



**HAL**  
open science

# Effects of extreme environmental conditions of the Bouraké lagoon on sponge *Rhabdastrella globostellata* metabolism

Federica Maggioni

► **To cite this version:**

Federica Maggioni. Effects of extreme environmental conditions of the Bouraké lagoon on sponge *Rhabdastrella globostellata* metabolism. Life Sciences [q-bio]. Université de la Nouvelle-Calédonie, 2023. English. NNT: 2023NCAL0001 . tel-04610309

**HAL Id: tel-04610309**

**<https://unc.hal.science/tel-04610309>**

Submitted on 13 Jun 2024

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

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



Distributed under a Creative Commons Attribution 4.0 International License

**UNIVERSITÉ DE LA NOUVELLE-CALÉDONIE**  
**ÉCOLE DOCTORALE DU PACIFIQUE (ED 469)**

*Thèse de Doctorat*

*Biologie des populations et écologie*

Federica MAGGIONI

**Effets des conditions environnementales extrêmes de la lagune  
de Bouraké sur le métabolisme de l'éponge *Rhabdastrella*  
*globostellata***

**Effects of extreme environmental conditions of the Bouraké  
lagoon on sponge *Rhabdastrella globostellata* metabolism**

*Soutenue le 13 Avril 2023 devant le jury composé de : – Defended on April 13, 2023  
in front of the jury composed of:*

Dr. Mathieu Pernice  
Dr. Thierry Perez  
Pr. Claude Payri  
Dr. Verena Schoepf  
Dr. Benjamin Mueller  
Pr. James J. Bell  
Pr. Yves Letourneur  
Dr. Riccardo Rodolfo-Metalpa

Rapporteur  
Rapporteur  
Examinatrice  
Examinatrice  
Examinateur  
Examinateur  
Directeur  
Encadrant



***“People protect what they love, they love what they understand and they understand what they are taught”***

*Jacques-Yves Cousteau*



## Abstract

Anthropogenic carbon dioxide emissions and other greenhouse gasses have increased exponentially since the industrial revolution, causing significant changes in the Earth's climate. The three main effects of climate change on marine ecosystems are warming, acidification, and oxygen decline. While most ecological and physiological research targets corals, the effects on sponges and their ecological role have yet to be further investigated. Sponges provide essential ecological functions, including contributions to nutrient and carbon cycling. They can uptake dissolved organic carbon and transform and release it in particulate organic carbon (i.e., detritus), which higher trophic levels can use. Understanding how future climatic conditions could alter the ecological role of sponges is critical for a better understanding of the future of coral reefs. This thesis addressed the gaps in our knowledge of how sponge nutrients and organic matter recycling might change under extreme environmental conditions (high temperature, low pH, and low dissolved oxygen).

Firstly (chapter 2), I performed a long-term physical, chemical, and benthic characterization of the Bouraké lagoon (New Caledonia). Data collected from 2016 to 2020 showed that this site is characterized by daily variation, driven by tide, of chemical and physical parameters, which reach extreme values during low tide (33°C, 7.2 pH<sub>T</sub>, and 1.9 mg O<sub>2</sub> L<sup>-1</sup>). Despite these extreme environmental conditions, a rich and healthy benthic community was found with 66 coral species, 28 macroalgae species, and 11 sponge species. My results demonstrate that the Bouraké lagoon is a unique site that can be used as a natural laboratory to study the effects of extreme environmental conditions on organisms.

After characterizing my study site, I performed 118 *in-situ* incubations, in the Bouraké lagoon, using respirometric benthic chambers to study the effects of extreme conditions on sponge carbon and nutrient cycling and the photosynthetic activity of their associated symbionts (chapter 3). The most abundant sponge species found in the Bouraké lagoon, *Rhabdastrella globostellata* (Carter, 1883), was chosen as a model species and incubated *in-situ* under both normal (high tide) and extreme (low tide) conditions. The responses of the sponge holobionts were examined by comparing the changes in physical and chemical seawater parameters at the beginning and the end of incubations. The results showed that the sponges stopped producing detritus (i.e., the sponge loop) under extreme conditions. My findings also suggest that high temperature (> 31°C) could be the main driver of this break in the sponge organic carbon recycling, particularly when combined with extreme acidification and deoxygenation levels.

From the results of the *in-situ* experiments, the temperature was hypothesized as the main factor affecting the sponge carbon cycle. *Ex-situ* aquarium experiments were then performed to explore the nitrogen and carbon recycling response of *R. globostellata*'s exposed to high temperatures (chapter 4). Specimens of *R. globostellata* from the Bouraké lagoon and a control site were exposed at 28°C and 32°C. The responses were assessed by tracking labeled  $^{13}\text{C}$  and  $^{15}\text{N}$  in sponge tissue and detritus production. The results show that the Bouraké sponges incorporated more  $^{13}\text{C}$  and  $^{15}\text{N}$ , assimilated as coral mucus into the tissues than released as detritus. These rates doubled when exposed at 32°C compared to 28°C. In contrast, sponges from the control site incorporate less  $^{13}\text{C}$  and  $^{15}\text{N}$  in their tissues and released more in detritus. The change in C and N allocation, with greater accumulation in the sponge tissues of Bouraké specimens, may be an adaptation to the extreme environmental conditions of the site.

In a world where climate change is accelerating, and many of the effects of these changes on organisms have yet to be studied, my research demonstrates how the sponge *R. globostellata* responds to different environmental conditions and shows that its metabolism changes to thrive in extreme environments. I provide the first evidence that exposure to elevated temperatures (+3°C) results in a change in the allocation and use of carbon and nitrogen between sponge tissue and detritus, also providing insight into the possible change in the availability and quality of food sources in a future coral reef. Finally, I showed evidences that sponges could develop a mechanism for adapting to different environmental conditions by making the most of it. My hope is that the suggested adaptation to future environmental conditions will be possible also for other taxa.

## Acknowledgments

First, I wish to thank my supervisor Dr. Riccardo Rodolfo-Metalpa, without whom none of this would have been possible. I still remember our first dive in Bouraké during my MSc thesis in 2018, where because of the visibility, I lost you after not even 1 minute and found myself on the other side of the lagoon carried by the strong current. I thought to myself: "who made me do that?!" It feels odd to end (for the moment) this adventure after so many years. Thank you for your support and patience. I also want to thank my supervisor Professor Yves Letourneur, for his constant guidance and support. I am very glad to have had the opportunity to learn from both of you!

Special thanks to Pr. Claude Payri, Dr. Verena Schoepf, Dr. Mathieu Pernice, Dr. Benjamin Mueller, Dr. Thierry Perez, Pr. James J. Bell, and for agreeing to be my examiners.

I wish to thank Professor James J. Bell for always being available to advise and support me with this research project. Thank you so much for your inspiration and the time you took to help me. I also thank Professor Carlo Cerrano who since my MSc has always followed me and given me advice over the years. Both of their thoughtful advice have constantly improved my thinking. It has certainly been a great pleasure for me to work with you, and I hope we will find ways to continue to share our ideas.

I owe a debt of gratitude to all of the people with that I have worked with who have influenced and supported me along the way. I wish to express my sincere gratitude to the IRD diver staff (Mahé, and Magali), boat captains (Miguel, Sam, Philippe, Jordi), and the ALIS staff for logistical support during all field trips and experiments. Thanks to the LAMA laboratory staff and the IFREMER staff (Adecap, Saint-Vincent) for their willingness and hospitality during the endless days of the laboratory. Thanks to Dr. Hugues Lemonnier for his advice and support. Special thanks to all the staff of "Aquarium de lagoon" for their support during my aquarium experience, particularly to Dr. Olivier Chateau who supported me at every step of the experimental design, even though sometimes my ideas might have been a bit crazy! I wish to sincerely thank Dr. Clarisse Majorel for her support during these years and assistance during laboratory experiments. Finally, thanks to all the researchers of IRD research center for always being helpful to me, and thanks to you Veronique Perrin for your constant help with administrative paperwork, and your delicious desserts. Special thanks for colleagues Dr. Veronique Berteaux-Lecellier and Dr. Gaël Lecellier who allowed me to find the site for the control sponge. Thanks also to my co-authors and collaborators who helped me throughout my thesis and projects: Mireille Pujó-Pay, Patrick Raimbault, James J. Bell, Lorenzo Bramanti, Carlo Cerrano, Philippe Jourand, Pierre-louis Stenger, Barbara Calcinai, Andreas Ravache, Olivier Chateau, Megan Shaffer, Hugues Lemonnier, Fanny Houlbreque, Francesca Benzoni, Claude Payri, and Jérôme Aucan.

During my PhD I have the chance to meet some researchers and spent time with them during fieldwork. I would like to thank each of them for the inspiration and the good times spent together. Thanks to Timothy Ravasi, Erina Kawai, Billy Moore, Greg Torda, Micheal Izumiyama, Celia Schunter, Sandra Ramirez Calero, Megan Shaffer, James J. Bell, Chris Cornwall. How amazing was the Supernatural 2 mission ? A memory I will have for a lifetime! I hope our collaborations, field trips, after-work drinks, and friendship will continue. Thanks also to Greg and Esme owners of “Chez Esme” in Bouraké, where I spent a lot of time during my fieldwork activity. Thanks for their hospitality, kind dinner and party during fieldwork, and of course the “kava” specialty!

A very special thanks go to my parents Marta e Dario for instilling in me the tenacity, drive, and motivation to get this completed, and for continuous encouragement. I know I have not always been very present, working on the other side of the world, but thank you for letting me live with my passion and making my own choices. Thanks also to my brother Filippo, who never often believed in me by giving me his continuous and fundamental moral support, and to my uncle Domenico for his support. Finally, thanks to Olivier, with whom I have been sharing my life for two years. Thank you for your support, positivity, patience, care, and love.

My experience in New Caledonia would have certainly been hard without the affection and support of my lab mates and friends (Cinzia, Thibault, Valentine and Clément), always ready for advice, 5 minutes of madness (well, maybe 15 minutes), a coffee break or a beer after work! Thanks also to the interns, the other PhD student became my friends and friends during these years (Camille, Laure, Maxime, Andreas, Tristan, Thomas, Nohé, Mahé, Magali, Sebastian, Hadrien, Laetitia, Perrine, Valentine, Hugo, Pauline, Hugo, Xavier, Juliette, Leo, Prisca). A special thanks also to my friends from Italy (Giulia, Giulia, Alice, Alice, Stefania, Alessandro, Federica, Enzo, Luca, Marika, and Davide), who despite the distance have always stayed by my side to listen and support me. This PhD would have been very hard without their infallible affection, morals, and smile.

Finally, I wish to express my sincere gratitude to my funding agency, the University of New Caledonia and “Ecole Doctoral du Pacific” which made this PhD possible through a Doctoral Scholarship. I would like also to thank the IRD center and Aquarium de lagoon for financial support during the field and experiments. Thanks to the 11<sup>th</sup> sponge scholarship supported my attendance at the 11<sup>th</sup> sponge conference in Leiden, where I meet all the amazing people of the sponge family.

Thank you to all to make this chapter of my life possible!

Tata

## Contributions and Publications

The data chapters that make up this thesis are based on four manuscripts. Chapters 2 and 3 are published in *Biogeosciences* and *Marine Pollution Bulletin*, respectively. Chapter 4 was submitted on *Limnology and Oceanography* journal. A further method article was submitted for publication in *Limnology and Oceanography Methods* (see **Appendix B** – Section B3.1 for article details).

### Chapter 2:

Publication: **Maggioni F.**, Pujo-Pay M., Aucan J., Cerrano C., Calcinai B., Payri C., Benzoni F., Letourneur Y., and Rodolfo-Metalpa R. The Bouraké semi-enclosed lagoon (New Caledonia) – a natural laboratory to study the lifelong adaptation of a coral reef ecosystem to extreme environmental conditions. *Biogeosciences*, 18, 5117–5140, 2021. <https://doi.org/10.5194/bg-18-5117-2021>

### Chapter 3:

Publication: **Maggioni, F.**, Bell, J. J., Pujo-Pay M., Shaffer M., Cerrano C., Lemonnier H., Letourneur Y., and Rodolfo-Metalpa R. Sponge organic matter recycling: Reduced detritus production under extreme environmental conditions. *Marine Pollution Bulletin*, 190: 114869, 2023. <https://doi.org/10.1016/j.marpolbul.2023.114869>

### Chapter 4:

Publication under review: **Maggioni F.**, Raimbault P., Chateau O., Pujo-Pay M., Letourneur Y., and Rodolfo-Metalpa, Carbon and nitrogen recycling shift in warm-adapted sponges. *Limnology and Oceanography*.

### Additional method article:

Publication in preparation: **Maggioni F.**, Ravache A., Pecorari F., and Rodolfo-Metalpa R. Testing the performance of 3D photogrammetry to calculate underwater sponge volume. *Limnology and Oceanography Method*.

**Beside my thesis, three other publications resulting from side projects were written in these three years:**

Publication under review: **Maggioni F.**, Stenger P. L., Jourand P., and Majorel C. The phylum Chloroflexi and their SAR202 clade dominate the microbiome of two marine sponges living in extreme environmental conditions. *Marine Ecology*.

Publication under review: **Maggioni F.**, Stenger P. L., Jourand P., Letourneur Y., and Majorel C. Bioaccumulation of heavy metals in two marine sponges living in the Bouraké lagoon (New Caledonia). *MDPI metals*.

Publication in preparation: Stenger P. L., **Maggioni F.**, Jourand P., and Majorel C. Selection of appropriate DNA markers to assess the hidden microbiome of marine sponges in a specific environment.



---

# TABLE OF CONTENTS

---

<b>Chapter 1: General introduction .....</b>	<b>1</b>
1.1 <i>Climate change implications for coral reefs .....</i>	2
1.2 <i>The sponge holobiont .....</i>	4
1.3 <i>Ecological roles of sponges in coral reefs .....</i>	7
1.4 <i>Impacts of climate change on sponges .....</i>	9
1.5 <i>The natural laboratories .....</i>	11
1.6 <i>Objectives of the study.....</i>	16
 <b>Chapter 2: The Bouraké semi-enclosed lagoon (New Caledonia) – a natural laboratory to study the lifelong adaptation of a coral reef ecosystem to extreme environmental conditions. 19</b>	
<b>Résumé .....</b>	<b>20</b>
<b>Abstract.....</b>	<b>23</b>
2.1 <i>Introduction.....</i>	25
2.2 <i>Methods .....</i>	27
2.2.1 <i>Study site .....</i>	27
2.2.2 <i>Environmental monitoring.....</i>	28
2.2.2.1 <i>Oceanographic sensors deployment and short- to long-term measurements .....</i>	28
2.2.2.2 <i>Phase averaged and tidal harmonic analyses for diurnal and semidiurnal oscillations .....</i>	29
2.2.2.3 <i>Diel cycles of carbonate chemistry and chemical parameters .....</i>	30
2.2.3 <i>Benthic community characterization and distribution.....</i>	32
2.2.4 <i>Stable isotopes analyses.....</i>	33
2.3 <i>Results .....</i>	35
2.3.1 <i>Variability of physical environmental parameters.....</i>	35
2.3.2 <i>Diel cycles of carbonate chemistry and chemical parameters .....</i>	42
2.3.3 <i>Benthic community distribution and species identification.....</i>	45
2.3.4 <i>Stable isotope signatures.....</i>	49
2.4 <i>Discussion .....</i>	51
2.4.1 <i>Physical and chemical characteristics of the Bouraké lagoon .....</i>	51
2.4.2 <i>Stable carbon and nitrogen isotope signature of the Bouraké lagoon .....</i>	54
2.4.3 <i>Effects of physical and chemical conditions on species distribution .....</i>	55
2.5 <i>Conclusion.....</i>	60
 <b>Chapter 3: Sponge organic matter recycling: reduced detritus production under extreme environmental conditions .....</b>	<b>63</b>
<b>Resumé.....</b>	<b>64</b>
<b>Abstract.....</b>	<b>67</b>

3.1 Introduction.....	68
3.2 Materials and methods.....	71
3.2.1 The sponge study model <i>Rhabdastrella globostellata</i> (Carter, 1883).....	71
3.2.2 Experimental design.....	71
3.2.3 Incubation set up.....	72
3.2.4 Data collection.....	73
3.2.5 Seawater analyses.....	73
3.2.6 Data analysis.....	74
3.3 Results.....	75
3.3.1 Environmental conditions at the start of the dark incubations.....	75
3.3.2 Metabolic activities of the sponge holobiont in the dark.....	76
3.3.3 Sponge holobiont photosynthetic activity.....	81
3.4 Discussion.....	83
3.4.1 Changes in sponge organic matter recycling.....	83
3.4.2 Effect of extreme conditions on sponge holobiont nutrient recycling and feeding.....	86
3.4.3 Effect of extreme conditions on the sponge photosynthetic activity.....	88
3.5 Conclusion.....	89
<b>Chapter 4: Carbon and nitrogen recycling shift in warm-adapted sponges.....</b>	<b>91</b>
<b>Resumé.....</b>	<b>92</b>
<b>Abstract.....</b>	<b>93</b>
4.1 Introduction.....	94
4.2 Materials and methods.....	95
4.2.1 Samples collection and maintenance.....	95
4.2.2 Acclimatization and experimental set-up.....	96
4.2.3 Corals labelling.....	97
4.2.4 Transfer and incorporation of coral mucus to sponges.....	98
4.2.5 Organic matter and seawater chemical measurements.....	99
4.2.6 Isotopic analyses.....	100
4.2.7 Statistical analyses.....	101
4.3 Results.....	102
4.3.1 Environmental parameters.....	102
4.3.2 Carbon and nitrogen consumption and production.....	102
4.3.3 Sponges and detritus enrichment.....	105
4.3.4 <sup>13</sup> C and <sup>15</sup> N total fluxes and incorporation in the sponge tissue and detritus.....	106
4.4 Discussion.....	108
<b>Chapter 5: General discussion.....</b>	<b>113</b>
5.1 <i>Rhabdastrella globostellata</i> shows different strategies to cope with extreme environmental conditions.....	114
5.2 Bouraké sponges: a model of adaptation for future coral reefs?.....	119

---

<i>5.3 Future perspectives</i> .....	121
<i>5.4 General conclusion</i> .....	122
<b>References</b> .....	<b>125</b>
<b>Appendices</b> .....	<b>150</b>
<i>Appendix A</i> .....	151
<i>Appendix B</i> .....	164
<i>Appendix C</i> .....	184
<b>French summary - résumé</b> .....	<b>190</b>
<b>Italian summary - sintesi</b> .....	<b>214</b>

## LIST OF FIGURES

- Figure 1.1.** Schematic representation of a sponge with pinacocytes (external epithelium, Pinacoderm), choanocytes (internal epithelium), the mesohyl with spicules and Amoebocyte cells. Arrows indicate the direction of water flow. .... 5
- Figure 1.2.** Example of different sponge morphologies based on environmental conditions (from Schönberg, 2021). .... 6
- Figure 1.3.** Cycling of dissolved organic carbon (DOC, blue), dissolved inorganic nitrogen and nutrients (DIN, green), and detritus (black) in Caribbean fore-reef environments (Pawlik and McMurray, 2020). The consumption of component organisms is shown in red. This figure summarizes essential components of the sponge loop (De Goeij et al., 2013) and the direct predation on sponge tissue (Pawlik et al., 2016; Mumby and Steneck, 2018). Evidence for seaweed production of labile DOC (1), which forms a major component of the diet of sponges (2), along with detritus (3). While the sponge-loop hypothesis proposes the transformation of DOC as detritus by cryptic sponges (4), this does not occur for emergent sponges, which return DOC to the ecosystem as food for spongivores (5) (Pawlik and McMurray, 2020). .... 8
- Figure 1.4.** Examples of extreme natural environments: (a) Nikko semi-enclosed bay; (b) Ambite Island CO<sub>2</sub> seep (Papua New Guinea, © Tom Shlesinger); (c) Bouraké semi-enclosed lagoon (New Caledonia, © Serge Andrefouet); and (d) Kimberley Bay (Australia, © Zoe Richards). .... 13
- Figure 1.5.** Examples of mangrove-coral assemblages. (a) Bocas del Toro, Panamá (© Heather A. Stewart), and (b) mangrove lagoon near the Great Barrier Reef in Australia (© Emma Camp). .... 15
- Figure 2.1.** Map of the study site (top panel) showing the semi-enclosed mangrove lagoon of Bouraké and reference reefs (R1 and R2). Photos a and b (bottom panels) are aerial pictures (taken at 130 m above the Bouraké lagoon) of the inner (bottom) and middle reefs (a) and the outer reef (b). White dots and yellow squares indicate the sampling stations used for physical and chemical environmental monitoring, respectively. Georep New Caledonia database (<https://georep.nc/>) and QGIS software were used to build the figure (top panel). .... 27
- Figure 2.2.** Temporal shift between the averaged tides recorded at the reference (St R1 and R2) and at the Bouraké reefs (St B1 and B2) and the predicted tide calculated for the study area (see SHOM: <http://data.shom.fr> for the Nouméa harbour). .... 35
- Figure 2.3.** Long-term temperature monitoring from October 2018 to April 2020 at the reference (St R1 and R2) and Bouraké reefs (St B1 and B2). Data are plotted using weekly averaged temperature (a) and weekly averaged diel changes (b). .... 36
- Figure 2.4.** Seawater pH<sub>T</sub> (a), (b) and dissolved oxygen (DO) (c), (d) variations recorded at the reference (St R1 and R2) and Bouraké reefs (St B1 and B2). Data were overlaid in a single tidal phase (12 h) (a), (c); and in a 24 h cycle (b), (d). Data are 22, 72, 31, and 72 semidiurnal tidal cycles for pH, and 36, 79, 34 and 42 semidiurnal tidal cycles for DO, for R1, R2, B1 and B2, respectively. ... 38
- Figure 2.5.** Spatial and depth-related short-term changes (24 h) in pH<sub>T</sub> at stations in the Bouraké lagoon (see **Figure 2.1** for the stations' location). Spatial variations were assessed: (a) within the inner

reef (St A-C); **(b)** between the inner and the outer reefs (St B1, B2); **(c)** between the outer reef (but still inside the Bouraké lagoon), and the reef outside the semi-enclosed lagoon (St U vs T). Depth-related variations were assessed between shallow reefs and the bottom of the channel: **(d)** at the inner reef (St R); and **(e)** at the outer reef (St S). Tide (black line) refers to St B2. .... 39

**Figure 2.6.** Spatial and depth-related short-term measurements (24 h) of dissolved oxygen (DO) at stations in the Bouraké lagoon (see Figure 1 for the location of the stations). Spatial variations were assessed on **(a)** the inner reef (St A-G), **(b)** the middle reef (St H-N), and **(c)** the outer reef (St O-Q). Depth-related variations between shallow reefs and bottom water within the Bouraké lagoon were assessed **(d)** at the inner reef (St R) and **(e, f)** at the middle reef (St I and N). Tide (black line) refers to St B2..... 41

**Figure 2.7.** Short-term salinity monitoring during **(a)** the winter of 2019 (from 15/07/2019 to 18/07/2019) at the reference (St R1 and R2) and Bouraké reefs (St B1 and B2); **(b)** the summer of 2020 (from 29/11/2020 to 04/12/2020) at the reference (St R1 and R2) and Bouraké reefs (St T and B2). Tide (black line) refers to St B2. .... 42

**Figure 2.8.** Seawater carbonate chemistry measured ( $\text{pH}_T$ ) and calculated (DIC and  $\Omega_{\text{ara}}$ ) during diel cycles in 2017 and 2019 (pooled data, see also Appendix A Figure A2.1.) at R1, R2 and the Bouraké reefs (outer, middle and inner) **(a-c)**. Boxes ( $n = 6-14$ ) represent the interquartile range (25th and 75th percentile); the horizontal line is the median, and the whiskers represent the data range (i.e., minimum and maximum). Changes are illustrated across a 12 h tidal phase in the Bouraké lagoon **(d-f)**. Linear regression lines are plotted for each falling (HT to LT) and rising tide (LT to HT). Shaded boxes have only a graphical significance and only suggest the tide changes during measurements..... 43

**Figure 2.9.** Orthosilicic acid, organic carbon and nitrogen parameters measured during diel cycles in 2017 and 2019 (pooled data, see also Appendix A Figure A2.2.) at R1, R2 and the Bouraké reefs (outer, middle and inner) **(a-d)**. Boxes ( $n = 6-14$ ) represent the interquartile range (25th and 75th percentile); the horizontal line is the median, and the whiskers represent the data range (i.e., minimum and maximum). Changes are illustrated across a 12 h tidal phase in Bouraké **(e-h)**. Linear regression lines are plotted for each falling (HT to LT) and rising tide (LT to HT). Shaded boxes have only a graphical significance and only suggest the tide changes during measurements. .... 45

**Figure 2.10.** Benthic community and bottom substrate characterization in the Bouraké lagoon. Hierarchical Ward's method cluster dendrogram (top) and non-metric multidimensional scaling (nMDS) of abiotic and biotic percent cover and species richness per transect (T1-T24) **(a)**. Colored circles represent clusters of transects identified in the dendrogram; circle size corresponds to species richness. Ellipses represent 95% confidence intervals. Dominant biotic and abiotic descriptors were used to describe the benthic community and bottom substrate for each cluster in **(b)**. The satellite image is from georep New Caledonia database (<https://georep.nc/>), and the QGIS software was used for transect georeferencing..... 47

**Figure 2.11.** Principal component analysis (PCA) between benthic community (macroalgae, sponges, corals, branching corals and massive corals) data and environmental parameters (PON, POC, DOC,  $\text{Si}(\text{OH})_4$ ,  $\text{PO}_4^{3-}$ ,  $\text{NO}_x$ , and  $\text{NH}_4^+$ ) measured for each transect ( $n = 24$ ). The length of the vectors indicates the contribution of each parameter. The first dimension explains 48.1% of the variance, and the second dimension explains 23.1% of the variance. Colours refer to the cluster subdivision (**Figure 2.10**). .... 48

- Figure 2.12.** Plot of  $\delta^{13}\text{C}$  versus  $\delta^{15}\text{N}$  (mean  $\pm$  SD when more than 1 sample) of macroalgae, sponges, fish, mangrove leaf and seagrasses collected at Bouraké outer (B02), Bouraké inner (B05), and Reference site (R1). Label with \* refer to sample collected in March 2019, while the other were collected in December 2020.....49
- Figure 3.1.** Dissolved oxygen consumption measured during the incubations in the dark in 2018 (in gray) at both LT (n = 9) and HT (n = 12), and 2020 (in blue) at both HT (n = 24) and LT (n = 28). Boxes are the interquartile range of data (25th and 75th percentiles); the horizontal line is the median, and the whiskers represent the data range (i.e., minimum and maximum)..... 76
- Figure 3.2.** Sponge nutrients and organic matter consumption/production measured during the incubations in the dark at HT and LT in 2018 and 2020. **(a)**  $\text{Si}(\text{OH})_4$ , **(b)**  $\text{NO}_x$ , **(c)**  $\text{PO}_4^{3-}$ , **(d)**  $\text{NH}_4^+$ , **(e)** Dissolved Organic Carbon (DOC), **(f)** Particulate Organic Carbon (POC), **(g)** Dissolved Inorganic Carbon (DIC), and **(h)** Bacteria abundance. Data are the difference between the beginning and the end of the incubations. Boxes represent the interquartile range (25th and 75th percentile); the horizontal line is the median, and the whiskers represent the data range (i.e., minimum and maximum). ..... 78
- Figure 3.3.** Sponge consumption/production measured during the incubations in the dark at HT and LT in 2020. **(a)** Detritus (POC-LPOC), **(b)** phytoplankton abundance, **(c)** Life Particulate Organic Carbon (LPOC). Data are the difference between the beginning and the end of the incubations. Boxes represent the interquartile range (25th and 75th percentile); the horizontal line is the median, and the whiskers represent the data range (i.e., minimum and maximum). ..... 79
- Figure 3.4.** Principal Component Analyses (PCA) on the parameters measured in the incubation medium **(a)** in 2018, **(b)** in 2020 at both HT and LT. Each dots in red indicates one single sponge incubation metabolism made during HT, while triangles in blue indicates incubations during LT. Arrows indicate the variable and their length approximates the variance of the variables. The ellipses represent the 95% interval confidence. Large triangles or dots indicate multiple incubations at the same point. .... 80
- Figure 3.5.** Principal Component Analyses (PCA) on Spearman correlation matrix on data collected in 2020 between the seawater chemical parameters measured at the beginning of the incubations, and the absolute values of their changes (i.e., consumption and production) during the incubations. Each dot indicates the sponge metabolism parameters while arrows indicate the variable, and their length approximates the variance of the variables. .... 81
- Figure 3.6.** Generalized additive models (GAM) on **(a)** relative ETR (rETR) and **(b)** the effective quantum yield ( $F_v/F_m$ ) versus irradiance (PAR). Data were obtained from dark-adapted sponges during the ex-situ incubations in 2020 under high tide (HT; n = 9 red) and low tide (LT; n = 9 black) conditions. The shaded areas indicate the 95% confidence intervals of the GAM model. Individual measurements were also reported. .... 82
- Figure 4.1.** Map of New Caledonia (top panel) with the two study sites and pictures of the fragments of *Rhabdastrella globostellata* freshly transplanted at the control site (Tupeti Island, **A**), and after one month in-situ in the Buraké lagoon (**B**). Within A and B, a graph reports a typical temperature variation measured during the summer at each study site. Georep New Caledonia database

