 OTUs for all samples. Two of these corresponded to clade C1, while the other two corresponded to clade D1a. Clade D1a was highly dominant in the Om colonies, whereas clade C1 was almost exclusive to the corals from NC (Fig. 3). In contrast to the bacterial community,

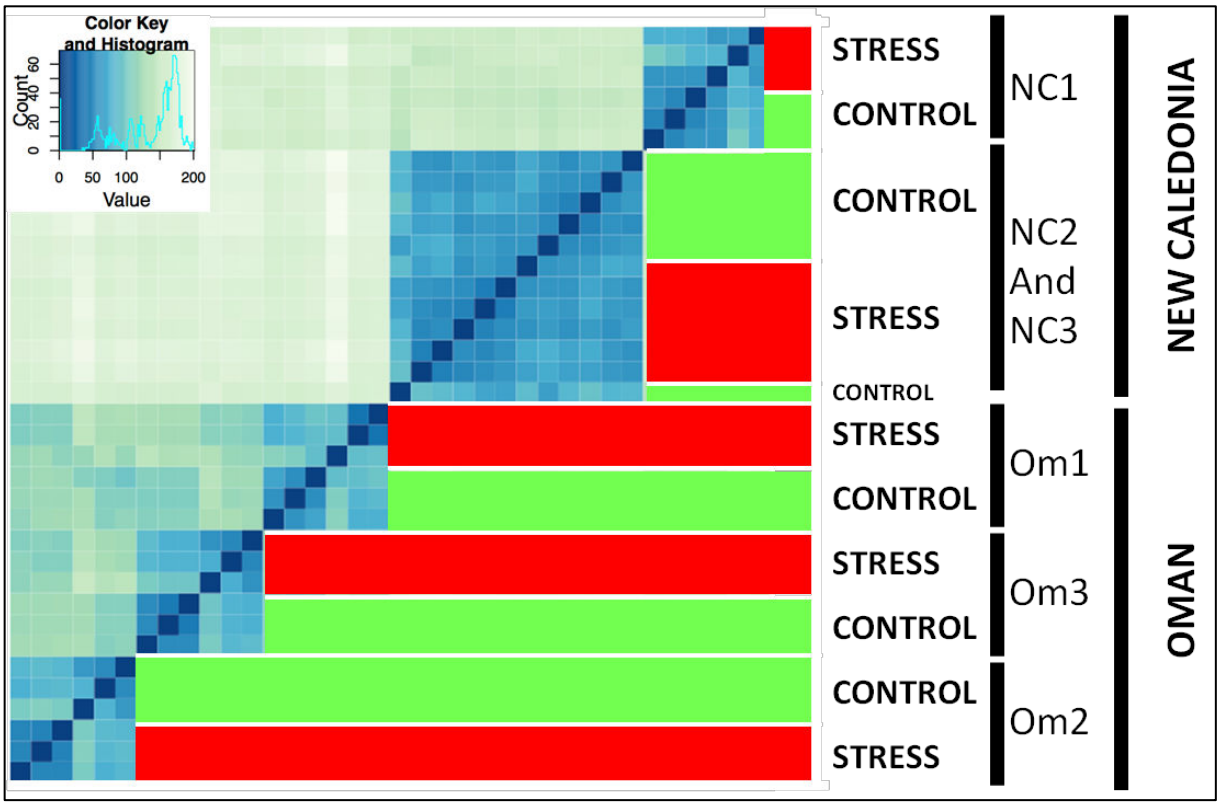


Figure 4 : Hierarchical clustering analyses performed using DESeq2 rlog-normalized RNA-seq data for the 36 transcriptomes: 2 conditions (control and thermal stress); 3 replicates per condition for each colony; 3 colonies per population; and 2 populations (the thermotolerant population from Om and the thermosensitive population from NC). The color (from white to dark blue) indicates the distance metric used for clustering (dark blue corresponds to the maximum correlation values).

the *Symbiodinium* community was very specific to each population, and remained stable during the transition from the field to the test tanks, and during stress exposure.

2.3.4. Host transcriptome analysis

We generated 36 transcriptomes corresponding to triplicate samples for 3 colonies for each population at the control (C) and stress (S) temperatures.

In total, the transcriptome sequencing of these 36 samples yielded 1,970,889,548 high quality Illumina paired reads of 100 bp. Depending on the sample, 40–70% of reads could be mapped to the *Pocillopora* sp. reference genome (40–64% for the Om population, and 59–70% for NC population).

The better alignment of the NC samples to the reference genome can be explained by the phylogenetic relationships between the different genotypes used in the present study and the one used for genome sequencing. The genotype of the NC colonies corresponded to clade 1, which is the same clade as that used for genome sequencing. The aligned reads could be assembled in 99,571 unique transcripts (TCONS), representing putative splicing variants of 26,600 genes identified as "XLOC" in the genome (FASTA sequences available in Additional File 3).

The transcriptomes from each population grouped together, providing further evidence of genetic differences between the NC and Om genotypes (Fig. 4). The transcriptomes also grouped by colony, indicating that the transcriptomes were genotype specific, and by condition replicates. An exception was the clustering of the transcriptomes for NC2 and NC3; for these the transcriptomes for the stress and control conditions clustered together, suggesting they may have been clones. As *Pocillopora* sp. can generate numerous clones over small spatial scale (Adjeroud et al. 2014), we analyzed the genotypes of these two colonies using five microsatellite markers (PV2, PV7 (Magalon et al. 2004), Pd3-002, Pd3-004, and Pd3-005 (Starger et al. 2008)) that have previously been shown to be polymorphic in *Pocillopora* sp. This microsatellite analysis confirmed that these two colonies share the same alleles (Additional File 4).

Differential gene expression analysis was performed using DEseq2, which enabled gene expression comparison between stress and control samples. For each population the 9 control samples and the 9 stress samples (3 replicates per temperature and per colony) were considered to be replicates. Full results of the comparisons between stressed and controls (log2-fold change and adjusted *p* values) are provided in Additional File 5. In total, 5287 genes were differentially expressed (adjusted *p* value <

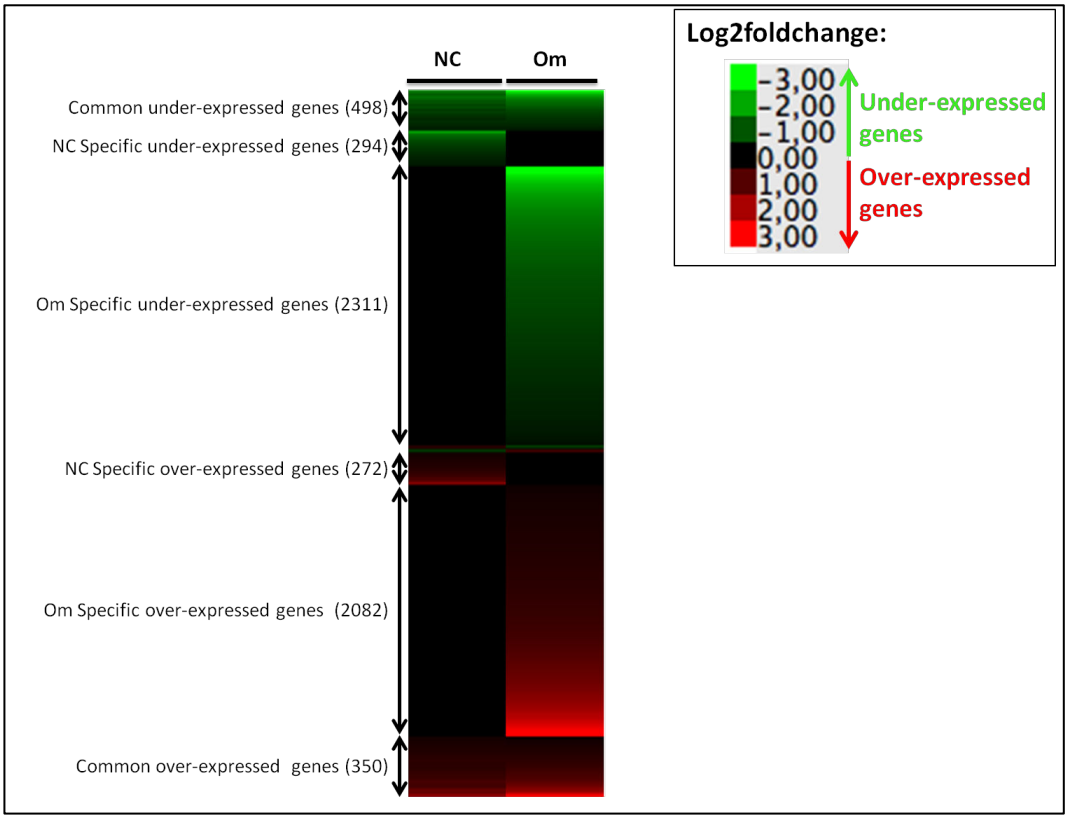


Figure 5 : Heatmap for significant differential gene expression in at least one comparison. Each gene is represented by a line that is colored red when the gene was overexpressed in the stress temperature treatment compared with the control, colored green when it was underexpressed, and colored black when the gene was not expressed or when there was no difference in gene expression between the control and temperature treatment.

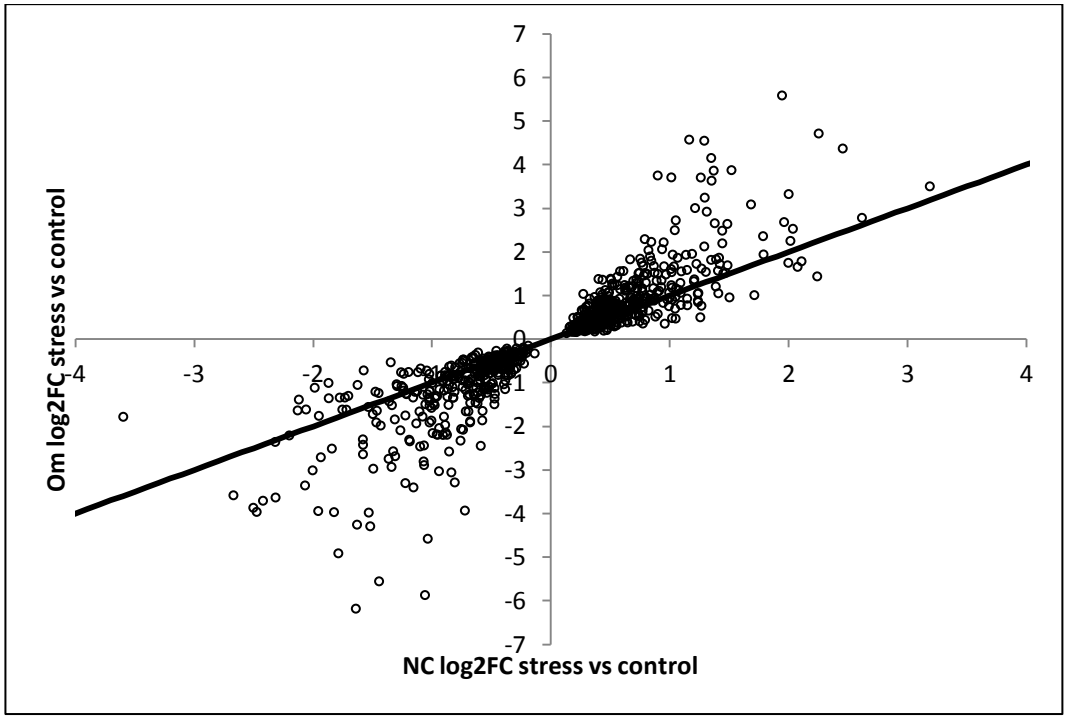


Figure 6 : Scatterplot of the log2-fold changes in gene expression in response to thermal stress in the Om population (y-axis) vs. the NC population (x-axis) for the 848 genes that were overexpressed (498 genes) or underexpressed (350 genes) in both populations. The line represents the 1:1 line.

0.05) in the Om population and 1460 were expressed in the NC population. The heatmap generated to visualize the expression pattern of each gene in the populations is shown in Figure 5.

Among these genes, 350 were overexpressed and 498 were underexpressed in both populations; 272 were overexpressed and 294 were underexpressed only in the NC population; 2082 were overexpressed and 2311 were underexpressed only in the Om population; 24 were overexpressed in the NC population but underexpressed in the Om population; and 22 were underexpressed in the NC population but overexpressed in the Om population.

Among those genes that were modulated following stress in both populations the differential expression level was significantly higher (Wilcoxon test; p value = 2.2×10^{-16}) for the Om corals (Fig. 6). Among the 498 overexpressed genes, 358 were more induced in coral samples from Om than those from NC (log2-fold change Om > NC; Fig. 6), and of the 350 underexpressed genes, 259 were more repressed in Om corals (Additional File 6). The mean of the log2-fold change for common overexpressed genes was 0.9 for Om samples *vs.* 0.6 for NC samples (variance: 0.6 for Om; 0.2 for NC), and for the common underexpressed genes was –1.2 for Om samples *vs*. –0.8 for NC samples (variance: 1 for Om; 0.3 for NC).

These first results revealed a greater transcriptome change in the Om population (4393 differentially expressed genes for the Om population *vs.* 566 genes for the NC population).

2.3.5. Discriminant Analysis of Principal Components (DAPC):

To quantify the transcriptome plasticity at the gene expression level we performed a DAPC analysis, which enabled identification of the axis in the multivariate space along which the difference between pre-specified sample groups was maximized. Multidimensional gene expression vectors for additional samples were then projected onto this axis to quantify genome-wide differences in gene expression in the context of the pre-specified contrast.

The DAPC analysis clearly discriminated the two populations based on their overall gene expression patterns (Fig. 7). We also found that populations significantly differed in their gene expression plasticity in response to heat stress (Wilcoxon tests; control p value = 0.73, stress p value < 0.01). This result was corroborated by the significance of the interaction term between the population and temperature effects in

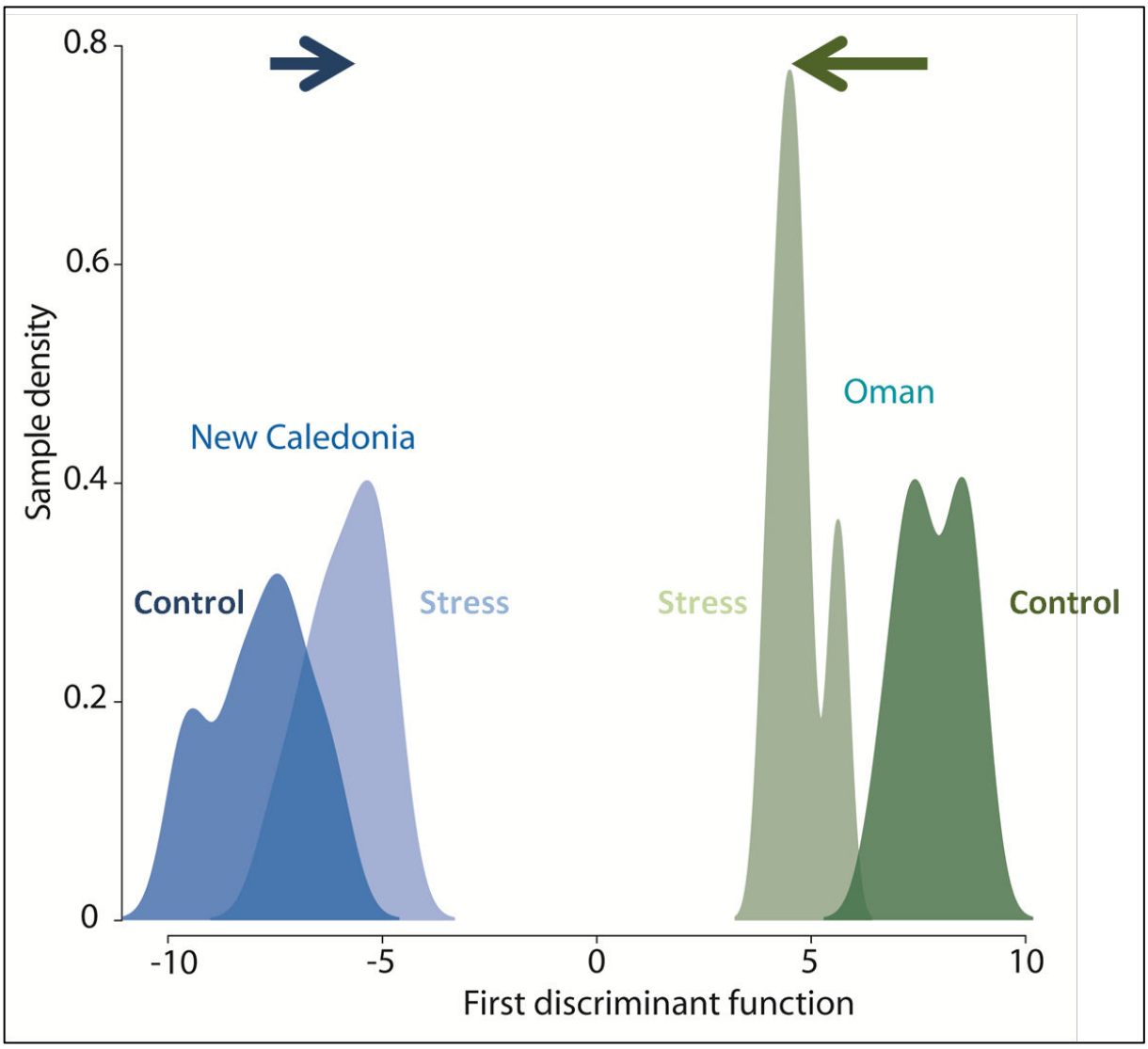


Figure 7 : Population level gene expression variation in response to thermal stress, based on DAPC analysis. The x axis is the first discriminant function of the DAPC along which the overall gene expression difference between individuals at both temperatures (stress and control) and from both populations (NC and Om) was maximized. This indicates the degree of similarity between the transcriptomes. The density plots obtained for the populations from NC and Om are represented in blue and green, respectively. Dark and light density plots correspond to individuals from the control and stress temperatures. The arrows above the density plots represent the direction of mean change in the gene expression profiles.

the GLM (p = 0.04), which indicated that the slope of the reaction norm was different between populations. More particularly, the Om population responded to a greater extent than the NC population, and thus showed significantly higher gene expression plasticity in response to thermal stress.

2.3.6. Analysis of gene function:

To investigate the functions associated with the differentially expressed genes we performed a blastx annotation of transcripts followed by a GO term annotation to determine to which protein each gene (XLOC) corresponded most closely, and the biological process, molecular function, and cell compartment localization of the protein. Differentially expressed genes associated with the control and stress conditions (adjusted p value < 0.05) clustered into 6 groups according to their expression patterns in both populations (see host transcriptome analysis section). We performed a Fischer exact test to determine what biological processes were significantly (FDR < 0.01) associated with each cluster, by comparison with the entire set of annotated genes (Additional File 7). We used REVIGO to visualize the enhanced biological processes (Supek et al. 2011).

For the 498 common overexpressed genes, 139 biological processes were enhanced compared with the entire set of annotated genes. The most significant biological process identified in the REVIGO analysis was stress response, which was associated with the lowest FDR value (2.1×10^{-68}). Following this sequentially were cellular metabolism (FDR = 3.7×10^{-49}), positive regulation of biological processes (FDR = 2.4×10^{-43}), cell death (FDR = 2.5×10^{-33}), cellular localization (FDR = 8.4×10^{-25}), and pigment metabolism (FDR = 2.1×10^{-21}). For the overexpressed genes in NC corals, 38 biological processes were enhanced and the REVIGO analysis showed that organic acid catabolism (FDR = 1.6×10^{-22}), protein transport (FDR = 1.8×10^{-16}), stress response (FDR = 4.8×10^{-13}), and cellular metabolism (FDR = 3×10^{-12}) were the four most significantly enhanced biological processes. Among the genes overexpressed in Om corals the REVIGO analysis showed that 160 enhanced biological processes grouped together, with the most significant being ncRNA metabolism (FDR = 8.9×10^{-303}), cellular metabolism (FDR = 4.4×10^{-70}), carbohydrate derivative biosynthesis (FDR = 5.9×10^{-64}), and organic substance transport (FDR = 2×10^{-44}).

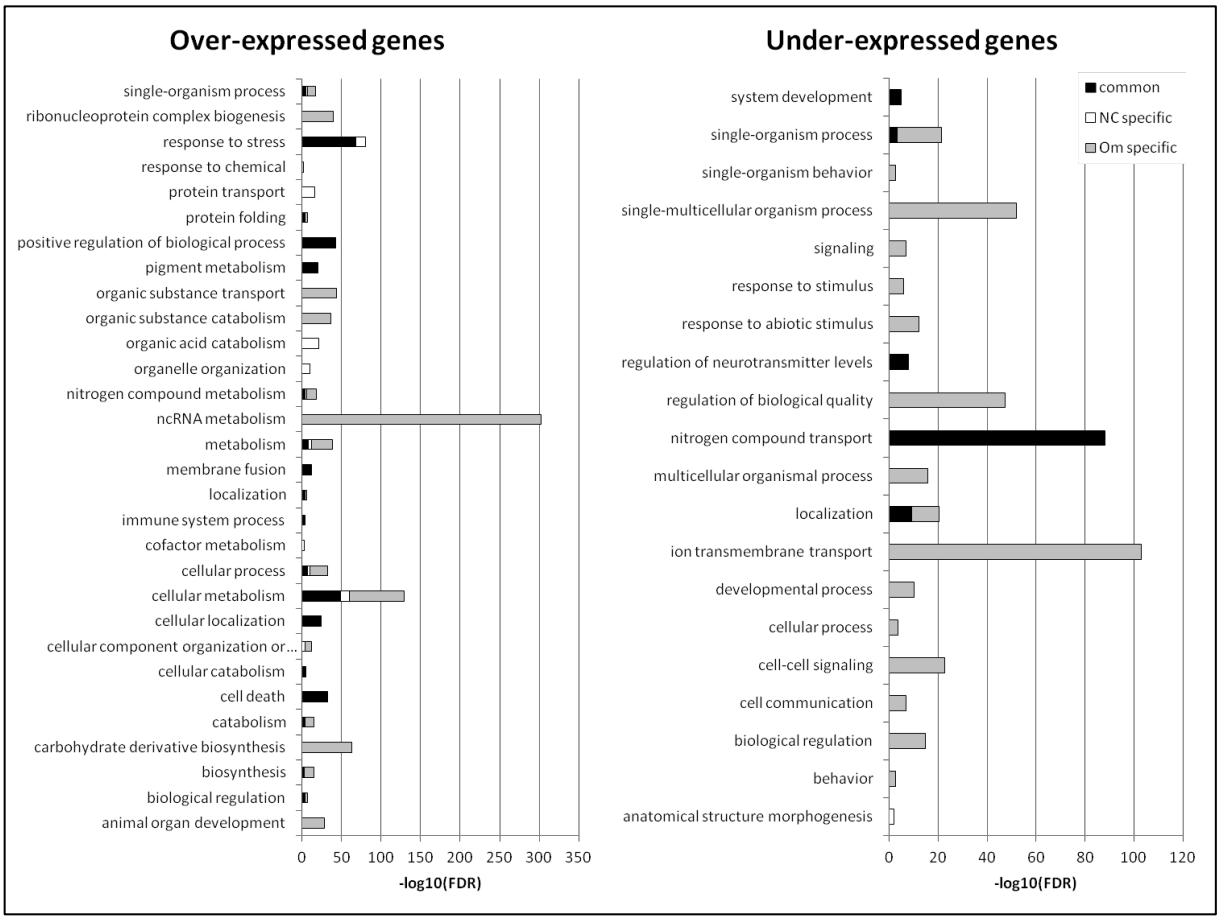


Figure 8 : Visualization and summary of the GO enrichment following analysis by REVIGO. Each enhanced biological process is represented by a bar proportional to the log10(FDR). The colors corresponds to the 3 categories of genes (common: black; Om-specific: grey; NC-specific: white) that were overexpressed (left panel) or underexpressed (right panel).

For the 350 common underexpressed genes, 48 biological processes were enhanced compared with the entire set of annotated genes. The REVIGO analysis grouped these genes into five biological processes: nitrogen compound transport (FDR = 5.4×10^{-89}), localization (FDR = 8.1×10^{-10}), regulation of neurotransmitter levels (FDR = 1.2×10^{-8}), system development (FDR = 8.8×10^{-6}), and single organism process (FDR = 0.0004). Among the underexpressed genes in NC corals, only one biological process (anatomical structure/morphogenesis) was found to be enhanced (FDR = 0.00937). Among the underexpressed genes in Om corals the REVIGO analysis grouped 139 enhanced biological processes, with the most significant being ion transmembrane transport (FDR = 7.6×10^{-104}), single multicellular organism process (FDR = 7.5×10^{-53}), regulation of biological quality (FDR = 6×10^{-48}), cell–cell signaling (FDR = 1.5×10^{-23}), single organism process (FDR = 1.1×10^{-18}), multicellular organism process (FDR = 1.5×10^{-16}), biological regulation (FDR = 2.3×10^{-15}), response to abiotic stimulus (FDR = 6.2×10^{-13}), and localization (FDR = 4.6×10^{-12}).

Regarding others Gene Ontology annotations, the common overexpressed genes were correlated with enhancement of 41 cellular components that were associated in the REVIGO analysis with cellular compartments including the mitochondria (FDR = 1.5×10^{-180}), cells (FDR = 1.5×10^{-19}), organelles (FDR = 2.5×10^{-15}), endomembrane systems (FDR = 6.5×10^{-7}), the membrane-enclosed lumen (FDR = 0.0002), the entire membrane (FDR = 0.0019), the membrane raft (FDR = 0.009), and the membrane (FDR = 0.009). The genes overexpressed in NC corals were associated in the REVIGO analysis with 23 cellular compartments, with the mitochondria (FDR = 2.5×10^{-82}), cells (FDR = 4.9×10^{-7}), organelles (FDR = 6.9×10^{-6}), and the envelope (FDR = 0.009) being the four most significant. The genes overexpressed in Om corals were associated in the REVIGO analysis with 70 cellular components, the most significant of which were the intracellular organelle lumen (FDR = 1×10^{-560}), organelles (FDR = 9.9×10^{-48}), cells (2.7×10^{-46}), the membrane-enclosed lumen (FDR = 1.2×10^{-19}), macromolecular complexes (FDR = 1×10^{-16}), the endomembrane system (FDR = 2.7×10^{-11}), the envelope (FDR = 3×10^{-9}), and the membrane (FDR = 0.0008).

The common underexpressed genes were associated in the REVIGO analysis with 13 cellular components including the plasma membrane (FDR = 1.1×10^{-44}), membrane (FDR = 2.4×10^{-11}), neurons (FDR = 0.0003), presynapse (FDR = 0.0004), and synapse (FDR = 0.007). The genes underexpressed in NC corals were associated in the REVIGO

analysis with 7 biological processes in cellular components amongst which the plasma membrane region (FDR = 8.5×10^{-9}), cell periphery (FDR = 0.001), the membrane (FDR = 0.002), and membrane part (FDR = 0.009) were the four most significant. The underexpressed genes in Om corals were associated in the REVIGO analysis with 26 cellular components, the most significant of which included the integral component of the plasma membrane (FDR = 3.1×10^{-81}), the periphery (FDR = 1.2×10^{-22}), the membrane (FDR =6.2×10^{-17}), the transmembrane transporter complex (FDR = 1.5×10^{-14}), the extracellular region (FDR = 4.5×10^{-6}), the cell (FDR = 9.5×10^{-6}), synapses (FDR = 1.9×10^{-5}), and the synapse part (FDR = 4.4×10^{-5}).

To investigate whether the thermotolerant population in our study displayed a frontloading strategy, as previously described in scleractinians (Barshis et al. 2013), we compared the constitutive expression levels (in control conditions) between the two populations for those genes that were overexpressed in the NC population (Additional File 8). This analysis showed that the basal expression level was often greater in the more thermotolerant population (Om). Among the 794 genes that were overexpressed in the NC population, 484 were constitutively more expressed in the Om than the NC control conditions. Among these genes, 301 were overexpressed in the Om population, 20 were underexpressed in the Om population, and 163 were not differentially expressed between the control and stress temperature in the Om population, reflecting true frontloading based on the definition of Barshis et al. 2013 (Barshis et al. 2013). These 3 categories of genes (overexpressed, down-regulated, and frontloaded) were submitted to enhancement analysis. No significant results were found for the down-regulated genes. The frontloaded genes were enhanced in the biological processes cellular respiration (FDR = 4.4×10^{-23}), cellular component organization (FDR = 0.002), homeostatic process (FDR = 0.005), cellular component organization or biogenesis (FDR = 0.007), cofactor metabolism (FDR = 0.009), and stress response (FDR = 0.009), and in the cellular compartments including the mitochondrion (FDR = 1.6×10^{-66}), envelope (FDR = 0.0002), cell (FDR = 0.0002), and organelle (FDR = 0.0009). Most interestingly, for genes associated with a higher basal expression level and overexpression in the Om colonies, the most enhanced biological processes included stress response (FDR = 1.2×10^{-26}), pigment metabolism (FDR = 5.1×10^{-24}), regulation of phosphate metabolism (FDR = 3.2×10^{-15}), cellular metabolism (FDR = 2.7×10^{-11}), and protein folding (FDR =

7.3 × 10^{-6}). Among the 43 overexpressed genes involved in the response to stress in the NC population, 23 were frontloaded in the Om population.

2.4. DISCUSSION

2.4.1. An ecologically realistic thermal stress

The aim of the present study was to characterize and compare the response to thermal stress of corals from populations from two contrasting temperature environments. The thermal stress applied was ecologically realistic, with the individuals of each population being subjected to the upper annual limit of the temperature ranges to which they were naturally exposed, up to a level just below that at which signs of physiological collapse were observed. To avoid other confounding effects the coral colonies from the two populations were transferred to tanks and acclimated for 6 months in standardized controlled conditions in artificial seawater. Consequently, the only variable in the stress experiments was temperature. During the temperature increase, the first observable modification of the phenotype (polyp closure) was observed for all genotypes of the two populations at the upper temperature they were subject to in their natural environments (30°C and 34°C for NC and Om corals, respectively). This result confirmed that our stress was ecologically realistic and that the physiological status of the corals was similar and comparable for both populations at the different temperatures. To assess the response of healthy corals at elevated temperature we performed a molecular analysis to compare the transcriptome of corals stressed at 29°C and 33°C (for the NC and Om colonies, respectively) with the transcriptomes of control corals maintained at the mean temperature of warmer months in their respective natural habitats (27°C and 29°C for the NC and Om colonies, respectively). Any change in the holobiont was therefore a response to the thermal stress and not because of disruption of the coral integrity.

2.4.2. Symbiotic community: bacterial and Symbiodinium composition

We used 16S and ITS2 metabarcoding to characterize the coral bacterial and *Symbiodinium* communities respectively. For the bacterial community we identified significant differences between populations and genotypes but no difference between the conditions (*in situ*, control, and stress). The microbiota composition of all samples was consistent with previous studies, showing a high proportion of Gammaproteobacteria and dominance of the symbiotic genus *Endozoicomonas* genus

(Bourne & Munn 2005; Kvennefors et al. 2012; Peixoto et al. 2017; Rohwer et al. 2001; Neave, Apprill, et al. 2016).

For the *Symbiodinium* community the ITS2 metabarcoding analysis enabled intra-clade resolution (Quigley et al. 2014) . Four OTUs dominated; these corresponded to the two distinct subclades D1a and C1, which represent the majority of sequences in the Om and NC corals, respectively. Nine genotype clades (A through I) have been identified in the genus *Symbiodinium* (Baker 2003). The *Symbiodinium* type can strongly influence the holobiont fitness, with clade D providing tolerance to higher temperatures (Berkelmans & van Oppen 2006) and C1 enhancing coral growth rates (Little et al. 2004). We found that subclade D1a was dominant in the Oman corals in the present study, which is consistent with the results of previous studies (Berkelmans & van Oppen 2006).

Although the microbial community (both bacterial and *Symbiodinium*) differed between the NC and Om populations, the composition did not change during transition from the field to the artificial seawater conditions, and also remained stable during the experimental temperature increase. This shows that the coral holobiont assemblage was stable over the course of the experiment, with no changes in the *Symbiodinium* community (no bleaching and no shift), and no change in the bacterial community leading to the occurrence of potential pathogens, as has been observed during thermal stress in *Acropora tenuis* (Littman et al. 2010).

2.4.3. Host transcriptomic response

2.4.3.1. Plasticity analyses:

Given the symbiotic community stability during the thermal stress, we assumed that most of the response was attributable to the coral host. We thus performed a qualitative and quantitative comparison of gene expression changes for both populations in control and thermal stress conditions. The coral samples from the Om population showed greater regulation of gene expression levels, having a much higher number of differentially expressed genes between the control and the stress conditions (5287 genes for Om corals *vs*. 1460 genes for NC corals). In addition, for the same set of genes differentially expressed in the same way in both populations, the absolute value of the log2-fold changes was higher in the Om population for 73% of those genes. The DAPC analysis showed that the thermotolerant Om corals exhibited a higher level of

transcriptome plasticity than those from the thermosensitive NC population. These findings are consistent with a recent study that identified greater transcriptome plasticity for a most thermotolerant off-shore population compared with an in-shore population, as predicted from the theory that greater plasticity is expected for populations in a more variable environment (Kenkel & Matz 2016b). A recently described alternative strategy for thermotolerance, termed frontloading, involves a constitutive and higher level of expression of genes specific to the response to thermal stress, with no or little regulation compared with the thermosensitive population (Barshis et al. 2013). In our study, among the 794 genes overexpressed in NC corals, 483 also had a higher basal level of expression (in control conditions) in Om corals than in NC corals, with 300 showing overexpression in Om corals (211 more than in NC corals) and 163 (20.5%) displaying a constant level of expression during the thermal stress (true frontloaded genes) (Barshis et al. 2013).

The thermotolerant Om corals thus exhibited both greater plasticity compared with the thermosensitive NC corals, as well as frontloading. It has been proposed that occurrence of plasticity *vs*. frontloading strategies can depend on the frequency of the stress, with frequent perturbations promoting frontloading strategies, and less frequent perturbations promoting plasticity strategies (Kenkel & Matz 2016b). In this study we observed the occurrence of both strategies. We hypothesize that frontloading could reflect assimilation of plasticity, whereby a plastic gene expression becomes fixed following very frequent stresses (Waddington 1953). In this context, the Om population would be in an intermediate state between plastic gene expression and frontloading, with assimilation in progress.

These two strategies are only useful if they facilitate a more efficient response. To investigate the role of plastic and frontloaded genes in the thermal stress response, we performed an enrichment analysis that highlighted numerous significant biological processes for which we inspected each gene to discuss hereafter those whose biological function would be relevant in this context. We thus aimed to identify the molecular mechanisms underlying greater thermotolerance in Om corals.

2.4.3.2. Functional analyses:

Response to stress:

Reactive Oxygen Species (ROS) detoxification:

One of the first consequences of thermal stress is the production of reactive oxygen species (ROS), which are associated with protein, membrane and DNA damage. These oxidative molecules are produced in excess by the symbiont and mitochondria during the respiration process, and cells produce several enzymes to detoxify them to limit cellular damage (Weis 2008). Down-regulation of the calcium-binding messenger protein calmodulin is a sign of oxidative stress (Schallreuter et al. 2007; Townsend et al. 2002); this protein had more isoforms that were differentially expressed in Om corals, together with one calcium calmodulin-dependent kinase. Quinone oxidoreductases were also specifically underexpressed in Om corals. These proteins are known to be involved in the generation of ROS (Porté et al. 2009). The thioredoxin gene was up-regulated in both populations, but with higher fold changes in Om corals. This enzyme detoxifies oxidized molecules and is often implicated in coral heat stress (DeSalvo et al. 2010; Maor-Landaw et al. 2014). Polyamine oxidase is a key component of the oxidative burst in plants, and is involved in the induction of apoptosis (Yoda 2006). Two genes, one underexpressed in Om corals and one overexpressed in NC corals, were found to be regulated in our experiment.

Apoptosis:

Among the differentially regulated genes we found were numerous genes involved in apoptosis, which is known to be implicated in coral responses to thermal stress (Ainsworth et al. 2011). Among these, TNFR and TRAF genes code for receptors and receptor-associated components of the tumor necrosis factor (TNF), which is known to participate in the apoptosis pathway. Ten TNFR genes and 3 TRAF genes were found to be overexpressed following thermal stress in both populations, and there was a higher basal level of expression in the Om corals for 10 of the 13 genes. A second set of 5 TNFR and 11 TRAF genes were specifically overexpressed in the Om corals, indicating more intense regulation of the apoptosis pathway in the thermotolerant corals. In a previous study TNFR genes were found among frontloaded genes, and TRAF genes were overexpressed in response to thermal stress, which is consistent with our results (Barshis et al. 2013). Furthermore, 4 TNFs were found to be specifically underexpressed

in the Om corals, whereas TNFAIP3 was specifically overexpressed. The TNFAIP3 gene (TNF alpha-induced protein 3) is known to inhibit the NFkB process (inflammatory response) and apoptosis (Opipari et al. 1990). This further suggests that Om corals are capable of greater regulation of apoptosis. Caspase 8 and caspase 3 were specifically overexpressed in the Om corals. Caspases constitute the effector core of the apoptotic process (Nicholson & Thornberry 1997), and caspase 3 has commonly been implicated in the heat stress response in corals (Maor-Landaw & Levy 2016). Interestingly, Fem-1 homolog B, which is involved in the apoptotic process as a death receptor-associated protein, was frontloaded in Om corals.

DNA repair:

Overproduction of ROS subsequent to thermal stress is responsible for cellular and DNA damage (Yakovleva et al. 2009; Jena 2012). Numerous genes coding for DNA repair enzymes including DNA excision repair, DNA ligase, and DNA polymerase were overexpressed in both population. Among these, in Om corals 3 showed higher basal expression levels and 2 were frontloaded.

HSPs:

Heat shock proteins (HSPs) are involved in protein folding and protein damage repair in all organisms. Several HSPs were found to be overexpressed in both coral populations (Hsp60, Hsp70, and Hsp71). Among these Hsp70 was more overexpressed (log2-fold change of 3.2 in Om corals *vs.* 1.3 for NC corals), and Hsp60 and Hsp71 showed higher basal expression levels in the Om corals. Furthermore, Hsp105, Hsp75, Hsp90, and Hsp71 were overexpressed only in Om corals. Hsp70 is one of the most documented protein chaperones in coral heat stress (Barshis et al. 2013), but Hsp60 (B. Brown et al. 2002), Hsp90 (Carpenter et al. 2010), and Hsp40 (Maor-Landaw & Levy 2016) are also known to be involved in stress response in these organisms. We also found that chaperones of the Hsp40 family (also known as DNAj) were also regulated during our heat stress experiment; 3 of these were overexpressed in both populations but there was a higher basal expression level in the Om corals, whereas 5 were overexpressed only in the Om corals. These proteins seem to be implicated in protein folding during early thermal stress in *Porites*, and are highly expressed prior to bleaching and tissue peeling (Maor-Landaw & Levy 2016).

We also found specific Om overexpression of the pyrexia gene, which codes for a transient receptor-activated cation channel, and had been shown to protect flies from high temperatures (Lee et al. 2005).

Energetic metabolism:

Mitochondrial functions:

A high number of regulated genes were mitochondrial (either mitochondria- or nuclear-encoded); this is evident in the cellular component enrichment histogram, especially for common and NC-specific genes. Nevertheless, the mitochondrial compartment was also well represented among genes displaying a higher overexpression level or frontloading in Om corals.

The mitochondria are central to numerous important biological processes including energy-generating respiration, but can be the source of oxidative molecules involved in apoptosis.

Among mitochondrial associated genes a number were specifically or more intensively regulated in Om corals, including those coding for 2-oxoglutarate dehydrogenase, which catalyzes the conversion of 2-oxoglutarate to succinyl-CoA and CO_2 in the Krebs cycle), and several monocarboxylate transporters for lactate or pyruvate, which have been shown to be essential for regulation of energy metabolism in *Aiptasia* (Halestrap & Meredith 2004; Lehnert et al. 2014).

Several other genes known to participate in energy metabolism were overexpressed, including galactose-1-phosphate uridylyltransferase (overexpressed in both populations) and succinate dehydrogenase [ubiquinone] flavo mitochondrial-like (overexpressed in NC corals and showing frontloading expression in Om corals). This suggests the involvement of energy metabolism and/or a changes in resource usage, as has been proposed in symbiotic *vs.* aposymbiotic *Aiptasia* (Lehnert et al. 2014). Energy metabolism in corals is highly dependent of their symbiotic algae, which is the major source of glucose generated by photosynthesis. During thermal stress the symbiotic algae can decrease in density and/or display a reduced capacity to generate energetic compounds, thus obliging the coral host to use alternative sources of energy, such as fatty acids (Karim et al. 2015).

In this context we also observed a high level of regulation of lipid metabolism in the Om corals. The apolipo A (a constituent of lipoprotein associated with lipid

dissolution) and dLp HDL-BGBP precursor (which has lipid transporter activity) genes were specifically overexpressed in the Om corals, and could facilitate the transport and assimilation of lipids.

Symbiont regulation:

Perturbation of energy metabolism and a decrease in the efficiency of energy production by symbiotic algae can be linked to the modulation of genes associated with symbiont maintenance. In our experiment three Rab-11 isoforms were found to be overexpressed, including: one having a higher basal expression level in both populations and a higher fold change in the Om corals; one that occurred in NC corals and showed frontloading expression in Om corals; and one induced only in the Om corals. This recycling regulatory protein has been shown to regulate phagosomes containing *Symbiodinium* cells in the *Aiptasia-Symbiodinium* symbiosis (Chen et al. 2005), suggesting a potential role in *Symbiodinium* exclusion during bleaching process.

Calumenin was also overexpressed in both populations, but was expressed at a higher rate in Om corals. This protein is known to be a signal intermediate for sym32, a signaling protein involved in symbiont recognition by the coral host (Ganot et al. 2011).

In addition, three lectins exhibited differential expression in the Om corals: one was overexpressed (with a log2-fold change of 4.1) and two were underexpressed (log2-fold change of –2.8 and –2.5). It has been reported that lectins regulate the symbiosome (Vidal-dupiol et al. 2009).

Morpho-anatomic processes and reproduction:

Skeleton and muscle cells:

Some enzymes potentially involved in skeleton formation and muscle contraction were down-regulated in corals from Om.

A single chain carbonic anhydrase and 4 carbonic anhydrase genes (1, 12, 2, and 7) were found to be underexpressed in Om corals. Carbonic anhydrases catalyze the interconversion of CO_2 and water to bicarbonate and protons, enabling maintenance of the acid–base balance (Tashian 1989). In hard corals, carbonic anhydrases are also involved in biomineralization processes involved in skeleton formation (Le Goff et al. 2016). Two hephaestin genes were also underexpressed in Om corals. These copper-dependent ferroxidases were identified in the skeleton of *Acropora millepora* (Gitschier et al. 1999), where they can be involved in the incorporation and regulation of iron in

the bicarbonate skeleton (Ramos-Silva et al. 2013). Several myosin related proteins similar to myosin light chain kinase or myosin regulatory light polypeptide 9 were found to be specifically underexpressed in the Om corals. Actin-binding LIM partial, potentially involved in Z-disk activity of muscles, and several genes having the LIM domain were also found to be underexpressed in Om corals. In eumetazoans including cnidarians, these protein are involved in regulation of muscle activity (for a review see (Leclère & Röttinger 2017)). In addition, the genes for the acetyl choline receptor "43 kDa receptor-associated of the synapse-like" and the "calcium-activated potassium channel subunit beta-like" proteins, both of which down-regulate muscle contraction, were overexpressed in Om, which is in accordance with a global decrease in functioning of the contractile apparatus.

We hypothesize that down-regulation of skeleton formation and muscle contraction could be a trade-off in energy allocation during the response to thermal stress.

Cnidocytes:

We observed a higher level of regulation of genes involved in the functioning of cnidocytes (also known as nematocytes) in Om corals. These ectodermal cells are specific to cnidarian, and are involved in environment sensing, defense, and predation.

Three cytosolic phospholipase A2 (cPLA2) genes were specifically overexpressed in Om corals. In cnidocytes, the corresponding proteins are involved in venom efficacy by promoting prey lysis (Argiolas & Pisano 1983),(Nevalainen et al. 2004). One MAC perforin domain-containing gene had a higher basal expression level in Om corals. This gene encodes for a membrane attack complex/perforin-domain containing protein, and is found in gland cells and nematocytes in *Nematostella*, suggesting a potential role in prey killing (Miller et al. 2007). Conversely, we found underexpression of a gigantoxin homolog, which is a cytolysin (actinoporin) found abundantly in cnidocytes (or nematocytes) (Hu et al. 2011; Frazão et al. 2012). Several polycystic kidney disease gene (PKD1 and 2) isoforms were also found to be underexpressed in the Om corals. These proteins are localized in tentacles in Hydra, and seem to be involved in cnidocyte discharge (McLaughlin 2017). These changes could have been promoted by notch signaling (with overexpression of one notch homolog 2); this pathway is essential for cnidogenesis in *Nematostella vectensis* (Marlow et al. 2012).

A switch to heterotrophic nutrition is consistent with a decrease in *Symbiodinium* cell numbers, and could be linked to higher levels of resistance to thermal stress, as has been described in other scleractinian corals (Hughes & Grottoli 2013; Kisten 2014; Aichelman et al. 2016).

Nervous system:

Many genes involved in nervous system functioning were differentially regulated, with some being overexpressed (e.g. neuronal acetylcholine receptor and VWFA, which regulate the calcium dependent voltage chain), and others being underexpressed, including a proton myo-inositol cotransporter, SCO-spondin (axon guidance), sodium- and chloride-dependent GABA and taurine transporters (neurotransmission), sodium-dependent phosphate transport 2B, and synaptotagmin. Many transcription factors that could be involved in morpho-anatomic integrity, including numerous forkhead box homologs implicated in cell growth, proliferation, and differentiation were overexpressed in Om corals. All these may be involved more globally in the regulation of morpho-anatomic processes (muscle contraction, cnidocytes).

circadian clock / reproduction:

Several photoreceptors were differentially expressed in the corals. A cryptochrome gene was frontloaded in Om. These flavoproteins are found in plants, animals, insects, and cnidarians, and in corals it has been shown that cryptochrome can synchronize the circadian clock and the reproductive system (Reitzel et al. 2013). Three melatonin receptors were also found to be regulated in Om corals (2 overexpressed and 1 underexpressed). As in vertebrates, where melatonin has been well characterized, in cnidarians it is also involved in the circadian clock (Peres et al. 2014). Similarly, 2 underexpressed and 3 overexpressed opsin-like genes were identified. The protein is a homolog of the photoreceptor melanopsin, and in *Acropora* is involved in circadian cycle regulation (Vize 2009).

Taken together, changes in morpho-anatomic regulation and perturbations in circadian cycles and reproduction could also reflect a trade-off mechanism in the Om stress response. This trade-off is consistent with greater plasticity, and intensive and not fully specific gene regulation activity.

Epigenetic processes:

Two histone acetyltransferase genes were overexpressed in Om corals. These enzymes, which are also found in the sea anemone *Nematostella*, are involved in epigenetic gene regulation by modifying the nucleosome structure, and thus the transcription of genes (Sterner & Berger 2000; Karmodiya et al. 2014). Several histone–lysine N-methyltransferases were also identified, 5 of which were underexpressed (four PRDM6 and one setd3 isoform) and 3 of which were overexpressed in Om corals. As with histone acetyl-transferase, these enzymes are involved in gene expression regulation through their influence on chromatin structure (Huang 2002; Vervoort et al. 2016). One lymphoid-specific helicase-like (HELLS) gene was frontloaded in Om corals. This protein is known to participate in epigenetic processes, and interacts with DNMT1 (cytosine methyl-transferase) for maintenance of or *de novo* methylation (Dabe et al. 2015; Myant et al. 2011). We identified underexpression of 2 SID-1 transmembrane family member genes involved in dsRNA regulation (Sapozhnikova & Barannik n.d.).

Many reverse transcriptase homologs, including RNA-directed DNA polymerase from a jockey-like mobile element, were differentially expressed or more intensely regulated during thermal stress in the Om corals. These reverse transcriptases are typical of the retro-transposon activity known to be activated or less controlled during general stress (Grandbastien 1998). The loss of inhibition of retro-transposon activity may reflect a trade-off with more important functions, but could also be an SOS survival mechanism, enabling rapid genome changes in a threatening environment (Wessler 1996; Maumus et al. 2009).

2.5. CONCLUSION

Comparison of the response of corals from two contrasting temperature environments to exposure to an ecologically realistic thermal stress shed light on the molecular basis of thermotolerance. We found that during stress exposure the symbiotic community was stable in both coral populations, but we identified major differences in gene regulation processes between the two populations. The more thermotolerant population displayed (i) a more plastic transcriptome response involving more differentially expressed genes and higher fold expression changes; and (ii) a constitutive and higher level of expression for a range of genes (frontloaded genes). If frontloading is a consequence of assimilation processes, plasticity could originate from epigenetic processes, which were clearly induced in the thermotolerant population. In the context

of global warming, which is predicted to cause abnormal and rapid temperature increase (Settele et al. 2014), phenotypic plasticity and capacity for rapid adaptation through assimilation could increase the chances of coral survival. Our results contribute to understanding of coral evolution under variable environments, and suggest that management measures including coral transplantation or hybridization between sensitive and resistant populations may be approaches to increasing the thermotolerance of coral populations as sea surface temperatures increase in the future.

2.6. ACKNOWLEDGEMENTS

We are grateful to the Genotoul bioinformatics platform, and the Toulouse Midi-Pyrenees and Sigenae group for providing help and computing resources (Galaxy instance; http://sigenae-workbench.toulouse.inra.fr). We also thank the TECNOVIV platform (University of Perpignan) and bioinformatics service for computing help and resources.

List of abbreviations

NC: New Caledonia
Om: Oman
DAPC: Discriminant Analysis of Principal Components
FDR: False Discovery Rate

Declarations

Ethics approval and consent to participate
Not applicable
Consent for publication
Not applicable
Availability of data and material
The datasets generated and analyzed during the current study are available in the SRA repository (http://www.ncbi.nlm.nih.gov/bioproject/399069).
Competing interests
The authors declare that they have no competing interests.
Funding
This project was funded by the ADACNI program of the French National Research Agency (ANR) (project no. ANR-12-ADAP-0016; http://adacni.imbe.fr) and the Campus France PHC program.
Authors' contributions
JVD, MA, DA, GM, and ET were involved in the study concept and design. KBR, LF, MC, and JVD were involved in the collection of samples. All authors were involved in data acquisition and analysis. KBR drafted the manuscript, and all authors contributed to critical revisions and approved the final manuscript.

Additional files

Additional File 1: Figure S1. Bacterial class composition (for the 24 most abundant) within each replicate for the two populations, the three colonies of each population, and three conditions per colony. In situ (dark arrows); control temperature (green arrows); stress temperature (red arrows).

Additional File 2: ANOVA results for alpha diversity (Shannon index) and beta diversity (Bray-Curtis distance) between populations, colonies, or conditions.

Additional File 3: List and sequences of the 26,600 genes (XLOC) generated during RNAseq alignment.

Additional File 4: Microsatellite results for the colonies NC2 and NC3 from the New Caledonia population.

Additional File 5: DEseq2 results for the log2-fold changes, and adjusted p values between stress and control conditions for each population.

Additional File 6: Comparison between the log2-fold change in each population of genes differentially underexpressed or overexpressed in the same way in both populations.

Additional File 7: GO enrichment results for biological processes, molecular functions, and cellular compartments for common, New Caledonia-specific, or Oman-specific overexpressed and underexpressed genes.

Additional File 8: Frontloaded genes in Oman corals among overexpressed genes from New Caledonia corals.

2.7. REFERENCES:

Abrego, D. et al., 2008. Species-specific interactions between algal endosymbionts and coral hosts define their bleaching response to heat and light stress. *Proceedings. Biological sciences / The Royal Society*, 275(1648), pp.2273–2282.

Adjeroud, M. et al., 2014. Genetic diversity, clonality and connectivity in the scleractinian coral Pocillopora damicornis: A multi-scale analysis in an insular, fragmented reef system. *Marine Biology*, 161(3), pp.531–541.

Adjeroud, M. & Tsuchiya, M., 1999. Genetic variation and clonality in the scleractinian coral Pocillopora damicornis in the Ryukyu Archipelago, southern Japan. *Marine Biology*, 134, pp.753–760.

Afgan, E. et al., 2016. The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2016 update. *Nucleic Acids Research*, 44(W1), pp.W3–W10. Available at: https://academic.oup.com/nar/article-lookup/doi/10.1093/nar/gkw343 [Accessed June 5, 2017].

Aichelman, H.E. et al., 2016. Heterotrophy mitigates the response of the temperate coral Oculina arbuscula to temperature stress. *Ecology and evolution*, 6(18), pp.6758–6769. Available at: http://www.ncbi.nlm.nih.gov/pubmed/27777745 [Accessed August 31, 2017].

Ainsworth, T.D. et al., 2011. Defining the tipping point. A complex cellular life/death balance in corals in response to stress. *Scientific Reports*, 1(14), pp.130–136.

Altschul, S.F. et al., 1990. Basic local alignment search tool. *Journal of Molecular Biology*, 215(3), pp.403–410. Available at: http://www.ncbi.nlm.nih.gov/pubmed/2231712 [Accessed May 30, 2017].

Anctil, M., 2009. Chemical transmission in the sea anemone Nematostella vectensis: A genomic perspective. *Comparative Biochemistry and Physiology - Part D: Genomics and Proteomics*, 4(4), pp.268–289. Available at: http://dx.doi.org/10.1016/j.cbd.2009.07.001.

Anders, S., Pyl, P.T. & Huber, W., 2015. HTSeq - A Python framework to work with high-throughput sequencing data. *Bioinformatics*, 31(2), pp.166–169. Available at: https://academic.oup.com/bioinformatics/article-lookup/doi/10.1093/bioinformatics/btu638 [Accessed May 30, 2017].

Anthony & Fabricius, 2000. Shifting roles of heterotrophy and autotrophy in coral energetics under varying turbidity. *Journal of experimental marine biology and ecology*, 252(2), pp.221–253. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10967335 [Accessed September 14, 2017].

Antonovics, J., 1987. The evolutionary dis-synthesis: which bottle for which wine? *The American Naturalist*, 129, pp.321–331. Available at: http://www.jstor.org/stable/2461683.

Argiolas, A. & Pisano, J.J., 1983. Facilitation of phospholipase A2 activity by mastoparans, a new class of mast cell degranulating peptides from wasp venom. *Journal of Biological Chemistry*, 258(22), pp.13697–13702.

Aubin-Horth, N. & Renn, S.C.P., 2009. Genomic reaction norms: Using integrative biology to understand molecular mechanisms of phenotypic plasticity. *Molecular Ecology*, 18(18), pp.3763–3780.

Baker, A.C. et al., 2004. Coral reefs: Corals' adaptive response to climate change. *Nature*, 430(7001), pp.741–741. Available at: http://www.nature.com/doifinder/10.1038/430741a [Accessed September 4, 2017].

Baker, A.C., 2001. Ecosystems: Reef corals bleach to survive change. *Nature*, 411(6839), pp.765–766. Available at: http://www.nature.com/doifinder/10.1038/35081151 [Accessed September 11, 2017].

Baker, A.C., 2003. Flexibility and Specificity in Coral-Algal Symbiosis: Diversity, Ecology, and Biogeography of *Symbiodinium*. *Annual Review of Ecology, Evolution, and Systematics*, 34(1), pp.661–689. Available at: http://www.annualreviews.org/doi/10.1146/annurev.ecolsys.34.011802.132417.

Baker, A.C., Glynn, P.W. & Riegl, B., 2008. Climate change and coral reef bleaching: An ecological assessment of long-term impacts, recovery trends and future outlook. *Estuarine, Coastal and Shelf Science*, 80(4), pp.435–471. Available at: http://dx.doi.org/10.1016/j.ecss.2008.09.003.

Banin, E. et al., 2003. Superoxide Dismutase Is a Virulence Factor Produced by the Coral Bleaching Pathogen Vibrio shiloi. *Current Microbiology*, 46(6), pp.418–422. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12732948 [Accessed September 11, 2017].

Barnes, D.J. & Chalker, B.E., 1990. Calcification and photosynthesis in reef-building corals and algae. *Ecosystems of the world*, 25, pp.109–131. Available at: http://www.refdoc.fr/Detailnotice?idarticle=19676197.

Barshis, D.J. et al., 2013. Genomic basis for coral resilience to climate change. *Proceedings of the National Academy of Sciences of the United States of America*, 110(4), pp.1387–92. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3557039&tool=pmcentrez&rendertype=abstract [Accessed March 7, 2017].

Barshis, D.J. et al., 2014. Lineage-specific transcriptional profiles of Symbiodinium spp. Unaltered by heat stress in a coral host. *Molecular Biology and Evolution*, 31(6), pp.1343–1352. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24651035 [Accessed September 6, 2017].

Bashiardes, S., Zilberman-Schapira, G. & Elinav, E., 2016. Use of Metatranscriptomics in Microbiome Research. *Bioinformatics and biology insights*, 10, pp.19–25. Available at: http://www.ncbi.nlm.nih.gov/pubmed/27127406 [Accessed September 2, 2017].

Bayer, T. et al., 2013. The microbiome of the Red Sea coral Stylophora pistillata is dominated by tissue-associated Endozoicomonas bacteria. *Applied and environmental microbiology*, 79(15), pp.4759–62. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23709513 [Accessed September 2, 2017].

Becker, M. et al., 2013. Hybridization may facilitate in situ survival of endemic species through periods of climate change. *Nature Clim. Change*, 3(12), pp.1039–1043.

Available at: http://dx.doi.org/10.1038/nclimate2027.

Bellantuono, A.J. et al., 2012. Coral Thermal Tolerance: Tuning Gene Expression to Resist Thermal Stress. *PLoS ONE*, 7(11), pp.1–14.

Bellantuono, A.J., Hoegh-Guldberg, O. & Rodriguez-Lanetty, M., 2011. Resistance to thermal stress in corals without changes in symbiont composition. *Proceedings of the Royal Society B: Biological Sciences*, 279(1731), pp.1100–1107. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21976690 [Accessed March 10, 2017].

Bellwood, D.R. et al., 2004. Confronting the coral reef crisis. *Nature*, 429(6994), pp.827–833. Available at: http://dx.doi.org/10.1038/nature02691.

Ben-Haim, Y. et al., 2003. Vibrio coralliilyticus sp. nov., a temperature-dependent pathogen of the coral Pocillopora damicornis. *International Journal of Systematic and Evolutionary Microbiology*, 53(1), pp.309–315.

Benjamini, Y. & Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc Ser B Methodol*, 57.

Bentis, C., Kaufman, L. & Golubic, S., 2000. Endolithic fungi in reef-building corals (Order : Scleractinia) are common, cosmopolitan, and potentially pathogenic. *The Biological Bulletin*, 198(2), pp.254–260. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10786945 [Accessed September 10, 2017].

Berkelmans, R., 2009. Bleaching and Mortality Thresholds: How Much is Too Much? In Springer, Berlin, Heidelberg, pp. 103–119. Available at: http://link.springer.com/10.1007/978-3-540-69775-6_7 [Accessed September 4, 2017].

Berkelmans, R. & 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. *Proceedings. Biological sciences / The Royal Society*, 273(1599), pp.2305–2312.

Bettarel, Y. et al., 2015. The versatile nature of coral-associated viruses. *Environmental microbiology*, 17(10), pp.3433–3439.

Blackall, L.L., Wilson, B. & van Oppen, M.J.H., 2015. Coral-the world’s most diverse symbiotic ecosystem. *Molecular Ecology*, 24(21), pp.5330–5347. Available at: http://www.ncbi.nlm.nih.gov/pubmed/26414414 [Accessed September 4, 2017].

Blankenberg, D. et al., 2010. Manipulation of FASTQ data with Galaxy. *Bioinformatics (Oxford, England)*, 26(14), pp.1783–5. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20562416 [Accessed May 30, 2017].

Bordenstein, S.R. et al., 2015. Host Biology in Light of the Microbiome: Ten Principles of Holobionts and Hologenomes M. K. Waldor, ed. *PLOS Biology*, 13(8), p.e1002226. Available at: http://dx.plos.org/10.1371/journal.pbio.1002226 [Accessed August 24, 2017].

Bosch, T.C.G.G. & McFall-Ngai, M.J., 2011. Metaorganisms as the new frontier. *Zoology*, 114(4), pp.185–190. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21737250 [Accessed September 4, 2017].

Boulotte, N.M. et al., 2016. Exploring the Symbiodinium rare biosphere provides evidence for symbiont switching in reef-building corals. *The ISME Journal*, 10(11), pp.2693–2701. Available at: http://www.nature.com/doifinder/10.1038/ismej.2016.54 [Accessed September 10, 2017].

Bourne, D. et al., 2008. Changes in coral-associated microbial communities during a bleaching event. *The ISME Journal*, 2(4), pp.350–363. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18059490 [Accessed September 11, 2017].