---

(<https://georep.nc/>, last access: November 2022) and Qgis software were used to build the figures. .... 96

**Figure 4.2.** Sponge dissolved organic carbon (DOC) and detritus consumption, and production during the incubations at 28°C and 32°C for both sponges from Bouraké and Control site (Tupeti). Boxes are the interquartile range of data (25th and 75th percentiles); the horizontal line is the median, and the whiskers represent the data range (i.e., minimum and maximum). .... 104

**Figure 4.3.** Carbon source uptake (%) by the sponge at 28°C and 32°C from Bouraké and Control (Tupeti) sites. Only consumed values (negative values) of bacteria, dissolved organic carbon (DOC), phytoplankton and particulate organic carbon (POC) as sources of carbon were considered. .... 105

**Figure 4.4.** Stable isotope enrichment of  $^{13}\text{C}$  and  $^{15}\text{N}$  in sponge tissue (**a-b**) and detritus (**c-d**) after 4 and 8 days of experiment, at 28°C and 32°C, for both sponges from Bouraké (dots) and Control (triangles). Non-enriched stable isotope signature as also reported as CTRL (square). Data as expressed as isotope tracer enrichment  $\Delta\delta^{13}\text{C}$  (‰) and  $\Delta\delta^{15}\text{N}$  (‰). Note that detritus enrichment was calculated on a day basis. .... 106

**Figure 4.5.** Sponge tissue and detritus incorporation of  $^{13}\text{C}$  (**a**) and  $^{15}\text{N}$  (**b**) at 28°C and 32°C, for both sponges from Bouraké and control site (Tupeti). Daily incorporation rates (mean  $\pm$  SD) of coral mucus are expressed in  $\mu\text{mol C or N g}^{-1} \text{d}^{-1}$ . .... 108

**Figure 5.1.** Synthetic scheme of the main strategies used by the Bouraké sponge *R. globostellata* to cope (i.e., adaptive response) with a change in temperature. .... 117

## LIST OF TABLES

<b>Table 2.1.</b> Summary of the statistical analyses applied to the seawater physical and chemical parameters collected between 2016 and 2020 at the Bouraké lagoon (St B1, B2, St A-R, outer, middle and inner) and at reference reefs (St R1 and R2). Differences in temperature, salinity and chemical parameters between the Bouraké lagoon and reference reefs and spatial and vertical differences in pH and DO inside the Bouraké lagoon. K-W: Kruskal-Wallis's test; W: Wilcoxon test; GLM: General Linear Model; D: Dunn's test; T: Tukey's test; C: Conover's test. ....	37
<b>Table 2.2.</b> Summary of the seawater physical and chemical data (mean $\pm$ SD) measured in July 2017 and June 2019 and calculated using the CO2SYS package. Data were pooled and averaged per station. nd = not determined. Reference reefs: St R1 and R2; Bouraké lagoon stations: outer, middle and inner. ....	44
<b>Table 2.3.</b> Summary of isotopic signature (mean $\pm$ SD, when more than 1 sample) in carbon ( $\delta^{13}\text{C}$ ‰) and nitrogen ( $\delta^{15}\text{N}$ ‰) for macroalgae, fish, sponges, mangrove leaf and seagrasses collected in March 2019 (Mar-19) and December 2020 (Dec-20) at R1, Bouraké outer (B02), and Bouraké inner (B05). ....	50
<b>Table 3.1.</b> Summary of the environmental parameters measured at the beginning of the incubations in 2018 and in 2020, both at HT and LT. Data are expressed as mean $\pm$ SD; number of replicates are in brackets; nd means not determined. ....	75
<b>Table 3.2.</b> Summary of the gross photosynthesis ( $P_g$ ), respiration ( $R_{\text{dark}}$ ), and their ratio (P:R) measured in-situ, and the initial photosynthetic efficiency ( $F_v/F_m$ ) and $r\text{ETR}_{\text{max}}$ measured ex-situ. For in-situ, $n = 9$ and $n = 8$ at HT and LT, respectively; and for ex-situ, $n = 9$ . Data are normalized and expressed as mean $\pm$ SD. ....	82
<b>Table 4.1.</b> Summary of two-way ANOVAs between temperature conditions (two levels, constant and variable), origin (two levels, control and Bouraké), and their interaction. <b>a)</b> the carbon and nitrogen consumption and/or production measured during 3h of incubation; <b>b)</b> carbon sources consumed during the incubations (only data showing carbon consumption were included); <b>c)</b> $^{13}\text{C}$ and $^{15}\text{N}$ enrichments ( $\Delta\delta^{13}\text{C}$ and $\Delta\delta^{15}\text{N}$ ) measured on both the sponge tissue and detritus at the end of incubations; <b>d)</b> $^{13}\text{C}$ and $^{15}\text{N}$ incorporation measured on both tissue and detritus; and <b>e)</b> $^{13}\text{C}:^{15}\text{N}$ ratio. When the interaction was significant, a Tukey HSD test was applied. B. is Bouraké and C. the control at $32^\circ\text{C}$ and $28^\circ\text{C}$ . ....	103

**CHAPTER 1:**  
GENERAL INTRODUCTION

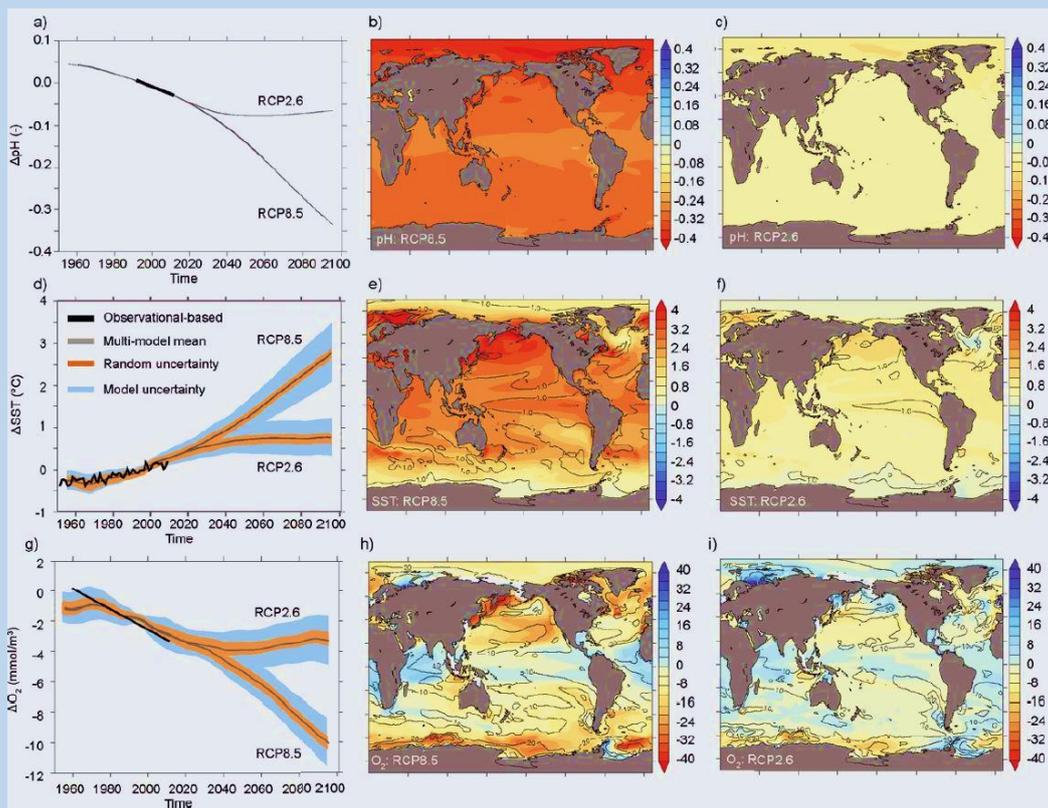
---

## 1.1 Climate change implications for coral reefs

Greenhouse gas emissions such as carbon dioxide or methane from human activities directly impact all ecosystems by altering the composition of communities and threatening species persistence (Chapin et al., 1997; Dinerstein et al., 2019). Atmospheric carbon dioxide ( $\text{CO}_2$  atm) is now 50% higher than pre-industrial levels exceeding 400 ppm (NOAA Mauna Loa website: <http://www.esrl.noaa.gov/gmd/ccgg/trends/mlo.html>). It is expected to increase at unprecedented rate reaching 1000 ppm by 2100 if no additional efforts are made to moderate greenhouse gas emissions (IPCC, 2014). It is estimated that, of the approximately 350 billion tons of  $\text{CO}_2$  that have been already emitted into the atmosphere, 25% has already been absorbed by the ocean (Canadell et al., 2007; Le Quéré et al., 2010; Heinze et al., 2015; Cao and Zhang, 2017). The  $\text{CO}_2$  absorbed in seawater causes an increase in the partial seawater pressure of  $\text{CO}_2$  ( $p\text{CO}_2$ ) and forms carbonic acid ( $\text{H}_2\text{CO}_3$ ), which is unstable and dissociates into hydrogen ( $\text{H}^+$ ) and bicarbonate ( $\text{HCO}_3^-$ ) ions and, at a lower rate, carbonate ions ( $\text{CO}_3^{2-}$ ). The increase in  $\text{H}^+$  affects the water chemistry causing an acidification shift in seawater (e.g., Caldeira and Wickett, 2003; Raven et al., 2005) and a decrease in the pH of the upper layers of the world's oceans (Pörtner and Farrell, 2008). This phenomenon is called ocean acidification (OA) (i.e., Langdon and Atkinson, 2005). Currently, the surface ocean pH ranges on average between 7.9 and 8.2 (Takahashi et al., 2014). The shift in the carbonate chemistry of the ocean has significant implications for marine organisms including reduced calcification rates and disruptions of acid-base physiology, causing general negative impacts on organism physiology, survival and ecosystem functioning (Fabry et al., 2008; Pörtner, 2008). Rising  $\text{CO}_2$  atm has increased global surface temperatures by  $\sim 0.2^\circ\text{C}$  per decade over the past 30 years (Hansen et al., 2006). Oceans absorb a significant amount of this excess energy (Hoegh-Guldberg and Bruno, 2010). The Intergovernmental Panel on Climate Change (IPCC) predicts that ocean warming (OW) resulting from this rising atm  $\text{CO}_2$  will cause an increase in the sea surface temperatures of  $1.1^\circ\text{C}$  to  $3.0^\circ\text{C}$  by the end of the century (IPCC, 2014). Organisms are acclimated to a range of temperatures, and, outside these optimum temperatures, biological processes are likely to be compromised, resulting in changes in individual metabolism, population survival, and species persistence (Hoegh-Guldberg and Bruno, 2010). Organisms' optimum temperatures drive the large-scale geographical distributions of marine organisms (Pörtner, 2008). Ocean warming also influences oceanic processes such as ocean mixing (Manucharyan et al., 2011) and ocean current dynamics, contributing to seawater's oxygen depletion (Winton et al., 2013). This phenomenon is called ocean deoxygenation (OD) (Shaffer et al., 2009), and impacts fundamental ecological processes. According to the IPCC (2014), dissolved oxygen (DO) will decrease by 2% to 4% by the end of the 21<sup>st</sup> century for the RCP 2.6 and RCP 8.5 scenarios, respectively (**Box 1 for RCP details**).

**Box 1: What are scenarios and RCPs? (Source: IPCC website glossary)**

In the context of the IPCC, scenarios aim to explore possible future pathways of GHG (Greenhouse gases) emissions, their main driving forces, and how they might be affected by policy interventions (IPCC, 2007). Based on these factors, four different 21<sup>st</sup> century pathways, the so-called “representative Concentration Pathways” (RCPs), are used for making projections of GHG emissions and atmospheric concentrations, air pollutant emissions and land-use (IPCC, 2007). The RCPs include a stringent mitigation scenario (RCP 2.6), two intermediate scenarios (RCP 4.5 and RCP 6.0), and one scenario with very high GHG emissions (RCP 8.5). Numbers correspond to the radiative forcing peaks of  $3 \text{ W m}^{-2}$ ,  $4.5 \text{ W m}^{-2}$ ,  $6.0 \text{ W m}^{-2}$ , and  $8.5 \text{ W m}^{-2}$ . By the end of the 21st century, in the worst-case scenario (RCP 8.5), pH is projected to decrease (in pH units) by  $0.29 \pm < 0.005$ , sea surface temperature (SST) is projected to increase by  $2.39 \pm 0.50^\circ\text{C}$ , and oxygen concentrations averaged over 100-600 m depth are projected to decrease by  $6.98 \pm 1.02 \text{ mmol m}^{-3}$  (IPCC, 2007). Those oxygen units are equivalent to  $\mu\text{mol L}^{-1}$  and  $\sim\mu\text{mol kg}^{-1}$  (**Box Figure 1.1**).



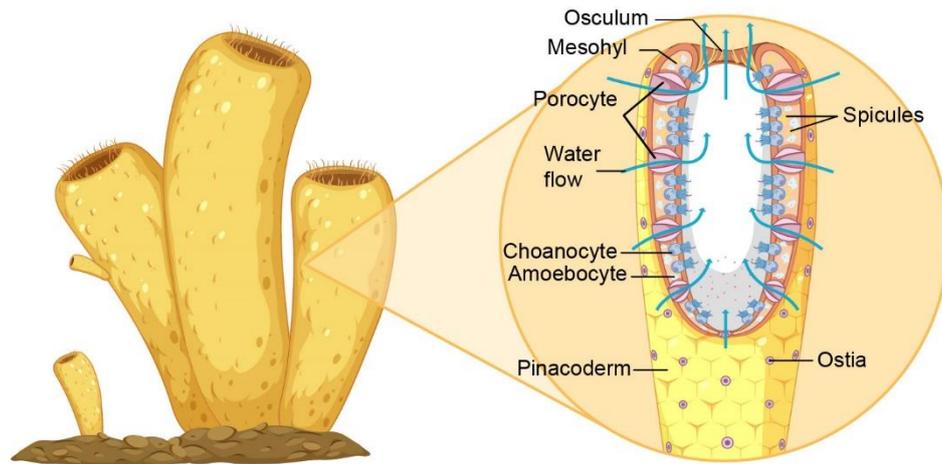
**Box Figure 1.1.** Time series and spatial patterns of simulated change in surface pH, sea surface temperature ( $^\circ\text{C}$ ), and oxygen concentration ( $\text{mmol m}^{-3}$ ) averaged over 100 to 600 m depth. Simulated spatial patterns of multi-model mean changes in year 2095 for the (middle column) RCP 8.5 scenario and right column RCP 2.6 scenario (From Laffoley, D. and Baxter, 2019).

These three main effects of climatic change (i.e., OA, OW, and OD) have impacts on reef structures and communities with consequences spanning from ecosystem shift to biodiversity loss (e.g., Fabricius et al., 2013; Sunday et al., 2017; Agostini et al., 2018). Reported acidification effects ranges from changes in cellular metabolism and organism physiology to population and ecosystem-level dynamics (Gattuso and Hansson, 2011). A decrease in the complexity, extent and species richness of biogenic reefs in all the biogeographic regions of the World was also reported as a consequence of OA (Hall-Spencer and Harvey, 2019). The OW has been demonstrated to cause shifts in species distribution, mass mortalities, reduction in ocean productivity, disruption in food web dynamics, and decline in habitat-forming species (e.g., Pörtner and Farrell, 2008; Hoegh-Guldberg and Bruno, 2010; Byrne and Przeslawski, 2013). Finally, OD has widespread effects on marine life, causing changes in the physiology of marine organisms (Seibel, 2011; Somero, 2015) and habitat reduction (Prince and Goodyear, 2006; Stramma et al., 2012). Although corals are the most studied organisms, when we want to study the effects of climate change on the coral reef ecosystem, we must consider other less studied organisms, such as sponges, whose role in the reefs' ecological processes is particularly relevant.

## **1.2 The sponge holobiont**

Sponges are the most ancient multicellular animals on Earth and the oldest metazoan group still extant on our planet (Li et al., 1998). They are the exclusive representatives of the phylum Porifera, which is divided into four classes: Calcarea, Hexantinellida, Homoscleromorpha and Demospongiae, with 83% of all known sponges belonging to the latter class (van Soest et al., 2012). Sponges have a simple level of organization since they are deprived of organs but have specialized cells with various life functions.

The sponge body architecture is arranged around the aquiferous system (**Figure 1.1**), which consists of a network of channels and chambers with flowing ambient water supporting sponge feeding. The sponges are made up of an external layer of flat cells called pinacocytes (external epithelium, pinacoderm), which isolates the sponge's internal structure (mesohyl) from the external environment, and an inner layer of flagella-bearing cells called choanocytes (internal epithelium, choanoderm), which generate the water currents necessary for filtering seawater. The pinacoderm present small inhalant apertures (ostia) and larger exhalant apertures (oscula) for the water flow (**Figure 1.1**).



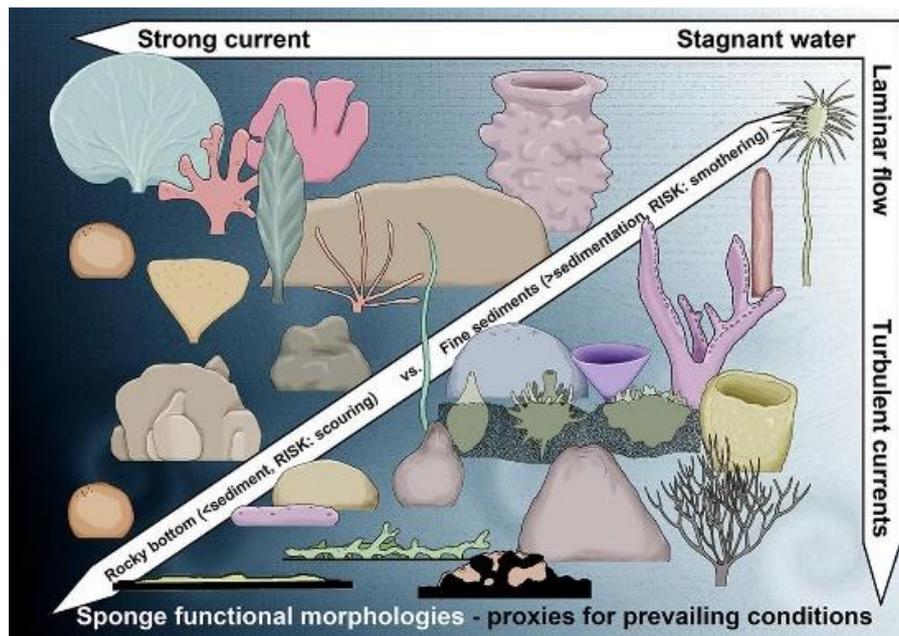
**Figure 1.1.** Schematic representation of a sponge with pinacocytes (external epithelium, Pinacoderm), choanocytes (internal epithelium), the mesohyl with spicules and Amoebocyte cells. Arrows indicate the direction of water flow (modified from istockphoto.com).

The aquiferous system is involved in the production and maintenance of water current flow within the sponge body. Its functional role is to support sponge feeding (pinocytosis/phagocytosis) and allow excretion and  $O_2/CO_2$  exchanges (by simple diffusion). The direction of ambient water flow is from dermal pores through an ostia canal to a choanocyte chamber, and then out an osculum canal (**Figure 1.1**). The mesohyl includes an extracellular matrix with jelly consistency, collagen fibrils and fibers, skeletal structures with mineral deposits (spicules), and cells (i.e., amoebocyte). Most body cells are totipotent, with a high degree of mobility and morpho-functional plasticity (De Vos et al., 1991). In the mesohyl, archeocytes cells are involved in food particle digestion via phagocytosis. These totipotent cells are capable of differentiating into any sponge cell type. The sponge skeleton is made of organic and inorganic materials that form a scaffold-like framework, which supports the various cell types. The organic material is composed of collagen microfibrils, which are referred to as spongin in demosponges. The inorganic materials increase stiffness and are composed of either silica ( $SiO_2$ ) or calcium carbonate ( $CaCO_3$ ). The distinction between the two inorganic materials has important taxonomic and phylogenetic consequences. Sponges belonging to the Class Demospongiae and Hexactinellida produce siliceous spicules with some exceptions in demosponges. For example, the Dictyoceratida have lost the inorganic component of the skeleton, while the Calcarea produce calcitic spicules forming the skeleton (Hill and Hill, 2009).

Sponge species have been reported to grow in distinct sizes and shapes due to the form of internal mineral and/or organic skeletons secreted by specialized cells. The shapes of sponges may vary between species and genera, mainly in response to environmental factors such as hydrodynamics,

light, and turbidity. For example, globular and fistular sponges are usually abundant in an environment with high sediment deposition, while sponges with much horizontal surface area often exclude in fine sediment deposition (Schönberg, 2021) (**Figure 1.2**).

This simple yet effective way of life has undoubtedly helped the persistence of sponges through historical mass extinction events, allowing them to persist today within benthic ecosystems of all aquatic (and mainly marine) environments, spanning temperate, tropical, and polar habitats (van Soest et al., 2012). Their survival under this extensive range of conditions is also due to their associated microbial communities. All macro-organisms (i.e., metazoans, fungi, plants, and algae) are associated with microbial communities and are called holobionts, which refers to a complex system consisting of the host, the microbiota, and the interactions among them (Pita et al., 2018). The microbial partners contribute to the nutrition (Nicholson et al., 2012), defense (Flórez et al., 2015), immunity (Eberl, 2010), and/or development (Koropatnick et al., 2004) of the host.



**Figure 1.2.** Example of different sponge morphologies based on environmental conditions (from Schönberg, 2021).

Marine sponges perfectly illustrate the idea of holobionts, given the exceptionally diverse microbial communities (about 40,000 operational taxonomic units or OTUs) housed within them (Thomas et al., 2016; Moitinho-Silva et al., 2017a). The sponge holobiont can be categorized into two nutritional types based on the sponge association with phototrophic (i.e., more than 50% of energy requirements are acquired from photosynthetically fixed carbon) or heterotrophic symbionts (i.e., primarily reliant on filter-feeding for carbon requirements, Cheshire and Wilkinson, 1991). Based on the abundance

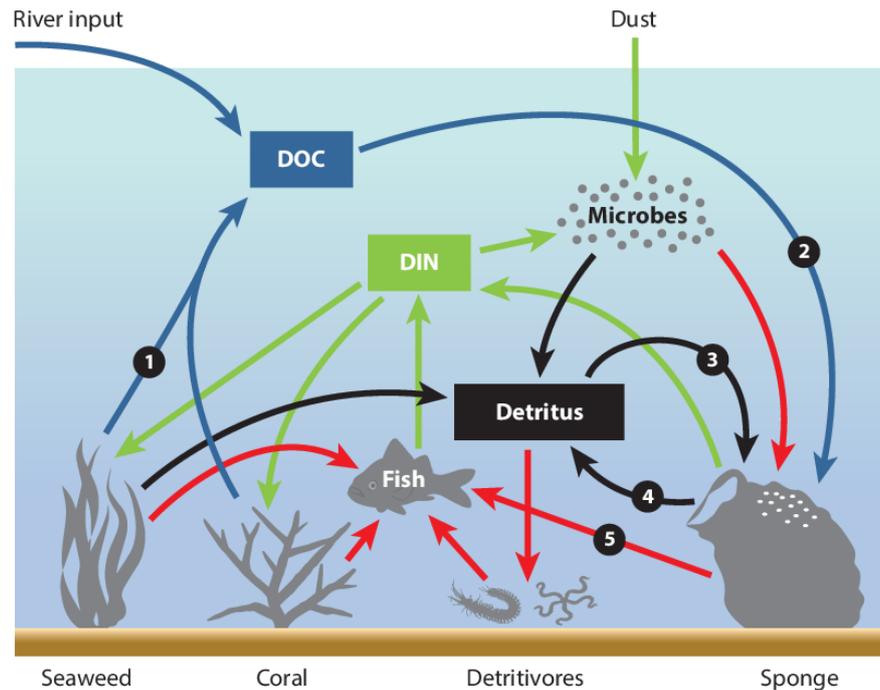
and diversity of microbes in tissues, sponges can be classified into high microbial abundance (HMA) or low microbial abundance (LMA) sponges. For example, the HMA sponges can contain  $10^{10}$  bacterial cells/g wet weight of sponge, which is 2-4 orders of magnitude higher than surrounding seawater (Hentschel et al., 2006). Sponges are efficient filter feeders vital to the health of all marine systems by linking nutrients of the open water column to benthic communities. Their wide distribution and often biomass dominance, besides their important functional roles, mean that their contribution to ecosystem functioning should not be overlooked, as their ecological role in the global ecosystem is likely higher than previously thought (Bell, 2008).

### **1.3 Ecological roles of sponges in coral reefs**

Sponges play an essential ecological role in the marine environment (Diaz and Rützler, 2001; Wulff, 2001; Bell, 2008). Although some rare sponge species are carnivorous (e.g., Chevaldonné et al., 2015), the vast majority are filter-feeding organisms, feeding primarily on nano- and picoplankton and heterotrophic bacteria in the water column. In addition, they also uptake dissolved organic carbon (DOC) and can contribute considerably to organic production in oligotrophic habitats (Reiswig, 1971; Riisgard et al., 1993; De Goeij et al., 2013; Hoytema et al., 2023). Consequently, sponges influence ecosystem functions by modifying biotic and abiotic factors (Bell, 2008) and are considered key ecosystem engineers for efficiently retaining, transforming, and allocating the nutrients and energy needed by coral reef ecosystems. Indeed, sponges have been reported to play a key role in benthic-pelagic couplings through complex biogeochemical processes, such as the carbon cycle (e.g., Maldonado et al., 2012; Mueller et al., 2014; Cathalot et al., 2015), the nitrogen cycle (e.g., Jiménez and Ribes, 2007; Maldonado et al., 2012; Fiore et al., 2013), the silicate cycle (e.g., Maldonado et al., 2005; Chu et al., 2011), and the phosphorus cycle (e.g., Maldonado et al., 2012; Colman, 2015).

In coral reef ecosystems, the largest available organic matter pools in seawater is in the form of dissolved organic matter (DOM). However, DOM remains largely inaccessible to most reef fauna (Benner et al., 1992). Bacterioplankton is known to process DOM and then return it to the classic food chain through planktonic grazing, a pathway known as the microbial loop (Azam et al., 1983). Essentially, by consuming DOM, bacteria remineralize nutrients that would otherwise be lost to the environment (Fenchel, 2008). In shallow tropical water, a similar DOM recycling pathway called the “sponge loop” was hypothesized by De Goeij et al. (2013). In the sponge loop, sponges transform DOM into a more readily available food source for other benthic reef fauna. Sponges convert DOM released by primary producers into particulate organic matter (POM) in the form of detritus and is more readily accessible to most sponge detritus-feeding associated fauna (e.g., De Goeij et al., 2013; Alexander et al., 2014; Rix et al., 2017; Archer et al., 2017). However, no detritus production has

been found for several massive sponges for which an alternative sponge-loop pathway was hypothesized. In this alternative pathway, it has been hypothesized that sponge-assimilated DOM is transferred to higher trophic levels *via* direct predation on sponge tissue (**Figure 1.3**) (e.g., Pawlik et al., 2016; McMurray et al., 2018; Pawlik and McMurray, 2020). Predators reported for sponges are spongivory fishes (e.g., angelfishes, parrotfishes, filefishes, and trunk-cowfishes), sea turtles, nudibranch, and starfish (Wulff, 1994; Pawlik, 1998; Wulff, 2006).