Bourne, D.G., Morrow, K.M. & Webster, N.S., 2016. Insights into the Coral Microbiome: Underpinning the Health and Resilience of Reef Ecosystems. *Annual Review of Microbiology*, 70(1), pp.317–340. Available at: http://www.annualreviews.org/doi/10.1146/annurev-micro-102215-095440.

Bourne, D.G. & Munn, C.B., 2005. Diversity of bacteria associated with the coral Pocillopora damicornis from the Great Barrier Reef. *Environmental microbiology*, 7(8), pp.1162–74. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16011753 [Accessed February 16, 2016].

Bradshaw, A.D., 1965. Evolutionary Significance of Phenotypic Plasticity in Plants. In pp. 115–155. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0065266008600486 [Accessed August 30, 2017].

Brown, B. et al., 2002. Exploring the basis of thermotolerance in the reef coral Goniastrea aspera. *Marine Ecology Progress Series*, 242, pp.119–129. Available at: http://www.int-res.com/abstracts/meps/v242/p119-129/ [Accessed August 17, 2017].

Brown, B.. et al., 2002. Preliminary evidence for tissue retraction as a factor in photoprotection of corals incapable of xanthophyll cycling. *Journal of Experimental Marine Biology and Ecology*, 277(2), pp.129–144. Available at: http://www.sciencedirect.com/science/article/pii/S0022098102003052 [Accessed

September 16, 2017].

Brown, B.E. & Bythell, J.C., 2005. Perspectives on mucus secretion in reef corals. *Marine Ecology Progress Series*, 296, pp.291–309. Available at: http://www.int-res.com/abstracts/meps/v296/p291-309/.

Brown, B.E., Le Tissier, M.D.A. & Bythell, J.C., 1995. Mechanisms of bleaching deduced from histological studies of reef corals sampled during a natural bleaching event. *Marine Biology*, 122(4), pp.655–663. Available at: http://link.springer.com/10.1007/BF00350687 [Accessed September 11, 2017].

Bruno, J.F. et al., 2007. Regional Decline of Coral Cover in the Indo-Pacific: Timing, Extent, and Subregional Comparisons R. Freckleton, ed. *PLoS ONE*, 2(8), p.e711. Available at: http://dx.plos.org/10.1371/journal.pone.0000711 [Accessed August 29, 2017].

Buddemeier, R.W. & Fautin, D.G., 1993. Coral Bleaching as an Adaptive Mechanism. *BioScience*, 43(5), pp.320–326. Available at: https://academic.oup.com/bioscience/article-lookup/doi/10.2307/1312064.

Burke, L.M., World Resources Institute., K., Spalding, M., et al., 2011. *Reefs at risk revisited*, World Resources Institute. Available at: http://agris.fao.org/agris-search/search.do;jsessionid=CD163B24A00FECF025AC12127C8868A6?request_locale=es&recordID=QW2016000348&sourceQuery=&query=&sortField=&sortOrder=&agrovocString=&advQuery=&centerString=&enableField=#.WZ70MNSGxvw.mendeley [Accessed August 24, 2017].

Burke, L.M., World Resources Institute., K., Spalding, M., et al., 2011. *Reefs at risk revisited*, World Resources Institute. Available at: http://agris.fao.org/agris-search/search.do;jsessionid=CD163B24A00FECF025AC12127C8868A6?request_locale=es&recordID=QW2016000348&sourceQuery=&query=&sortField=&sortOrder=&agrovocString=&advQuery=&centerString=&enableField=#.WZ70MNSGxvw.mendeley [Accessed August 24, 2017].

Cahill, A.E. et al., 2013. How does climate change cause extinction? *Proceedings of the Royal Society B: Biological Sciences*, 280(1750), pp.20121890–20121890. Available at: http://rspb.royalsocietypublishing.org/cgi/doi/10.1098/rspb.2012.1890.

Canestrelli, D. et al., 2017. Climate change promotes hybridisation between deeply divergent species. *PeerJ*, 5, p.e3072. Available at: https://peerj.com/articles/3072.

Capinha, C. et al., 2013. Effects of Climate Change, Invasive Species, and Disease on the

Distribution of Native European Crayfishes. *Conservation Biology*, 27(4), pp.731–740. Available at: http://doi.wiley.com/10.1111/cobi.12043 [Accessed August 28, 2017].

Carpenter, L.W., Patterson, M.R. & Bromage, E.S., 2010. Water flow influences the spatiotemporal distribution of heat shock protein 70 within colonies of the scleractinian coral Montastrea annularis (Ellis and Solander, 1786) following heat stress: Implications for coral bleaching. *Journal of Experimental Marine Biology and Ecology*, 387(1–2), pp.52–59. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0022098110000742 [Accessed August 17, 2017].

Ceh, J., Van Keulen, M. & Bourne, D.G., 2011. Coral-associated bacterial communities on Ningaloo Reef, Western Australia. *FEMS Microbiology Ecology*, 75(1), pp.134–144. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21044100 [Accessed September 1, 2017].

Cervino, J.M. et al., 2008. The *Vibrio* core group induces yellow band disease in Caribbean and Indo-Pacific reef-building corals. *Journal of Applied Microbiology*, 105(5), pp.1658–1671. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18798767 [Accessed September 11, 2017].

Chen, C.-P. et al., 2011. The dynamics of microbial partnerships in the coral Isopora palifera. *The ISME Journal*, 5(4), pp.728–740. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20962876 [Accessed September 1, 2017].

Chen, I.-C. et al., 2011. Rapid range shifts of species associated with high levels of climate warming. *Science (New York, N.Y.)*, 333(6045), pp.1024–6. Available at: http://www.sciencemag.org.gate1.inist.fr/content/333/6045/1024.full [Accessed July 9, 2014].

Chen, M.-C. et al., 2005. ApRab11, a cnidarian homologue of the recycling regulatory protein Rab11, is involved in the establishment and maintenance of the Aiptasia–Symbiodinium endosymbiosis. *Biochemical and Biophysical Research Communications*, 338(3), pp.1607–1616. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0006291X05024101 [Accessed August 11, 2017].

Chen, Z.J., 2005. Ubiquitin signalling in the NF-κB pathway. *Nature Cell Biology*, 7(8), pp.758–765. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16056267 [Accessed

September 19, 2017].

Chevin, L.M., Collins, S. & Lefèvre, F., 2013. Phenotypic plasticity and evolutionary demographic responses to climate change: Taking theory out to the field. *Functional Ecology*, 27(4), pp.967–979.

Chmielewski, F.M. & Rötzer, T., 2002. Annual and spatial variability of the beginning of growing season in Europe in relation to air temperature changes. *Climate Research*, 19(3), pp.257–264.

Cikala, M. et al., 1999. Identification of caspases and apoptosis in the simple metazoan Hydra. *Current biology : CB*, 9(17), pp.959–62. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10508589 [Accessed August 17, 2017].

Coffroth, M.A. & Santos, S.R., 2005. Genetic diversity of symbiotic dinoflagellates in the genus Symbiodinium. *Protist*, 156(1), pp.19–34.

Coles, S.L. & Riegl, B.M., 2013. Thermal tolerances of reef corals in the Gulf: A review of the potential for increasing coral survival and adaptation to climate change through assisted translocation. *Marine Pollution Bulletin*, 72(2), pp.323–332. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23058810 [Accessed September 4, 2017].

Colley, N.J. & Trench, R.K., 1983. Selectivity in phagocytosis and persistence of symbiotic algae in the scyphistoma stage of the jellyfish Cassiopeia xamachana. *Proceedings of the Royal Society of London. Series B, Biological sciences*, 219(1214), pp.61–82. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22470960 [Accessed September 10, 2017].

Conesa, A. et al., 2005. Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. *Bioinformatics*, 21(18), pp.3674–3676. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16081474 [Accessed May 30, 2017].

Cooney, R.P. et al., 2002. Characterization of the bacterial consortium associated with black band disease in coral using molecular microbiological techniques. *Environmental microbiology*, 4(7), pp.401–13. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12123476 [Accessed September 11, 2017].

Costanza, R. et al., 2014. Changes in the global value of ecosystem services. *Global Environmental Change*, 26, pp.152–158. Available at: http://www.sciencedirect.com.inee.bib.cnrs.fr/science/article/pii/S0959378014000685 [Accessed August 28, 2017].

Cramer, W. et al., 2014. Chapter 18 - Detection and attribution of observed impacts. *Climate*

Change 2014: Impacts, Adaptation and Vulnearability. Part A: Global and Sectoral Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, pp.979–1037.

Cróquer, A. et al., 2013. Bacterial assemblages shifts from healthy to yellow band disease states in the dominant reef coral *Montastraea faveolata*. *Environmental Microbiology Reports*, 5(1), pp.90–96. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23757136 [Accessed August 24, 2017].

Crozier, L.G. & Hutchings, J.A., 2014. Plastic and evolutionary responses to climate change in fish. *Evolutionary Applications*, 7(1), pp.68–87. Available at: http://doi.wiley.com/10.1111/eva.12135 [Accessed August 26, 2017].

Cumbo, V.R., Baird, A.H. & van Oppen, M.J.H., 2013. The promiscuous larvae: flexibility in the establishment of symbiosis in corals. *Coral Reefs*, 32(1), pp.111–120. Available at: http://link.springer.com/10.1007/s00338-012-0951-7 [Accessed September 10, 2017].

Cunning, R., Glynn, P.W. & Baker, A.C., 2013. Flexible associations between Pocillopora corals and Symbiodinium limit utility of symbiosis ecology in defining species. *Coral Reefs*, 32(3), pp.795–801.

Dabe, E.C. et al., 2015. DNA Methylation in Basal Metazoans: Insights from Ctenophores. *Integrative and Comparative Biology*, 55(6), pp.1096–1110.

Debiasse, M.B. & Kelly, M.W., 2016. Plastic and Evolved Responses to Global Change: What Can We Learn from Comparative Transcriptomics? *Journal of Heredity*, pp.71–81. Available at: https://oup.silverchair-cdn.com/oup/backfile/Content_public/Journal/jhered/107/1/10.1093_jhered_esv073/2/esv073.pdf?Expires=1494949310&Signature=d0MmUUDcgEfHASsGZw-VS~3tMz52TbvzPXE-logH6dovkcivsfVxu2zG~qTE9aO9eBw5nZS30sOIo8kR~CON8FDuSwhtDVRlurqavCIkx-1gPZuuhc~qzeCS9jg4gKtd4l7rlxMUbS-S9WcrJ-4eAh0Z~m1lf24prXWJjGZrcig8Up-RRqgiAujhThSYWSf5DhuqVudr9qQgHtO74n84AgQk-SDO9hoVUUzWQla-~INtTGKqVyypTslkdy4i5cRcvbVfRdQagzjAgDRPCjEhbPDKXWQhTIPi1PK0xXHdZ4LfrJUYA22RtFmVmasnWgoX6vHnXo-lfSdwqN-nULH01A__&Key-Pair-Id=APKAIUCZBIA4LVPAVW3Q [Accessed May 15, 2017].

DeJong, G., 2005. Evolution of phenotypic plasticity: patters of plasticity and the emergence of ecotypes. *New Phytologist*, 166(2003), pp.101–118.

Denner, E.B.M. et al., 2003. Aurantimonas coralicida gen. nov., sp. nov., the causative agent of white plague type II on Caribbean scleractinian corals. *INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY*, 53(4), pp.1115–1122. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12892136 [Accessed September 11, 2017].

Desalvo, M.K. et al., 2008. Differential gene expression during thermal stress and bleaching in the Caribbean coral Montastraea faveolata. *Molecular Ecology*, 17(17), pp.3952–3971. Available at: http://doi.wiley.com/10.1111/j.1365-294X.2008.03879.x [Accessed June 6, 2017].

DeSalvo, M.K. et al., 2010. Coral host transcriptomic states are correlated with Symbiodinium genotypes. *Molecular Ecology*, 19(6), pp.1174–1186.

Dixon, B., 2015. Tolerance Across Latitudes. , 348(6242), pp.2014–2016.

Dobin, A. et al., 2013. STAR: ultrafast universal RNA-seq aligner. *Bioinformatics*, 29(1), pp.15–21. Available at: https://academic.oup.com/bioinformatics/article-lookup/doi/10.1093/bioinformatics/bts635 [Accessed May 30, 2017].

Douglas, A., 1998. Host benefit and the evolution of specialization in symbiosis. *Heredity*, 81(6), pp.599–603.

Downs, C.. et al., 2002. Oxidative stress and seasonal coral bleaching. *Free Radical Biology and Medicine*, 33(4), pp.533–543. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0891584902009073 [Accessed September 11, 2017].

Downs, C.A. et al., 2009. Symbiophagy as a cellular mechanism for coral bleaching. *Autophagy*, 5(2), pp.211–6. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19066451 [Accessed September 11, 2017].

Duncan, E.J., Gluckman, P.D. & Dearden, P.K., 2014. Epigenetics, plasticity, and evolution: How do we link epigenetic change to phenotype? *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution*, 322(4), pp.208–220. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24719220 [Accessed September 5, 2017].

Dykens, J.A. et al., 1992. Oxygen Radical Production in the Sea Anemone Anthopleura Elegantissima and its Endosymbiotic Algae. *Journal of Experimental Biology*, 168(1). Available at: http://jeb.biologists.org/content/168/1/219 [Accessed September 11, 2017].

Efrony, R. et al., 2007. Phage therapy of coral disease. *Coral Reefs*, 26(1), pp.7–13. Available

at: http://link.springer.com/10.1007/s00338-006-0170-1 [Accessed September 2, 2017].

Efrony, R., Atad, I. & Rosenberg, E., 2009. Phage Therapy of Coral White Plague Disease: Properties of Phage BA3. *Current Microbiology*, 58(2), pp.139–145. Available at: http://link.springer.com/10.1007/s00284-008-9290-x [Accessed September 2, 2017].

Escudié, F. et al., 2015. *FROGS: Find Rapidly OTU with Galaxy Solution*,

Ewert, M.A., Lang, J.W. & Nelson, C.E., 2005. Geographic variation in the pattern of temperature-dependent sex determination in the American snapping turtle (Chelydra serpentina). *Journal of Zoology*, 265(1), pp.81–95. Available at: http://doi.wiley.com/10.1017/S0952836904006120 [Accessed August 28, 2017].

Fabricius, K.E. et al., 2004. Identity and diversity of coral endosymbionts (zooxanthellae) from three Palauan reefs with contrasting bleaching, temperature and shading histories. *Molecular Ecology*, 13(8), pp.2445–2458. Available at: http://doi.wiley.com/10.1111/j.1365-294X.2004.02230.x [Accessed September 4, 2017].

Félix, M. & Wagner, a, 2008. Robustness and evolution: concepts, insights and challenges from a developmental model system. *Heredity*, 100(2), pp.132–140.

Ferrer, L.M. & Szmant, A.M., 1988. Nutrient regeneration by the endolithic community in coral skeletons. *Proceedings of the 6th International Coral Reef Symposium*, 3.

Finnegan, D.J., 2012. Retrotransposons. *Current Biology*, 22(11), pp.R432–R437. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0960982212004459 [Accessed September 5, 2017].

Fisher, L. et al., 2009. Real-time coral stress observations before, during, and after beach nourishment dredging offshore southeast Florida, USA. *Proceedings of the 11th International Coral Reef Symposium*, (1), pp.34–37.

Flot, J.-F. & Tillier, S., 2007. The mitochondrial genome of Pocillopora (Cnidaria: Scleractinia) contains two variable regions: The putative D-loop and a novel ORF of unknown function. *Gene*, 401(1), pp.80–87. Available at: http://www.sciencedirect.com/science/article/pii/S0378111907003666 [Accessed May 31, 2017].

Franks, S.J., Weber, J.J. & Aitken, S.N., 2014. Evolutionary and plastic responses to climate change in terrestrial plant populations. *Evolutionary Applications*, 7(1), pp.123–139.

Available at: http://doi.wiley.com/10.1111/eva.12112 [Accessed August 28, 2017].

Frazão, B., Vasconcelos, V. & Antunes, A., 2012. Sea anemone (cnidaria, anthozoa, actiniaria) toxins: An overview. *Marine Drugs*, 10(8), pp.1812–1851.

Frydenborg, B.R. et al., 2014. Temperature-Dependent Inhibition of Opportunistic Vibrio Pathogens by Native Coral Commensal Bacteria. *Microbial Ecology*, 67(2), pp.392–401. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24370863 [Accessed September 14, 2017].

Galindo-González, L. et al., 2017. LTR-retrotransposons in plants: Engines of evolution. *Gene*, 626, pp.14–25. Available at: http://www.ncbi.nlm.nih.gov/pubmed/28476688 [Accessed September 5, 2017].

Ganot, P. et al., 2011. Adaptations to endosymbiosis in a Cnidarian-Dinoflagellate association: Differential gene expression and specific gene duplications. *PLoS Genetics*, 7(7).

Gates, R.D., Baghdasarian, G. & Muscatine, L., 1992. Temperature Stress Causes Host Cell Detachment in Symbiotic Cnidarians: Implications for Coral Bleaching. *The Biological Bulletin*, 182(3), pp.324–332. Available at: http://www.journals.uchicago.edu/doi/10.2307/1542252 [Accessed September 11, 2017].

Ghalambor, C.K. et al., 2007. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Functional Ecology*, 21(3), pp.394–407.

Gibert, J.M., 2017. The flexible stem hypothesis: evidence from genetic data. *Development Genes and Evolution*, (1911), pp.1–11.

Gienapp, P. et al., 2008. Climate change and evolution: Disentangling environmental and genetic responses. *Molecular Ecology*, 17(1), pp.167–178.

Gignoux-Wolfsohn, S.A. & Vollmer, S. V., 2015. Identification of Candidate Coral Pathogens on White Band Disease-Infected Staghorn Coral C. R. Voolstra, ed. *PLOS ONE*, 10(8), p.e0134416. Available at: http://www.ncbi.nlm.nih.gov/pubmed/26241853 [Accessed September 14, 2017].

Gitschier, J. et al., 1999. Hephaestin, a ceruloplasmin homologue implicated in intestinal iron transport, is defective in the sla mouse. *Nature Genetics*, 21(2), pp.195–199. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9988272 [Accessed August 17, 2017].

Glasl, B., Herndl, G.J. & Frade, P.R., 2016. The microbiome of coral surface mucus has a key role in mediating holobiont health and survival upon disturbance. *Isme J*, pp.1–13. Available at: http://dx.doi.org/10.1038/ismej.2016.9%5Cnhttp://10.1038/ismej.2016.9.

Gleason, D.F. & Wellington, G.M., 1993. Ultraviolet radiation and coral bleaching. *Nature*, 365(6449), pp.836–838. Available at: http://www.nature.com/doifinder/10.1038/365836a0 [Accessed September 14, 2017].

Glynn, P.W. et al., 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. *Bulletin of Marine Science*, 69(1), pp.79–109.

Glynn, P.W. & D'Croz, L., 1990. Experimental evidence for high temperature stress as the cause of El Nifio-coincident coral mortality. *Coral Reefs*, 8, pp.181–191.

Le Goff, C. et al., 2016. Carbonic anhydrases in cnidarians: Novel perspectives from the octocorallian corallium rubrum. *PLoS ONE*, 11(8), pp.1–22.

Goodier, J.L., 2016. Restricting retrotransposons: a review. *Mobile DNA*, 7, p.16. Available at: http://www.ncbi.nlm.nih.gov/pubmed/27525044 [Accessed September 5, 2017].

Goreau, T.F., 1964. Mass Expulsion of Zooxanthellae from Jamaican Reef Communities after Hurricane Flora. *Science*, 145(3630), pp.383–386. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17816975 [Accessed September 14, 2017].

Granados-Cifuentes, C. et al., 2013. High natural gene expression variation in the reef-building coral Acropora millepora: potential for acclimative and adaptive plasticity. *BMC genomics*, 14(1), p.228. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3630057&tool=pmcentrez&rendertype=abstract.

Grandbastien, M., 1998. Activation of plant retrotransposons under stress conditions. *Trends in Plant Science*, 3(5), pp.181–187. Available at: http://linkinghub.elsevier.com/retrieve/pii/S1360138598012321 [Accessed August 18, 2017].

Grottoli, A.G., Rodrigues, L.J. & Palardy, J.E., 2006. Heterotrophic plasticity and resilience in bleached corals. *Nature*, 440(7088), pp.1186–1189. Available at: http://www.nature.com/doifinder/10.1038/nature04565 [Accessed September 14, 2017].

Guerrero, R., Margulis, L. & Berlanga, M., 2013. Symbiogenesis: the holobiont as a unit of

evolution. *International microbiology : the official journal of the Spanish Society for Microbiology*, 16(3), pp.133–43. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24568029 [Accessed August 24, 2017].

Guest, J.R. et al., 2012. Contrasting Patterns of Coral Bleaching Susceptibility in 2010 Suggest an Adaptive Response to Thermal Stress M. V. Matz, ed. *PLoS ONE*, 7(3), p.e33353. Available at: http://dx.plos.org/10.1371/journal.pone.0033353 [Accessed September 4, 2017].

Halestrap, A.P. & Meredith, D., 2004. The SLC16 gene family?from monocarboxylate transporters (MCTs) to aromatic amino acid transporters and beyond. *Pfl�gers Archiv European Journal of Physiology*, 447(5), pp.619–628. Available at: http://link.springer.com/10.1007/s00424-003-1067-2 [Accessed August 17, 2017].

Harrison, P. & Wallace, C., 1990. Reproduction, dispersal and recruitment of scleractinian corals. *Coral reefs*. Available at: http://epubs.scu.edu.au/esm_pubs/3077 [Accessed September 17, 2017].

Hashimoto, K. et al., 2004. Isolation and characterization of stress-responsive genes from the scleractinian coral Pocillopora damicornis. *Coral Reefs*, 23(4), pp.485–491. Available at: http://link.springer.com/10.1007/s00338-004-0410-1 [Accessed July 19, 2017].

Hegarty, M.J., 2012. Invasion of the hybrids. *Molecular Ecology*, 21(19), pp.4669–4671. Available at: http://doi.wiley.com/10.1111/j.1365-294X.2012.05720.x [Accessed August 28, 2017].

Hernandez-Agreda, A., Gates, R.D. & Ainsworth, T.D., 2017. Defining the Core Microbiome in Corals' Microbial Soup. *Trends in Microbiology*, 25(2), pp.125–140. Available at: http://www.ncbi.nlm.nih.gov/pubmed/27919551 [Accessed August 24, 2017].

Heron, S.., Eakin, C.M. & Douvere, F., 2017. Impacts of Climate Change on World Heritage Coral Reefs: A First Global Scientific Assessment. *UNESCO World Heritage Centre*.

Hoegh-Guldberg, O. et al., 2007. Coral Reefs Under Rapid Climate Change and Ocean Acidification. *Science*, 318(2007), pp.1737–1742. Available at: http://science.sciencemag.org/content/318/5857/1737.abstract.

Hoeksema, B., 2017. World Register of Marine Species: Pocillopora damicornis (Linnaeus, 1758). Available at: http://www.marinespecies.org/aphia.php?p=taxdetails&id=206953 [Accessed August 31, 2017].

Hoffmann, A.A., Sgro, C.M. & Sgrò, C.M., 2011. Climate change and evolutionary adaptation.

Nature, 470(7335), pp.479–485. Available at: http://www.nature.com/doifinder/10.1038/nature09670.

Hofmann, G.E. & Todgham, A.E., 2010. Living in the now: physiological mechanisms to tolerate a rapidly changing Environment. *Annual Review of Physiology*, 72(1), pp.127–145. Available at: http://www.annualreviews.org/doi/abs/10.1146/annurev-physiol-021909-135900.

de Hoon, M.J.L. et al., 2004. Open source clustering software. *Bioinformatics*, 20(9), pp.1453–1454. Available at: http://www.ncbi.nlm.nih.gov/pubmed/14871861 [Accessed June 8, 2017].

Houlbrèque, F. & Ferrier-Pagès, C., 2009. Heterotrophy in Tropical Scleractinian Corals. *Biological Reviews*, 84(1), pp.1–17. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19046402 [Accessed September 14, 2017].

Hsu, C.-M. et al., 2012. Temporal and Spatial Variations in Symbiont Communities of Catch Bowl Coral Isopora palifera (Scleractinia: Acroporidae) on Reefs in Kenting National Park, Taiwan. *Zoological studies*, 51(8), pp.1343–1353. Available at: http://zoolstud.sinica.edu.tw/Journals/51.8/1343.pdf [Accessed September 10, 2017].

Hu, B. et al., 2011. Purification and characterization of gigantoxin-4, a new actinoporin from the sea anemone Stichodactyla gigantea. *International Journal of Biological Sciences*, 7(6), pp.729–739.

Huang, H. et al., 2013. Diversity of free-living and symbiotic Symbiodinium in the coral reefs of Sanya , South China Sea. *Marine Biology Research*, 9(2), pp.117–128.

Huang, S., 2002. Histone methyltransferases, diet nutrients and tumour suppressors. *Nature Reviews Cancer*, 2(6), pp.469–476. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12189389 [Accessed August 17, 2017].

Hughes, A.D. & 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(11), p.e81172. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24278392 [Accessed August 31, 2017].

Hume, B.C.C. et al., 2015. Symbiodinium thermophilum sp. nov., a thermotolerant symbiotic alga prevalent in corals of the world's hottest sea, the Persian/Arabian Gulf. *Scientific Reports*, 5, p.8562.

Inouye, D.W. et al., 2000. Climate change is affecting altitudinal migrants and hibernating

species. *Proceedings of the National Academy of Sciences*, 97(4), pp.1630–1633. Available at: http://www.pnas.org/cgi/doi/10.1073/pnas.97.4.1630.

IPCC, 2013. IPCC Factsheet: What is the IPCC? Available at: http://www.ipcc.ch/news_and_events/docs/factsheets/FS_what_ipcc.pdf [Accessed August 22, 2017].

Jaenisch, R. & Bird, A., 2003. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. *Nature Genetics*, 33(3s), pp.245–254. Available at: http://www.nature.com/doifinder/10.1038/ng1089 [Accessed September 5, 2017].

Janzen, F.J., 1994. Climate change and temperature-dependent sex determination in reptiles. *Proceedings of the National Academy of Sciences of the United States of America*, 91(16), pp.7487–90. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8052608 [Accessed August 28, 2017].

Jena, N.R., 2012. DNA damage by reactive species: Mechanisms, mutation and repair. *Journal of biosciences*, 37(3), pp.503–17. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22750987 [Accessed August 18, 2017].

Jombart, T., Devillard, S. & Balloux, F., 2010. Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC Genetics*, 11(1), p.94. Available at: http://dx.doi.org/10.1186/1471-2156-11-94.

Jones, A.. et al., 2008. A community change in the algal endosymbionts of a scleractinian coral following a natural bleaching event: field evidence of acclimatization. *Proceedings of the Royal Society B: Biological Sciences*, 275(1641), pp.1359–1365. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18348962 [Accessed September 10, 2017].

Jones, A. & Berkelmans, R., 2010. Potential Costs of Acclimatization to a Warmer Climate: Growth of a Reef Coral with Heat Tolerant vs. Sensitive Symbiont Types R. DeSalle, ed. *PLoS ONE*, 5(5), p.e10437. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20454653 [Accessed September 7, 2017].

Kaplan, J.M. & Pigliucci, M., 2001. Genes “for” phenotypes: A modern history view. *Biology and Philosophy*, 16(2), pp.189–213.

Karell, P. et al., 2011. Climate change drives microevolution in a wild bird. *Nature communications*, 2, p.208. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21343926 [Accessed August 24, 2017].

Karim, W., Nakaema, S. & Hidaka, M., 2015. Temperature effects on the growth rates and photosynthetic activities of Symbiodinium cells. *J. Mar. Sci. Eng.*, 3, pp.368–381.

Karmodiya, K. et al., 2014. Camello, a novel family of Histone Acetyltransferases that acetylate histone H4 and is essential for zebrafish development. *Scientific reports*, 4(iii), p.6076. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4133703&tool=pmcentrez&rendertype=abstract.

Kazandjian, A. et al., 2008. Isolation of symbiosomes and the symbiosome membrane complex from the zoanthid Zoanthus robustus. *Phycologia*, 47(May), pp.294–306. Available at: http://www.phycologia.org/doi/abs/10.2216/PH07-23.1%5Cnhttp://www.phycologia.org/perlserv/?request=get-abstract&doi=10.2216%252FPH07-23.1.

Kellogg, C.A., 2004. Tropical Archaea: Diversity associated with the surface microlayer of corals. *Marine Ecology Progress Series*, 273, pp.81–88. Available at: http://pubs.er.usgs.gov/publication/70026947.

Kenkel, C.D. & Matz, M. V, 2016a. Enhanced gene expression plasticity as a mechanism of adaptation to a variable environment in a reef-building coral. *Nature Ecology & Evolution*, 1(3), p.14. Available at: http://www.nature.com/articles/s41559-016-0014.

Kenkel, C.D. & Matz, M. V, 2016b. Gene expression plasticity as a mechanism of coral adaptation to a variable environment. *Nature Ecology & Evolution*, 1(1), p.14. Available at: http://www.nature.com/articles/s41559-016-0014.

Kenkel, C.D., Meyer, E. & Matz, M. V., 2013. Gene expression under chronic heat stress in populations of the mustard hill coral (Porites astreoides) from different thermal environments. *Molecular Ecology*, 22(16), pp.4322–4334.

Kimes, N.E. et al., 2013. The *Montastraea faveolata* microbiome: ecological and temporal influences on a Caribbean reef-building coral in decline. *Environmental Microbiology*, 15(7), pp.2082–2094. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23750924 [Accessed September 1, 2017].

Kisten, Y., 2014. The influence of heterotrophy on the resilience of hard coral Pocillopora damicornis to thermal stress and bleaching. Available at: https://researchspace.ukzn.ac.za/handle/10413/11212 [Accessed August 31, 2017].

Klindworth, A. et al., 2013. Evaluation of general 16S ribosomal RNA gene PCR primers for

classical and next-generation sequencing-based diversity studies. *Nucleic Acids Research*, 41(1), pp.1–11.

Knowlton, N. & Rohwer, F., 2003. Multispecies Microbial Mutualisms on Coral Reefs: The Host as a Habitat. *The American Naturalist*, 162(S4), pp.S51–S62. Available at: http://www.ncbi.nlm.nih.gov/pubmed/14583857 [Accessed September 14, 2017].

Koh, E.G.L., 1997. Do Scleractinian Corals Engage in Chemical Warfare Against Microbes? *Journal of Chemical Ecology*, 23(2), pp.379–398. Available at: http://link.springer.com/10.1023/B:JOEC.0000006366.58633.f4 [Accessed September 1, 2017].

Kooperman, N. et al., 2007. Coral mucus-associated bacterial communities from natural and aquarium environments. *FEMS Microbiology Letters*, 276(1), pp.106–113. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17937669 [Accessed September 10, 2017].

Koren, O. & Rosenberg, E., 2006. Bacteria associated with mucus and tissues of the coral Oculina patagonica in summer and winter. *Applied and Environmental Microbiology*, 72(8), pp.5254–5259. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16885273 [Accessed September 1, 2017].

Krämer, W.E. et al., 2012. Dynamic Regulation of Photoprotection Determines Thermal Tolerance of Two Phylotypes of Symbiodinium Clade A at Two Photon Fluence Rates. *Photochemistry and Photobiology*, 88(2), pp.398–413. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22117932 [Accessed September 7, 2017].

Kvennefors, E.C.E. et al., 2012. Regulation of Bacterial Communities Through Antimicrobial Activity by the Coral Holobiont. *Microbial Ecology*, 63(3), pp.605–618.

Lajeunesse, T.C., 2005. “Species” radiations of symbiotic dinoflagellates in the Atlantic and Indo-Pacific since the Miocene-Pliocene transition. *Molecular Biology and Evolution*, 22(3), pp.570–581.

LaJeunesse, T.C. et al., 2003. Low symbiont diversity in southern Great Barrier Reef corals relative to those of the Caribbean. *Limnology and Oceanography*, 48(5), pp.2046–2054.

Lajeunesse, T.C. & Trench, R.K., 2000. Biogeography of two species of Symbiodinium (Freudenthal) inhabiting the intertidal sea anemone Anthopleura elegantissima (Brandt). *Biological Bulletin*, 199(2), pp.126–134.

Leclère, L. & Röttinger, E., 2017. Diversity of Cnidarian Muscles: Function, Anatomy, Development and Regeneration. *Frontiers in Cell and Developmental Biology*,

4(January), p.157. Available at: http://journal.frontiersin.org/article/10.3389/fcell.2016.00157/full.

Lee, S.T.M. et al., 2016. Mucus Sugar Content Shapes the Bacterial Community Structure in Thermally Stressed Acropora muricata. *Frontiers in microbiology*, 7, p.371. Available at: http://www.ncbi.nlm.nih.gov/pubmed/27047481 [Accessed September 14, 2017].

Lee, Y. et al., 2005. Pyrexia is a new thermal transient receptor potential channel endowing tolerance to high temperatures in Drosophila melanogaster. *Nat Genet*, 37(3), pp.305–310. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15731759.

Leggat, W. et al., 2011. Differential Responses of the Coral Host and Their Algal Symbiont to Thermal Stress C. R. Voolstra, ed. *PLoS ONE*, 6(10), p.e26687. Available at: http://dx.plos.org/10.1371/journal.pone.0026687 [Accessed September 5, 2017].

Lehnert, E.M. et al., 2014. Extensive differences in gene expression between symbiotic and aposymbiotic cnidarians. *G3 (Bethesda, Md.)*, 4(2), pp.277–95. Available at: http://www.g3journal.org/lookup/ [Accessed August 8, 2017].

Lema, K.A., Willis, B.L. & Bourne, D.G., 2012. Corals form characteristic associations with symbiotic nitrogen-fixing bacteria. *Applied and environmental microbiology*, 78(9), pp.3136–44. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22344646 [Accessed September 14, 2017].

Lesser, M.P. et al., 2004. Discovery of Symbiotic Nitrogen-Fixing Cyanobacteria in Corals. *Science*, 305(5686), pp.997–1000. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15310901 [Accessed September 1, 2017].

Lesser, M.P., 1996. Elevated temperatures and ultraviolet radiation cause oxidative stress and inhibit photosynthesis in ymbiotic dinoflagellates. *Limnology and Oceanography*, 41(2), pp.271–283. Available at: http://doi.wiley.com/10.4319/lo.1996.41.2.0271 [Accessed September 11, 2017].

Levin, R.A. et al., 2016. Sex, Scavengers, and Chaperones: Transcriptome Secrets of Divergent Symbiodinium Thermal Tolerances. *Molecular Biology and Evolution*, 33(9), pp.2201–2215. Available at: https://watermark.silverchair.com/api/watermark?token=AQECAHi208BE49Ooan9kkhW_Ercy7Dm3ZL_9Cf3qfKAc485ysgAAAdIwggHOBgkqhkiG9w0BBwagggG_MIIBuwIBADCCAbQGCSqGSIb3DQEHATAeBglghkgBZQMEAS4wEQQMH58QZzp2c3L9POndAgEQgIIBhVCd3LYm5MBSXDHiQ-jCl3AZxPVm--kUw_xFy6gORC49h [Accessed September 5, 2017].

Li, J. et al., 2014. Bacterial dynamics within the mucus, tissue and skeleton of the coral Porites lutea during different seasons. *Scientific Reports*, 4, p.7320. Available at: http://www.nature.com/srep/2014/141205/srep07320/full/srep07320.html%5Cnhttp://www.nature.com/doifinder/10.1038/srep07320.

Liefting, M., Hoffmann, A.A. & Ellers, J., 2009. Plasticity versus environmental canalization: Population differences in thermal responses along a latitudinal gradient in drosophila serrata. *Evolution*, 63(8), pp.1954–1963. Available at: http://doi.wiley.com/10.1111/j.1558-5646.2009.00683.x [Accessed May 12, 2017].

Little, A.F., van Oppen, M.J.H. & Willis, B.L., 2004. Flexibility in algal endosymbioses shapes growth in reef corals. *Science (New York, N.Y.)*, 304(5676), pp.1492–1494. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15178799 [Accessed September 7, 2017].

Littman, R.A., Bourne, D.G. & Willis, B.L., 2010. Responses of coral-associated bacterial communities to heat stress differ with Symbiodinium type on the same coral host. *Molecular Ecology*, 19(9), pp.1978–1990. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20529072 [Accessed September 1, 2017].

Littman, R., Willis, B.L. & Bourne, D.G., 2011. Metagenomic analysis of the coral holobiont during a natural bleaching event on the Great Barrier Reef. *Environmental Microbiology Reports*, 3(6), pp.651–660. Available at: http://doi.wiley.com/10.1111/j.1758-2229.2010.00234.x [Accessed September 15, 2017].

Logan, M.L., Cox, R.M. & Calsbeek, R., 2014. Natural selection on thermal performance in a novel thermal environment. *Proceedings of the National Academy of Sciences of the United States of America*, (19), pp.1–5. Available at: http://www.ncbi.nlm.nih.gov/pubmed/25225361.

Love, M.I., Huber, W. & Anders, S., 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*, 15(12), p.550. Available at: http://dx.doi.org/10.1186/s13059-014-0550-8.

Luna, G.M. et al., 2009. Vibrio harveyi as a causative agent of the White Syndrome in tropical stony corals. *Environmental Microbiology Reports*, 2(1), pp.120–127. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23766006 [Accessed September 11, 2017].

Magalon, H. et al., 2004. Development of coral and zooxanthella-specific microsatellites in three species of Pocillopora (Cnidaria, Scleractinia) from French Polynesia. *Molecular Ecology Notes*, 4(2), pp.206–208.

Magalon, H., Adjeroud, M. & Veuille, M., 2005. Patterns of genetic variation do not correlate with geographical distance in the reef-building coral *Pocillopora meandrina* in the South Pacific. *Molecular Ecology*, 14(October), pp.1861–1868.

Magoc, T. & Salzberg, S.L., 2011. FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics*, 27(21), pp.2957–2963. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21903629 [Accessed August 31, 2017].

Mao-Jones, J. et al., 2010. How Microbial Community Composition Regulates Coral Disease Development C. Roberts, ed. *PLoS Biology*, 8(3), p.e1000345. Available at: http://dx.plos.org/10.1371/journal.pbio.1000345 [Accessed September 11, 2017].

Maor-Landaw, K. et al., 2014. Gene expression profiles during short-term heat stress in the red sea coral *Stylophora pistillata*. *Global Change Biology*, 20(10), pp.3026–3035. Available at: http://doi.wiley.com/10.1111/gcb.12592 [Accessed August 17, 2017].

Maor-Landaw, K. & Levy, O., 2016. Gene expression profiles during short-term heat stress; branching vs. massive Scleractinian corals of the Red Sea. *PeerJ*, 4, p.e1814. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4824894&tool=pmcentrez&rendertype=abstract.

Margulis, L., 1993. *Symbiosis in Cell Evolution: Microbial Communities in the Archean and Proterozoic Eons*, Freeman. Available at: https://books.google.fr/books?id=8ZiFQgAACAAJ.

Margulis, L. & Fester, R., 1991. *Symbiosis as a source of evolutionary innovation : speciation and morphogenesis*, MIT Press. Available at: https://mitpress.mit.edu/books/symbiosis-source-evolutionary-innovation [Accessed August 24, 2017].

Marlow, H. et al., 2012. Functional Roles of Notch Signaling in the cnidarian Nematostella vectensis. *Dev Biol.*, 76(October 2009), pp.211–220.

Martin, M., 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.journal*, 17(1), p.10. Available at: http://journal.embnet.org/index.php/embnetjournal/article/view/200 [Accessed May 30, 2017].

Maumus, F. et al., 2009. Potential impact of stress activated retrotransposons on genome evolution in a marine diatom. *BMC genomics*, 10, p.624. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20028555%5Cnhttp://www.pubmedcentral.nih.

gov/articlerender.fcgi?artid=PMC2806351.

Mayfield, A.B. et al., 2014. Compartment-specific transcriptomics in a reef-building coral exposed to elevated temperatures. *Molecular Ecology*, 23(23), pp.5816–5830. Available at: http://doi.wiley.com/10.1111/mec.12982 [Accessed September 15, 2017].

Mayfield, A.B. et al., 2017. The proteomic response of the reef coral Pocillopora acuta to experimentally elevated temperatures. Available at: https://peerj.com/preprints/3252/ [Accessed September 15, 2017].

Maynard, J.A. et al., 2008. Major bleaching events can lead to increased thermal tolerance in corals. *Marine Biology*, 155(2), pp.173–182. Available at: http://link.springer.com/10.1007/s00227-008-1015-y [Accessed September 4, 2017].

McDevitt-Irwin, J.M. et al., 2017. Responses of Coral-Associated Bacterial Communities to Local and Global Stressors. *Frontiers in Marine Science*, 4, p.262. Available at: http://journal.frontiersin.org/article/10.3389/fmars.2017.00262/full [Accessed September 1, 2017].

McFadden, C.S., 1997. Contributions of sexual and asexual reproduction to population structure in the clonal soft coral, alcyonium rudyi. *Evolution*, 51(1), pp.112–126. Available at: http://www.ncbi.nlm.nih.gov/pubmed/28568799 [Accessed September 15, 2017].

McFall-Ngai, M. et al., 2013. Animals in a bacterial world, a new imperative for the life sciences. *Proceedings of the National Academy of Sciences*, 110(9), pp.3229–3236. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23391737 [Accessed August 24, 2017].

McFall-Ngai, M.J., Henderson, B. & Ruby, E.G., 2005. *The Influence of Cooperative Bacteria on Animal Host Biology*, Cambridge University Press. Available at: https://books.google.fr/books?id=BfwalYMlmlEC.

McGinley, M. et al., 2012. Symbiodinium spp. in colonies of eastern Pacific Pocillopora spp. are highly stable despite the prevalence of low-abundance background populations. *Marine Ecology Progress Series*, 462, pp.1–7. Available at: http://www.int-res.com/abstracts/meps/v462/p1-7/ [Accessed September 10, 2017].

McLaughlin, S., 2017. Evidence that polycystins are involved in Hydra cnidocyte discharge. *Invertebrate Neuroscience*, 17(1), pp.1–14.

McMurdie, P.J. et al., 2013. phyloseq: An R Package for Reproducible Interactive Analysis

and Graphics of Microbiome Census Data M. Watson, ed. *PLoS ONE*, 8(4), p.e61217. Available at: http://dx.plos.org/10.1371/journal.pone.0061217 [Accessed August 31, 2017].

Merilä, J. & Hendry, A.P., 2014. Climate change, adaptation, and phenotypic plasticity: the problem and the evidence. *Evolutionary Applications*, 7(1), pp.1–14. Available at: http://doi.wiley.com/10.1111/eva.12137 [Accessed August 26, 2017].

Meyer, J.L. et al., 2016. Epimicrobiota Associated with the Decay and Recovery of Orbicella Corals Exhibiting Dark Spot Syndrome. *Frontiers in Microbiology*, 7, p.893. Available at: http://www.ncbi.nlm.nih.gov/pubmed/27375605 [Accessed August 24, 2017].

Mieog, J.C. et al., 2007. Real-time PCR reveals a high incidence of Symbiodinium clade D at low levels in four scleractinian corals across the Great Barrier Reef: Implications for symbiont shuffling. *Coral Reefs*, 26(3), pp.449–457. Available at: http://link.springer.com/10.1007/s00338-007-0244-8 [Accessed March 7, 2017].

Miller, D.J. et al., 2007. The innate immune repertoire in cnidaria--ancestral complexity and stochastic gene loss. *Genome biology*, 8(4), p.R59. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17437634 [Accessed August 8, 2017].

Miller, T.R. et al., 2004. Chemotaxis of Silicibacter sp. strain TM1040 toward dinoflagellate products. *Applied and environmental microbiology*, 70(8), pp.4692–701. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15294804 [Accessed September 14, 2017].

Mitchelmore, C.L., Schwarz, J.A. & Weis, V.M., 2002. Development of symbiosis-specific genes as biomarkers for the early detection of cnidarian-algal symbiosis breakdown. *Marine environmental research*, 54(3–5), pp.345–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12408586 [Accessed September 14, 2017].

MØller, A.P. & Merilä, J., 2004. Analysis and Interpretation of Long-Term Studies Investigating Responses to Climate Change. In pp. 111–130. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0065250404350063 [Accessed July 11, 2017].

Morrow, K.M. et al., 2012. Bacterial associates of two Caribbean coral species reveal species-specific distribution and geographic variability. *Applied and environmental microbiology*, 78(18), pp.6438–49. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22773636 [Accessed September 14, 2017].

Muhlfeld, C.C. et al., 2014. Invasive hybridization in a threatened species is accelerated by

climate change. *Nature Clim. Change*, 4(7), pp.620–624. Available at: http://dx.doi.org/10.1038/nclimate2252.

Muller-Parker, G., D'Elia, C.F. & Cook, C.B., 2015. Interactions Between Corals and Their Symbiotic Algae. In *Coral Reefs in the Anthropocene*. Dordrecht: Springer Netherlands, pp. 99–116. Available at: http://link.springer.com/10.1007/978-94-017-7249-5_5 [Accessed August 29, 2017].

Munn, C.B., 2015. The Role of Vibrios in Diseases of Corals. *Microbiology Spectrum*, 3(4), pp.1–12. Available at: http://www.asmscience.org/content/journal/microbiolspec/10.1128/microbiolspec.VE-0006-2014.

Muscatine, L. & Porter, J.W., 1977. Reef Corals: Mutualistic Symbioses Adapted to Nutrient-Poor Environments. *BioScience*, 27(7), pp.454–460. Available at: https://academic.oup.com/bioscience/article-lookup/doi/10.2307/1297526 [Accessed May 15, 2017].

Myant, K. et al., 2011. LSH and G9a/GLP complex are required for developmentally programmed DNA methylation. *Genome Research*, 21(1), pp.83–94. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21149390 [Accessed August 17, 2017].

Neave, M.J., Rachmawati, R., et al., 2016. Differential specificity between closely related corals and abundant Endozoicomonas endosymbionts across global scales. *The ISME Journal*, 11(1), pp.1–15. Available at: http://www.nature.com/doifinder/10.1038/ismej.2016.95 [Accessed May 30, 2017].

Neave, M.J., Apprill, A., et al., 2016. Diversity and function of prevalent symbiotic marine bacteria in the genus Endozoicomonas. *Applied Microbiology and Biotechnology*, 100(19), pp.8315–8324. Available at: http://link.springer.com/10.1007/s00253-016-7777-0 [Accessed August 24, 2017].

Neave, M.J. et al., 2014. Whole-Genome Sequences of Three Symbiotic Endozoicomonas Strains. *Genome Announcements*, 2(4), pp.e00802-14-e00802-14. Available at: http://www.ncbi.nlm.nih.gov/pubmed/25125646 [Accessed September 2, 2017].

Nevalainen, T.J. et al., 2004. Phospholipase A2 in Cnidaria. *Comparative Biochemistry and Physiology - B Biochemistry and Molecular Biology*, 139(4), pp.731–735.

Nguyen-Kim, H. et al., 2014. High occurrence of viruses in the mucus layer of scleractinian corals. *Environmental Microbiology Reports*, 6(6), pp.675–682. Available at:

http://doi.wiley.com/10.1111/1758-2229.12185 [Accessed September 14, 2017].

Nicholson, D.W. & Thornberry, N.A., 1997. Caspases: Killer proteases. *Trends in Biochemical Sciences*, 22(8), pp.299–306.

Obura, D.O., 2009. Reef corals bleach to resist stress. *Marine Pollution Bulletin*, 58(2), pp.206–212. Available at: http://dx.doi.org/10.1016/j.marpolbul.2008.10.002.

Odum, H.T. & Odum, E.P., 1955. Trophic Structure and Productivity of a Windward Coral Reef Community on Eniwetok Atoll. *Ecological Monographs*, 25(3), pp.291–320. Available at: http://doi.wiley.com/10.2307/1943285 [Accessed September 10, 2017].

Olofsson, H., Ripa, J. & Jonzen, N., 2009. Bet-hedging as an evolutionary game: the trade-off between egg size and number. *Proceedings of the Royal Society B: Biological Sciences*, 276(1669), pp.2963–2969. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19474039 [Accessed September 5, 2017].

Opipari, a W., Boguski, M.S. & Dixit, V.M., 1990. The A20 cDNA induced by tumor necrosis factor alpha encodes a novel type of zinc finger protein. *The Journal of Biological Chemistry*, 265(25), pp.14705–14708. Available at: http://www.jbc.org/content/265/25/14705.long.

Oppen, M. van, 2001. In vitro establishment of symbiosis in Acropora millepora planulae. *Coral Reefs*, 20(3), pp.200–200. Available at: http://link.springer.com/10.1007/s003380100167 [Accessed August 29, 2017].

Ormond, R.F.G. & Roberts, C.M., 1997. The biodiversity of coral reef fishes. *Marine biodiversity: patterns and processes*.

Palumbi, S.R. et al., 2014. Mechanisms of reef coral resistance to future climate change. *Science*, 344(6186), pp.895–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24762535.

Pantos, O. et al., 2015. Habitat-specific environmental conditions primarily control the microbiomes of the coral Seriatopora hystrix. *The ISME Journal*, 9(9), pp.1–12. Available at: http://www.nature.com/doifinder/10.1038/ismej.2015.3.

Parmesan, C., 2006. Ecological and Evolutionary Responses to Recent Climate Change. *Annual Review of Ecology, Evolution, and Systematics*, 37(1), pp.637–669. Available at: http://www.annualreviews.org/doi/10.1146/annurev.ecolsys.37.091305.110100.

Parmesan, C., 2007. Influences of species, latitudes and methodologies on estimates of phenological response to global warming. *Global Change Biology*, 13(9), pp.1860–1872.

Parmesan, C. et al., 1999. Poleward shifts in geographical ranges of butterfly species associated with regional warming. *Nature*, 399(June), pp.579–583. Available at: http://eprints.whiterose.ac.uk/54320/.

Patterson, K.L. et al., 2002. The etiology of white pox, a lethal disease of the Caribbean elkhorn coral, Acropora palmata. *Proceedings of the National Academy of Sciences of the United States of America*, 99(13), pp.8725–30. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12077296 [Accessed September 11, 2017].

Pearse, V.B. & Muscatine, L., 1971. ROLE OF SYMBIOTIC ALGAE (ZOOXANTHELLAE) IN CORAL CALCIFICATION. *The Biological Bulletin*, 141(2), pp.350–363. Available at: http://www.journals.uchicago.edu/doi/10.2307/1540123 [Accessed September 10, 2017].

Peixoto, R.S. et al., 2017. Beneficial Microorganisms for Corals (BMC): Proposed Mechanisms for Coral Health and Resilience. *Frontiers in Microbiology*, 8, p.341. Available at: http://journal.frontiersin.org/article/10.3389/fmicb.2017.00341/full [Accessed May 11, 2017].

Penin, L., Vidal-Dupiol, J. & Adjeroud, M., 2013. Response of coral assemblages to thermal stress: are bleaching intensity and spatial patterns consistent between events? *Environmental Monitoring and Assessment*, 185(6), pp.5031–5042. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23054287 [Accessed September 4, 2017].

Peres, R. et al., 2014. Developmental and light-entrained expression of melatonin and its relationship to the circadian clock in the sea anemone Nematostella vectensis. *EvoDevo*, 5, p.26. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4169136&tool=pmcentrez&rendertype=abstract.

Perez, S. & Weis, V., 2006. Nitric oxide and cnidarian bleaching: an eviction notice mediates breakdown of a symbiosis. *Journal of Experimental Biology*, 209(14), pp.2804–2810. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16809471 [Accessed September 11, 2017].

Perry, A.L., 2005. Climate Change and Distribution Shifts in Marine Fishes. *Science*, 308(5730), pp.1912–1915. Available at: http://www.sciencemag.org/cgi/doi/10.1126/science.1111322.

Pettay, D.T. & LaJeunesse, T.C., 2009. Microsatellite loci for assessing genetic diversity,

dispersal and clonality of coral symbionts in “stress-tolerant” clade D Symbiodinium. *Molecular Ecology Resources*, 9(3), pp.1022–1025. Available at: http://doi.wiley.com/10.1111/j.1755-0998.2009.02561.x [Accessed September 7, 2017].

Pinzon, J.H. & LaJeunesse, T.C., 2011. Species delimitation of common reef corals in the genus Pocillopora using nucleotide sequence phylogenies, population genetics and symbiosis ecology. *Molecular Ecology*, 20(2), pp.311–325. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21091563 [Accessed September 4, 2017].

Planton, S., 2013. Annex III: Glossary, In: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*, pp.1447–1466.

Pochon, X. et al., 2001. High genetic diversity and relative specificity among Symbiodinium-like endosymbiotic dinoflagellates in soritid foraminiferans. *Marine Biology*, 139(6), pp.1069–1078.

Pochon, X. et al., 2006. Molecular phylogeny, evolutionary rates, and divergence timing of the symbiotic dinoflagellate genus Symbiodinium. *Molecular Phylogenetics and Evolution*, 38(1), pp.20–30.

Pochon, X. & Gates, R.D., 2010. A new Symbiodinium clade (Dinophyceae) from soritid foraminifera in Hawai’i. *Molecular Phylogenetics and Evolution*, 56(1), pp.492–497. Available at: http://dx.doi.org/10.1016/j.ympev.2010.03.040.

Pochon, X., LaJeunesse, T.C. & Pawlowski, J., 2004. Biogeographic partitioning and host specialization among foraminiferan dinoflagellate symbionts (Symbiodinium; Dinophyta). *Marine Biology*, 146(1), pp.17–27.

Porté, S. et al., 2009. Three-dimensional structure and enzymatic function of proapoptotic human p53-inducible quinone oxidoreductase PIG3. *The Journal of biological chemistry*, 284(25), pp.17194–205. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19349281 [Accessed August 17, 2017].

Pratlong, M. et al., Identifying adaptive loci in a context of high genetic struc- ture, local adaptation to depth in the red coral (Corallium rubrum). *Molecular Ecology*.

Pratlong, M., 2016. *Le défi évolutif du changement climatique, processus adaptatifs chez le*

corail rouge (Corallium rubrum). Aix-Marseille Université.

Putnam, H.M. et al., 2012. Endosymbiotic flexibility associates with environmental sensitivity in scleractinian corals. *Proceedings of the Royal Society B: Biological Sciences*, 279(1746), pp.4352–4361. Available at: http://rspb.royalsocietypublishing.org/content/royprsb/279/1746/4352.full.pdf [Accessed May 30, 2017].

Putnam, H.M. & Gates, R.D., 2015. Preconditioning in the reef-building coral Pocillopora damicornis and the potential for trans-generational acclimatization in coral larvae under future climate change conditions. *Journal of Experimental Biology*, 218(15). Available at: http://jeb.biologists.org/content/218/15/2365 [Accessed September 4, 2017].

Quéré, C. Le & Raupach, M., 2009. Trends in the sources and sinks of carbon dioxide. *Nature Geoscience*, (November), pp.1–6. Available at: http://www.nature.com/ngeo/journal/vaop/ncurrent/full/ngeo689.html.

Quigley, K.M. et al., 2014. Deep-Sequencing Method for Quantifying Background Abundances of Symbiodinium Types: Exploring the Rare Symbiodinium Biosphere in Reef-Building Corals. *PLoS ONE*, 9(4), p.e94297. Available at: http://dx.plos.org/10.1371/journal.pone.0094297.

Rädecker, N. et al., 2015. Nitrogen cycling in corals: The key to understanding holobiont functioning? *Trends in Microbiology*, 23(8), pp.490–497. Available at: http://www.ncbi.nlm.nih.gov/pubmed/25868684 [Accessed September 2, 2017].

Raina, J.-B. et al., 2010. Do the organic sulfur compounds DMSP and DMS drive coral microbial associations? *Trends in Microbiology*, 18(3), pp.101–108. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20045332 [Accessed September 2, 2017].

Ramos-Silva, P. et al., 2013. The skeletal proteome of the coral acropora millepora: The evolution of calcification by co-option and domain shuffling. *Molecular Biology and Evolution*, 30(9), pp.2099–2112.

Reaka-Kudla, M.L., 1997. The Global Biodiversity of Coral Reefs: A comparison with Rain Forests. *Biodiversity II: Understanding and Protecting Our Biological Resources*, pp.83–108. Available at: http://www.nap.edu/openbook/0309052270/html/83.html.

Regan, H.M. et al., 2001. The currency and tempo of extinction. *The American naturalist*, 157(1), pp.1–10.

Reitzel, A.M., Tarrant, A.M. & Levy, O., 2013. Circadian clocks in the cnidaria: Environmental entrainment, molecular regulation, and organismal outputs. *Integrative and Comparative Biology*, 53(1), pp.118–130.

Reshef, L. et al., 2006. The Coral Probiotic Hypothesis. *Environmental Microbiology*, 8(12), pp.2068–2073. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17107548 [Accessed January 30, 2016].

Reynolds, J.M. et al., 2008. Enhanced photoprotection pathways in symbiotic dinoflagellates of shallow-water corals and other cnidarians. *Proceedings of the National Academy of Sciences of the United States of America*, 105(36), pp.13674–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18757737 [Accessed March 7, 2017].

Rhen, T. & W . Lang, J., 1998. Among-Family Variation for Environmental Sex Determination in Reptiles. *Evolution*, 52(5), pp.1514–1520. Available at: http://www.jstor.org/stable/2411322.

Richier, S. et al., 2006. Oxidative stress and apoptotic events during thermal stress in the symbiotic sea anemone, Anemonia viridis. *FEBS Journal*, 273(18), pp.4186–4198. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16907933 [Accessed July 19, 2017].

Richier, S. et al., 2005. Symbiosis-induced adaptation to oxidative stress. *Journal of Experimental Biology*, 208(2), pp.277–285. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15634847 [Accessed September 11, 2017].

Richmond, R.H., 1987. Energetic Relationships and Biogeographical Differences among Fecundity, Growth and Reproduction in the Reef Coral Pocillopora Damicornis. *Bulletin of Marine Science*, 41(2), pp.594–604. Available at: http://www.ingentaconnect.com/content/umrsmas/bullmar/1987/00000041/00000002/art00044 [Accessed September 15, 2017].

Richmond, R.H., 1987. Energetics, competency, and long-distance dispersal of planula larvae of the coral Pocillopora damicornis. *Marine Biology*, 93(4), pp.527–533. Available at: http://link.springer.com/10.1007/BF00392790 [Accessed August 29, 2017].

Richmond, R.H. & Hunter, C.L., 1990. Reproduction and recruitment of corals: comparisons among the Caribbean, the Tropical Pacific, and the Red Sea. *Marine Ecology Progress Series*, 60, pp.185–203. Available at: http://www.jstor.org/stable/24842589 [Accessed September 15, 2017].

Richmond, R.H. & Jokiel, P.L., 1984. Lunar periodicity in larva release in the reef coral

pocillopora damicornis at enewetak and hawaii. *Coral reef paper*, 34(2), pp.280–287.

Riegl, B.M. et al., 2011. Present Limits to Heat-Adaptability in Corals and Population-Level Responses to Climate Extremes C. R. Voolstra, ed. *PLoS ONE*, 6(9), p.e24802. Available at: http://dx.plos.org/10.1371/journal.pone.0024802 [Accessed August 24, 2017].

Ritchie, K., 2006. Regulation of microbial populations by coral surface mucus and mucus-associated bacteria. *Marine Ecology Progress Series*, 322, pp.1–14. Available at: http://www.int-res.com/abstracts/meps/v322/p1-14/ [Accessed September 1, 2017].

Ritchie, K.B. & Smith, G.W., 1997. Physiological comparison of bacterial communities from various species of scleractinian corals. In *Proc 8th Int Coral Reef Symp*. pp. 521–526.

Rodrigues, L.J., Grottoli, A.G. & Lesser, M.P., 2008. Long-term changes in the chlorophyll fluorescence of bleached and recovering corals from Hawaii. *Journal of Experimental Biology*, 211(15). Available at: http://jeb.biologists.org/content/211/15/2502.eLetters [Accessed August 29, 2017].

Roff, D.A. & Fairbairn, D.J., 2007. The evolution of trade-offs: Where are we? *Journal of Evolutionary Biology*, 20(2), pp.433–447.

Rognes, T. et al., 2016. VSEARCH: a versatile open source tool for metagenomics. Available at: https://peerj.com/preprints/2409/ [Accessed August 31, 2017].

Rohwer, F. et al., 2002. Diversity and distribution of coral-associated bacteria. *Marine Ecology Progress Series*, 243, pp.1–10.

Rohwer, F. et al., 2001. Diversity of bacteria associated with the Caribbean coral Montastraea franksi. *Coral Reefs*, 20(1), pp.85–91. Available at: http://dx.doi.org/10.1007/s003380100138.

Rosenberg, E. et al., 2007. The role of microorganisms in coral health, disease and evolution. *Nature Reviews Microbiology*, 5(5), pp.355–362. Available at: http://www.nature.com/doifinder/10.1038/nrmicro1635 [Accessed February 16, 2016].

Rosenberg, E. & Falkovitz, L., 2004. The *Vibrio shiloi* / *Oculina patagonica* Model System of Coral Bleaching. *Annual Review of Microbiology*, 58(1), pp.143–159. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15487933 [Accessed September 11, 2017].

Rosenberg, E. & Loya, Y., 2004. *Coral Health and Disease*, Springer Berlin Heidelberg.

Roth, M.S., Goericke, R. & Deheyn, D.D., 2012. Cold induces acute stress but heat is ultimately more deleterious for the reef-building coral Acropora yongei. *Scientific reports*, 2, p.240. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22355753

[Accessed September 4, 2017].

Rouzé, H. et al., 2017. An updated assessment of *Symbiodinium* spp. that associate with common scleractinian corals from Moorea (French Polynesia) reveals high diversity among background symbionts and a novel finding of clade B. *PeerJ*, 5, p.e2856. Available at: http://www.ncbi.nlm.nih.gov/pubmed/28168100 [Accessed September 7, 2017].

Rouzé, H. et al., 2016. Symbiodinium clades A and D differentially predispose Acropora cytherea to disease and Vibrio spp. colonization. *Ecology and Evolution*, 6(2), pp.560–572. Available at: http://www.ncbi.nlm.nih.gov/pubmed/26843939 [Accessed September 4, 2017].

Rowan, R., 2004. Coral bleaching: thermal adaptation in reef coral symbionts. *Nature*, 430(7001), p.742. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15306800 [Accessed September 4, 2017].

Rowan, R. et al., 1997. Landscape ecology of algal symbionts creates variation in episodes of coral bleaching. *Nature*, 388(6639), pp.265–269. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9230434 [Accessed September 11, 2017].

Rowan, R. & Knowlton, N., 1995. Intraspecific diversity and ecological zonation in coral-algal symbiosis. *Proceedings of the National Academy of Sciences of the United States of America*, 92(7), pp.2850–2853.

Rowan, R. & Powers, D., 1991. Molecular genetic identification of symbiotic dinoflagellates (zooxanthellae) . *Marine Ecology Progress Series*, 71, pp.65–73.

Rowan, R. & Powers, D. a, 1992. Ribosomal RNA sequences and the diversity of symbiotic dinoflagellates (zooxanthellae). *Proceedings of the National Academy of Sciences of the United States of America*, 89(8), pp.3639–3643.

Saldanha, A.J., 2004. Java Treeview--extensible visualization of microarray data. *Bioinformatics*, 20(17), pp.3246–3248. Available at: https://academic.oup.com/bioinformatics/article-lookup/doi/10.1093/bioinformatics/bth349 [Accessed June 8, 2017].

Sapozhnikova, V.O. & Barannik, T. V, EVOLUTIONARY ANALYSIS OF ARCHITECTURE AND EXTRACELLULAR DOMAIN SEQUENCE OF SID-1 FAMILY PROTEINS. , p.93.

Sato, Y., Willis, B.L. & Bourne, D.G., 2010. Successional changes in bacterial communities during the development of black band disease on the reef coral, Montipora hispida. *The*

ISME Journal, 4(2), pp.203–214. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19776765 [Accessed August 24, 2017].

Schallreuter, K.U. et al., 2007. Hydrogen peroxide-mediated oxidative stress disrupts calcium binding on calmodulin: More evidence for oxidative stress in vitiligo. *Biochemical and Biophysical Research Communications*, 360(1), pp.70–75. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17592724 [Accessed August 16, 2017].

Schlichter, D., Zscharnack, B. & Krisch, H., 1995. Transfer of photoassimilates from endolithic algae to coral tissue. *Naturwissenschaften*, 82(12), pp.561–564. Available at: http://link.springer.com/10.1007/BF01140246 [Accessed September 1, 2017].

Schmidt-roach, S. et al., 2014. With eyes wide open: A revision of species within and closely related to the Pocillopora damicornis species complex (Scleractinia; Pocilloporidae) using morphology and genetics. *Zoological Journal of the Linnean Society*, 170(1), pp.1–33.

Schmidt-Roach, S. et al., 2012. Broadcast Spawning by Pocillopora Species on the Great Barrier Reef. *PLoS ONE*, 7(12), pp.1–7.

Seneca, F.O. & Palumbi, S.R., 2015. The role of transcriptome resilience in resistance of corals to bleaching. , pp.1467–1484. Available at: http://content.ebscohost.com/ContentServer.asp?EbscoContent=dGJyMNLe80SeqK44yOvqOLCmr0%2BeprZSsKi4SLaWxWXS&ContentCustomer=dGJyMOzpsE21p7JOuePfgeyx9Yvf5ucA&T=P&P=AN&S=R&D=mnh&K=25728233 [Accessed July 20, 2017].

Settele, J. et al., 2014. Chapter 4 - Terrestrial and inland water systems. *Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part A: Global and Sectoral Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*, pp.271–359. Available at: http://www.ipcc.ch/pdf/assessment-report/ar5/wg2/WGIIAR5-Chap4_FINAL.pdf.

Shakun, J.D. et al., 2012. Global warming preceded by increasing carbon dioxide concentrations during the last deglaciation. Available at: http://www.atm.damtp.cam.ac.uk/mcintyre/shakun-co2-temp-lag-nat12.pdf [Accessed May 8, 2017].

Shashar, N. et al., 1997. Coral Endolithic Algae: Life in a Protected Environment! *Pacific Science*, 51(2), pp.167–173. Available at: http://hl-128-171-57-22.library.manoa.hawaii.edu/bitstream/10125/3107/1/v51n2-167-173.pdf [Accessed

September 10, 2017].

Shnit-Orland, M. & Kushmaro, A., 2009. Coral mucus-associated bacteria: a possible first line of defense. *FEMS Microbiology Ecology*, 67(3), pp.371–380. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19161430 [Accessed September 10, 2017].

Silverstein, R.N., Cunning, R. & Baker, A.C., 2017. Tenacious D: *Symbiodinium* in clade D remain in reef corals at both high and low temperature extremes despite impairment. *The Journal of Experimental Biology*, 220(7), pp.1192–1196. Available at: http://www.ncbi.nlm.nih.gov/pubmed/28108671 [Accessed September 4, 2017].

Spalding, M., Ravilious, C. & Green, E.P. (Edmund P., 2001. *World atlas of coral reefs*, University of California Press. Available at: https://archive.org/details/worldatlasofcora01spal [Accessed August 28, 2017].

Starger, C.J. et al., 2008. Ten polymorphic STR loci in the cosmopolitan reef coral, Pocillopora damicornis. *Molecular Ecology Resources*, 8(3), pp.619–621. Available at: http://doi.wiley.com/10.1111/j.1471-8286.2007.02017.x [Accessed May 16, 2017].

Stat, M. et al., 2013. The distribution of the thermally tolerant symbiont lineage (Symbiodinium clade D) in corals from Hawaii: correlations with host and the history of ocean thermal stress. *Ecology and evolution*, 3(5), pp.1317–29. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23762518 [Accessed September 7, 2017].

Stat, M. & Gates, R.D., 2011. Clade D *Symbiodinium* in Scleractinian Corals: A “Nugget” of Hope, a Selfish Opportunist, an Ominous Sign, or All of the Above? *Journal of Marine Biology*, 2011, pp.1–9. Available at: http://www.hindawi.com/journals/jmb/2011/730715/ [Accessed September 4, 2017].

Steen, R.G. & Muscatine, L., 1987. Low temperature evokes rapid exocytosis of symbiotic algae by a sea anemone. *The Biological Bulletin*, 172(2), pp.246–263. Available at: http://www.journals.uchicago.edu/doi/10.2307/1541797 [Accessed September 11, 2017].

Sterner, D.E. & Berger, S.L., 2000. Acetylation of histones and transcription-related factors. *Microbiology and molecular biology reviews : MMBR*, 64(2), pp.435–59. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10839822 [Accessed August 17, 2017].

Stocker, T.F. et al., 2013. Summary for Policymakers. In Intergovernmental Panel on Climate Change, ed. *Climate Change 2013 - The Physical Science Basis*. Cambridge: Cambridge University Press, pp. 1–30. Available at:

http://ebooks.cambridge.org/ref/id/CBO9781107415324A009.

Stocker & T.F., D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V.B. and P.M.M. (eds. ., 2015. Summary for Policymakers. In: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. *CEUR Workshop Proceedings*, 1542, pp.33–36.

Sunagawa, S. et al., 2015. Structure and function of the global ocean microbiome. *Science*, 348(6237), pp.1261359–1261359. Available at: http://www.sciencemag.org/cgi/doi/10.1126/science.1261359.

Supek, F. et al., 2011. REVIGO Summarizes and Visualizes Long Lists of Gene Ontology Terms. *Plos One*, 6(7). Available at: http://journals.plos.org/plosone/article/file?id=10.1371/journal.pone.0021800&type=printable [Accessed July 26, 2017].

Suzuki, Y. & Nijhout, H.F., 2006. Evolution of a Polyphenism by Genetic Accommodation. *Science*, 311(5761), pp.650–652. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16456077 [Accessed August 30, 2017].

Sweet, M.J., Croquer, A. & Bythell, J.C., 2011. Bacterial assemblages differ between compartments within the coral holobiont. *Coral Reefs*, 30(1), pp.39–52. Available at: http://link.springer.com/10.1007/s00338-010-0695-1 [Accessed September 10, 2017].

T. Banaszak, A., LaJeunesse, T.C. & Trench, R.K., 2000. The synthesis of mycosporine-like amino acids (MAAs) by cultured, symbiotic dinoflagellates. *Journal of Experimental Marine Biology and Ecology*, 249(2), pp.219–233. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0022098100001921 [Accessed September 11, 2017].

Tamura, K. et al., 2013. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution*, 30(12), pp.2725–2729. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24132122 [Accessed May 31, 2017].

Tashian, R.E., 1989. The carbonic anhydrases: Widening perspectives on their evolution, expression and function. *BioEssays*, 10(6), pp.186–192. Available at: http://doi.wiley.com/10.1002/bies.950100603 [Accessed August 24, 2017].

Tchernov, D. et al., 2004. Membrane lipids of symbiotic algae are diagnostic of sensitivity to thermal bleaching in corals. *Proceedings of the National Academy of Sciences of the*

United States of America, 101(37), pp.13531–5. Available at: http://www.scopus.com/inward/record.url?eid=2-s2.0-4544249149&partnerID=tZOtx3y1.

Teplitski, M. & Ritchie, K., 2009. How feasible is the biological control of coral diseases? *Trends in ecology & evolution*, 24(7), pp.378–85. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19406502 [Accessed September 2, 2017].

Teplitsky, C. et al., 2008. Bergmann's rule and climate change revisited: Disentangling environmental and genetic responses in a wild bird population. *Proceedings of the National Academy of Sciences*, 105(36), pp.13492–13496. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18757740 [Accessed August 26, 2017].

Thackeray, S.J. et al., 2010. Trophic level asynchrony in rates of phenological change for marine, freshwater and terrestrial environments. *Global Change Biology*, 16(12), pp.3304–3313.

Thurber, R.V. et al., 2009. Metagenomic analysis of stressed coral holobionts. *Environmental Microbiology*, 11(8), pp.2148–2163. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19397678 [Accessed September 15, 2017].

Toller, W.W., Rowan, R. & Knowlton, N., 2001. Repopulation of Zooxanthellae in the Caribbean corals Montastraea annularis and M. faveolata following experimental and disease-associated bleaching. *The Biological bulletin*, 201(3), pp.360–73. Available at: http://www.journals.uchicago.edu/doi/10.2307/1543614 [Accessed September 11, 2017].

Toller, W.W., Rowan, R. & Knowlton, N., 2001. Zooxanthellae of the *Montastraea annularis* Species Complex: Patterns of Distribution of Four Taxa of *Symbiodinium* on Different Reefs and Across Depths. *The Biological Bulletin*, 201(3), pp.348–359. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11751247 [Accessed September 10, 2017].

Torda, G. et al., 2017. Rapid adaptive responses to climate change in corals. *Nature Climate Change*, 7(9), pp.627–636. Available at: http://www.nature.com/doifinder/10.1038/nclimate3374.

Tout, J. et al., 2015. Increased seawater temperature increases the abundance and alters the structure of natural Vibrio populations associated with the coral Pocillopora damicornis. *Frontiers in Microbiology*, 6(MAY).

Townsend, J.P. et al., 2002. Effects of temperature on gene expression in embryos of the

coral Montastraea faveolata. *Genome Biology*, 3(12), p.research0071.1. Available at: http://genomebiology.biomedcentral.com/articles/10.1186/gb-2002-3-12-research0071 [Accessed August 16, 2017].

Trapnell, C. et al., 2010. Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. *Nat Biotech*, 28(5), pp.511–515. Available at: http://dx.doi.org/10.1038/nbt.1621.

Traylor-Knowles, N. et al., 2011. Production of a reference transcriptome and transcriptomic database (PocilloporaBase) for the cauliflower coral, Pocillopora damicornis. *BMC Genomics*, 12(1), p.585. Available at: http://www.biomedcentral.com/1471-2164/12/585.

Tripp, H.J. et al., 2008. SAR11 marine bacteria require exogenous reduced sulphur for growth. *Nature*, 452(7188), pp.741–744. Available at: http://www.nature.com/doifinder/10.1038/nature06776 [Accessed September 14, 2017].

Tsang, R.H.L. & Ang, P., 2015. Cold temperature stress and predation effects on corals: their possible roles in structuring a nonreefal coral community. *Coral Reefs*, 34(1), pp.97–108. Available at: http://link.springer.com/10.1007/s00338-014-1210-x [Accessed September 4, 2017].

Ulstrup, K.E. & Van Oppen, M.J.H., 2003. Geographic and habitat partitioning of genetically distinct zooxanthellae (Symbiodinium) in Acropora corals on the Great Barrier Reef. *Molecular Ecology*, 12(12), pp.3477–3484.

Vargas-Angel, B. et al., 2006. An experimental histopathological rating scale of sedimentation stress in the Caribbean coral Montastraea cavernosa. *Proc 10th Int. Coral Reef Symp. {…}*, 1173, pp.1168–1173. Available at: http://scholar.google.com/scholar?hl=en%7B&%7DbtnG=Search%7B&%7Dq=intitle:An+experimental+histopathological+rating+scale+of+sedimentation+stress+in+the+Caribbean+coral+Montastraea+cavernosa%7B#%7D0.

Vega Thurber, R.L. et al., 2008. Metagenomic analysis indicates that stressors induce production of herpes-like viruses in the coral Porites compressa. *Proceedings of the National Academy of Sciences*, 105(47), pp.18413–18418. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19017800 [Accessed September 14, 2017].

Veron, J.E.N. & Stafford-Smith, M., 2000. *Corals of the world*, Australian Institute of Marine

Science. Available at: https://books.google.fr/books?id=m5zwAAAAMAAJ.

Vervoort, M. et al., 2016. Evolution of Prdm Genes in Animals: Insights from Comparative Genomics. *Molecular biology and evolution*, 33(3), pp.679–96. Available at: http://www.ncbi.nlm.nih.gov/pubmed/26560352 [Accessed August 17, 2017].

Vidal-dupiol, J. et al., 2009. Coral bleaching under thermal stress: putative involvement of host/symbiont recognition mechanisms. *BMC physiology*, 9, p.14. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19653882 [Accessed August 31, 2017].

Vidal-dupiol, J., 2011. Stress environnementaux chez le corail scléractiniaire Pocillopora damicornis : Du modèle expérimental à l'identification de marqueurs fonctionnels du stress. *Thesis*.

Vidal-Dupiol, J. et al., 2013. Genes Related to Ion-Transport and Energy Production Are Upregulated in Response to CO2-Driven pH Decrease in Corals: New Insights from Transcriptome Analysis S. Dupont, ed. *PLoS ONE*, 8(3), p.e58652. Available at: http://dx.plos.org/10.1371/journal.pone.0058652 [Accessed September 15, 2017].

Vidal-Dupiol, J. et al., 2014. Thermal stress triggers broad Pocillopora damicornis transcriptomic remodeling, while Vibrio coralliilyticus infection induces a more targeted immuno-suppression response. *PloS one*, 9(9), p.e107672. Available at: http://www.ncbi.nlm.nih.gov/pubmed/25259845 [Accessed August 31, 2017].

Vimal, J., 2007. Physiopathologie des Coraux. *These*, Docteur Vé, p.127. Available at: http://www2.vet-lyon.fr/bib/fondoc/th_sout/th_pdf/2007lyon025.pdf.

Vize, P.D., 2009. Transcriptome Analysis of the Circadian Regulatory Network in the Coral Acropora millepora. *Biol Bull*, 216(2), pp.131–137. Available at: http://www.biolbull.org/cgi/content/abstract/216/2/131.

Voolstra, C.R. et al., 2009. Effects of temperature on gene expression in embryos of the coral Montastraea faveolata. *BMC genomics*, 10, p.627. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20030803.

Waddington, C.H., 1953. Genetic Assimilation of an Acquired Character. *Evolution*, 7(2), p.118. Available at: http://www.jstor.org/stable/2405747?origin=crossref [Accessed July 20, 2017].

Wakefield, T.S. & Kempf, S.C., 2001. Development of Host- and Symbiont-Specific Monoclonal Antibodies and Confirmation of the Origin of the Symbiosome Membrane in a Cnidarian–Dinoflagellate Symbiosis. *The Biological Bulletin*, 200(2), pp.127–143.

Available at: http://www.ncbi.nlm.nih.gov/pubmed/11341574 [Accessed September 10, 2017].

Warner, M.E., Fitt, W.K. & Schmidt, G.W., 1999. Damage to photosystem II in symbiotic dinoflagellates: a determinant of coral bleaching. *Proceedings of the National Academy of Sciences of the United States of America*, 96(14), pp.8007–12. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10393938 [Accessed September 11, 2017].

Webster, N.S. & Bourne, D., 2007. Bacterial community structure associated with the Antarctic soft coral, Alcyonium antarcticum. *FEMS Microbiology Ecology*, 59(1), pp.81–94. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17233746 [Accessed August 24, 2017].

Webster, N.S. & Reusch, T.B., 2017. Microbial contributions to the persistence of coral reefs Coral reefs under climate change. *The ISME Journal advance online publication*, doi(10). Available at: http://eprints.uni-kiel.de/38011/1/Webster_Reusch_2017_ismej.pdf [Accessed September 1, 2017].

Wegley, L. et al., 2007. Metagenomic analysis of the microbial community associated with the coral Porites astreoides. *Environmental Microbiology*, 9(11), pp.2707–2719. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17922755 [Accessed September 10, 2017].

Weis, V. et al., 2001. Host-symbiont specificity during onset of symbiosis between the dinoflagellates Symbiodinium spp. and planula larvae of the scleractinian coral Fungia scutaria. *Coral Reefs*, 20(3), pp.301–308. Available at: http://link.springer.com/10.1007/s003380100179 [Accessed September 4, 2017].

Weis, V.M., 2008. Cellular mechanisms of Cnidarian bleaching: stress causes the collapse of symbiosis. *Journal of Experimental Biology*, 211(19), pp.3059–3066. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18805804 [Accessed July 19, 2017].

Welsh, R.M. et al., 2015. Alien vs. Predator: Pathogens open niche space for opportunists, unless controlled by predators. Available at: https://peerj.com/preprints/1537/ [Accessed September 14, 2017].

Wessler, S.R., 1996. Plant retrotransposons: Turned on by stress. *Current Biology*, 6(8), pp.959–961.

Wild, C., Woyt, H. & Huettel, M., 2005. Influence of coral mucus on nutrient fluxes in carbonate sands. *MARINE ECOLOGY PROGRESS SERIES Mar Ecol Prog Ser*, 287, pp.87–

98. Available at: http://www.int-res.com/articles/meps2005/287/m287p087.pdf [Accessed September 15, 2017].

Wilkinson, C. & Network, G.C.R.M., 2008. Status of Coral Reefs of the World: 2008. *Status of Coral Reefs of the World: 2008*, pp.5–19.

Wilson, W. et al., 2001. Temperature induction of viruses in symbiotic dinoflagellates. *Aquatic Microbial Ecology*, 25(1), pp.99–102. Available at: http://www.int-res.com/abstracts/ame/v25/n1/p99-102/ [Accessed September 14, 2017].

Wilson, W.H. et al., 2005. An enemy within? Observations of virus-like particles in reef corals. *Coral Reefs*, 24(1), pp.145–148. Available at: http://link.springer.com/10.1007/s00338-004-0448-0 [Accessed September 14, 2017].

van Woesik, R. et al., 2011. Revisiting the winners and the losers a decade after coral bleaching. *Marine Ecology Progress Series*, 434, pp.67–76. Available at: http://www.int-res.com/abstracts/meps/v434/p67-76/ [Accessed June 5, 2017].

Van Woesik, R., De Vantier, L. & Glazebrook, J., 1995. Effects of Cyclone “Joy” on nearshore coral communities of the Great Barrier Reef. *Marine Ecology Progress Series*, 128, pp.261–270. Available at: http://www.int-res.com/abstracts/meps/v128/p261-270/ [Accessed September 14, 2017].

Yakovleva, I.I.M. et al., 2009. Algal symbionts increase oxidative damage and death in coral larvae at high temperatures. *Marine Ecology Progress Series*, 378, pp.105–112. Available at: http://www.int-res.com/abstracts/meps/v378/p105-112/ [Accessed August 18, 2017].

Yeoh, S.-R. & Dai, C.-F., 2010. The production of sexual and asexual larvae within single broods of the scleractinian coral, Pocillopora damicornis. *Marine Biology*, 157(2), pp.351–359. Available at: http://link.springer.com/10.1007/s00227-009-1322-y [Accessed September 17, 2017].

Yoda, H., 2006. Polyamine Oxidase Is One of the Key Elements for Oxidative Burst to Induce Programmed Cell Death in Tobacco Cultured Cells. *Plant Physiology*, 142(1), pp.193–206. Available at: http://www.plantphysiol.org/cgi/doi/10.1104/pp.106.080515.

Yuan, C. et al., 2016. Effects of elevated ammonium on the transcriptome of stony coral Pocillopora damicornis. *Marine Pollution Bulletin*, 114(1), pp.46–52. Available at: http://dx.doi.org/10.1016/j.marpolbul.2016.08.036.

Ziegler, M. et al., 2017. Bacterial community dynamics are linked to patterns of coral heat

tolerance. *Nature Communications*, 8, pp.1–8. Available at: http://dx.doi.org/10.1038/ncomms14213.

Zilber-Rosenberg, I. & Rosenberg, E., 2008. Role of microorganisms in the evolution of animals and plants: The hologenome theory of evolution. *FEMS Microbiology Reviews*, 32(5), pp.723–735.

3. CHAPITRE 2 : ANALYSE DE L'INFLUENCE DU CLADE HOTE, DE LA GÉOGRAPHIE ET DU RÉGIME THERMIQUE SUR LES COMMUNAUTÉS BACTÉRIENNES ET DE *SYMBIODINIUM* DU CORAIL SCLÉRACTINIAIRE *POCILLOPORA DAMICORNIS SENSU LATO*.

Ce chapitre correspond à un article soumis à Microbiome, numéro spécial « Host-microbiota interactions: from holobiont theory to analysis » (2017)

D'après la théorie évolutive de l'hologénome, tous les compartiments de l'holobionte, c'est à dire l'hôte et les microorganismes symbiotiques associés, jouent un rôle sur le phénotype de l'hôte et donc sur sa valeur sélective. C'est d'autant plus vrai chez les coraux scléractiniaires, chez qui l'influence des assemblages de *Symbiodinium* sur la thermo-tolérance, la photo-tolérance et la croissance a été montrée dans de nombreuses études. Considérer l'holobionte comme la véritable unité d'évolution implique une coévolution entre l'hôte et ses symbiotes. L'évolution d'un organisme dans un environnement fluctuant et les conséquences sur son adaptabilité, prédite pour être favorisée dans de tels environnements, dépendrait donc de l'hôte mais également de sa communauté symbiotique. Dans le chapitre précédent, nous avons montré une plus grande plasticité transcriptomique de l'hôte corallien chez la population la plus thermo-tolérante. Bien que les communautés symbiotiques (bactéries et *Symbiodinium*) aient montré de la spécificité pour chacune des populations (voire même des génotypes pour la communauté bactérienne), ces expérimentations ne permettent pas de conclure quant à leur influence sur la thermo-tolérance de l'hôte corallien. Dans ce second chapitre, nous avons cherché à identifier quels paramètres biotiques (haplotype de l'hôte) ou abiotiques (géographie ou régime thermique) pouvaient influencer la distribution des communauté bactériennes et de *Symbiodinium* chez *Pocillopora damicornis sansu lato*. Pour cela, nous avons comparé les communautés microbiennes (bactéries et *Symbiodinium*) en milieu naturel (échantillons de colonies de morphotype *Pocillopora damicornis)* provenant de quatre régions géographiques aux régimes thermiques très contrastés : la Polynésie française, la Nouvelle-Calédonie, Djibouti et Taïwan. Le séquençage de l'haplotype mitochondrial a révélé deux clades d'hôtes : clade 1 pour

Taïwan, Polynésie Française et Nouvelle-Calédonie et clade 2 pour Djibouti La composition des communautés bactériennes, analysée en métabarcoding 16S, s'est révélée très variable entre les échantillons, mais un core bactérien émerge dans l'ensemble des populations. Les bactéries du genre *Endozoicomonas* (endosymbionte de nombreux organismes marins) sont majoritaires, tel que décrit chez de nombreuses espèces de corail. Les genres *Acinetobacter* et *Arcobacter* qui sont également présents dans tous les échantillons, pourraient être d'après la littérature des bactéries opportunistes lors de pathogénicité. Les analyses statistiques montrent que c'est l'haplotype de l'hôte qui contraint le plus la composition des communautés bactériennes, tandis que les température ont un effet plus faible. En ce qui concerne l'assemblage des *Symbiodinium*, on retrouve un seul clade majoritaire pour chacun des échantillons avec des différences très marquées entre populations : le clade D1 est dominant à Djibouti et en Polynésie Française, alors que le clade C1 est le plus abondant à Taïwan et en Nouvelle-Calédonie. On observe également une grande diversité de clades très faiblement abondants (<1%) dans toutes les populations. Pour la première fois, nous avons montré que les communautés de *Symbiodinium* sont principalement influencées par les températures minimales. On observe ainsi par exemple une corrélation négative entre l'association avec le clade D, connu pour être thermo-tolérant, et les températures minimales.

Ce travail suggère donc que les communautés microbiennes et notamment de *Symbiodinium* participent aux capacités d'adaptation de *Pocillopora damicornis* à des modifications de température. En revanche la grande diversité des *Symbiodinium* « cryptiques » pourrait permettre des changements de thermo-tolérances de l'hôte en fonction des variations de l'environnement via le mécanisme du blanchissement adaptatif, et ainsi participer à la plasticité de l'hôte. Bien que les communautés bactériennes soient, elles aussi, faiblement corrélées avec les températures moyennes, des études supplémentaires sur la réponse au stress thermique des différents microbiomes sont nécessaires pour conclure sur l'influence du microbiome sur la thermo-tolérance de l'hôte. De plus, nous n'avons pas mis en évidence d'effet de la géographie sur la composition des communautés microbiennes alors qu'elle est corrélée à l'haplotype hôte, ce qui soutient l'hypothèse d'une coévolution entre l'hôte et ses partenaires symbiotiques.

THERMAL REGIME AND HOST CLADE, RATHER THAN GEOGRAPHY, DRIVE SYMBIODINIUM AND BACTERIAL ASSEMBLAGES IN THE SCLERACTINIAN CORAL POCILLOPORA DAMICORNIS SENSU LATO.

Brener-Raffalli Kelly[1], Clerissi Camille[1], Vidal-Dupiol Jeremie[1], Adjeroud Mehdi[2], Bonhomme François[3], Pratlong Marine[4], Aurelle Didier[4], Mitta Guillaume[1], Toulza Eve[1]*.

1: Univ. Perpignan Via Domitia, IHPE UMR 5244, CNRS, IFREMER, Univ. Montpellier, F-66860 Perpignan, France. kelly.brener@univ-perp.fr, camille.clerissi@univ-perp.fr, Jeremie.Vidal.Dupiol@ifremer.fr, mitta@univ-perp.fr, eve.toulza@univ-perp.fr

2: Institut de Recherche pour le Développement, UMR 9220 ENTROPIE & Laboratoire d'Excellence CORAIL, Université de Perpignan, 52 avenue Paul Alduy, 66860 Perpignan, France. mehdi.adjeroud@ird.fr

3: CNRS, Institut des Sciences de l'Évolution, UMR 5554 CNRS-UM-IRD-EPHE, Sète, France. francois.bonhomme@univ-montp2.fr.

4: Aix Marseille Université, Institut Méditerranéen de la Biodiversité et d'Ecologie marine et continentale, UMR 7263 CNRS, IRD, Avignon Université, Marseille, France. marine.pratlong@imbe.fr, didier.aurelle@imbe.fr

* : corresponding author

ABSTRACT

Background : Although the term holobiont has been popularized in corals with the advent of the hologenome theory of evolution, the underlying concepts are still matter of debate. Indeed, the relative contribution of host and environment in shaping the microbial communities should be examined carefully to evaluate the potential role of symbionts for holobiont adaptation in the context of global changes. As a sessile, long-lived, symbiotic and environmentally sensitive organism, the reef-building coral *Pocillopora damicornis* is a good species to address these issues.

Results : We sampled *Pocillopora damicornis* colonies corresponding to two different mitochondrial lineages in different geographic areas displaying different thermal regimes: Djibouti, French Polynesia, New Caledonia and Taïwan. The *Symbiodinium* and bacteria communities were characterized using high throughput sequencing of Internal Transcribed Spacer ITS2 and 16S rRNA gene. Bacterial microbiota was very diverse with high prevalence of *Endozoicomonas*, *Arcobacter* and *Acinetobacter* in all samples. While *Symbiodinium* clade C1 was dominant in Taïwan and New Caledonia, D1 was highly abundant in Djibouti and French Polynesia. Moreover, we also identified a high background diversity (i.e. with abundances <1%) of A1, C3, C15 and G subclades. Using redundancy analyses, we found that the effect of geography was very low for both communities, and that host genotypes and temperatures differently influenced *Symbiodinium* and bacterial microbiota. Indeed, while the constraint of host haplotype was higher than temperatures on bacterial composition, we showed for the first time a strong relationship between the composition of *Symbiodinium* communities and minimal sea surface temperatures.

Conclusion : Because *Symbiodinium* assemblages are more related to thermal regimes than bacterial communities, we propose that their contribution to adaptive capacities of the holobiont to thermal regimes might be higher than the influence of bacterial microbiota. Moreover, the link between *Symbiodinium* communities and minimal temperatures suggested low relative fitness of clade D at lower temperatures. This observation is particularly relevant in the context of climate change, since corals will face increasing temperatures, as well as much frequent abnormal cold episodes in some areas of the world.

Keywords: coral holobiont, microbiota, bacterial communities, *Symbiodinium* assemblages, thermal adaptation.

3.1. BACKGROUND

All partners (bionts) involved in a stable symbiosis, and thus being part of the entire organism, constitute the holobiont (Margulis & Fester, 1991). A decade after this term has been defined, it has been popularized in corals (Rohwer, Seguritan, & Azam, 2002), and subsequently led to the hologenome theory of evolution (Jefferson, 1994; Rosenberg, Koren, Reshef, Efrony, & Zilber-Rosenberg, 2007) (Zilber-Rosenberg & Rosenberg, 2008). The hologenome is defined as the sum of the genetic information of the host and its symbiotic microorganisms. In this context, phenotypes are the product of the collective genomes of the holobiont partners, being the true unit of biological organization and thus the object of natural selection (McFall-Ngai et al., 2013) (Bordenstein & Theis, 2015; Guerrero, Margulis, & Berlanga, 2013). This concept has gained increased attention for many issues on the functioning, homeostasis or evolution of living organisms, extending our knowledge of microbial community associated to them (see (Bosch & McFall-Ngai, 2011) for a review on metaorganisms).

Microbial communities of corals are considered as the most diverse studied to date (Blackall, Wilson, & van Oppen, 2015), forming a complex consortium composed by the cnidarian host, as well as microbial eukaryotes (including the dinoflagellate endosymbiont *Symbiodinium*), prokaryotes (bacteria and archae), and viruses. The symbiosis between scleractinian corals and dinoflagellate algae of the genus *Symbiodinium* provides the foundation for the ecological success of coral reefs over millions of years (Muscatine & Porter, 1977). In this phototrophic and potentially mutualistic association, the coral host provides inorganic nutrients in exchange of photosynthetically fixed carbon (photosynthates), and amino acids from the algal symbiont (Muscatine & Porter, 1977) (DeSalvo et al., 2010) (Davy, Allemand, & Weis, 2012). Algae from the *Symbiodinium* genus are classified into nine clades (from A to I) (Baker, 2003) (Coffroth & Santos, 2005), and the physiology of the coral holobiont is affected by the clade of the symbiont (Mieog, van Oppen, Cantin, & Stam, 2007; Rouzé, Lecellier, Saulnier, & Berteaux-Lecellier, 2016). Although the establishment of a specific symbiosis occurs during early stages of host larvae colonization (Weis, Reynolds, deBoer, & Krupp, 2001), some coral species can switch endosymbiotic algae during their lifetime especially after bleaching events (Baker, Starger, McClanahan, & Glynn, 2004a) (Jones, Berkelmans, van Oppen, Mieog, & Sinclair, 2008), although the long-term persistence of such changes is matter of debate (Thornhill, Lajeunesse, Kemp, & Fitt,

Table 1 : Samples of *Pocillopora damicornis sensu lato* used in this study (n=94)

Region	Locality	Code	Latitude	Longitude	Nb
Djibouti	Moucha Island	DJMI	11°43'49.27"N	43°13'26.69"E	7
	Ras Korali	DJRK	11°46'46.15"N	42°55'27.65"E	8
	Sable Blanc	DJSB	11°34'57.41"N	42°47'45.86"E	12
French Polynesia	Moorea - Papetoai	FPMP	17°29'31.72"S	149°52'8.34"O	6
	Moorea - Tiahura	FPMT	17°29'22.90"S	149°53'48.65"O	5
	Raiatea - Avera	FPRA	16°47'22.00"S	151°23'30.63"O	8
	Tahaa - Tapuamu	FPTT	16°36'51.03"S	151°32'33.91"O	5
	Tahaa - Vaitoare	FPTV	16°40'36.26"S	151°27'18.92"O	9
	Tahiti Papeete	FPTP	17°34'27.93"S	149°37'11.07"O	7
New-Caledonia	Baie de Ste Marie	NCBS	22°17'35.86"S	166°28'14.44"E	4
	Baie de Ma'a	NCBM	22°11'45.17"S	166°20'23.67"E	4
	Grande Rade	NCGR	22°14'38.63"S	166°25'7.58"E	6
Taiwan	Outlet	TWOU	21°55'48.4"N	120°44'41.9"E	6
	Hobihu	TWHO	21°56'18.6"N	120°44'45.7"E	4
	Wanliton	TWWA	21°59'37.7"N	120°42'22.7"E	3

2006). Bacterial communities associated to corals have also been extensively studied, comparing different species (Littman et al., 2009; Rohwer et al., 2002), disease states (Cróquer, Bastidas, Elliott, & Sweet, 2013; Meyer, Rodgers, Dillard, Paul, & Teplitski, 2016; Sato, Willis, & Bourne, 2009), or environmental conditions (Bourne et al., 2007) {Pantos:2015co} (see also (Hernandez-Agreda, Gates, & Ainsworth, 2016) for a review).

Despite this large corpus of studies, none addressed the effect of natural thermal regimes on microbial assemblages in stony corals. However, sea surface temperature increase is the main factor of ongoing climate changes affecting reef-building corals, (32.8% of species being considered at risk of extinction) (Carpenter, Abrar, Aeby, & Aronson, 2008) with massive bleaching and mortality events (Hughes et al., 2017).

In this study, we investigated the effect of thermal regimes, as well as host clade and geographical distances on the bacterial and *Symbiodinium* assemblages in the complex *Pocillopora damicornis sensus lato* (Veron & Pichon 1976), a functional group of environmentally sensitive scleractinian corals (van Woesik, Sakai, Ganase, & Loya, 2011) that was recently split into 5 clades (Pinzón et al., 2013; Schmidt-Roach, Miller, Lundgren, & Andreakis, 2014). High-throughput metabarcoding allowed us to access for the first time the whole diversity of both bacterial communities and *Symbiodinium* assemblages.

3.2. METHODS

3.2.1. Sampling sites and study design

Colonies of *Pocillopora damicornis sensus lato* growing between 1 to 5 m depth were sampled by snorkeling within four regions (Djibouti, Taiwan, New Caledonia and French Polynesia (Adjeroud et al., 2013)) in 15 localities (Table 1). The tip (1-2 cm) from one healthy branch of each colony was cut and disposed individually in a plastic bag held in seawater during the sampling cruise. Samples were subsequently transferred into modified CHAOS buffer (4M guanidium thiocyanate, 0.5% N-lauryl sarcosine sodium 25mM Tris-HCl pH 8, 0.1M b-mercaptoethanol) as already described (Flot, 2007).

Thermal regime descriptors were compiled from weekly mean sea surface temperature data collected from IGOSS: Integrated Global Ocean Services System Products Bulletin (http://iridl.ldeo.columbia.edu/SOURCES/.IGOSS/) for quadrats of 1° longitude X 1° latitude and from 1982 to the year of sampling (2008-2009).

3.2.2. DNA extraction

DNA extraction was performed using CTAB (Cetyl TrimethylAmmonium Bromide)-based extraction method (Winnepenninckx, Backeljau, & De Wachter, 1993).

3.2.3. Host haplotype identification

The mitochondrial variable open reading frame (ORF) was amplified with FATP6.1 and RORF primers (Flot & Tillier, 2007), and submitted to sanger sequencing (GenBank Accession Numbers ###). Protein-coding sequences were analyzed using MEGA version 6 (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013). Sequence alignment was performed using muscle. The best model (Kimura-2 parameters with uniform substitution rates) was selected for the lowest BIC (Bayesian Information Criterion). Maximum-likelihood tree was computed with the best model and the robustness of the tree was tested with 1000 bootstrap replicates.

3.2.4. 16S rRNA gene and Internal Transcribed Spacer ITS2 metabarcoding

For bacterial communities, 16S rRNA gene libraries were generated using the 341F (5'-CCTACGGGNGGCWGCAG-3') and 805R (5'-GACTACHVGGGTATCTAATCC-3') primers targeting the variable V3V4 loops (Klindworth et al., 2012).

Symbiodinium assemblages were analyzed using ITS2 (internal transcribed spacer of the ribosomal RNA gene) amplicon libraries with specific primers targeting a ~350 bp sequence: ITS2-F (5'-GTGAATTGCAGAACTCCGTG-3') and ITS2-R (5'-CCTCCGCTTACTTATATGCTT-3') (Lajeunesse & Trench, 2000; Quigley et al., 2014).

For both 16S and ITS2 markers, paired-end sequencing with 250 bp read length was performed on the MiSeq system (Illumina) using the v2 chemistry according to the manufacturer's protocol. Sequencing was performed at the McGill University in the Génome Québec Innovation Centre, Montréal, Canada.

3.2.5. Sequence analysis of metabarcoding datasets

The FROGS pipeline (Find Rapidly OTU with Galaxy Solution) implemented on a galaxy instance (http://sigenae-workbench.toulouse.inra.fr/galaxy/) was used for data processing (Escudié et al., 2015). In brief, paired reads were merged using FLASH (Magoč & Salzberg, 2011). After denoising and primer/adapters removal with cutadapt (Martin, 2011), *de novo* clustering was done using SWARM that uses a local clustering threshold, with aggregation distance d=3 after denoising. Chimera were removed using VSEARCH (Rognes, Flouri, Nichols, Quince, & Mahé, 2016) . We filtered the dataset for

singletons and performed affiliation using Blast+ against the Silva database (release 128, september 2016) for 16S amplicons. For ITS2 metabarcoding, the *Symbiodinium* clade was assessed using blastn best hit against the nr/nt database of the NCBI (Altschul, Gish, Miller, Myers, & Lipman, 1990). To confirm blast identification, we performed phylogenetic analysis using MAFFT to produce sequence alignment and FastTree (GTR+CAT model) to compute tree with the approximately maximum likelihood method. Finally, OTU tables were produced in standard BIOM format for subsequent analyses.

3.2.6. Statistical analyses

All statistical analyses were done using R v3.3.1 ((Team, 2012) http://www.R-project.org). We used the phyloseq R package for community composition analysis (McMurdie & Holmes, 2013) to infer alpha diversity metrics at the OTU level, as well as beta diversity (between sample distance) from the OTU table. Community similarity was assessed by Principal Coordinate Analysis (PCoA) using the Bray-Curtis dissimilarities.

We performed one-way ANOVA or non-parametric Kruskal-Wallis tests (when normality of residuals and homogenity of variances were rejected (Shapiro and Bartlett test, respectively)) to compare alpha diversity metrics. When ANOVA or Kruskal-Wallis tests were significant, we then computed pairwise comparisons between group levels (post-hoc analyses) with Bonferroni corrections for multiple testing using pairwise-t-tests or Dunn test, respectively.

Redundancy analysis (Ramette, 2007) (hereafter named RDA) was used to investigate the variations of the different bacterial OTU/*Symbiodinium* clades under the constraint of the environmental variables. Bacterial OTUs and *Symbiodinium* clades were Hellinger-transformed before performing RDAs (Legendre & Gallagher, 2001) on datasets of geography, temperature and host genotypes (Supplementary Table S1). Then significant variables (i.e. variables that significantly explained changes in the distribution of OTUs/clades) were identified using a forward-selection procedure (999 permutations), implemented in the R "vegan" package and in the "rda", "ordiR2step" functions. For all analyses, the threshold significance level was set at 0.05.

3.3. RESULTS

3.3.1. Sampling sites display diverse and contrasted thermal regimes

To have a precise view of the thermal regimes in the different sampling sites, we extracted several descriptors over a long-term continuous monitoring (weekly) of mean

Table 2 : Thermal regime descriptors of the four sampled regions.

	Djibouti	French Polynesia	New Caledonia	Taiwan
Mean temperature (°C)	28.60	27.67	24.77	26.56
Max temperature (°C)	32.20	29.95	28.78	30.83
Min temperature (°C)	25.11	25.25	21.28	21.40
Mean of the warmer months (°C)	30.46	28.75	26.82	29.14
Mean of the colder months (°C)	26.32	26.51	22.81	23.88
Variance (°C)	3.17	1.05	3.04	4.56

sea surface temperature data. We computed annual mean temperature, minimum and maximum temperatures, as well as mean temperatures for the three warmer or colder months over these time-series records, and annual temperature variance (Table 2). We had very contrasted thermal regimes between the sampled regions with high and low annual variations in Taiwan and French Polynesia, respectively. The minimal temperatures were lower in New Caledonia and Taiwan compared to Djibouti and French Polynesia. Maximum temperatures were less contrasted with a more continuous increase from New Caledonia to French Polynesia, then Taiwan and Djibouti.

3.3.2. *Pocillopora damicornis sensu lato* morphotypes corresponded to 2 different clades

Analysis of mitochondrial ORF for all samples revealed two clades corresponding to *Pocillopora* types 3c 3e, 3g, 3h and 7a (clade 2) for all samples from Djibouti, and *Pocillopora* type 5 (clade 1) for samples from New Caledonia, Taiwan and French Polynesia (Pinzón et al., 2013) (Schmidt-Roach et al., 2014) (Supplementary Figure S1 and Supplementary Table S1). This is consistent with the known geographical distribution of *Pocillopora* lineages with a wide distribution of clade 1 *i.e.* types 4 and 5 namely *Pocillopora damicornis* and *Pocillopora acuta* (respectively) throughout the Pacific Ocean, and the presence of type 3 and 7 (*P. damicornis sensu lato* morphotype) in the Western Indian Ocean (Schmidt-Roach et al., 2014) (Gélin, Postaire, Fauvelot, & Magalon, 2017b).

3.3.3. *Symbiodinium* assemblages revealed high specificity as well as high background diversity

The relative abundances of *Symbiodinium* types in each coral sample were analyzed using high-throughput sequencing method. ITS2 amplicon sequencing with MiSeq yielded 45,000 informative clusters per sample in average. After clustering and filtering for OTUs containing less than 100 sequence tags, 53 clusters of ITS2 were obtained (Supplementary Table S2). Taxonomic affiliation was performed using blastn comparison to NT followed by phylogenetic analysis. Maximum-Likelihood trees confirmed the presence of clades A1, C1, C3, C15, D1 and G with largely unresolved polytomies in the clade C (Supplementary Figure S2), such as observed previously (Lajeunesse, 2004). Nevertheless, the topology is congruent with previous works on the molecular taxonomy of *Symbiodinium* (Pochon, Montoya-Burgos, Stadelmann, & Pawlowski, 2006).

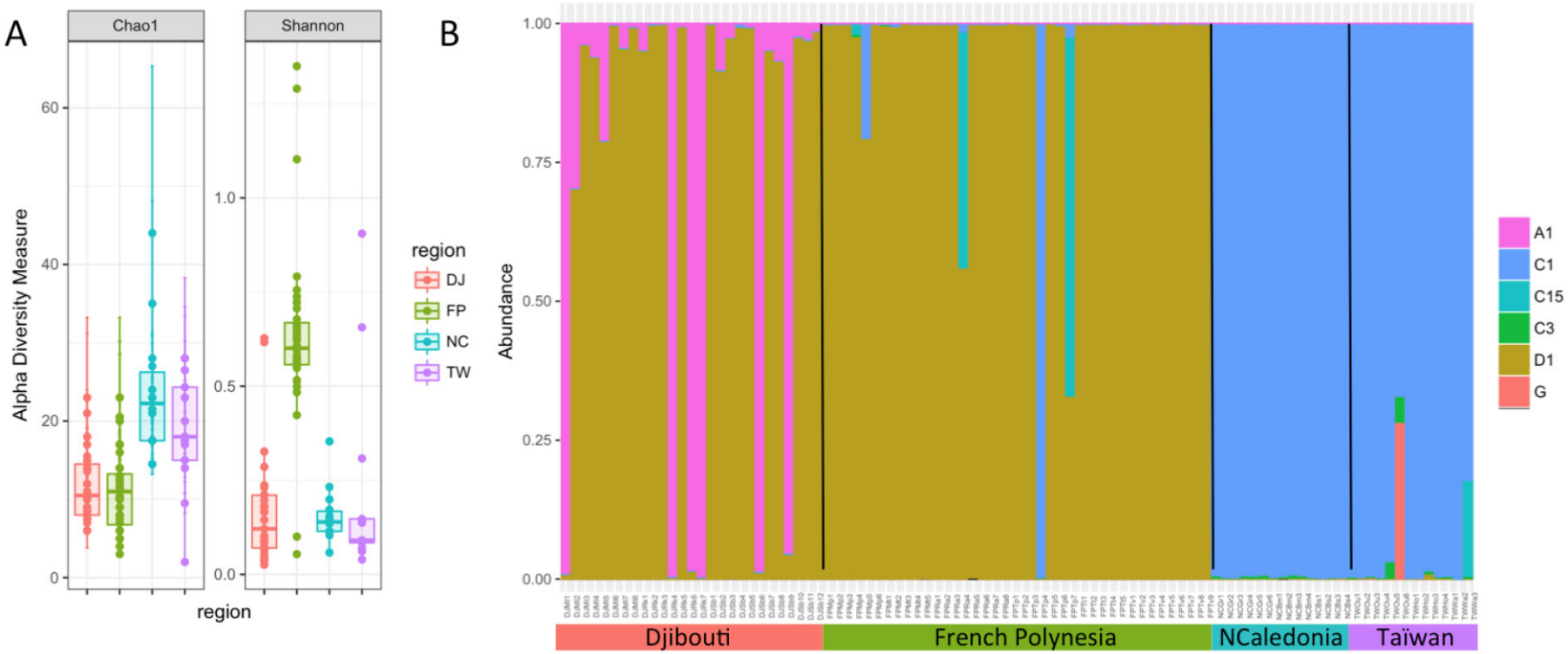


Figure 1 : *Symbiodinium* diversity:
A) **Alpha-diversity (Chao1 and Shannon) index comparison between samples from the 4 regions based on ITS2; DJ: Djibouti, FP: French Polynesia, NC: New Caledonia, TW: Taiwan.**
B) Proportion of *Symbiodinium* subclades per sample.

OTU richness assessed by Chao1 index was slightly higher in New Caledonia and Taiwan. Moreover, Shannon diversity index was significantly higher for Djibouti compared to the others regions (Figure 1A). Only one *Symbiodinium* clade was highly abundant per sample (accounting for more than 95% of sequences) (Figure 1B), suggesting high specificity for each association. In New Caledonia and Taiwan, *Symbiodinium* clade C1 was almost exclusively present in all samples. Clade D1 was dominant in French Polynesia samples (except for 2 samples), whereas a more diverse pattern is observed in Djibouti with a majority of clade D1 as well as a substantial proportion of clade A1, either as the main *Symbiodinium* clade or in association with D1. Accordingly, principal coordinate analysis based on Bray-Curtis dissimilarities followed this partitioning with two main groups (French Polynesia and Djibouti *vs.* Taiwan and New-Caledonia) separated along the first axis (which explained 56.1% of the total variation) (Figure 2). Samples from Djibouti forming a separate group along the second axis are hosting *Symbiodinium* clade A1. Samples from different host clades were overlapping on both axes.

3.3.4. Bacterial microbiota was highly diverse with a few number of core taxa

The bacterial microbiota of each coral sample was analyzed using 16S (V3V4) amplicon sequencing with MiSeq. It yielded 70,000 informative clusters per sample in average. After singleton filtering, we obtained 33,649 OTUs (representing a total of 7,108,067 sequences) that could be annotated using the Silva 16S database. After filtering for chloroplast and non-bacterial sequences, we obtained 31,076 OTUs (Supplementary Table S3) representing 6,569,797 sequences. Notably, the 100 most abundant OTUs represented more than 90% of the whole sequences. OTU richness assessed by Chao1 index was higher in French Polynesia and slightly higher in Djibouti compared to samples of New Caledonia and Taiwan (Figure 3A, Supplementary File S4). Moreover, Shannon diversity index was significantly higher for Djibouti compared to the others regions. These results indicated a high number of rare OTUs in French Polynesia and higher evenness in Djibouti.

Phylum-level assignment of bacterial OTUs indicated the dominance of Proteobacteria in all samples (Figure 3B). Within this phylum, the majority of sequences were affiliated to the genus *Endozoicomonas* (Family Hahellaceae) representing 66.9% of the overall sequences (Supplementary File S5). Three other genera, *Arcobacter*,

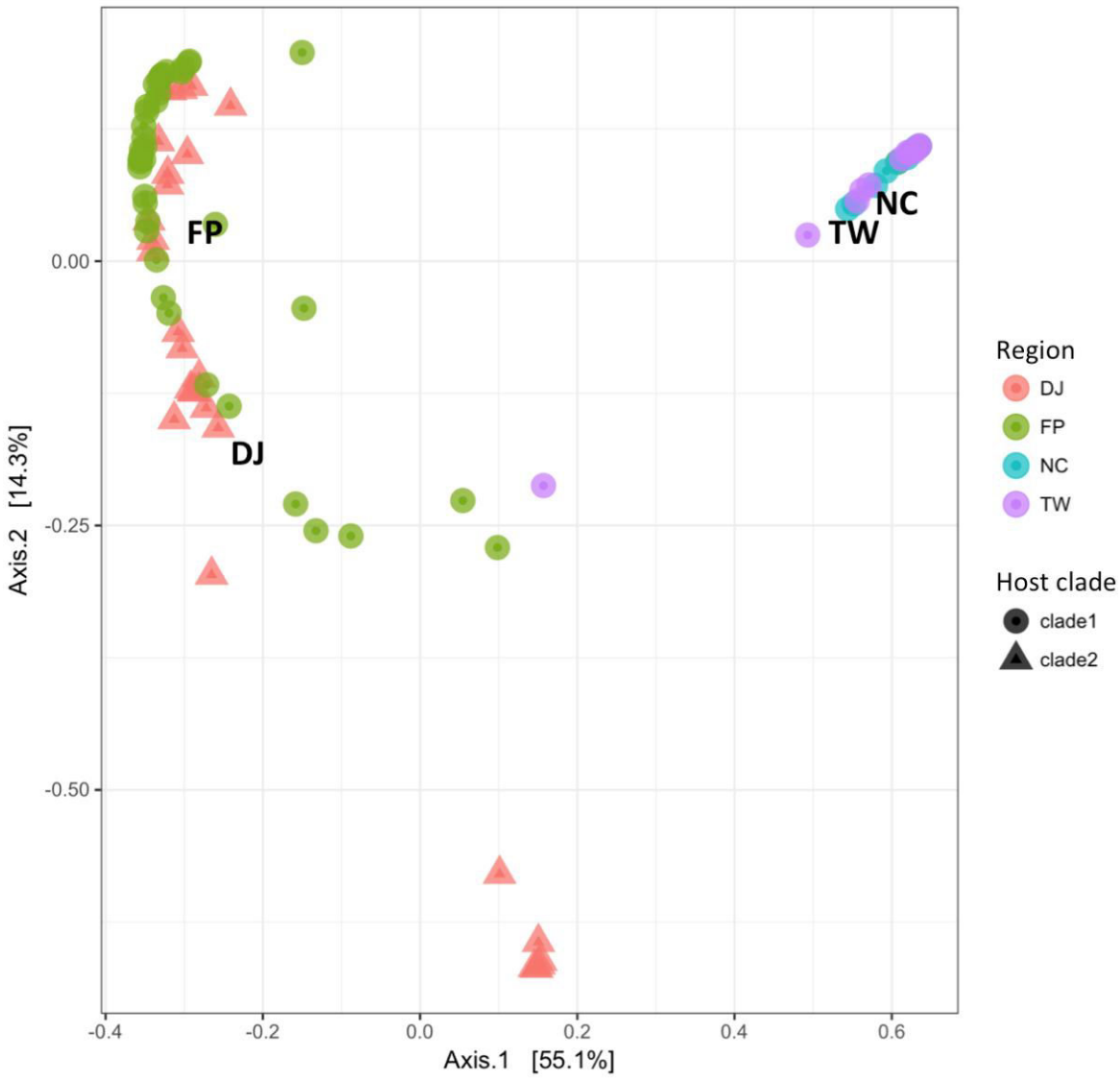


Figure 2 : Principal coordinate analysis of Bray-Curtis dissimilarities (ITS2) between all pairs of samples (colored by region of origin and shaped by *Pocillopora* host haploplype). DJ: Djibouti, FP: French Polynesia, NC: New Caledonia, TW: Taiwan. These axes represent the two synthetic variables explaining the most variation of the dataset.

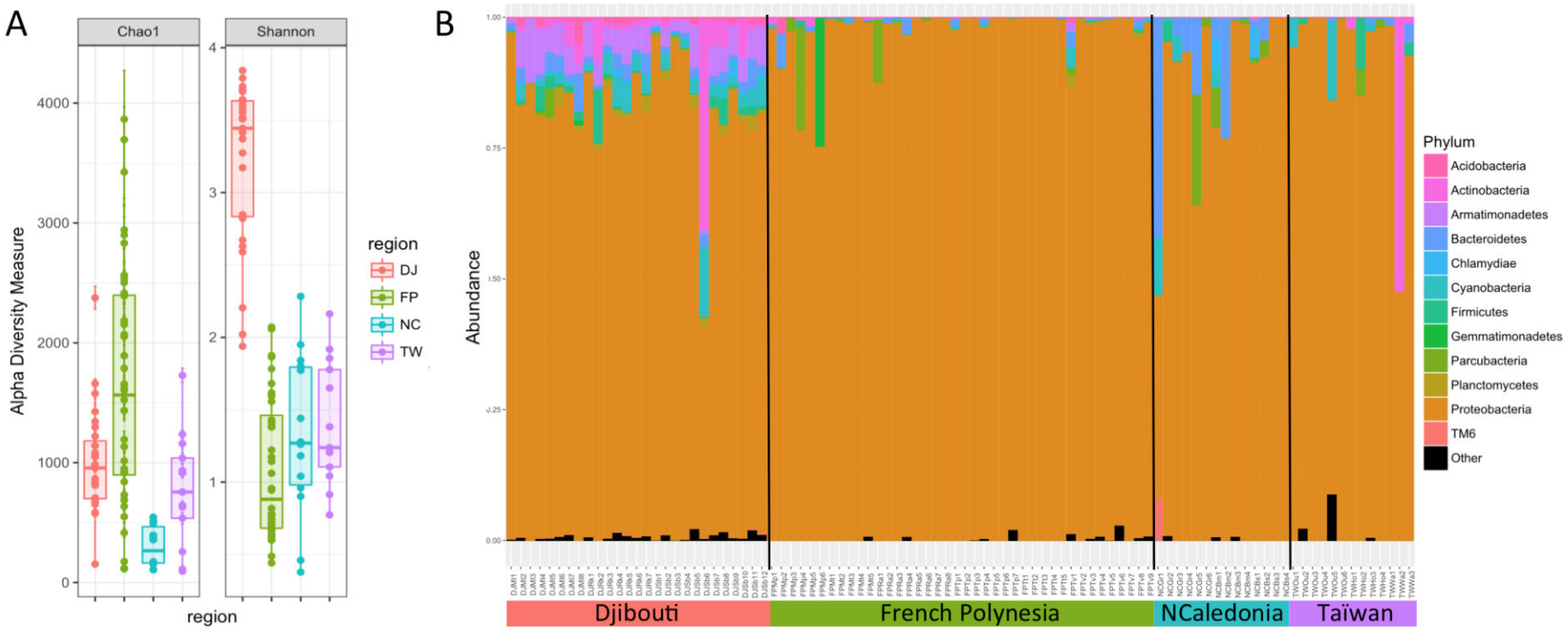


Figure 3 : Bacterial diversity:
A) **Alpha-diversity (Chao1 and Shannon) index comparison between samples from the 4 regions based on 16S ; DJ: Djibouti, FP: French Polynesia, NC: New-Caledonia, TW: Taiwan.**
B) Phylum-level community composition per sample.

Table 3 : Number of samples for each region for core taxa (shared by at least 50% of samples within each region and 50% of the overall samples) at the family and genus levels. The total number of samples for each region is indicated into brackets. The family ranks also corresponding to the core genus are indicated in bold.

Rank	Taxa	DJ (27)	FP (40)	NC (14)	TW (13)	Total (94)
Family	***Hahellaceae***	27	40	14	13	94
	Moraxellaceae	25	37	13	12	87
	Comamonadaceae	26	36	13	10	85
	Campylobacteraceae	25	34	13	12	84
	Rhodobacteraceae	25	36	11	9	81
	Caulobacteraceae	24	30	9	7	70
	Sphingomonadaceae	23	26	9	8	66
Genus	*Endozoicomonas*	27	40	14	13	94
	Acinetobacter	26	35	13	12	86
	Arcobacter	27	33	12	12	84

Acinetobacter and *Sphingomonas* were present at appreciable relative abundance (6.6%, 1.9% and 1% of total phyla tags, respectively). *Arcobacter* (Campylobacteraceae) was particularly abundant in samples from Djibouti (25% of sequence tags), whereas *Acinetobacter* (Moraxellaceae) represented 9.4% of the sequence tags in New Caledonia.

Among 227 families and 513 genera in the whole dataset, only the genus *Endozoicomonas* was common to all samples. We also considered as core phylotypes (at the family and genus level) the taxa shared by 50% of the individuals within each region (Table 3). Two additionnal genera, *Arcobacter* and *Acinetobacter*, fall into this category in addition to *Endozoicomonas*. At the family level, 4 supplementary families namely Comamonadaceae, Rhodobacteraceae, Caulobacteraceae and Sphingomonadaceae were shared by at least 50% of samples. In all cases, core taxa were among the 10 most abundant in the whole dataset (14 families and 6 genera were shared by at least 50% of the overall samples) (Supplementary File S5).

Considering each region independently, strikingly much more taxa were shared between at least 50% of samples from Djibouti (27 families and 46 genera) (Supplementary File S5), which could be linked to higher equitability. Twelve families and 5 genera were core to French Polynesia samples, 12 families and 4 genera for New Caledonia, whereas only 7 families and 3 genera were shared by at least 50% of samples from Taiwan (Supplementary File S5).

Lastly, principal coordinate analysis based on Bray-Curtis dissimilarities showed a partitioning of bacterial communities by both host haplotype and region (Figure 4). Samples from Djibouti (*Pocillopora* clade 2) and New Caledonia (*Pocillopora* clade 1) formed separate groups, whereas Taiwan and French Polynesia samples (clade 1) overlapped on the two principal axes.

3.3.5. *Symbiodinium* communities were mostly influenced by minimal temperatures, whereas bacterial community structure was associated to host haplotype and mean temperatures.