**Figure 1.3.** Cycling of dissolved organic carbon (DOC, blue), dissolved inorganic nitrogen and nutrients (DIN, green), and detritus (black) in Caribbean fore-reef environments (Pawlik and McMurray, 2020). The consumption of component organisms is shown in red. This figure summarizes essential components of the sponge loop (De Goeij et al., 2013) and the direct predation on sponge tissue (Pawlik et al., 2016; Mumby and Steneck, 2018). Evidence for seaweed production of labile DOC (1), which forms a major component of the diet of sponges (2), along with detritus (3). While the sponge-loop hypothesis proposes the transformation of DOC as detritus by cryptic sponges (4), this does not occur for emergent sponges, which return DOC to the ecosystem as food for spongivores (5) (Pawlik and McMurray, 2020).

The assimilation of DOC by sponge holobionts is supported mainly by the sponge itself (De Goeij et al., 2013; Rix et al., 2016; 2017), accounting for up 90% of the holobiont's total heterotrophic carbon uptake (Yahel et al., 2003; Hoer et al., 2018); the remainder is due to the microbes associated with the sponge. Through these two pathways, sponges contribute to the control of the marine top-down (Pawlik et al., 2013), such as sponge predation, and bottom-up, such as food availability in the coral reefs (Lesser and Slattery, 2013).

Another important role of sponges is their impact on benthic substrates. Both bioeroders and consolidators/stabilizers are important roles that sponges can play in tropical environments and, in some cases, even temperate regions (Cebrian and Uriz, 2006). For example, studies have shown that some sponges contribute to the consolidation of the reef structure by binding corals to the reef and discouraging excavation by other organisms (Wulff, 2001), thereby increasing coral survival. In addition, sponges provide important microhabitats for coral reef organisms (Bell, 2008) and often host a significant diversity of crustaceans, mollusks, bryozoans, polychaetes, cnidarians, echinoderms, and even fish. Finally, various chemical and physical adaptations make sponges efficient competitors for space in reef ecosystems (Hill, 1998; Diaz and Rützler, 2001, Rützler, 2002). Despite the fundamental role of sponges in coral reefs, most studies are focused on stress response/tolerance to future environmental conditions (e.g., Bennet et al., 2017; Bell et al., 2018a; 2022) rather than ecological processes, such as carbon and nutrient cycling. Therefore, filling these gaps and understanding how future conditions may affect their role in the entire reef becomes important.

#### **1.4 Impacts of climate change on sponges**

Sponges are both winners and losers in future climate scenarios with species-specific responses. Some studies identified sponges as more tolerant to OW and OA than several other benthic groups, and they likely will benefit from changing ocean conditions through increased productivity and reduced spatial competition (Bell et al., 2013; 2018b). Globally, there have been numerous reports of increased sponge abundance on coral reefs as the coral cover has declined (i.e., Aronson et al., 2002; McMurray et al., 2010; Bell et al., 2013; Kelmo et al., 2014). In the Caribbean, the giant barrel sponge *Xestospongia muta* has increased up to 50% in some reefs since 2000, while the mean coral cover has decreased (McMurray et al., 2010). Contrary to reef-building corals, in which all species rely primarily upon photosynthetic dinoflagellates to satisfy nutritional requirements (Muscatine and Porter, 1977), not all sponges are phototrophic (Taylor et al., 2007). Phototrophic sponges comprise only half of the total sponge biomass on the outer reefs of the Great Barrier Reef (Taylor et al., 2007), and slightly lower percentages of phototrophic species have been reported on Caribbean reefs (Vicente, 1990). This suggests that the impacts of OW and OA may be less important on the sponges' metabolism than for corals. Studies assessing the combined effects of OW and OA have predominantly focused on the response of bioeroding sponge species, such as *Cliona celata* (Duckworth and Peterson, 2013), *C. orientalis* (Fang et al., 2013; Wisshak et al., 2012) and *C. varians* (Stubler et al., 2015). For instance, sponge bio-erosion rates have been reported to increase by 17% and 61% under future OW and OA scenarios compared to present-day conditions (e.g., Wisshak et

al., 2012; Duckworth and Peterson, 2013; Fang et al., 2013). Studies on non-bioeroding sponges also suggest a degree of sponge tolerance to the effects of mainly OA and OW (Duckworth et al., 2012; Lesser et al., 2016; Vicente et al., 2016). Research exposing six sponge species to OW and OA conditions suggested no impact on growth, survival, or secondary metabolite biosynthesis (Duckworth et al., 2012). Furthermore, OW and OA have only marginal negative effects on spicule biomineralization and little effects on the overall survival or growth rates of the Hawaiian sponge *Mycale grandis* (Vicente et al., 2016).

Several authors have reported the positive effects of OA on sponges. For example, Wisshak et al. (2012) found an increase in bioerosion rates of up to 61% in *Cliona orientalis* exposed to experimentally manipulated seawater (pH 7.9 and 7.6). Both Goodwin et al., (2014) and Morrow et al., (2015) reported high tolerances of some sponge species to low pH near volcanic CO<sub>2</sub> vents. In these studies, the two sponges *Coelocarteria singaporensis* and *Cinachyra* sp. showed an increase in photosynthetic microbial activity, providing nutritional benefit to the sponge, and increased potential for growth under future climate scenarios (Morrow et al., 2015). A general tolerance to reduced pH was also reported in New Zealand for temperate sponges exposed to a range of pH (7.6-8.0) for four weeks (Bates and Bell, 2018). Overall, sponges appear generally insensitive to OA (Leung et al., 2022), and their responses to elevated *p*CO<sub>2</sub> are species-specific. The fitness and success of the host could also be due to the stability and flexibility of the microbial partnerships (Morrow et al., 2015).

In contrast, a decline in sponge abundance was also reported (e.g., Cebrian et al., 2011; Goodwin et al., 2014). The combined effects of OW and OA on *Xestospongia muta* have shown that the phototrophic cyanobacterial symbiont productivity declines with exposure to elevated temperature and *p*CO<sub>2</sub>. However, no evidence of associated host stress was reported and this despite a reduction in both holobiont carbohydrate levels and stability of the sponge microbiome (i.e.,  $\beta$ -diversity) (Lesser et al., 2016). A study on the effects of OA in sponges reported that the percentage of sponge cover decreases significantly at volcanic CO<sub>2</sub> vents (Fabricius et al., 2011). Since OA has been shown to affect most species with carbonate skeletons, directly altering their calcification and increasing dissolution, only sponges of the class Calcarea, which have calcium carbonate skeletons and represent 8% of all sponges (van Soest et al., 2012), will likely be affected. However, it is also hypothesized that OA can impact more dominant species with siliceous skeletons because sponges have a limited ability for acid-base regulation (Philp, 1997; Pörtner, 2008). Studies focusing exclusively on the effects of OW have demonstrated that some sponges are sensitive to temperature predicted under future climate change scenarios. For example, the Great Barrier Reef sponge *Rhopaloides odorabile* has a thermal threshold of 32°C (Webster et al., 2008; Pantile and Webster, 2011; Massaro et al.,

2012) when host-symbiont molecular interactions start to breakdown, eventually leading to death (Fan et al., 2013). In addition, some sponge disease outbreaks have been correlated with elevated seawater temperatures (Sutherland et al., 2004). In the Mediterranean, several reports of sponge loss have been caused by increased sea temperature (Cerrano et al., 2000). Recent work has also shown that *Cliona* sp. cannot survive ocean temperatures projected for 2100 (Achlati et al., 2017; Ramsby et al., 2018b).

Depending on local environmental characteristics, species-specific responses, and the environment in which the sponge lives, sponges could be advantaged or disadvantaged in a future coral reef. For a scenario where sponges will become dominant on the reef, the balance between accretion and erosion of the reef will be altered, especially considering that both abundances of bio-eroding sponges and bio-erosion rates would increase at higher  $p\text{CO}_2$  (e.g., Wisshak et al., 2014). However, while sponge reefs might still provide some three-dimensional habitat, the overall complexity will be considerably reduced, which may drastically reduce the overall biodiversity (Bell et al., 2013). Future reefs, dominated primarily by sponges, would also cause the benthic community to shift from primarily comprised of phototrophic organisms to predominately heterotrophic species (Bell et al., 2018a). Initially, this would impact the organisms that are obligate coral feeders (Pratchett et al., 2006) or taxa that rely on coral-derived DOM and/or POM (Wild et al., 2004). However, it is possible that these species could acclimatize to utilize exclusively DOC from increased microalgae production. In general, coral reefs are complex systems, and it is difficult to accurately predict how a reef might function if sponges become the dominant fauna. However, increased sponge abundance is likely to directly influence some key processes, such as bio-erosion and water filtering (including nutrient and carbon cycling), which are the most important functional roles of sponges on reefs (Bell, 2008), and both are predicted to increase in sponge-dominated systems. In contrast, if sponges decline in future coral reefs, important players in carbonate balance and coral reef health will disappear (reviews in Diaz and Rützler, 2001; Wulff, 2001).

Despite the ecological importance of sponges, little is known about the effects of environmental changes and adaptive sponge capacities to cope with changing ocean conditions. Most studies rely on replicating these conditions in controlled environments and/or assessing these parameters separately, which gives an unrealistic view of the response of organisms to the multiple effects of climate change occurring concurrently in their natural environments.

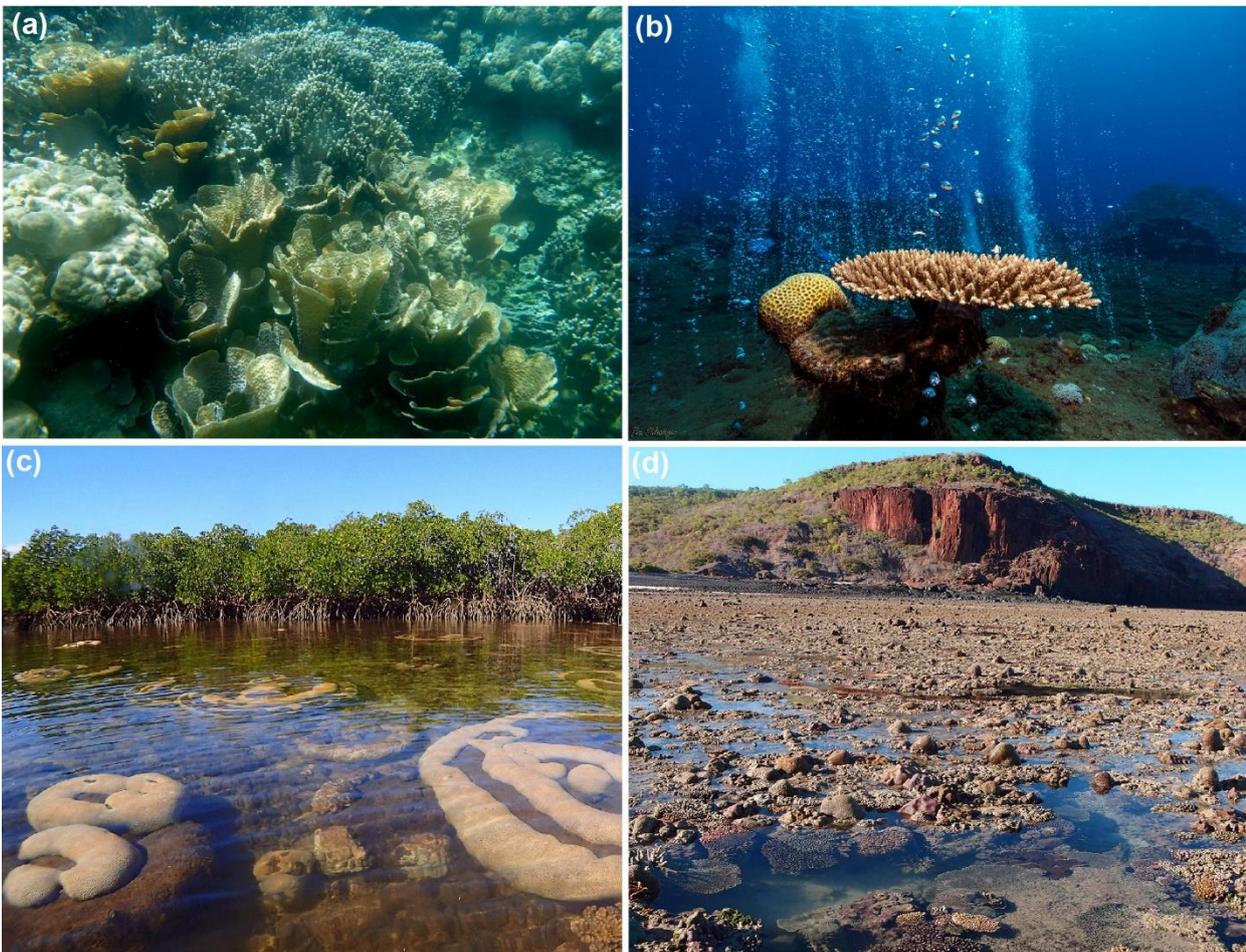
## **1.5 The natural laboratories**

Climate change effects (warming and acidification) have been studied over the past 15 years, mainly in the laboratory (*in vitro*), where it has been possible to consider the effect of individual parameters

and controlled conditions over time. *In vitro* experiments have been fundamental to understanding how reef organisms might respond to future climates. Advantages of using aquaria include the possibility of studying one factor at a time, the possibility of changing several parameters under time-controlled conditions, and the possibility of repeating experiments. However, it is impossible to replicate the complex ecological and biogeochemical interactions that govern coral reef systems using aquaria, especially if co-limiting factors such as nutrients, currents, and irradiance are considered. Furthermore, some experiments can be difficult to simulate (Atkinson and Cuet, 2008), and most are too short for complete acclimatization of the organisms to take place.

The discovery of these natural laboratories, where organisms are already living under future-like conditions, makes it possible to study the multiple long-term effects of future climate change conditions directly *in-situ* in the context of natural variability. These natural sites are becoming important tools to study the actual response of organisms exposed to future-like conditions during their whole life. Studies of populations or communities that persist under extreme and marginal physical and chemical conditions in nature have thus become increasingly popular for ecosystem-scale predictions of future coral reefs. Here, an extreme environment is referred to as a habitat characterized by harsh environmental conditions beyond the optimal range for the development of organisms as reviewed by Camp et al., (2018).

Natural laboratories such as shallow-water volcanic CO<sub>2</sub> seeps, low-pH upwelling areas, mangrove habitats and semi-enclosed bays are study systems where the surrounding seawater is subjected to a localized or widespread increase in either *p*CO<sub>2</sub> and/or temperature, and in some cases decrease in dissolved oxygen (Camp et al., 2018) (**Figure 1.4**). For example, around CO<sub>2</sub> vents, the community structure changes, and only some acidification-tolerant species thrive in the presence of high *p*CO<sub>2</sub> (e.g., Hall-Spencer et al., 2008; Kroeker et al., 2011, 2013; Fabricius et al., 2011, 2015; Hall-Spencer et al., 2022). The low biodiversity observed at these sites is linked to the loss of habitat-forming corals (Fabricius et al., 2013; Sunday et al., 2017). Some studies also showed that the structural integrity of any reef framework is compromised due to a loss of architectural complexity (Fabricius et al., 2011), increased rates of bioerosion (Enochs et al., 2016), lower coral growth (Fabricius et al., 2011) and reduced skeletal density (Fantazzini et al., 2015; Strahl et al., 2016). The ability of corals to maintain calcification under high *p*CO<sub>2</sub> conditions appears to be species-specific (Rodolfo-Metalpa et al., 2011; Inoue et al., 2013).



**Figure 1.4.** Examples of extreme natural environments: (a) Nikko semi-enclosed bay; (b) Ambite Island CO<sub>2</sub> seep (Papua New Guinea, © Tom Shlesinger); (c) Bouraké semi-enclosed lagoon (New Caledonia, © Serge Andreffouet); and (d) Kimberley Bay (Australia, © Zoe Richards).

Upwelling environments have the potential to be OA hot spots (Feely et al., 2008; Manzello, 2010). For instance, corals of the Galápagos Islands and nearby Panamá reefs are also frequently exposed to the El Niño -Southern Oscillation (ENSO, +3-4°C during ENSO events for > 2 months), creating a unique opportunity to study the short-term effects of low pH (< 8.0) and thermal stress events (+1-3°C) on corals (Manzello et al., 2014). Manzello et al., (2014) showed that corals maintain their linear extension rates but experience a reduction in skeletal density. However, upwelling sites can be seasonal, meaning that the physicochemical conditions representative of future scenarios may be limited in time.

Mangrove habitats can also be important natural laboratories. These widespread reef-associated environments have recently received attention for their potential to minimize the negative impacts of elevated temperature and  $p\text{CO}_2$  via increased primary production and carbon storage (Yates et al.,

2014; Camp et al., 2016; Sultana et al., 2016). They play a key role in the global carbon budget due to their high productivity, and intense carbon processing (e.g., Borges et al., 2003; Dittmar et al., 2006; Bouillon et al., 2014) and are considered one of the most important CO<sub>2</sub> sinks (Donato et al., 2012). Although mangrove forests represent critical habitat for many organisms (Yates et al., 2014; Stewart et al., 2021), they can also be an extreme habitat, with low pH and very variable temperature. This habitat could host selects stress-tolerant species and expose resident species to very different physico-chemical conditions compared to neighboring coral reefs (Camp et al., 2017; 2018; 2019). For example, in the Caribbean, Yates et al., (2014) suggested that mangroves could be a refuge for corals through physical shading and elevation of downstream total alkalinity (TA) because of carbonate-sediment dissolution (i.e., a buffering potential similar to seagrasses habitats). An increasing number of empirical studies document nested mangrove coral assemblages with corals establishing long-term and extensive communities within mangrove habitats (Rogers and Herlan, 2012). These include locations in the Caribbean (Rogers, 2017; Stewart et al., 2021), such as the extensive nested mangrove coral assemblage in Panama (Stewart et al., 2022), the Australian Great Barrier Reef (Camp et al., 2019), and sites in the Seychelles and Sulawesi (Camp et al., 2016) (**Figure 1.5**).

Sponges can also be very abundant in mangrove systems, providing a particular opportunity to study sponge responses to multiple climate change factors. Most of what is known about sponges in mangrove ecosystems is from distribution, and taxonomic studies in the Caribbean (Diaz and Rützler, 2009; Guerra-Castro et al., 2011; Diaz, 2012). Only a few studies are available on mangrove sponges in the Indo-Pacific; for example, 115 sponge species have been recorded in the Berau region (Indonesia), 74 of which were attached to mangrove roots (Becking et al., 2013). Caribbean mangrove sponge communities contain species specifically adapted to survive “extreme” levels of salinity, temperature, and sedimentation (Rützler, 1995; Wulff, 2000, 2005; Engel and Pawlik, 2005; Pawlik et al., 2007; Nagelkerken et al., 2008). Mangrove-derived organic matter, leaching from roots and decaying litter, is the main source of carbon for sponges living in mangrove habitats (Granek et al., 2009), although oceanic particulate or dissolved organic matter may enter into the system (Letourneur et al., 2018).



**Figure 1.5.** Examples of mangrove-coral assemblages. (a) Bocas del Toro, Panamá (© Heather A. Stewart), and (b) mangrove lagoon near the Great Barrier Reef in Australia (© Emma Camp).

Finally, semi-enclosed bays are also considered natural laboratories for studying future like climatic conditions. For example, the Nikko semi-enclosed bay in Palau, which is characterized by low pH (range 7.65-8.02) and high-temperature conditions (31.8°C), has high coral coverage (> 60%) and species diversity (21 scleractinian genera) (Van Woesik et al., 2012; Shamberger et al., 2014; Golbuu et al., 2016). In Nikko Bay, Kurihara et al., (2020) hypothesized that the corals had developed mechanisms to adapt to the site-specific conditions and maintain their fitness in the bay's warmer, more acidic conditions. The semi-enclosed bay of Bouraké in New Caledonia was first described as a regular mangrove system (e.g., Thollot, 1992; Thollot et al., 1999), without mentioning the presence of corals and other benthic organisms living in it and without reporting the incredible environmental variability that these species experience. The particularities of the Bouraké lagoon were highlighted by the study of Camp et al. (2017), thanks to some short-term monitoring and the study of the responses of the corals living there. Camp et al., (2017) described the Bouraké lagoon as a site characterized by the diel variation of temperature, pH, and oxygen, resulting in a warmer (> 33.0°C), acidified (< 7.3 pH<sub>T</sub>) and deoxygenated (< 2.3 mg L<sup>-1</sup>) seawater compared to the lagoon outside.

Camp et al. (2017) found that some of the 32 species of corals showed metabolic plasticity to cope with extreme environmental parameters. The study revealed the actual value of the Bouraké lagoon as a natural laboratory to study the responses of organisms to extreme environmental conditions. The recent studies in the Bouraké lagoon span from fish patterns and distribution (Komornicki, 1988; Dubuc et al., 2019a; 2019b) to coral physiology and recruitment (Jacquemont et al., 2022; Tanvet et al., 2022).

## 1.6 Objectives of the study

Sponges have a key role in coral reefs, supporting higher trophic levels through the conversion of DOM into detritus (sponge loop) or sponge biomass that becomes food for other reef organisms (e.g., De Goeij et al., 2013; McMurray et al., 2018; Rix et al., 2018). However, the effects that climate change may have on these ecological processes and, thus, on the reef as a whole still need to be better understood. This thesis aimed to address the gaps in our knowledge of the effects of extreme environmental conditions on sponge ecological responses (i.e., sponge carbon and nutrient cycling) and to provide insights into the consequences for future reefs. To this end, I used the natural laboratory of Bouraké, where sponges are particularly abundant and where environmental conditions are close to or even worse than projected for the future. It was essential to give a detailed physico-chemical description of this natural laboratory for which only a short monitoring was available (Camp et al. 2017). Then, in a series of experiments carried out *in-situ*, I exposed the sponge species *Rhabdastrella globostellata* (Carter, 1883) to both ‘normal’ and ‘extreme’ environmental conditions, which occur naturally in the lagoon of Bouraké during high and low tide, respectively. Finally, to distinguish the effects of warming from other environmental factors on the sponge carbon and nitrogen recycling, I carried out labeled  $^{13}\text{C}$  and  $^{15}\text{N}$  *ex-situ* experiments at the “Aquarium des lagons” (Nouméa, New Caledonia). The specific objectives of this thesis are divided into three main chapters:

### *1. Long-term physico-chemical characterization and assessment of the distribution of benthic communities in the Bouraké natural laboratory*

In this chapter (**chapter 2**), I fully characterized the physico-chemical parameters and the distribution of the benthic communities. I also analyzed the stable isotopes signature of the Bouraké natural laboratory. Based on data collected from 2016 to 2020, I performed a long-term meta-analysis of temperature,  $\text{pH}_T$ , and DO, as well as nutrients and organic matter concentrations, comparing the Bouraké stations and two reference sites. Photo-quadrat transects were used to assess the distribution of major benthic taxa living at the Bouraké lagoon. Finally, I measured the stable isotope signatures of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  to highlight differences in the composition of carbon and nitrogen sources between

the outermost and innermost zone of the Bouraké lagoon, in selected benthic organisms, such as sponges and macroalgae. Part of this work began with my Master's thesis (Maggioni, 2018). This chapter provides a full description of the study site, which was recognized as a natural laboratory for studying the effects of extreme environmental conditions on marine organisms (Maggioni et al., 2021).

## 2. *Effects of extreme environmental conditions on R. globostellata nutrients and organic matter recycling, as well as its phototrophic activity*

In this chapter (**chapter 3**), I performed 70 incubations *in-situ* using benthic respirometric chambers to assess the metabolic responses (i.e., nutrients and organic matter recycling) and the photosynthetic activity of *R. globostellata* during summer. Incubations were performed at high and low tides, representing “normal” and extreme environmental conditions. Changes in seawater nutrients and organic matter contents at the beginning and the end of incubations were used to evaluate their production and/or consumption in sponge metabolism. Dark incubations were performed during summer in both 2018 and 2020, the latter being the warmer, while dark/light incubations were only performed in 2020. This chapter provides a first information on the metabolic response of the sponge *R. globostellata* subjected to different environmental conditions such as those extreme (Maggioni et al., 2023).

## 3. *Effects of temperature on R. globostellata carbon and nitrogen recycling*

In this chapter (**chapter 4**), I performed an *ex-situ* experiment based on the results found in chapter 3, where summer temperature (> 31°C) was hypothesized as the main factor affecting the sponge carbon cycle. To specifically study the effect of temperature on sponge carbon and nitrogen recycling, I monitored the incorporation of labeled <sup>13</sup>C and <sup>15</sup>N into sponge tissue and detritus produced by the sponge during two temperature experiments at 28°C and 32°C. The discovery of the sponge *R. globostellata* outside the Bouraké lagoon (in 2021) allowed me to compare a “normal” population under constant temperature conditions with the presumably adapted sponges of Bouraké, which are subject to fluctuating temperature conditions that can reach the extreme value of 33°C. This chapter provides a first assessment of the consequences of high temperature on carbon (C) and nitrogen (N) recycling in the sponge *R. globostellata* adapted to high temperatures (Bouraké) and those that are not (control) (Maggioni et al., *under review in Limnology and Oceanography*).

In the last chapter (**chapter 5**), I first summarize the key findings that make the Bouraké lagoon one of the most suitable natural laboratory to study the responses of organisms to extreme environmental conditions. I discuss how the physiological and ecological processes of the sponge *R. globostellata*

might be affected under extreme environmental conditions, especially under high temperatures. I then focus on the effects of these conditions on the main ecological processes in sponges, such as the recycling of nutrients and organic matter. I outline the main strategies used by the sponge *R. globostellata* to adapt and thrive in such environmental conditions. I use my findings to better describe the ecological processes of sponges from a global perspective, assessing the future consequences for their role and, thus, for trophic interactions in future coral reefs.

## **CHAPTER 2:**

### **THE BOURAKÉ SEMI-ENCLOSED LAGOON (NEW CALEDONIA) – A NATURAL LABORATORY TO STUDY THE LIFELONG ADAPTATION OF A CORAL REEF ECOSYSTEM TO EXTREME ENVIRONMENTAL CONDITIONS**

---



The semi-enclosed lagoon of Bouraké (Nouméa, New Caledonia): the natural laboratory characterized and used in this study.

## Résumé

Selon les données expérimentales actuelles, les récifs coralliens pourraient être très fortement impactés, voire possiblement disparaître, d'ici la fin du siècle si les émissions de CO<sub>2</sub> restent inchangées. Toutefois, les récentes découvertes de récifs diversifiés et à forte couverture de corail vivant, dans des conditions extrêmes suggèrent que certains coraux pourraient bien se développer dans une eau de mer chaude, à forte *p*CO<sub>2</sub> et désoxygénée. Les cheminées volcaniques sous-marines de CO<sub>2</sub>, les lagons semi-fermés et les estuaires de mangrove sont des sites d'étude où un ou plusieurs paramètres écologiques importants pour la vie dans les océans sont proches ou même dépassent ceux actuellement prévus pour l'année 2100. Bien qu'ils ne reproduisent pas parfaitement les conditions futures, ces « laboratoires naturels » offrent des possibilités uniques d'explorer les mécanismes que les espèces récifales pourraient utiliser pour suivre le rythme du changement climatique. Pour y parvenir, il est essentiel de caractériser leur environnement dans son ensemble et de prendre en compte de manière précise tous les facteurs environnementaux possibles qui pourraient différer de ce qui est prévu dans le futur, modifiant éventuellement la réponse de l'écosystème.