To disentangle the influence of biogeography, temperature and host haplotype (Supplementary Table S1) on coral communities of both *Symbiodinium* and bacteria, we computed redundancy analysis (RDA) and forward-selection procedures. First, we performed RDAs on the whole dataset (geography, thermal regime and host haplotype). A very high proportion (85%) of *Symbiodinium* clade distribution was explained by the

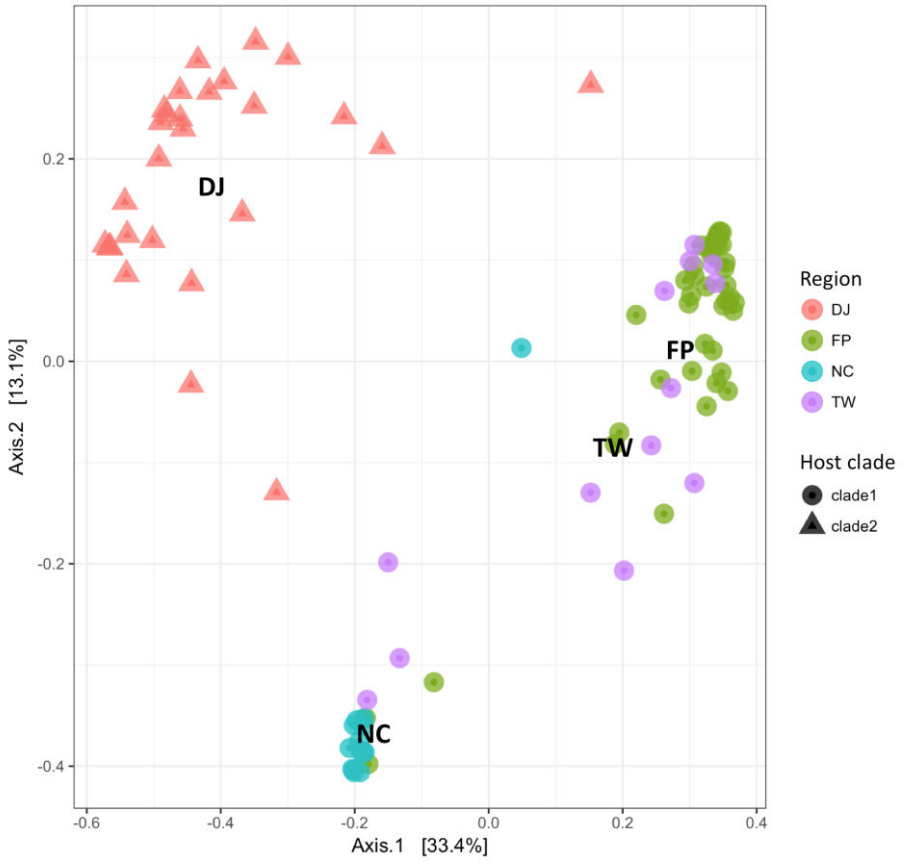


Figure 4 : Principal coordinate analysis of Bray-Curtis dissimilarities (16S) between all pairs of samples (colors and shapes indicate region of origin and *Pocillopora* host haploplype, respectively). Axes represent the two synthetic variables explaining most variation in the dataset (about 46 %).

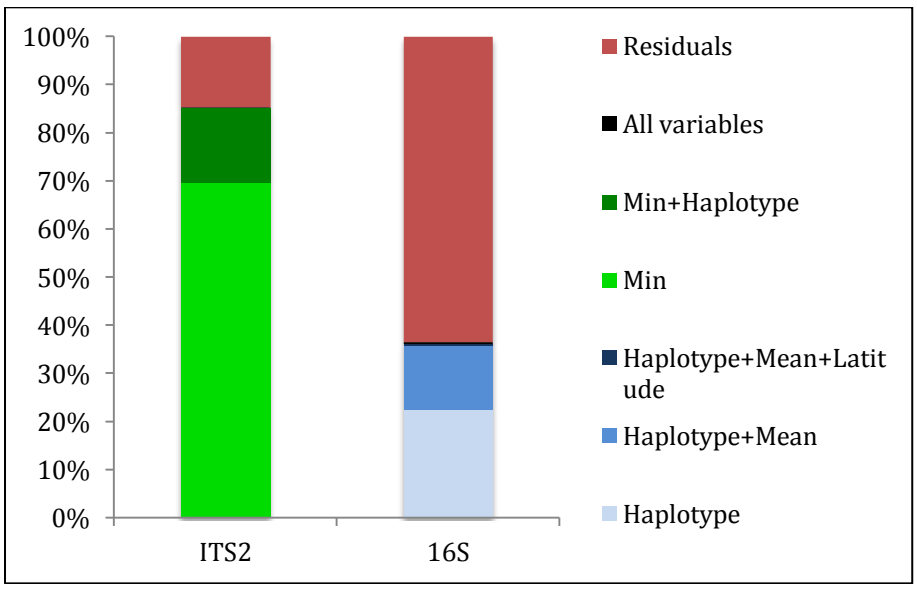


Figure 5 : Cumulative variations explained by the addition of significant variables for the reduced models identified using RDA and forward selection procedures. Min: Minimal temperature; Mean: Mean temperature; Haplotype: coral haplotype.

variables used in this study; this proportion was lower although important (36%) for the bacterial OTU distributions (Figure 5, Supplementary Table S6). Forward selection procedures were carried-out on the whole dataset to identify the significant variables (Supplementary Table S1) constraining the distribution of both communities. This study highlighted (i) that minimal temperature followed by host haplotype mostly constrained *Symbiodinium* distribution in the 94 samples (p = 0.001), and (ii) that in contrast, host haplotype followed by mean temperature then latitude mostly constrained bacteria distribution (p = 0.001).

3.4. Discussion

3.4.1. Diversity of P. damicornis sensu lato samples

In this study, we sampled *P. damicornis sensu lato* colonies in four regions and phylogenetic analyses revealed a complex pattern. Samples from French Polynesia, New Caledonia and Taiwan corresponded to *Pocillopora* clade 1 (more precisely type 5, newly assigned to *Pocillopora acuta sensu stricto* (Schmidt-Roach et al., 2014) (Pinzón et al., 2013)). Samples from Djibouti corresponded to *Pocillopora* clade 2 (types 3 and 7) later assigned to SSH12 and SSH13 and all consistent with *Pocillopora damicornis* morphotype (Gélin, Postaire, Fauvelot, & Magalon, 2017b). Morphological characters have been shown to be insufficient to discriminate species in the *Pocillopora damicornis* complex and genetic markers are necessary to discriminate the species (Pinzón et al., 2013). This species complex displays in addition high phenotypic plasticity and cryptic lineages (Gélin, Postaire, Fauvelot, & Magalon, 2017b). Nevertheless, as we used a single mitochondrial marker for *Pocillopora* clade identification, we cannot completely rule out the possibility of mitochondrial introgression or hybridization that seem to be common among this genus (Combosch & Vollmer, 2015; Combosch, Guzman, Schuhmacher, & Vollmer, 2008). Finally, although it was proposed that *Symbiodinium* assemblages may be useful for integrative taxonomy of *Pocillopora* species (Pinzón & LaJeunesse, 2010), we found in this study that *Symbiodinium* communities did not discriminate *Pocillopora* haplotypes.

3.4.2. Diversity of coral microbiota

The core microbiome can be broadly defined as the stable and consistent taxonomic groups associated to a particular habitat (Shade & Handelsman, 2011). These commonly associated microbial communities are likely of ecological and functional

importance in the holobiont fitness. The definition of the core micobiome in corals is variable among authors, ranging from 30% to 100% of shared bacterial phylotypes at all different taxonomic levels from Kingdom to strain (reviewed in (Hernandez-Agreda et al., 2016)). These studies already highlighted the high diversity and variability of microbial communities in corals. We defined here the core microbiome as taxa (OTUs/genera/families) present in at least 50% of samples within each population, and thus reflecting stable associations with *Pocillopora damicornis sensu lato*.

All core taxa of bacteria were among the most abundant in the dataset, which is in contradiction with previous study identifying rare taxa as ubiquitous endosymbionts (D Ainsworth et al., 2015). Bacteria of the *Endozoicomonas* genus were present in all samples and were the most abundant in the whole dataset, making up from 33.3% of the total number of sequence tags in Djibouti (consistent with a higher evenness) to 87.4% in French Polynesia where rare OTUs were the most diverse. This group has been described for the first time only recently in marine slugs (Kurahashi & Yokota, 2007). So far, they have been identified in numerous coral species and are now considered as ubiquitous endosymbionts of many marine host species (see (Neave, Apprill, Ferrier-Pages, & Voolstra, 2016a) for a review). Notably, comparative genomic analysis of different strains of *Endozoicomonas* from different hosts suggest a common role in carbohydrate cycling with potential specificities in amino-acid synthesis (Neave, Michell, Apprill, & Voolstra, 2017). In addition, *Acinetobacter* and *Arcobacter* were respectively present in 86 and 84 samples over 94, and were the third and second most abundant genus after *Endozoicomonas*. Although considered as a terrestrial bacteria, *Acinetobacter* (gamma-proteobacteria) can be dominant in bleached corals (Koren & Rosenberg, 2008). It has also been identified in healthy corals where it can be abundant (C.-P. Chen, Tseng, Chen, & Tang, 2011) (Morrow, Moss, Chadwick, & Liles, 2012) (Li et al., 2014), but its function remains elusive. Bacteria of the genus *Arcobacter* (epsilon-proteobacteria) are associated to a wide range of habitats, as free-living or pathogenic, and is especially abundant in marine environments (W. G. Miller et al., 2006) and have been identified specifically in diseased corals in some studies (Frias-Lopez, Zerkle, Bonheyo, & Fouke, 2002), sometimes also in healthy samples (de Castro et al., 2010), and has been show to increase during pathogenesis (Séré et al., 2015; Sweet & Bythell, 2012). *Arcobacter* has also been found in high abundances in necrotic sponges (L. Fan, Liu, Simister, Webster, & Thomas, 2013) as well as moribund oysters (Lokmer & Mathias

Wegner, 2014) where it is associated with decreased bacterial diversity and may act as an opportunistic pathogen. Although detectable at background abundances in the majority of samples, *Arcobacter* was highly abundant in all samples from Djibouti (25% of sequence tags *vs.* less than 1% in the other regions), indicating that this genus can also be abundant in healthy hosts, and moreover associated with high bacterial diversity.

Metabarcoding targeting ITS2 provides highly sensitive and quantitative measurements of *Symbiodinium* diversity (Quigley et al. 2014). It was used with 454-pyrosequencing (Boulotte et al., 2016; L. Thomas, Kendrick, Kennington, Richards, & Stat, 2014) and more recently with Illumina MiSeq sequencing (Ziegler, Seneca, Yum, Palumbi, & Voolstra, 2017). Although we did not find ubiquitous clades for the whole samples, we showed using MiSeq sequencing striking differences in terms of *Symbiodinium* associations between populations. Clade D1 was dominant in French Polynesia. This is consistent with a recent study in Moorea, that showed association with *Symbiodinium* clade D and few clade C (Rouzé et al., 2017).

Colonies from Djibouti were also mainly associated with clade D1, while clade C1 was dominant in Taiwan and New Caledonia. To our knowledge, this is the first study of symbiotic communities associated with *Pocillopora* in Djibouti, New Caledonia and Taiwan. A comparative study of *Symbiodinium* clades associated with hard corals from the Persian Gulf with annual temperatures from 16°C up to 36°C revealed the dominance of clade C3 associated with a lower proportion of A1 and minor quantities of C15, whereas clade D was not detected (Hume et al., 2013).

Even if one single clade was dominant in almost all samples, we detected background abundances of the other clades in all regions constituting the so called "*Symbiodinium* rare biosphere" (Boulotte et al., 2016). The cryptic diversity that we reported here is actually very high (H' Shannon index up to 0.6 for Djibouti samples, Figure 1A) and falls into a similar range such as reported in the latter work for two Pocilloporid species as well as for *P. verrucosa* (Weber, DeForce, & Apprill, 2017). Such high diversity can potentially confer higher adaptive potential to their hosts through redundant or complementary symbiotic functions, and/or potential for symbiont switching or shuffling (Fabina, Putnam, Franklin, Stat, & Gates, 2013). High-throughput methods (ITS metabarcoding using 454) applied to the generalist and environmentally sensitive *Acropora* species also revealed the same pattern across Western Australia with

clade C being dominant in all samples with background abundances of other clades (L. Thomas et al., 2014).

Finally, bacterial communities in corals may also be shaped by the presence of photosymbionts (Bourne et al., 2013) through the use of DMSP released by the algae (Raina, Dinsdale, Willis, & Bourne, 2010), but at a finer scale we could not find any relationship for the same host species between *Symbiodinium* type and bacterial communities.

3.4.3. Influence of geography on coral microbiota is very low

Because we found different microbiota composition between samples, we used redundancy analyses in order to identify variables that significantly constrained their composition. We first found that the influence of geography was low, since longitude had no significant effect and the effect of latitude in our dataset was null for *Symbiodinium* assemblages and was marginal on bacterial communities (less than 1% when added to the variance explained by host haplotype and mean temperature). In contradiction with this result, geographic clustering of symbionts is expected to be high in brooding species with vertical transmission, compared to horizontal transmission that would in contrary favor settlement of locally adapted symbionts. *Pocillopora damicornis* is a hermaphrodite brooder (T. Y. Fan, Lin, Kuo, Soong, & Liu, 2006; Harrison & Wallace, 1990) thus transmitting its symbionts vertically (Baird, Guest, & Willis, 2009), and is able to release sexual as well as parthenogenetic larvae (Whitaker, 2006; Yeoh & Dai, 2009). However, these results are similar to others studies showing that bacterial communities associated with different coral species are stable through space and time (Rohwer et al. 2002). Thus, we hypothesize that low parental effect on symbiont communities may be explained by the relatively high connectivity and long-range dispersal for this species compared to other brooders (Adjeroud et al., 2013) (Gélin, Fauvelot, Mehn, Bureau, Rouzé, et al., 2017a), which would mitigate the effect of geography and allow higher symbiont mixing with the local microbial environment.

3.4.4. Influence of host haplotype is higher for bacterial communities than for *Symbiodinium*

Although we did not find significant link between microbiota and geography, we highlighted links with host haplotypes, particularly for bacterial microbiota. As the two host clades sampled in this study were from potentially different microbial environments, we cannot rule out a confounding effect between host haplotype and environmental communities present in the seawater at the time of larvae settlement and bacterial microbiota acquisition. Nevertheless, others studies observed that host genotypes might influence bacterial microbiota, since (i) different coral species harboured different microbiota composition (Rohwer et al. 2002), (ii) differential specificity between closely related corals and abundant *Endozoicomonas* endosymbionts were found across global scales (Neave et al., 2016b).

In contrast, we found a significant but less important link between *Symbiodinium* and host haplotype. This observation might also be linked to the adaptive bleaching hypothesis, which proposes that coral bleaching is an adaptive mechanism through the modification of *Symbiodinium* composition (Buddemeier & Fautin, 1993). For example, endosymbiotic flexibility (the ability of a coral species to associate with multiple *Symbiodinium* clades) was observed to be associated with resistance (Baker, 2003). In addition, *Pocillopora* is a generalist host, displaying a high intraspecific and interspecific flexibility in terms of *Symbiodinium* assemblages (mainly among clades A, C and D) (Putnam, Stat, Pochon, & Gates, 2012).

3.4.5. Influence of temperature is higher for *Symbiodinium* than for bacterial microbiota

Although host genotypes mostly constrained bacterial assemblages, we also found a significant link with mean temperatures. Interestingly, reciprocal transplants of *Acropora* corals at a reduced geographic scale revealed as well that their microbiome were different across thermally variable habitats and changed over time after transplantation (Ziegler et al., 2017). As a consequence, bacterial communities might be linked to heat-tolerance of their coral hosts, but further studies of their dynamics during a thermal stress are needed to understand their contribution to the holobiont response.

Strikingly, redundancy analysis revealed that most variation in *Symbiodinium* communities was actually explained by annual minimal temperatures (70%), whereas we observed no significant correlation with mean, maximal or annual fluctuations of

temperatures. In *Acropora*, it has been shown experimentally that *Symbiodinium* type alters larvae settlement in a temperature-dependent fashion (Winkler, Pandolfi, & Sampayo, 2015). In the latter work, lower temperatures and not higher temperatures adversely affected recruitment by reducing larval survival and settlement. Accordingly, we propose that host / *Symbiodinium* associations are stable in space at a regional scale, and are actually more sensitive to a minimal, threshold temperature, than to annual variations or to maximal temperatures.

In particular, clade D is common in all areas colonized by scleractinian corals (Knowlton & Rohwer, 2003) and many studies have shown that it confers higher thermotolerance to its host through photoprotection (Jones & Berkelmans, 2012; Rowan, 2004b), and that it increases in proportion after bleaching events (Baker, Starger, McClanahan, & Glynn, 2004b; Fabricius, Mieog, Colin, Idip, & van Oppen, 2004; Rowan, 2004a). Clade D is thus commonly considered as stress-resistant and potentially opportunistic for compromised corals facing stressful conditions (Stat & Gates, 2011). Nevertheless and in accordance to the link we observed with minimal temperatures, some studies have revealed that cold stress may be detrimental for coral harboring this symbiont (Roth, Goericke, & Deheyn, 2012; Tsang & Ang, 2015). More recently, it has been shown experimentally that clade D1a suffered more photodamages at low temperatures than clade C3 (Silverstein, Cunning, & Baker, 2017). Thus, it seems that clade D may not display higher plasticity in terms of thermal range, but rather that it is only adapted to hot thermal environments. Because climate changes will not only be associated with global increase of sea surface temperatures, but also to extreme thermal events including cold episodes (Kim et al., 2013), further studies are required to understand the potential response of coral reef in the future, particularly for regions with highly fluctuating thermal regimes and low temperature thresholds.

3.5. CONCLUSIONS

The hologenome theory of evolution reboots elements of Lamarckian evolution and has been thus matter of much debate (reviewed in (Theis et al., 2016) (Bordenstein & Theis, 2015)). Although the host genome follows a Mendelian framework, potential changes in microbial assemblages may be inherited to the next generation. Conversely, for the selection to operate on holobiont phenotypes at evolutionary scales, specificity of the interaction through coevolution between partners would be expected.

Using combined high throughput barcoding approaches for both bacterial and *Symbiodinium* communities, we showed that variation in *Symbiodinium* composition is mostly explained by thermal regime especially minimal temperatures, whereas bacterial communities are much less related to temperature modifications. In this context, we propose that *Symbiodinium* types might confer more enhanced adaptive capacities to temperature modifications than the bacterial microbiota. However, *Symbiodinium* clade D was known to be adapted to high temperature, and we found a negative relationship with low temperatures, suggesting low plasticity for this clade. Such low plasticity might limit the adaptive capabilities of coral associated to clade D and living in highly variable thermal regime. However, a high background diversity of *Symbiodinium* was also observed, providing the potential for coral colonies to adapt or acclimatize to future environmental changes via symbiont shuffling (*i.e.* changes in the relative abundance of *Symbiodinium* types constituting the within host community).

Our study may thus contribute to new insights into the importance of microbial (*i.e.* bacterial and *Symbiodinium*) communities for holobiont functioning as well as the relative importance of host and environmental factors in shaping the interaction.

3.6. ACKNOWLEDGEMENTS:

We would like to thank Lorenzo Bramanti, Pauline Bosserelle and Tung-Yung Fan for their help in coral sampling. We are grateful to the genotoul bioinformatics platform Toulouse Midi-Pyrenees and Sigenae group for providing help and computing resources thanks to Galaxy instance http://sigenae-workbench.toulouse.inra.fr.

Declarations :

Ethics approval and consent to participate:

Not applicable.

Consent for publication:

Not applicable.

Availability of data and materials:

The datasets generated during the current study are available in the Sequence Read Archive repository under BioProject ID PRJNA393088, BioSample ID SUB2829485 (to be released upon publication).

Competing interests:

The authors declare that they have no competing interests.

Funding:

This project has been funded by the ADACNI programme of the French National Research Agency (ANR) (project no. ANR-12-ADAP-0016; http://adacni.imbe.fr)

Authors' contributions:

ET, JVD, MA, DA, FB and GM were involved in the study concept and design. JVD and MA were involved in the collection of samples. KBR, CC, FB, DA, MP and ET were involved in data

acquisition and analysis. ET drafted the manuscript and all authors contributed to critical revisions and approved the final manuscript.

Supplementary files :

Supplementary Figure S1: Maximum-likelihood tree of the mitochondrial ORF defining *Pocillopora* types. Numbers are bootstraps (%) reflecting clade support.

Supplementary Figure S2: Maximum-likelihood tree of the 53 *Symbiodinium* OTUs based on ITS2, together with GenBank representatives of each identified clade. Numbers are bootstraps (%) reflecting clade support.

Supplementary Table S1: Sample metadata including geographic and abiotic variables (temperature descriptors) as well as *Pocillopora* haplotype identification.

Supplementary Table S2: *Symbiodinium* OTU table with sequence tag counts per sample and taxonomic affiliation

Supplementary Table S3: Bacterial OTU table with sequence tag counts per sample and taxonomic affiliation.

Supplementary File S4: Diversity indices calculated on bacterial diversity for each sample and statistical analyses of differences between regions.

Supplementary File S5: Number of bacterial sequences and positive samples for each region at the family and genus levels. Taxa shared by at least 50% of samples in one population are colored in light grey, whereas taxa shared y at least 50% of the overall samples are colored in dark grey. We considered as core microbiota the taxa shared by 50% of samples for each of the 4 populations (regions) studied.

Supplementary Table S6: Variations in bacterial (16S) and *Symbiodinium* (ITS2) communities explained by the addition of significant variables for the reduced models identified using RDA and forward selection procedures.

3.7. REFERENCES

Adjeroud, M., Guérécheau, A., Vidal-Dupiol, J., Flot, J.-F., Arnaud-Haond, S., & Bonhomme, F. (2013). Genetic diversity, clonality and connectivity in the scleractinian coral Pocillopora damicornis: a multi-scale analysis in an insular, fragmented reef system. Marine Biology, 161(3), 531–541. http://doi.org/10.1007/s00227-013-2355-9

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local search alignment tool. J. Mol. Biol.

Baird, A. H., Guest, J. R., & Willis, B. L. (2009). Systematic and Biogeographical Patterns in the Reproductive Biology of Scleractinian Corals. Annual Review of Ecology, Evolution, and Systematics, 40(1), 551–571. http://doi.org/10.1146/annurev.ecolsys.110308.120220

Baker, A. C. (2003). Flexibility and specificity in coral-algal symbiosis: diversity, ecology, and biogeography of Symbiodinium. Annual Review of Ecology. http://doi.org/10.1146/132417

Baker, A. C., Starger, C. J., McClanahan, T. R., & Glynn, P. W. (2004a). Coral reefs: Corals' adaptive response to climate change. Nature ..., 430(7001), 741–741. http://doi.org/10.1016/0304-3800(94)00132-4

Baker, A. C., Starger, C. J., McClanahan, T. R., & Glynn, P. W. (2004b). Corals' adaptive response to climate change.

Blackall, L. L., Wilson, B., & van Oppen, M. J. H. (2015). Coral-the world's most diverse symbiotic ecosystem. Molecular Ecology, 24(21), 5330–5347. http://doi.org/10.1111/mec.13400

Bordenstein, S. R., & Theis, K. R. (2015). Host Biology in Light of the Microbiome: Ten Principles of Holobionts and Hologenomes. PLoS Biology, 13(8), e1002226. http://doi.org/10.1371/journal.pbio.1002226

Bosch, T. C. G., & McFall-Ngai, M. J. (2011). Metaorganisms as the new frontier. Zoology, 114(4), 185–190. http://doi.org/10.1016/j.zool.2011.04.001

Boulotte, N. M., Dalton, S. J., Carroll, A. G., Harrison, P. L., Putnam, H. M., Peplow, L. M., & van Oppen, M. J. (2016). Exploring the Symbiodinium rare biosphere provides evidence for symbiont switching in reef-building corals. The ISME Journal, 10(11), 2693–2701. http://doi.org/10.1038/ismej.2016.54

Bourne, D. G., Bourne, D. G., Dennis, P. G., Dennis, P. G., Uthicke, S., Uthicke, S., et al. (2013). Coral reef invertebrate microbiomes correlate with the presence of photosymbionts. The ISME Journal. http://doi.org/10.1038/ismej.2012.172

Bourne, D., Bourne, D., Iida, Y., Iida, Y., Uthicke, S., Uthicke, S., et al. (2007). Changes in coral-associated microbial communities during a bleaching event. The ISME Journal, 2(4), 350–363. http://doi.org/10.1038/ismej.2007.112

Buddemeier, R. W., & Fautin, D. G. (1993). Coral bleaching as an adaptive mechanism. Bioscience.

Carpenter, K. E., Abrar, M., Aeby, G., & Aronson, R. B. (2008). One-third of reef-building corals face elevated extinction risk from climate change and local impacts. ….

Chen, C.-P., Tseng, C.-H., Chen, C. A., & Tang, S.-L. (2011). The dynamics of microbial partnerships in the coral Isopora palifera. The ISME Journal, 5(4), 728–740. http://doi.org/10.1038/ismej.2010.151

Coffroth, M. A., & Santos, S. R. (2005). Genetic Diversity of Symbiotic Dinoflagellates in the Genus Symbiodinium. Protist, 156(1), 19–34. http://doi.org/10.1016/j.protis.2005.02.004

Combosch, D. J., & Vollmer, S. V. (2015). Trans-Pacific RAD-Seq population genomics confirms introgressive hybridization in Eastern Pacific Pocillopora corals. Molecular Phylogenetics and Evolution. http://doi.org/10.1016/j.ympev.2015.03.022

Combosch, D. J., Guzman, H. M., Schuhmacher, H., & Vollmer, S. V. (2008). Interspecific hybridization and restricted trans-Pacific gene flow in the Tropical Eastern Pacific Pocillopora. Molecular Ecology, 17(5), 1304–1312. http://doi.org/10.1111/j.1365-294X.2007.03672.x

Cróquer, A., Bastidas, C., Elliott, A., & Sweet, M. (2013). Bacterial assemblages shifts from healthy to yellow band disease states in the dominant reef coral Montastraea faveolata. Environmental Microbiology Reports, 5(1), 90–96. http://doi.org/10.1111/j.1758-2229.2012.00397.x

D Ainsworth, T., Krause, L., Bridge, T., Torda, G., Raina, J.-B., Zakrzewski, M., et al. (2015). The coral core microbiome identifies rare bacterial taxa as ubiquitous endosymbionts. The ISME Journal, 9(10), 2261–2274. http://doi.org/10.1038/ismej.2015.39

Davy, S. K., Allemand, D., & Weis, V. M. (2012). Cell biology of cnidarian-dinoflagellate symbiosis. Microbiology and Molecular Biology Reviews, 76(2), 229–261. http://doi.org/10.1128/MMBR.05014-11

de Castro, A. P., Araújo, S. D., Reis, A. M. M., Moura, R. L., Francini-Filho, R. B., Pappas, G., et al. (2010). Bacterial community associated with healthy and diseased reef coral Mussismilia hispida from eastern Brazil. Microbial Ecology, 59(4), 658–667. http://doi.org/10.1007/s00248-010-9646-1

DeSalvo, M. K., Sunagawa, S., Fisher, P. L., Voolstra, C. R., Iglesias-Prieto, R., & Medina, M. (2010). Coral host transcriptomic states are correlated with Symbiodiniumgenotypes. Molecular Ecology, 19(6), 1174–1186. http://doi.org/10.1111/j.1365-294X.2010.04534.x

Escudié, F., Auer, L., Bernard, M., Cauquil, L., Vidal, K., Maman, S., et al. (2015). FROGS : Find Rapidly OTUs with Galaxy. The Environmental Genomics Conference, Montpellier, France.

Fabina, N. S., Putnam, H. M., Franklin, E. C., Stat, M., & Gates, R. D. (2013). Symbiotic specificity, association patterns, and function determine community responses to global changes: defining critical research areas for coral-Symbiodiniumsymbioses. Global Change Biology, 335, n/a–n/a. http://doi.org/10.1111/gcb.12320

Fabricius, K. E., Mieog, J. C., Colin, P. L., Idip, D., & van Oppen, M. J. H. (2004). Identity and diversity of coral endosymbionts (zooxanthellae) from three Palauan reefs

with contrasting bleaching, temperature and shading histories. Molecular Ecology, 13(8), 2445–2458. http://doi.org/10.1111/j.1365-294X.2004.02230.x

Fan, L., Liu, M., Simister, R., Webster, N. S., & Thomas, T. (2013). Marine microbial symbiosis heats up: the phylogenetic and functional response of a sponge holobiont to thermal stress. The ISME Journal, 7(5), 991–1002. http://doi.org/10.1038/ismej.2012.165

Fan, T. Y., Lin, K. H., Kuo, F. W., Soong, K., & Liu, L. L. (2006). Diel patterns of larval release by five brooding scleractinian corals. … Ecology Progress Series.

Flot, J. F. (2007). Vers une taxonomie moléculaire des coraux du genre Pocillopora.

Flot, J.-F., & Tillier, S. (2007). The mitochondrial genome of Pocillopora (Cnidaria: Scleractinia) contains two variable regions: the putative D-loop and a novel ORF of unknown function. Gene, 401(1-2), 80–87. http://doi.org/10.1016/j.gene.2007.07.006

Frias-Lopez, J., Zerkle, A. L., Bonheyo, G. T., & Fouke, B. W. (2002). Partitioning of bacterial communities between seawater and healthy, black band diseased, and dead coral surfaces. Applied and Environmental Microbiology, 68(5), 2214–2228.

Gélin, P., Fauvelot, C., Mehn, V., Bureau, S., Rouzé, H., & Magalon, H. (2017a). Superclone Expansion, Long-Distance Clonal Dispersal and Local Genetic Structuring in the Coral Pocillopora damicornis Type β in Reunion Island, South Western Indian Ocean. PloS One, 12(1), e0169692. http://doi.org/10.1371/journal.pone.0169692

Gélin, P., Postaire, B., Fauvelot, C., & Magalon, H. (2017b). Reevaluating species number, distribution and endemism of the coral genus Pocillopora Lamarck, 1816 using species delimitation methods and microsatellites. Molecular Phylogenetics and Evolution. http://doi.org/10.1016/j.ympev.2017.01.018

Guerrero, R., Margulis, L., & Berlanga, M. (2013). Symbiogenesis: the holobiont as a unit of evolution. International Microbiology, 16(3), 133–143. http://doi.org/10.2436/20.1501.01.188

Harrison, P. L., & Wallace, C. C. (1990). Reproduction, dispersal and recruitment of scleractinian corals. Ecosystems of the world.

Hernandez-Agreda, A., Gates, R. D., & Ainsworth, T. D. (2016). Defining the Core Microbiome in Corals' Microbial Soup. Trends in Microbiology. http://doi.org/10.1016/j.tim.2016.11.003

Hughes, T. P., Kerry, J. T., Álvarez-Noriega, M., Álvarez-Romero, J. G., Anderson, K. D., Baird, A. H., et al. (2017). Global warming and recurrent mass bleaching of corals. Nature …, 543(7645), 373–377. http://doi.org/10.1038/nature21707

Hume, B., D'Angelo, C., Burt, J., Baker, A. C., Riegl, B., & Wiedenmann, J. (2013). Corals from the Persian/Arabian Gulf as models for thermotolerant reef-builders: Prevalence of clade C3 Symbiodinium, host fluorescence and ex situ temperature tolerance. Marine Pollution Bulletin, 72(2), 313–322. http://doi.org/10.1016/j.marpolbul.2012.11.032

Jefferson, R. (1994). The Hologenome. … World: A Future of PCR. Cold Spring Harbor.

Jones, A. M., Berkelmans, R., van Oppen, M. J. H., Mieog, J. C., & Sinclair, W. (2008). A community change in the algal endosymbionts of a scleractinian coral following a natural bleaching event: field evidence of acclimatization. Proceedings of the Royal Society B-Biological Sciences, 275(1641), 1359–1365. http://doi.org/10.1046/j.1365-294X.2003.01988.x

Jones, A., & Berkelmans, R. (2012). The photokinetics of thermo-tolerance in Symbiodinium. Marine Ecology.

Kim, B.-M., Son, S.-W., Min, S.-K., Jeong, J.-H., Kim, S.-J., Zhang, X., et al. (2013). Weakening of the stratospheric polar vortex by Arctic sea-ice loss. Nature Communications, 5, 4646–4646. http://doi.org/10.1038/ncomms5646

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., & Glockner, F. O. (2012). Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Research, 41(1), e1–e1. http://doi.org/10.1093/nar/gks808

Knowlton, N., & Rohwer, F. (2003). Multispecies Microbial Mutualisms on Coral Reefs: The Host as a Habitat. The American Naturalist, 162(s4), S51–S62. http://doi.org/10.1086/378684

Koren, O., & Rosenberg, E. (2008). Bacteria associated with the bleached and cave coral Oculina patagonica. Microbial Ecology, 55(3), 523–529. http://doi.org/10.1007/s00248-007-9297-z

Kurahashi, M., & Yokota, A. (2007). Endozoicomonas elysicola gen. nov., sp. nov., a gamma-proteobacterium isolated from the sea slug Elysia ornata. Systematic and Applied Microbiology, 30(3), 202–206. http://doi.org/10.1016/j.syapm.2006.07.003

Lajeunesse, T. C. (2004). “Species” Radiations of Symbiotic Dinoflagellates in the Atlantic and Indo-Pacific Since the Miocene-Pliocene Transition. Molecular Biology and Evolution, 22(3), 570–581. http://doi.org/10.1093/molbev/msi042

Lajeunesse, T. C., & Trench, R. K. (2000). Biogeography of two species of Symbiodinium (Freudenthal) inhabiting the intertidal sea anemone Anthopleura elegantissima (Brandt). The Biological Bulletin, 199(2), 126–134. http://doi.org/10.2307/1542872

Legendre, P., & Gallagher, E. D. (2001). Ecologically meaningful transformations for ordination of species data. Oecologia, 129(2), 271–280. http://doi.org/10.1007/s004420100716

Li, J., Chen, Q., Long, L.-J., Dong, J.-D., Yang, J., & Zhang, S. (2014). Bacterial dynamics within the mucus, tissue and skeleton of the coral Porites lutea during different seasons. Scientific Reports, 4, 7320. http://doi.org/10.1038/srep07320

Littman, R., Singh, Y., Willis, B. L., Ahmad, J., Pfeffer, C., Musarrat, J., et al. (2009). Diversities of coral-associated bacteria differ with location, but not species, for three acroporid corals on the Great Barrier Reef. FEMS Microbiology Ecology, 68(2), 152–163. http://doi.org/10.1111/j.1574-6941.2009.00666.x

Lokmer, A., & Mathias Wegner, K. (2014). Hemolymph microbiome of Pacific oysters in response to temperature, temperature stress and infection. The ISME Journal, 9(3), 670–682. http://doi.org/10.1038/ismej.2014.160

Magoč, T., & Salzberg, S. L. (2011). FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics.

Margulis, L., & Fester, R. (1991). Symbiosis as a Source of Evolutionary Innovation. MIT Press.

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet Journal.

McFall-Ngai, M., Hadfield, M. G., Bosch, T. C., Carey, H. V., Domazet-Lošo, T., Douglas, A. E., et al. (2013). Animals in a bacterial world, a new imperative for the life sciences. Proceedings of the National Academy of Sciences of the United States of America, 110(9), 3229–3236. http://doi.org/10.1073/pnas.1218525110/-/DCSupplemental

McMurdie, P. J., & Holmes, S. (2013). phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. PloS One, 8(4), e61217. http://doi.org/10.1371/journal.pone.0061217.s002

Meyer, J. L., Rodgers, J. M., Dillard, B. A., Paul, V. J., & Teplitski, M. (2016). Epimicrobiota Associated with the Decay and Recovery of Orbicella Corals Exhibiting Dark Spot Syndrome. Frontiers in Microbiology, 7, 893. http://doi.org/10.3389/fmicb.2016.00893

Mieog, J. C., van Oppen, M., Cantin, N. E., & Stam, W. T. (2007). Real-time PCR reveals a high incidence of Symbiodinium clade D at low levels in four scleractinian corals across the Great Barrier Reef: implications for …. Coral Reefs.

Miller, W. G., Parker, C. T., Rubenfield, M., Mendz, G. L., Wösten, M. M. S. M., Ussery, D. W., et al. (2006). The complete genome sequence and analysis of the epsilonproteobacterium Arcobacter butzleri. PloS One, 2(12), e1358–e1358. http://doi.org/10.1371/journal.pone.0001358

Morrow, K. M., Moss, A. G., Chadwick, N. E., & Liles, M. R. (2012). Bacterial associates of two Caribbean coral species reveal species-specific distribution and geographic variability. Applied and Environmental Microbiology, 78(18), 6438–6449. http://doi.org/10.1128/AEM.01162-12

Muscatine, L., & Porter, J. W. (1977). Reef corals: mutualistic symbioses adapted to nutrient-poor environments. Bioscience.

Neave, M. J., Apprill, A., Ferrier-Pages, C., & Voolstra, C. R. (2016a). Diversity and function of prevalent symbiotic marine bacteria in the genus Endozoicomonas. Applied Microbiology and Biotechnology, 100(19), 8315–8324. http://doi.org/10.1007/s00253-016-7777-0

Neave, M. J., Michell, C. T., Apprill, A., & Voolstra, C. R. (2017). Endozoicomonas genomes reveal functional adaptation and plasticity in bacterial strains symbiotically associated with diverse marine hosts. Scientific Reports, 7, 40579. http://doi.org/10.1038/srep40579

Neave, M. J., Rachmawati, R., Xun, L., Michell, C. T., Bourne, D. G., Apprill, A., & Voolstra, C. R. (2016b). Differential specificity between closely related corals and abundant Endozoicomonas endosymbionts across global scales. The ISME Journal, 11(1), 186–200. http://doi.org/10.1007/BF00431399

Pinzón, J. H., & LaJeunesse, T. C. (2010). Species delimitation of common reef corals in the genus Pocillopora using nucleotide sequence phylogenies, population genetics and symbiosis ecology. Molecular Ecology, 20(2), 311–325. http://doi.org/10.1111/j.1365-294X.2010.04939.x

Pinzón, J. H., Sampayo, E., Cox, E., Chauka, L. J., Chen, C. A., Voolstra, C. R., & LaJeunesse, T. C. (2013). Blind to morphology: genetics identifies several widespread ecologically common species and few endemics among Indo-Pacific cauliflower corals (Pocillopora, Scleractinia). Journal of Biogeography, 40(8), 1595–1608. http://doi.org/10.1111/jbi.12110

Pochon, X., Montoya-Burgos, J. I., Stadelmann, B., & Pawlowski, J. (2006). Molecular phylogeny, evolutionary rates, and divergence timing of the symbiotic dinoflagellate genus Symbiodinium. Molecular Phylogenetics and Evolution, 38(1), 20–30. http://doi.org/10.1016/j.ympev.2005.04.028

Putnam, H. M., Stat, M., Pochon, X., & Gates, R. D. (2012). Endosymbiotic flexibility associates with environmental sensitivity in scleractinian corals. Proceedings of the Royal Society B-Biological Sciences, 279(1746), 4352–4361. http://doi.org/10.1371/journal.pone.0020434

Quigley, K. M., Quigley, K. M., Davies, S. W., Davies, S. W., Kenkel, C. D., Kenkel, C. D., et al. (2014). Deep-Sequencing Method for Quantifying Background Abundances of Symbiodinium Types: Exploring the Rare Symbiodinium Biosphere in Reef-Building Corals. PloS One, 9(4), e94297. http://doi.org/10.1371/journal.pone.0094297.s005

Raina, J.-B., Dinsdale, E. A., Willis, B. L., & Bourne, D. G. (2010). Do the organic sulfur compounds DMSP and DMS drive coral microbial associations? Trends in Microbiology, 18(3), 101–108. http://doi.org/10.1016/j.tim.2009.12.002

Ramette, A. (2007). Multivariate analyses in microbial ecology. FEMS Microbiology Ecology.

Rognes, T., Flouri, T., Nichols, B., Quince, C., & Mahé, F. (2016). VSEARCH: a versatile open source tool for metagenomics. PeerJ.

Rohwer, F., Seguritan, V., & Azam, F. (2002). Diversity and distribution of coral-associated bacteria. Marine Ecology Progress ….

Rosenberg, E., Koren, O., Reshef, L., Efrony, R., & Zilber-Rosenberg, I. (2007). The role of microorganisms in coral health, disease and evolution. Nature Reviews Microbiology, 5(5), 355–362. http://doi.org/10.1038/nrmicro1635

Roth, M. S., Goericke, R., & Deheyn, D. D. (2012). Cold induces acute stress but heat is ultimately more deleterious for the reef-building coral Acropora yongei. Scientific Reports.

Rouzé, H., Lecellier, G. J., Saulnier, D., Planes, S., Gueguen, Y., Wirshing, H. H., & Berteaux-Lecellier, V. (2017). Data S2: Symbiodinium28S DNA sequences mentioned in the paper. PeerJ, 5(9), e2856. http://doi.org/10.7717/peerj.2856/supp-9

Rouzé, H., Lecellier, G., Saulnier, D., & Berteaux-Lecellier, V. (2016). Symbiodinium clades A and D differentially predispose Acropora cythereato disease and Vibrio spp. colonization. Ecology and Evolution, 6(2), 560–572. http://doi.org/10.1002/ece3.1895

Rowan, R. (2004a). Coral bleaching: thermal adaptation in reef coral symbionts. Nature …, 430(7001), 742–742. http://doi.org/10.1038/430742a

Rowan, R. (2004b). Thermal adaptation in reef coral symbionts.

Sato, Y., Willis, B. L., & Bourne, D. G. (2009). Successional changes in bacterial communities during the development of black band disease on the reef coral, Montipora hispida. The ISME Journal, 4(2), 203–214. http://doi.org/10.1038/ismej.2009.103

Schmidt-Roach, S., Miller, K. J., Lundgren, P., & Andreakis, N. (2014). With eyes wide open: a revision of species within and closely related to the Pocillopora damicornisspecies complex (Scleractinia; Pocilloporidae) using morphology and genetics. Zoological Journal of the Linnean Society, 170(1), 1–33. http://doi.org/10.1111/zoj.12092

Séré, M., Wilkinson, D. A., Schleyer, M. H., Chabanet, P., Quod, J.-P., & Tortosa, P. (2015). Characterisation of an atypical manifestation of black band disease on Porites lutea in the Western Indian Ocean. PeerJ, 4, e2073–e2073. http://doi.org/10.7717/peerj.2073

Shade, A., & Handelsman, J. (2011). Beyond the Venn diagram: the hunt for a core microbiome. Environmental Microbiology, 14(1), 4–12. http://doi.org/10.1111/j.1462-2920.2011.02585.x

Silverstein, R. N., Cunning, R., & Baker, A. C. (2017). Tenacious D: Symbiodiniumin clade D remain in reef corals at both high and low temperature extremes despite impairment. The Journal of Experimental Biology, 220(7), 1192–1196. http://doi.org/10.1242/jeb.148239

Stat, M., & Gates, R. D. (2011). Clade D Symbiodiniumin Scleractinian Corals: A ?Nugget? of Hope, a Selfish Opportunist, an Ominous Sign, or All of the Above? Journal of Marine Biology, 2011(3774), 1–9. http://doi.org/10.1007/s00227-009-1263-5

Sweet, M., & Bythell, J. (2012). Ciliate and bacterial communities associated with White Syndrome and Brown Band Disease in reef-building corals. Environmental Microbiology.

Team, R. C. (2012). R: A Language and Environment for Statistical Computing. 2012, Vienna: R Foundation for Statistical Computing. Google Scholar.

Theis, K. R., Dheilly, N. M., Klassen, J. L., Brucker, R. M., Baines, J. F., Bosch, T. C. G., et al. (2016). Getting the Hologenome Concept Right: an Eco-Evolutionary Framework for Hosts and Their Microbiomes. mSystems, 1(2), e00028–16. http://doi.org/10.1128/mSystems.00028-16

Thomas, L., Kendrick, G. A., Kennington, W. J., Richards, Z. T., & Stat, M. (2014). Exploring Symbiodiniumdiversity and host specificity in Acroporacorals from geographical extremes of Western Australia with 454 amplicon pyrosequencing. Molecular Ecology, 23(12), 3113–3126. http://doi.org/10.1111/mec.12801

Thornhill, D. J., Lajeunesse, T. C., Kemp, D. W., & Fitt, W. K. (2006). Multi-year, seasonal genotypic surveys of coral-algal symbioses reveal prevalent stability or post-bleaching reversion. Marine Biology.

Tsang, R., & Ang, P., Jr. (2015). Cold temperature stress and predation effects on corals: their possible roles in structuring a nonreefal coral community. Coral Reefs.

van Woesik, R., Sakai, K., Ganase, A., & Loya, Y. (2011). Revisiting the winners and the losers a decade after coral bleaching. Marine Ecology Progress Series, 434, 67–76. http://doi.org/10.3354/meps09203

Weber, L., DeForce, E., & Apprill, A. (2017). Optimization of DNA extraction for advancing coral microbiota investigations. Microbiome, 5(1), 18. http://doi.org/10.1186/s40168-017-0229-y

Weis, V., Reynolds, W., deBoer, M., & Krupp, D. (2001). Host-symbiont specificity during onset of symbiosis between the dinoflagellates Symbiodinium spp. and planula larvae of the scleractinian coral Fungia scutaria. Coral Reefs, 20(3), 301–308. http://doi.org/10.1007/s003380100179

Whitaker, K. (2006). Genetic evidence for mixed modes of reproduction in the coral Pocillopora damicornis and its effect on population structure. Marine Ecology Progress Series.

Winkler, N. S., Pandolfi, J. M., & Sampayo, E. M. (2015). Symbiodinium identity alters the temperature-dependent settlement behaviour of Acropora millepora coral larvae before the onset of symbiosis. Proceedings. Biological Sciences, 282(1801), 20142260. http://doi.org/10.1098/rspb.2014.2260

Winnepenninckx, B., Backeljau, T., & De Wachter, R. (1993). Extraction of high molecular weight DNA from molluscs. Trends in Genetics : TIG, 9(12), 407.

Yeoh, S.-R., & Dai, C.-F. (2009). The production of sexual and asexual larvae within single broods of the scleractinian coral, Pocillopora damicornis. Marine Biology, 157(2), 351–359. http://doi.org/10.1007/s00227-009-1322-y

Ziegler, M., Seneca, F. O., Yum, L. K., Palumbi, S. R., & Voolstra, C. R. (2017). Bacterial community dynamics are linked to patterns of coral heat tolerance. Nature Communications, 8, 14213. http://doi.org/10.1038/ncomms14213

Zilber-Rosenberg, I., & Rosenberg, E. (2008). Role of microorganisms in the evolution of animals and plants: the hologenome theory of evolution. FEMS Microbiology Reviews, 32(5), 723–735. http://doi.org/10.1111/j.1574-6976.2008.00123.x

4. DISCUSSION GÉNÉRALE :

4.1. ÉVOLUTION ET IMPLICATION DU MICROBIOTE BACTÉRIEN DANS LA THERMO-TOLÉRANCE CHEZ *POCILLOPORA DAMICORNIS* :

L'implication du microbiote dans la fitness de l'hôte, au travers de différents apports (apport de matière organique, azote et antibiotiques), n'est plus à démontrer, notamment pour le genre *Endozoicomonas* qui est prédominant dans les coraux scléractiniaires sains. Il produirait des antibiotiques et participerait au recyclage des matières azotées (Bourne et al. 2016; Bayer et al. 2013; Neave, Rachmawati, et al. 2016; Neave et al. 2014; Rohwer et al. 2002). Celui-ci est d'ailleurs retrouvé en très grande proportion dans nos deux études (33,3 à 87,4%).

En revanche, le rôle direct du microbiote dans la thermo-tolérance des coraux fait encore débat. On sait que l'impact d'un stress thermique sur le microbiote des coraux thermo-sensibles induit un profond changement dans la composition du microbiote (augmentation de l'abondance de plusieurs taxons, souvent pathogènes et diminution du genre *Endozoicomonas*), menant souvent à l'émergence de maladies (Bourne *et al.*, 2007; Mao-Jones *et al.,* 2010). D'après une revue de 2017 résumant 45 articles s'intéressant à l'impact des changements globaux sur l'holobionte corallien, il a été montré que la richesse bactérienne augmentait dans 60% des cas lors d'un stress environnemental. Ceci suggère une invasion de pathogènes non-résidents, initialement, facilitée par la fragilisation du microbiome commensal (dont la diversité beta augmente, ce qui signifie un changement dans la composition et les proportions des taxons bactériens, signe d'une déstabilisation), plutôt qu'une augmentation de bactéries à pouvoir pathogène déjà présentes dans le microbiome (McDevitt-Irwin et al. 2017; Welsh et al. 2015). Malheureusement, malgré les nombreuses données récoltées ces cinq dernières années, il est encore très difficile de déterminer si ce bouleversement du microbiote est la cause ou la conséquence d'un affaiblissement et d'une diminution des capacités immunitaires de l'hôte, accompagnée de la colonisation des tissus du corail par des pathogènes.

Par ailleurs, il a été montré que le microbiote bactérien était très différent en composition et abondance de la composition bactérienne de la colonne d'eau (Bourne & Munn 2005; Koren & Rosenberg 2006; Glasl et al. 2016) et pouvait être spécifique à une

espèce, une population voir même une colonie (C.-P. Chen et al. 2011; Morrow et al. 2012; Rohwer et al. 2002). On pourrait en déduire une sélection active de la part du corail ainsi qu'une différence de niche écologique, avec certaines espèces bactériennes se multipliant plus facilement dans le mucus que dans l'eau et *vice versa*.

Nous n'avons pas pu recueillir le microbiote de la colonne d'eau pour nous en assurer, mais étant donné la généralité de ce phénomène, on peut supposer que le microbiote de nos échantillons ne soit pas identique à celui de la colonne d'eau, ne serait-ce que par rapport aux nombreuses différences de conditions qu'offrent ces deux niches écologiques (Wild et al. 2005). En revanche, dans nos deux études, on constate bien une spécificité du microbiote pour son espèce, son clade et même sa colonie. Cette spécificité, retrouvée notamment dans l'étude de Chen *et al.* (2011), nous donne des indications quant aux questions soulevées précédemment (C.-P. Chen et al. 2011). En effet, cette spécificité suggère une coévolution entre l'hôte et son microbiote. La perturbation du microbiote lors d'un stress thermique, pourrait donc être, au moins en partie, due à l'hôte corallien qui n'est alors plus apte à réguler son microbiote.

Dans notre étude présentée au chapitre 2, nous avons également relevé une influence du régime thermique (et plus précisément, la température moyenne dans la région échantillonnée) sur la composition du microbiote. Dans l'étude de la thermo-tolérance de l'holobionte corallien (chapitre 1), on ne constate aucun changement du microbiote lors de la montée en température pour les deux populations coralliennes portant chacune des microbiotes différents et étant issues de régimes thermiques différents. La température, à cette échelle de temps, n'a donc pas eu d'influence sur la composition du microbiote. L'influence de la température n'est pas toujours constatée dans la littérature (C.-P. Chen et al. 2011; Ziegler et al. 2017), et pourrait dépendre de l'amplitude des différences de température que l'on compare. Dans notre analyse (chapitre 2), nous avons testé la corrélation entre le régime thermique et le microbiote, mais peut-être qu'à beaucoup plus petite échelle, si nous avions pu tester le lien avec les températures au moment de l'échantillonnage, nous aurions eu des résultats plus marquants. Enfin, se pose la question de la réponse fonctionnelle du microbiote. En effet, nous nous sommes uniquement intéressés au cours de ce travail de thèse aux variations des communautés bactériennes au niveau taxonomique. Or, dans le contexte de l'hologénome, ce n'est pas uniquement l'espèce des bactéries qui a un intérêt pour la physiologie de l'holobionte, mais aussi et surtout les différents gènes qu'elles expriment

au cours du temps et des stress et dont l'activité va induire différents phénotypes (Zilber-Rosenberg & Rosenberg 2008). Plusieurs études se sont intéressées aux gènes contenus dans le microbiote bactériens via une approche métagénomique, révélant des activités métaboliques différentes entre corail sain et corail stressé (Littman et al. 2011; Thurber et al. 2009). Un shift des communautés, aussi bien bactéries que virus, archeae, champignons et micro-algue, est alors observé accompagné d'une augmentation des gènes de virulences (Littman et al. 2011; Thurber et al. 2009) et d'un passage au mode hétérotrophe plutôt qu'autotrophe en association avec les métabolismes des acides gras, protéines, carbohydrates, phosphore et sulfure (Littman et al. 2011; Thurber et al. 2009). En revanche, dans ces deux dernières études, l'activité du microbiote est analysée au cours du collapse physiologique que subit l'holobionte corallien au cours d'un stress, c'est-à-dire au moment du blanchissement et donc de la rupture de l'homéostasie. Il serait intéressant d'étudier de la même façon la composition du microbiote et son activité avant l'apparition des collapses afin de décoder tout le processus qui mène à la rupture de la symbiose et à l'apparition des pathogènes.

D'autres expériences pourraient répondre aux nombreuses questions que l'on se pose encore sur l'interaction entre le corail et son microbiote dans un environnement changeant :

- Obtenir, à plus grande échelle, le microbiote des coraux pour avoir une plus grande puissance statistique et établir davantage de corrélations entre facteurs biotiques et abiotiques et communauté bactérienne liée aux coraux. Dans cette optique, les résultats de la mission TARA Pacific sont très attendus, en plus de ceux déjà publiés sur le microbiome des océans (Sunagawa et al. 2015).
- Manipuler le microbiote : via l'utilisation de larves axéniques qu'on associerait avec différents sets de bactéries (Peixoto et al. 2017), ou bien en utilisant des antibiotiques ou des bactériophages qui sont capables d'éliminer les bactéries résidentes comme cela a déjà été fait sur les pathogènes *Vibrio coralliilyticus* et *Thallassomonas loyana,* respectivement responsables de blanchissements chez *Pocillopora damicornis* et de la maladie des plaques blanches chez *Favia favus* (Efrony et al. 2007; Efrony et al. 2009; Teplitski & Ritchie 2009). Ces techniques nous permettraient d'étudier la réponse de l'hôte face au stress thermique avec ou sans son microbiote ou avec un microbiote différent. Déterminer la gamme de températures

auxquelles il peut survivre pourrait nous renseigner sur le rôle direct potentiel du microbiote dans la thermo-tolérance de l'holobionte.

- Utiliser la métatranscriptomique en parallèle de la métagénomique afin de caractériser la réponse fonctionnelle du microbiote lors d'expériences de stress thermique et définir les mécanismes qui permettent le maintien des interactions entre l'hôte et son microbiote (Bashiardes et al. 2016; Littman et al. 2011; Thurber et al. 2009).

La thermo-tolérance de la communauté bactérienne et la stabilité de sa composition durant un stress thermique peuvent également être influencée par le clade de *Symbiodinium* hébergé par l'hôte. En effet, dans son étude utilisant des larves d'*Acropora tenuis* infectée par les clades C1 ou D, Littman montre que le microbiote des coraux en symbiose avec le clade C est différent et plus stable durant un stress thermique que celui de ceux hébergeant le clade D (Littman et al. 2010). En revanche, il est important de signaler que le clade C est habituellement plus avantageux pour cette espèce corallienne, car il lui procure une croissance plus rapide ainsi qu'une meilleure thermo-tolérance. La stabilité du microbiote en fonction du clade de zooxanthelle pourrait donc être la conséquence de la physiologie de l'hôte qui aurait plus d'énergie à allouer à la gestion de son microbiote lorsqu'il est associé au clade C1 plutôt qu'au clade D. Dans notre étude (chapitre 2), nous n'avons pas trouvé de corrélation entre le clade de *Symbiodinium* et le microbiote, mais le clade de la zooxanthelle ayant son influence sur l'hôte, il est important de prendre en compte chacun des compartiments de l'holobionte lorsque l'on s'intéresse à sa thermo-tolérance et sa fitness en général.

4.2. ÉVOLUTION ET IMPLICATION DE L'ASSEMBLAGE DES ZOOXANTHELLES DANS LA THERMO-TOLÉRANCE CHEZ *POCILLOPORA DAMICORNIS* :

Parmi les populations de *Pocillopora damicornis* que nous avons étudiées dans nos deux chapitres, nous avons identifié les clades majoritaires suivants:

- Le clade C1 de *Symbiodinium* chez les coraux de Nouvelle-Calédonie (chapitre 1 et 2) et de Taïwan qui appartiennent à l'haplotype du clade 1 de *Pocillopora damicornis*.
- Le clade D1 en Polynésie Française où les coraux appartiennent tous à l'haplotype du clade 1.
- Le clade D1 à Oman et Djibouti où les coraux appartiennent au clade 2 de *Pocillopora damicornis*.

La présence des clades C1 et D1 chez *Pocillopora damicornis* est cohérente avec la littérature, puisque cette espèce peut héberger les clades A, B, C, D et G (Putnam et al. 2012; Blackall et al. 2015; Cunning et al. 2013; Pinzon & LaJeunesse 2011). Dans le chapitre 1, étant donné le nombre de populations étudiées et le plan expérimental, nous ne pouvions pas établir une quelconque corrélation entre les paramètres biotiques ou abiotiques et l'assemblage de *Symbiodinium*. Néanmoins, le clade D étant reconnu comme augmentant la thermo-tolérance chez *Pocillopora damicornis* (Cunning et al. 2013), ces deux combinaisons (population thermo-tolérante en symbiose avec le clade D et population thermo-sensible en symbiose avec le clade C) sont également cohérentes avec la littérature (Pinzon & LaJeunesse 2011). Au vu des résultats du chapitre 2, on se rend bien compte que les combinaisons ne sont pas si binaires, puisque les deux régions dont le régime thermique est le plus variable (Djibouti et Taïwan) ont des assemblages très différents, avec le clade D1 majoritaire pour l'une et le clade C1 majoritaire pour l'autre. De même, on retrouve le clade D1 majoritaire dans une population expérimentant de faibles variations mais avec une température moyenne élevée (Polynésie Française). Les variations annuelles de températures ne semblent donc pas avoir d'influence sur la répartition des zooxanthelles. D'après les analyses statistiques de corrélation du chapitre 2, ce serait en fait, la température basse qui influencerait le plus la répartition des clades de *Symbiodinium*. La corrélation négative entre les températures basses et le clade D thermo-résistant pourrait paraitre étonnante. En effet, le clade D confère une thermo-tolérance accrue même en petites proportions chez de nombreuses espèces scléractiniaires, et il présente un fort opportunisme à la suite d'épisodes de blanchissement (Baker et al. 2004; Rowan 2004; Fabricius et al. 2004; Stat & Gates 2011; Rouzé et al. 2016). C'est donc un clade avec un grand spectre d'hôte et une tolérance importante pour les changements environnementaux, ce qui lui vaut la place de favori pour la résistance au stress thermique (Stat et al. 2013; Pettay & LaJeunesse 2009). Cependant d'autres études ont montré une sensibilité des coraux abritant le clade D face à des températures basses [défaut de croissance (Roth et al. 2012; Tsang & Ang 2015), photosensibilité (Silverstein et al. 2017), défaut de croissance des larves (Weis et al. 2001)]. Le clade D ne présenterait donc pas plus de plasticité en terme de régime thermique mais serait seulement mieux adapté aux hautes températures.

Ce résultat soutient le fait que chaque clade permet à son hôte d'acquérir des compétences diverses en fonction de son environnement : le clade C1 aura tendance à améliorer la croissance dans un environnement thermiquement stable (Little et al. 2004; Jones & Berkelmans 2010), le D aura tendance à améliorer la thermo-tolérance pour les températures anormalement hautes (Stat & Gates 2011), le clade A produit un pigment particulier permettant une photo-protection chez les coraux fortement exposés (Reynolds et al. 2008; Krämer et al. 2012). D'ailleurs à la suite d'un blanchissement et de la résilience du corail, comme déjà mentionné plus haut, la proportion des différents clades aura tendance à changer via le mécanisme de blanchissement adaptatif (Buddemeier & Fautin 1993). Ainsi après un blanchissement, le corail aura la possibilité d'être colonisé par un clade différent (« symbiont switching »), ou bien par un clade déjà présent mais à l'état cryptique (« symbiont shuffling ») qui lui confèrera des capacités physiologiques différentes et il sera mieux adapté au nouvel environnement (Bosch & McFall-Ngai 2011; Baker 2003; Rouzé et al. 2017). C'est d'autant plus vrai pour *Pocillopora*, qui est un hôte généraliste abritant une grande diversité de *Symbiodinium* (Putnam et al. 2012; Blackall et al. 2015). Dans nos différentes populations, on a d'ailleurs une grande diversité de clades cryptiques (détectés à moins de 1% d'abondance) notamment pour la population de Djibouti qui pourrait ainsi avoir une meilleure plasticité dans l'assemblage des *Symbiodinium*. Il est également important de signaler que pour l'étude de la thermo-tolérance, au chapitre 1, les coraux des populations d'Oman et de Nouvelle-Calédonie, ont été maintenus pendant plusieurs mois dans un même aquarium et que malgré cette cohabitation et les stress subis par ces coraux (transport par avion de plus de 48h), aucune variation de l'assemblage des *Symbiodinium* pour aucune des populations n'a été observée. Dans notre expérience de stress thermique, nous n'avons pas atteint le blanchissement, ce qui pourrait expliquer pourquoi il n'y a pas eu de changement de clades au sein des populations. Il serait intéressant d'utiliser ces deux mêmes populations pour expérimenter des stress thermiques récurrents lors desquels nous irions jusqu'au collapse physiologique (blanchissement). À la suite de cela, nous laisserions le temps aux coraux de résilier avant de les resoumettre à un stress thermique. Cette expérience nous permettrait d'étudier la mise en place de la thermotolérance au sein des deux populations et, via des analyses de type métabarcoding ITS2, d'observer une éventuelle modification des assemblages en relation avec une augmentation de la thermotolérance.

Comme pour le microbiote, on retrouve également une corrélation significative entre les *Symbiodinium* et le clade hôte pouvant révéler une spécificité d'association (chapitre 2). *P. damicornis* étant un corail hermaphrodite « brooder », les planulas émises suite à la reproduction sexuée contiennent déjà leur algue symbiotique. On a donc ici une transmission verticale du symbionte qui pourrait favoriser la spécificité et la co-évolution entre les deux partenaires (Douglas 1998), ce qui est cohérent avec le résultat obtenu. Les populations thermo-tolérantes d'Oman et Djibouti ont un profil similaire en termes d'haplotype hôte et d'assemblage de *Symbiodinium*. Il est difficile d'en conclure un profil d'holobionte thermo-tolérant car ces deux populations ne sont pas géographiquement éloignées. On peut supposer que des échanges larvaires de *Pocillopora damicornis* et de ses symbiontes ont pu avoir lieu étant donnée la grande capacité de dispersion larvaire de cette espèce (R. H. Richmond 1987). Une étude publiée par Cunning *et al.* en 2013 s'est intéressée, pour l'espèce *Pocillopora damicornis,* au sein de différents récifs de l'est du pacifique tropical, à la répartition des clades de *Symbiodinium* en fonction de l'haplotype et de la thermo-tolérance du corail hôte (susceptibilité au blanchissement) (Cunning et al. 2013). D'après leurs résultats, le type 1 [correspondant au clade 3 (Schmidt-roach et al. 2014)] de *Pocillopora damicornis* était majoritaire dans le site qui avait subi le plus grand nombre de stress thermiques (événements de blanchissement), suggérant que le type 1 serait plus thermo-tolérant et aurait été sélectionné au cours des perturbations thermiques successives. Les autres sites présentaient 77% et 37% de type 1, le reste correspondant au type 3 (correspondant au clade 2 (Schmidt-roach et al. 2014)). En ce qui concerne les *Symbiodinium*, ils ont retrouvé les clade C et D associés aux deux haplotypes, tour à tour majoritaires ou uniques, mais sans réelle spécificité contrairement à ce qui avait pu être montré par Pinzon et LaJeunesse en 2011, c'est à dire une association préférentielle du type 1 de *Pocillopora* avec le clade D de *Symbiodinium* (Pinzon & LaJeunesse 2011).

Au travers de notre travail et des données de la littérature, on se rend bien compte de toute la complexité des associations entre corail hôte et *Symbiodinium,* notamment pour *Pocillopora*. Malgré la spécificité du symbionte pour son hôte observée dans nos travaux, l'histoire de vie et notamment les variations anormales de température (voir d'autres stress environnementaux) ont pu altérer cette spécificité en faveur du mécanisme de blanchissement adaptatif. L'interaction entre ces deux mécanismes complique alors l'interprétation des données et met en avant l'importance

d'un suivi sur le long terme des populations afin de collecter un maximum de données sur l'histoire de vie de chacune d'elle. Il est donc difficile de prédire avec quel clade de *Symbiodinium* un corail thermo-tolérant sera plus particulièrement associé, tant de nombreux paramètres biotiques et abiotiques peuvent influencer cette association. En revanche, le génotype de l'hôte peut avoir une grande influence sur la thermo-tolérance de l'holobionte. Le clade 2 retrouvé dans les régions d'Oman et Djibouti, présente d'ailleurs toutes les spécificités requises comme candidat potentiel à la thermo-tolérance : présent dans des environnements très variables et en symbiose avec un clade de *Symbiodinium* résistant aux fortes augmentations de température.

4.3. ÉVOLUTION DE LA THERMO-TOLÉRANCE DE *POCILLOPORA DAMICORNIS* DANS UN ENVIRONNEMENT FLUCTUANT :

Nous avons vu dans les deux sections précédentes que chacun des compartiments microbiens de l'holobionte pouvait avoir un lien avec le génotype de l'hôte, ainsi qu'avec l'environnement thermique. Néanmoins, lors d'un stress écologiquement réaliste, la structure des communautés de bactéries et zooxanthelles reste stable. La stabilité des micro-organismes symbiotiques est un paramètre important pour l'état de santé de l'holobionte, car lorsque cette stabilité est rompue (blanchissement ou dysbiose bactérienne), l'état de santé se dégrade avec une perte de l'homéostasie et des nécroses se développent liées (ou pas) à un processus infectieux.

Dans notre étude de la thermo-tolérance de l'holobionte corallien, nous avons comparé la réponse au stress thermique de deux populations, l'une provenant d'un environnement thermiquement stable (Nouvelle-Calédonie, avec des températures allant de 22 à 29°C), l'autre soumise à un environnement plus variable (Oman, avec des températures allant de 22 à 33°C). La réponse au stress a ensuite été analysée pour chaque population, à la suite d'une montée en température jusqu'aux limites supérieures de la thermo-tolérance de chacune de ces populations. A l'issue de ce stress thermique, l'étude des communautés symbiotiques a montré une stabilité, c'est-a-dire pas de changement de la composition des communautés de bactéries et de *Symbiodinium*. Même si les réponses fonctionnelles du microbiote restent à étudier, cette stabilité suggère que l'hôte devrait jouer un rôle dans la réponse au stress thermique.

Plusieurs autres travaux suggèrent que la thermo-tolérance serait principalement due à l'hôte corallien lui-même plutôt qu'à sa communauté de micro-organismes

symbiotiques et notamment de *Symbiodinium*. Il a été montré chez *Pocillopora* dans le Pacifique tropical, que l'environnement le plus variable abrite spécifiquement un haplotype de *Pocillopora damicornis* (le clade 3), lequel n'est pas associé à un clade de *Symbiodinium* particulier (soit C soit D pour un même environnement thermique) (Cunning et al. 2013). D'autre part, le pré-conditionnement à une température haute, mais non stressante, génère une thermo-tolérance à de plus hautes températures chez le corail *Acropora millepora* (Bellantuono et al. 2011). Or, aucun changement de la communauté symbiotique (aussi bien bactéries que *Symbiodinium*) n'est constaté entre colonies pré-conditionnées et non conditionnées. Ce résultat suggère que l'hôte corallien est au moins un des artisans de la thermo-tolérance.

Chez *Porites astreoides,* la comparaison de l'activité transcriptomique de l'hôte et des zooxanthelles n'a révélé aucune réponse spécifique face au stress thermique chez les *Symbiodinium* des coraux les plus thermotolérants (Kenkel & Matz 2016a). Une forte réponse de l'hôte avec une quasi-absence de réponse du symbionte a également été décrite chez *Acropora aspera* durant une expérience de blanchissement (Leggat et al. 2011). De même, l'expression des gènes de deux clades de *Symbiodinium* (C et D) au sein de l'hôte corallien *Acropora hyacinthus* n'a révélé aucun changement au cours d'un stress thermique (Barshis et al. 2014). Les zooxanthelles sont pourtant capables d'exprimer une réponse au stress comme le montre l'étude du transcriptome de *Symbiodinium* en culture en condition de stress, avec une induction de ROS scavenger et de chaperones, deux familles de protéines impliquées dans la réponse au stress thermique (Levin et al. 2016). Pour expliquer ce résultat, Kenkel et Matz suggèrent que le maintien de l'activité des zooxanthelles en cas de stress thermique serait due à l'activité de l'hôte corallien et non pas à des mécanismes intrinsèques de réponse au stress du symbionte (Kenkel & Matz 2016a).

À travers l'étude de l'expression des gènes de l'hôte corail, nous avons pu montrer une plus forte plasticité transcriptomique (plus de gènes différentiellement exprimés, et pour un gène donné, une régulation plus forte) chez la population thermo-tolérante par rapport à la population thermo-sensible, ainsi que du frontloading (une expression constitutive et plus forte chez la population thermo-tolérante des gènes induits chez la population thermosensible). Ces deux mécanismes, considérés comme mutuellement exclusifs (Barshis et al. 2013; Kenkel & Matz 2016a), ont donc été mis en évidence au sein de la même population au cours de ce travail. D'après Kenkel et Matz

(2016), la plasticité transcriptomique serait mise en place chez les populations expérimentant un environnement stressant, mais dont la fréquence des stress est faible (annuelle) et le frontloading serait mis en place chez les populations expérimentant des stress plus fréquents. Notre hypothèse pour expliquer la présence de ces deux mécanismes au sein de notre population thermo-tolérante est que le frontloading résulterait d'une assimilation permettant de réduire le cout de la régulation des gènes dans un environnement où ils sont régulièrement sollicités (Waddington 1953; Gibert 2017). Il faut noter que les mécanismes impliqués restent à découvrir.

En ce qui concerne la fonction des gènes plastiques ou frontloadés, la population thermo-tolérante montre une induction des réponses au stress classiquement mises en évidence chez les coraux (Voolstra et al. 2009; Desalvo et al. 2008; Seneca & Palumbi 2015; Palumbi et al. 2014; Kenkel & Matz 2016a): une induction de gènes codant des protéines anti-oxydantes (la thioredoxine, par exemple) et une répression de gènes codants des protéines participant à la production de ROS (quinone oxydoreductase) (DeSalvo et al. 2010) ; une induction de la régulation de l'apoptose (TNFR, TRAF), car le stress thermique et les molécules oxydantes induisent des dommages irréversibles sur les membranes et l'ADN, ce qui nécessite une élimination des cellules endommagées (Barshis et al. 2013); une induction des protéines chaperonnes de type HSPs, qui permettent de réduire la dénaturation des protéines par la chaleur (Maor-Landaw & Levy 2016; B. Brown et al. 2002); et finalement une induction des gènes impliqués dans la réparation de l'ADN (DNA ligase, DNA polymerase) suite aux dommages provoqués par les molécules oxydantes (Yakovleva et al. 2009). Quelques fonctions qui participent à la physiologie de base sont en revanche réprimées, comme la synthèse du squelette calcaire (carbonic anhydrase (Le Goff et al. 2016)) ou le développement des muscles [myosin related protein (Leclère & Röttinger 2017)] probablement via un mécanisme de trade-off (Roff & Fairbairn 2007). Toutes ces fonctions caractéristiques de la réponse au stress chez les coraux suggèrent fortement que la plasticité transcriptomique et le frontloading mis en évidence dans cette étude sont adaptatifs et non pas le fruit du hasard (Ghalambor et al. 2007). En effet, on pourrait observer une plus grande plasticité transcriptomique sans que celle-ci ne permette une réponse plus efficace. Dans ce cas, elle serait la résultante d'une stratégie de « bet hedging » (Olofsson et al. 2009) mais pas d'une adaptation à l'environnement

thermique. Compte tenu du nombre important de gènes modulés ne rentrant pas dans les catégories sus-cités, cette seconde hypothèse de « bet hedging » ne peut être écartée.

Parmi les gènes différentiellement exprimés chez la population thermo-tolérante un certain nombre participent aux mécanismes de régulation épigénétiques. En effet, on a pu montrer une régulation accrue de gènes impliqués dans les modifications d'histones et dans la méthylation de l'ADN, deux mécanismes connus comme pouvant moduler l'expression des gènes dans de nombreux modèles (Jaenisch & Bird 2003). Ces mécanismes de régulation des gènes pourraient être en partie responsables de la plasticité transcriptomique que l'on observe chez la population thermo-tolérante (Duncan et al. 2014). Nous avons également identifié chez la population thermo-tolérante un grand nombre de transcrits codant des transcriptase inverses de rétro-transposons (Finnegan 2012). L'activité des rétro-transposons a longtemps été considérée comme néfaste car ces fragments d'ADN ont la capacité de se copier et de s'intégrer n'importe où dans le génome, pouvant induire l'inactivation ou la modification de l'expression de gènes. Chez les plantes, c'est toutefois un mécanisme qui peut également apporter une résistance au stress en générant de la variabilité phénotypique (Galindo-González et al. 2017). D'après ces différents constats, on peut émettre plusieurs hypothèses :

- L'activité accrue des rétro-transposons en réponse au stress thermique permettrait de générer de la variabilité phénotypique et de multiplier les chances d'adaptation à un futur environnement stressant par une sorte de « Bet-Hedging transcriptionnel » (Olofsson et al. 2009).
- Les rétro-transposons auraient pu induire la surexpression de certains gènes, se retrouvant ainsi eux-mêmes constitutivement exprimés, ce qui pourrait être à l'origine du frontloading (Galindo-González et al. 2017).
- La structure chromatinienne étant plus permissive pour permettre la régulation plastique des gènes, l'activité des rétro-transposons s'en trouve par conséquent accélérée ou, du moins, moins réprimée sans réel intérêt pour l'organisme (Goodier 2016).

Il serait intéressant de tester ces différentes hypothèses, mutuellement non exclusives, par des expériences de stress thermiques récurrents qui permettraient de suivre la mise en place de la thermo-tolérance et d'en comprendre les mécanismes:

- Est-ce que des modifications épigénétiques pourraient permettre la mise en place rapide de la thermo-tolérance (étude de la méthylation, des modifications d'histones et des rétro-transposons) ?
- Est-ce qu'une sélection s'opère au fur et à mesure des stress entre différents génotypes potentiels au sein des mêmes colonies, c'est-à-dire via par exemple des modifications des gènes par les rétro-transposons et/ou des mutations spontanées?
- Est-ce qu'on observe un changement des communautés symbiotiques au cours des stress récurrents (metabarcoding 16S et ITS2) ?
- Est-ce que les larves issues des parents exposés au stress sont plus thermo-tolérantes (mécanisme de plasticité trans-générationnelle) (Putnam & Gates 2015; Torda et al. 2017).

D'un point de vue génétique, il apparait que la population thermo-tolérante d'Oman appartient au clade 2, qui correspond à une population plus thermo-tolérante avec une réponse plus efficace face au stress thermique que le clade 1 de Nouvelle-Calédonie. D'après nos résultats sur la biogéographie des différents haplotypes de *Pocillopora damicornis* au travers des différents régimes thermiques, le clade 2 est retrouvé dans deux populations à fortes variations de températures (Oman et Djibouti) et dont la température moyenne haute est la plus forte (31,27°C avec un maximum de 33,15°C pour Oman et 30,46°C avec un maximum de 32,20°C pour Djibouti); le clade 1 est lui retrouvé en Nouvelle-Calédonie, en Polynésie-Française et à Taïwan, trois régions aux régimes thermiques contrastés (faible et forte variations), mais dont la température ne dépasse pas 30,8°C (maximum de Taïwan). Ceci pourrait suggérer que la répartition des clades de Pocillopora pourrait dépendre des régimes thermiques mais ce serait la température haute plutôt que les variations de température qui influencerait cette répartition (Taïwan présente une variance supérieure à celle de Djibouti). En revanche, dans l'étude qui a permis la classification des différentes espèces de Pocillopora en quatre clades, on retrouve chacun de ces deux clades (1 et 2) dans toutes les régions que nous avons étudiés. Il n'y aurait donc pas de clade plus thermo-tolérant que les autres. Les différentes espèces de *Pocillopora* ont un niveau de différenciation spécifique sur la base du marqueur moléculaire mitochondrial et ont donc pris des trajectoires d'évolution indépendantes. Cependant, la faible divergence nucléaire indique que des

hybridations introgressives doivent avoir eu lieu, même entre espèces éloignées. Cela suggère, comme décrit dans l'article de Torda *et al.* (2017), qu'une propagation d'allèles adaptative (ici liées à la thermo-tolérance) ait pu avoir lieu, permettant d'augmenter les capacités d'adaptation de certaines espèces à la hausse des températures et d'en réduire le risque d'extinction (Schmidt-roach et al. 2014; Torda et al. 2017). Cette observation nous permet d'émettre l'hypothèse qu'un ou plusieurs gènes, communs à toutes les populations thermo-tolérantes de *Pocillopora damicornis*, pourraient être à l'origine de leur capacité à résister au stress thermique.

Pour identifier ces gènes qui pourraient être associés à la thermo-tolérance chez *Pocillopora damicornis*, une recherche de signaux d'adaptation locale a été entreprise. Un échantillonnage de masse a été réalisé sur différentes populations de *Pocillopora damicornis* expérimentant un environnement thermique contrasté et, sur plusieurs sites par population, trente fragments distants d'une dizaine de mètres ont été prélevés et blanchis afin d'en extraire l'ADN. Le but de cet échantillonnage est ensuite de mettre en évidence, via une analyse RAD-séquençage (qui permet de séquencer un grand nombre de fragments d'ADN générés par une enzyme de restriction pour mettre en évidence des SNPs), un ou plusieurs loci sous sélection, communs à l'ensemble des échantillons des populations soumises aux mêmes régimes thermiques. L'analyse des résultats de cette expérience n'a pas encore commencée mais une étude similaire a été effectuée chez l'espèce *Corallium rubrum*, un octocoralliaire de Méditerranée. Des populations de surface (régime thermique variable) et de profondeur (régime thermique plus stable) sur différents sites ont été échantillonnées afin d'étudier de potentiels loci sélectionnés ((Pratlong et al. n.d.) soumis). Des marqueurs potentiels d'adaptation locale à la profondeur ont été identifiés pour certains sites, mais aucun n'est commun à toutes les populations profondes (article soumis, Pratlong M et al 2017). Cela s'explique par le faible potentiel de dispersion de *Corallium rubrum*, ce qui n'est pas le cas pour *Pocillopora damicornis*.

Finalement, même si l'hôte semble avoir une incidence majeure sur la réponse de l'holobionte face à un stress thermique et sur la sélection des micro-organismes symbiotiques, beaucoup d'expériences sont encore nécessaires pour élucider le poids de chacun des compartiments de l'holobionte dans la thermo-tolérance et sur sa mise en

place dans le modèle corallien *Pocillopora damicornis*. Le poids relatif des mécanismes génétiques et épigénétiques sous-jacents devra également être étudié.

5. CONCLUSION GÉNÉRALE :

Comme nous l'avons vu tout au long de ce travail de thèse, les coraux scléractiniaires, et en particulier *Pocillopora damicornis*, sont des modèles biologiques aussi complexes qu'intéressants. Que ce soit en matière d'échantillonnage, de maintenance ou d'analyse moléculaire, beaucoup de mises au point ont du être réalisées afin de mener à bien notre projet. La première année de thèse a permis de lever un certain nombre de verrous méthodologiques et nous avons été confronté à des problèmes inattendus pour récupérer le populations à étudier. En effet, nous avons perdu à trois reprises les coraux échantillonnés sur le terrain, soit lors du transit vers le laboratoire, soit à cause de problèmes liés à la maintenance en aquarium.

La complexité de *P. damicornis* va bien au delà de son utilisation en tant que modèle biologique. Il est complexe de par la multitude de paramètres biotiques (*Symbiodinium*, bactéries, virus, champignons et autre invertébrés commensaux) et abiotiques (luminosité, température, pH, sédiments, polluants) avec lesquels il interagit pour créer, avec les nombreuses autres espèces de coraux scléractiniaires, ces immenses structures bioconstruites abritant une multitude d'espèces de poissons, de mollusques et d'invertébrés marins. Et c'est aussi ce qui en fait un modèle intéressant, car malgré sa simplicité en tant qu'organisme diploblastique, ses multiples symbioses lui ont permis d'acquérir de nombreuses fonctionnalités : l'algue unicellulaire du genre *Symbiodinium* lui apporte l'énergie nécessaire à la construction de son squelette calcaire et sa communauté bactérienne lui apporte protection contre les pathogènes, nutrition et dégradation des déchets.

C'est pour toutes ces particularités, que l'étude de ce modèle est primordiale, notamment dans le contexte du réchauffement climatique, qui a cruellement impacté les récifs coralliens.

Lors de ce travail de thèse, je me suis intéressée tout particulièrement au modèle *Pocillopora damicornis* et aux paramètres biotiques et abiotiques qui pouvaient favoriser sa thermo-tolérance. J'ai pu montrer que:

- La thermo-tolérance dépendait au moins en partie de l'hôte corallien. Ce dernier montre une grande plasticité dans la régulation de ses gènes ainsi qu'une expression constitutive de certains autres probablement régulés grâce à des mécanismes épigénétiques et une forme d'assimilation dont les mécanismes devront être étudiés.
- Ces mécanismes de régulation des gènes mis en place au cours de l'évolution dans un environnement fluctuant sont adaptatifs puisqu'ils permettaient une meilleure gestion de la réponse au stress.
- La composition des communautés symbiotiques, *Symbiodinium* et bactéries était influencée par l'haplotype de l'hôte.
- La répartition géographique des différents clades de *Symbiodinium* était influencée par la température minimale des régions étudiées.

Ces différents résultats nous permettent d'en savoir un peu plus sur les mécanismes qui permettent une meilleure thermo-tolérance chez *Pocillopora damicornis*, mais des études complémentaires devraient nous apporter des éléments d'information cruciaux:

⇨ L'utilisation de stress thermiques récurrents sur clones afin d'étudier la réponse de l'hôte corallien (génétique, transcriptomique et épigénétique) et de ses symbiontes (génétique et transcriptomique) au cours des stress et de la mise en place de la thermo-tolérance. En effet, d'après plusieurs études sur l'évolution des récifs coralliens face au stress thermiques récurrents, induits par le réchauffement climatique, on observe la mise en place d'une acclimatation/adaptation des différents récifs qui se constate par la diminution de l'amplitude des blanchissements (Coles & Riegl 2013; Guest et al. 2012; Maynard et al. 2008; Berkelmans 2009; Penin et al. 2013). Cette expérience de stress thermique répétés a été réalisée au sein de notre laboratoire sur une colonie unique de *Pocillopora damicornis*. Elle a consisté à amener les coraux jusqu'au blanchissement grâce à un stress thermique, à les laisser résilier pendant une année, puis à les soumettre à nouveau à un stress thermique jusqu'au blanchissement, et ce, sur deux années consécutives. Plusieurs échantillonnages ont été réalisés durant l'expérience permettant d'accéder à l'hologénome par séquençage du génome corallien et métabarcoding ITS2, mais aussi à son activité transcriptomique par RNA-seq ainsi qu'aux marques épigénétiques par séquençage bisulfite (méthylation de l'ADN). Cette expérience

a permis de démontrer l'augmentation de la thermotolérance après des stress récurrents sur une même colonie, mais les mécanismes sous-jacents sont encore à l'étude (Vidal-Dupiol et al, en préparation).

- ⇨ L'étude des marqueurs de l'adaptation locale qui a consisté à séquencer des fragments d'ADN pour mettre en évidence des SNPs chez deux populations de *Pocillopora damicornis* expérimentant des environnements thermiques contrastés. Cette technique devrait permettre de mettre en évidence des *loci* sous sélection dans les environnements favorisant la thermo-tolérance.

Dans un contexte plus large de l'étude de l'évolution des cnidaires, j'ai également participé au cours de ma thèse, à plusieurs projets d'études sur le modèle *Corallium rubrum* dans le cadre du projet ANR ADACNI (Processus adaptatifs chez les cnidaires: étude intégrative de la réponse au stress thermique et au changement climatique, des gènes aux populations) et dont une partie des travaux sont développés dans la thèse de Marine Pratlong (Pratlong 2016). Le corail rouge est un cnidaire de la classe des anthozoaires et de la sous-classe des octocoralliaires (contrairement à *Pocillopora* qui est un hexacoralliaire). Séparé évolutivement de près de 250 millions d'années, ces deux sous-classes sont éloignées phylogénétiquement et physiologiquement car le corail rouge n'est pas « zooxanthellé » et vit en zone tempérée. On le retrouve essentiellement dans le bassin méditerranéen, et jusqu'à 800 m de profondeur. Il est malgré tout intéressant de comparer ces deux modèles, car ce sont tous deux des cnidaires impactés par le réchauffement climatique, qui présentent le même phénotype de collapse physiologique face au stress thermique, c'est-à-dire la fermeture des polypes suivi de nécroses (et/ou de blanchissement pour *Pocillopora*), et les mêmes techniques d'étude ont été employées (analyse des marqueurs d'adaptation locale par RAD-séquençage et analyse de la plasticité transcriptomique par RNA-seq suite à un stress thermique écologiquement réaliste). Que les résultats soient similaires ou non, la comparaison de ces deux modèles apportera des informations essentielles sur les capacités d'adaptation au réchauffement climatique des cnidaires.

Vous trouverez en annexe les articles publiés ou soumis correspondant aux différentes études réalisées sur *Corallium rubrum*. .

6. BIBLIOGRAPHIE :

Abrego, D. et al., 2008. Species-specific interactions between algal endosymbionts and coral hosts define their bleaching response to heat and light stress. *Proceedings. Biological sciences / The Royal Society*, 275(1648), pp.2273–2282.

Adjeroud, M. et al., 2014. Genetic diversity, clonality and connectivity in the scleractinian coral Pocillopora damicornis: A multi-scale analysis in an insular, fragmented reef system. *Marine Biology*, 161(3), pp.531–541.

Adjeroud, M. & Tsuchiya, M., 1999. Genetic variation and clonality in the scleractinian coral Pocillopora damicornis in the Ryukyu Archipelago, southern Japan. *Marine Biology*, 134, pp.753–760.

Ainsworth, T.D. et al., 2011. Defining the tipping point. A complex cellular life/death balance in corals in response to stress. *Scientific Reports*, 1(14), pp.130–136.

Anctil, M., 2009. Chemical transmission in the sea anemone Nematostella vectensis: A genomic perspective. *Comparative Biochemistry and Physiology - Part D: Genomics and Proteomics*, 4(4), pp.268–289. Available at: http://dx.doi.org/10.1016/j.cbd.2009.07.001.

Anthony & Fabricius, 2000. Shifting roles of heterotrophy and autotrophy in coral energetics under varying turbidity. *Journal of experimental marine biology and ecology*, 252(2), pp.221–253. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10967335 [Accessed September 14, 2017].

Antonovics, J., 1987. The evolutionary dis-synthesis: which bottle for which wine? *The American Naturalist*, 129, pp.321–331. Available at: http://www.jstor.org/stable/2461683.

Aubin-Horth, N. & Renn, S.C.P., 2009. Genomic reaction norms: Using integrative biology to understand molecular mechanisms of phenotypic plasticity. *Molecular Ecology*, 18(18), pp.3763–3780.

Baker, A.C. et al., 2004. Coral reefs: Corals' adaptive response to climate change. *Nature*, 430(7001), pp.741–741. Available at: http://www.nature.com/doifinder/10.1038/430741a [Accessed September 4, 2017].

Baker, A.C., 2001. Ecosystems: Reef corals bleach to survive change. *Nature*, 411(6839), pp.765–766. Available at: http://www.nature.com/doifinder/10.1038/35081151 [Accessed September 11, 2017].

Baker, A.C., 2003. Flexibility and Specificity in Coral-Algal Symbiosis: Diversity, Ecology, and Biogeography of *Symbiodinium*. *Annual Review of Ecology, Evolution, and Systematics*, 34(1), pp.661–689. Available at: http://www.annualreviews.org/doi/10.1146/annurev.ecolsys.34.011802.132417.

Baker, A.C., Glynn, P.W. & Riegl, B., 2008. Climate change and coral reef bleaching: An ecological assessment of long-term impacts, recovery trends and future outlook. *Estuarine, Coastal and Shelf Science*, 80(4), pp.435–471. Available at: http://dx.doi.org/10.1016/j.ecss.2008.09.003.

Banin, E. et al., 2003. Superoxide Dismutase Is a Virulence Factor Produced by the Coral Bleaching Pathogen Vibrio shiloi. *Current Microbiology*, 46(6), pp.418–422. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12732948 [Accessed September 11, 2017].

Barnes, D.J. & Chalker, B.E., 1990. Calcification and photosynthesis in reef-building corals and algae. *Ecosystems of the world*, 25, pp.109–131. Available at: http://www.refdoc.fr/Detailnotice?idarticle=19676197.

Barshis, D.J. et al., 2013. Genomic basis for coral resilience to climate change. *Proceedings of the National Academy of Sciences of the United States of America*, 110(4), pp.1387–92. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3557039&tool=pmcentrez&rendertype=abstract [Accessed March 7, 2017].

Barshis, D.J. et al., 2014. Lineage-specific transcriptional profiles of Symbiodinium spp. Unaltered by heat stress in a coral host. *Molecular Biology and Evolution*, 31(6), pp.1343–1352. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24651035 [Accessed September 6, 2017].

Bashiardes, S., Zilberman-Schapira, G. & Elinav, E., 2016. Use of Metatranscriptomics in Microbiome Research. *Bioinformatics and biology insights*, 10, pp.19–25. Available at: http://www.ncbi.nlm.nih.gov/pubmed/27127406 [Accessed September 2, 2017].

Bay-Nouailhat, A. & Bay-Nouailhat, W., 2017. Mer et littoral - La biologie des Cnidaires - Hydraires, Méduses, Alcyons, Gorgones, Anémones, Coraux. Available at: http://www.mer-littoral.org/05/hexacoralliaires.php [Accessed September 10, 2017].

Bayer, T. et al., 2013. The microbiome of the Red Sea coral Stylophora pistillata is dominated by tissue-associated Endozoicomonas bacteria. *Applied and environmental microbiology*, 79(15), pp.4759–62. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23709513 [Accessed September 2, 2017].

Becker, M. et al., 2013. Hybridization may facilitate in situ survival of endemic species through periods of climate change. *Nature Clim. Change*, 3(12), pp.1039–1043. Available at:

http://dx.doi.org/10.1038/nclimate2027.
Bellantuono, A.J. et al., 2012. Coral Thermal Tolerance: Tuning Gene Expression to Resist Thermal Stress. *PLoS ONE*, 7(11), pp.1–14.
Bellantuono, A.J., Hoegh-Guldberg, O. & Rodriguez-Lanetty, M., 2011. Resistance to thermal stress in corals without changes in symbiont composition. *Proceedings of the Royal Society B: Biological Sciences*, 279(1731), pp.1100–1107. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21976690 [Accessed March 10, 2017].
Ben-Haim, Y. et al., 2003. Vibrio coralliilyticus sp. nov., a temperature-dependent pathogen of the coral Pocillopora damicornis. *International Journal of Systematic and Evolutionary Microbiology*, 53(1), pp.309–315.
Bentis, C., Kaufman, L. & Golubic, S., 2000. Endolithic fungi in reef-building corals (Order : Scleractinia) are common, cosmopolitan, and potentially pathogenic. *The Biological Bulletin*, 198(2), pp.254–260. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10786945 [Accessed September 10, 2017].
Berkelmans, R., 2009. Bleaching and Mortality Thresholds: How Much is Too Much? In Springer, Berlin, Heidelberg, pp. 103–119. Available at: http://link.springer.com/10.1007/978-3-540-69775-6_7 [Accessed September 4, 2017].
Berkelmans, R. & 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. *Proceedings. Biological sciences / The Royal Society*, 273(1599), pp.2305–2312.
Bettarel, Y. et al., 2015. The versatile nature of coral-associated viruses. *Environmental microbiology*, 17(10), pp.3433–3439.
Blackall, L.L., Wilson, B. & van Oppen, M.J.H., 2015. Coral-the world's most diverse symbiotic ecosystem. *Molecular Ecology*, 24(21), pp.5330–5347. Available at: http://www.ncbi.nlm.nih.gov/pubmed/26414414 [Accessed September 4, 2017].
Bosch, T.C.G.G. & McFall-Ngai, M.J., 2011. Metaorganisms as the new frontier. *Zoology*, 114(4), pp.185–190. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21737250 [Accessed September 4, 2017].
Boulotte, N.M. et al., 2016. Exploring the Symbiodinium rare biosphere provides evidence for symbiont switching in reef-building corals. *The ISME Journal*, 10(11), pp.2693–2701. Available at: http://www.nature.com/doifinder/10.1038/ismej.2016.54 [Accessed September 10, 2017].
Bourne, D. et al., 2008. Changes in coral-associated microbial communities during a bleaching event. *The ISME Journal*, 2(4), pp.350–363. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18059490 [Accessed September 11, 2017].
Bourne, D.G., Morrow, K.M. & Webster, N.S., 2016. Insights into the Coral Microbiome: Underpinning the Health and Resilience of Reef Ecosystems. *Annual Review of Microbiology*, 70(1), pp.317–340. Available at: http://www.annualreviews.org/doi/10.1146/annurev-micro-102215-095440.
Bourne, D.G. & Munn, C.B., 2005. Diversity of bacteria associated with the coral Pocillopora damicornis from the Great Barrier Reef. *Environmental microbiology*, 7(8), pp.1162–74. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16011753 [Accessed February 16, 2016].
Bradshaw, A.D., 1965. Evolutionary Significance of Phenotypic Plasticity in Plants. In pp. 115–155. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0065266008600486 [Accessed August 30, 2017].
Brown, B. et al., 2002. Exploring the basis of thermotolerance in the reef coral Goniastrea aspera. *Marine Ecology Progress Series*, 242, pp.119–129. Available at: http://www.int-res.com/abstracts/meps/v242/p119-129/ [Accessed August 17, 2017].
Brown, B.. et al., 2002. Preliminary evidence for tissue retraction as a factor in photoprotection of corals incapable of xanthophyll cycling. *Journal of Experimental Marine Biology and Ecology*, 277(2), pp.129–144. Available at: http://www.sciencedirect.com/science/article/pii/S0022098102003052 [Accessed September 16, 2017].
Brown, B.E. & Bythell, J.C., 2005. Perspectives on mucus secretion in reef corals. *Marine Ecology Progress Series*, 296, pp.291–309. Available at: http://www.int-res.com/abstracts/meps/v296/p291-309/.
Brown, B.E., Le Tissier, M.D.A. & Bythell, J.C., 1995. Mechanisms of bleaching deduced from histological studies of reef corals sampled during a natural bleaching event. *Marine Biology*, 122(4), pp.655–663. Available at: http://link.springer.com/10.1007/BF00350687 [Accessed September 11, 2017].
Bruno, J.F. et al., 2007. Regional Decline of Coral Cover in the Indo-Pacific: Timing, Extent, and Subregional Comparisons R. Freckleton, ed. *PLoS ONE*, 2(8), p.e711. Available at: http://dx.plos.org/10.1371/journal.pone.0000711 [Accessed August 29, 2017].
Buddemeier, R.W. & Fautin, D.G., 1993. Coral Bleaching as an Adaptive Mechanism. *BioScience*, 43(5), pp.320–326. Available at: https://academic.oup.com/bioscience/article-lookup/doi/10.2307/1312064.

Burke, L.M. et al., 2011. *Reefs at risk revisited*, World Resources Institute. Available at: http://agris.fao.org/agris-search/search.do;jsessionid=CD163B24A00FECF025AC12127C8868A6?request_locale=es&recordID=QW2016000348&sourceQuery=&query=&sortField=&sortOrder=&agrovocString=&advQuery=&centerString=&enableField=#.WZ70MNSGxvw.mendeley [Accessed August 24, 2017].

Cahill, A.E. et al., 2013. How does climate change cause extinction? *Proceedings of the Royal Society B: Biological Sciences*, 280(1750), pp.20121890–20121890. Available at: http://rspb.royalsocietypublishing.org/cgi/doi/10.1098/rspb.2012.1890.

Canestrelli, D. et al., 2017. Climate change promotes hybridisation between deeply divergent species. *PeerJ*, 5, p.e3072. Available at: https://peerj.com/articles/3072.

Capinha, C. et al., 2013. Effects of Climate Change, Invasive Species, and Disease on the Distribution of Native European Crayfishes. *Conservation Biology*, 27(4), pp.731–740. Available at: http://doi.wiley.com/10.1111/cobi.12043 [Accessed August 28, 2017].

Carpenter, L.W., Patterson, M.R. & Bromage, E.S., 2010. Water flow influences the spatiotemporal distribution of heat shock protein 70 within colonies of the scleractinian coral Montastrea annularis (Ellis and Solander, 1786) following heat stress: Implications for coral bleaching. *Journal of Experimental Marine Biology and Ecology*, 387(1–2), pp.52–59. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0022098110000742 [Accessed August 17, 2017].

Ceh, J., Van Keulen, M. & Bourne, D.G., 2011. Coral-associated bacterial communities on Ningaloo Reef, Western Australia. *FEMS Microbiology Ecology*, 75(1), pp.134–144. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21044100 [Accessed September 1, 2017].

Cervino, J.M. et al., 2008. The *Vibrio* core group induces yellow band disease in Caribbean and Indo-Pacific reef-building corals. *Journal of Applied Microbiology*, 105(5), pp.1658–1671. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18798767 [Accessed September 11, 2017].

Chen, C.-P. et al., 2011. The dynamics of microbial partnerships in the coral Isopora palifera. *The ISME Journal*, 5(4), pp.728–740. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20962876 [Accessed September 1, 2017].

Chen, I.-C. et al., 2011. Rapid range shifts of species associated with high levels of climate warming. *Science (New York, N.Y.)*, 333(6045), pp.1024–6. Available at: http://www.sciencemag.org.gate1.inist.fr/content/333/6045/1024.full [Accessed July 9, 2014].

Chevin, L.M., Collins, S. & Lefèvre, F., 2013. Phenotypic plasticity and evolutionary demographic responses to climate change: Taking theory out to the field. *Functional Ecology*, 27(4), pp.967–979.

Chmielewski, F.M. & Rötzer, T., 2002. Annual and spatial variability of the beginning of growing season in Europe in relation to air temperature changes. *Climate Research*, 19(3), pp.257–264.

Cikala, M. et al., 1999. Identification of caspases and apoptosis in the simple metazoan Hydra. *Current biology : CB*, 9(17), pp.959–62. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10508589 [Accessed August 17, 2017].

Coffroth, M.A. & Santos, S.R., 2005. Genetic diversity of symbiotic dinoflagellates in the genus Symbiodinium. *Protist*, 156(1), pp.19–34.

Coles, S.L. & Riegl, B.M., 2013. Thermal tolerances of reef corals in the Gulf: A review of the potential for increasing coral survival and adaptation to climate change through assisted translocation. *Marine Pollution Bulletin*, 72(2), pp.323–332. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23058810 [Accessed September 4, 2017].

Colley, N.J. & Trench, R.K., 1983. Selectivity in phagocytosis and persistence of symbiotic algae in the scyphistoma stage of the jellyfish Cassiopeia xamachana. *Proceedings of the Royal Society of London. Series B, Biological sciences*, 219(1214), pp.61–82. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22470960 [Accessed September 10, 2017].

Cooney, R.P. et al., 2002. Characterization of the bacterial consortium associated with black band disease in coral using molecular microbiological techniques. *Environmental microbiology*, 4(7), pp.401–13. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12123476 [Accessed September 11, 2017].

Costanza, R. et al., 2014. Changes in the global value of ecosystem services. *Global Environmental Change*, 26, pp.152–158. Available at: http://www.sciencedirect.com.inee.bib.cnrs.fr/science/article/pii/S0959378014000685 [Accessed August 28, 2017].

Cramer, W. et al., 2014. Chapter 18 - Detection and attribution of observed impacts. *Climate Change 2014: Impacts, Adaptation and Vulnearability. Part A: Global and Sectoral Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*, pp.979–1037.

Crozier, L.G. & Hutchings, J.A., 2014. Plastic and evolutionary responses to climate change in fish.

Evolutionary Applications, 7(1), pp.68–87. Available at: http://doi.wiley.com/10.1111/eva.12135 [Accessed August 26, 2017].

Cumbo, V.R., Baird, A.H. & van Oppen, M.J.H., 2013. The promiscuous larvae: flexibility in the establishment of symbiosis in corals. *Coral Reefs*, 32(1), pp.111–120. Available at: http://link.springer.com/10.1007/s00338-012-0951-7 [Accessed September 10, 2017].

Cunning, R., Glynn, P.W. & Baker, A.C., 2013. Flexible associations between Pocillopora corals and Symbiodinium limit utility of symbiosis ecology in defining species. *Coral Reefs*, 32(3), pp.795–801.

Debiasse, M.B. & Kelly, M.W., 2016. Plastic and Evolved Responses to Global Change: What Can We Learn from Comparative Transcriptomics? *Journal of Heredity*, pp.71–81. Available at: https://oup.silverchair-cdn.com/oup/backfile/Content_public/Journal/jhered/107/1/10.1093_jhered_esv073/2/esv073.pdf?Expires=1494949310&Signature=d0MmUUDcgEfHASsGZw-VS~3tMz52TbvzPXE-logH6dovkcivsfVxu2zG~qTE9aO9eBw5nZS30sOIo8kR~CON8FDuSwhtDVRlurqavCIkx-1gPZuuhc~qzeCS9jg4gKtd4l7rlxMUbS-S9WcrJ-4eAh0Z~m1lf24prXWJjGZrcig8Up-RRqgiAujhThSYWSf5DhuqVudr9qQgHtO74n84AgQk-SDO9hoVUUzWQla-~INtTGKqVyypTslkdy4i5cRcvbVfRdQagzjAgDRPCjEhbPDKXWQhTIPi1PK0xXHdZ4LfrJUYA22RtFmVmasnWgoX6vHnXo-lfSdwqN-nULH01A__&Key-Pair-Id=APKAIUCZBIA4LVPAVW3Q [Accessed May 15, 2017].

DeJong, G., 2005. Evolution of phenotypic plasticity: patters of plasticity and the emergence of ecotypes. *New Phytologist*, 166(2003), pp.101–118.

Denner, E.B.M. et al., 2003. Aurantimonas coralicida gen. nov., sp. nov., the causative agent of white plague type II on Caribbean scleractinian corals. *INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY*, 53(4), pp.1115–1122. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12892136 [Accessed September 11, 2017].

Desalvo, M.K. et al., 2008. Differential gene expression during thermal stress and bleaching in the Caribbean coral Montastraea faveolata. *Molecular Ecology*, 17(17), pp.3952–3971. Available at: http://doi.wiley.com/10.1111/j.1365-294X.2008.03879.x [Accessed June 6, 2017].

DeSalvo, M.K. et al., 2010. Coral host transcriptomic states are correlated with Symbiodinium genotypes. *Molecular Ecology*, 19(6), pp.1174–1186.

Douglas, A., 1998. Host benefit and the evolution of specialization in symbiosis. *Heredity*, 81(6), pp.599–603.

Downs, C.. et al., 2002. Oxidative stress and seasonal coral bleaching. *Free Radical Biology and Medicine*, 33(4), pp.533–543. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0891584902009073 [Accessed September 11, 2017].

Downs, C.A. et al., 2009. Symbiophagy as a cellular mechanism for coral bleaching. *Autophagy*, 5(2), pp.211–6. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19066451 [Accessed September 11, 2017].

Duncan, E.J., Gluckman, P.D. & Dearden, P.K., 2014. Epigenetics, plasticity, and evolution: How do we link epigenetic change to phenotype? *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution*, 322(4), pp.208–220. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24719220 [Accessed September 5, 2017].

Dykens, J.A. et al., 1992. Oxygen Radical Production in the Sea Anemone Anthopleura Elegantissima and its Endosymbiotic Algae. *Journal of Experimental Biology*, 168(1). Available at: http://jeb.biologists.org/content/168/1/219 [Accessed September 11, 2017].

Efrony, R. et al., 2007. Phage therapy of coral disease. *Coral Reefs*, 26(1), pp.7–13. Available at: http://link.springer.com/10.1007/s00338-006-0170-1 [Accessed September 2, 2017].

Efrony, R., Atad, I. & Rosenberg, E., 2009. Phage Therapy of Coral White Plague Disease: Properties of Phage BA3. *Current Microbiology*, 58(2), pp.139–145. Available at: http://link.springer.com/10.1007/s00284-008-9290-x [Accessed September 2, 2017].

El-Awady, N., 2015. Corals get by with a little help from a friend. *Nature Middle East*. Available at: http://www.natureasia.com/en/nmiddleeast/article/10.1038/nmiddleeast.2015.191 [Accessed September 11, 2017].

Ewert, M.A., Lang, J.W. & Nelson, C.E., 2005. Geographic variation in the pattern of temperature-dependent sex determination in the American snapping turtle (Chelydra serpentina). *Journal of Zoology*, 265(1), pp.81–95. Available at: http://doi.wiley.com/10.1017/S0952836904006120 [Accessed August 28, 2017].

Fabricius, K.E. et al., 2004. Identity and diversity of coral endosymbionts (zooxanthellae) from three Palauan reefs with contrasting bleaching, temperature and shading histories. *Molecular Ecology*, 13(8), pp.2445–2458. Available at: http://doi.wiley.com/10.1111/j.1365-294X.2004.02230.x

[Accessed September 4, 2017].
Félix, M. & Wagner, a, 2008. Robustness and evolution: concepts, insights and challenges from a developmental model system. *Heredity*, 100(2), pp.132–140.
Ferrer, L.M. & Szmant, A.M., 1988. Nutrient regeneration by the endolithic community in coral skeletons. *Proceedings of the 6th International Coral Reef Symposium*, 3.
Field, C.. et al., 2014. Changements climatiques 2014: Incidences, adaptation et vulnérabilité – Résumé à l'intention des décideurs. Contribution du Groupe de travail II au cinquième Rapport d'évaluation du Groupe d'experts intergouvernemental sur l'évolution du climat. , p.34.
Finnegan, D.J., 2012. Retrotransposons. *Current Biology*, 22(11), pp.R432–R437. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0960982212004459 [Accessed September 5, 2017].
Fisher, L. et al., 2009. Real-time coral stress observations before, during, and after beach nourishment dredging offshore southeast Florida, USA. *Proceedings of the 11th International Coral Reef Symposium*, (1), pp.34–37.
Franks, S.J., Weber, J.J. & Aitken, S.N., 2014. Evolutionary and plastic responses to climate change in terrestrial plant populations. *Evolutionary Applications*, 7(1), pp.123–139. Available at: http://doi.wiley.com/10.1111/eva.12112 [Accessed August 28, 2017].
Frydenborg, B.R. et al., 2014. Temperature-Dependent Inhibition of Opportunistic Vibrio Pathogens by Native Coral Commensal Bacteria. *Microbial Ecology*, 67(2), pp.392–401. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24370863 [Accessed September 14, 2017].
Galindo-González, L. et al., 2017. LTR-retrotransposons in plants: Engines of evolution. *Gene*, 626, pp.14–25. Available at: http://www.ncbi.nlm.nih.gov/pubmed/28476688 [Accessed September 5, 2017].
Garrett, T.A. et al., 2013. Comparative Lipid Profiling of the Cnidarian Aiptasia pallida and Its Dinoflagellate Symbiont C. Wicker-Thomas, ed. *PLoS ONE*, 8(3), p.e57975. Available at: http://dx.plos.org/10.1371/journal.pone.0057975 [Accessed September 13, 2017].
Gates, R.D., Baghdasarian, G. & Muscatine, L., 1992. Temperature Stress Causes Host Cell Detachment in Symbiotic Cnidarians: Implications for Coral Bleaching. *The Biological Bulletin*, 182(3), pp.324–332. Available at: http://www.journals.uchicago.edu/doi/10.2307/1542252 [Accessed September 11, 2017].
Ghalambor, C.K. et al., 2007. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Functional Ecology*, 21(3), pp.394–407.
Gibert, J.M., 2017. The flexible stem hypothesis: evidence from genetic data. *Development Genes and Evolution*, (1911), pp.1–11.
Gienapp, P. et al., 2008. Climate change and evolution: Disentangling environmental and genetic responses. *Molecular Ecology*, 17(1), pp.167–178.
Gignoux-Wolfsohn, S.A. & Vollmer, S. V., 2015. Identification of Candidate Coral Pathogens on White Band Disease-Infected Staghorn Coral C. R. Voolstra, ed. *PLOS ONE*, 10(8), p.e0134416. Available at: http://www.ncbi.nlm.nih.gov/pubmed/26241853 [Accessed September 14, 2017].
Glasl, B., Herndl, G.J. & Frade, P.R., 2016. The microbiome of coral surface mucus has a key role in mediating holobiont health and survival upon disturbance. *Isme J*, pp.1–13. Available at: http://dx.doi.org/10.1038/ismej.2016.9%5Cnhttp://10.1038/ismej.2016.9.
Gleason, D.F. & Wellington, G.M., 1993. Ultraviolet radiation and coral bleaching. *Nature*, 365(6449), pp.836–838. Available at: http://www.nature.com/doifinder/10.1038/365836a0 [Accessed September 14, 2017].
Glynn, P.W. et al., 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. *Bulletin of Marine Science*, 69(1), pp.79–109.
Glynn, P.W. & D'Croz, L., 1990. Experimental evidence for high temperature stress as the cause of El Nifio-coincident coral mortality. *Coral Reefs*, 8, pp.181–191.
Le Goff, C. et al., 2016. Carbonic anhydrases in cnidarians: Novel perspectives from the octocorallian corallium rubrum. *PLoS ONE*, 11(8), pp.1–22.
Goodier, J.L., 2016. Restricting retrotransposons: a review. *Mobile DNA*, 7, p.16. Available at: http://www.ncbi.nlm.nih.gov/pubmed/27525044 [Accessed September 5, 2017].
Goreau, T.F., 1964. Mass Expulsion of Zooxanthellae from Jamaican Reef Communities after Hurricane Flora. *Science*, 145(3630), pp.383–386. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17816975 [Accessed September 14, 2017].
Granados-Cifuentes, C. et al., 2013. High natural gene expression variation in the reef-building coral Acropora millepora: potential for acclimative and adaptive plasticity. *BMC genomics*, 14(1), p.228. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3630057&tool=pmcentrez&rendertyp

e=abstract.
Grottoli, A.G., Rodrigues, L.J. & Palardy, J.E., 2006. Heterotrophic plasticity and resilience in bleached corals. *Nature*, 440(7088), pp.1186–1189. Available at: http://www.nature.com/doifinder/10.1038/nature04565 [Accessed September 14, 2017].
Guest, J.R. et al., 2012. Contrasting Patterns of Coral Bleaching Susceptibility in 2010 Suggest an Adaptive Response to Thermal Stress M. V. Matz, ed. *PLoS ONE*, 7(3), p.e33353. Available at: http://dx.plos.org/10.1371/journal.pone.0033353 [Accessed September 4, 2017].
Harrison, P. & Wallace, C., 1990. Reproduction, dispersal and recruitment of scleractinian corals. *Coral reefs*. Available at: http://epubs.scu.edu.au/esm_pubs/3077 [Accessed September 17, 2017].
Hashimoto, K. et al., 2004. Isolation and characterization of stress-responsive genes from the scleractinian coral Pocillopora damicornis. *Coral Reefs*, 23(4), pp.485–491. Available at: http://link.springer.com/10.1007/s00338-004-0410-1 [Accessed July 19, 2017].
Hegarty, M.J., 2012. Invasion of the hybrids. *Molecular Ecology*, 21(19), pp.4669–4671. Available at: http://doi.wiley.com/10.1111/j.1365-294X.2012.05720.x [Accessed August 28, 2017].
Heron, S.., Eakin, C.M. & Douvere, F., 2017. Impacts of Climate Change on World Heritage Coral Reefs: A First Global Scientific Assessment. *UNESCO World Heritage Centre*.
Hoegh-Guldberg, O. et al., 2007. Coral Reefs Under Rapid Climate Change and Ocean Acidification. *Science*, 318(2007), pp.1737–1742. Available at: http://science.sciencemag.org/content/318/5857/1737.abstract.
Hoeksema, B., 2017. World Register of Marine Species: Pocillopora damicornis (Linnaeus, 1758). Available at: http://www.marinespecies.org/aphia.php?p=taxdetails&id=206953 [Accessed August 31, 2017].
Hoffmann, A.A., Sgro, C.M. & Sgrò, C.M., 2011. Climate change and evolutionary adaptation. *Nature*, 470(7335), pp.479–485. Available at: http://www.nature.com/doifinder/10.1038/nature09670.
Hofmann, G.E. & Todgham, A.E., 2010. Living in the now: physiological mechanisms to tolerate a rapidly changing Environment. *Annual Review of Physiology*, 72(1), pp.127–145. Available at: http://www.annualreviews.org/doi/abs/10.1146/annurev-physiol-021909-135900.
Houlbrèque, F. & Ferrier-Pagès, C., 2009. Heterotrophy in Tropical Scleractinian Corals. *Biological Reviews*, 84(1), pp.1–17. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19046402 [Accessed September 14, 2017].
Hsu, C.-M. et al., 2012. Temporal and Spatial Variations in Symbiont Communities of Catch Bowl Coral Isopora palifera (Scleractinia: Acroporidae) on Reefs in Kenting National Park, Taiwan. *Zoological studies*, 51(8), pp.1343–1353. Available at: http://zoolstud.sinica.edu.tw/Journals/51.8/1343.pdf [Accessed September 10, 2017].
Huang, H. et al., 2013. Diversity of free-living and symbiotic Symbiodinium in the coral reefs of Sanya , South China Sea. *Marine Biology Research*, 9(2), pp.117–128.
Inouye, D.W. et al., 2000. Climate change is affecting altitudinal migrants and hibernating species. *Proceedings of the National Academy of Sciences*, 97(4), pp.1630–1633. Available at: http://www.pnas.org/cgi/doi/10.1073/pnas.97.4.1630.
IPCC, 2013. IPCC Factsheet: What is the IPCC? Available at: http://www.ipcc.ch/news_and_events/docs/factsheets/FS_what_ipcc.pdf [Accessed August 22, 2017].
Jaenisch, R. & Bird, A., 2003. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. *Nature Genetics*, 33(3s), pp.245–254. Available at: http://www.nature.com/doifinder/10.1038/ng1089 [Accessed September 5, 2017].
Janzen, F.J., 1994. Climate change and temperature-dependent sex determination in reptiles. *Proceedings of the National Academy of Sciences of the United States of America*, 91(16), pp.7487–90. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8052608 [Accessed August 28, 2017].
Jones, A.. et al., 2008. A community change in the algal endosymbionts of a scleractinian coral following a natural bleaching event: field evidence of acclimatization. *Proceedings of the Royal Society B: Biological Sciences*, 275(1641), pp.1359–1365. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18348962 [Accessed September 10, 2017].
Jones, A. & Berkelmans, R., 2010. Potential Costs of Acclimatization to a Warmer Climate: Growth of a Reef Coral with Heat Tolerant vs. Sensitive Symbiont Types R. DeSalle, ed. *PLoS ONE*, 5(5), p.e10437. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20454653 [Accessed September 7, 2017].
Kaplan, J.M. & Pigliucci, M., 2001. Genes "for" phenotypes: A modern history view. *Biology and Philosophy*, 16(2), pp.189–213.
Karell, P. et al., 2011. Climate change drives microevolution in a wild bird. *Nature communications*, 2, p.208. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21343926 [Accessed August 24, 2017].
Kazandjian, A. et al., 2008. Isolation of symbiosomes and the symbiosome membrane complex from the

zoanthid Zoanthus robustus. *Phycologia*, 47(May), pp.294–306. Available at: http://www.phycologia.org/doi/abs/10.2216/PH07-23.1%5Cnhttp://www.phycologia.org/perlserv/?request=get-abstract&doi=10.2216%252FPH07-23.1.

Kellogg, C.A., 2004. Tropical Archaea: Diversity associated with the surface microlayer of corals. *Marine Ecology Progress Series*, 273, pp.81–88. Available at: http://pubs.er.usgs.gov/publication/70026947.

Kenkel, C.D. & Matz, M. V, 2016. Enhanced gene expression plasticity as a mechanism of adaptation to a variable environment in a reef-building coral. *Nature Ecology & Evolution*, 1(3), p.14. Available at: http://www.nature.com/articles/s41559-016-0014.

Kenkel, C.D., Meyer, E. & Matz, M. V., 2013. Gene expression under chronic heat stress in populations of the mustard hill coral (Porites astreoides) from different thermal environments. *Molecular Ecology*, 22(16), pp.4322–4334.

Kimes, N.E. et al., 2013. The *Montastraea faveolata* microbiome: ecological and temporal influences on a Caribbean reef-building coral in decline. *Environmental Microbiology*, 15(7), pp.2082–2094. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23750924 [Accessed September 1, 2017].

Knowlton, N. & Rohwer, F., 2003. Multispecies Microbial Mutualisms on Coral Reefs: The Host as a Habitat. *The American Naturalist*, 162(S4), pp.S51–S62. Available at: http://www.ncbi.nlm.nih.gov/pubmed/14583857 [Accessed September 14, 2017].

Koh, E.G.L., 1997. Do Scleractinian Corals Engage in Chemical Warfare Against Microbes? *Journal of Chemical Ecology*, 23(2), pp.379–398. Available at: http://link.springer.com/10.1023/B:JOEC.0000006366.58633.f4 [Accessed September 1, 2017].

Kooperman, N. et al., 2007. Coral mucus-associated bacterial communities from natural and aquarium environments. *FEMS Microbiology Letters*, 276(1), pp.106–113. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17937669 [Accessed September 10, 2017].

Koren, O. & Rosenberg, E., 2006. Bacteria associated with mucus and tissues of the coral Oculina patagonica in summer and winter. *Applied and Environmental Microbiology*, 72(8), pp.5254–5259. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16885273 [Accessed September 1, 2017].

Krämer, W.E. et al., 2012. Dynamic Regulation of Photoprotection Determines Thermal Tolerance of Two Phylotypes of Symbiodinium Clade A at Two Photon Fluence Rates. *Photochemistry and Photobiology*, 88(2), pp.398–413. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22117932 [Accessed September 7, 2017].

LaJeunesse, T., Parkinson, J.E. & Trench, R.K., 2012. Symbiodinium. *The Three of Life*. Available at: http://tolweb.org/Symbiodinium/126705/2012.07.04 [Accessed September 13, 2017].

Lajeunesse, T.C., 2005. "Species" radiations of symbiotic dinoflagellates in the Atlantic and Indo-Pacific since the Miocene-Pliocene transition. *Molecular Biology and Evolution*, 22(3), pp.570–581.

LaJeunesse, T.C. et al., 2003. Low symbiont diversity in southern Great Barrier Reef corals relative to those of the Caribbean. *Limnology and Oceanography*, 48(5), pp.2046–2054.

Leclère, L. & Röttinger, E., 2017. Diversity of Cnidarian Muscles: Function, Anatomy, Development and Regeneration. *Frontiers in Cell and Developmental Biology*, 4(January), p.157. Available at: http://journal.frontiersin.org/article/10.3389/fcell.2016.00157/full.

Lee, S.T.M. et al., 2016. Mucus Sugar Content Shapes the Bacterial Community Structure in Thermally Stressed Acropora muricata. *Frontiers in microbiology*, 7, p.371. Available at: http://www.ncbi.nlm.nih.gov/pubmed/27047481 [Accessed September 14, 2017].

Leggat, W. et al., 2011. Differential Responses of the Coral Host and Their Algal Symbiont to Thermal Stress C. R. Voolstra, ed. *PLoS ONE*, 6(10), p.e26687. Available at: http://dx.plos.org/10.1371/journal.pone.0026687 [Accessed September 5, 2017].

Lehnert, E.M. et al., 2014. Extensive differences in gene expression between symbiotic and aposymbiotic cnidarians. *G3 (Bethesda, Md.)*, 4(2), pp.277–95. Available at: http://www.g3journal.org/lookup/ [Accessed August 8, 2017].

Lema, K.A., Willis, B.L. & Bourne, D.G., 2012. Corals form characteristic associations with symbiotic nitrogen-fixing bacteria. *Applied and environmental microbiology*, 78(9), pp.3136–44. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22344646 [Accessed September 14, 2017].

Lesser, M.P. et al., 2004. Discovery of Symbiotic Nitrogen-Fixing Cyanobacteria in Corals. *Science*, 305(5686), pp.997–1000. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15310901 [Accessed September 1, 2017].

Lesser, M.P., 1996. Elevated temperatures and ultraviolet radiation cause oxidative stress and inhibit photosynthesis in ymbiotic dinoflagellates. *Limnology and Oceanography*, 41(2), pp.271–283. Available at: http://doi.wiley.com/10.4319/lo.1996.41.2.0271 [Accessed September 11, 2017].

Levin, R.A. et al., 2016. Sex, Scavengers, and Chaperones: Transcriptome Secrets of Divergent

Symbiodinium Thermal Tolerances. *Molecular Biology and Evolution*, 33(9), pp.2201–2215. Available at: https://watermark.silverchair.com/api/watermark?token=AQECAHi208BE49Ooan9kkhW_Ercy7Dm3ZL_9Cf3qfKAc485ysgAAAdIwggHOBgkqhkiG9w0BBwagggG_MIIBuwIBADCCAbQGCSqGSIb3DQEHATAeBglghkgBZQMEAS4wEQQMH58QZzp2c3L9POndAgEQgIIBhVCd3LYm5MBSXDHiQ-jCl3AZxPVm--kUw_xFy6gORC49h [Accessed September 5, 2017].

Li, J. et al., 2014. Bacterial dynamics within the mucus, tissue and skeleton of the coral Porites lutea during different seasons. *Scientific Reports*, 4, p.7320. Available at: http://www.nature.com/srep/2014/141205/srep07320/full/srep07320.html%5Cnhttp://www.nature.com/doifinder/10.1038/srep07320.