Cette chapitre se concentre sur le lagon semi-fermé de Bouraké (Nouvelle-Calédonie, océan Pacifique Sud-Ouest) où un écosystème récifal sain prospère dans une eau chaude, à pH réduit et appauvrie en O<sub>2</sub>. Nous avons utilisé une approche multi-échelle pour caractériser les principaux paramètres physico-chimiques, cartographier la composition de la communauté benthique (i.e., coraux, les éponges et les macroalgues), et fournir des informations préliminaires sur la caractérisation isotopique des sources de matière organique (matière organique sédimentaire, macroalgues, phanérogames et feuilles de palétuviers) et d'organismes sessiles et de poissons en analysant les valeurs de δ<sup>13</sup>C et de δ<sup>15</sup>N dans la lagune de Bouraké. Les données ont révélé que la plupart des paramètres physiques et chimiques sont régulés par la marée, fluctuent fortement 3 à 4 fois par jour et sont entièrement prévisibles. Le pH de l'eau de mer et l'oxygène dissous diminuent à marée descendante et atteignent des valeurs extrêmement faibles à marée basse (7,2 pH<sub>T</sub> et 1,9 mg O<sub>2</sub> L<sup>-1</sup> à Bouraké, contre 7,9 pH<sub>T</sub> et 5,5 mg O<sub>2</sub> L<sup>-1</sup> sur les récifs de référence). L'oxygène dissous, la température et le pH fluctuent en fonction de la marée jusqu'à 4,91 mg O<sub>2</sub> L<sup>-1</sup>, 6,50°C et 0,69 pH<sub>T</sub> en un seul jour. De plus, la concentration de la plupart des paramètres chimiques était jusqu'à cinq fois plus élevée dans la lagune de Bouraké, en particulier pour le carbone organique et inorganique et l'azote, mais aussi pour certains nutriments, notamment les silicates. De manière surprenante, malgré les conditions environnementales extrêmes et la composition chimique altérée de l'eau de mer mesurée à Bouraké, les résultats révèlent une communauté diversifiée et à couverture élevée de macroalgues, d'éponges et de coraux comptant respectivement 28, 11 et 66 espèces. De façon générale, les différents organismes ont montré une signature plus élevée en δ<sup>13</sup>C et plus faible en δ<sup>15</sup>N dans la partie intérieure

de la lagune de Bouraké que dans la partie extérieure. La variabilité environnementale et le déséquilibre des nutriments pourraient contribuer à leur survie dans des conditions environnementales aussi extrêmes. La dynamique naturelle de l'écosystème de Bouraké et sa pertinence a été décrit en tant que laboratoire naturel pour étudier les réponses adaptatives des organismes benthiques à de multiples conditions environnementales extrêmes.

Ce chapitre a fait l'objet d'une publication dans la revue « *Biogeosciences* » (cf. ci-dessous). Seule la partie sur les isotopes stables a été ajoutée dans ce thèse, du fait de résultats encore non acquis lors de la publication de ces travaux.

Biogeosciences, 18, 5117–5140, 2021

<https://doi.org/10.5194/bg-18-5117-2021>

© Author(s) 2021. This work is distributed under the Creative Commons Attribution 4.0 License.



## **The Bouraké semi-enclosed lagoon (New Caledonia) – a natural laboratory to study the lifelong adaptation of a coral reef ecosystem to extreme environmental conditions**

**Federica Maggioni<sup>1</sup>, Mireille Pujo-Pay<sup>2</sup>, Jérôme Aucan<sup>1,3</sup>, Carlo Cerrano<sup>4</sup>, Barbara Calcinai<sup>4</sup>, Claude Payri<sup>1</sup>, Francesca Benzoni<sup>5</sup>, Yves Letourneur<sup>1</sup>, and Riccardo Rodolfo-Metalpa<sup>1</sup>**

<sup>1</sup>ENTROPIE, IRD, Université de la Réunion, CNRS, IFREMER, Université de Nouvelle-Calédonie, Nouméa 98800, New Caledonia

<sup>2</sup>CNRS, UMR 7621, Laboratoire d’Océanographie Microbienne, Observatoire Océanologique de Banyuls, Sorbonne Université, Paris, France

<sup>3</sup>Pacific Community Center for Ocean Science (SPC), Nouméa, New Caledonia

<sup>4</sup>Department of Life and Environmental Sciences (DiSVA), Polytechnic University of Marche, Ancona, Italy

<sup>5</sup>Red Sea Research Center, Division of Biological and Environmental Science and Engineering, King Abdullah University of Science and Technology, 23955-6900 Thuwal, Saudi Arabia

**Correspondence:** Federica Maggioni ([federica.maggioni@ird.fr](mailto:federica.maggioni@ird.fr))

## Abstract

According to current experimental evidence, coral reefs could disappear within the century if CO<sub>2</sub> emissions remain unabated. However, recent discoveries of diverse and high cover reefs that already live under extreme conditions suggest that some corals might thrive well under hot, high *p*CO<sub>2</sub>, and deoxygenated seawater. Volcanic CO<sub>2</sub> vents, semi-enclosed lagoons and mangrove estuaries are unique study sites where one or more ecologically relevant parameters for life in the oceans are close or even worse than currently projected for the year 2100. Although they do not perfectly mimic future conditions, these natural laboratories offer unique opportunities to explore the mechanisms that reef species could use to keep pace with climate change. To achieve this, it is essential to characterize their environment as a whole, and accurately consider all possible environmental factors that may differ from what is expected in the future, possibly altering the ecosystem response.

This chapter focuses on the semi-enclosed lagoon of Bouraké (New Caledonia, SW Pacific Ocean) where a healthy reef ecosystem thrives in warm, acidified and deoxygenated water. A multi-scale approach was used to characterize the main physical-chemical parameters, mapped the benthic community composition (i.e., corals, sponges, and macroalgae), provide preliminary information on the isotopic characterization of organic matter sources (sedimentary organic matter, macroalgae, phanerogams, and mangrove leaves), sessile organisms and fish by analyzing  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values in the Bouraké lagoon. The data revealed that most physical and chemical parameters are regulated by the tide, strongly fluctuate 3 to 4 times a day, and are entirely predictable. The seawater pH and dissolved oxygen decrease during falling tide and reach extreme low values at low tide (7.2 pH<sub>T</sub> and 1.9 mg O<sub>2</sub> L<sup>-1</sup> at Bouraké, *vs* 7.9 pH<sub>T</sub> and 5.5 mg O<sub>2</sub> L<sup>-1</sup> at reference reefs). Dissolved oxygen, temperature, and pH fluctuates according to the tide of up to 4.91 mg O<sub>2</sub> L<sup>-1</sup>, 6.50°C, and 0.69 pH<sub>T</sub> units on a single day. Furthermore, the concentration of most of the chemical parameters was one to five times higher at the Bouraké lagoon, particularly for organic and inorganic carbon and nitrogen, but also for some nutrients, notably silicates. Surprisingly, despite extreme environmental conditions and altered seawater chemical composition measured at Bouraké, our results reveal a diverse and high cover community of macroalgae, sponges and corals accounting for 28, 11 and 66 species, respectively. In general, the different organisms showed a higher signature in  $\delta^{13}\text{C}$  and lower in  $\delta^{15}\text{N}$  in the inner part of the Bouraké lagoon than in the outer part. Both environmental variability and nutrient imbalance might contribute to their survival under such extreme environmental conditions. The natural dynamics of the Bouraké ecosystem and its relevance as a natural laboratory were described to investigate the benthic organism's adaptive responses to multiple extreme environmental conditions.

Chapter 2: The Bouraké semi-enclosed lagoon (New Caledonia) – a natural laboratory to study the  
lifelong adaptation of a coral reef ecosystem to extreme environmental conditions

---

This chapter has been published in the journal "*Biogeosciences*" (see below). Only the part on stable isotopes has been added in this thesis, because of results not yet acquired at the time of the publication of this work.

## 2.1 Introduction

Marginal and extreme environments, where some species persist under sub-optimal environmental conditions, have become a precious tool to investigate the potential resilience of marine organisms in the face of climate change (Camp et al., 2017, 2018). These sites may be used as natural laboratories where at least one or more environmental parameters naturally mimic extreme environmental conditions over a large area of the ecosystem. They provide an opportunity to simultaneously investigate changes in species responses and their ability to acclimatize and adapt to global environmental changes (Soares, 2020; Kurihara et al., 2020). General observation suggest ecosystem-level consequences of life-long exposure to extreme conditions, such as reduced biological diversity, especially among calcifying organisms, decreased rates of coral calcification, and high rates of bioerosion (e.g., Hall-Spencer et al., 2008; Manzello et al., 2008; Fabricius et al., 2011; Crook et al., 2013; Kroeker et al., 2011; Iglesias-Prieto et al., 2014; Milazzo et al., 2014; Paytan et al., 2014). However, some natural laboratories can host very rich reef communities. Examples of such sites have been documented in Palau (Golbuu et al., 2016; Barkley et al., 2017; Shamberger et al., 2018; Kurihara et al., 2021), Papua New Guinea (Pichler et al., 2019), the Kimberly region, Australia (Dandan et al., 2015; Schoepf et al., 2015), and in mangrove lagoons of New Caledonia (Camp et al., 2017) and the US Virgin Islands (Yates et al., 2014).

These natural laboratories have become a common experimental asset in climate change research. However, the lack of empirical characterization of the physical and biogeochemical conditions, including diurnal and seasonal fluctuations, have been argued to bias the interpretation of the biological mechanisms that trigger the responses of organisms (e.g., Vizzini et al., 2013; Camp et al., 2018; Aiuppa et al., 2021). Using limited environmental descriptors makes it difficult to unequivocally identify the main driver(s) of the biological response among the primary factors (i.e., acidification, warming and/or deoxygenation), the potential secondary factors (e.g., pollution, water flow, tide, seawater nutrients and organic content, turbidity, etc.), and their combination. Water temperature, pH and dissolved oxygen can also co-vary negatively or positively and combine with other secondary factors, acting synergistically or antagonistically with unknown effects on benthic community responses. Their extreme values and the extent to which organisms are exposed are crucial in shaping biological responses (Boyd et al., 2016; Rivest et al., 2017). For instance, early studies suggest that temperature fluctuations due to diel or tidal variations could expose corals to stressful temperatures long enough to induce acclimatization or adaptation, but short enough to avoid coral mortality (Craig et al., 2001; Oliver and Palumbi, 2011; Castillo et al., 2012; Palumbi et al., 2014; DeCarlo et al., 2019).

Coral reef organisms from such thermally variable environments are expected to respond positively to future heat events (Rivest et al., 2017). Besides, corals naturally subjected to high  $p\text{CO}_2$  variability have shown immune defences when experimentally exposed to high temperatures, thus buffering the magnitude of thermal stress during heat waves (Wall et al., 2020). The extreme tidal range in the Kimberly region (Northwest of Australia) exposes corals to short-term temperature maxima of up to  $37^\circ\text{C}$ , and fluctuations of up to  $7^\circ\text{C}$  daily. Despite the high temperature, also combined with strong currents and turbid waters, diverse and probably resilient coral reefs have been described there (Dandan et al., 2015; Schoepf et al., 2015). Overall, only parts of these natural laboratories' physical and biogeochemical parameters have been quantified and only during short periods of fieldwork due to logistic constraints. Long-term monitoring of seawater parameters and their fluctuation is essential to better understand the mechanisms used by resilient reef organisms in these natural laboratories and interpret how marine taxa will respond to future biogeochemical changes in the environment.

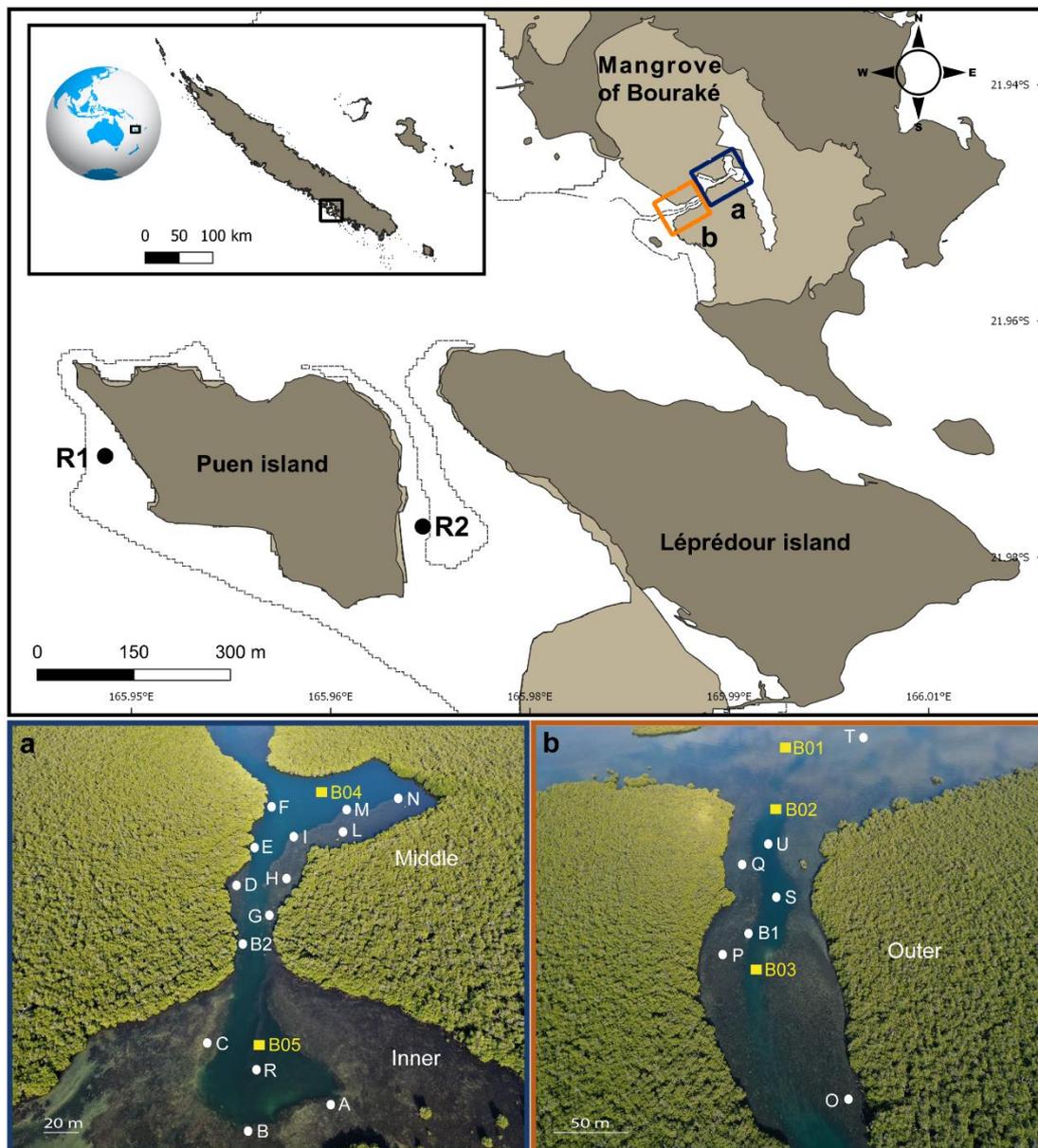
The semi-enclosed lagoon of Bouraké (New Caledonia, SW Pacific Ocean) has been considered one of the most suitable natural laboratories for future extreme environmental conditions (Camp et al., 2019). In a preliminary study, Camp et al., (2017) reported a series of compelling short-term data of seawater carbonate chemistry and a general survey of the coral populations. In this first assessment of the Bouraké lagoon, the authors measured diel fluctuations of temperature (ranging from  $25.9$  to  $33.1^\circ\text{C}$ ), pH (ranging from  $7.24$  to  $7.91$   $\text{pH}_T$  units) and dissolved oxygen (DO) (ranging from  $1.80$  to  $6.97$   $\text{mg L}^{-1}$ ) regulated by a one-meter tide. They revealed that about 20 species of corals were exposed 44% of the time to a  $\text{pH}_T$  of  $7.7$ - $7.8$ , and 71% of the time to temperatures predicted for the end of the century under the IPCC scenario RCP4.5 (IPCC, 2014). These striking preliminary findings qualified the Bouraké lagoon as a unique site where potentially adapted corals withstand extreme environmental conditions.

The extent to which the Bouraké species are exposed to sub optimal conditions remains unclear and the best way to assess these very encouraging findings is to start with fully characterizing the main environmental parameters and the daily and seasonal fluctuations to which reef species have been subjected during their entire life. In this chapter a multi-scale approach was used to map and describe the benthic community living in the Bouraké lagoon and report on new evidence based on three years of data. Because of the size of the area, the close vicinity of a dense mangrove forest, and the demonstrated tide effect on the local environmental conditions, it has been hypothesized that (1) environmental conditions fluctuate regularly but are spatially heterogeneous throughout the Bouraké lagoon, and (2) only a limited number of species can resist to the extreme physical and chemical conditions at the study site, when compared to adjacent bay-sheltered reefs.

## 2.2 Methods

### 2.2.1 Study site

Starting in February 2016, the semi-enclosed coral reef lagoon of Bouraké (South Province, Grande Terre, New Caledonia) and adjacent reference reefs (**Figure 2.1**) were studied. The mangrove of Bouraké is located in a semi-arid geographical area, and it lacks rivers input typical of mangrove estuaries.



**Figure 2.1.** Map of the study site (top panel) showing the semi-enclosed mangrove lagoon of Bouraké and reference reefs (R1 and R2). Photos **a** and **b** (bottom panels) are aerial pictures (taken at 130 m above the Bouraké lagoon) of the inner (bottom) and middle reefs (**a**) and the outer reef (**b**). White dots and yellow squares indicate the sampling stations used for physical and chemical environmental monitoring, respectively. Georep New Caledonia database (<https://georep.nc/>) and QGIS software were used to build the figure (top panel).

In Bouraké, a channel of more than 80 m wide and 0.5 m to 6 m deep penetrates a dense mangrove forest made by *Avicennia marina* and *Rhizophora stylosa*. It expands into side pools and a large reservoir in the inner part of the mangrove system. A preliminary calculation of its surface and seawater volume of the main area (i.e., without the large seawater reservoir covered by the mangrove forest) using the 3ds Max Model gives underestimated values of 192,100 m<sup>2</sup> and 246,060 m<sup>3</sup>, respectively (Rodolfo-Metalpa et al., in prep.). Terraces extend from the mangrove, on both sides of the channel and form diverse and compact reefs at their ends. Coral species are less abundant and diverse near the mangrove roots, where the bottom substrate is composed of fine sediment and mud. There, reefs are exposed to air only at low tide during the spring tides (1.1 m maximum tidal range). We subdivided the lagoon into three main areas: an external, an intermediate, and an inner reef (hereafter called outer, middle and inner reefs; panels a & b in **Figure 2.1**). A series of sampling stations were selected, and some of the main seawater chemical and physical environmental parameters were measured at the study site and two adjacent reference reefs, namely R1, a typical fringing reef, and R2, a semi-enclosed shallow and relatively large bay. Both reefs were located 4.8 km and 3.2 km from the entrance of the Bouraké lagoon, respectively.

## 2.2.2 Environmental monitoring

### 2.2.2.1 Oceanographic sensors deployment and short- to long-term measurements

From February 2016 to December 2020 up to eight YSI 600 OMS-M, three Seabird SeaFET<sup>TM</sup> pH loggers, and four Hobo water temperature Pro V2 were set at 10-min logging intervals, and used individually or in combination to assess short-, medium-, or long-term variations across depth (as a proxy of the tide), temperature (T°C), dissolved oxygen concentration (DO, mg L<sup>-1</sup>), seawater pH<sub>T</sub> (total scale), and salinity (see **Appendix A, Table A2.1**). YSI dissolved oxygen optical sensors were calibrated against zero and 100% saturated seawater at 25°C. Two SeaFETs were calibrated by the manufacturer, while the third was corrected before deployment by measuring its deviation from the two others in the same seawater.

Short-term (i.e., 24h) spatial and depth-related variations in pH and DO were simultaneously measured at several stations in the Bouraké lagoon in March and April 2018 (**Figure 2.1**). Spatial variations were assessed *i*) within the inner (St A-C for pH, and A-G for DO), the middle (St H-N for DO), and the outer (St O-Q for DO) reefs, *ii*) between the outer and the inner reefs (St B1, B2 for pH), and *iii*) between the outer and the reef outside the semi-enclosed lagoon (St U vs T for pH). In the Bouraké lagoon, we recorded differences between the surface (shallow) and the bottom water: *i*)

at the outer reef (St S for pH), *ii*) at the middle reef (St I and N for DO), and *iii*) at the inner reef (St R for pH, and DO). Salinity was measured in July 2019 (St R1, R2, B1 and B2) and December 2020 (St R1, R2, T and B2).

Medium-term measurements (i.e., 2-3 weeks) of the DO and pH were recorded at the reference (St R1, R2) and Bouraké reefs (St B1, B2) between 2016 and 2019.

Long-term measurements (>1 year) of seawater temperature were recorded at R1 and R2 starting from January 2019 and September 2017, respectively, and at B1 and B2 from October 2018 and September 2017, respectively. Only temperature data between October 2018 and April 2020 were compared between sites.

Short-term pH and DO data and long-term temperature data were compared between stations using General Linear Modelling (GLM), and the Tukey' HSD *post hoc* test was used when significant factor effects were found. When data did not conform to normality or homogeneity of variance, the Kruskal-Wallis test followed by the Dunn's multiple comparisons test (Bonferroni-adjusted) or the Wilcoxon test were performed. Statistical analyses were carried out using either Statistica® or R version 3.4.4 (R Core Team, 2018), the latter using "stats", "FSA", and "MASS" packages.

#### **2.2.2.2 Phase averaged and tidal harmonic analyses for diurnal and semidiurnal oscillations**

Medium-term pH and DO changes were investigated by averaging time and tidal phases for diurnal and semi-diurnal oscillations. To do this, all data were overlaid on a daily period and a tidal phase. First, we calculated a predicted tide for the study area using the Nouméa harbour tide (50 km south of our study site) modified with coefficients from the Naval Hydrographic and Oceanographic Service (SHOM; <http://data.shom.fr>). The predicted tide was used to assign a semidiurnal tidal phase (12 h) to each sampling time, and the data were averaged for each of these tidal phases. Similarly, the data were averaged for each hour of the day (24 h). Because tides at sea are a sequence of sinusoidal harmonic components that are different for each location, we performed a harmonic tidal analysis on the DO and pH data. The "UTide"-`ut_solv()` tidal analysis package (Codiga, 2011) with the principal semidiurnal lunar constituent ( $M_2$ ), principal semidiurnal solar constituent ( $S_2$ ), and solar diurnal constituent ( $S_1$ ) was used. For each parameter, the amplitudes of the tidal harmonics  $M_2$  (12.4 h),  $S_2$  (12 h), and  $S_1$  (24 h) were calculated with a 95% confidence interval based on the 200 Monte-Carlo simulations.

### 2.2.2.3 *Diel cycles of carbonate chemistry and chemical parameters*

Surface water samples were collected across a diel cycle in June 2017, and July 2019 for pH, total alkalinity ( $A_T$ ), dissolved inorganic carbon (DIC), nutrients (orthosilicic acid [ $\text{Si}(\text{OH})_4$ ], nitrogen oxide [ $\text{NO}_x$ ], ammonium [ $\text{NH}_4^+$ ], phosphate [ $\text{PO}_4^{3-}$ ], dissolved organic carbon (DOC), particulate organic carbon (POC), and particulate organic nitrogen (PON) (see **Appendix A- Table A2.2** for a summary of data collected). A summary of the total sampling is given in Supplementary Table S2. Dissolved inorganic nitrogen (DIN) and total organic carbon (TOC) were calculated as [ $\text{NO}_x$ ] + [ $\text{NH}_4^+$ ], and DOC + POC, respectively. The most important ratios were calculated (e.g., DOC:TOC;  $\text{Si}(\text{OH})_4$ :DIN) and contrasted between stations to evaluate the availability of nutrients and organic matter in the Bouraké lagoon (Jacquet et al., 2006; Leopold et al., 2017).

In 2017, during three consecutive days (from May 31<sup>st</sup> to June 02<sup>nd</sup>), seawater was sampled six times: twice during both high and low tide and once at both rising and falling tide. In total, we sampled one reference station (R2), three stations at the outer reef of the Bouraké lagoon (Outer: St B01-B03), one at the middle reef (Middle: St B04), and one at the inner reef inside the lagoon (Inner: St B05) (**Figure 2.1**). The whole collection lasted about 30 minutes. In 2019, during three consecutive days (from July 16<sup>th</sup> to 18<sup>th</sup>), sampling was carried out every hour from 8 am to 3 pm. The B1 and B2 stations were sampled on the first day, R1 on the second and R2 on the third day.

During diel cycles, at each station and sampling time, pH and temperature were measured at the surface (0.5 m deep) using a portable pH-meter (913, Metrohm) calibrated with TRIS buffer (Dickson lab, batch #T28). A subsample (50 mL) was filtered through 0.45  $\mu\text{m}$  Whatman<sup>TM</sup> Puradisc CA filters using a syringe and poisoned with 20  $\mu\text{L}$  saturated  $\text{HgCl}_2$  to further measure  $A_T$ . Two 20 mL subsamples were analysed using an auto titrator (EcoTitrator, Metrohm), and  $A_T$  was calculated from the Gran function. Results were corrected against  $A_T$  standards (A. Dickson, batch #155, Scripps, USA). The seawater carbonate parameters  $p\text{CO}_2$ ,  $\text{CO}_3^{2-}$ , and aragonite saturation state ( $\Omega_{\text{ara}}$ ) were then calculated from the  $\text{pH}_T$ ,  $A_T$ , temperature, and mean salinity (35) using the free-access  $\text{CO}_2\text{SYS}$  package (Pierrot et al., 2006).

Ammonium concentration was determined on a 40 mL subsample of unfiltered seawater, collected using a 60 mL Schott bottle and stored in the dark. Samples were processed using a fluorimeter (Turner Designs) between six and 18 h after two mL of OPA reagent (o-phthaldialdehyde) was added (Holmes et al., 1999).

The sampling of nutrients was performed using two replicate 20 mL polypropylene vials, rinsed three times using filtered seawater (Whatman<sup>TM</sup> Puradisc CA syringe filters 0.45  $\mu\text{m}$ ), filled with the

sample and immediately poisoned with 20  $\mu\text{L}$  saturated  $\text{HgCl}_2$ . Measurements of  $\text{PO}_4^{3-}$ ,  $\text{NO}_x$ , and  $\text{Si}(\text{OH})_4$  nutrients were performed by colourimetry (Seal Analytical).

Seawater samples for DIC were collected in two replicate glass vials (20 mL), filled with unfiltered water and poisoned with 10  $\mu\text{L}$  saturated  $\text{HgCl}_2$ . The vials were immediately closed, the absence of bubbles was visually checked, and the samples were stored in the dark at room temperature for later analysis on a Shimadzu TOC-L analyser (Non-Dispersive Infrared, NDIR). Typical analytical precision was less than  $\pm 2 \mu\text{mol kg}^{-1}$ . The accuracy was verified using regular measurements of reference material (CRM) from A. Dickson's laboratory.

Seawater samples for DOC were collected in two pre-combusted (4 h at  $450^\circ\text{C}$ ) glass ampoules filled with water filtered using a glass syringe filtration system (SGE<sup>TM</sup>) with two pre-combusted 25-mm GF/F Whatman<sup>TM</sup> filters. Samples were then acidified with ultrapure orthophosphoric acid ( $\text{H}_3\text{PO}_4$ ), sealed, and stored in the dark at room temperature for later analysis by high-temperature catalytic oxidation (HTCO) (Sugimura and Suzuki, 1988; Cauwet, 1994) on a Shimadzu TOC-L analyser. Typical analytical precision was  $\pm 0.1\text{-}0.5 \mu\text{M C}$  (SD). Consensus reference materials (<http://www.rsmas.miami.edu/groups/biogeochem/CRM.html>) were injected every 12 to 17 samples to ensure stable operating conditions. DOC concentrations are only available for the 2017 sampling because of a sample's pollution in 2019.