Liefting, M., Hoffmann, A.A. & Ellers, J., 2009. Plasticity versus environmental canalization: Population differences in thermal responses along a latitudinal gradient in drosophila serrata. *Evolution*, 63(8), pp.1954–1963. Available at: http://doi.wiley.com/10.1111/j.1558-5646.2009.00683.x [Accessed May 12, 2017].

Little, A.F., van Oppen, M.J.H. & Willis, B.L., 2004. Flexibility in algal endosymbioses shapes growth in reef corals. *Science (New York, N.Y.)*, 304(5676), pp.1492–1494. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15178799 [Accessed September 7, 2017].

Littman, R.A., Bourne, D.G. & Willis, B.L., 2010. Responses of coral-associated bacterial communities to heat stress differ with Symbiodinium type on the same coral host. *Molecular Ecology*, 19(9), pp.1978–1990. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20529072 [Accessed September 1, 2017].

Littman, R., Willis, B.L. & Bourne, D.G., 2011. Metagenomic analysis of the coral holobiont during a natural bleaching event on the Great Barrier Reef. *Environmental Microbiology Reports*, 3(6), pp.651–660. Available at: http://doi.wiley.com/10.1111/j.1758-2229.2010.00234.x [Accessed September 15, 2017].

Logan, M.L., Cox, R.M. & Calsbeek, R., 2014. Natural selection on thermal performance in a novel thermal environment. *Proceedings of the National Academy of Sciences of the United States of America*, (19), pp.1–5. Available at: http://www.ncbi.nlm.nih.gov/pubmed/25225361.

Luna, G.M. et al., 2009. Vibrio harveyi as a causative agent of the White Syndrome in tropical stony corals. *Environmental Microbiology Reports*, 2(1), pp.120–127. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23766006 [Accessed September 11, 2017].

Magalon, H., Adjeroud, M. & Veuille, M., 2005. Patterns of genetic variation do not correlate with geographical distance in the reef-building coral *Pocillopora meandrina* in the South Pacific. *Molecular Ecology*, 14(October), pp.1861–1868.

Mao-Jones, J. et al., 2010. How Microbial Community Composition Regulates Coral Disease Development C. Roberts, ed. *PLoS Biology*, 8(3), p.e1000345. Available at: http://dx.plos.org/10.1371/journal.pbio.1000345 [Accessed September 11, 2017].

Maor-Landaw, K. et al., 2014. Gene expression profiles during short-term heat stress in the red sea coral *Stylophora pistillata*. *Global Change Biology*, 20(10), pp.3026–3035. Available at: http://doi.wiley.com/10.1111/gcb.12592 [Accessed August 17, 2017].

Maor-Landaw, K. & Levy, O., 2016. Gene expression profiles during short-term heat stress; branching vs. massive Scleractinian corals of the Red Sea. *PeerJ*, 4, p.e1814. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4824894&tool=pmcentrez&rendertype=abstract.

Margulis, L., 1993. *Symbiosis in Cell Evolution: Microbial Communities in the Archean and Proterozoic Eons*, Freeman. Available at: https://books.google.fr/books?id=8ZiFQgAACAAJ.

Margulis, L. & Fester, R., 1991. *Symbiosis as a source of evolutionary innovation : speciation and morphogenesis*, MIT Press. Available at: https://mitpress.mit.edu/books/symbiosis-source-evolutionary-innovation [Accessed August 24, 2017].

Mayfield, A.B. et al., 2014. Compartment-specific transcriptomics in a reef-building coral exposed to elevated temperatures. *Molecular Ecology*, 23(23), pp.5816–5830. Available at: http://doi.wiley.com/10.1111/mec.12982 [Accessed September 15, 2017].

Mayfield, A.B. et al., 2017. The proteomic response of the reef coral Pocillopora acuta to experimentally elevated temperatures. Available at: https://peerj.com/preprints/3252/ [Accessed September 15, 2017].

Maynard, J.A. et al., 2008. Major bleaching events can lead to increased thermal tolerance in corals. *Marine Biology*, 155(2), pp.173–182. Available at: http://link.springer.com/10.1007/s00227-008-1015-y [Accessed September 4, 2017].

McDevitt-Irwin, J.M. et al., 2017. Responses of Coral-Associated Bacterial Communities to Local and Global

Stressors. *Frontiers in Marine Science*, 4, p.262. Available at: http://journal.frontiersin.org/article/10.3389/fmars.2017.00262/full [Accessed September 1, 2017].
McFadden, C.S., 1997. Contributions of sexual and asexual reproduction to population structure in the clonal soft coral, alcyonium rudyi. *Evolution*, 51(1), pp.112–126. Available at: http://www.ncbi.nlm.nih.gov/pubmed/28568799 [Accessed September 15, 2017].
McFall-Ngai, M. et al., 2013. Animals in a bacterial world, a new imperative for the life sciences. *Proceedings of the National Academy of Sciences*, 110(9), pp.3229–3236. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23391737 [Accessed August 24, 2017].
McFall-Ngai, M.J., Henderson, B. & Ruby, E.G., 2005. *The Influence of Cooperative Bacteria on Animal Host Biology*, Cambridge University Press. Available at: https://books.google.fr/books?id=BfwalYMlmlEC.
McGinley, M. et al., 2012. Symbiodinium spp. in colonies of eastern Pacific Pocillopora spp. are highly stable despite the prevalence of low-abundance background populations. *Marine Ecology Progress Series*, 462, pp.1–7. Available at: http://www.int-res.com/abstracts/meps/v462/p1-7/ [Accessed September 10, 2017].
Merilä, J. & Hendry, A.P., 2014. Climate change, adaptation, and phenotypic plasticity: the problem and the evidence. *Evolutionary Applications*, 7(1), pp.1–14. Available at: http://doi.wiley.com/10.1111/eva.12137 [Accessed August 26, 2017].
Mieog, J.C. et al., 2007. Real-time PCR reveals a high incidence of Symbiodinium clade D at low levels in four scleractinian corals across the Great Barrier Reef: Implications for symbiont shuffling. *Coral Reefs*, 26(3), pp.449–457. Available at: http://link.springer.com/10.1007/s00338-007-0244-8 [Accessed March 7, 2017].
Miller, T.R. et al., 2004. Chemotaxis of Silicibacter sp. strain TM1040 toward dinoflagellate products. *Applied and environmental microbiology*, 70(8), pp.4692–701. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15294804 [Accessed September 14, 2017].
Mitchelmore, C.L., Schwarz, J.A. & Weis, V.M., 2002. Development of symbiosis-specific genes as biomarkers for the early detection of cnidarian-algal symbiosis breakdown. *Marine environmental research*, 54(3–5), pp.345–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12408586 [Accessed September 14, 2017].
MØller, A.P. & Merilä, J., 2004. Analysis and Interpretation of Long-Term Studies Investigating Responses to Climate Change. In pp. 111–130. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0065250404350063 [Accessed July 11, 2017].
Morrow, K.M. et al., 2012. Bacterial associates of two Caribbean coral species reveal species-specific distribution and geographic variability. *Applied and environmental microbiology*, 78(18), pp.6438–49. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22773636 [Accessed September 14, 2017].
Muhlfeld, C.C. et al., 2014. Invasive hybridization in a threatened species is accelerated by climate change. *Nature Clim. Change*, 4(7), pp.620–624. Available at: http://dx.doi.org/10.1038/nclimate2252.
Muller-Parker, G., D'Elia, C.F. & Cook, C.B., 2015. Interactions Between Corals and Their Symbiotic Algae. In *Coral Reefs in the Anthropocene*. Dordrecht: Springer Netherlands, pp. 99–116. Available at: http://link.springer.com/10.1007/978-94-017-7249-5_5 [Accessed August 29, 2017].
Munn, C.B., 2015. The Role of Vibrios in Diseases of Corals. *Microbiology Spectrum*, 3(4), pp.1–12. Available at: http://www.asmscience.org/content/journal/microbiolspec/10.1128/microbiolspec.VE-0006-2014.
Muscatine, L. & Porter, J.W., 1977. Reef Corals: Mutualistic Symbioses Adapted to Nutrient-Poor Environments. *BioScience*, 27(7), pp.454–460. Available at: https://academic.oup.com/bioscience/article-lookup/doi/10.2307/1297526 [Accessed May 15, 2017].
Neave, M.J. et al., 2016. Differential specificity between closely related corals and abundant Endozoicomonas endosymbionts across global scales. *The ISME Journal*, 11(1), pp.1–15. Available at: http://www.nature.com/doifinder/10.1038/ismej.2016.95 [Accessed May 30, 2017].
Neave, M.J. et al., 2014. Whole-Genome Sequences of Three Symbiotic Endozoicomonas Strains. *Genome Announcements*, 2(4), pp.e00802-14-e00802-14. Available at: http://www.ncbi.nlm.nih.gov/pubmed/25125646 [Accessed September 2, 2017].
Nguyen-Kim, H. et al., 2014. High occurrence of viruses in the mucus layer of scleractinian corals. *Environmental Microbiology Reports*, 6(6), pp.675–682. Available at: http://doi.wiley.com/10.1111/1758-2229.12185 [Accessed September 14, 2017].
NOAA Sites, 2017. NOAA National Ocean Service Education: Global distribution of coral reefs. Available at: https://oceanservice.noaa.gov/education/kits/corals/media/supp_coral05a.html [Accessed September 9, 2017].

Obura, D.O., 2009. Reef corals bleach to resist stress. *Marine Pollution Bulletin*, 58(2), pp.206–212. Available at: http://dx.doi.org/10.1016/j.marpolbul.2008.10.002.

Odum, H.T. & Odum, E.P., 1955. Trophic Structure and Productivity of a Windward Coral Reef Community on Eniwetok Atoll. *Ecological Monographs*, 25(3), pp.291–320. Available at: http://doi.wiley.com/10.2307/1943285 [Accessed September 10, 2017].

Olofsson, H., Ripa, J. & Jonzen, N., 2009. Bet-hedging as an evolutionary game: the trade-off between egg size and number. *Proceedings of the Royal Society B: Biological Sciences*, 276(1669), pp.2963–2969. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19474039 [Accessed September 5, 2017].

Oppen, M. van, 2001. In vitro establishment of symbiosis in Acropora millepora planulae. *Coral Reefs*, 20(3), pp.200–200. Available at: http://link.springer.com/10.1007/s003380100167 [Accessed August 29, 2017].

Ormond, R.F.G. & Roberts, C.M., 1997. The biodiversity of coral reef fishes. *Marine biodiversity: patterns and processes*.

Palumbi, S.R. et al., 2014. Mechanisms of reef coral resistance to future climate change. *Science*, 344(6186), pp.895–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24762535.

Pantos, O. et al., 2015. Habitat-specific environmental conditions primarily control the microbiomes of the coral Seriatopora hystrix. *The ISME Journal*, 9(9), pp.1–12. Available at: http://www.nature.com/doifinder/10.1038/ismej.2015.3.

Parmesan, C., 2007. Influences of species, latitudes and methodologies on estimates of phenological response to global warming. *Global Change Biology*, 13(9), pp.1860–1872.

Parmesan, C. et al., 1999. Poleward shifts in geographical ranges of butterfly species associated with regional warming. *Nature*, 399(June), pp.579–583. Available at: http://eprints.whiterose.ac.uk/54320/.

Patterson, K.L. et al., 2002. The etiology of white pox, a lethal disease of the Caribbean elkhorn coral, Acropora palmata. *Proceedings of the National Academy of Sciences of the United States of America*, 99(13), pp.8725–30. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12077296 [Accessed September 11, 2017].

Pearse, V.B. & Muscatine, L., 1971. ROLE OF SYMBIOTIC ALGAE (ZOOXANTHELLAE) IN CORAL CALCIFICATION. *The Biological Bulletin*, 141(2), pp.350–363. Available at: http://www.journals.uchicago.edu/doi/10.2307/1540123 [Accessed September 10, 2017].

Peixoto, R.S. et al., 2017. Beneficial Microorganisms for Corals (BMC): Proposed Mechanisms for Coral Health and Resilience. *Frontiers in Microbiology*, 8, p.341. Available at: http://journal.frontiersin.org/article/10.3389/fmicb.2017.00341/full [Accessed May 11, 2017].

Penin, L., Vidal-Dupiol, J. & Adjeroud, M., 2013. Response of coral assemblages to thermal stress: are bleaching intensity and spatial patterns consistent between events? *Environmental Monitoring and Assessment*, 185(6), pp.5031–5042. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23054287 [Accessed September 4, 2017].

Perez, S. & Weis, V., 2006. Nitric oxide and cnidarian bleaching: an eviction notice mediates breakdown of a symbiosis. *Journal of Experimental Biology*, 209(14), pp.2804–2810. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16809471 [Accessed September 11, 2017].

Perry, A.L., 2005. Climate Change and Distribution Shifts in Marine Fishes. *Science*, 308(5730), pp.1912–1915. Available at: http://www.sciencemag.org/cgi/doi/10.1126/science.1111322.

Pettay, D.T. & LaJeunesse, T.C., 2009. Microsatellite loci for assessing genetic diversity, dispersal and clonality of coral symbionts in "stress-tolerant" clade D Symbiodinium. *Molecular Ecology Resources*, 9(3), pp.1022–1025. Available at: http://doi.wiley.com/10.1111/j.1755-0998.2009.02561.x [Accessed September 7, 2017].

Pinzon, J.H. & LaJeunesse, T.C., 2011. Species delimitation of common reef corals in the genus Pocillopora using nucleotide sequence phylogenies, population genetics and symbiosis ecology. *Molecular Ecology*, 20(2), pp.311–325. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21091563 [Accessed September 4, 2017].

Planton, S., 2013. Annex III: Glossary, In: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*, pp.1447–1466.

Pochon, X. et al., 2001. High genetic diversity and relative specificity among Symbiodinium-like endosymbiotic dinoflagellates in soritid foraminiferans. *Marine Biology*, 139(6), pp.1069–1078.

Pochon, X. et al., 2006. Molecular phylogeny, evolutionary rates, and divergence timing of the symbiotic dinoflagellate genus Symbiodinium. *Molecular Phylogenetics and Evolution*, 38(1), pp.20–30.

Pochon, X. & Gates, R.D., 2010. A new Symbiodinium clade (Dinophyceae) from soritid foraminifera in

Hawai'i. *Molecular Phylogenetics and Evolution*, 56(1), pp.492–497. Available at: http://dx.doi.org/10.1016/j.ympev.2010.03.040.
Pochon, X., LaJeunesse, T.C. & Pawlowski, J., 2004. Biogeographic partitioning and host specialization among foraminiferan dinoflagellate symbionts (Symbiodinium; Dinophyta). *Marine Biology*, 146(1), pp.17–27.
Pratlong, M. et al., Identifying adaptive loci in a context of high genetic struc- ture, local adaptation to depth in the red coral (Corallium rubrum). *Molecular Ecology*.
Pratlong, M., 2016. *Le défi évolutif du changement climatique, processus adaptatifs chez le corail rouge (Corallium rubrum)*. Aix-Marseille Université.
Putnam, H.M. et al., 2012. Endosymbiotic flexibility associates with environmental sensitivity in scleractinian corals. *Proceedings of the Royal Society B: Biological Sciences*, 279(1746), pp.4352–4361. Available at: http://rspb.royalsocietypublishing.org/content/royprsb/279/1746/4352.full.pdf [Accessed May 30, 2017].
Putnam, H.M. & Gates, R.D., 2015. Preconditioning in the reef-building coral Pocillopora damicornis and the potential for trans-generational acclimatization in coral larvae under future climate change conditions. *Journal of Experimental Biology*, 218(15). Available at: http://jeb.biologists.org/content/218/15/2365 [Accessed September 4, 2017].
Quéré, C. Le & Raupach, M., 2009. Trends in the sources and sinks of carbon dioxide. *Nature Geoscience*, (November), pp.1–6. Available at: http://www.nature.com/ngeo/journal/vaop/ncurrent/full/ngeo689.html.
Rädecker, N. et al., 2015. Nitrogen cycling in corals: The key to understanding holobiont functioning? *Trends in Microbiology*, 23(8), pp.490–497. Available at: http://www.ncbi.nlm.nih.gov/pubmed/25868684 [Accessed September 2, 2017].
Raina, J.-B. et al., 2010. Do the organic sulfur compounds DMSP and DMS drive coral microbial associations? *Trends in Microbiology*, 18(3), pp.101–108. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20045332 [Accessed September 2, 2017].
Regan, H.M. et al., 2001. The currency and tempo of extinction. *The American naturalist*, 157(1), pp.1–10.
Reshef, L. et al., 2006. The Coral Probiotic Hypothesis. *Environmental Microbiology*, 8(12), pp.2068–2073. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17107548 [Accessed January 30, 2016].
Reynolds, J.M. et al., 2008. Enhanced photoprotection pathways in symbiotic dinoflagellates of shallow-water corals and other cnidarians. *Proceedings of the National Academy of Sciences of the United States of America*, 105(36), pp.13674–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18757737 [Accessed March 7, 2017].
Rhen, T. & W . Lang, J., 1998. Among-Family Variation for Environmental Sex Determination in Reptiles. *Evolution*, 52(5), pp.1514–1520. Available at: http://www.jstor.org/stable/2411322.
Richier, S. et al., 2006. Oxidative stress and apoptotic events during thermal stress in the symbiotic sea anemone, Anemonia viridis. *FEBS Journal*, 273(18), pp.4186–4198. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16907933 [Accessed July 19, 2017].
Richier, S. et al., 2005. Symbiosis-induced adaptation to oxidative stress. *Journal of Experimental Biology*, 208(2), pp.277–285. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15634847 [Accessed September 11, 2017].
Richmond, R.H., 1987. Energetic Relationships and Biogeographical Differences among Fecundity, Growth and Reproduction in the Reef Coral Pocillopora Damicornis. *Bulletin of Marine Science*, 41(2), pp.594–604. Available at: http://www.ingentaconnect.com/content/umrsmas/bullmar/1987/00000041/00000002/art00004 [Accessed September 15, 2017].
Richmond, R.H., 1987. Energetics, competency, and long-distance dispersal of planula larvae of the coral Pocillopora damicornis. *Marine Biology*, 93(4), pp.527–533. Available at: http://link.springer.com/10.1007/BF00392790 [Accessed August 29, 2017].
Richmond, R.H. & Hunter, C.L., 1990. Reproduction and recruitment of corals: comparisons among the Caribbean, the Tropical Pacific, and the Red Sea. *Marine Ecology Progress Series*, 60, pp.185–203. Available at: http://www.jstor.org/stable/24842589 [Accessed September 15, 2017].
Richmond, R.H. & Jokiel, P.L., 1984. Lunar periodicity in larva release in the reef coral pocillopora damicornis at enewetak and hawaii. *Coral reef paper*, 34(2), pp.280–287.
Riegl, B.M. et al., 2011. Present Limits to Heat-Adaptability in Corals and Population-Level Responses to Climate Extremes C. R. Voolstra, ed. *PLoS ONE*, 6(9), p.e24802. Available at: http://dx.plos.org/10.1371/journal.pone.0024802 [Accessed August 24, 2017].
Ritchie, K., 2006. Regulation of microbial populations by coral surface mucus and mucus-associated

bacteria. *Marine Ecology Progress Series*, 322, pp.1–14. Available at: http://www.int-res.com/abstracts/meps/v322/p1-14/ [Accessed September 1, 2017].
Ritchie, K.B. & Smith, G.W., 1997. Physiological comparison of bacterial communities from various species of scleractinian corals. In *Proc 8th Int Coral Reef Symp*. pp. 521–526.
Rodrigues, L.J., Grottoli, A.G. & Lesser, M.P., 2008. Long-term changes in the chlorophyll fluorescence of bleached and recovering corals from Hawaii. *Journal of Experimental Biology*, 211(15). Available at: http://jeb.biologists.org/content/211/15/2502.eLetters [Accessed August 29, 2017].
Roff, D.A. & Fairbairn, D.J., 2007. The evolution of trade-offs: Where are we? *Journal of Evolutionary Biology*, 20(2), pp.433–447.
Rohwer, F. et al., 2002. Diversity and distribution of coral-associated bacteria. *Marine Ecology Progress Series*, 243, pp.1–10.
Rosenberg, E. et al., 2007. The role of microorganisms in coral health, disease and evolution. *Nature Reviews Microbiology*, 5(5), pp.355–362. Available at: http://www.nature.com/doifinder/10.1038/nrmicro1635 [Accessed February 16, 2016].
Rosenberg, E. & Falkovitz, L., 2004. The *Vibrio shiloi* / *Oculina patagonica* Model System of Coral Bleaching. *Annual Review of Microbiology*, 58(1), pp.143–159. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15487933 [Accessed September 11, 2017].
Rosenberg, E. & Loya, Y., 2004. *Coral Health and Disease*, Springer Berlin Heidelberg.
Roth, M.S., Goericke, R. & Deheyn, D.D., 2012. Cold induces acute stress but heat is ultimately more deleterious for the reef-building coral Acropora yongei. *Scientific reports*, 2, p.240. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22355753 [Accessed September 4, 2017].
Rouzé, H. et al., 2017. An updated assessment of *Symbiodinium* spp. that associate with common scleractinian corals from Moorea (French Polynesia) reveals high diversity among background symbionts and a novel finding of clade B. *PeerJ*, 5, p.e2856. Available at: http://www.ncbi.nlm.nih.gov/pubmed/28168100 [Accessed September 7, 2017].
Rouzé, H. et al., 2016. Symbiodinium clades A and D differentially predispose Acropora cytherea to disease and Vibrio spp. colonization. *Ecology and Evolution*, 6(2), pp.560–572. Available at: http://www.ncbi.nlm.nih.gov/pubmed/26843939 [Accessed September 4, 2017].
Rowan, R., 2004. Coral bleaching: thermal adaptation in reef coral symbionts. *Nature*, 430(7001), p.742. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15306800 [Accessed September 4, 2017].
Rowan, R. et al., 1997. Landscape ecology of algal symbionts creates variation in episodes of coral bleaching. *Nature*, 388(6639), pp.265–269. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9230434 [Accessed September 11, 2017].
Rowan, R. & Knowlton, N., 1995. Intraspecific diversity and ecological zonation in coral-algal symbiosis. *Proceedings of the National Academy of Sciences of the United States of America*, 92(7), pp.2850–2853.
Rowan, R. & Powers, D., 1991. Molecular genetic identification of symbiotic dinoflagellates (zooxanthellae) . *Marine Ecology Progress Series*, 71, pp.65–73.
Rowan, R. & Powers, D. a, 1992. Ribosomal RNA sequences and the diversity of symbiotic dinoflagellates (zooxanthellae). *Proceedings of the National Academy of Sciences of the United States of America*, 89(8), pp.3639–3643.
runkle, 2017. 3 different reefs. Available at: http://www.runkle.org/Student Gallery/07-7th-iSearch-Projects/coco- coral reefs/3 kinds.htm [Accessed September 10, 2017].
Schlichter, D., Zscharnack, B. & Krisch, H., 1995. Transfer of photoassimilates from endolithic algae to coral tissue. *Naturwissenschaften*, 82(12), pp.561–564. Available at: http://link.springer.com/10.1007/BF01140246 [Accessed September 1, 2017].
Schmidt-roach, S. et al., 2014. With eyes wide open: A revision of species within and closely related to the Pocillopora damicornis species complex (Scleractinia; Pocilloporidae) using morphology and genetics. *Zoological Journal of the Linnean Society*, 170(1), pp.1–33.
Schmidt-Roach, S. et al., 2012. Broadcast Spawning by Pocillopora Species on the Great Barrier Reef. *PLoS ONE*, 7(12), pp.1–7.
Seneca, F.O. & Palumbi, S.R., 2015. The role of transcriptome resilience in resistance of corals to bleaching. , pp.1467–1484. Available at: http://content.ebscohost.com/ContentServer.asp?EbscoContent=dGJyMNLe80SeqK44yOvqOLCmr0%2BeprZSsKi4SLaWxWXS&ContentCustomer=dGJyMOzpsE21p7JOuePfgeyx9Yvf5ucA&T=P&P=AN&S=R&D=mnh&K=25728233 [Accessed July 20, 2017].
Settele, J. et al., 2014. Chapter 4 - Terrestrial and inland water systems. *Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part A: Global and Sectoral Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*, pp.271–359. Available at: http://www.ipcc.ch/pdf/assessment-report/ar5/wg2/WGIIAR5-Chap4_FINAL.pdf.

Shakun, J.D. et al., 2012. Global warming preceded by increasing carbon dioxide concentrations during the last deglaciation. Available at: http://www.atm.damtp.cam.ac.uk/mcintyre/shakun-co2-temp-lag-nat12.pdf [Accessed May 8, 2017].

Shashar, N. et al., 1997. Coral Endolithic Algae: Life in a Protected Environment! *Pacific Science*, 51(2), pp.167–173. Available at: http://hl-128-171-57-22.library.manoa.hawaii.edu/bitstream/10125/3107/1/v51n2-167-173.pdf [Accessed September 10, 2017].

Shnit-Orland, M. & Kushmaro, A., 2009. Coral mucus-associated bacteria: a possible first line of defense. *FEMS Microbiology Ecology*, 67(3), pp.371–380. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19161430 [Accessed September 10, 2017].

Silverstein, R.N., Cunning, R. & Baker, A.C., 2017. Tenacious D: *Symbiodinium* in clade D remain in reef corals at both high and low temperature extremes despite impairment. *The Journal of Experimental Biology*, 220(7), pp.1192–1196. Available at: http://www.ncbi.nlm.nih.gov/pubmed/28108671 [Accessed September 4, 2017].

Spalding, M., Ravilious, C. & Green, E.P. (Edmund P., 2001. *World atlas of coral reefs*, University of California Press. Available at: https://archive.org/details/worldatlasofcora01spal [Accessed August 28, 2017].

Stat, M. et al., 2013. The distribution of the thermally tolerant symbiont lineage (Symbiodinium clade D) in corals from Hawaii: correlations with host and the history of ocean thermal stress. *Ecology and evolution*, 3(5), pp.1317–29. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23762518 [Accessed September 7, 2017].

Stat, M. & Gates, R.D., 2011. Clade D *Symbiodinium* in Scleractinian Corals: A "Nugget" of Hope, a Selfish Opportunist, an Ominous Sign, or All of the Above? *Journal of Marine Biology*, 2011, pp.1–9. Available at: http://www.hindawi.com/journals/jmb/2011/730715/ [Accessed September 4, 2017].

Steen, R.G. & Muscatine, L., 1987. Low temperature evokes rapid exocytosis of symbiotic algae by a sea anemone. *The Biological Bulletin*, 172(2), pp.246–263. Available at: http://www.journals.uchicago.edu/doi/10.2307/1541797 [Accessed September 11, 2017].

Stocker, T.F. et al., 2013. Summary for Policymakers. In Intergovernmental Panel on Climate Change, ed. *Climate Change 2013 - The Physical Science Basis*. Cambridge: Cambridge University Press, pp. 1–30. Available at: http://ebooks.cambridge.org/ref/id/CBO9781107415324A009.

Sunagawa, S. et al., 2015. Structure and function of the global ocean microbiome. *Science*, 348(6237), pp.1261359–1261359. Available at: http://www.sciencemag.org/cgi/doi/10.1126/science.1261359.

Suzuki, Y. & Nijhout, H.F., 2006. Evolution of a Polyphenism by Genetic Accommodation. *Science*, 311(5761), pp.650–652. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16456077 [Accessed August 30, 2017].

Sweet, M.J., Croquer, A. & Bythell, J.C., 2011. Bacterial assemblages differ between compartments within the coral holobiont. *Coral Reefs*, 30(1), pp.39–52. Available at: http://link.springer.com/10.1007/s00338-010-0695-1 [Accessed September 10, 2017].

T. Banaszak, A., LaJeunesse, T.C. & Trench, R.K., 2000. The synthesis of mycosporine-like amino acids (MAAs) by cultured, symbiotic dinoflagellates. *Journal of Experimental Marine Biology and Ecology*, 249(2), pp.219–233. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0022098100001921 [Accessed September 11, 2017].

Tchernov, D. et al., 2004. Membrane lipids of symbiotic algae are diagnostic of sensitivity to thermal bleaching in corals. *Proceedings of the National Academy of Sciences of the United States of America*, 101(37), pp.13531–5. Available at: http://www.scopus.com/inward/record.url?eid=2-s2.0-4544249149&partnerID=tZOtx3y1.

Teplitski, M. & Ritchie, K., 2009. How feasible is the biological control of coral diseases? *Trends in ecology & evolution*, 24(7), pp.378–85. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19406502 [Accessed September 2, 2017].

Teplitsky, C. et al., 2008. Bergmann's rule and climate change revisited: Disentangling environmental and genetic responses in a wild bird population. *Proceedings of the National Academy of Sciences*, 105(36), pp.13492–13496. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18757740 [Accessed August 26, 2017].

Thackeray, S.J. et al., 2010. Trophic level asynchrony in rates of phenological change for marine, freshwater and terrestrial environments. *Global Change Biology*, 16(12), pp.3304–3313.

Thurber, R.V. et al., 2009. Metagenomic analysis of stressed coral holobionts. *Environmental Microbiology*, 11(8), pp.2148–2163. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19397678 [Accessed September 15, 2017].

Toller, W.W., Rowan, R. & Knowlton, N., 2001. Repopulation of Zooxanthellae in the Caribbean corals Montastraea annularis and M. faveolata following experimental and disease-associated bleaching. *The Biological bulletin*, 201(3), pp.360–73. Available at: http://www.journals.uchicago.edu/doi/10.2307/1543614 [Accessed September 11, 2017].

Toller, W.W., Rowan, R. & Knowlton, N., 2001. Zooxanthellae of the *Montastraea annularis* Species Complex: Patterns of Distribution of Four Taxa of *Symbiodinium* on Different Reefs and Across Depths. *The Biological Bulletin*, 201(3), pp.348–359. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11751247 [Accessed September 10, 2017].

Torda, G. et al., 2017. Rapid adaptive responses to climate change in corals. *Nature Climate Change*, 7(9), pp.627–636. Available at: http://www.nature.com/doifinder/10.1038/nclimate3374.

Tout, J. et al., 2015. Increased seawater temperature increases the abundance and alters the structure of natural Vibrio populations associated with the coral Pocillopora damicornis. *Frontiers in Microbiology*, 6(MAY).

Townsend, J.P. et al., 2002. Effects of temperature on gene expression in embryos of the coral Montastraea faveolata. *Genome Biology*, 3(12), p.research0071.1. Available at: http://genomebiology.biomedcentral.com/articles/10.1186/gb-2002-3-12-research0071 [Accessed August 16, 2017].

Traylor-Knowles, N. et al., 2011. Production of a reference transcriptome and transcriptomic database (PocilloporaBase) for the cauliflower coral, Pocillopora damicornis. *BMC Genomics*, 12(1), p.585. Available at: http://www.biomedcentral.com/1471-2164/12/585.

Tripp, H.J. et al., 2008. SAR11 marine bacteria require exogenous reduced sulphur for growth. *Nature*, 452(7188), pp.741–744. Available at: http://www.nature.com/doifinder/10.1038/nature06776 [Accessed September 14, 2017].

Tsang, R.H.L. & Ang, P., 2015. Cold temperature stress and predation effects on corals: their possible roles in structuring a nonreefal coral community. *Coral Reefs*, 34(1), pp.97–108. Available at: http://link.springer.com/10.1007/s00338-014-1210-x [Accessed September 4, 2017].

Ulstrup, K.E. & Van Oppen, M.J.H., 2003. Geographic and habitat partitioning of genetically distinct zooxanthellae (Symbiodinium) in Acropora corals on the Great Barrier Reef. *Molecular Ecology*, 12(12), pp.3477–3484.

Vargas-Angel, B. et al., 2006. An experimental histopathological rating scale of sedimentation stress in the Caribbean coral Montastraea cavernosa. *Proc 10th Int. Coral Reef Symp. {…}*, 1173, pp.1168–1173. Available at: http://scholar.google.com/scholar?hl=en%7B&%7DbtnG=Search%7B&%7Dq=intitle:An+experimental+histopathological+rating+scale+of+sedimentation+stress+in+the+Caribbean+coral+Montastraea+cavernosa%7B#%7D0.

Vega Thurber, R.L. et al., 2008. Metagenomic analysis indicates that stressors induce production of herpes-like viruses in the coral Porites compressa. *Proceedings of the National Academy of Sciences*, 105(47), pp.18413–18418. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19017800 [Accessed September 14, 2017].

Vidal-dupiol, J. et al., 2009. Coral bleaching under thermal stress: putative involvement of host/symbiont recognition mechanisms. *BMC physiology*, 9, p.14. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19653882 [Accessed August 31, 2017].

Vidal-dupiol, J., 2011. Stress environnementaux chez le corail scléractiniaire Pocillopora damicornis : Du modèle expérimental à l'identification de marqueurs fonctionnels du stress. *Thesis*.

Vidal-Dupiol, J. et al., 2013. Genes Related to Ion-Transport and Energy Production Are Upregulated in Response to CO2-Driven pH Decrease in Corals: New Insights from Transcriptome Analysis S. Dupont, ed. *PLoS ONE*, 8(3), p.e58652. Available at: http://dx.plos.org/10.1371/journal.pone.0058652 [Accessed September 15, 2017].

Vidal-Dupiol, J. et al., 2014. Thermal stress triggers broad Pocillopora damicornis transcriptomic remodeling, while Vibrio coralliilyticus infection induces a more targeted immuno-suppression response. *PloS one*, 9(9), p.e107672. Available at: http://www.ncbi.nlm.nih.gov/pubmed/25259845 [Accessed August 31, 2017].

Vimal, J., 2007. Physiopathologie des Coraux. *These*, Docteur Vé, p.127. Available at: http://www2.vet-lyon.fr/bib/fondoc/th_sout/th_pdf/2007lyon025.pdf.

Voolstra, C.R. et al., 2009. Effects of temperature on gene expression in embryos of the coral Montastraea faveolata. *BMC genomics*, 10, p.627. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20030803.

Waddington, C.H., 1953. Genetic Assimilation of an Acquired Character. *Evolution*, 7(2), p.118. Available at: http://www.jstor.org/stable/2405747?origin=crossref [Accessed July 20, 2017].

Wakefield, T.S. & Kempf, S.C., 2001. Development of Host- and Symbiont-Specific Monoclonal Antibodies

and Confirmation of the Origin of the Symbiosome Membrane in a Cnidarian–Dinoflagellate Symbiosis. *The Biological Bulletin*, 200(2), pp.127–143. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11341574 [Accessed September 10, 2017].

Warner, M.E., Fitt, W.K. & Schmidt, G.W., 1999. Damage to photosystem II in symbiotic dinoflagellates: a determinant of coral bleaching. *Proceedings of the National Academy of Sciences of the United States of America*, 96(14), pp.8007–12. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10393938 [Accessed September 11, 2017].

Webster, N.S. & Reusch, T.B., 2017. Microbial contributions to the persistence of coral reefs Coral reefs under climate change. *The ISME Journal advance online publication*, doi(10). Available at: http://eprints.uni-kiel.de/38011/1/Webster_Reusch_2017_ismej.pdf [Accessed September 1, 2017].

Wegley, L. et al., 2007. Metagenomic analysis of the microbial community associated with the coral Porites astreoides. *Environmental Microbiology*, 9(11), pp.2707–2719. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17922755 [Accessed September 10, 2017].

Weis, V. et al., 2001. Host-symbiont specificity during onset of symbiosis between the dinoflagellates Symbiodinium spp. and planula larvae of the scleractinian coral Fungia scutaria. *Coral Reefs*, 20(3), pp.301–308. Available at: http://link.springer.com/10.1007/s003380100179 [Accessed September 4, 2017].

Weis, V.M., 2008. Cellular mechanisms of Cnidarian bleaching: stress causes the collapse of symbiosis. *Journal of Experimental Biology*, 211(19), pp.3059–3066. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18805804 [Accessed July 19, 2017].

Welsh, R.M. et al., 2015. Alien vs. Predator: Pathogens open niche space for opportunists, unless controlled by predators. Available at: https://peerj.com/preprints/1537/ [Accessed September 14, 2017].

Wild, C., Woyt, H. & Huettel, M., 2005. Influence of coral mucus on nutrient fluxes in carbonate sands. *MARINE ECOLOGY PROGRESS SERIES Mar Ecol Prog Ser*, 287, pp.87–98. Available at: http://www.int-res.com/articles/meps2005/287/m287p087.pdf [Accessed September 15, 2017].

Wilson, W. et al., 2001. Temperature induction of viruses in symbiotic dinoflagellates. *Aquatic Microbial Ecology*, 25(1), pp.99–102. Available at: http://www.int-res.com/abstracts/ame/v25/n1/p99-102/ [Accessed September 14, 2017].

Wilson, W.H. et al., 2005. An enemy within? Observations of virus-like particles in reef corals. *Coral Reefs*, 24(1), pp.145–148. Available at: http://link.springer.com/10.1007/s00338-004-0448-0 [Accessed September 14, 2017].

Van Woesik, R., De Vantier, L. & Glazebrook, J., 1995. Effects of Cyclone "Joy" on nearshore coral communities of the Great Barrier Reef. *Marine Ecology Progress Series*, 128, pp.261–270. Available at: http://www.int-res.com/abstracts/meps/v128/p261-270/ [Accessed September 14, 2017].

Yakovleva, I.I.M. et al., 2009. Algal symbionts increase oxidative damage and death in coral larvae at high temperatures. *Marine Ecology Progress Series*, 378, pp.105–112. Available at: http://www.int-res.com/abstracts/meps/v378/p105-112/ [Accessed August 18, 2017].

Yeoh, S.-R. & Dai, C.-F., 2010. The production of sexual and asexual larvae within single broods of the scleractinian coral, Pocillopora damicornis. *Marine Biology*, 157(2), pp.351–359. Available at: http://link.springer.com/10.1007/s00227-009-1322-y [Accessed September 17, 2017].

Yoda, H., 2006. Polyamine Oxidase Is One of the Key Elements for Oxidative Burst to Induce Programmed Cell Death in Tobacco Cultured Cells. *Plant Physiology*, 142(1), pp.193–206. Available at: http://www.plantphysiol.org/cgi/doi/10.1104/pp.106.080515.

Yuan, C. et al., 2016. Effects of elevated ammonium on the transcriptome of stony coral Pocillopora damicornis. *Marine Pollution Bulletin*, 114(1), pp.46–52. Available at: http://dx.doi.org/10.1016/j.marpolbul.2016.08.036.

Ziegler, M. et al., 2017. Bacterial community dynamics are linked to patterns of coral heat tolerance. *Nature Communications*, 8, pp.1–8. Available at: http://dx.doi.org/10.1038/ncomms14213.

Zilber-Rosenberg, I. & Rosenberg, E., 2008. Role of microorganisms in the evolution of animals and plants: The hologenome theory of evolution. *FEMS Microbiology Reviews*, 32(5), pp.723–735.

ANNEXE 1 : IDENTIFICATION DE LOCUS D'ADAPTATION LOCALE DANS UN CONTEXTE DE FORTE STRUCTURE GÉNÉTIQUE

TITLE : Identifying adaptive loci in a context of strong genetic structure: local adaptation to depth in the red coral *Corallium rubrum*

RUNNING TITLE: Local adaptation to depth in the red coral

AUTHORS: Pratlong M.[1,2], Brener-Raffalli K.[3], Haguenauer A.[1], Mitta G.[3], Toulza E.[3], Bonabaud M.[4], Rialle S.[4] Garrabou J.[5,6], Bensoussan N.[7], Pontarotti P.[2]* & Aurelle D[1]*

AFFILIATIONS

1. Aix Marseille Univ, Avignon Université, CNRS, IRD, IMBE, 13007 Marseille, France

2. Aix Marseille Univ, CNRS, Centrale Marseille, I2M, Marseille, France, Equipe Evolution Biologique et Modélisation, Marseille, France

3. Perpignan Via Domitia Univ., IHPE UMR 5244, CNRS, IFREMER, Montpellier Univ., Perpignan, France

4. UMS BioCampus- MGX Montpellier GenomiX, Institut de Génomique Fonctionelle, 141, rue de la Cardonnille, 34094, Montpellier Cedex 05, France

5. Institute of Marine Sciences (ICM-CSIC), Barcelona, 08003, Spain

6. Aix-Marseille Université, Mediterranean Institute of Oceanography (M I O), Marseille, Université de Toulon, CNRS /IRD, France

7. IPSO FACTO, SCOPArl, Pole Océanologie, Marseille, 13001, France

* These authors jointly supervised this work.

Corresponding author: Marine Pratlong (marine.pratlong@gmail.com)

ABSTRACT

The study of local adaptation is of major concern in a context of global change. Genome scans are promising approaches in this context. In the marine environment they have been applied to various species characterized by very low levels of genetic structure. No such empirical study has been developed yet on marine species with strong genetic structure, despite their theoretical interest in this context. The red coral (*Corallium rubrum*, Cnidaria) is a highly genetically structured species and a promising model for the study of local adaptation along a depth gradient. Here, we used RAD-Sequencing in order to identify signals of local adaptation to depth and thermal regime in the red coral. We designed a sampling scheme with (i) pairs of 'shallow vs deep' populations for which local adaptation may occur according to previous studies of thermotolerance in this species and (ii) three geographical regions as replicates. Our results showed significant genomic differentiation among locations and among depths. The tests of association between genetics and temperature enabled to identify candidate loci under selection but with a potentially high rate of false positives. We discuss the methodological obstacles and biases encountered for the detection of selected loci in such a strongly genetically structured species. On this basis, we discuss the robustness of the candidates for local adaptation detected in each geographical region.

KEYWORDS: Local adaptation, Cnidarians, Population genomics, Ecology

MAIN TEXT

Introduction

Different mechanisms may explain the life and evolution in contrasted environments of species with large ecological range. The balance between environmental conditions, biological traits and evolutionary constrains will shape the output of the interaction between genetic and plastic responses for each species facing different environmental conditions. In the absence of other evolutionary pressures (migration, genetic drift), and if the environmental gradient is persistent for an extended period of time, each local population submitted to local selection could become genetically adapted to the corresponding local environment (Gagnaire & Gaggiotti, 2016; Kawecki & Ebert, 2004). In the case of plasticity or acclimatization, a given genotype can develop during its lifetime morphological or physiological responses allowing an increase in fitness in different environments (DeWitt et al., 1998; Pigliucci, 2001). Although particular situations favoring local adaptation or acclimatization are documented, it is often difficult to disentangle the effects of these two mechanisms and establish their relative contributions to adaptability (Palumbi et al., 2014). In addition, understanding these mechanisms has a fundamental interest in the current context of climate change for improving predictive models and proposing management strategies (Gagnaire & Gaggiotti, 2016; Mumby et al., 2011).

Gene flow is generally considered to be a key factor opposing local adaptation through the input in a population of potentially maladapted individuals (migration load; Lenormand, 2002). This consideration, based on the hypothesis of random gene flow, does not take into account the possibility of habitat selection through which individuals can choose to settle in habitats that maximize their fitness (Edelaar & Bolnick, 2012; Edelaar et al., 2008; Ravigné et

al., 2009; Szulkin et al., 2016). Furthermore, several theoretical studies have shown that gene flow can counteract the effect of genetic drift and promote local adaptation (Alleaume-Benharira et al., 2006; Felsenstein, 1975; Hastings & Rohlf, 1974; Nagylaki, 1978; Slatkin & Maruyama, 1975). These results were confirmed in several studies in both marine and terrestrial environments, where signals of local adaptation have been detected in presence of high gene flow (Conover et al., 2006; Gagnaire & Gaggiotti, 2016; Muir, et al., 2014; Nielsen et al., 2009; Pespeni & Palumbi, 2010; Szulkin et al., 2016). Contrary to expectations, no study were dedicated to the search of local adaptation signal in marine species with very strong genetic structure, based on genome scan methods. Indeed, several such studies have been conducted at very large scale on highly dispersive teleost species (Bernardi et al., 2016; Bradbury et al., 2010; Guo et al., 2016; Limborg et al., 2012; Milano et al., 2014; Wang et al., 2013), while others focused on benthic invertebrates with a highly dispersive, planctonic larvae stage (Araneda et al., 2016; Bay & Palumbi, 2014; Benestan et al., 2016; Chu et al., 2014). The study of local adaptation in a context of high genetic structure may be difficult from a methodological point of view: high average F_{ST} values can lead to a high number of false positives in outliers tests for the detection of selection by the corresponding increase in the variance of F_{ST} values (Bierne et al., 2013; Hoban et al., 2016). Furthermore, in a context of high average genomic differentiation, it could be difficult to identify selected loci with a higher differentiation than expected under the neutral model. Finally, if genetic drift is strong, it can generate outlier loci with apparent correlation with an environmental variable outside any selective effect (Coop et al., 2010; Hofer et al., 2009; Kawecki & Ebert, 2004). Therefore the empirical study of local adaptation in this case remain often unexplored and challenging.

Marine coastal environments offer particularly interesting conditions for studies of local

adaptation, because of the gradual changes in environmental conditions along coastline at small scale, the more or less gradual vertical changes from shallow to deep water and the patchy distribution of contrasted habitats at different scales (Lundgren et al., 2013; Sanford & Kelly, 2011; Wrange et al., 2014). Recently, following worries on global change and the increase in mortality events potentially linked to anthropogenic pressures (Doney et al., 2012; Reusch & Wood, 2007; Somero, 2010; Somero, 2012; Walther et al., 2002), studies of local adaptation became more frequent in coastal ecosystems (Ayre, 1995; Barshis et al., 2010; Barshis et al., 2013; Bay & Palumbi, 2014; Bongaerts et al., 2011; D'Croz & Maté, 2004; Haguenauer et al., 2013; Jin et al., 2016; Kersting et al., 2013; Ledoux et al., 2015; Lundgren et al., 2013; Mayfield et al., 2012; Oliver & Palumbi, 2011; Palumbi et al., 2014; Pivotto et al., 2015; Potts, 1984; Pratlong et al., 2015; Sherman & Ayre, 2008; Smith et al., 2007; Ulstrup & Van Oppen, 2003; Walsh & Somero, 1981; Ziegler et al., 2014). By studying the genetic basis of the adaptation to different current environmental conditions, one might expect understanding the possibility of adaptation to expected future climate conditions. However, few examples confirmed the role of local adaptation in the observed physiological differences between individuals from contrasted environments and the role of individual acclimatization can rarely be excluded (Barshis et al., 2013; Bay & Palumbi, 2014; Jin et al., 2016; Ledoux et al., 2015; Lundgren et al., 2013). In this context, genome scans are powerful approaches to explore adaptive processes in natural populations (Manel et al., 2016).

The red coral (*Corallium rubrum*) is an asymbiotic temperate octocoral distributed from 5 to 1 000 m depth in the Mediterranean sea and the near Atlantic (Boavida et al., 2016, Costantini et al., 2011). It is a sessile and long-living species (more than 100 years), with low growth and recruitments rates (Marschal et al., 2004; Santangelo et al., 2012). The red coral is a

112 particularly low disperser, with populations separated by fewer than 10 m detected as
113 genetically differentiated (Ledoux et al., 2010a; but see Martínez-Quintana et al., 2015). The
114 shallowest populations, above the seasonal thermocline, are submitted to high maximum
115 temperatures and to frequent and intense thermal fluctuations in summer (Haguenauer et al.,
116 2013). The intensity and frequency of extreme thermal events decrease with depth, and the
117 deepest populations are submitted to stable thermal regimes. Only individuals below the
118 maximum depth of seasonal thermocline are expected to be completely naive concerning heat
119 stress. Since the observation of mass mortality events affecting this species during thermal
120 anomalies occuring during summer 1999 and 2003, the relationship between the red coral and
121 its thermal environment has been intensively studied in the region of Marseille (France)
122 (Garrabou et al., 2009; Garrabou et al., 2001). Common garden experiments performed in
123 aquarium highlighted differences in polyp activities, calcification rate, necrosis response and
124 expression of HSP70 between shallow and deep individuals facing thermal stress
125 (comparisons 11 / 40 m in Torrents et al., (2008), 20 / 40 m in Ledoux et al., (2015) and 5 /
126 20 / 40 m in Haguenauer et al., (2013)). Ledoux et al., (2015) conducted reciprocal transplant
127 between individuals from 20 m and 40 m around Marseille and observed a local adaptation
128 signal with a response validating the “local vs. foreign criterion” (Kawecki & Ebert, 2004)
129 The same trend was not observed in Corsica. Finally, transcriptomes of individuals from 5 and
130 40 m in Marseille were compared after a common garden acclimatization and several genes
131 were detected as differentially expressed in a basal state (without the application of any stress;
132 Pratlong et al., 2015).
133 Together, these studies highlighted thermotolerance (i.e. phenotypic) differences between
134 individuals from different depths in Marseille, but we still do not know if these differences are

the result of local adaptation or of individual acclimatization, or both. Previous works on this species enabled us to have a precise idea of the geographic scale at which local adaptation may occur, and will be useful to optimize our sampling design. Because populations from different regions may have evolved similar responses to thermal stress, through similar or different genetic basis, it is interesting to investigate local adaptation in pairs of 'shallow vs deep' populations submitted to contrasted thermal regimes in geographical distinct regions (Hoban et al., 2016; Jones et al., 2012). Finally, the study of neutral genetic structure is essential for a good interpretation of outliers tests for the detection of selection, considering the biases highlighted above.

Here we applied Restriction site Associated DNA sequencing (RAD-seq) to individuals from pairs of 'shallow *vs.* deep' populations in three geographical regions of the Mediterranean Sea and corresponding to distinct genetic clusters. The goal of this study was to characterize the neutral and adaptive genomic variation in this species and to test the possibility of local adaptation to depth through a genome scan approach. Our results enable us to discuss first the neutral genetic structure of the red coral. In a second time, we highlight the methodological obstacles expected in the detection of local adaptation in the context of such genetic structure and how to handle them. Finally, we discuss the robustness of the candidates of local adaptation detected in each geographical region.

Material and methods

Sampling and DNA extraction

Corallium rubrum colonies were collected by scuba diving at two depths of two sites in three geographical regions (Marseille, Banyuls, Corsica) between February and August 2013

(Fig. S1, Table 1). The two depths of each sites presented contrasted thermal regimes (surveys from March 2012 to October 2014; Table 2). The three geographical regions presented different annual variations of temperatures between the two studied depths: a difference of 3.8 °C between the maximum observed at the two depths in Marseille, 1.7 °C in Corsica and 0.5 °C in Banyuls (Table 2). Thirty individuals per site and depth were collected (total 360 individuals), preserved in 95 % ethanol and stored at -20 °C until DNA extraction. Total genomic DNA was extracted according to the protocol of Sambrook et al., (1989), followed by a purification using Qiagen DNeasy blood and tissue spin columns (Qiagen). Genomic DNA concentration was quantified using a Qubit 2.0 Fluorometer (Life Technologies).

RAD-Sequencing

Twelve RAD libraries were prepared according to the protocol described in Etter et al., (2011), with small modifications. Briefly, 1 µg of genomic DNA for each sample was digested using high-fidelity PstI during 60 min at 37 °C. P1 adapters, with 4-6 bp individual barcodes were then ligated to each sample using 0.5 µL of T4 DNA ligase (NEB), 0.5 µL of rATP 100 mM (Promega), 1 µL of DTT 500 mM (Promega), 1 µL of 10X T4 ligase buffer (NEB) and incubated during 60 min at 22 °C, 10 min at 65 °C and 1 min at 64 °C. DNA samples were pooled by 32 (generally by location), sheared, size selected and P2-barcoded. Final PCR for RAD-tags enrichment were performed with 16 cycles and primers dimers were removed during a final AMPure Beads Purification (Agencourt). Libraries were sequenced on an Illumina HiSeq2000 using 100 bp single-end reads, at the Biology Institute of Lille (IBL, UMR 8199 CNRS) and at the MGX sequencing platform in Montpellier (France).

The STACKS pipeline (Catchen et al., 2013; Catchen et al., 2011) was used for the loci *de*

novo assembly and genotyping. Quality filtering and demultiplexing were performed with the *process_radtags* module with default parameters which enables us in particular to remove any read with uncalled base and to performe a phred-33 quality filtering of raw reads. Exact-matching RAD loci (putative orthologous tags) were individually assembled using *ustacks* with a minimum depth of coverage of five reads per allele (m = 5) and a maximum of five nucleotide mismatches between allele (M = 5). *Cstacks* was used to build a catalogue of consensus loci from all individuals, with five mismatches allowed between individuals at the same locus (n = 5). Matches of individual RAD loci to the catalogue of loci were searched using *sstacks*. Finally, the *population* module was used to obtain the loci that were successfully genotyped in at least 75 % of individuals from all populations. Because of the increased of error rates toward the end of the reads, we observed an increase in the number of SNPs from position 86 bp to 91 bp and we removed these positions from the analysis. In order to filter for poor-quality SNPs and artifacts due paralogous sequences, we used VCFtools (Danecek et al., 2011) to remove SNPs that were not at the Hardy-Weinberg equilibrium within at least one of the 12 populations with a p-value threshold of 0.01. SNPs with a minor allele frequencies below 0.01 were removed using VCFTools. Individuals with more than 30 % of missing genotypes were discarded. Finally, only the first SNP of each RAD-tag was kept for further analysis.

Diversity and neutral genetic structure

Global F_{IS} over alleles and gene diversity were estimated using GENEPOP and ARLEQUIN v.3.5 (Excoffier & Lischer, 2010; Rousset, 2008). The *C. rubrum* genetic structure was first analyzed by principal component analysis (PCA) using the package adegenet in R (Jombart,

2008; R Core Team, 2016). This analysis was performed on the total dataset (12 populations) and inside each of the three studied geographical regions (four populations in each). The dataset was centered and missing data were replaced by the mean allele frequency for each locus. In a second step, we performed a Bayesian population clustering implemented in the program STRUCTURE v.2.3.4 (Falush et al., 2003, 2007; Hubisz et al., 2009; Pritchard et al., 2000). We performed ten independent replicates from $K = 1$ to 10 with a burn-in of 50 000 and a number of MCMC iterations after burn-in of 100 000, with the model allowing for admixture and correlated allele frequencies between clusters. We calculated the ΔK statistic of Evanno et al., (2005) to help in the choice of the most appropriate number of genetic clusters. We used CLUMPAK to summarize the STRUCTURE results from the ten independent runs (Kopelman et al., 2015). The global and pairwise populations F_{ST} and exact tests for population differentiation were calculated with GENEPOP (Rousset, 2008). The correlation between the spatial distance between the two depths of the same site and the corresponding population pairwise F_{ST} was tested with the correlation test of Spearman implemented in R (R Core Team, 2016). Finally, we conducted an analysis of molecular variance (AMOVA) in ARLEQUIN v.3.5 (Excoffier & Lischer, 2010) with 10 000 permutations. The hierarchy for this analysis was chosen to follow the three geographical regions of our samples (Marseille, Corsica and Banyuls). This choice was justified by the PCA on the overall dataset. Finally, we performed the PCA and F_{ST} calculation using a dataset comprising only putatively neutral SNPs (without the SNPs detected as outliers by F_{ST} outlier methods, see below).

Detection of local adaptation

In order to search for loci potentially involved in local adaptation, we first used

BAYESCENV (Villemereuil & Gaggiotti, 2015). This method identifies F_{ST} outlier loci that show a relationship between genetic differentiation and environmental differentiation. Runs were performed using default parameters, except the number of pilot runs that was set at 40. The maximal temperature recorded in each site was used as environmental variable (Table 2). We tested other descriptors of the thermal regime and we got similar results (data not shown). The convergence of runs was checked with the Gelman and Rubin's diagnostic using the R package coda (Plummer et al., 2006).

Second, we searched for F_{ST} outliers among red coral populations using ARLEQUIN v.3.5 (Excoffier & Lischer, 2010; Hofer et al., 2009). Because hierarchical genetic structures are known to lead to a high number of false positives in the search of outlier loci (Hofer et al., 2009), we performed this analysis independently in the three geographical regions in order to down a level in the structure. With this method, a distribution of F_{ST} across loci as a function of heterozygosity between populations is obtained by performing simulations under a hierarchical island model that is more appropriate than the island model in the case of the genetic structure of the red coral (two depths in one site and two sites in one geographical region). Outliers were identified as loci being in the tails of the generated distribution ($p < 0.01$). We selected among these candidate loci, those linked with depth differentiation by searching, inside each geographical regions, loci with significant differences in genotypic frequencies between depths ($p < 0.01$). We corrected the obtained p-values using a false discovery rate of 0.05 (Benjamini & Hochberg, 1995)

Finally, we used the R package pcadapt to search for outliers loci by taking into account population structure and individual admixture (Luu et al., 2017). This method is recommended in cases of hierarchical genetic structure for a better control of the false

positive rate. By identifying outliers loci linked with a particular principal component, pcadapt enabled us to focus on candidates linked with our biological question. From the pcadapt analyses, we selected outliers candidates linked with the relevant principal components with a q-value cutoff of 0.01.

Functional annotation and enrichment tests

The RAD tags were aligned on the red coral transcriptome (Pratlong et al., 2015) using the Burrows-Wheeler Alignment Tool (BWA) (Li & Durbin, 2009). Blast2GO was used for the annotation of resulting contigs and functional enrichment analyses (Conesa et al., 2005). First, a blastp was first performed on the NCBI nr database with an e-value threshold of 10^{-10} (Altschul et al., 1990). Then, Blast2GO retrieved Gene Ontology (GO) terms associated with the obtained BLAST hits. Finally, in order to identify function potentially over-represented in outliers, we performed an enrichment analysis using a Fisher's exact test corrected using a false discovery rate of 0.05 (Benjamini & Hochberg, 1995).

Results

RAD-Sequencing and genotyping

An average of 191 ± 21 millions of reads by library was obtained after sequencing. After the demultiplexing and cleaning processes of the STACKS's *process_radtags* module, an average of 180 ± 22 millions of reads by library was obtained. From these reads, we were able to assemble 138 810 unique consensus RAD-tags present in at least 75 % of our 360 individuals. After all quality filter steps (Table 3), 27 461 SNPs were available. Finally, we removed six individuals presenting more than 30 % of missing data (one individual from the

MEJ40 population, two from the BANN40 population, two from the GAL20 population and one from the GAL40 population). Our final dataset used for further analysis consisted in 354 individuals genotyped on 27 461 SNPs.

Genetic diversity

Multilocus values of the F_{IS} ranged between 0.005 (ELV12) and 0.065 (BANS40) (Table 4). Expected heterozygosity varied from 0.12 (GAL20) to 0.18 (all populations of Marseille) (Table 4). Populations of Marseille had higher values of expected heterozygosity than populations from Corsica and Banyuls ($p = 0.02$, Wilcoxon–Mann–Whitney test).

Population structure analysis

The positioning of individuals with respect to the first two principal components (PCA1 and PCA2) reflected the geographical and depth origin of the individuals (Fig. 1A). Individuals from Marseille and from Banyuls formed two clear and homogeneous clusters while individuals from the two sites of Corsica formed two different clusters with an important distance between them on the second axis. The first PCA axis (PCA1) explained 7.44 % of the total genotypic variance and separated individuals from Marseille from individuals from Banyuls and Corsica. The second axis (PCA2) explained 4.61 % of the total genotypic variance and separated individuals from the Porto site in Corsica from other individuals. Interestingly the fifth axis of the PCA separated all individuals according to their sex, independently from their geographical origin (Pratlong et al., 2017). Concerning PCA inside geographical regions, individuals from the two sites of Marseille and Corsica (north and south) were separated along the first axis (7.02 % and 14.09 % of the total genetic variance

296 respectively; Fig. 1B and 1C). The second axis (2.85 % of the total genetic variance)
297 separated populations from the two depths of the south site of Marseille (FIG-MOR), and
298 individuals from the two depths of the north site (ELV-MEJ). In Corsica and Banyuls, no PCA
299 axis showed evident association with depth. Individuals from the two depths of the Galeria
300 (GAL) site of Corsica were separated along the second axis (4.13 % of the total genetic
301 variance) but this was not the case for individuals from the two depths of the Porto site
302 (POR). Finally, individuals from Banyuls showed much less structure than individuals from
303 Marseille and Corsica (Fig. 1D). The first axis (3.11 % of the total genetic variance) separated
304 individuals from the two sites (north and south). The second axis (2.33 % of the total genetic
305 variance) separated individuals according to their sex (Pratlong et al., 2017). The PCA on the
306 overall dataset and inside each geographical region were identical to those results when only
307 putatively neutral SNPs were considered (Fig. S2).
308 The delta(K) criterion (Evanno et al., 2005) indicated K = 2 as the best number of clusters for
309 the STRUCTURE analysis. We present here the results for K = 2 to K = 4 which captured the
310 main information of the results (Fig. 2). In all cases, all clusters corresponded the main
311 geographical boundaries and the two depths of each sites were always clustered together. For
312 K = 2, a clear separation between the Marseille regions and the Corsica/Banyuls regions was
313 observed, confirming the separation of populations along the first PCA axis (Fig. 1). K = 3
314 separated the three geographical regions in 7/10 replicates, and the remaining replicates
315 grouped either one or the other Corsican sites with Banyuls populations (Fig. S3). Finally,
316 K = 4 separated the two Corsican sites.
317 The overall F_{ST} of the total dataset was 0.13. Pairwise F_{ST} values ranged from 0.01 (BANS20
318 vs BANS40, BANS20 vs BANN40 and BANN40 vs BANS40) to 0.24 (ELV12 vs GAL20

and FIG8 vs GAL20) (Table 5). Genetic differentiation was highly significant for all pairwise comparisons ($p < 0.001$), even for populations separated by 10 m ($F_{ST} = 0.022$ for BANN20 vs BANN40, $F_{ST} = 0.012$ for BANS20 vs BANS40 and $F_{ST} = 0.10$ for GAL20 vs GAL40). Considering the between-depths F_{ST}, high F_{ST} values can be observed for different loci and different samples comparisons (Fig. S4). The average F_{ST} between the two depths of the same site was 0.04 in Marseille, 0.08 in Corsica and 0.02 in Banyuls (0.04 for the total dataset). Considering only putatively neutral loci (see below for outliers loci), the overall F_{ST} of the total dataset was 0.12 and pairwise F_{ST} values ranged from 0.01 (BANS20 vs BANS40, BANS20 vs BANN40 and BANN40 vs BANS40) to 0.23 (ELV12 vs GAL20) (Table S1). There was no correlation between the distance between two depths of the same site and the corresponding population pairwise F_{ST} ($p = 0.75$). We obtained the same result ($p = 1$) if we removed the four populations of Marseille whose sampling sites for the two considered depths were not exactly the same (653 m between FIG8 and MOR40 and 995 m between ELV12 and MEJ40). Finally, the F_{ST} between the two shallow sites inside a geographical region was in all three cases higher than those between the two deep sites of the same region (0.10 vs 0.058 in Marseille, 0.20 vs 0.14 in Corsica and 0.025 vs 0.014 in Banyuls, $p < 1.10^{-16}$ in all three comparisons). In a similar way, the F_{ST} between two shallow sites of two different geographical regions were in all cases higher than those between the two corresponding deep sites, except for the comparisons between the Porto sites and the Banyuls sites ($p = 0.38$ and $p = 0.02$ for the POR/BANN and POR/BANS comparisons respectively; $p < 1.10^{-16}$ for the other comparisons).

The AMOVA indicated a similar percentage of the molecular variance explicated by differences among group and within groups (7.8 and 7.07 % respectively) and approximately

342 85 % of variance explicated by differences within populations (Table 6). There was significant
343 genetic differentiation at the three studied levels (F_{ST} = 0.15, F_{SC} = 0.08, F_{CT} = 0.08 ; $p < 0.001$
344 in the three cases).
345
346 Outliers SNPs
347 We identified 82 outliers with BAYESCENV. However, we noticed that all these outliers
348 seemed to be driven by the divergence between particular populations, with one allele being
349 always fixed in one or several populations without logical association with depth.
350 ARLEQUIN detected 563 loci potentially under selection in Marseille, 869 in Corsica and
351 397 in Banyuls. Among these SNPs, all were potentially under divergent selection in
352 Marseille and Banyuls and 207 of the 869 were potentially under balanced selection in
353 Corsica (the remaining 662 were potentially under divergent selection). Considering only
354 these outliers, the overall F_{ST} of the total dataset was 0.25 and pairwise F_{ST} values ranged from
355 0.02 (BANS20 vs BANS40) to 0.42 (GAL20 vs POR40) (Table S2). The 207 loci potentially
356 under balanced selection in Corsica were linked with sex differentiation and were not further
357 analyzed here (Pratlong et al., 2017). Eight outlier SNPs were detected both in Marseille and
358 Banyuls, 12 both in Marseille and Corsica and 12 both in Corsica and Banyuls. No SNP was
359 detected as potentially under divergent selection and common in the three regions. The
360 complementary chi2 test of genotypic frequencies differences between depths inside each
361 geographical region detected 162 candidate loci in Marseille, 1 371 in Corsica and 3 in
362 Banyuls, of which 35, 248 and 2 where also respectively detected with the ARLEQUIN
363 analysis. These numbers of candidate loci were correlated with the variance and the average
364 of F_{ST} values inside each geographical regions (correlation coefficient of 0.97). The second

axis of the PCA using the Marseille individuals showed apparent association with depth and appeared to be influenced by the variation of the candidates for local adaptation to depth detected by ARLEQUIN: 51 % of these loci were in the top 1 % of the axis contributions, and 86 % were in the top 5 %.

Because the Marseille region is the only one presenting a principal component linked with the depth (Fig. 1B), the pcadapt results obtained for the Corsica et Banyuls regions have poor biological relevance for our biological question. We chose thus to present only the pcadapt results obtained for the Marseille region. pcadapt detected 58 outliers loci linked with the second PCA axis, the one which was linked to depth differentiation. All these candidates were detected by the ARLEQUIN analyses and 20 were also common with the chi2 test presented above.

Functional annotation

Among the 27 461 analyzed RAD-tags, 6 376 had hits on the red coral transcriptome (23.2 %). Concerning SNPs detected as outliers by ARLEQUIN and contributing to the depth divergence, 8 on the 35 detected in Marseille had hits on the transcriptome, 46 on the 248 detected in Corsica and 2 on the 2 detected in Banyuls (Table S3). We did not observed any GO term enriched in coding regions among candidates SNPs, nor any functional enrichment in these outliers.

Discussion

Genetic diversity and structure

Our results confirm the high genetic structure of the red coral, which was already observed

388 with microsatellite loci (Costantini et al., 2007; Ledoux et al., 2010b). These results could be
389 the consequence of reduced mean larval dispersal distance (but see Martínez-Quintana et al.,
390 (2015). Genetic incompatibilities could also contribute to the observed differentiation at least
391 for some loci (see discussion on outliers below; Kulmuni & Westram, 2017). Our analysis of
392 the genetic structure of the red coral revealed several clusters mainly corresponding to the
393 geographical distributions of individuals. This structure is close to the one evidenced by
394 Ledoux et al. (2010b) by using microsatellites with similar sampling locations, and suggested
395 that it may partly be the result of the absence of suitable habitats between these regions and of
396 reduced dispersal (bathymetric barrier between Corsica and the continent and few shallow
397 rocky substrates between Marseille and Banyuls). The relative and unexpected proximity of
398 the populations from Banyuls and the two populations of Porto (POR20 and POR40)
399 according to the first component of our PCA was also suggested in the STRUCTURE analysis
400 of microsatellite data (Ledoux et al., 2010b). We now confirm this at the genomic level. A
401 high differentiation was observed here with PCA and STRUCTURE between the two sites of
402 Corsica separated by around 22 km. This was observed with microsatellites as well, but for
403 populations separated by around 15 km by Ledoux et al. (2010b). This pattern of genetic
404 structure could be explained by a putative barrier to gene flow (e.g. currents or lack of
405 suitable habitats) between Porto and the Punta Mucchilina in the south of the natural reserve
406 of Scandola. Another hypothesis would be that different lineages are present in this area. Such
407 lineages could have evolved in allopatry during past climatic fluctuations. This could explain
408 that the differentiation in Corsica was higher than the differentiation observed between the
409 two sites of Marseille despite a similar distance at sea. In addition the partial genetic
410 similarity between Porto and Banyuls could be the result of sporadic gene flow between the

411 two clusters but which did not extend further than the putative barrier in Corsica.
412 We reported here a significant vertical genetic structure between the two depths of the same
413 site (populations separated by less than 20 m). This fine-scale genetic structure has been
414 repetitively reported for the red coral with microsatellites (Costantini et al., 2011; Ledoux et
415 al., 2010a; b). Contrary to Prada et al., (2008) who showed the existence of two cryptic
416 lineages at two different depths in a tropical octocoral, the populations of red coral from the
417 two studied depths clearly correspond here to the same species, as the differentiation between
418 depth was lower than the differentiation between sites and regions. This structure may be the
419 result of both inherent life history traits (negative-phototropism of larvae) and environmental
420 variables. Weinberg (1979) has described a negative geotropism for the planulae of *C.*
421 *rubrum*, and Martínez-Quintana et al., (2015) demonstrated that this was an active behavior.
422 Depending on the orientation of the substrate, this might limit the connectivity between
423 depths. A physical barrier could also hinder the vertical connectivity: this could be the result
424 of a seasonal stratification during the larval emission (which can occur from June to
425 September in the Marseille region, Haguenauer A., pers. comm., Santangelo et al., 2003).
426 Differences in the spawning period have been proposed as potentially contributing to the
427 vertical differentiation as well (Costantini et al., 2011). Nevertheless even if the local thermal
428 conditions induce such shifts, if larvae dispersed randomly according to depth, this would not
429 lead to an important and stable differentiation. According to these hypotheses, the larval
430 behavior and putative vertical barriers should lead to a genome-wide neutral differentiation
431 between depths. The strength of this genetic structure between two depths of the same site
432 was more or less pronounced according to the considered site, without correlation with the
433 spatial distance between the two depths. Thus, even if inherent life history traits (dispersal

abilities, larval behavior) may have a significant impact on the genetic differentiation of such close populations, it seems that the differentiation between the two depths was influenced partly by local environmental constraints.

The horizontal genetic differentiation between the two shallow sites was higher than those between the two deep sites, at the scale of a geographical region, but also for comparisons between regions. This suggests a higher connectivity or lower rate of genetic drift for deep populations compared to shallow ones. Low effective population size, repeated bottlenecks and founder events are known to lead to an impoverishment of gene diversity and higher genetic structure (Brussard, 1984; Hoffmann & Blows, 1994). The repeated colonization of shallow depths from deeper ones or the higher harvesting pressure on shallow populations compared to deep one could lead to higher genetic drift and differentiation (Cannas et al., 2016; Rossi et al., 2008). Nevertheless we did not observe here a reduction in gene diversity for shallow populations. Connectivity differences then seem to be more probable in explaining the observed differences of genetic differentiation. Interestingly, Rossi *et al.* (2008) observed a higher frequency of patches of red coral below 50 m compared to above 50 m: if such pattern is present in other areas, then it could modify the connectivity levels through stepping stones migration. In a dedicated study of the vertical genetic structure of red coral in two western Mediterranean sites (Cap de Creus, Spain and Portofino, Italia), Costantini et al., (2011) observed a drop in connectivity around 40 – 50 m depth with a level of genetic diversity declining with depth. Our sampling scheme did not allow us to specifically test the same hypotheses but it would be interesting to extend our RAD Seq study along a depth gradient to better understand the genetic structure of this species.

Potential biases in the search of outlier loci

High F_{ST} values can be observed in our dataset for different loci and different sample comparisons, and not only for comparisons between depths (Fig. S4). It is highly improbable that all these loci and comparisons are shaped by divergent selection, because adaptive polymorphism is expected to be quite rare at the genome scale. It therefore indicates that neutral processes (i.e. mainly drift) alone generate high differentiation and high variance in F_{ST} in the red coral. Such patterns can then easily lead to false signals of divergent selection. The methods of detection of outlier loci used here are well suited for the detection of loci presenting high allele shifts between populations (adaptive traits controlled by few genes with large effects; Pritchard & Di Rienzo (2010), Gagnaire & Gaggiotti (2016)). The high number of outliers detected here in the three geographical regions by these methods raised thus concern on the biological relevance of these candidates. Apart from this effect, both the hierarchical genetic structure and the average level of differentiation of the red coral are known to lead to a high number of false positives and make the interpretation of results difficult (Bierne et al., 2013; Hoban et al., 2016). In this study, we set up a sampling design of pairs of geographically close 'shallow vs deep' populations in order to reduce signals from loci not under selection and to limit the effects of strong genetic structure on the detection of outliers (as recommended by Hoban et al. (2016)). Thanks to this sampling we could demonstrate a positive correlation between the number of outliers detected and both the variance and average of F_{ST} values inside each geographical region, with the strongest effect in Corsica. Such approach is then useful to envision the putative impact of false positive in this context.

The use of reduced representation approach such as RAD-seq raises another difficulty: the

markers density may be insufficient to detect a RAD-tag in linkage disequilibrium with a selected locus (Lowry et al., 2016, Catchen et al., 2017, McKinney et al., 2016). With 138 810 detected SNPs and a genome size of about 500 Mb, we expected in the case of the red coral, a SNP sampling of 1 for 3,60 kb (278 SNPs per Mb) and 1 for 18 kb after the SNPs filtering steps (55 SNPs per Mb). Without a reliable estimation of the linkage disequilibrium in our species, we are not able to estimate properly the proportion of the genome covered by our RAD-tags. The level of linkage disequilibrium is unknwon in the red coral, but the reduced gene flow and high genetic drift probably lead to much higher linkage disequilibrium (maybe a few kb) than in most other marine invertebrates. The genomic architecture of adaptation is also unknown but considering these observations, we expected that our RAD-tags may at least detect a signal of genetic adaptation, even if we did not detect a certain number of genomic regions under selection.

The strength of differences in allele frequencies between populations from different depths as a result of divergent selection should be correlated to the strength of divergent selective pressures between the two environments. In the red coral, it seems that the number of days spent above a threshold temperature is an appropriate variable to predict mortality events (Coma et al., 2009, Crisci et al., 2011). We did not report this variable in Table 2 because it is highly correlated with the standard deviation (Pearson correlation of 0.76, p value = 0.0045). Thus, if the temperature is a selective pressure in red coral population, it seems reasonable to consider its standard deviation as a crude estimate of its strength. In our case, the differences of standard deviation of temperature between the studied depths were higher in the Marseille sites than in the Corsican ones, and were very low in the Banyuls sites (Table 2). If, as hypothesized here, the local adaptation to depth in the red coral is driven by the thermal

environment of individuals, we expected therefore to observe a stronger signal of selection in Marseille than in Corsica and Banyuls, as it is the case here. Thus, the number of candidates detected in the three geographical regions could be related to the rate of false positives, which is correlated to the global genetic structure (see above); but also to the difference in selective pressure between the two studied depths. Unfortunately, in our study, the ecological and genetic distances were paired in such a way that we couldn't precisely evaluate the respective impact of false positives and true positives in the number of outliers detected.

Considering these limits, and in order to identify the most promising candidate genes, we applied a combination of different methods and a careful evaluation of the general structure and of the loci shaping differences between depths. But other approaches could be used which would take into account genomic information for the study of adaptation (Manel et al., 2016). Daub et al., (2013) developed a method to study polygenic adaptation: this could be useful here as it may be less sensitive to single locus stochastic effects. Nevertheless the genomic information on red coral are not precise enough for such approach. Studying the genomic distribution of parameters such as F_{ST} or nucleotide diversity, or the link between candidate SNPs and genes, would also be interesting complementary approaches (e.g. Hohenlohe et al., 2010) but a reference genome is still lacking for the red coral.

Apart from the detection of selected loci, the observed levels of genetic structure raises an interrogation on the evolution of local adaptation. Indeed whereas the red coral displays life history traits potentially favorable to the evolution of local adaptation (i.e. reduced dispersal limiting gene swamping; Lenormand (2002), but see Alleaume-Benharir et al., (2006)), its genetic characteristics hinder the detection of adaptive loci: this species probably displays metapopulations with small, isolated, local populations. In each local population the reduced

effective size could lead to important genetic drift (Ledoux et al., 2010b). Genetic drift can counteract the effects of local selection and could limit differences in allele frequencies for low to moderately selected loci. High genetic drift has been proposed to limit local adaptation to temperature in the Mediterranean octocoral *Paramuricea clavata* which displays a genetic structure similar to red coral (Crisci et al., 2017; Mokhtar-Jamaï et al., 2011). A less discussed point is that among the candidate loci detected here, some could be linked not to local adaptation but to intrinsic genetic incompatibilities whose allelic frequencies could be coupled with environmental barriers (Bierne et al., 2011). This could especially be the case between the two sites in Corsica. The frequency of genetic incompatibilities in marine populations is largely unknown and probably under-estimated (Plough et al., 2016). Even if not directly linked to local adaptation, such loci are part of the genomic landscape of this species along heterogeneous environments.

Local adaptation to depth in the red coral

By keeping in mind the aforementioned potential biases, we focused on candidate loci meeting the following criteria: i) detection with ARLEQUIN and pcadapt, ii) presence of a strong signal of differentiation between depth, iii) function relevant to the adaptation to thermal regime. These are the most promising loci for the study of genetic adaptation

The absence of candidate SNPs common to the three geographical regions could indicate that the adaptation to comparable shallow environmental pressures in these independent regions are based on different genetic pathways, or on non-genetic mechanisms (Putnam & Gates, 2015). However, the RAD-Sequencing enables to sequence a reduced proportion of the genome and the probability to observe convergent positions is small, simply because they

549 may not be sequenced. Furthermore, differences in the strength of selective pressure in the
550 three regions could also explain the differences in the detected loci. In Marseille we evidenced
551 a clear signal of differentiation between depths according to multivariate analysis, which was
552 confirmed by outlier loci. This detection of a signal of local adaptation in the Marseille region
553 is consistent with the observations from studies of thermotolerance differences in this region
554 (Haguenauer et al., 2013; Ledoux et al., 2015; Pratlong et al., 2015; Torrents et al., 2008). In
555 the case of the Marseille region there are then strong evidences of the existence of adaptive
556 differentiation at a scale of few tens of meters only. Concerning Corsican populations, Ledoux
557 *et al*. (2015) reported no phenotypic signal of local adaptation after reciprocal transplant
558 experiment. However, Ledoux et al., (2015) studied a phenotypic trait, diameter growth,
559 which may not be linked to adaptive abilities in all regions and genetic contexts. The most
560 promising candidate for the adaptation to thermal regime detected in Corsica, is an
561 homologous to an allene oxide synthase-lipoxygenase which is known to be involved in the
562 response to thermal stress in octocorals (Lõhelaid et al., 2015). Finally, the detection of only
563 two candidates to local adaptation to depth in the Banyuls region was consistent with the
564 selective pressure that should be weaker in this region (see above, Table 2). Moreover, the
565 Banyuls populations were also the ones with the lowest genetic structure between populations
566 from the two depths, a strongest effect of migration load can thus also limit the evolution of
567 local adaptation and allelic differences on selected loci (but see Alleaume-Benharira et al.,
568 (2005)).
569 The life in different environments can also rely on acclimatization. Until now, the role of local
570 acclimatization has mostly been highlighted in thermotolerance differences studies in
571 Cnidaria, probably because of the high plasticity abilities often observed in coral species

572 together with the difficulty to demonstrate the impact of genetic adaptation (Haguenauer et
573 al., 2013). Here, we showed that local adaptation seems to play a role in the observed
574 thermotolerance differences between individuals separated by only several meters in the red
575 coral. Local adaptation could lead to different acclimatization abilities between populations as
576 well. A similar pattern of correlation between gene sequences and thermal environment has
577 been discovered by Bay & Palumbi (2014) in *Acropora hyacinthus*, between non
578 differentiated sites of American Samoa but none of the functions highlighted were detected in
579 our study. We conducted common garden experiment and transcriptome sequencing of
580 individuals from two depths of a site in the Marseille region (Pratlong et al., 2015). We
581 detected several genes differentially expressed and others presenting alleles differentially
582 fixed between individuals from the two depths, but none of these genes were common with
583 the present analysis. However, differentially expressed genes may result from acclimatization
584 differences between individuals linked to epigenetic differences, sequence polymorphisms in
585 a control region or related to a transcription factor as well as sequence polymorphism in the
586 coding regions. Without a sequenced genome, we couldn't infer the potential physical
587 association between outlier loci and genes (i.e. are they inside or near coding or regulatory
588 regions), unless it is positioned in the coding region, which is rare.

Conclusion

To our knowledge, the red coral presented the highest level of differentiation among studies of local adaptation thought genome scans approaches in marine environment (Araneda et al., 2016; Bay & Palumbi, 2014; Benestan et al., 2016; Bernardi et al., 2016; Bradbury et al., 2010; Chu et al., 2014; Guo et al., 2016; Limborg et al., 2012; Milano et al., 2014; Wang et al., 2013). This study enabled us to empirically emphasize the limitations in the detection and the interpretation of signals of local adaptation using usual statistical methods in this strongly structured species. Both neutral an adaptive processes studied here highlighted the genetically singularity of shallow populations of the red coral, especially in the Marseille region were the shallowest populations are found. Together, the strong genetic structure we observed between shallow populations, the low dispersal abilities of the red coral and the local adaptation of these individuals to the highly variable thermal conditions they experiment raised strong concerns about the evolution of shallow populations and the possibility of loss of adaptive variations in case of local extinctions (Garrabou et al., 2001; Garrabou et al., 1998; Garrabou & Team, 2003; Torrents et al., 2005). From a methological point of view, although the extend of linkage disequilibrium should be considered in this type of study, it cannot be the only parameter invoked to decide whether or not to conduct a local adaptation experiment as other biological and genomic characteristics obviously play a significant role in the complexity of the analysis (Catchen et al., 2017).

608 Acknowledgements:

609 This work is a contribution to the Labex OT-Med (n° ANR-11-LABX-0061) funded by the
610 French Government “Investissements d’Avenir” program of the French National Research
611 Agency (ANR) through the A*MIDEX project (n° ANR-11-IDEX-0001-02). This project has
612 been funded by the ADACNI program of the French National Research Agency (ANR)
613 (project n°ANR-12-ADAP-0016; http://adacni.imbe.fr). We thank ECCOREV Research
614 Federation (FR 3098) for the financial support of part of this study. We thank Nicolas
615 Fernandez and Béatrice Loriod from the Marseille TGML platform for their invaluable help
616 and advice with the preparation of the RAD libraries; Véronique Dhennin from the Biology
617 Institute of Lille. We thank the molecular biology service of the IMBE, the informatic service
618 of the Pytheas Institute (especially Maurice Libes and Christophe Yohia) and Frédéric Zuberer
619 of the Pytheas Institute for his support in sampling. We thank the Scandola Natural Reserve,
620 especially Jean-Marie Dominici. We thank Manuela Carenzi for her help with statistical
621 aspects of the detection of local adaptation. We thank Pierre-Alexandre Gagnaire, François
622 Bonhomme and Nicolas Bierne for stimulating discussions.

References

Alleaume-Benharira, M., Pen, I. R., & Ronce, O. (2006). Geographical patterns of adaptation within a species' range: interactions between drift and gene flow. *Journal of Evolutionary Biology*, *19*(1), 203–215. doi: 10.1111/j.1420-9101.2005.00976.x

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, *215*(3), 403–410. doi: 10.1016/S0022-2836(05)80360-2

Araneda, C., Larraín, M. A., Hecht, B., & Narum, S. (2016). Adaptive genetic variation distinguishes Chilean blue mussels (*Mytilus chilensis*) from different marine environments. *Ecology and Evolution*, *6*(11), 3632–3644. doi: 10.1002/ece3.2110

Ayre, D. J. (1995). Localized adaptation of sea anemone clones: evidence from transplantation over two spatial scales. *Journal of Animal Ecology, 64(2)*, 186–196. doi: 10.2307/5754

Barshis, D. J., Ladner, J. T., Oliver, T. A., Seneca, F. O., Traylor-Knowles, N., & Palumbi, S. R. (2013). Genomic basis for coral resilience to climate change. *Proceedings of the National Academy of Sciences*, *110*(4), 1387–1392. doi: 10.1073/pnas.1210224110

Barshis, D. J., Stillman, J. H., Gates, R. D., Toonen, R. J., Smith, L. W., & Birkeland, C. (2010). Protein expression and genetic structure of the coral *Porites lobata* in an environmentally extreme Samoan back reef: does host genotype limit phenotypic plasticity? *Molecular Ecology*, *19*(8), 1705–1720. doi: 10.1111/j.1365-294X.2010.04574.x

Bay, R. A., & Palumbi, S. R. (2014). Multilocus adaptation associated with heat resistance in reef-building corals. *Current Biology*, *24*(24), 2952–2956. doi: 10.1016/j.cub.2014.10.044

Benestan, L., Quinn, B. K., Maaroufi, H., Laporte, M., Rochette, R., & Bernatchez, L. (2016). Seascape genomics provides evidence for thermal adaptation and current-mediated population structure in American lobster (*Homarus americanus*). *Molecular Ecology*, *25*(20), 5073-5092. doi: 10.1111/mec.13811

Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society. Series B (Methodological)*, *57*(1), 289–300. doi: 10.2307/2346101

Bernardi, G., Azzurro, E., Golani, D., & Miller, M. R. (2016). Genomic signatures of rapid adaptive evolution in the bluespotted cornetfish, a Mediterranean Lessepsian invader. *Molecular Ecology, 25(14), 3384-339*6. doi:10.1111/mec.13682

Bierne, N., Roze, D., & Welch, J. J. (2013). Pervasive selection or is it…? why are FST outliers sometimes so frequent? *Molecular Ecology*, *22*(8), 2061–2064. doi: 10.1111/mec.12241

Boavida J, Paulo D, Aurelle D, Arnaud-Haond S, Marschal C, et al. (2016) A well-kept treasure at depth: precious red coral rediscovered in Atlantic deep coral gardens (SW Portugal) after 300 Years. PLOS ONE 11(1): e0147228. doi: 10.1371/journal.pone.0147228.

Bongaerts, P., Riginos, C., Hay, K. B., Oppen, M. J. van, Hoegh-Guldberg, O., & Dove, S. (2011). Adaptive divergence in a scleractinian coral: physiological adaptation of *Seriatopora hystrix* to shallow and deep reef habitats. *BMC Evolutionary Biology*, *11*(1), 303. doi: 10.1186/1471-2148-11-303

Bradbury, I. R., Hubert, S., Higgins, B., Borza, T., Bowman, S., Paterson, I. G., … Bentzen, P. (2010). Parallel adaptive evolution of Atlantic cod on both sides of the Atlantic Ocean

669 in response to temperature. *Proceedings of the Royal Society of London B: Biological*
670 *Sciences*, *277*(1701), 3725–3734. doi: 10.1098/rspb.2010.0985
671 Brussard, P. F. (1984). Geographic patterns and environmental gradients: the central-marginal
672 model in *Drosophila* revisited. *Annual Review of Ecology and Systematics*, *15*(1), 25–
673 64. doi: 10.1146/annurev.es.15.110184.000325
674 Cannas, R., Sacco, F., Cau, A., Cuccu, D., Follesa, M. C., & Cau, A. (2016). Genetic
675 monitoring of deep-water exploited banks of the precious Sardinia coral *Corallium*
676 *rubrum* (L., 1758): useful data for a sustainable management: genetic monitoring of
677 exploited banks of the precious sardinia coral. *Aquatic Conservation: Marine and*
678 *Freshwater Ecosystems*, *26*(2), 236–250. doi: 10.1002/aqc.2522
679 Catchen, J., Hohenlohe, P. A., Bassham, S., Amores, A., & Cresko, W. A. (2013). Stacks: an
680 analysis tool set for population genomics. *Molecular Ecology*, *22*(11), 3124–3140. doi:
681 10.1111/mec.12354
682 Catchen, J. M., Hohenlohe, P. A., Bernatchez, L., Funk, W. C., Andrews, K. R., & Allendorf,
683 F. W. (2017). Unbroken: RADseq remains a powerful tool for understanding the
684 genetics of adaptation in natural populations. *Molecular Ecology Resources*, *17*(3),
685 362-365. doi: 10.1111/1755-0998.12669
686 Catchen, J. M., Amores, A., Hohenlohe, P., Cresko, W., & Postlethwait, J. H. (2011). Stacks:
687 building and genotyping loci de novo from short-read sequences. *G3: Genes,*
688 *Genomes, Genetics*, *1*(3), 171–182. doi: 10.1534/g3.111.000240
689 Chu, N. D., Kaluziak, S. T., Trussell, G. C., & Vollmer, S. V. (2014). Phylogenomic analyses
690 reveal latitudinal population structure and polymorphisms in heat stress genes in the
691 North Atlantic snail *Nucella lapillus*. *Molecular Ecology*, *23*(7), 1863–1873. doi:
692 10.1111/mec.12681
693 Coma, R., Ribes, M., Serrano, E., Jiménez, E., Salat, J., & Pascual, J. (2009). Global
694 warming-enhanced stratification and mass mortality events in the Mediterranean.
695 *Proceedings of the National Academy of Sciences*, 106(15), 6176-6181. doi:
696 10.1073/pnas.0805801106
697 Conesa, A., Götz, S., García-Gómez, J. M., Terol, J., Talón, M., & Robles, M. (2005).
698 Blast2GO: a universal tool for annotation, visualization and analysis in functional
699 genomics research. *Bioinformatics*, *21*(18), 3674–3676. doi:
700 10.1093/bioinformatics/bti610
701 Conover, D. O., Clarke, L. M., Munch, S. B., & Wagner, G. N. (2006). Spatial and temporal
702 scales of adaptive divergence in marine fishes and the implications for conservation.
703 *Journal of Fish Biology*, *69*(sc), 21–47. doi: 10.1111/j.1095-8649.2006.01274.x
704 Coop, G., Witonsky, D., Rienzo, A. D., & Pritchard, J. K. (2010). Using environmental
705 correlations to identify loci underlying local adaptation. *Genetics*, *185*(4), 1411–1423.
706 doi: 10.1534/genetics.110.114819
707 Costantini, F., Fauvelot, C., & Abbiati, M. (2007). Genetic structuring of the temperate
708 gorgonian coral (*Corallium rubrum*) across the western Mediterranean Sea revealed
709 by microsatellites and nuclear sequences. *Molecular Ecology*, *16*(24), 5168–5182. doi:
710 10.1111/j.1365-294X.2007.03579.x
711 Costantini, F., Rossi, S., Pintus, E., Cerrano, C., Gili, J.-M., & Abbiati, M. (2011). Low
712 connectivity and declining genetic variability along a depth gradient in *Corallium*
713 *rubrum* populations. *Coral Reefs*, *30*(4), 991–1003. doi: 10.1007/s00338-011-0771-1
714 Crisci, C., Bensoussan, N., Romano, J. C., & Garrabou, J. (2011). Temperature anomalies and

715 mortality events in marine communities: insights on factors behind differential
716 mortality impacts in the NW Mediterranean. *PLoS One*, *6*(9), e23814. doi:
717 10.1371/journal.pone.0023814
718 Crisci, C., Ledoux, J. B., Mokhtar-Jamaï, K., Bally, M., Bensoussan, N., Aurelle, D., ... &
719 Linares, C. (2017). Regional and local environmental conditions do not shape the
720 response to warming of a marine habitat-forming species. *Scientific Reports*, *7*(1),
721 5069. doi: 10.1038/s41598-017-05220-4
722 Danecek, P., Auton, A., Abecasis, G., Albers, C. A., Banks, E., DePristo, M. A., … Durbin, R.
723 (2011). The variant call format and VCFtools. *Bioinformatics*, *27*(15), 2156–2158. doi:
724 10.1093/bioinformatics/btr330
725 Daub, J. T., Hofer, T., Cutivet, E., Dupanloup, I., Quintana-Murci, L., Robinson-Rechavi, M.,
726 & Excoffier, L. (2013). Evidence for polygenic adaptation to pathogens in the human
727 genome. *Molecular biology and evolution*, *30*(7), 1544-1558. doi:
728 10.1093/molbev/mst080
729 D'Croz, L., & Maté, J. L. (2004). Experimental responses to elevated water temperature in
730 genotypes of the reef coral *Pocillopora damicornis* from upwelling and non-upwelling
731 environments in Panama. *Coral Reefs*, *23*(4), 473–483. doi: 10.1007/s00338-004-
732 0397-7
733 DeWitt, T. J., Sih, A., & Wilson, D. S. (1998). Costs and limits of phenotypic plasticity.
734 *Trends in Ecology & Evolution*, *13*(2), 77–81. doi: 10.1016/S0169-5347(97)01274-3
735 Doney, S. C., Ruckelshaus, M., Duffy, J. E., Barry, J. P., Chan, F., English, C. A., ... &
736 Talley, L. D. (2011). Climate change impacts on marine ecosystems. *Annual Review of*
737 *Marine Science*, *4*, 11-37. doi: 10.1146/annurev-marine-041911-111611
738 Edelaar, P., & Bolnick, D. I. (2012). Non-random gene flow: an underappreciated force in
739 evolution and ecology. *Trends in Ecology & Evolution*, *27*(12), 659–665. doi:
740 10.1016/j.tree.2012.07.009
741 Edelaar, P., Siepielski, A. M., & Clobert, J. (2008). Matching habitat choice causes directed
742 gene flow: a neglected dimension in evolution and ecology. *Evolution*, *62*(10), 2462–
743 2472. doi: 10.1111/j.1558-5646.2008.00459.x
744 Etter, P. D., Bassham, S., Hohenlohe, P. A., Johnson, E. A., & Cresko, W. A. (2011). SNP
745 discovery and genotyping for evolutionary genetics using RAD sequencing.
746 *Molecular Methods for Evolutionary Genetics*, 772, 157–178. doi: 10.1007/978-1-
747 61779- 228-1_9
748 Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals
749 using the software STRUCTURE: a simulation study. *Molecular Ecology*, *14*(8),
750 2611–2620. doi: 10.1111/j.1365-294X.2005.02553.x
751 Excoffier, L., & Lischer, H. E. L. (2010). Arlequin suite ver 3.5: a new series of programs to
752 perform population genetics analyses under Linux and Windows. *Molecular Ecology*
753 *Resources*, *10*(3), 564–567. doi: 10.1111/j.1755-0998.2010.02847.x
754 Falush, D., Stephens, M., & Pritchard, J. K. (2003). Inference of population structure using
755 multilocus genotype data: linked loci and correlated allele frequencies. *Genetics*,
756 *164*(4), 1567–1587. doi: 10.1111/j.1471-8286.2007.01758.x
757 Falush, D., Stephens, M., & Pritchard, J. K. (2007). Inference of population structure using
758 multilocus genotype data: dominant markers and null alleles. *Molecular Ecology*
759 *Notes*, *7*(4), 574–578. doi: 10.1111/j.1471-8286.2007.01758.x
760 Felsenstein, J. (1975). Genetic drift in clines which are maintained by migration and natural-

761 selection. *Genetics*, *81*(1), 191–207. doi: 10.1534/genetics.106.059881
762 Gagnaire, P.-A., & Gaggiotti, O. E. (2016). Detecting polygenic selection in marine
763 populations by combining population genomics and quantitative genetics approaches.
764 *Current Zoology*, 62(6), 603-616. doi: 10.1093/cz/zow088
765 Garrabou, J., Coma, R., Bensoussan, N., Bally, M., Chevaldonné, P., Cigliano, M., …
766 Cerrano, C. (2009). Mass mortality in Northwestern Mediterranean rocky benthic
767 communities: effects of the 2003 heat wave. *Global Change Biology*, *15*(5), 1090–
768 1103. doi: 10.1111/j.1365-2486.2008.01823.x
769 Garrabou, J., Perez, T., Sartoretto, S., & Harmelin, J. G. (2001). Mass mortality event in red
770 coral *Corallium rubrum* populations in the Provence region (France, NW
771 Mediterranean). *Marine Ecology Progress Series*, *217*, 263–272. doi:
772 10.3354/meps217263
773 Garrabou, J., Sala, E., Arcas, A., & Zabala, M. (1998). The impact of diving use on marine
774 rocky sublittoral communities: a case study of a Mediterranean bryozoan population.
775 *Conservation Biology*, *12*(2), 1–12. doi: 10.1111/j.1523-1739.1998.96417.x
776 Garrabou, J., & Team, D. (2003). Is global change a real threat for conservation of the NW
777 Mediterranean marine biodiversity? In *EGS-AGU-EUG Joint Assembly* (Vol. 1, p.
778 10522). doi: 10.1111/j.1466-8238.2011.00697.x
779 Guo, B., Li, Z., & Merilä, J. (2016). Population genomic evidence for adaptive differentiation
780 in the Baltic Sea herring. *Molecular Ecology*, *25*(12), 2833–2852. doi:
781 10.1111/mec.13657
782 Haguenauer, A., Zuberer, F., Ledoux, J.-B., & Aurelle, D. (2013). Adaptive abilities of the
783 Mediterranean red coral *Corallium rubrum* in a heterogeneous and changing
784 environment: from population to functional genetics. *Journal of Experimental Marine*
785 *Biology and Ecology*, *449*, 349–357. doi: 10.1016/j.jembe.2013.10.010
786 Hastings, A., & Rohlf, F. J. (1974). Gene flow: effect in stochastic models of differentiation.
787 *The American Naturalist*, *108*(963), 701–705. doi: 10.1086/282947
788 Hoban, S., Kelley, J. L., Lotterhos, K. E., Antolin, M. F., Bradburd, G., Lowry, D. B., …
789 Winn, A. A. (2016). Finding the genomic basis of local adaptation: pitfalls, practical
790 solutions, and future directions. *The American Naturalist*, 188(4), 379-397. doi:
791 10.1086/688018
792 Hofer, T., Ray, N., Wegmann, D., & Excoffier, L. (2009). Large allele frequency differences
793 between human continental groups are more likely to have occurred by drift during
794 range expansions than by selection. *Annals of Human Genetics*, *73*(1), 95–108. doi:
795 10.1111/j.1469-1809.2008.
796 Hoffmann, A. A., & Blows, M. W. (1994). Species borders: ecological and evolutionary
797 perspectives. *Trends in Ecology & Evolution*, *9*(6), 223–227. doi: 10.1016/0169-
798 5347(94)90248-8
799 Hohenlohe, P. A., Bassham, S., Etter, P. D., Stiffler, N., Johnson, E. A., & Cresko, W. A.
800 (2010). Population genomics of parallel adaptation in threespine stickleback using
801 sequenced RAD tags. *PLoS genetics*, *6*(2), e1000862. doi:
802 10.1371/journal.pgen.1000862
803 Hubisz, M. J., Falush, D., Stephens, M., & Pritchard, J. K. (2009). Inferring weak population
804 structure with the assistance of sample group information. *Molecular Ecology*
805 *Resources*, *9*(5), 1322–1332. doi: 10.1111/j.1755-0998.2009.02591.x
806 Jin, Y. K., Lundgren, P., Lutz, A., Raina, J.-B., Howells, E. J., Paley, A. S., … Oppen, M. J. H.

van. (2016). Genetic markers for antioxidant capacity in a reef-building coral. *Science Advances*, *2*(5), e1500842. doi: 10.1126/sciadv.1500842

Jombart, T. (2008). Adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics*, *24*(11), 1403–1405. doi: 10.1093/bioinformatics/btn129

Jones, F. C., Grabherr, M. G., Chan, Y. F., Russell, P., Mauceli, E., Johnson, J., … Kingsley, D. M. (2012). The genomic basis of adaptive evolution in threespine sticklebacks. *Nature*, *484*(7392), 55–61. doi: 10.1038/nature10944

Kawecki, T. J., & Ebert, D. (2004). Conceptual issues in local adaptation. *Ecology Letters*, *7*(12), 1225–1241. doi: 10.1111/j.1461-0248.2004.00684.x

Kersting, D. K., Bensoussan, N., & Linares, C. (2013). Long-term responses of the endemic reef-builder *Cladocora caespitosa* to mediterranean warming. *PLoS ONE*, *8*(8), e70820. doi: 10.1371/journal.pone.0070820

Kopelman, N. M., Mayzel, J., Jakobsson, M., Rosenberg, N. A., & Mayrose, I. (2015). Clumpak: a program for identifying clustering modes and packaging population structure inferences across K. *Molecular Ecology Resources*, *15*(5), 1179–1191. doi: 10.1111/1755-0998.12387

Kulmuni, J., & Westram, A. M. (2017). Intrinsic incompatibilities evolving as a by-product of divergent ecological selection: Considering them in empirical studies on divergence with gene flow. *Molecular Ecology*, *26*(12), 3093-3103. doi: 10.1111/mec.14147

Ledoux, J.-B., Aurelle, D., Bensoussan, N., Marschal, C., Féral, J.-P., & Garrabou, J. (2015). Potential for adaptive evolution at species range margins: contrasting interactions between red coral populations and their environment in a changing ocean. *Ecology and Evolution*, *5*(6), 1178–1192. doi: 10.1002/ece3.1324

Ledoux, J.-B., Garrabou, J., Bianchimani, O., Drap, P., Féral, J.-P., & Aurelle, D. (2010). Fine-scale genetic structure and inferences on population biology in the threatened Mediterranean red coral, *Corallium rubrum*. *Molecular Ecology*, *19*(19), 4204–4216. doi: 10.1111/j.1365-294X.2010.04814.x

Ledoux, J.-B., Mokhtar-Jamaï, K., Roby, C., Féral, J.-P., Garrabou, J., & Aurelle, D. (2010). Genetic survey of shallow populations of the Mediterranean red coral [*Corallium rubrum* (Linnaeus, 1758)]: new insights into evolutionary processes shaping nuclear diversity and implications for conservation. *Molecular Ecology*, *19*(4), 675–690. doi: 10.1111/j.1365-294X.2009.04516.x

Lenormand, T. (2002). Gene flow and the limits to natural selection. *Trends in Ecology & Evolution*, *17*(4), 183–189. doi: 10.1016/S0169-5347(02)02497-7

Li, H., & Durbin, R. (2009). Fast and accurate short read alignment with Burrows–Wheeler transform. *Bioinformatics*, *25*(14), 1754–1760. doi: 10.1093/bioinformatics/btp324

Limborg, M. T., Helyar, S. J., de Bruyn, M., Taylor, M. I., Nielsen, E. E., Ogden, R. O. B., … Bekkevold, D. (2012). Environmental selection on transcriptome-derived SNPs in a high gene flow marine fish, the Atlantic herring (*Clupea harengus*). *Molecular Ecology*, *21*(15), 3686–3703. doi: 10.1111/j.1365-294X.2012.05639.x

Lõhelaid, H., Teder, T., & Samel, N. (2015). Lipoxygenase-allene oxide synthase pathway in octocoral thermal stress response. *Coral Reefs*, *34*(1), 143–154. doi: 10.1007/s00338-014-1238-y

Lowry, D. B., Hoban, S., Kelley, J. L., Lotterhos, K. E., Reed, L. K., Antolin, M. F., & Storfer, A. (2017). Breaking RAD: an evaluation of the utility of restriction site-associated DNA sequencing for genome scans of adaptation. *Molecular ecology resources*, *17*(2),

142-152. doi: 10.1111/1755-0998.12635
Lundgren, P., Vera, J. C., Peplow, L., Manel, S., & Oppen, M. J. van. (2013). Genotype – environment correlations in corals from the Great Barrier Reef. *BMC Genetics*, *14*(1), 9. doi: 10.1186/1471-2156-14-9
Luu, K., Bazin, E., & Blum, M. G. B. (2017). pcadapt: an R package to perform genome scans for selection based on principal component analysis. *Molecular Ecology Resources*, *17*(1), 67–77. doi: 10.1111/1755-0998.12592
McKinney, G. J., Larson, W. A., Seeb, L. W., & Seeb, J. E. (2017). RADseq provides unprecedented insights into molecular ecology and evolutionary genetics: comment on Breaking RAD by Lowry et al. (2016). *Molecular ecology resources*, *17*(3), 356-361. doi: 10.1111/1755-0998.12649
Manel, S., Perrier, C., Pratlong, M., Abi-Rached, L., Paganini, J., Pontarotti, P., & Aurelle, D. (2016). Genomic resources and their influence on the detection of the signal of positive selection in genome scans. *Molecular Ecology*, *25*(1), 170–184. doi: 10.1111/mec.13468
Marschal, C., Garrabou, J., Harmelin, J. G., & Pichon, M. (2004). A new method for measuring growth and age in the precious red coral *Corallium rubrum* (L.). *Coral Reefs*, *23*(3), 423–432. doi: 10.1007/s00338-004-0398-6
Martínez-Quintana, A., Bramanti, L., Viladrich, N., Rossi, S., & Guizien, K. (2015). Quantification of larval traits driving connectivity: the case of *Corallium rubrum* (L. 1758). *Marine Biology*, *162*(2), 309–318. doi: 10.1007/s00227-014-2599-z
Mayfield, A. B., Chan, P.-H., Putnam, H. M., Chen, C.-S., & Fan, T.-Y. (2012). The effects of a variable temperature regime on the physiology of the reef-building coral *Seriatopora hystrix*: results from a laboratory-based reciprocal transplant. *The Journal of Experimental Biology*, *215*(23), 4183–4195. doi: 10.1242/jeb.071688
Milano, I., Babbucci, M., Cariani, A., Atanassova, M., Bekkevold, D., Carvalho, G. R., … Bargelloni, L. (2014). Outlier SNP markers reveal fine-scale genetic structuring across European hake populations (*Merluccius merluccius*). *Molecular Ecology*, *23*(1), 118–135. doi: 10.1111/mec.12568
Mokhtar-Jamaï, K., Pascual, M., Ledoux, J. B., Coma, R., FÉRAL, J. P., Garrabou, J., & Aurelle, D. (2011). From global to local genetic structuring in the red gorgonian *Paramuricea clavata*: the interplay between oceanographic conditions and limited larval dispersal. *Molecular Ecology*, *20*(16), 3291-3305. doi: 10.1111/j.1365-294X.2011.05176.x
Muir, A. P., Biek, R., Thomas, R., & Mable, B. K. (2014). Local adaptation with high gene flow: temperature parameters drive adaptation to altitude in the common frog (*Rana temporaria*). *Molecular Ecology*, *23*(3), 561–574. doi: 10.1111/mec.12624
Mumby, P. J., Elliott, I. A., Eakin, C. M., Skirving, W., Paris, C. B., Edwards, H. J., … Stevens, J. R. (2011). Reserve design for uncertain responses of coral reefs to climate change. *Ecology Letters*, *14*(2), 132–140. doi: 10.1111/j.1461-0248.2010.01562.x
Nagylaki, T. (1978). Random genetic drift in a cline. *Proceedings of the National Academy of Sciences of the United States of America*, *75*(1), 423–426. doi: 10.1073/pnas.75.1.423
Nielsen, E. E., Hemmer-Hansen, J., Poulsen, N. A., Loeschcke, V., Moen, T., Johansen, T., … Carvalho, G. R. (2009). Genomic signatures of local directional selection in a high gene flow marine organism; the Atlantic cod (*Gadus morhua*). *BMC Evolutionary Biology*, *9*(1), 276. doi: 10.1186/1471-2148-9-276

899 Oliver, T. A., & Palumbi, S. R. (2011). Do fluctuating temperature environments elevate coral
900 thermal tolerance? *Coral Reefs*, *30*(2), 429–440. doi: 10.1007/s00338-011-0721-y
901 Palumbi, S. R., Barshis, D. J., Traylor-Knowles, N., & Bay, R. A. (2014). Mechanisms of reef
902 coral resistance to future climate change. *Science*. doi: 10.1126/science.1251336
903 Pespeni, M. H., & Palumbi, S. R. (2010). The purple sea urchin genome suggests local
904 adaptation along a latitudinal gradient despite high gene flow. In *Integrative and*
905 *Comparative Biology* (Vol. 50, pp. E136–E136). Oxford Univ Press Inc Journals Dept,
906 2001 Evans Rd, Cary, NC 27513 USA. doi: 10.1098/rspb.2011.1823
907 Pigliucci, M. (2001). Phenotypic plasticity: beyond nature and nurture. JHU Press. doi:
908 10.1038/sj.hdy.6800153
909 Pivotto, I. D., Nerini, D., Masmoudi, M., Kara, H., Chaoui, L., & Aurelle, D. (2015). Highly
910 contrasted responses of Mediterranean octocorals to climate change along a depth
911 gradient. *Royal Society Open Science*, *2*(5), 140493. doi: 10.1098/rsos.140493
912 Plough, L. V., Shin, G., & Hedgecock, D. (2016). Genetic inviability is a major driver of type
913 III survivorship in experimental families of a highly fecund marine bivalve. *Molecular*
914 *ecology*, *25*(4), 895-910. doi: 10.1111/mec.13524
915 Plummer, M., Best, N., Cowles, K., & Vines, K. (2006). CODA: Convergence diagnosis and
916 output analysis for MCMC. *R News*, *6*(1), 7–11.
917 Potts, D. C. (1984). Natural selection in experimental populations of reef-building corals
918 (*Scleractinia*). *Evolution*, *38*(5), 1059–1078. doi: 10.1111/j.1558-5646.1984.tb00376.x
919 Prada, C., Schizas, N. V., & Yoshioka, P. M. (2008). Phenotypic plasticity or speciation? A
920 case from a clonal marine organism. *BMC Evolutionary Biology*, *8*(1), 47. doi:
921 10.1186/1471-2148-8-47
922 Pratlong, M., Haguenauer, A., Chabrol, O., Klopp, C., Pontarotti, P., & Aurelle, D. (2015).
923 The red coral (*Corallium rubrum*) transcriptome: a new resource for population
924 genetics and local adaptation studies. *Molecular Ecology Resources*, *15*(5), 1205–
925 1215. doi: 10.1111/1755-0998.12383
926 Pratlong, M., Haguenauer, A., Chenesseau, S., Brener, K., Mitta, G., Toulza, E., ... &
927 Pontarotti, P. (2017). Evidence for a genetic sex determination in Cnidaria, the
928 Mediterranean red coral (*Corallium rubrum*). *Royal Society Open Science*, *4*(3),
929 160880. doi: 10.1098/rsos.160880
930 Pritchard, J. K., & Di Rienzo, A. (2010). Adaptation–not by sweeps alone. *Nature reviews.*
931 *Genetics*, *11*(10), 665. doi: 10.1038/nrg2880
932 Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using
933 multilocus genotype data. *Genetics*, *155*(2), 945–959. doi: 10.1111/j.1471-
934 8286.2007.01758.x
935 Putnam, H. M., & Gates, R. D. (2015). Preconditioning in the reef-building coral *Pocillopora*
936 *damicornis* and the potential for trans-generational acclimatization in coral larvae
937 under future climate change conditions. *Journal of Experimental Biology*, *218*(15),
938 2365–2372. doi: 10.1242/jeb.123018
939 R Core Team. (2016). R: a language and environment for statistical computing. Vienna,
940 Austria: R Foundation for Statistical Computing.
941 Ravigné, V., Dieckmann, U., & Olivieri, I. (2009). Live where you thrive: joint evolution of
942 habitat choice and local adaptation facilitates specialization and promotes diversity.
943 *The American Naturalist*, *174*(4), E141–E169. doi: 10.1086/605369
944 Reusch, T. B., & Wood, T. E. (2007). Molecular ecology of global change. *Molecular*

Ecology, *16*(19), 3973–3992. doi: 10.1111/j.1365-294X.2007.03454.x
Rossi, S., Tsounis, G., Orejas, C., Padrón, T., Gili, J.-M., Bramanti, L., … Gutt, J. (2008). Survey of deep-dwelling red coral (*Corallium rubrum*) populations at Cap de Creus (NW Mediterranean). *Marine Biology*, *154*(3), 533–545. doi: 10.1007/s00227-008-0947-6
Rousset, F. (2008). genepop'007: a complete re-implementation of the genepop software for Windows and Linux. *Molecular Ecology Resources*, *8*(1), 103–106. doi: 10.1111/j.1471-8286.2007.01931.x
Sambrook, J., Fritsch, E. F., Maniatis, T., & others. (1989). *Molecular cloning* (Vol. 2). Cold spring harbor laboratory press New York.
Sanford, E., & Kelly, M. W. (2011). Local adaptation in marine invertebrates. *Annual Review of Marine Science*, *3*(1), 509–535. doi: 10.1146/annurev-marine-120709-142756
Santangelo, G., Bramanti, L., Rossi, S., Tsounis, G., Vielmini, I., Lott, C., & Gili, J. M. (2012). Patterns of variation in recruitment and post-recruitment processes of the Mediterranean precious gorgonian coral *Corallium rubrum*. *Journal of Experimental Marine Biology and Ecology*, *411*, 7–13. doi: 10.1016/j.jembe.2011.10.030
Santangelo, G., Carletti, E., Maggi, E., & Bramanti, L. (2003). Reproduction and population sexual structure of the overexploited Mediterranean red coral *Corallium rubrum*. *Marine Ecology Progress Series*, *248*, 99–108. doi: 10.3354/meps248099
Sherman, C. D. H., & Ayre, D. J. (2008). Fine-scale adaptation in a clonal sea anemone. *Evolution; International Journal Of Organic Evolution*, *62*(6), 1373–1380. doi: 10.1111/j.1558-5646.2008.00375.x
Slatkin, M., & Maruyama, T. (1975). Genetic drift in a cline. *Genetics*, *81*(1), 209–222.
Smith, L. W., Barshis, D., & Birkeland, C. (2007). Phenotypic plasticity for skeletal growth, density and calcification of *Porites lobata* in response to habitat type. *Coral Reefs*, *26*(3), 559–567. doi: 10.1007/s00338-007-0216-z
Smith, L. W., Barshis, D., & Birkeland, C. (2007). Phenotypic plasticity for skeletal growth, density and calcification of *Porites lobata* in response to habitat type. *Coral Reefs*, *26*(3), 559-567. doi: 10.1007/s00338-007-0216-z
Somero, G. N. (2010). The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine 'winners' and 'losers.' *The Journal of Experimental Biology*, *213*(6), 912–920. doi: 10.1242/jeb.037473
Somero, G. N. (2012). The physiology of global change: linking patterns to mechanisms. *Annual Review of Marine Science*, *4*, 39–61. doi: 10.1146/annurev-marine-120710-100935
Szulkin, M., Gagnaire, P.-A., Bierne, N., & Charmantier, A. (2016). Population genomic footprints of fine-scale differentiation between habitats in Mediterranean blue tits. *Molecular Ecology*, *25*(2), 542–558. doi: 10.1111/mec.13486
Torrents, O., Garrabou, J., Marschal, C., & Harmelin, J. G. (2005). Age and size at first reproduction in the commercially exploited red coral *Corallium rubrum* (L.) in the Marseilles area (France, NW Mediterranean). *Biological Conservation*, *121*(3), 391–397. doi: 10.1016/j.biocon.2004.05.010
Torrents, O., Tambutté, E., Caminiti, N., & Garrabou, J. (2008). Upper thermal thresholds of shallow vs. deep populations of the precious Mediterranean red coral *Corallium rubrum* (L.): Assessing the potential effects of warming in the NW Mediterranean. *Journal of Experimental Marine Biology and Ecology*, *357*(1), 7–19. doi:

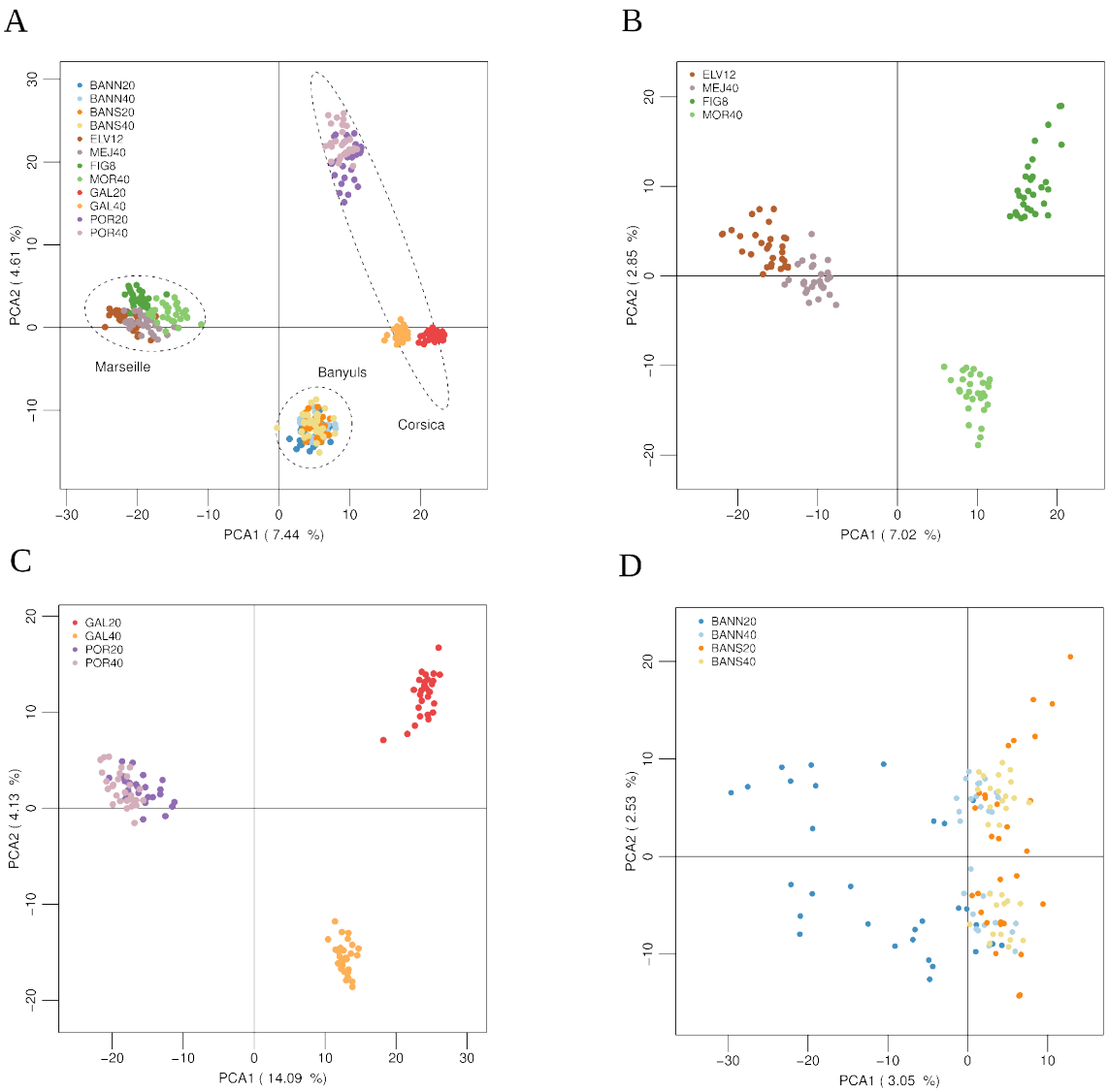
10.1016/j.jembe.2007.12.006
Ulstrup, K. E., & Van Oppen, M. J. H. (2003). Geographic and habitat partitioning of genetically distinct zooxanthellae (*Symbiodinium*) in *Acropora* corals on the Great Barrier Reef. *Molecular Ecology*, *12*(12), 3477–3484. doi: 10.1046/j.1365-294X.2003.01988.x
Villemereuil, P., & Gaggiotti, O. E. (2015). A new FST-based method to uncover local adaptation using environmental variables. *Methods in Ecology and Evolution*, *6*(11), 1248–1258. doi: 10.1111/2041-210X.12418
Walsh, P. J., & Somero, G. N. (1981). Temperature adaptation in sea anemones: physiological and biochemical variability in geographically separate populations of *Metridium senile*. *Marine Biology*, *62*(1), 25–34. doi: 10.1007/BF00396948
Walther, G.-R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T. J., … Bairlein, F. (2002). Ecological responses to recent climate change. *Nature*, *416*(6879), 389–395. doi: 10.1038/416389a
Wang, L., Liu, S., Zhuang, Z., Guo, L., Meng, Z., & Lin, H. (2013). Population genetic studies revealed local adaptation in a high gene-flow marine fish, the small yellow croaker (*Larimichthys polyactis*). *PloS One*, *8*(12), e83493. doi: 10.1371/journal.pone.0083493
Weinberg, S. (1979). The light-dependent behaviour of planula larvae of Eunicella singularis and Corallium rubrum and its implication for octocorallian ecology. *Bijdragen tot de Dierkunde*, *49*(1), 16-30.
Wrange, A.-L., André, C., Lundh, T., Lind, U., Blomberg, A., Jonsson, P. J., & Havenhand, J. N. (2014). Importance of plasticity and local adaptation for coping with changing salinity in coastal areas: a test case with barnacles in the Baltic Sea. *BMC Evolutionary Biology*, *14*, 156. doi: 10.1186/1471-2148-14-156
Ziegler, M., Roder, C. M., Büchel, C., & Voolstra, C. R. (2014). Limits to physiological plasticity of the coral *Pocillopora verrucosa* from the central Red Sea. *Coral Reefs*, *33*(4), 1115–1129. doi: 10.1007/s00338-014-1192-8

Data accessibility

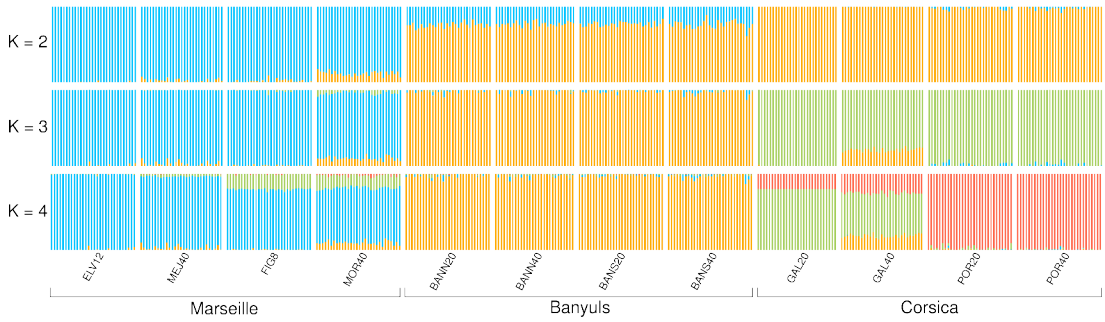
- Raw DNA sequences: short Read Archive (SRA) database under accession no. SRR5186771-SRR5187129.