Finally, one litre of unfiltered seawater was collected in a borosilicate glass bottle and stored on ice during sampling for later measurement of POC and PON contents. In the lab, particulate matter was collected on pre-combusted (4 h at  $450^\circ\text{C}$ ) Whatman<sup>TM</sup> GF/F filters using a Nalgene<sup>®</sup> vacuum system. The filters were dried at  $60^\circ\text{C}$  in the oven for 24 h and stored in airtight glass vials at  $4^\circ\text{C}$  in the dark until analysis on a CHN Perkin Elmer 2400. All glass bottles and vials used were pre-combusted, washed with HCl solutions (10%) and rinsed using milliQ water.

Seawater chemistry data were pooled by sampling area (R1, R2, Outer, Middle, Inner), and differences were tested using the Kruskal-Wallis test followed the Conover Multiple comparison test (Benjamini-Hochberg-adjusted). The effect of the tidal phases (i.e., falling and rising tide) on the seawater chemical composition in the Bouraké lagoon only was assessed by attributing each sample a tidal phase between 0 h (high tide) and 6 h (low tide), and between 6 h and 12 h (high tide). Multiple linear regression was used to assess the adjusted  $R^2$  and significance ( $p < 0.05$ ) of the data from 0 h to 6 h (falling tide) and from 6 h to 12 h (rising tide) separately. Statistical analyses were performed using either Statistica<sup>®</sup> or R (version 3.2.4, R Core Team, 2018), the latter using the "FSA", "stats", and "Conover.test" packages.

### 2.2.3 Benthic community characterization and distribution

The benthic community and bottom substrate of the Bouraké lagoon, referred hereafter as biotic and abiotic descriptors, respectively, were assessed in April 2018. Twenty-four 30 m-long geo-referenced transects (T1-T24) were laid in the lagoon along the terraces' edge at similar depths (i.e., ~1 m), targeting coral dominated benthic assemblages. On each transect, a 0.5 m x 0.5 m PVC quadrat was placed every meter, and a picture was taken with a waterproof photo-camera (Nikon AW 130) parallel to the substrate. A general description of the bottom (i.e., the various substrates) and a list of the most common and identifiable sessile species for each transect were made. For each of the 835 pictures collected, the cover of abiotic (i.e., mud, sand, rock, rubble, dead corals and unreadable) and biotic descriptors (i.e., branching, massive and soft corals, sponges, macroalgae and "others") with photoQuad software both by automatic multi-scale image segmentation regions and manual grid cell counts when necessary was estimated.

Photos of quadrats, the many other pictures collected during fieldwork, and laboratory morphological observations on collected samples were used to produce a non-exhaustive species list of corals, macroalgae and sponges. Corals were comprehensively sampled throughout the Bouraké lagoon and on the reference reef R2, while dominant macroalgal and sponges species were collected in the Bouraké lagoon alone and only if they were encountered at least three times along a transect, likely leading to an underestimation of their diversity. Coral diversity was assessed through photographic and sampling during time-based open search swims and SCUBA dives (Hill and Wilkinson, 2004). Whenever possible, scleractinian corals were identified to species level *in-situ* by photographic sampling only. When identification was doubtful *in vivo*, or when taxa were characterized by small corallite size (< 1 mm in diameter) and required additional morphological examination of the skeleton to confirm identification (e.g. genera *Acropora*, *Montipora* and *Porites*), a fragment of the colony was collected, tagged, cleaned in sodium hypochlorite overnight, rinsed in freshwater and dried. The reference collection is housed at IRD Nouméa. Microscopic examination of the skeletal features allowed species-level identification following the reference literature (Veron, and Wallace, 1984; Wallace, 1999; Veron, 2000). Sponges were identified based on their spicules' morphological characteristics (i.e., shape, length and width), or using a series of morphological descriptors (e.g., shape, size, colour, texture, surface ornamentations, fibres) for species without spicules. In the lab, a subsample of the collected sponges was immediately digested using HNO<sub>3</sub>, and spicules were measured with an optical stereomicroscope. For species without spicules, hand-cut sections of the choanosome and ectosome were observed under a stereomicroscope. Species were identified using the taxonomical keys of Rützler (1978), Hooper and Van Soest (2002), and Pons et al. (2017).

Macroalgae and corals identification were based on morphological and anatomical observations following the dedicated literature and referring to specimens housed at IRD Noumea.

Abiotic and biotic cover percentages averaged per transect, and species richness, calculated as the number of species in the transect, were plotted using non-metric multidimensional scaling (nMDS) based on Bray-Curtis dissimilarities (“vegan” package in R) of square-root transformed data. Finally, the best number of clusters for the whole Bouraké lagoon was determined using the gap statistic method (“cluster” and “factorextra” packages in R) and used for the hierarchical clustering representation (Ward, 1963). The cluster separation was verified with a two-way analysis of similarity ANOSIM. Within each cluster, the benthic community and bottom substrate were averaged between transects, and the dominant biotic and abiotic descriptors were selected and used to define the cluster.

Principal component analysis (PCA) was used to visualize the correlation between environmental parameters and the benthic descriptors of transects inside the Bouraké lagoon. The analysis was performed in R (package “FactorMineR”, version 3.2.4, R Core Team, 2018) using data of biotic descriptors (averaged per transect) and the seawater parameters averaged for each sampling area (i.e., outer, middle and inner reefs).

#### **2.2.4 Stable isotopes analyses**

Stable isotope analysis of  $^{13}\text{C}$  and  $^{15}\text{N}$  was performed to provide information on organic matter sources and trophic relationships. Carbon and nitrogen stable isotopes ratios were used to highlight differences in the composition of carbon source and nitrogen between the outermost (B02) and innermost (B05) zone of the Bouraké lagoon, in some benthic organisms.

Samples for stable isotopes analyses were collected in the summer season (March 2019 and December 2020). In 2019, 6 fishes species (tot samples = 19), 5 macroalgae species (tot samples = 6), 1 sponge species (*Rhabdastrella globostellata*) were sampled in the Bouraké inner zone (B05). Only for fish, 5 of the 6 species were collected at the reference site R1. Specifically, 3 *Dascyllus aruanus* in site B02 and 2 in R1, 2 *Amblygyphidon ternatensis* at R1, 3 *Chaetodon lunulatus* at R1, 1 *Chaetodon bennetti* at R1, 5 *Stegastes nigricans* at R1, and 3 *Apogon cf. crassiceps* at R1. Macroalgae are 1 *Caulerpa serrulata*, 1 *Sargassum* sp., 2 *Styopodium* sp., 1 *Halimeda discoidea* and 1 *H. distorta*. In 2020, 4 different species of macroalgae (*Turbinaria conoides*, *Styopodium* sp., *Sargassum* sp., *Dyctiota* sp.) and 4 different species of sponges (*Callyspongia* sp., *Haliclona* sp., *Dysidea* sp., and *Hyrtios* sp.) with two sample per species were sampled in both the outer (B02) and the inner (B05) zone. *Padina* sp. (n samples = 2), 4 mangrove leaf, and 1 undertermined seagrass species (n samples = 2) were only sampled at B05.

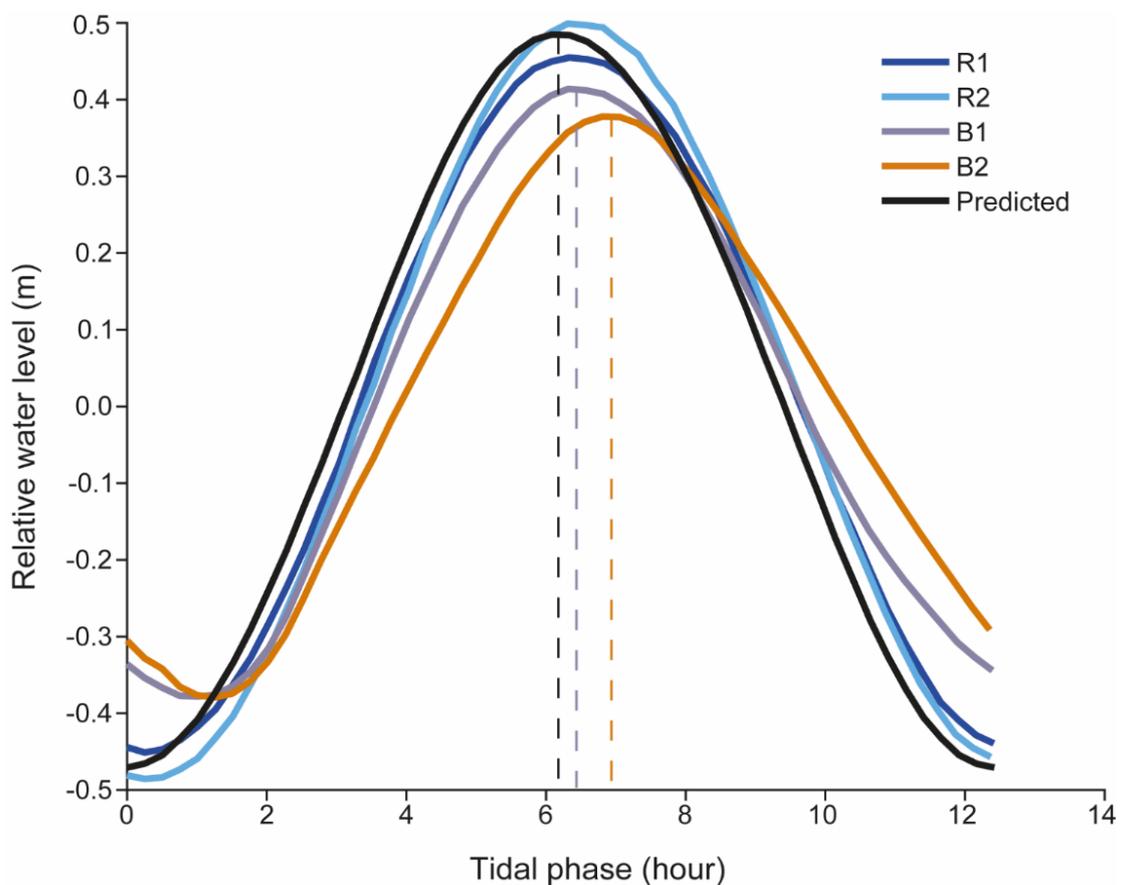
Samples were freeze-dried and ground into a fine powder using a mortar pestle. Sample (1 mg) of macroalgae, sponges and fish were analysed with no prior treatment. Samples with calcareous components, such as *Halimeda* sp. is treated for  $^{13}\text{C}$  analyses after acidification by 1% HCL solution to remove carbonates, rinsed with distilled water and oven-dried at 40°C for 24 h. The  $^{13}\text{C}:^{12}\text{C}$  and  $^{15}\text{N}:^{14}\text{N}$  ratios were analysed by continuous- flow isotope-ratio mass spectrometry. The spectrometer (IRMS Sercon, UAR IMAGO, Nouméa, New Caledonia) was used for isotopes analysis. The analytical precision was 0.4‰ for N and 0.15‰ for C, estimated from standards analysed along with the samples. Isotope ratios were expressed as parts per 1000 (‰) differences from a standard reference material:  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N} = [(R_{\text{sample}} / R_{\text{standard}}) - 1] \times 1000$ ; where R sample and R standard are the ratio of  $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$  in the sample and in the standard reference materials, respectively. The international standard references are Vienna Pee Dee Belemnite for carbon and atmospheric  $\text{N}_2$  for nitrogen.

These data were represented using point graph to get an overview of the different isotopic signatures in fish, sponges and macroalgae between the outer and inner zone of Bouraké and R1. Given the small number of samples, only for sampling in 2020, isotopic signatures for all sponge species and macroalgae species were pooled and differences between Bouraké outer (B02) and inner (B05) were compared, as well as average isotopic signatures of *Dascyllus aruanus* between Bouraké and reference reef (R1). Differences were tested by Wilcoxon test using R package “stats” (R Core Team, 2019).

## 2.3 Results

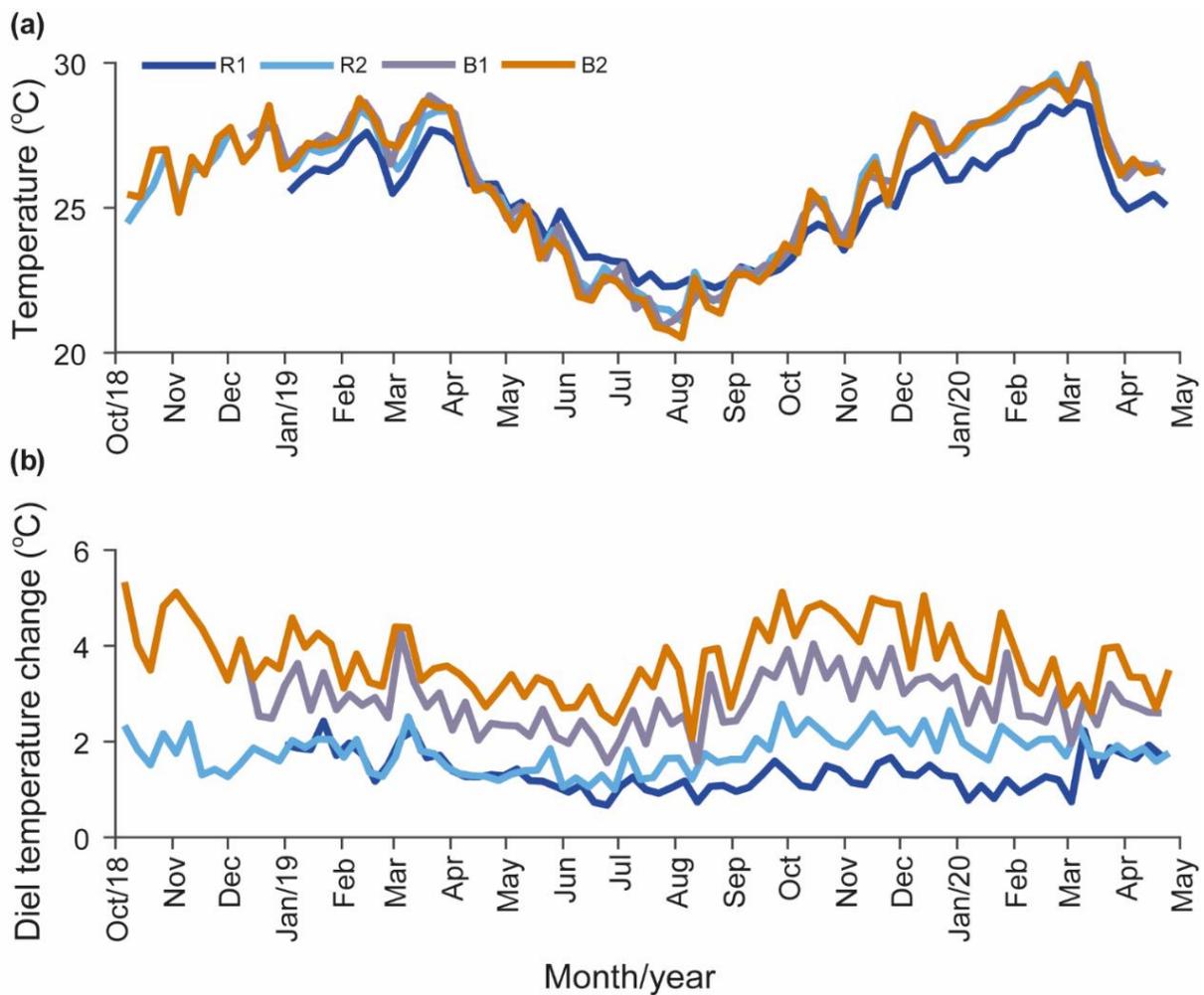
### 2.3.1 Variability of physical environmental parameters

*Tidal phases.* The predicted tide in the study area (SHOM data) is close to what measured at R1 and R2 (**Figure 2.2**). It is semidiurnal with a diurnal inequality ( $M_2 = 0.44$  m,  $S_2 = 0.16$  m) and has a small diurnal component ( $S_1 = 0.15$  m). It varies between 0.4 m and 1.7 m, depending on the phase of the moon, with a mean of 1.1 m. The tidal range inside the Bouraké lagoon is lower and varies between 0.4 m and 1.2 m, with a mean of 0.9 m. At B1 and B2, the tidal signal lags the predicted and measured tide at the reference stations by an average of 1.5 h for low tide and up to 45 min for high tide.



**Figure 2.2.** Temporal shift between the averaged tides recorded at the reference (St R1 and R2) and at the Bouraké reefs (St B1 and B2) and the predicted tide calculated for the study area (see SHOM: <http://data.shom.fr> for the Nouméa harbour).

*Temperature.* Results indicate that the water temperature has an annual cycle with lower values during winter (May-October) and higher values in summer (November-April). Daily averaged temperatures at R2, B1 and B2 were not significantly different, while the temperature at R1 was cooler in summer and warmer in winter (**Figure 2.3a; Table 2.1**) compared to the other stations. On a weekly basis, the averaged diel temperature variation was significantly different between stations:  $1.34 \pm 0.39^{\circ}\text{C}$  at R1 and  $3.73 \pm 0.74^{\circ}\text{C}$  at B2 (**Figure 2.3b; Table 2.1**). In a single day, we recorded temperature fluctuations of up to  $3.4^{\circ}\text{C}$  at R1 and  $6.5^{\circ}\text{C}$  at B2.



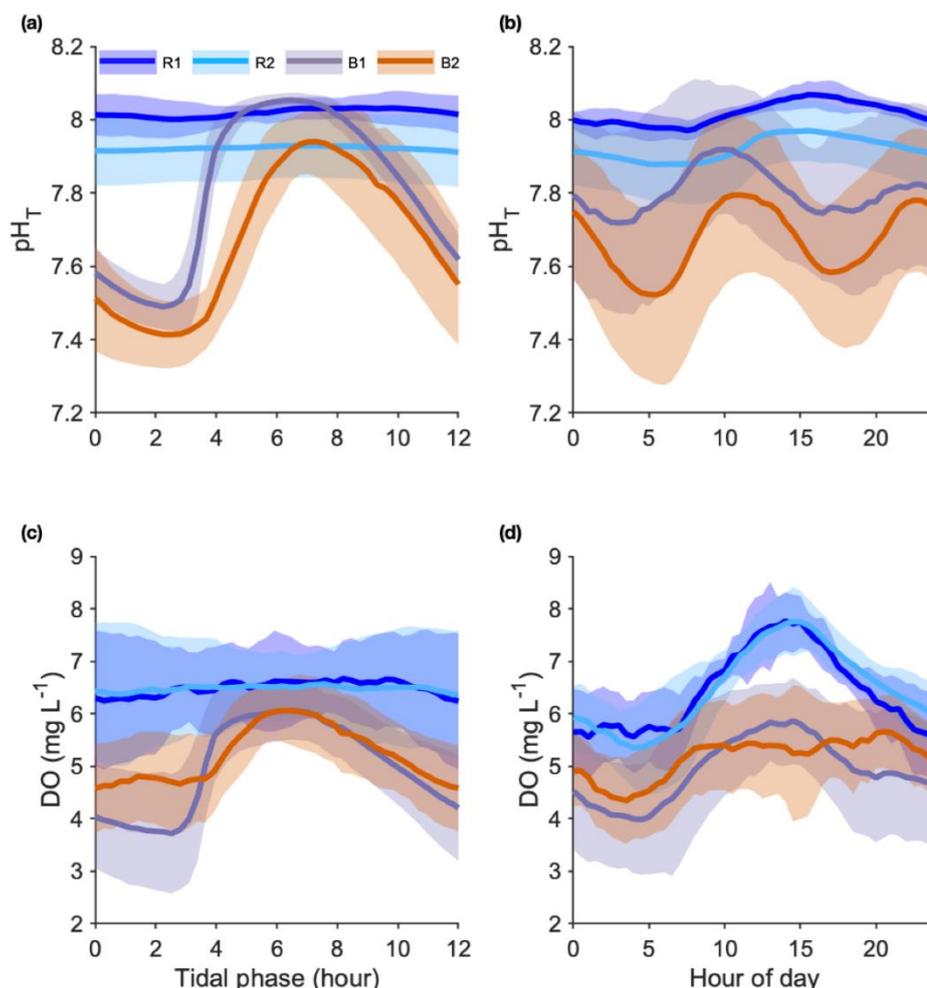
**Figure 2.3.** Long-term temperature monitoring from October 2018 to April 2020 at the reference (St R1 and R2) and Bouraké reefs (St B1 and B2). Data are plotted using weekly averaged temperature (a) and weekly averaged diel changes (b).

Chapter 2: The Bouraké semi-enclosed lagoon (New Caledonia) – a natural laboratory to study the lifelong adaptation of a coral reef ecosystem to extreme environmental conditions

**Table 2.1.** Summary of the statistical analyses applied to the seawater physical and chemical parameters collected between 2016 and 2020 at the Bouraké lagoon (St B1, B2, St A-R, outer, middle and inner) and at reference reefs (St R1 and R2). Differences in temperature, salinity and chemical parameters between the Bouraké lagoon and reference reefs and spatial and vertical differences in pH and DO inside the Bouraké lagoon. K-W: Kruskal-Wallis's test; W: Wilcoxon test; GLM: General Linear Model; D: Dunn's test; T: Tukey's test; C: Conover's test.

Parameters	(n)	K-W	W	GLM	p	post hoc (test)
<i>Seawater temperature (St R1, R2, B1, B2)</i>						
Daily means	2968	68.46			< 0.001	(D) $p < 0.001$ R1 < R2 = B1 = B2
Weekly oscillations	305	234.63			< 0.001	(D) $p < 0.01$ R1 < R2 < B1 < B2
<i>Seawater pH (Stations)</i>						
Spatial INNER (A-C)	711			5.32	0.005	(T) $p < 0.01$ A < C
Spatial (B1 vs B2)	1820			17.62	< 0.001	
Spatial (U vs T)	7280			189.8	< 0.001	
Surface vs deep (R)	664			26.88	< 0.001	
Surface vs deep (S)692				41.48	< 0.001	
<i>Seawater DO (Stations)</i>						
Spatial INNER (A-G)	1031			1.145	0.330	
Spatial MIDDLE (H-N)	700			0.636	0.636	
Spatial OUTER (O-Q)	399			1.636	0.196	
Surface vs deep (I)	280			0.000	0.994	
Surface vs deep (N)	280			22.05	< 0.001	
Surface vs deep (R)	258			0.443	0.839	
<i>Seawater Salinity (Stations)</i>						
Winter (R1, R2, B1, B2)	1732			463.5	< 0.001	(T) $p < 0.01$ R1 < R2 < B1 < B2
Summer (R1, R2, T, B2)	5105			1583	< 0.001	(T) $p < 0.01$ R1 < R2 < T < B2
Winter vs Summer (R1)	1872		287264		< 0.001	
Winter vs Summer (R2)	1728		253060		< 0.001	
Winter vs Summer (B2)	1872		150175		< 0.001	
<i>Seawater carbonate chemistry (St R1, R2, outer, middle, and inner)</i>						
pH <sub>T</sub>	66	36.48			< 0.001	(C) $p < 0.05$ R1 > R2 > Inn = Mid = Out
pCO <sub>2</sub>	66	36.48			< 0.001	(C) $p < 0.01$ R1 = R2 < Inn = Mid = Out
CO <sub>3</sub> <sup>2-</sup>	66	33.54			< 0.001	(C) $p < 0.05$ R1 > R2 > Inn = Mid = Out
Ω <sub>ara</sub>	66	33.27			< 0.001	(C) $p < 0.05$ R1 > R2 > Inn = Mid = Out
DIC	66	25.95			< 0.001	(C) $p < 0.01$ R1 = R2 < Inn = Mid = Out
A <sub>T</sub>	66	20.94			< 0.001	(C) $p < 0.05$ All ≠; Mid = R1; Mid = R2; Out = Inn; R1 = R2
<i>Seawater chemical parameters (St R1, R2, outer, middle, inner)</i>						
SiOH <sub>4</sub>	66	45.37			< 0.001	(C) $p < 0.05$ R1 < R2 < Out = Mid = Inn; Inn > Out
NO <sub>x</sub>	66	26.46			< 0.001	(C) $p < 0.05$ ; R1 < R2 = Inn = Mid; Inn < Out; Out > R1; Out > R2
NH <sub>4</sub> <sup>+</sup>	66	26.46			< 0.001	(C) $p < 0.01$ R1 < Inn = Mid = Out = R2
PO <sub>4</sub> <sup>3-</sup>	66	29.43			< 0.001	(C) $p < 0.05$ All ≠; R2 = Mid
DOC	36	16.12			0.001	(C) $p < 0.05$ All ≠; Mid = Out
POC	66	52.46			< 0.001	(C) $p < 0.05$ R1 = R2 < Out < Mid < Inn
PON	66	43.79			< 0.001	(C) $p < 0.05$ R1 = R2 < Out < Mid < Inn
DIN	66	20.13			< 0.001	(C) $p < 0.01$ R2 < R1 < Inn = Mid = Out
TOC	36	19.48			< 0.001	(C) $p < 0.01$ All ≠; Mid = Out
DIC:DIN	66	17.40			< 0.001	(C) $p < 0.01$ R1 > R2 = Inn = Mid = Out
POC:PON	66	44.87			< 0.001	(C) $p < 0.01$ R1 = R2 < Inn = Mid = Out
POC:DOC	36	18.47			< 0.001	(C) $p < 0.05$ All ≠; Inn = Mid
DOC TOC	36	18.21			< 0.001	(C) $p < 0.01$ R2 < Out < Inn < Mid; Inn = Mid; Inn = Out
Si(OH) <sub>4</sub> :DIN	66	43.77			< 0.001	(C) $p < 0.01$ R2 < Inn > Mid; Mid = Out; R1 = R2
DIN:DIP	65	24.59			< 0.001	(C) $p < 0.05$ R1 < R2 > Inn; R2 = Out = Mid; Inn = Mid
Si(OH) <sub>4</sub> :DIP	65	36.62			< 0.001	(C) $p < 0.01$ R1 < R2 < Inn = Mid = Out

*Seawater pH*. During the entire study period, pH was measured during 22, 72, 31, and 72 semidiurnal tidal cycles at R1, R2, B1, and B2, respectively. We overlaid all data on a single tidal phase of 12 h (**Figure 2.4a**) and a 24 h cycle (**Figure 2.4b**).

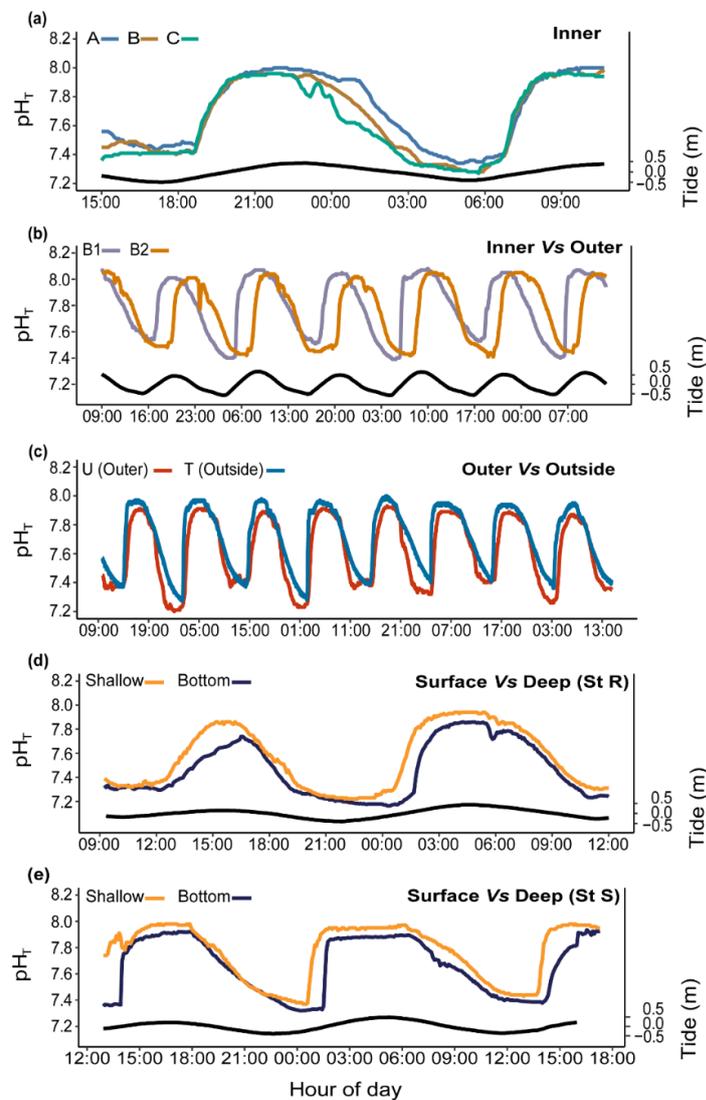


**Figure 2.4.** Seawater pH<sub>T</sub> (a), (b) and dissolved oxygen (DO) (c), (d) variations recorded at the reference (St R1 and R2) and Bouraké reefs (St B1 and B2). Data were overlaid in a single tidal phase (12 h) (a), (c); and in a 24 h cycle (b), (d). Data are 22, 72, 31, and 72 semidiurnal tidal cycles for pH, and 36, 79, 34 and 42 semidiurnal tidal cycles for DO, for R1, R2, B1 and B2, respectively.

The pH differed significantly between stations. During the studied period, we recorded means of  $8.01 \pm 0.04$  and  $7.89 \pm 0.08$  pH<sub>T</sub> units at R1 and R2, and  $7.80 \pm 0.22$  and  $7.67 \pm 0.23$  pH<sub>T</sub> units at B1 and B2, respectively. At both B1 and B2, pH was strongly correlated with the tidal cycle (**Figure 2.4a**, 82% and 73% of the total variance were explained by the tidal harmonic analysis, respectively). In contrast, it was only marginally correlated with the 24 h cycle (**Figure 2.4b**). During each tidal phase, the pH changed on average by about 0.6 units and reached a minimum of 7.23 and a maximum of 8.06 at B2 at low and high tide, respectively. The pH oscillations were mainly semidiurnal ( $M_2 = 0.27$

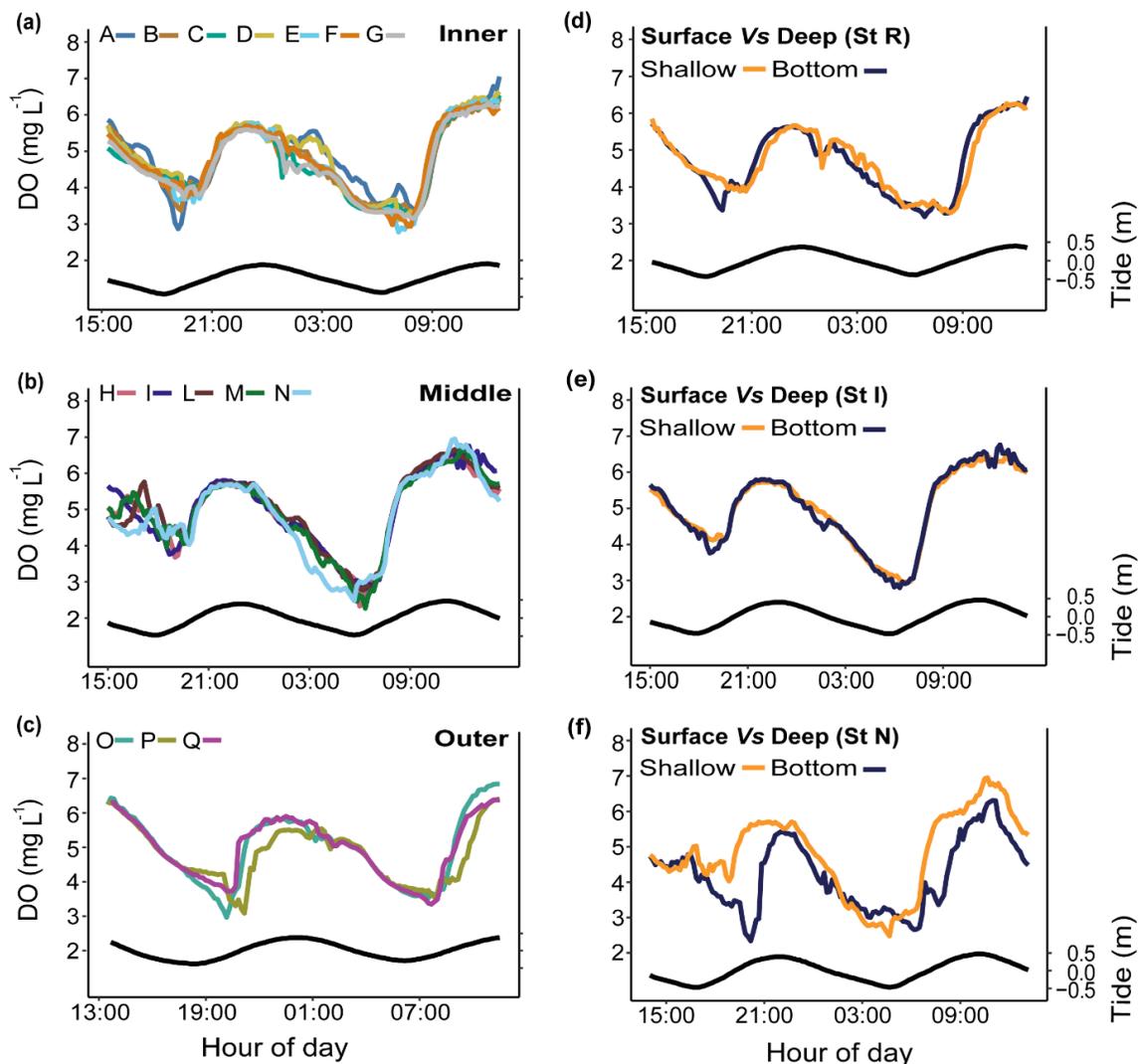
$\pm 0.01$ ,  $S_2 = 0.05 \pm 0.01$  pH<sub>T</sub> units, for B1;  $M_2 = 0.24 \pm 0.01$ ,  $S_2 = 0.09 \pm 0.01$  pH<sub>T</sub> units for B2). At stations R1 and R2, pH changed on average by about 0.1 pH<sub>T</sub> units and was mostly dependent on the 24 h cycle (**Figure 2.4b**,  $S_1 = 0.058 \pm 0.004$  and  $S_1 = 0.049 \pm 0.007$  pH<sub>T</sub> units for R1 and R2, respectively).

Simultaneous short-term pH measurements showed significant spatial differences (**Figure 2.5**; **Table 2.1**), between *i*) stations A and C, *ii*) stations B1 and B2, and *iii*) the outer reef in the Bouraké lagoon (St U) and the station outside the system (St T). There were also significant depth-related differences between shallow reefs and bottom water at stations R and S. In both spatial and depth-related analyses, differences were approximately 0.05 to 0.1 pH<sub>T</sub> units, and we found the lowest values at stations C and B2 and the bottom of the channel (St R and S).



**Figure 2.5.** Spatial and depth-related short-term changes (24 h) in pH<sub>T</sub> at stations in the Bouraké lagoon (see **Figure 2.1** for the stations' location). Spatial variations were assessed: **(a)** within the inner reef (St A-C); **(b)** between the inner and the outer reefs (St B1, B2); **(c)** between the outer reef (but still inside the Bouraké lagoon), and the reef outside the semi-enclosed lagoon (St U vs T). Depth-related variations were assessed between shallow reefs and the bottom of the channel: **(d)** at the inner reef (St R); and **(e)** at the outer reef (St S). Tide (black line) refers to St B2.

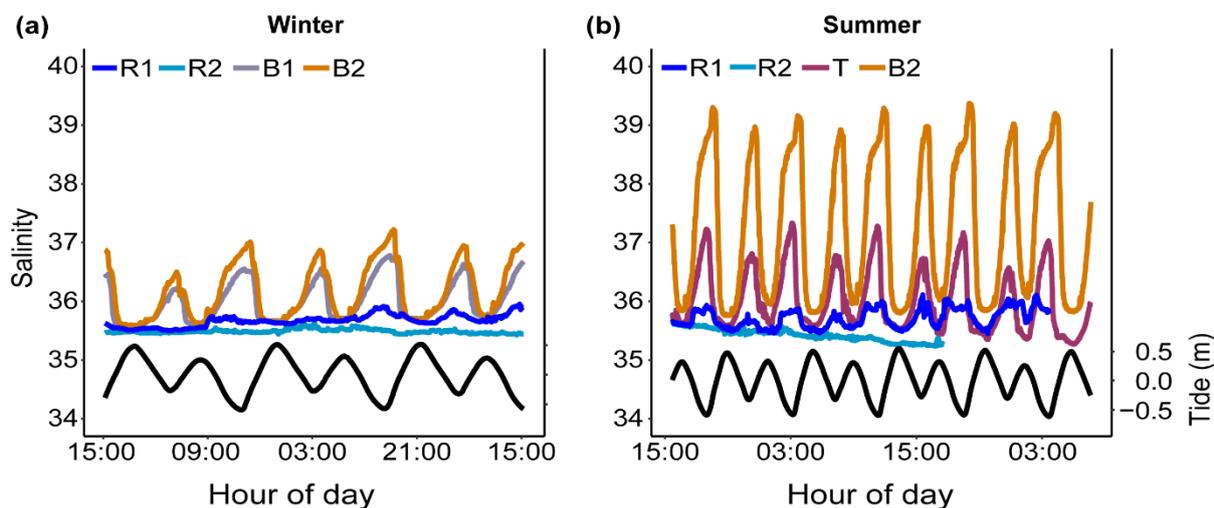
*Dissolved oxygen (DO)*. DO was measured during 36, 79, 34 and 42 semidiurnal tidal cycles at R1, R2, B1 and B2, respectively. All data were overlaid on a single tidal phase of 12 h (**Figure 2.4c**) and a 24 h cycle (**Figure 2.4d**). As with pH, the mean diel DO was higher at the reference reefs than in the Bouraké lagoon. During the studied period, mean DO values were  $4.89 \pm 1.18$  and  $5.23 \pm 0.89$  mg L<sup>-1</sup> at B1 and B2, respectively, and  $6.45 \pm 0.95$  and  $6.48 \pm 1.05$  mg L<sup>-1</sup> at R1 and R2, respectively. At stations B1 and B2, DO was strongly correlated with the tidal cycle (**Figure 2.4c**, 82 and 72% of the total DO variance were explained by the tidal harmonic analysis, respectively), and only marginally with the 24 h cycle (**Figure 2.4d**). DO oscillations were mainly semidiurnal ( $M_2 = 1.17 \pm 0.08$  mg L<sup>-1</sup>, and  $M_2 = 0.83 \pm 0.09$  mg L<sup>-1</sup> for B1 and B2, respectively) with a substantial diurnal component ( $S_1 = 1.12 \pm 0.04$  mg L<sup>-1</sup>, and  $S_1 = 0.681 \pm 0.04$  mg L<sup>-1</sup> for B1 and B2, respectively). During a semidiurnal tidal cycle, DO was lower at low tide (**Figure 2.4c**, 3.7 mg L<sup>-1</sup> and 4.6 mg L<sup>-1</sup> at B1 and B2, respectively) and higher at high tide (**Figure 2.4c**, 6.0 mg L<sup>-1</sup> and 6.1 mg L<sup>-1</sup> at B1 and B2, respectively). The minimum (1.89 mg L<sup>-1</sup>), and the maximum (7.24 mg L<sup>-1</sup>) DO value were both measured at B1 during low and high tide, respectively. During a 24 h cycle, DO was lower in the early morning (**Figure 2.4d**, 4.0 and 4.3 mg L<sup>-1</sup> at B1 and B2, respectively) and higher in the middle of the day (**Figure 2.4d**, 5.8 and 5.4 mg L<sup>-1</sup> at B1 and B2, respectively). In a single day, DO fluctuations were up to 6.37 mg L<sup>-1</sup> at R1 and 4.91 mg L<sup>-1</sup> at B2. At stations R1 and R2, DO was mostly dependent on the 24-hour cycle ( $S_1 = 1.34 \pm 0.06$  mg L<sup>-1</sup> and  $S_1 = 1.53 \pm 0.09$  mg L<sup>-1</sup> for R1 and R2, respectively) with lower values during the night (**Figure 2.4d**, 5.5 mg L<sup>-1</sup> at both stations), and higher values in the middle of the day (**Figure 2.4d**, 7.8 mg L<sup>-1</sup>, both stations). Simultaneous short-term DO measurements (**Figure 2.6; Table 2.1**) did not show significant differences over a 24 h cycle at the inner (St A-G; except for some specific deviations, e.g., St A), the middle (St H-N) or the outer reef (St O-Q). Dissolved oxygen did not change on a vertical gradient at stations R and I, but the bottom DO was significantly lower at station N.



**Figure 2.6.** Spatial and depth-related short-term measurements (24 h) of dissolved oxygen (DO) at stations in the Bouraké lagoon (see Figure 1 for the location of the stations). Spatial variations were assessed on (a) the inner reef (St A-G), (b) the middle reef (St H-N), and (c) the outer reef (St O-Q). Depth-related variations between shallow reefs and bottom water within the Bouraké lagoon were assessed (d) at the inner reef (St R) and (e, f) at the middle reef (St I and N). Tide (black line) refers to St B2.

**Salinity (S).** Salinity was measured only during two short-term periods in winter 2019 and summer 2020 (**Figure 2.7**). Salinity variations in the Bouraké lagoon were strongly correlated with the tidal cycle, and the highest mean values were measured during both winter and summer. Salinity was lower at reference reefs during both the winter and summer seasons (**Figure 2.7a-b**). The mean salinity during the winter of 2019 was  $35.49 \pm 0.04$  at R1,  $35.67 \pm 0.10$  at R2,  $36.00 \pm 0.35$  at B1, and  $36.17 \pm 0.47$  at B2 with significant differences between stations (**Table 2.1**). Salinity in the Bouraké lagoon peaked at 37.22 at B2 during low tide, while it was 35.65 at R1. During the summer of 2020, the mean salinity increased significantly in the Bouraké lagoon, with  $37.22 \pm 0.53$  at B2 reaching 39.37, while it remained lower at R1 with  $35.42 \pm 0.10$  and R2 with  $35.71 \pm 0.16$ . During summer 2020, we

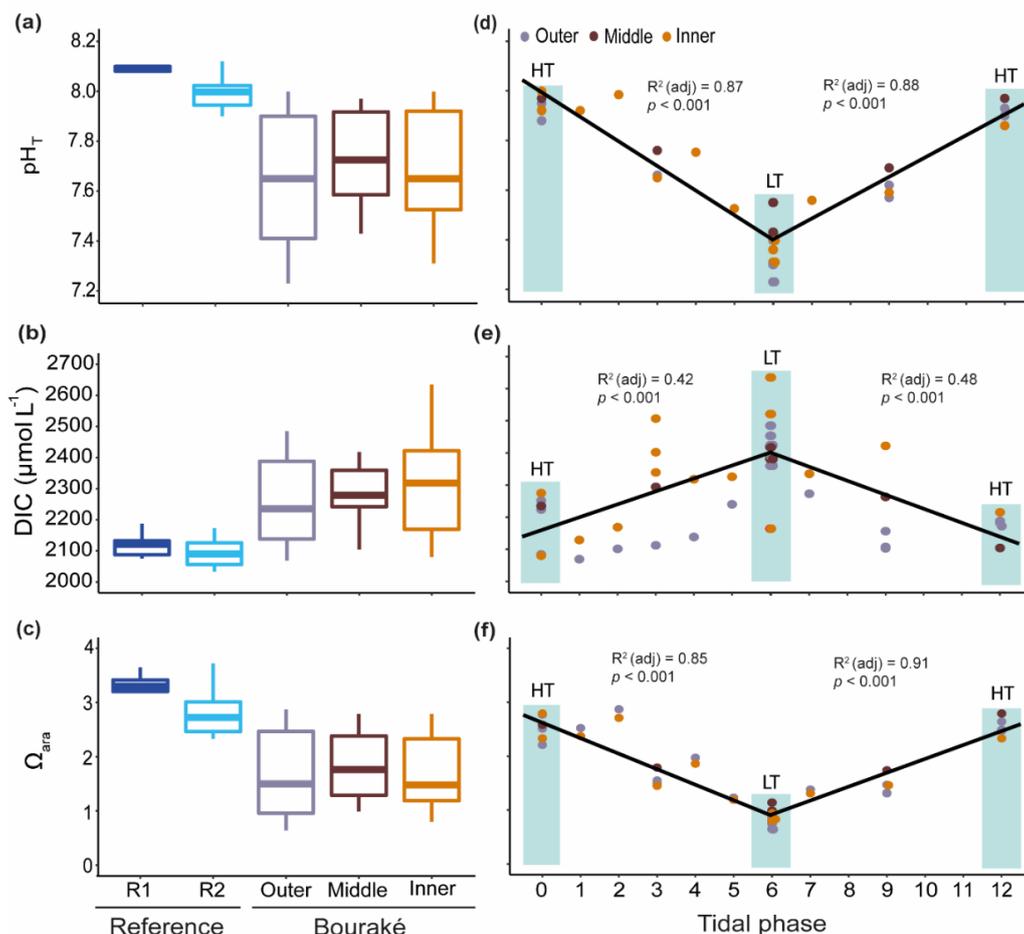
also measured short-term variations in salinity at station T, which is outside the Bouraké system. Although its distance from the lagoon entrance, the water mass discharged during falling tide increased seawater salinity also at this station where a maximum value of 37.33 during low tide was measured.



**Figure 2.7.** Short-term salinity monitoring during (a) the winter of 2019 (from 15/07/2019 to 18/07/2019) at the reference (St R1 and R2) and Bouraké reefs (St B1 and B2); (b) the summer of 2020 (from 29/11/2020 to 04/12/2020) at the reference (St R1 and R2) and Bouraké reefs (St T and B2). Tide (black line) refers to St B2.

### 2.3.2 Diel cycles of carbonate chemistry and chemical parameters

We monitored diel cycles in June 2017 and July 2019 at two reference reefs and three reefs within the semi-enclosed lagoon of Bouraké (i.e., the outer, the middle and the inner reefs). The seawater carbonate chemistry differed significantly between the reference and the Bouraké lagoon reefs (**Figure 2.8a-c; Table 2.1, Table 2.2; Appendix A- Figure A2.1**). The reference reefs R1 and R2 had higher pH,  $\text{CO}_3^{2-}$  and  $\Omega_{\text{ara}}$ , and lower DIC and  $p\text{CO}_2$  than the outer, middle or inner reefs in the Bouraké lagoon. The range of values in the lagoon was similar to previous measurements (see above), with levels of  $p\text{CO}_2 > 2000 \mu\text{atm}$  and levels of  $\Omega_{\text{ara}} < 1$ . By assigning a tidal phase (from 0 to 12 h) to each sample taken in the Bouraké lagoon (all reefs combined), the diel measurements showed significant regressions, either positive or negative, depending on the seawater carbonate parameter and the tide phase (**Figure 2.8d-f; Appendix A- Figure A2.1; Table A2.3**). For example, the DIC reached the highest value of  $2635 \mu\text{mol L}^{-1}$  during low tide on the inner reef with an average of  $2315.1 \pm 168.1 \mu\text{mol L}^{-1}$ , while  $\text{CO}_3^{2-}$  reached the lowest value of  $50.10 \mu\text{mol kg}^{-1}$  during low tide on the inner reef with an average of  $109.02 \pm 45.59 \mu\text{mol kg}^{-1}$ .

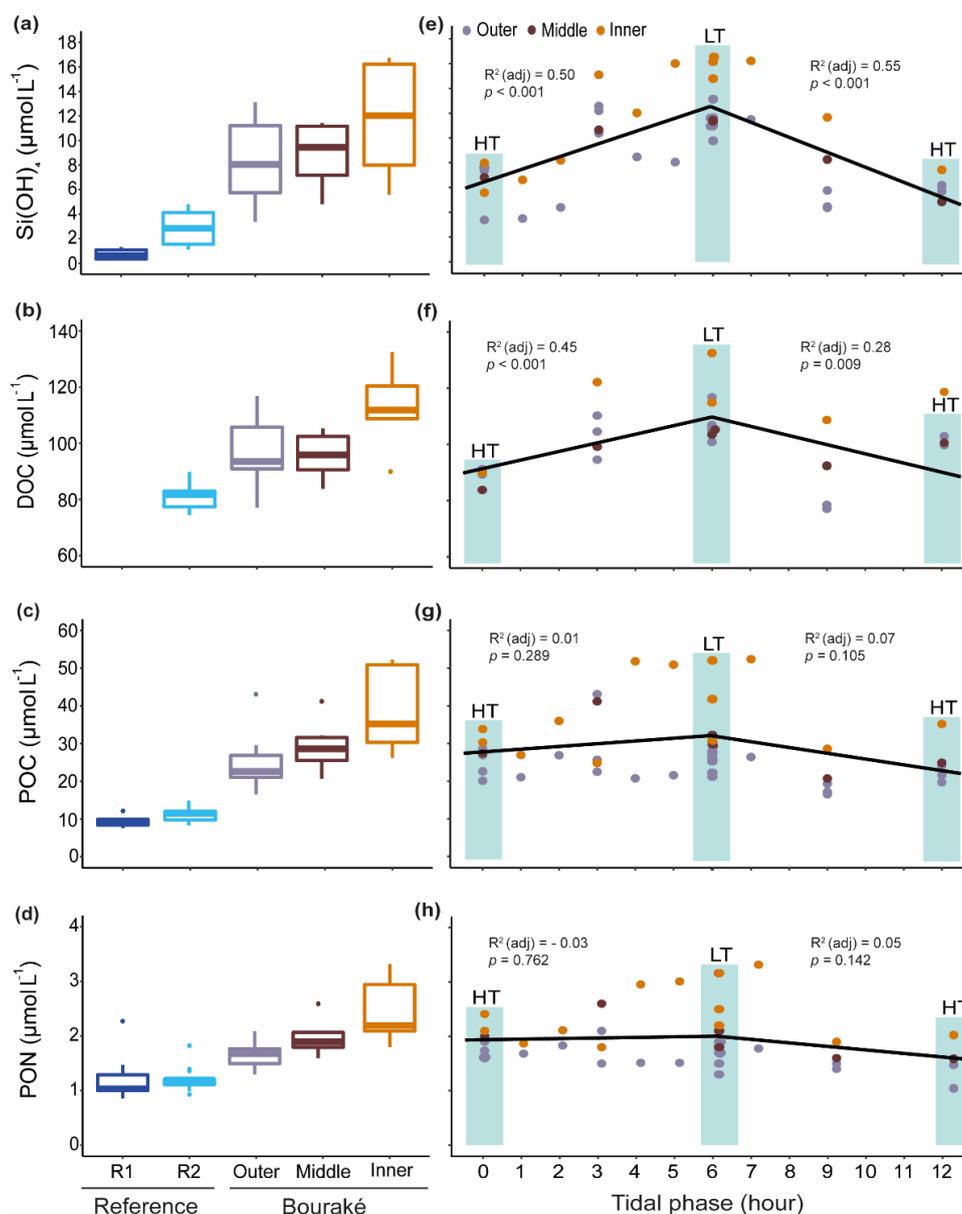


**Figure 2.8.** Seawater carbonate chemistry measured ( $pH_T$ ) and calculated (DIC and  $\Omega_{\text{ara}}$ ) during diel cycles in 2017 and 2019 (pooled data, see also Appendix A Figure A2.1.) at R1, R2 and the Bouraké reefs (outer, middle and inner) (a-c). Boxes ( $n = 6-14$ ) represent the interquartile range (25th and 75th percentile); the horizontal line is the median, and the whiskers represent the data range (i.e., minimum and maximum). Changes are illustrated across a 12 h tidal phase in the Bouraké lagoon (d-f). Linear regression lines are plotted for each falling (HT to LT) and rising tide (LT to HT). Shaded boxes have only a graphical significance and only suggest the tide changes during measurements.

**Table 2.2.** Summary of the seawater physical and chemical data (mean  $\pm$  SD) measured in July 2017 and June 2019 and calculated using the CO2SYS package. Data were pooled and averaged per station. nd = not determined. Reference reefs: St R1 and R2; Bouraké lagoon stations: outer, middle and inner.

	R1	R2	Outer	Middle	Inner
<i>Measured</i>					
Temp (°C)	22.54 $\pm$ 0.31	22.69 $\pm$ 1.50	23.57 $\pm$ 1.31	24.05 $\pm$ 1.20	23.00 $\pm$ 1.46
pH <sub>T</sub>	8.09 $\pm$ 0.01	8.02 $\pm$ 0.07	7.66 $\pm$ 0.25	7.73 $\pm$ 0.22	7.68 $\pm$ 0.24
DO (mg L <sup>-1</sup> )	7.20 $\pm$ 0.64	6.91 $\pm$ 1.01	6.11 $\pm$ 0.68	nd	5.98 $\pm$ 0.67
A <sub>T</sub> (μmol kg <sup>-1</sup> )	2294.1 $\pm$ 0.3	2261.2 $\pm$ 0.04	2357.8 $\pm$ 0.1	2256.3 $\pm$ 0.06	2393.7 $\pm$ 0.08
Si(OH) <sub>4</sub> (μmol L <sup>-1</sup> )	0.71 $\pm$ 0.42	2.86 $\pm$ 1.41	8.22 $\pm$ 3.14	8.88 $\pm$ 2.72	11.93 $\pm$ 4.27
NO <sub>x</sub> (μmol L <sup>-1</sup> )	0.21 $\pm$ 0.05	0.51 $\pm$ 0.16	0.65 $\pm$ 0.20	0.54 $\pm$ 0.09	0.54 $\pm$ 0.12
PO <sub>4</sub> <sup>3-</sup> (μmol L <sup>-1</sup> )	0.63 $\pm$ 0.08	0.19 $\pm$ 0.05	0.26 $\pm$ 0.13	0.19 $\pm$ 0.01	0.42 $\pm$ 0.23
NH <sub>4</sub> <sup>+</sup> (μmol L <sup>-1</sup> )	0.08 $\pm$ 0.06	0.23 $\pm$ 0.09	0.27 $\pm$ 0.12	0.34 $\pm$ 0.12	0.23 $\pm$ 0.14
DOC (μmol L <sup>-1</sup> )	nd	81.15 $\pm$ 5.49	96.89 $\pm$ 10.78	95.70 $\pm$ 8.38	112.82 $\pm$ 14.41
DIC (μmol L <sup>-1</sup> )	2117.7 $\pm$ 39.4	2092.4 $\pm$ 42.9	2251.1 $\pm$ 132.2	2282.5 $\pm$ 112.0	2315.1 $\pm$ 168.1
POC (μmol L <sup>-1</sup> )	9.28 $\pm$ 1.47	11.15 $\pm$ 1.97	24.07 $\pm$ 5.31	29.37 $\pm$ 7.03	38.11 $\pm$ 10.43
PON (μmol L <sup>-1</sup> )	1.24 $\pm$ 0.46	1.21 $\pm$ 0.22	1.64 $\pm$ 0.22	1.98 $\pm$ 0.35	2.43 $\pm$ 0.52
<i>Calculated</i>					
pCO <sub>2</sub> (μatm)	353.42 $\pm$ 7.24	467.76 $\pm$ 112.03	1402.92 $\pm$ 896.42	1047.06 $\pm$ 599.07	1318.95 $\pm$ 819.79
CO <sub>3</sub> <sup>2-</sup> (μmol kg <sup>-1</sup> )	211.92 $\pm$ 9.79	176.80 $\pm$ 30.05	105.84 $\pm$ 46.46	115.57 $\pm$ 46.18	109.02 $\pm$ 45.59
Ω <sub>aragonite</sub>	3.35 $\pm$ 0.16	2.80 $\pm$ 0.49	1.68 $\pm$ 0.73	1.84 $\pm$ 0.73	1.72 $\pm$ 0.71
TOC (μmol L <sup>-1</sup> )	nd	93.22 $\pm$ 6.18	121.63 $\pm$ 14.07	125.07 $\pm$ 13.32	144.72 $\pm$ 17.5
DIN (μmol L <sup>-1</sup> )	0.29 $\pm$ 0.07	0.74 $\pm$ 0.24	0.85 $\pm$ 0.33	0.88 $\pm$ 0.12	0.77 $\pm$ 0.24
DIC:DIN	7371.9 $\pm$ 1940.8	3132.9 $\pm$ 999.1	4068.5 $\pm$ 4621.8	2625.3 $\pm$ 318.8	3276.9 $\pm$ 1031.0
POC:PON	8.10 $\pm$ 2.41	9.26 $\pm$ 1.07	14.64 $\pm$ 2.04	14.70 $\pm$ 1.42	15.55 $\pm$ 1.33
POC:DOC	nd	0.15 $\pm$ 0.02	0.26 $\pm$ 0.06	0.31 $\pm$ 0.06	0.28 $\pm$ 0.05
DOC:TOC	nd	0.87 $\pm$ 0.01	0.80 $\pm$ 0.03	0.77 $\pm$ 0.02	0.78 $\pm$ 0.03
Si(OH) <sub>4</sub> :DIN	2.43 $\pm$ 1.31	4.14 $\pm$ 2.18	12.17 $\pm$ 8.99	9.90 $\pm$ 2.10	16.34 $\pm$ 6.38
DIN:DIP	0.47 $\pm$ 0.12	4.22 $\pm$ 1.28	nd	4.65 $\pm$ 0.52	2.78 $\pm$ 1.21
Si(OH) <sub>4</sub> :DIP	1.11 $\pm$ 0.63	15.11 $\pm$ 6.36	37.01 $\pm$ 17.16	46.79 $\pm$ 14.23	37.51 $\pm$ 24.46

Most of the chemical parameters were, in general, more concentrated (up to 5-times) in the Bouraké lagoon than at the reference reefs R1 and R2, and increased from the outer to the inner reef (**Figure 2.9a-d; Table 2.1, Table 2.2; Appendix A -Figure A2.2**). In addition, despite the Bouraké lagoon receives new seawater during the rising tide, only Si(OH)<sub>4</sub> had a decent regression coefficient showing either positive and negative correlation with the falling and the rising tide (**Figure 2.9e-h; Appendix A - Figure A2.2; Table A2.3**). It reached the highest value of 16.74 μmol L<sup>-1</sup> during low tide on the inner reef with an average of 11.93  $\pm$  4.27 μmol L<sup>-1</sup>, and the lowest value of 3.38 μmol L<sup>-1</sup> during high tide on the outer reef with an average of 8.22  $\pm$  3.14 μmol L<sup>-1</sup>, 3- to 6-time higher than at the reference stations R2 and R1. The lack of a clear effect of the tide for the other chemicals causes their accumulation inside the lagoon system. Ratios of organic and inorganic carbon, nitrogen, and nutrients showed significant differences between the Bouraké lagoon and the reference reefs (**Table 2.1, Table 2.2**) with higher average POC:PON, POC:DOC, Si(OH)<sub>4</sub>:DIN, Si(OH)<sub>4</sub>:DIP and DIN:DIP (PO<sub>4</sub><sup>3-</sup>), and lower average DIC:DIN and DOC:TOC in the Bouraké lagoon compared to reference reefs.