- Filtered SNP file: Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.rs7bm.

1023 Tables and Figures
1024



1025 Figure 1. Principal component analysis (Axes 1 and 2) of A) the 12 red coral populations
1026 (n = 354 individuals, 27 461 SNPs), B) the four red coral populations from Marseille (n = 119
1027 individuals, 27 461 SNPs), C) the four red coral populations from Corsica (n = 117
1028 individuals, 27 461 SNPs), D) the four red coral populations from Banyuls (n = 118
1029 individuals, 27 461 SNPs).



1030 Figure 2. Results from Bayesian individual clustering with STRUCTURE for K = 2 to K = 4.
1031 For K = 2 and K = 4, all ten replicates produced the same structure. For K = 3, the major
1032 mode presented here was the result of 7/10 replicates. Minor modes are presented in Fig. S3.

1033 Table 1. Characteristics of red coral sampling sites.

Population	Geographic region	Site	Depth (m)	GPS	GPS
FIG8	Marseille	Marseille South	8	43° 12.330'N	5° 26.790'E
MOR40	Marseille	Marseille South	40	43° 12.060'N	5° 27.100'E
ELV12	Marseille	Marseille North	12	43° 19.780'N	5° 14.210'E
MEJ40	Marseille	Marseille North	40	43° 19.700'N	5° 13.480'E
BANN20	Banyuls	Banyuls North	25	42° 26.890'N	3° 10.330'E
BANN40	Banyuls	Banyuls North	35	42° 26.890'N	3° 10.330'E
BANS20	Banyuls	Banyuls South	26	42° 26.390'N	3° 10.790'E
BANS40	Banyuls	Banyuls South	36	42° 26.390'N	3° 10.790'E
POR20	Corsica	Porto	21	42° 16.292'N	8° 41.255'E
POR40	Corsica	Porto	33	42° 16.292'N	8° 41.255'E
GAL20	Corsica	Galeria	26	42° 28.210'N	8° 38.950'E
GAL40	Corsica	Galeria	36	42° 28.210'N	8° 38.950'E

1034

1035 Table 2. Temperatures characteristics of the red coral sampling sites from March 2012 to
1036 October 2014.

	Minimum	Maximum	Mean	Standard Deviation
BANN20	12.22	24.29	17.20	2.63
BANN40	9.41	23.83	14.49	2.45
BANS20	12.22	24.29	17.20	2.63
BANS40	9.41	23.83	14.49	2.45
ELV12	11.81	26.70	16.60	3.24
MEJ40	11.86	22.87	15.29	2.18
FIG8	12.63	26.92	17.03	3.52
MOR40	12.73	23.06	15.40	2.11
GAL20	12.46	25.09	17.13	3.13
GAL40	12.56	23.83	16.26	2.45
POR20	12.51	25.91	17.51	3.41
POR40	12.56	23.83	16.26	2.45

1037

1038 Table 3. Counts of SNP loci after each step of filtering.

Step	Number of SNPs	Software
After assembly raw data	138 810	Stacks (Catchen et al., 2013; Catchen et al., 2011)
Excluding loci not in within population HWE	86 520	VCFtools (Danecek et al., 2011)
MAF 1 %	56 844	VCFtools (Danecek et al., 2011)
One SNPs per RAD-tag	27 461	

1039

1040 Table 4. Measures of F_{IS} and gene diversity of the red coral populations based on 27 461
1041 SNPs.

Population	F_{IS}	Gene diversity
BANN20	0.018	0.15
BANN40	0.012	0.15
BANS20	0.019	0.15
BANS40	0.065	0.13
ELV12	0.005	0.17
MEJ40	0.053	0.18
FIG8	0.005	0.18
MOR40	0.036	0.18
GAL20	0.013	0.09
GAL40	0.019	0.13
POR20	0.023	0.13
POR40	0.009	0.15

1042

1043 Table 5. Pairwise F_{ST} estimates. All comparisons were highly significant. Intra-region comparisons are highlighted.

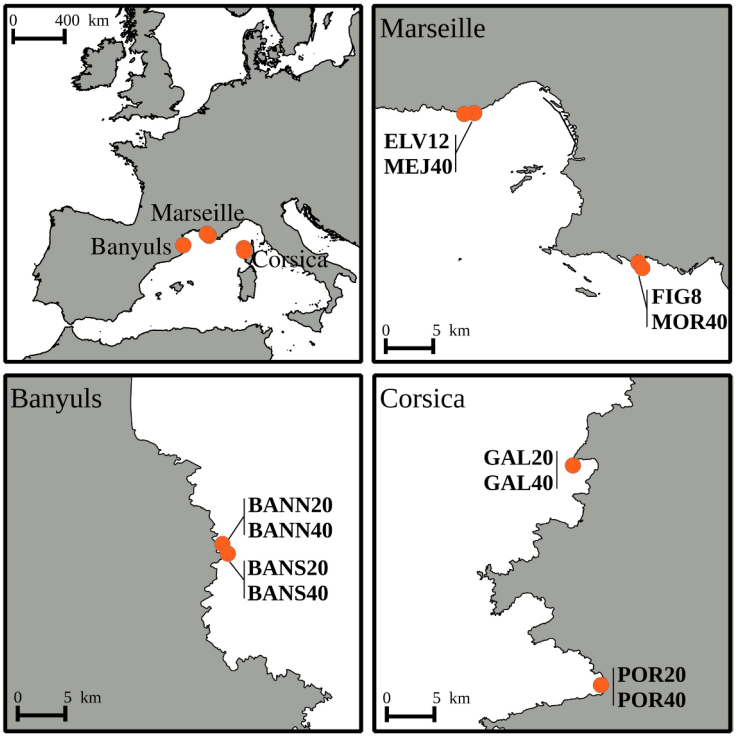
	BANN20	BANN40	BANS20	BANS40	ELV12	MEJ40	FIG8	MOR40	GAL20	GAL40	POR20
BANN40	0.02										
BANS20	0.03	0.01									
BANS40	0.03	0.01	0.01								
ELV12	0.13	0.13	0.13	0.13							
MEJ40	0.11	0.11	0.11	0.11	0.03						
FIG8	0.14	0.13	0.13	0.13	0.10	0.08					
MOR40	0.11	0.10	0.10	0.10	0.08	0.06	0.05				
GAL20	0.18	0.17	0.17	0.17	0.24	0.22	0.24	0.21			
GAL40	0.11	0.10	0.10	0.10	0.19	0.16	0.18	0.15	0.10		
POR20	0.14	0.13	0.13	0.13	0.17	0.16	0.17	0.14	0.20	0.13	
POR40	0.14	0.13	0.13	0.13	0.17	0.15	0.17	0.14	0.21	0.14	0.05

1044

1045 Table 6. Percent of the variation explained by grouping populations according to their
1046 geographical region on the analysis of molecular variance (performed with ARLEQUIN).

Source of variation	d.f.	Percentage of variation
Among groups	2	7.80
Among populations within groups	9	7.07
Within populations	696	85.13

1047



1048 Figure S1. Location of the sampling sites of the red coral among the three studied
1049 geographical regions.

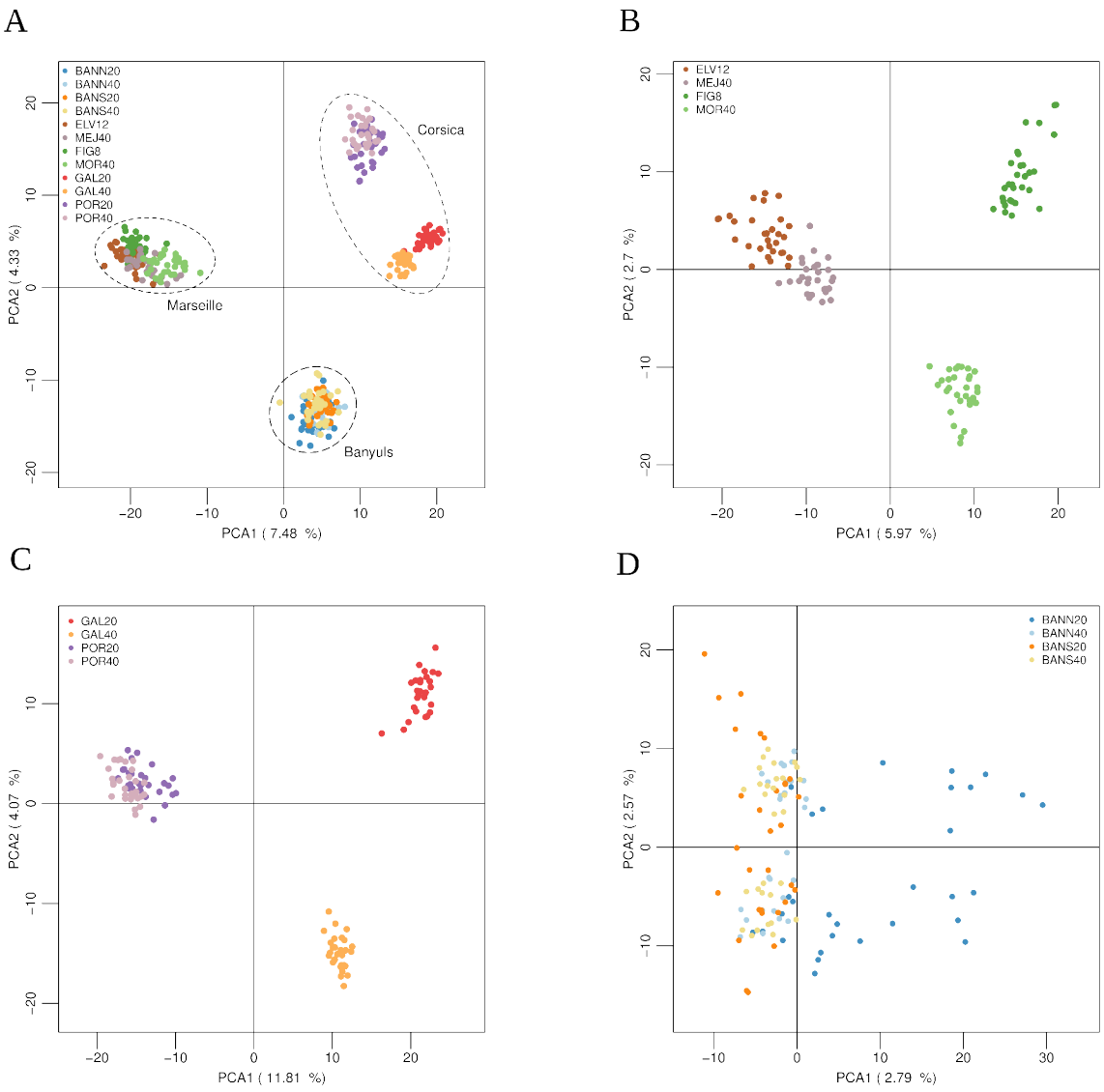
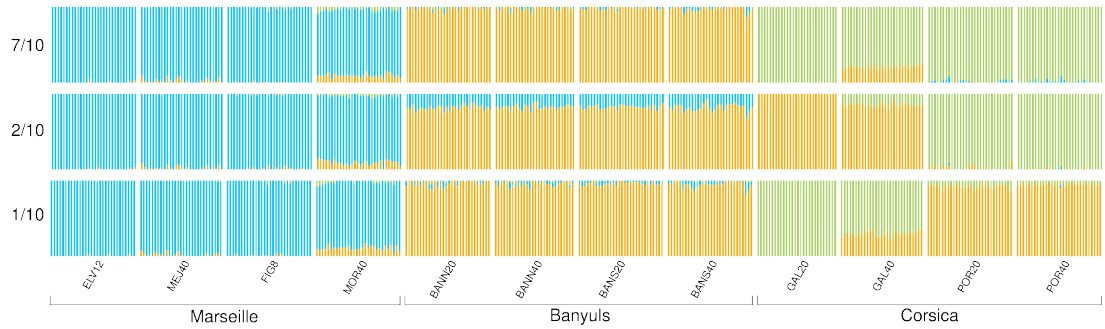
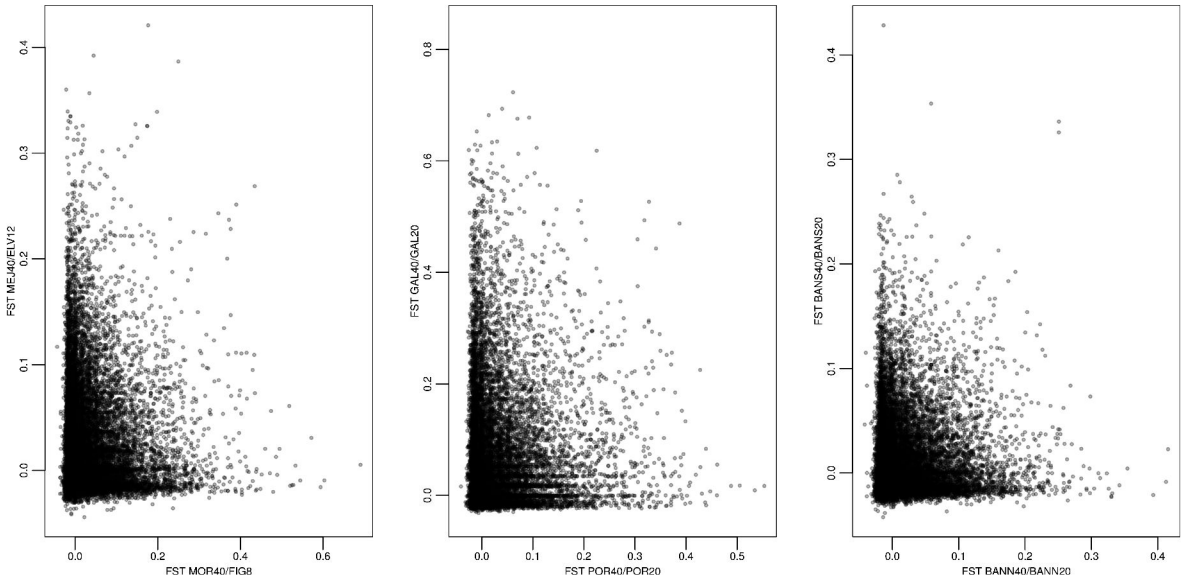


Figure S2. Principal component analysis (Axes 1 and 2), using only putative neutral SNPs, of the A) 12 red coral populations (n = 354 individuals, 25 669 SNPs), B) four red coral populations from Marseille (n = 119 individuals, 26 898 SNPs), C) four red coral populations from Corsica (n = 117 individuals, 26 592 SNPs), D) four red coral populations from Banyuls (n = 118 individuals, 27 069 SNPs).



1056 Figure S3. Results from Bayesian individual clustering with STRUCTURE for K = 3. The
1057 three figures correspond to major and minor modes detected.
1058



1059 Figure S4. Joint distribution of between-depths F_{ST} in the three geographical regions.

Table S1. Pairwise F_{ST} estimates using only putatively neutral SNPs. All comparisons were highly significants. Intra-region comparisons are highlighted.

	BANN20	BANN40	BANS20	BANS40	ELV12	MEJ40	FIG8	MOR40	GAL20	GAL40	POR20
BANN40	0.02										
BANS20	0.02	0.01									
BANS40	0.02	0.01	0.01								
ELV12	0.13	0.12	0.12	0.12							
MEJ40	0.11	0.10	0.10	0.10	0.03						
FIG8	0.13	0.12	0.12	0.12	0.09	0.07					
MOR40	0.10	0.10	0.09	0.09	0.07	0.05	0.04				
GAL20	0.17	0.16	0.16	0.16	0.23	0.21	0.22	0.20			
GAL40	0.11	0.09	0.09	0.09	0.18	0.16	0.18	0.15	0.10		
POR20	0.13	0.12	0.12	0.12	0.17	0.15	0.16	0.14	0.17	0.11	
POR40	0.13	0.12	0.12	0.12	0.16	0.15	0.16	0.14	0.18	0.12	0.04

Table S2. Pairwise F_{ST} estimates using only outlier SNPs from the ARLEQUIN analysis. All comparisons were highly significants. Intra-region comparisons are highlighted.

	BANN20	BANN40	BANS20	BANS40	ELV12	MEJ40	FIG8	MOR40	GAL20	GAL40	POR20
BANN40	0.04										
BANS20	0.06	0.03									
BANS40	0.06	0.03	0.02								
ELV12	0.19	0.18	0.19	0.18							
MEJ40	0.16	0.14	0.15	0.15	0.04						
FIG8	0.19	0.17	0.18	0.18	0.23	0.19					
MOR40	0.14	0.12	0.13	0.13	0.19	0.14	0.08				
GAL20	0.27	0.27	0.28	0.27	0.37	0.35	0.36	0.32			
GAL40	0.15	0.13	0.15	0.13	0.26	0.24	0.26	0.22	0.17		
POR20	0.23	0.21	0.21	0.21	0.26	0.22	0.22	0.18	0.41	0.30	
POR40	0.24	0.23	0.24	0.23	0.26	0.23	0.23	0.19	0.42	0.31	0.07

ANNEXE 2 : MISE EN ÉVIDENCE D'UN DÉTERMINISME GÉNÉTIQUE DU SEXE CHEZ LE CORAIL ROUGE

ROYAL SOCIETY OPEN SCIENCE

rsos.royalsocietypublishing.org

Research

Cite this article: Pratlong M *et al*. 2017 Evidence for a genetic sex determination in Cnidaria, the Mediterranean red coral (*Corallium rubrum*). *R. Soc. open sci.* **4**: 160880. http://dx.doi.org/10.1098/rsos.160880

Received: 3 November 2016
Accepted: 1 February 2017

Subject Category:
Genetics

Subject Areas:
evolution/genomics/ecology

Keywords:
genetic sex determination, *Corallium rubrum*, RAD-sequencing, Cnidaria

Authors for correspondence:
M. Pratlong
e-mail: marine.pratlong@gmail.fr
D. Aurelle
e-mail: didier.aurelle@univ-amu.fr

†These authors contributed equally to this study.

Electronic supplementary material is available online at https://dx.doi.org/10.6084/m9.figshare.c.3699073.

THE ROYAL SOCIETY PUBLISHING

Evidence for a genetic sex determination in Cnidaria, the Mediterranean red coral (*Corallium rubrum*)

M. Pratlong[1,2], A. Haguenauer[1], S. Chenesseau[1], K. Brener[3], G. Mitta[3], E. Toulza[3], M. Bonabaud[4], S. Rialle[4], D. Aurelle[1,†] and P. Pontarotti[2,†]

[1]Aix Marseille Univ, Avignon Université, CNRS, IRD, IMBE, Marseille, France
[2]Aix Marseille Univ, CNRS, Centrale Marseille, I2M, Marseille, France
[3]Perpignan Via Domitia Univ, IHPE UMR 5244, CNRS, IFREMER, Montpellier Université, Perpignan, France
[4]UMS BioCampus- MGX Montpellier GenomiX, Institut de Génomique Fonctionelle, 141 rue de la Cardonnille, 34094 Montpellier Cedex 05, France

MP, 0000-0002-3406-9829; PP, 0000-0001-7202-3648

Sexual reproduction is widespread among eukaryotes, and the sex-determining processes vary greatly among species. While genetic sex determination (GSD) has been intensively described in bilaterian species, no example has yet been recorded among non-bilaterians. However, the quasi-ubiquitous repartition of GSD among multicellular species suggests that similar evolutionary forces can promote this system, and that these forces could occur also in non-bilaterians. Studying sex determination across the range of Metazoan diversity is indeed important to understand better the evolution of this mechanism and its lability. We tested the existence of sex-linked genes in the gonochoric red coral (*Corallium rubrum*, Cnidaria) using restriction site-associated DNA sequencing. We analysed 27 461 single nucleotide polymorphisms (SNPs) in 354 individuals from 12 populations including 53 that were morphologically sexed. We found a strong association between the allele frequencies of 472 SNPs and the sex of individuals, suggesting an XX/XY sex-determination system. This result was confirmed by the identification of 435 male-specific loci. An independent test confirmed that the amplification of these loci enabled us to identify males with absolute certainty. This is the first demonstration of a GSD system among non-bilaterian species and a new example of its convergence in multicellular eukaryotes.

1. Introduction

Sexual reproduction is ubiquitous among eukaryotes [1], and there is a wealth of literature on the evolutionary advantages of sex [2]. Although sex is widely shared, the corresponding mechanisms and the sex-determination systems vary greatly among species [3,4]. In gonochoric species, the sexual identity of individuals is defined by sex-determination systems, going from purely genetic sex determination (GSD), to purely environmental sex determination (ESD) where the same genotype can produce both male and female phenotypes depending on environmental conditions [3]. GSD is observed, for example, in mammals, where sex chromosomes are present. The echiurian *Bonellia viridis* gives an example of ESD, where larvae recruiting on a female will develop into males, but otherwise become female [5]. While GSD has been intensively studied in bilaterian species, one example has been recorded so far among non-bilaterians such as Porifera, Cnidaria and Ctenophora [3,4]. However, the quasi-ubiquitous repartition of GSD among eukaryotes suggests that similar evolutionary forces repeatedly led to the evolution of GSD, and these forces could occur also in non-bilaterians. This lack of evidence of GSD in non-bilaterians is probably the consequence of a reduced number of model organisms in these groups, and sometimes of the difficulty to identify separate sexes. Nevertheless, studying sex determination along the range of Metazoan diversity is important to understand better the evolution of this mechanism and its lability. For example, understanding the ancestral state of sex-determination systems in Metazoans requires studying them in the main branches of the phylogenetic tree.

Cnidarians display various sexual systems, from hermaphrodism (simultaneous or sequential) to gonochorism, and sexual reproduction can take place at different stages, polyp or medusae [6]. The corresponding system determinations are poorly known: a few ESD examples have been reported but no examples of GSD have so far been confirmed among non-bilaterians [6]. A cytogenetic analysis has shown a clear evidence of potential sex chromosomes in a scleractinian [7] but the role of these chromosomes in sex determination remains to be studied as this species is hermaphroditic. Gonochorism is highly predominant in octocorals (89% of the species [8]), even if cases of rapid transition between gonochorism and hermaphrodism have been demonstrated in the genus *Alcyonium* [9]. The relative stability of gonochorism in octocorals makes them interesting models for the study of sex-determination systems. This could, for example, correspond to an evolutionary trap, which would stabilize a sex-determination system [4], or, conversely, to a variety of sex-determination systems, but with a selective pressure for gonochorism.

The red coral (*Corallium rubrum*) is a long-lived gonochoric octocoral, with an age at first reproduction of around 7–10 years [10,11]. This harvested species is the object of ecological and population genetic studies [12,13] for conservation and management purposes. Although hermaphrodite colonies have been mentioned in the first description of the reproduction of this species [14], it is considered to be gonochoric. Red coral individuals from the two sexes are morphologically identical at the macroscopic scale, and the sex can be identified microscopically after dissection only during the period of gametogenesis (from May to September) [15]. Elucidating the sex-determination factors in this species would be useful for a better understanding of its biology and of its potential response to environmental change. It would also widen our knowledge of sex-determination systems in cnidarians. The identification of GSD may be difficult in groups where cytogenetic analyses are problematic to implement, as is often the case in non-model species, or when species lack visually heteromorphic sex chromosomes [16,17]. Thus, our goal here was to test the existence of sex-linked genes in the precious red coral, with a population genomic approach. We used restriction site-associated DNA sequencing (RAD-Seq) applied to sexed individuals, as proposed in [6]. Our results point to an XX/XY sex-determination system in this species, and we developed a polymerase chain reaction (PCR)-based protocol for sexing.

2. Material and methods

2.1. Sampling and DNA extraction

Corallium rubrum colonies were collected by scuba diving at two depths of two sites in three geographical regions of the Mediterranean Sea (Marseille, Banyuls, Corsica) between February and August 2013 (electronic supplementary material, figure S1 and table S1). Thirty individuals per site and depth were collected (360 individuals), preserved in 95% ethanol and stored at −20 °C. To validate our results, 40 additional individuals were collected in one site near Marseille in June 2016 and conserved in both 95%

ethanol and formaldehyde. Total genomic DNA was extracted according to the protocol of [18], followed by a purification using Qiagen DNeasy blood and tissue spin columns.

2.2. Morphological sex identification

Samples fixed in 95% ethanol from the Corsica populations were decalcified in 10% EDTA adjusted to a pH of 7.4 with NaOH for 48 h and dehydrated in graded alcohols, cleared in xylene substitute (Neo-clear VWR) and embedded in paraffin wax. Sections (7 µm) were cut using a rotary microtome. Sections were stained using trichrome of Masson variant of Goldner protocols and examined using a Leica DMLB. The sex of individuals from the Marseille population collected in June 2016 and preserved in formaldehyde was identified under a dissecting microscope after dissection.

2.3. Restriction site-associated DNA sequencing

Twelve RAD libraries were prepared according to the protocol described in [19], with small modifications (see the electronic supplementary material, methods). Libraries were sequenced on an Illumina HiSeq2000 using 100 bp single-end reads, at the Biology Institute of Lille (IBL, UMR 8199 CNRS) and at the MGX sequencing platform in Montpellier (France). The Stacks pipeline [20,21] was used for the loci de novo assembly and genotyping. We applied several filters to the resulting dataset in order to filter for poor-quality single nucleotide polymorphisms (SNPs) and artefacts due paralogous sequences (electronic supplementary material, table S2 and methods).

2.4. Identification of sex-linked loci

The presence of sex-linked loci, i.e. loci common to the two sex chromosomes but presenting differences in allele frequencies between sex (figure 1*a*), was explored by performing a principal component analysis (PCA) using the package adegenet in R [22,23]. This analysis was performed on the total dataset (12 populations). The dataset was centred and missing data were replaced by the mean allele frequency for each locus.

2.5. Identification of sex-specific loci

We filtered the Stacks catalogue in order to search among morphologically sexed individuals for loci present in all individuals from one sex (one read or more by individual) and absent in all individuals from the other sex (no read detected) (i.e. sex-specific loci; figure 1*a*).

2.6. Real-time polymerase chain reaction

Primers were designed for six putative male-specific sequences using IDT online tool (http://eu.idtdna.com/Primerquest/Home/Index) (primer sequences in electronic supplementary material, table S3). To avoid a specific hybridization, we selected loci presenting no blast hit results other than themselves against the Stacks catalogue. The presence or absence of target genomic sequences was assessed by real-time PCR on 1 µl of the same DNA extracts of the 58 morphologically sexed individuals from Corsica used for RAD-Seq, and for the 39 supplementary morphologically sexed individuals from Marseille (see the electronic supplementary material, methods). The difference of amplification between males and females was tested with a Wilcoxon–Mann–Whitney test in R [23].

3. Results and discussion

3.1. Morphological identification of sex

The sexing of the 58 individuals from four populations in Corsica was undertaken on the basis of gonad analysis. Among them, 25 males and 28 females were formally identified (hereafter called morphological males and females). Five individuals were sexually undetermined because of the quality of the tissues, or due to a sexually immature stage of development. Among the 40 individuals from the sampling of June 2016 in Marseille, we identified 15 males and 24 females (one individual was sexually undetermined).

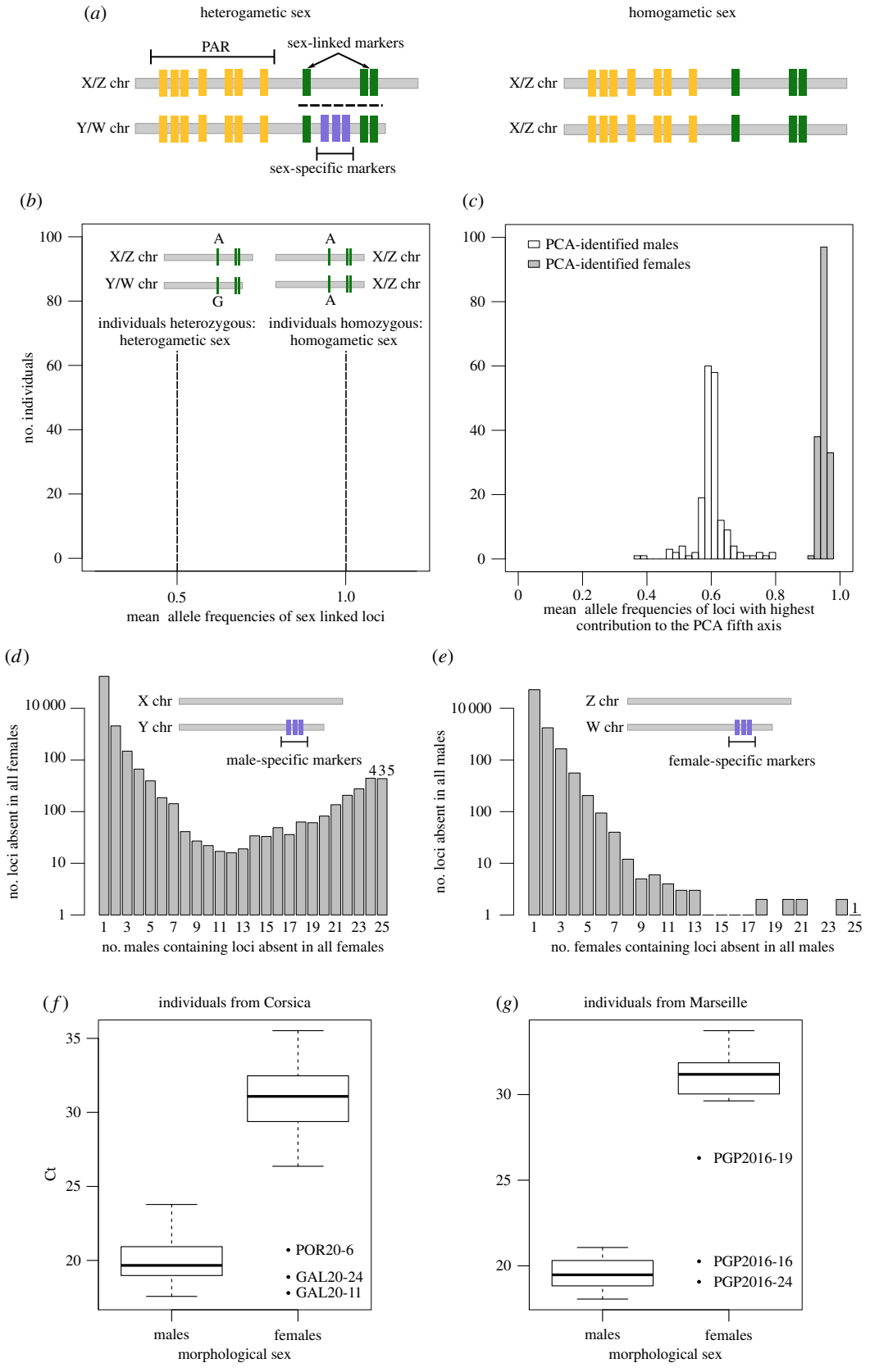


Figure 1. (*Caption opposite*.)

Figure 1. (*Opposite*.) (*a*) Example of a genetic sex-determination system. Sex-specific markers (blue) are present on the Y chromosomes in the XX/XY system and in the W chromosome in the ZW/ZZ system. Sex-linked markers (green) are common to the two sex chromosomes but should present differences in allele frequencies between sex because of the recombination arrest (adapted from [15]). (*b*) Mean allele frequencies expected in the case of sex-linked markers fixed on the X/Z and on the Y/W chromosomes. For those markers, individuals of the heterogametic sex (males in the XX/XY system, females in the ZW/ZZ system) should be heterozygous and individuals of the homogametic sex (females in the XX/XY system, males in the ZW/ZZ system) should be homozygous. (*c*) Mean allele frequencies of loci with the highest contribution to the PCA fifth axis observed in PCA-identified males and females. (*d*,*e*) Distribution of loci absent in all morphologically sexed individuals of one sex in morphologically sexed individuals of the opposite sex. (*f*) Results of the real-time PCR amplification (threshold cycle Ct) of one male-specific locus (Locus_139082) on morphologically sexed males and females from the Corsica populations. The Ct indicates the number of PCR cycles necessary to reach a threshold value; a low Ct value indicates a high amplification rate from the corresponding sample. Results for the six loci tested were similar (electronic supplementary material, figure S3). (*g*) Result of the real-time PCR amplification (threshold cycle Ct) of one male-specific locus (Locus_139082) on morphologically sexed males and females from the Marseille population. Results for the six loci tested were similar (electronic supplementary material, figure S4).

3.2. RAD-tag sequencing and quality filtering

RAD-tag sequencing generated an average of 187 ± 21 million reads per library before any quality filtering. The quality filtering step enabled us to remove an average of 2.02% of reads without a correct restriction enzyme cut site, an average of 1.69% of reads with ambiguous barcodes and an average of 1.61% of reads with low quality score. An average of 183 ± 22 million reads per library (95% of total reads) were retained (electronic supplementary material, table S4) with an average of 5.7 million reads per individual. After assembly (see details in electronic supplementary material, methods), 138 810 SNPs were successfully genotyped in at least 75% of individuals from all populations (electronic supplementary material, table S2). Individuals were sequenced with a mean coverage of 37 reads per individual per locus. Morphologically sexed females presented a mean coverage of 37.9 reads per individual per locus, and males 45.6 reads per individual per locus. A total of 27 461 SNPs remained after several steps of filtration (electronic supplementary material, table S2 and methods).

3.3. Identification of sex-linked loci

While the first four principal components of the PCA highlighted neutral population genetic differences [24], the fifth axis (1.58% of explained variance) separated individuals from all populations in two clear groups that matched the male/female repartition of morphologically sexed individuals (figure 2). Only three individuals that were morphologically female appeared among males in the PCA. From this PCA, we identified potential females and males among individuals for which sex determination was not performed on the basis of the visible separation in two groups. Considering this separation, we postulate that the dataset comprised 169 females and 185 males, corresponding to a balanced sex ratio ($p = 0.40$, electronic supplementary material, table S5). The sex ratio was balanced also inside each population, except in ELV12, where it was significantly biased towards male individuals (70% of males; $p = 0.03$, electronic supplementary material, table S5). There were 472 SNPs with a contribution to the fifth axis of the PCA higher than 1% (electronic supplementary material, figure S2); these loci displayed the same allele fixed in almost all morphological females, and were at the heterozygous state in almost all morphological males. This observation was confirmed with PCA-identified males and females (figure 1*b*,*c*). The genotypes of these 472 SNPs enabled the sexing of 95% of individuals for the whole dataset. Among these SNPs, when considering only morphologically sexed individuals, 379 SNPs were fixed in all females, 59 were at the heterozygous state in all males (the remaining SNPs were at the heterozygous state in almost all males). Fifty-five SNPs were in these two categories and were therefore diagnostic of sex for the morphologically sexed individuals. Identification of such SNPs that were homozygous in females and heterozygous in males suggested an XX/XY sex-determination system in the red coral, with a non-recombining XY-like region. Of the SNPs leading the fifth PCA axis, 347 were fixed in all PCA-identified females. However, none of these SNPs was at the heterozygous state in 100% of PCA-identified males. SNPs that were strictly homozygous in females and heterozygous in males were found inside each population and geographical region, but none was common to the three geographical regions. Furthermore, even if we did not observe markers that were heterozygous in 100% of males, these markers remain at the heterozygous state in the majority of males: 303 of these 472 sex-linked markers were at the heterozygous state in more than 70% of all males. The absence of

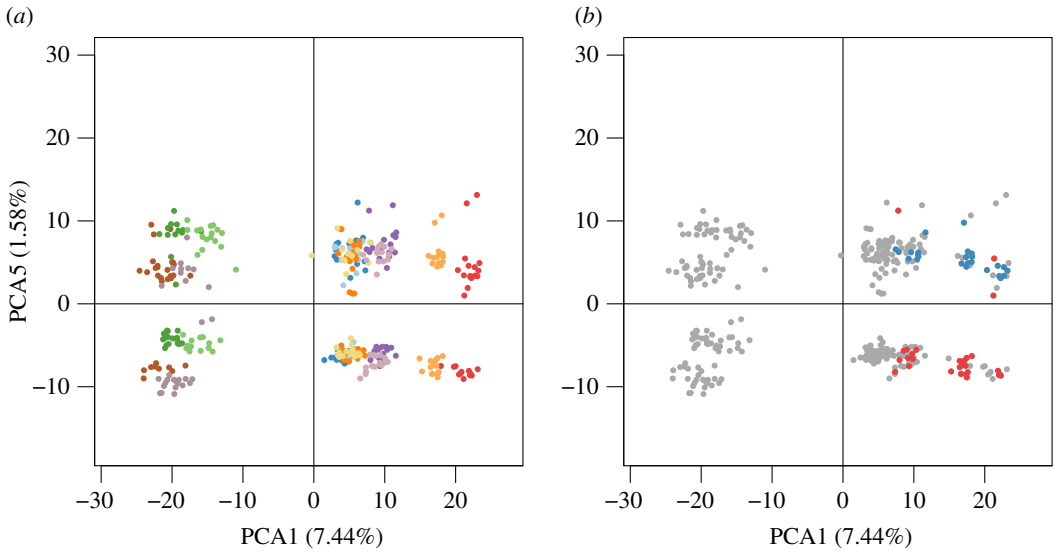


Figure 2. Principal component analysis (axes 1 and 5) of the 12 red coral populations ($n = 354$ individuals, 27 461 SNPs). Colours correspond to the (*a*) population of individuals (light blue: BANN20, dark blue: BANN40, dark orange: BANS20, yellow: BANS40, brown: ELV12, grey: MEJ40, dark green: FIG8, light green: MOR40, red: GAL20, light orange: GAL40, dark purple: POR20, light purple: POR40; see electronic supplementary material, figure S1 and table S1) and (*b*) sex of individuals determined morphologically (red: females, blue: males).

markers diagnostic of sex in the overall dataset may indicate that these loci were submitted to a low but non-null recombination rate, as is the case when sexual chromosomes have recently diverged, or near the boundary of pseudoautosomal regions, where recombination is more frequent than in fully sex-linked regions [25,26]. It could also be the result of polymorphism within the restriction sites on the Y chromosome (i.e. allele dropout) making it impossible to observe the Y allele with RAD-Seq in some populations [27]. A low rate of mutation, recombination or allele dropout could suffice to make a marker go from diagnostic (heterozygous in 100% of males) to sex-linked but not diagnostic (heterozygous in less than 100% of males). For these different reasons, it may be difficult to identify strictly diagnostic loci common to all populations for such species with a strong genetic structure. Nevertheless, even if diagnostic markers of sex were not found at the scale of the overall dataset, the multilocus analysis enabled us to identify clearly males and females. Sex-linked polymorphisms identified by RAD-Seq have been found in the pistachio (*Pistacia vera*) and the salmon louse (*Lepeophtheirus salmonis*), where authors identified markers being heterozygous in females and homozygous in males, and suggested a ZW/ZZ system [28,29]. These polymorphisms have also been found in the Atlantic halibut (*Hippoglossus hippoglossus*), the Nile tilapia (*Oreochromis niloticus*) and the date palm (*Phoenix dactylifera*), where an XX/XY system was suggested [30–32]. Here, we highlighted a strong signal of sex-linked markers in our population genomic dataset. Considering the pattern of allele frequencies of these loci, they may be detected as being under balanced selection, which can easily lead to a misinterpretation of the signal when these loci are not expected (especially in species whose sex-determining system is unknown).

3.4. Identification of sex-specific loci

We identified 435 loci present in all 25 morphological males and absent in the 28 morphological females (figure 1*a*,*d*,*e*). To avoid any bias caused by an eventual misidentification of the sex of the three misclassified females, these individuals have not been taken into account for this analysis. To confirm the male specificity of these 435 loci, we targeted six of them in real-time PCR of morphologically sexed individuals. The six loci could be amplified in 100% of morphologically sexed males. Almost no amplification signal was observed in 100% of morphologically sexed females (figure 1*f*; electronic supplementary material, figure S3; $p < 0.01$ in all tests, electronic supplementary material, table S6). The presence of male-specific loci supports our previous hypothesis of a system with male heterogamety (i.e. XX/XY) [16,17,33]. Finally, in order to confirm that the male-specific markers identified from only one geographical region were not the result of random divergence between sexes, we applied the real-time PCR test to the 40 individuals from the additional June 2016 sampling in Marseille. The six

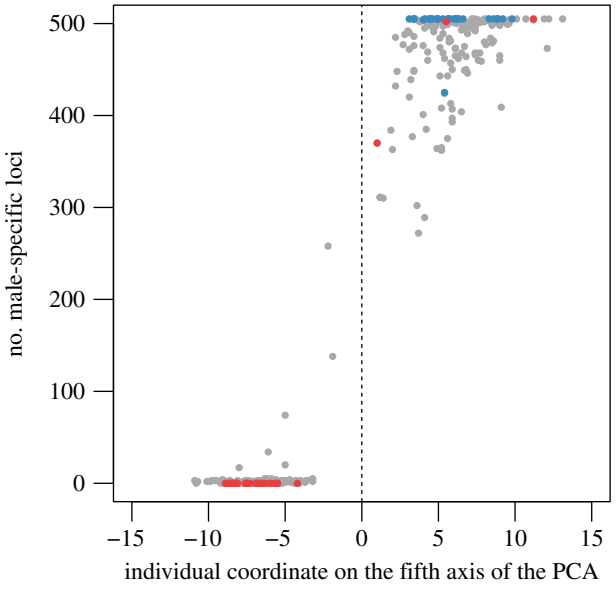


Figure 3. Plot of the number of male-specific loci possessed by an individual as a function of the individual coordinate on the fifth axis of the PCA. Morphologically sexed individuals are indicated (red: females, blue: males).

male-specific primer pairs enabled us to amplify 100% of morphologically sexed males by real-time PCR. Almost no signal was observed in 22 of the morphologically sexed females (figure 1*g*; electronic supplementary material, figure S4; $p < 0.01$ in all tests, electronic supplementary material, table S6). Two females presented an amplification profile of the six markers similar to that of the males, as was also the case for three females from Corsica. This confirms that these loci are male-specific, and that they are conserved between the two distant populations.

3.5. Cross-validation

Finally, as a validation test, we crossed the results obtained by PCA and by sex-specific loci by searching the presence of the 435 male-specific loci in all 354 individuals (figure 3). Twenty-four per cent of PCA-identified males possessed all 435 male-specific loci and 80% of the individuals possessed 90% of these loci. Some of these loci may have been lost during library preparation and sequencing, or due to allele dropout, thereby explaining their absence in some of the males. Furthermore, the male-specific loci have been found from 25 individuals, and it is likely that some of them have been detected erroneously if the pattern of technical missing data for a locus followed by chance the male/female distribution. All of the 435 male-specific loci were absent in 40% of PCA-identified females, and 97% of PCA-identified females contained less than 1% of male-specific loci. Figure 3 illustrates the correlation between the three methods of sex identification presented here (coordinate on the fifth axis of the PCA, number of male-specific loci and morphological identification). We observed an extremely good correlation between these three methods, the groups of males and females being well defined in each case. The three misclassified females identified from the PCA also possessed a high number of male-specific loci, and seemed to be genetically male. Such incongruence between sex genotype and phenotype may result from an environmental sexual reversal during sexual differentiation [34], or from the existence of females with the XY genotype, as already described in several mammals [35]. Finally, four individuals that have not been sexed morphologically presented female genotypes but between 15 and 65 male-specific loci. Further analysis is needed to determine the sexual identity of these individuals.

The number of sex-specific markers should depend on the size of the non-recombining region and on the divergence between X and Y, from a minimal Y-specific region for homomorphic sex chromosomes to several sex-specific markers in heteromorphic sex chromosomes [33]. The 435 male-specific loci detected here suggested at least 218 male-specific PstI sites, which is far higher than the number of sex-specific loci in other similar studies based on RAD-Seq [17,33,36]. However, considering the specificity of each study (genome size, frequency of the restriction site and parameters used for the loci assembly), we could not directly compare the number of sex-linked markers or the number of male-specific markers.

Finally, the functional annotation of loci in the Stacks catalogue (see the electronic supplementary material, methods) enabled us to identified a homologue of a double-sex and mab3-related transcription factor (*Dmrt*). The *Dmrt* family of transcription factors is involved in sex determination in numerous metazoans, and the conservation of this function in cnidarians has recently been shown [37,38]. In our case, this gene was neither a sex-specific marker nor a sex-linked marker, which remains compatible with a role in sexual differentiation.

4. Conclusion

In summary, the exploration both of sex-linked polymorphisms and of sex-specific loci enabled us to identify an XX/XY genetic sex-determination system in the red coral. This is the first time such an identification has been made for non-bilaterian species, and is a new example of the evolution of GSD in multicellular organisms [3,39]. As gonochorism is predominant among octocorals [8], they constitute a promising group for further exploration of GSD among Cnidaria. In hexacorals, the sister-group of octocorals [40,41], there is apparently a higher diversity of reproduction systems than in octocorals [6]. Extending our study to other anthozoans would be useful in order to test scenarios of evolution of reproduction systems in this group [6]. Furthermore, our study has shown that signal of GSD, if it is unexpected, can be misinterpreted in non-model organisms (for example because sex-linked markers could be detected as being under balanced selection). Our research also emphasizes that population genomic datasets should be analysed and interpreted by taking into account the possibility of GSD in non-model organisms. We provide here real-time PCR primers that will enable the identification of red coral males and females, and that will facilitate the monitoring of the population dynamics of this emblematic species, which is being increasingly submitted to anthropic pressures such as harvesting and global change [42–44].

Ethics. The experiments performed during this study are in agreement with the French law on animal experiments (law no. R 214-87 to 90 of 'Code rural'). The sampling was authorized by the French Ministry of Ecology, Sustainable Development, Transports and Housing (authorization no. 009, date: January 2013; authorization no. 943, date: 17 December 2013; authorization no. 150, date: 12 March 2013).

Data accessibility. The raw DNA sequences are available in the Short Read Archive (SRA) database under accession no. SRR5186771-SRR5187129 [45]. The filtered SNP file is available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.rs7bm [46].

Authors' contributions. D.A., P.P., A.H., G.M. and E.T. conceived the project. A.H. and M.P. performed library preparation. M.B. performed the library sequencing. S.R. performed the bioinformatic quality control of sequenced libraries. A.H. performed the real-time PCR. S.C. morphologically determined the sex of individuals. M.P. analysed the data. M.P. wrote the paper. A.H., D.A., E.T., G.M., K.B., M.P., P.P. and S.C. discussed the results and contributed to the revision of the final manuscript.

Competing interests. We declare we have no competing interests.

Funding. This project has been funded by the ADACNI programme of the French National Research Agency (ANR) (project no. ANR-12-ADAP-0016; http://adacni.imbe.fr) and ECCOREV Research Federation (FR 3098). This work is a contribution to the Labex OT-Med (no. ANR-11-LABX-0061) funded by the French Government 'Investissements d'Avenir' programme of the French National Research Agency (ANR) through the A*MIDEX project (no. ANR-11-IDEX-0001-02).

Acknowledgements. We thank Nicolas Fernandez and Béatrice Loriod from the Marseille TGML platform for their invaluable help and advice with the preparation of the RAD libraries; Véronique Dhennin from the Biology Institute of Lille; and the team of the MGX platform for the sequencing of the RAD libraries. We thank the molecular biology service of the IMBE and Frédéric Zuberer of the Pytheas Institute for their support in sampling. We thank the Scandola Natural Reserve, especially Jean-Marie Dominici, and also thank Alexander Ereskovsky for his advice in red coral sexing.

References

1. Speijer D, Lukeš J, Eliáš M. 2015 Sex is a ubiquitous, ancient, and inherent attribute of eukaryotic life. *Proc. Natl Acad. Sci. USA* **112**, 8827–8834. (doi:10.1073/pnas.1501725112)
2. Otto SP. 2009 The evolutionary enigma of sex. *Am. Nat.* **174**, S1–S14. (doi:10.1086/599084)
3. Beukeboom LW, Perrin N. 2014 *The evolution of sex determination*. Oxford, UK: Oxford University Press.
4. Bachtrog D *et al.* 2014 Sex determination: why so many ways of doing it? *PLoS Biol.* **12**, e1001899. (doi:10.1371/journal.pbio.1001899)
5. Berec L, Schembri PJ, Boukal DS. 2005 Sex determination in *Bonellia viridis* (Echiura: Bonelliidae): population dynamics and evolution. *Oikos* **108**, 473–484. (doi:10.1111/j.0030-1299.2005.13350.x)
6. Siebert S, Juliano CE. In press. Sex, polyps, and medusae: determination and maintenance of sex in cnidarians. *Mol. Reprod. Dev.* (doi:10.1002/mrd.22690)
7. Taguchi T *et al.* 2014 Molecular cytogenetic analysis of the scleractinian coral *Acropora solitaryensis* Veron & Wallace 1984. *Zool. Sci.* **31**, 89–94. (doi:10.2108/zsj.31.89)

8. Kahng SE, Benayahu Y, Lasker HR. 2011 Sexual reproduction in octocorals. *Mar. Ecol. Prog. Ser.* **443**, 265–283. (doi:10.3354/meps09414)
9. McFadden CS, Donahue R, Hadland BK, Weston R. 2001 A molecular phylogenetic analysis of reproductive trait evolution in the soft coral genus *Alcyonium*. *Evolution* **55**, 54–67. (doi:10.1111/j.0014-3820.2001.tb01272.x)
10. Torrents O, Garrabou J, Marschal C, Harmelin J. 2005 Age and size at first reproduction in the commercially exploited red coral *Corallium rubrum* (L.) in the Marseilles area (France, NW Mediterranean). *Biol. Conserv.* **121**, 391–397. (doi:10.1016/j.biocon.2004.05.010)
11. Marschal C, Garrabou J, Harmelin J, Pichon M. 2004 A new method for measuring growth and age in the precious red coral *Corallium rubrum* (L.). *Coral Reefs* **23**, 423–432. (doi:10.1007/s00338-004-0398-6)
12. Ledoux J-B, Mokhtar-Jamaï K, Roby C, Féral J-P, Garrabou J, Aurelle D. 2010 Genetic survey of shallow populations of the Mediterranean red coral [*Corallium rubrum* (Linnaeus, 1758)]: new insights into evolutionary processes shaping nuclear diversity and implications for conservation. *Mol. Ecol.* **19**, 675–690. (doi:10.1111/j.1365-294X.2009.04516.x)
13. Santangelo G, Bramanti L, Iannelli M. 2007 Population dynamics and conservation biology of the over-exploited Mediterranean red coral. *J. Theor. Biol.* **244**, 416–423. (doi:10.1016/j.jtbi.2006.08.027)
14. de Lacaze-Duthiers H. 1864 Histoire naturelle du corail: organisation, reproduction, pêche en Algérie, industrie et commerce. J.-B. Baillière et fils.
15. Santangelo G, Carletti E, Maggi E, Bramanti L. 2003 Reproduction and population sexual structure of the overexploited Mediterranean red coral *Corallium rubrum*. *Mar. Ecol. Prog. Ser.* **248**, 99–108. (doi:10.3354/meps248099)
16. Gamble T, Zarkower D. 2014 Identification of sex-specific molecular markers using restriction site-associated DNA sequencing. *Mol. Ecol. Resour.* **14**, 902–913. (doi:10.1111/1755-0998.12237)
17. Gamble T. 2016 Using RAD-seq to recognize sex-specific markers and sex chromosome systems. *Mol. Ecol.* **25**, 2114–2116. (doi:10.1111/mec.13648)
18. Sambrook J, Fritsch EF, Maniatis T, others. 1989 *Molecular cloning*, vol. 2. New York, NY: Cold Spring Harbor Laboratory Press.
19. Etter PD, Bassham S, Hohenlohe PA, Johnson EA, Cresko WA. 2011 SNP discovery and genotyping for evolutionary genetics using RAD sequencing. *Mol. Methods Evol. Genet.* **772**, 157–178. (doi:10.1007/978-1-61779-228-1_9)
20. Catchen J, Hohenlohe PA, Bassham S, Amores A, Cresko WA. 2013 Stacks: an analysis tool set for population genomics. *Mol. Ecol.* **22**, 3124–3140. (doi:10.1111/mec.12354)
21. Catchen JM, Amores A, Hohenlohe P, Cresko W, Postlethwait JH. 2011 *Stacks*: building and genotyping loci *de novo* from short-read sequences. *G3 Genes Genomes Genet.* **1**, 171–182. (doi:10.1534/g3.111.000240)
22. Jombart T. 2008 adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics* **24**, 1403–1405. (doi:10.1093/bioinformatics/btn129)
23. R Development Core Team. 2011 R 2.14. 1. R project for statistical computing, Vienna, Austria.
24. Pratlong M *et al.* In preparation. Identifying adaptive loci in a context of high genetic structure, local adaptation to depth in the red coral.
25. Qiu S, Bergero R, Guirao-Rico S, Campos JL, Cezard T, Gharbi K, Charlesworth D. 2016 RAD mapping reveals an evolving, polymorphic and fuzzy boundary of a plant pseudoautosomal region. *Mol. Ecol.* **25**, 414–430. (doi:10.1111/mec.13297)
26. Bergero R, Qiu S, Forrest A, Borthwick H, Charlesworth D. 2013 Expansion of the pseudo-autosomal region and ongoing recombination suppression in the *Silene latifolia* sex chromosomes. *Genetics* **194**, 673–686. (doi:10.1534/genetics.113.150755)
27. Gautier M, Gharbi K, Cezard T, Foucaud J, Kerdelhué C, Pudlo P, Cornuet J-M, Estoup A. 2013 The effect of RAD allele dropout on the estimation of genetic variation within and between populations. *Mol. Ecol.* **22**, 3165–3178. (doi:10.1111/mec.12089)
28. Kafkas S, Khodaeiaminjan M, Güney M, Kafkas E. 2015 Identification of sex-linked SNP markers using RAD sequencing suggests ZW/ZZ sex determination in *Pistacia vera* L. *BMC Genomics* **16**, 98. (doi:10.1186/s12864-015-1326-6)
29. Carmichael SN *et al.* 2013 Identification of a sex-linked SNP marker in the salmon louse (*Lepeophtheirus salmonis*) using RAD sequencing. *PLoS ONE* **8**, e77832. (doi:10.1371/journal.pone.0077832)
30. Palaiokostas C *et al.* 2013 Mapping the sex determination locus in the Atlantic halibut (*Hippoglossus hippoglossus*) using RAD sequencing. *BMC Genomics* **14**, 566. (doi:10.1186/1471-2164-14-566)
31. Palaiokostas C, Bekaert M, Khan MG, Taggart JB, Gharbi K, McAndrew BJ, Penman DJ, Orban L. 2013 Mapping and validation of the major sex-determining region in Nile tilapia (*Oreochromis niloticus* L.) using RAD sequencing. *PLoS ONE* **8**, e68389. (doi:10.1371/journal.pone.0068389)
32. Cherif E *et al.* 2013 Male-specific DNA markers provide genetic evidence of an XY chromosome system, a recombination arrest and allow the tracing of paternal lineages in date palm. *New Phytol.* **197**, 409–415. (doi:10.1111/nph.12069)
33. Gamble T, Coryell J, Ezaz T, Lynch J, Scantlebury DP, Zarkower D. 2015 Restriction site-associated DNA sequencing (RAD-seq) reveals an extraordinary number of transitions among gecko sex-determining systems. *Mol. Biol. Evol.* **32**, 1296–1309. (doi:10.1093/molbev/msv023)
34. Bianchi NO. 2002 Akodon sex reversed females: the never ending story. *Cytogenet. Genome Res.* **96**, 60–65. (doi:10.1159/000063029)
35. Saunders PA, Franco T, Sottas C, Maurice T, Ganem G, Veyrunes F. 2016 Masculinised behaviour of XY females in a mammal with naturally occuring sex reversal. *Sci. Rep.* **6**, 22881. (doi:10.1038/srep22881)
36. Fowler BL, Buonaccorsi VP. 2016 Genomic characterization of sex-identification markers in *Sebastes carnatus* and *S. chrysomelas* rockfishes. *Mol. Ecol.* **25**, 2165–2175. (doi:10.1111/mec.13594)
37. Chen C-J, Shikina S, Chen W-J, Chung Y-J, Chiu Y-L, Bertrand JAM, Lee Y-H, Chang C-F. 2016 A novel female-specific and sexual reproduction-associated Dmrt gene discovered in the stony coral, *Euphyllia ancora*. *Biol. Reprod.* **94**, 40. (doi:10.1095/biolreprod.115.133173)
38. Traylor-Knowles NG, Kane EG, Sombatsaphay V, Finnerty JR, Reitzel AM. 2015 Sex-specific and developmental expression of Dmrt genes in the starlet sea anemone, *Nematostella vectensis*. *EvoDevo* **6**, 13. (doi:10.1186/s13227-015-0013-7)
39. Bergero R, Charlesworth D. 2009 The evolution of restricted recombination in sex chromosomes. *Trends Ecol. Evol.* **24**, 94–102. (doi:10.1016/j.tree.2008.09.010)
40. Pratlong M, Rancurel C, Pontarotti P, Aurelle D. In press. Monophyly of Anthozoa (Cnidaria): why do nuclear and mitochondrial phylogenies disagree? *Zool. Scrip*. (doi:10.1111/zsc.12208)
41. Zapata F *et al.* 2015 Phylogenomic analyses support traditional relationships within Cnidaria. *PLoS ONE* **10**, e0139068. (doi:10.1371/journal.pone.0139068)
42. Bramanti L *et al.* 2013 Detrimental effects of ocean acidification on the economically important Mediterranean red coral (*Corallium rubrum*). *Glob. Change Biol.* **19**, 1897–1908. (doi:10.1111/gcb.12171)
43. Garrabou J, Perez T, Sartoretto S, Harmelin JG. 2001 Mass mortality event in red coral *Corallium rubrum* populations in the Provence region (France, NW Mediterranean). *Mar. Ecol. Prog. Ser.* **217**, 263–272. (doi:10.3354/meps217263)
44. Priori C, Erra F, Angiolillo M, Santangelo G. 2015 Effects of gastropod predation on the reproductive output of an overexploited deep octocoral. *Coral Reefs* **34**, 59–63. (doi:10.1007/s00338-014-1223-5)
45. Pratlong M *et al.* 2016 Data from: Evidence for a genetic sex determination in Cnidaria, the Mediterranean red coral (*Corallium rubrum*). Short Read Archive (SRA) database. Accessions no. SRR5186771-SRR5187129.
46. Pratlong M *et al.* 2017 Data from: Evidence for a genetic sex determination in Cnidaria, the Mediterranean red coral (*Corallium rubrum*). Dryad Digital Repository. (doi:10.5061/dryad.rs7bm)

RÉSUMÉ

Dans le contexte du réchauffement climatique, les récifs coralliens subissent des stress thermiques de plus en plus fréquents et intenses. Dans le but de mieux comprendre les mécanismes de la thermo-tolérance des coraux, j'ai développé une approche intégrative sur l'holobionte corallien (méta-organisme composé de l'hôte corallien, son algue symbiotique et son microbiote). Pour cela, j'ai réalisé une expérience de stress thermique écologiquement réaliste sur une espèce de corail, *Pocillopora damicornis*. Cette espèce étant présente dans l'ensemble de l'Indo-Pacifique, j'ai pu comparer la réponse de deux populations dont la thermotolérance est différente puisqu'elles sont soumises à des régimes thermiques contrastés. J'ai analysé, pour chacune d'entre-elles, la réponse de l'hôte corallien (par RNAseq), ainsi que la structure et les changements au niveau des microbiotes algaux et bactériens (par métabarcoding). Les résultats obtenus montrent qu'alors que la structure du microbiote n'est pas influencée par le stress, le corail y répond de façon très différente selon la population étudiée. La population issue d'un environnement plus fluctuant met en place une réponse plus efficace et plus plastique, probablement grâce à l'intervention de mécanismes épigénétiques. Une autre étude réalisée sur différentes populations de *P. damicornis* dans le cadre de cette thèse montre que la composition du microbiote est influencée par le génome de l'hôte ainsi que par le régime thermique. Un des clades de l'algue symbiotique connu pour améliorer la thermo-tolérance de l'hôte corallien semble plus sensible aux basses températures que les autres.

SUMMARY

In the context of global warming, coral reefs are experiencing thermal stresses which are becoming more frequent and intense. In order to get a better understanding of the mechanisms of coral thermo-tolerance, I developed an integrative approach on the coral holobiont (meta-organism composed of the coral host, its symbiotic algae and microbiota). For this, I performed an ecologically realistic thermal stress experiment on a coral species, *Pocillopora damicornis*. This species is widespread in the Indo-Pacific area. I compared the response of two populations whose thermotolerance is different since they are subjected to contrasting thermal regimes. I analyzed, for each of them, the response of the coral host (by RNAseq), as well as the structure and changes in the algal and bacterial microbiota (by metabarcoding). The results show that, while the structure of the microbiota is not influenced by stress, coral responds very differently depending on the population studied. The population from a more fluctuating environment displays a more effective and more plastic response, probably thanks to the involvement of epigenetic mechanisms. Another study carried out on different populations of *P. damicornis* showed that the composition of the microbiota is influenced by the host genome and the thermal regime. One of the clades of the symbiotic algae, known to improve the heat-tolerance of the coral host, appears more sensitive to low temperatures than the others.