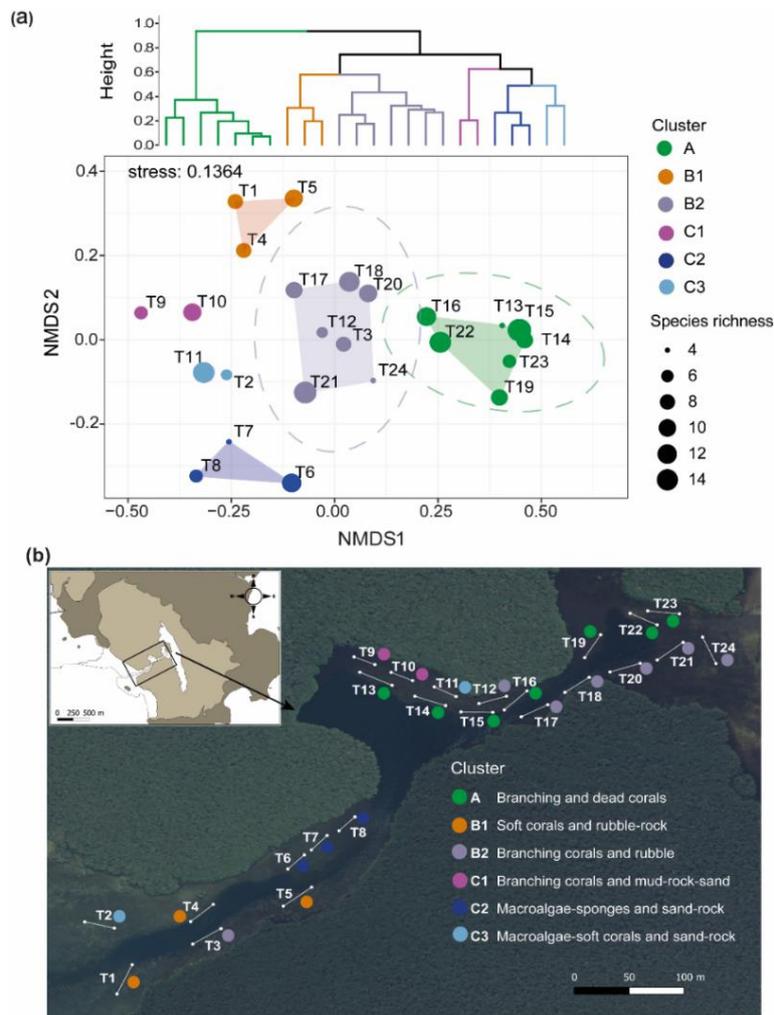


**Figure 2.9.** Orthosilicic acid, organic carbon and nitrogen parameters measured during diel cycles in 2017 and 2019 (pooled data, see also Appendix A Figure A2.2.) at R1, R2 and the Bouraké reefs (outer, middle and inner) (a-d). Boxes ( $n = 6-14$ ) represent the interquartile range (25th and 75th percentile); the horizontal line is the median, and the whiskers represent the data range (i.e., minimum and maximum). Changes are illustrated across a 12 h tidal phase in Bouraké (e-h). Linear regression lines are plotted for each falling (HT to LT) and rising tide (LT to HT). Shaded boxes have only a graphical significance and only suggest the tide changes during measurements.

### 2.3.3 Benthic community distribution and species identification

**Benthic community distribution.** The cluster analysis and nMDS allowed grouping the 24 transects in the Bouraké lagoon in six clusters corresponding to habitat descriptors, namely clusters A, B1, B2,

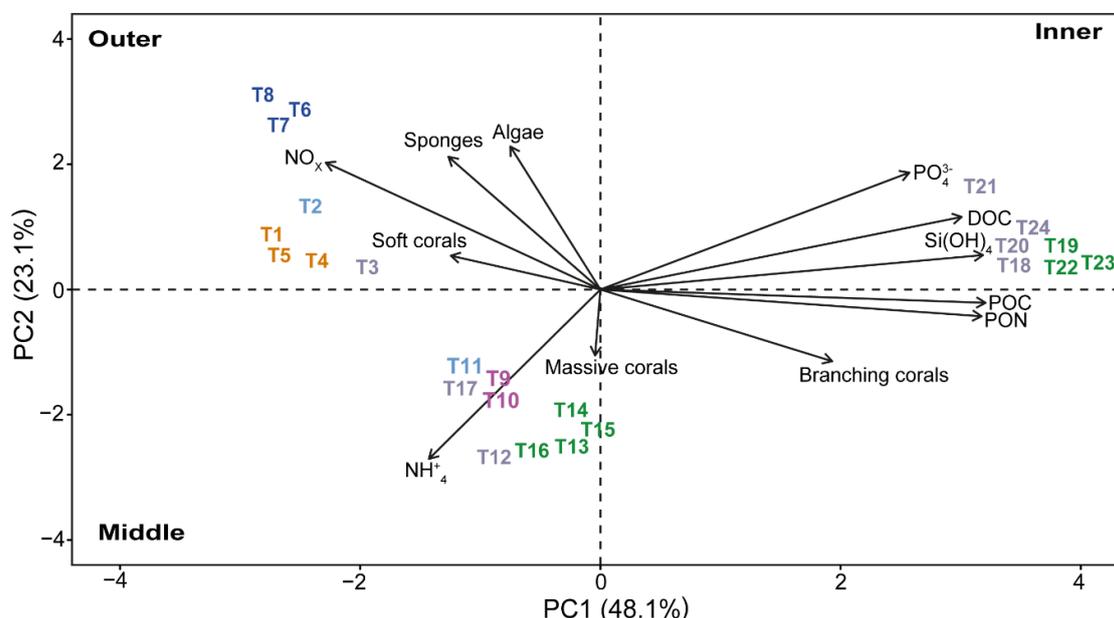
C1, C2, and C3 (**Figure 2.10a**; **Appendix A - Figure A2.3** for details of the community descriptors, and **Table A2.4** for transect averaged data). The nMDS produced an exhaustive representation of the Bouraké lagoon benthic communities and abiotic features (2-dimensional stress = 0.136) confirmed by ANOSIM (Global  $R = 0.948$ ,  $p = 0.001$ ). For the benthic community distribution, a clear separation (95% confidence interval represented by the ellipse in **Figure 2.10a**) was found for cluster A and B2, including most transects located on the middle and inner reefs (**Figure 2.10b**). For both, branching corals were the dominant biotic descriptor with an average of 81% and 31% coverage, respectively (**Appendix A - Figure A2.3**), with a maximum of 96% in cluster A (T23, **Appendix A - Table A2.4**). Among branching corals, the two most abundant genera were *Acropora* and *Montipora*. For the abiotic features of the substrate, dead corals and rubbles characterized transects in clusters A and B2, respectively. Species richness (**Figure 2.10a**) was high in all transects of the Bouraké lagoon, except T13 and T24, both found at the middle and inner reefs' limits. The abiotic substrate of cluster C1 was characterized by 66% mud, 12% rocks and 10% sand (**Appendix A - Table A2.4** for detailed cover data per transect). Only a few branching corals (< 10%) were found, but species richness was relatively high (6 at T9 and 10 at T10). Cluster C2 was distinct, which is not surprising due to its location in a relatively shallow convergence zone that divides the lagoon into two parts. There, the substrate is made of coarse sand (13%) and rocks (12%) and is mainly colonized by macroalgae and sponges (31% and 32%, respectively). Species richness in the area was heterogeneous and ranged from 4 to 12. *Dictyota* spp. and *Halimeda discoidea* were the main macroalgal species, while *Rhabdastrella globostellata* was the dominant sponge species in the area. Cluster B1, located on the outer reef, is characterized by an abundance of soft corals (48%) and rubbles (21%), and high biological richness (**Figure 2.10a**).



**Figure 2.10.** Benthic community and bottom substrate characterization in the Bouraké lagoon. Hierarchical Ward's method cluster dendrogram (top) and non-metric multidimensional scaling (nMDS) of abiotic and biotic percent cover and species richness per transect (T1-T24) (a). Colored circles represent clusters of transects identified in the dendrogram; circle size corresponds to species richness. Ellipses represent 95% confidence intervals. Dominant biotic and abiotic descriptors were used to describe the benthic community and bottom substrate for each cluster in (b). The satellite image is from georep New Caledonia database (<https://georep.nc/>), and the QGIS software was used for transect georeferencing.

Cluster C3 is characterized by coarse sand (49%), rocks (17%) and a few benthic organisms such as macroalgae (10%) and soft corals (8%). Principal component analysis (PCA) reduced the multicollinearity problem and the first two Principal Components accounted for a cumulative 71.2% of the dataset variance. In particular, the PCA plot (Figure 2.11) allowed to assign the transects to three distinct groups depending on their position in the study area. The distinction among transects groups in the biplot is clear and they are grouped based on their location in the outer, middle or inner lagoon reefs. The outer reef sites are characterized by sponges, macroalgae and soft corals, which appear to correlate with NO<sub>x</sub>. Conversely, branching corals are the shared and characteristic feature

of the middle and inner reef sites. However, these two reef groups differ in their major correlation to  $\text{NH}_4^+$  for middle reef sites and POC, DOC,  $\text{Si}(\text{OH})_4$ , and  $\text{PO}_4^{3-}$  for the inner sites.

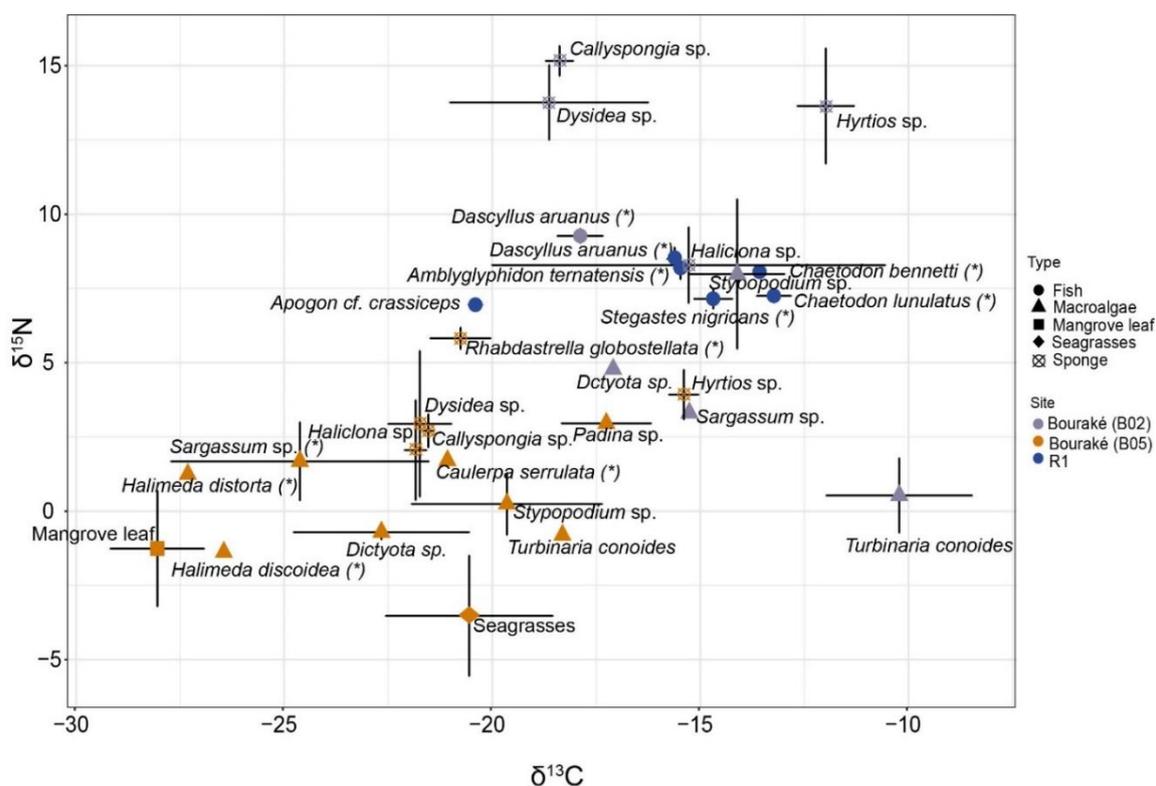


**Figure 2.11.** Principal component analysis (PCA) between benthic community (macroalgae, sponges, corals, branching corals and massive corals) data and environmental parameters ( $\text{PON}$ ,  $\text{POC}$ ,  $\text{DOC}$ ,  $\text{Si}(\text{OH})_4$ ,  $\text{PO}_4^{3-}$ ,  $\text{NO}_x$ , and  $\text{NH}_4^+$ ) measured for each transect ( $n = 24$ ). The length of the vectors indicates the contribution of each parameter. The first dimension explains 48.1% of the variance, and the second dimension explains 23.1% of the variance. Colours refer to the cluster subdivision (**Figure 2.10**).

**Species identification.** A total of 66 coral species were identified from the Bouraké lagoon (**Appendix A - Table A2.5**). Most of the species belong to the family Acroporidae (17 *Acropora* species, 2 *Anacropora* and 4 *Montipora*) and Merulinidae (15 species). In total, 58 species were identified from the reference reef R2, 29 of which were also found in the Bouraké lagoon. Overall, the hard coral fauna within the lagoon was characterized by species commonly found around the Grande Terre, with some of them typically found in turbid coastal environments such as *Pseudosiderastrea tayamai* and *Heliofungia actiniformis*. They were not encountered at the reference site. Remarkably, two New Caledonia endemic coral species, *Cantharellus noumeae* and *Polycyathus fulvus*, both described from coastal habitats characterized by terrigenous sediment inputs (Noumea lagoon and Prony Bay, respectively), were common in the innermost reef of the Bouraké lagoon. The Bouraké lagoon also had 28 species of macroalgae (14 Phaeophyceae, 9 Chlorophyta and 5 Rhodophyta), including the crustose coralline algae *Lithothamnium* sp., *Lithophyllum* sp., and *Hydrolithon reinboldii*, and 11 sponge species belonging to eight families of the class Demospongiae (**Appendix A - Tables A2.5; A2.6**), which were found mainly in the coral matrix and sediment.

### 2.3.4 Stable isotope signatures

Mean  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values were significantly different between Bouraké outer (B02) and Bouraké inner (B05) considering pooled macroalgae species ( $\delta^{13}\text{C}$ :  $W = 1, p < 0.01$ ;  $\delta^{15}\text{N}$ :  $W = 6, p < 0.05$ ) and pooled sponges species ( $\delta^{13}\text{C}$ :  $W = 10, p < 0.05$ ;  $\delta^{15}\text{N}$ :  $W = 0, p < 0.001$ ). Samples from Bouraké (B05) showed  $^{13}\text{C}$ -depleted and  $^{15}\text{N}$ -depleted values and were mostly plotted in the bottom left of the plot, while sample from Bouraké (B02) showed  $^{13}\text{C}$ -enriched and  $^{15}\text{N}$ -enriched and were mostly plotted in the top right of the plot (**Figure 2.12**).



**Figure 2.12.** Plot of  $\delta^{13}\text{C}$  versus  $\delta^{15}\text{N}$  (mean  $\pm$  SD when more than 1 sample) of macroalgae, sponges, fish, mangrove leaf and seagrasses collected at Bouraké outer (B02), Bouraké inner (B05), and Reference site (R1). Label with \* refer to sample collected in March 2019, while the other were collected in December 2020.

For fish, the  $\delta^{13}\text{C}$  values ranged from  $-20.37 \pm 0.17$  ‰ to  $-13.20 \pm 0.42$  ‰, while  $\delta^{15}\text{N}$  values ranged from  $6.95 \pm 0.14$  ‰ to  $8.53 \pm 0.35$  ‰ depending on species. For *Dascyllus aruanus*, differences between R1 and B02 were relatively moderate (**Table 2.3, Figure 2.12**) without statistical significant differences ( $\delta^{13}\text{C}$ :  $W = 6, p = 0.2$ ;  $\delta^{15}\text{N}$ :  $W = 0, p = 0.2$ ). Considering all sponge species the  $\delta^{13}\text{C}$  signature ranged from  $-18.70 \pm 2.39$  ‰ to  $-11.95 \pm 0.70$  ‰ at Bouraké (B02), while from  $-21.81 \pm 0.26$  ‰ to  $-15.36 \pm 0.36$  ‰ at Bouraké (B05), while sponge  $\delta^{15}\text{N}$  signature ranged from  $8.29 \pm 1.28$  ‰ to  $15.16 \pm 0.52$  ‰ at Bouraké (B02), while from  $2.01 \pm 1.69$  ‰ to  $3.93 \pm 0.84$  ‰ at Bouraké (B05)

(Table 2.3). The  $\delta^{15}\text{N}$  signature of *Hyrtios* sp. *Callyspongia* sp. and *Dysidea* sp., at B02 showed also higher values compared to fish in the same zone and at reference reef R1 (Table 2.3). Macroalgae  $\delta^{13}\text{C}$  signature ranged from  $-17.03\text{‰}$  to  $-10.19 \pm 1.76\text{‰}$  at Bouraké (B02), while from  $-22.63 \pm 2.11\text{‰}$  to  $-17.22 \pm 1.08\text{‰}$  at Bouraké (B05), while Macroalgae  $\delta^{15}\text{N}$  signature ranged from  $0.53 \pm 1.26\text{‰}$  to  $7.98 \pm 2.53\text{‰}$  at Bouraké (B02), while from  $-0.49\text{‰}$  to  $3.10\text{‰}$  at Bouraké (B05) (Table 2.3). Mangrove leafs showed the lowest  $\delta^{13}\text{C}$  values with  $-28.02 \pm 1.13\text{‰}$  (Table 2.3, Figure 2.12).

**Table 2.3.** Summary of isotopic signature (mean  $\pm$  SD, when more than 1 sample) in carbon ( $\delta^{13}\text{C}$  ‰) and nitrogen ( $\delta^{15}\text{N}$  ‰) for macroalgae, fish, sponges, mangrove leaf and seagrasses collected in March 2019 (Mar-19) and December 2020 (Dec-20) at R1, Bouraké outer (B02), and Bouraké inner (B05).

Year	Site	Type	Genus/Species	$\delta^{15}\text{N}$ ‰	$\delta^{13}\text{C}$ ‰
Mar-19	R1	Fish	<i>Stegastes nigricans</i> (n = 5)	$7.15 \pm 0.33$	$-14.66 \pm 0.46$
Mar-19	R1	Fish	<i>Amblyglyphidon ternatensis</i> (n = 2)	$8.19 \pm 0.39$	$-15.44 \pm 0.17$
Mar-19	R1	Fish	<i>Chaetodon lunulatus</i> (n = 3)	$7.25 \pm 0.23$	$-13.20 \pm 0.42$
Mar-19	R1	Fish	<i>Dascyllus aruanus</i> (n = 2)	$8.53 \pm 0.35$	$-15.58 \pm 0.12$
Mar-19	B02	Fish	<i>Dascyllus aruanus</i> (n = 3)	$7.25 \pm 0.23$	$-13.20 \pm 0.42$
Mar-19	R1	Fish	<i>Apogon</i> cf. <i>crassiceps</i> (n = 3)	$6.95 \pm 0.14$	$-20.37 \pm 0.17$
Mar-19	R1	Fish	<i>Chaetodon bennetti</i> (n = 1)	8.06	-13.54
Mar-19	B05	Sponge	<i>Rhabdastrella globostellata</i> (n = 3)	$5.28 \pm 0.37$	$-20.73 \pm 0.73$
Mar-19	B05	Macroalgae	<i>Caulerpa serrulata</i> (n = 1)	1.70	-21.04
Mar-19	B05	Macroalgae	<i>Sargassum</i> sp. (n = 2)	$0.97 \pm 0.70$	$-26.37 \pm 0.58$
Mar-19	B05	Macroalgae	<i>Stypopodium</i> sp. (n = 1)	0.98	-21.24
Mar-19	B05	Macroalgae	<i>Halimeda discoidea</i> (n = 1)	-1.38	-26.42
Mar-19	B05	Macroalgae	<i>Halimeda distorta</i> (n = 1)	1.24	-27.28
Dec-20	B02	Sponge	<i>Hyrtios</i> sp. (n = 2)	$13.64 \pm 1.96$	$-11.95 \pm 0.70$
Dec-20	B02	Sponge	<i>Callyspongia</i> sp. (n = 2)	$15.16 \pm 0.52$	$-18.35 \pm 0.35$
Dec-20	B02	Sponge	<i>Haliclona</i> sp. (n = 2)	$8.29 \pm 1.28$	$-15.25 \pm 4.73$
Dec-20	B02	Sponge	<i>Dysidea</i> sp. (n = 2)	$13.76 \pm 1.26$	$-18.70 \pm 2.39$
Dec-20	B05	Sponge	<i>Hyrtios</i> sp. (n = 2)	$3.93 \pm 0.84$	$-15.36 \pm 0.36$
Dec-20	B05	Sponge	<i>Callyspongia</i> sp. (n = 3)	$2.70 \pm 0.56$	$-21.50 \pm 0.14$
Dec-20	B05	Sponge	<i>Haliclona</i> sp. (n = 2)	$2.01 \pm 1.69$	$-21.81 \pm 0.26$
Dec-20	B05	Sponge	<i>Dysidea</i> sp. (n = 2)	$2.94 \pm 2.46$	$-21.71 \pm 0.77$
Dec-20	B02	Macroalgae	<i>Stypopodium</i> sp. (n = 2)	$7.98 \pm 2.53$	$-14.08 \pm 1.15$
Dec-20	B02	Macroalgae	<i>Turbinaria conoides</i> (n = 2)	$0.53 \pm 1.26$	$-10.19 \pm 1.76$
Dec-20	B02	Macroalgae	<i>Sargassum</i> sp. (n = 1)	3.31	-15.23
Dec-20	B02	Macroalgae	<i>Dictyota</i> sp. (n = 1)	4.78	-17.06
Dec-20	B05	Macroalgae	<i>Stypopodium</i> sp. (n = 1)	3.10	-21.04
Dec-20	B05	Macroalgae	<i>Turbinaria conoides</i> (n = 1)	-0.81	-18.28
Dec-20	B05	Macroalgae	<i>Sargassum</i> sp. (n = 1)	-0.49	-17.99
Dec-20	B05	Macroalgae	<i>Dictyota</i> sp. (n = 3)	$-0.71 \pm 0.25$	$-22.63 \pm 2.11$
Dec-20	B05	Macroalgae	<i>Padina</i> sp. (n = 2)	$2.95 \pm 0.11$	$-17.22 \pm 1.08$
Dec-20	B05	Mangrove leaf	Mangrove leaf (n = 4)	$-1.26 \pm 1.95$	$-28.02 \pm 1.13$
Dec-20	B05	Plant	Seagrass (n = 2)	$-4.61 \pm 1.07$	$-21.67 \pm 0.39$

## 2.4 Discussion

### 2.4.1 Physical and chemical characteristics of the Bouraké lagoon

The Bouraké lagoon covers an estimated area of *ca.* 20 ha that penetrates a mangrove forest, large enough to assess the combined effects of extreme environmental conditions on an established coral reef ecosystem. The multi-scale approach confirmed previous findings (Camp et al., 2017), showing that the Bouraké lagoon is hottest (ranging from 17.5 to 33.8°C), deoxygenated (ranging from 1.87 to 7.24 mg L<sup>-1</sup>), and acid (ranging from 7.23 to 7.92 pH<sub>T</sub> units) when compared to neighboring reefs. Besides, salinity was significantly higher than at the reference reefs during both winter and summer (with maxima of 37.22 and 39.37, respectively).

Several marked differences in the environmental conditions between the Bouraké lagoon and the reference reefs, both in the absolute range, and in the variability of the measured environmental parameters. First, the seawater temperature is higher in summer in the Bouraké lagoon (**Figure 2.3**), but it is also colder during winter, resulting in an annual temperature range of 17.5-33.8°C. We compared temperatures recorded at Bouraké lagoon to those of the reference St R2, which showed the most typical temperature range for shallow water temperatures in the south of New Caledonia (i.e., 22-28°C, Varillon et al., 2021). We notice that in Bouraké, temperatures were 40% of the time above 28°C during the summer of 2020, while winter temperatures were on average 46.5% of the time lower than 22°C. While warming is considered the main threat for coral reefs, low temperatures (< 20°C) can cause coral bleaching by inducing responses similar to high temperatures, including a reduction in the *Symbiodiniaceae* cell density and chlorophyll *a* content (e.g., Saxby et al., 2003; Hoegh-Guldberg and Fine, 2004; Hoegh-Guldberg et al., 2005; Kemp et al., 2011; Bellworthy and Fine, 2021). The negative effect of cold temperatures is even more substantial during neap tides when colonies on the reef crest are exposed to air for hours at low temperatures during cold winters. For example, Porter et al. (1982) and Davis (1982) reported > 90% coral mortality in shallow (< 2 m) reefs of the Dry Tortugas following the winter of 1976-1977 when temperatures reached 14°C. This is consistent with observations (data not shown) during the cold winter of 2019 when the upper 12-20 cm of several massive and branching corals had died.

Second, in the Bouraké lagoon, benthic assemblages are continuously exposed to large fluctuations in the main environmental parameters, toward sub-optimal values. Some of these environmental fluctuations are entirely predictable. For instance, marine organisms are exposed to a temperature fluctuation of about twice the reference reefs' amplitude (up to 6.5°C vs 3.5°C) in a single day. Dissolved oxygen fluctuations were similar between stations but in a significantly different range: 3.7 to 6.8 mg L<sup>-1</sup> at B2 and 5.4 to 7.8 mg L<sup>-1</sup> at the reference stations. According to the organisms

tolerance to DO fluctuation, which is quite unknown in corals, low DO concentrations can change fish tidal migration in the mangrove (Dubuc et al., 2019a). Besides, tidal modelling revealed that, at the reference reefs, pH, DO (**Figure 2.4**), and temperatures slightly increased in the afternoon and decreased during the night. This finding agrees with what should be expected from reef metabolic activities and daily cycles but, in the Bouraké lagoon, these parameters, including salinity (**Figure 2.7**), are entirely driven by tides. Here, seawater pH and DO varied between extremely low values at low tide and close-to-normal values during high tide (see also **Figure 2.8a-c** for pH). Finally, the timing of the tide was out of phase between sites, with a delay of about 45 minutes at high tide and 1.5 h at low tide in the Bouraké lagoon (**Figure 2.2**).

The unique environmental conditions measured in the Bouraké lagoon are linked to its unique topographical and geomorphological characteristics, the resulting water circulation and the direction of the tide. New water from the lagoon enters through the channel at each rising tide and flows into the semi-enclosed lagoon towards the large mangrove area behind it. This water initially had ambient values of pH, temperature and DO, but, during the trip, it mixes with the acidic, warm and deoxygenated water in the system and the mangrove area, therefore gradually changing from its original values. Inside the mangrove forest, we hypothesize that the water chemistry further changes due to the metabolic reactions in the sediments, and mangrove roots (e.g., Alongi et al., 2004; Bouillon et al., 2007; Gleeson et al., 2013; Call et al., 2015). Conversely, on a falling tide, the seawater becomes gradually more acidic, hot and oxygen-depleted because the water that resided in the mangrove area gradually drains out of the system. This takes about 6 h, during which the vast reservoir of shallow mangrove water continues to be chemically altered, becoming increasingly acidic, oxygen-depleted and hot. As a result, significant spatial differences in pH between the outer reef (the entry of the lagoon) and the inner reef (near the mangrove forest), as well as a considerable delay in the synchronization of the tidal shift were measured (**Figure 2.5b**). Interestingly, because the volume of seawater discharged in 6 h is so large, it affects also the area outside the system where we measured similar seawater conditions as inside, even if it mixes with the main lagoon's water (see **Figure 2.5c, St U vs St T**). It means that the area (and the organisms) affected by the suboptimal parameters is larger than previously thought. The species living in this area have likely developed specific mechanisms to withstand the very fluctuating environmental conditions, and as such, they warrant further attention. Since the fluctuations are linked to tidal phases, it could be argued that organisms living in the Bouraké lagoon may benefit from periods of normal conditions at high tide, during which they can recover from the stress they have experienced at low tide (e.g., Rivest et al., 2017). While this could be partially the case for species living on the outer reef, close to the main

lagoon, the pH and DO inside the Bouraké lagoon rarely reach normal values, and persist longer as the low tide is delayed by 1.5 h compared to the reference reef (**Figure 2.2**).

Preliminary results from a hydrodynamic model of the study site suggest that tide-associated water mass movements are spatially heterogeneous and likely to play an essential role in shaping coral resilience to extreme conditions (see discussion below). Indeed, one can imagine a single water mass moving with the same physical characteristics from the mangrove area towards the outer reefs or in the opposite direction depending on the tide. However, the complex geomorphology of the Bouraké lagoon, its bottom topography and the complex web of coral reefs and mangrove trees on the edges deviate and probably change the seawater physical and chemical properties. Significant spatial differences in pH within each reef area (inner, middle and outer reefs; **Figure 2.5a, b**), as well as throughout the water column were measured (i.e., between the surface and the bottom; **Figure 2.5d, e**). In general, bottom seawater was 0.1-0.2 pH<sub>T</sub> units lower than the surface, probably due to a pumping mechanism by the water mass of more acidic pore water from the sediments. The pH also differed spatially within the inner reef, by up to 0.3 pH<sub>T</sub> units (for instance St. A vs St. C; **Figure 2.5a**), perhaps due to the influence of stagnant water on the mangrove edges or a more intense metabolic activity by the local benthic community. Dissolved oxygen concentrations between the surface and the bottom were only significantly different at St. N on the middle reef (**Figure 2.6f**). This station is mainly characterized by mud as the current is lower than anywhere else in the Bouraké lagoon. Here, the sediment's biological activity possibly traps the oxygen making it less available to the water column.

Throughout the Bouraké lagoon, organisms are exposed to extreme and fluctuating suboptimal physical parameters, such as pH, and DO which are more pronounced on the bottom and last longer, and with more extreme values, on the inner reefs. It is also true for some of the seawater chemical parameters measured, which show higher concentrations in the Bouraké lagoon than on the reference reefs (see **Table 2.2**). For instance, orthosilicic acid, phosphate, dissolved and particulate organic carbon, and particulate organic nitrogen were 1.4 to 4.2-fold more concentrated on the inner reef than at station R2. Within the Bouraké lagoon in general, chemicals increased from the outer to the inner reef, and except for [NO<sub>x</sub>], [NH<sub>4</sub>]<sup>+</sup>, [PO<sub>4</sub>]<sup>3-</sup>, they never return to “normal” values despite the Bouraké system receives new seawater during the rising tide. The high concentrations in orthosilicic acid and organic compounds, both dissolved and particulate are due to the combined effect of more acidic and organic-rich water coming out of the mangrove forest during a falling tide (**Figure 2.9**) and the system's particular morphology, limiting the complete renewal of the seawater, especially at the inner reefs.

#### **2.4.2 Stable carbon and nitrogen isotope signature of the Bouraké lagoon**

Light intensity, temperature or nutrient concentrations (and their incorporation rates) may change productivity rates of primary producers and, therefore, their  $\delta^{13}\text{C}$  signatures. At the same time, variation in ammonium and nitrate concentrations in seawater can modify  $\delta^{15}\text{N}$  signatures of the organic matter sources (Hemminga and Mateo, 1996, Briand et al., 2015; Fey et al., 2021). Similarly, spatial fluctuation on isotopic signatures of sponges and macroalgae in the Bouraké lagoon, between the outer zone (B02) and the inner zone (B05) was found. In the specific, sponges and macroalgae in the outer zone of the Bouraké lagoon were  $^{15}\text{N}$ -enriched and  $^{13}\text{C}$ -enriched compared to the inner zone. This trend could be due to recycling of N between the host and the symbionts (e.g., Weisz et al., 2007; Southwell et al., 2008; Mohamed et al., 2010), utilization of other nitrogen sources (i.e., bacteria, phytoplankton, Fey et al., 2021), or higher capacity to take up organic N from POM (Sitnikova et al., 2016). Differences between inner and outer in Bouraké could also be explained by the different concentration of nutrients in the site. Although with not significant differences,  $[\text{NO}_x]$  and  $[\text{NH}_4]^+$  were more concentrated in the outer part of the lagoon, while organic matter such as DOC and POC were significantly more concentrated in the inner part of the lagoon. This suggest that the inner zone was more influenced by terrestrial and mangroves inputs, while the outer zone were more influenced by marine sources. This hypothesis is supported by a recent study showing higher enrichment in  $^{15}\text{N}$  for macroalgae, which incorporate more marine nitrogen sources depending on seasonal variations in nitrogen stock characteristics (Fey et al., 2021). Although, no spatial and/or temporal differences were found in invertebrate and fish between estuary and mouth of the mangrove forest at Foa (New Caleodnia) (Letourneur et al., 2018), this result strengthen with study showing strong spatial variability in  $\delta^{13}\text{C}$  (higher) and  $\delta^{15}\text{N}$  (lower) in intermediate reefs in New Caledonia (Briand et al., 2015). Furthermore, in agreement with the results of this chapter, a gradient in C and N isotopic values was also found from the entrance to the end of two bay in French Polynesia, where lower  $\delta^{13}\text{C}$  values and higher  $\delta^{15}\text{N}$  values were measured at the end of each bay, compared with the entrance for POM and algae turf (Letourneur et al., 2013). Similarly, macroalgae in the inner zone of the Bouraké lagoon incorporated dissolved nutrients of terrestrial and mangrove origin, and sponges filtered mainly detritus and enriched seawater carbon. This mangrove inputs could also confirm by the stable signature of mangrove leaf in Bouraké lagoon, which are  $^{13}\text{C}$  depleted and  $^{15}\text{N}$  depleted. Although stable isotopes should be used with caution in this study due to the moderate sampling effort, they provided a first global assessment of OM sources and how various organisms in such an extreme coral reefs ecosystem could incorporate this OM.

### 2.4.3 Effects of physical and chemical conditions on species distribution

Extreme environmental conditions, such as those measured in the Bouraké lagoon, are known to strongly affect the metabolism, growth and even survival of several marine organisms, particularly those tolerating only a narrow range of environmental changes such as scleractinian corals (e.g., Coles and Jokiel, 1977; Hoegh-Guldberg and Smith, 1989; Hoegh-Guldberg, 1999; Fitt et al., 2001). For instance, *in-situ* studies at volcanic CO<sub>2</sub> seeps have shown that chronic exposure to ocean acidification (near-future *p*CO<sub>2</sub> levels) can cause a reduction in coral diversity and lower the recruitment and abundances of structurally complex hermatypic corals. Moreover, shifts in competitive interactions between taxa and a decrease in cover and richness of soft corals and sponges were also observed (e.g., Fabricius et al., 2011; Enochs et al., 2015; Sunday et al., 2017; Agostini et al., 2018). However, the Bouraké lagoon features high coral, sponge, macroalgae and CCA cover and species richness, adding at the check-list proposed by Camp et al. (2017) an additional 46 coral species (totaling 66 species and 33 genera), 28 species of macroalgae and 11 species of sponges (**Appendix A-Table A2.5**). Such a high number of species has never been reported for mangrove systems characterized by marginal conditions, at least concerning the three major benthic groups taxa investigated here (e.g., Yates et al., 2014; Camp et al., 2019).

In general, given the extensive literature demonstrating the negative effects of suboptimal abiotic conditions on corals, these findings are unexpected and suggest that corals in the Bouraké lagoon may have developed unique survival and adaptive mechanisms. Some studies have reported similar findings, but none has ever reported such high coral species diversity (e.g., Yates et al., 2014; Schoepf et al., 2015; Shamberger et al., 2018; Camp et al., 2019). For instance, in Palau (Micronesia), the highest coral cover (> 60%) and species diversity (21 scleractinian genera) were found at the lowest pH study site of the Nikko Bay station (Barkley et al., 2015). Studies in the Virgin Islands' mangrove system have reported over 30 coral species growing in marginal conditions (Yates et al., 2014; Rogers, 2017). Similarly, in a recent study of two Australian mangrove lagoons, characterized by relatively extreme environmental conditions (low pH, low oxygen and high temperature), Camp et al., (2019) identified 12 and 29 scleractinian coral species in the Woody Isles and at Howick Island, respectively. Among the 66 species identified, 29 of the most abundant coral species in New Caledonia were found at both the reference reef and in the Bouraké lagoon, indicating that species living at our study site are not very different from a typical bay-sheltered fringing reef. There is not an apparent selection in the coral form since massive (e.g., *Porites* spp.), corymbose (e.g., *Pocillopora digitata*), phaceloid (*Galaxea fascicularis*) and branching (*Acropora* spp.) species are equally abundant, as well as in their thermal and pH tolerance. Further experiments are underway at both the

molecular and phenotypic level to better understand the mechanisms of resilience used by the corals from Bouraké.

Remarkably, the two coral species, currently considered New Caledonia endemics, thrive in the innermost benthic assemblages of the Bouraké lagoon, making it not only a unique natural laboratory as well as a potential conservation priority site. In the Bouraké lagoon, benthos species richness was very high throughout the system and the distribution of branching and massive corals was spatially heterogeneous (**Figure 2.10b**; **Appendix A - Figure A2.3**). Coral cover decreased near the mangrove, where the accumulation of fine sediments and exposure to air during low tide do not allow corals' survival (**Appendix A - Figure A2.3**). Branching corals were particularly abundant on the inner and middle reefs, with the highest cover of 96% in the inner reef (T23, **Appendix A - Table A2.4**). They became sparse on the outer reef, even absent at the system's entrance where soft and massive corals dominated. Macroalgae and sponges, including CCA, were quite cryptic throughout the Bouraké lagoon but found almost everywhere in the coral matrix and buried in the sediment. They are particularly abundant in a shallow area that divides the lagoon into two parts (transects T6-T8). There, a Venturi effect generates a strong current, and the substrate consists of coarse sand and rocks. The high flow and the bottom characteristics are both likely to promote macroalgae and sponge occurrences (averaged 30% and 32% cover, respectively). The sponge *Rhabdastrella globostellata* is particularly abundant (up to 40% cover) and forms massive banks embedding adjacent coral colonies. In general, macroalgae and sponges diversity was not particularly rich, with only 28 and 11 species, respectively, likely due to the sampling method. However, in the Indo-pacific mangrove systems, sponge diversity is, in general, not extremely high, especially if compared to the Caribbean (Nagelkerken et al., 2008). Few studies are available from Indo-Pacific mangrove sponges. The highest diversity was reported in a study in the mangrove of Banka Island (North Sulawesi, Indonesia), where 19 species were found (Calcinai et al., 2017). These findings on mangrove sponges in such a unique mangrove area in New Caledonia add to the limited knowledge of sponge species diversity in Pacific mangrove systems.

Water flow may play a critical role in the response of organisms to acidification (Cornwall et al., 2014; Hurd, 2015; Comeau et al., 2019), warming (Schoepf et al., 2018) and deoxygenation (Hughes et al., 2020). It has been reported to affect the diffusion boundary layer (i.e., a thin layer of stagnant water located around aquatic organisms) of corals, CCA and other calcareous macroalgae, altering their ability to calcify at low pH and to excrete metabolic wastes (reviewed in Nelson and Altieri, 2019). Knowing that flow speed could play a critical role and that deserves further investigations and measurements at the study site, two hypotheses can be made to explain the high diversity of the

Bouraké species and their resilience to suboptimal parameters: *i*) the species may benefit from the constant fluctuations of the physical and chemical parameters, *ii*) the species may benefit from heterotrophic inputs of the mangrove (in term of nutrients and organic matter).

Regarding environmental fluctuations, averaged daily variations in temperature, DO, and pH of about 3.79°C, 3.80 mg L<sup>-1</sup>, and 0.63 pH<sub>T</sub> units, respectively. The temperature fluctuated by up to 6.55°C in a single day. Frequent exposure to stressful temperatures can induce acclimatization or adaptation in corals if the period of exposure is short enough to avoid mortality (Oliver and Palumbi, 2011; Palumbi et al., 2014; Schoepf et al., 2015; Rivest et al., 2017; Safaie et al., 2018). For example, in the Kimberly region (Australia), corals regularly exposed to temperatures up to 37°C, with daily variations of up to 7°C, appear less prone to bleaching and grow and calcify at rates comparable to corals in more thermally stable environments (Dandan et al., 2015; Schoepf et al., 2015; 2020). However, they are not immune to extreme heat stress events (Le Nohaïc et al., 2017). Another example is the corals in the back reef and shallow pools around the island of Ofu (American Samoa), which despite occasional daily fluctuations of up to 6°C, and an average daily temperature of 29°C in summer (Piniak and Brown, 2009; Oliver and Palumbi, 2011) sustain reasonable levels of coral cover (25-26%) and high diversity (Craig et al., 2001). Some studies tend to indicate that corals depended on the *a priori* 'frontloading' of genes involved in heat resistance in the coral host and/or the host's ability to adjust its physiology during short-term (weeks) environmental changes (Barshis et al., 2013; Palumbi et al., 2014). However, physiological adjustments have an energetic cost that corals cannot sustain in the long term, affecting other metabolic functions such as calcification. It could explain in part the lower calcification rates observed in some coral species, as seems to be the case for corals in the Bouraké lagoon (e.g., Camp et al., 2017).

There is also evidence that exposure to short-term oscillations in low pH, alone or in combination with high temperature, can mitigate the impact of extreme environmental stressors on corals (Warner et al., 1996; Oliver and Palumbi, 2011; Dufault et al., 2012; Schoepf et al., 2015; Safaie et al., 2018). Species in the Bouraké lagoon appear to be thriving despite the large seawater pH variability (up to 0.6 pH<sub>T</sub> units). In general, species exposed to pCO<sub>2</sub> fluctuations above 500 µatm in their natural environment display enhanced plasticity to future ocean acidification scenarios (Vargas et al., 2017; Carstensen and Duarte, 2019). For example, fluctuations in pH could increase CCA tolerance to ocean acidification by providing respite periods at ambient pH (Rivest et al., 2017), during which organisms can calcify at a standard rate, compensating for decreased calcification during periods of lower pH (Comeau et al., 2013; Cornwall et al., 2018). Diurnal variability in pH, typical of more variable pH habitats, may confer tolerance to resident calcifying species via the selection of individuals better

adapted to survive in these environments (Rivest et al., 2017; Kapsenberg and Cyronak, 2019), although transgenerational acclimatization is required (Cornwall et al., 2020).

In addition to changes in temperature and pH, which are the two most commonly tested environmental parameters, oxygen must also be considered. Despite the impact of reduced oxygen levels and its natural fluctuations on coral reefs has yet to be established, low DO (2-4 mg L<sup>-1</sup>) seems to increase the sensitivity of branching corals, resulting in a decline in coral health, bleaching, and tissue loss (Haas et al., 2014; Hughes et al., 2020; Alderdice et al., 2021). This chapter shows that although the Bouraké system can reach conditions close to hypoxia for several coral species (< 3 mg L<sup>-1</sup>; **Figure 2.4**), these latter seem to have promoted compensation mechanisms that allow them to survive in these conditions. The natural laboratory of Bouraké, where DO fluctuates with the tide, in combination with other environmental stressors, offers a perfect setting to test the practically unknown effects of deoxygenation in reef-building corals exposed to acid and hot conditions (Nelson and Altieri, 2019; Hughes et al., 2020).

Although corals appear to possess cellular mechanisms to counteract short-term osmotic changes (Mayfield and Gates, 2007), high and fluctuating salinity is possible additional stress that corals living in the Bouraké lagoon have to face daily, adding up to the already long list of suboptimal environmental parameters. *In-situ* studies have demonstrated that acute and prolonged decrease in salinity can affect the coral photosynthetic efficiency, resulting in a reduction of the amount of energy transferred to corals (Muthiga and Szmant, 1987; Manzello and Lirman, 2003) and induce coral death (e.g., Jokiel et al., 1993). The response of corals to a change in salinity is related to the strength and duration of the hypo-hypersaline exposure and the species tolerance. For example, *Stylophora pistillata* seems able to acclimate more effectively to hypo- rather than to hyper-saline conditions (Ferrier-Pagès et al., 1999). Further experiments are needed to assess the effect of high and fluctuating salinity on the physiology of corals in Bouraké.

Besides the hypothesis that environmental variability improves the metabolism of organisms, particularly their resilience to extreme conditions, a series of other physical (e.g., current flow) and chemical parameters (e.g., organic matter) in the Bouraké lagoon may work in combination to offset or enhance these effects. Mangrove habitats are highly productive ecosystems and are sites of intense carbon processing, with a high potential impact on the global carbon budget (e.g., Borges et al., 2003; Dittmar et al., 2006; Bouillon et al., 2014). In the Bouraké lagoon, benthic communities might have access to a range of heterotrophic inputs, nutrients, carbon, and nitrogen sources. These sources can be metabolized by the species to increase their energy budget and cope with the suboptimal parameters, but they can also become toxic if too concentrated or depleted, leading to functional

limitations. Exceptionally, high concentrations of organic and inorganic carbon and nitrogen were measured, but also of some nutrients, notably silicates and phosphorus, and we confirmed the potential contribution of the mangrove in those inputs, especially during the falling tide (**Figure 2.8 and Figure 2.9; Table 2.2; Appendix A- Figure A2.1 and Figure A2.2**). Dissolved organic carbon contributes significantly to the TOC pool (POC+DOC), with a concentration increase between the reference sites and the Bouraké lagoon, reaching maximum values on the inner reef. High OM content can increase DOC availability to corals, providing the sustainable energy to withstand extreme environmental conditions (Levas et al., 2015). Some studies showed that the high OM of turbid reefs can support elevated coral heterotrophy that can facilitate energy maintenance during periods of stress (Anthony and Fabricius, 2000).

Nutrients could partially explain the distribution of organisms throughout the Bouraké lagoon (**Figure 2.11**). Indeed,  $\text{NO}_x$  concentrations were higher on the outer reef, dominated by sponges, macroalgae and soft corals, while  $\text{NH}_4^+$  was high on the middle reef and POC, DOC,  $\text{Si(OH)}_4$ , and  $\text{PO}_4^{3-}$  were higher on the inner reef. Both the middle and inner reefs are characterized by the highest branching coral cover. Nitrates can accumulate inside the host cells, possibly favoring sponges and macroalgae, while ammonium is the preferred source used by coral symbionts (Raven et al., 1992). However, the nutrient negative or positive effect on corals' physiology is difficult to demonstrate experimentally (Atkinson et al., 1995; Szmant, 2002; Bongiorni et al., 2003). Van De Waal et al., (2009) have shown that the performance of organisms depends on concentrations of, and ratios between, different nutrients, and between organic and inorganic matter/components and the possible imbalance due to environmental changes. Bouraké lagoon is mostly N-limited ( $\text{DIN:DIP} < 10$  and  $\text{Si(OH)}_4:\text{DIN} > 1$ ), which confirms the findings of Justić et al. (1995) and is similar to the conditions observed in other New Caledonian mangroves (Jacquet et al., 2006). Nutrients limitation has been demonstrated to lower the temperature effect at which coral bleaching occurs (Wiedenmann et al., 2013; Ezzat et al., 2016b; 2019), which contrasts with the resilience of Bouraké corals to the warming in the summer of 2016 (10-20% bleaching only) compared to other reefs in New Caledonia (up to 90% bleaching) (Benzoni et al., 2017). Corals symbionts recycle their host's metabolic wastes and take up dissolved inorganic nitrogen (DIN) and phosphorus (DIP) from seawater (Grover et al., 2003; Pernice et al., 2012; Rosset et al., 2015), both of which are used to produce vital organic molecules. Nutrient starvation can occur when the availability of one type of essential nutrient decreases, resulting in an imbalanced N:P ratio of inorganic nutrients in seawater (Wiedenmann et al., 2013; D'Angelo and Wiedenmann, 2014). The N:P ratio in the Bouraké lagoon was 2.8:1 and 4.6:1 at the middle and the reference reefs, respectively, which is lower than the range calculated for average reef waters of 4.3:1 to 7.2:1 (Crossland et al., 1984, Furnas et al., 1995). Although these data should be considered

cautiously, they suggest that the Bouraké lagoon seawater is not limited in phosphorus, an essential nutrient in coral resilience to bleaching (Ezzat et al., 2016a; Rosset et al., 2017). Seawater in the Bouraké lagoon was not limited in Si ( $\text{Si(OH)}_4\text{:DIP} > 10$  and  $\text{Si(OH)}_4\text{:DIN} > 1$ ; Justić et al., 1995), and Si was six times more abundant on the inner reef than elsewhere in the system (**Figure 2.9; Table 2.2**). Silicates are an important source of nutrition and skeletal construction for primary producers such as sponges. Orthosilicic acid is a biologically available form of silicon that is poorly soluble at a very low pH. In the Bouraké lagoon, pH fluctuates with the tide, and one can expect that orthosilicic acid would rise during the falling tide. This elevated concentration of orthosilicic acid could be involved in the growth and persistence of the Bouraké large banks of *Rhabdastrella globostellata*, as previously reported for other sponges in the shallow hydrothermal vents in the Pacific Ocean (Maldonado et al., 1999; Cárdenas and Rapp, 2013; Bertolino et al., 2017).

Information about light, turbidity, current, bacteria biomass, phytoplankton sources as well as other biological communities still lacking to fully understand this complex and dynamic system and the functioning of this complex and dynamic coral ecosystem. However, data of this chapter already demonstrate that the Bouraké lagoon's uniqueness as a natural laboratory for studying the adaptive responses of corals and other reef species to the combination of multiple suboptimal environmental parameters, which are, to some extent, worse than those projected for the future. The investigations of this chapter indicate that the geomorphology of this unique site has not changed for the last 80 to 100 years, certainly longer. With this in mind, at least in the previous century, the environmental conditions remained unchanged, and the corals of the Bouraké lagoon could have experienced the current extreme conditions for several generations. Most importantly, the species found in the Bouraké lagoon are not unique to the mangrove habitat. They are common throughout New Caledonia and the SW Pacific region, suggesting that they have used specific strategies to cope with the suboptimal environmental conditions.

## 2.5 Conclusion

A multi-scale approach was used to characterize the physical and chemical environmental parameters of one of the most realistic natural laboratory for extreme environmental conditions, the semi-enclosed lagoon of Bouraké (New Caledonia), and accurately map its benthic community for the first time. In this chapter several physical and chemical parameters such as pH, DO, temperature, and salinity, but also nutrients and organic matter were studied and result showed that: *i*) they fluctuate between low and high tides, ranging from suboptimal-extreme to near normal values; *ii*) although predictable according to the tide, they differed spatially, and *iii*) suboptimal values persisted longer and were more acute on the inner reef. Results clearly show that fluctuations are: *i*) predictable, at

least for some of the physical parameters for which enough data were collected (i.e., pH, DO, temperature and salinity); *ii*) mainly driven by the tide; and *iii*) that seawater nutrient imbalance and organic inputs increase during the falling tide and originate from the mangrove forest and associated sediments. Although several studies suggest that ocean acidification, warming and, to some extent, deoxygenation will lead to a reduction in biodiversity, increase in bleaching and reef dissolution, in the Bouraké lagoon, a healthy reef with high coral cover and species richness, but also sponges and macroalgae (including CCA) was found. It was beyond the scope of this study to assess the contribution of environmental variability and nutrient imbalance to the organism' stress tolerance under extreme conditions. However, both coexist in the Bouraké lagoon, suggesting evidence of their contribution to the survival of organisms in extreme environmental conditions. This chapter provides evidence that this is possible in existing natural habitats, giving a glimmer of hope for the future of coral reefs. Further experiments are needed to reveal the mechanisms involved in the organisms' resilience to such conditions. Finally, the compelling basis for using this site as a natural laboratory for study the multitude of complex stressors acting together on life-long adapted coral reefs were provided.



# CHAPTER 3:

## SPONGE ORGANIC MATTER RECYCLING: REDUCED DETRITUS PRODUCTION UNDER EXTREME ENVIRONMENTAL CONDITIONS

---



The fieldwork in the Bouraké lagoon, where all *in-situ* sponge incubations were undertaken

## Resumé

Le chapitre précédent a mis en évidence les caractéristiques du site unique de la lagune de Bouraké en Nouvelle-Calédonie, caractérisé par des fluctuations du pH (7.23-8.10 unités), de la température (17.5-33.8°C) et de l'oxygène (1.87-7.24 mg L<sup>-1</sup>), qui atteignent des valeurs extrêmes à marée basse. Malgré ces caractéristiques extrêmes, la lagune abrite 66 espèces de coraux, 11 espèces d'éponges et 28 espèces de macroalgues. Ce site est désormais reconnu pour l'étude des réponses des organismes aux conditions extrêmes. Parmi les différentes éponges qui peuplent Bouraké, une en particulier et qui est de loin la plus abondante, *Rhabdsastrella globostellata* s'avère être la plus massive. Cette éponge a été choisie comme modèle d'étude pour comprendre les mécanismes qui lui permettent d'être aussi abondante malgré des conditions extrêmes. Ce troisième chapitre met en évidence la manière dont le métabolisme de l'éponge évolue en fonction des conditions environnementales, notamment la forte température, l'acidification et la désoxygénation.

Les éponges constituent un élément clé des écosystèmes coralliens et jouent un rôle important dans les cycles du carbone et des nutriments. En convertissant le carbone organique dissous (COD) en débris cellulaires (i.e., la «boucle de l'éponge»), et en assimilant le COD en biomasse, les éponges soutiennent le fonctionnement des récifs coralliens, et maintiennent une productivité et une biodiversité élevées dans un environnement oligotrophe. Malgré l'importance de cette «boucle», on sait peu de choses sur la façon dont ces cycles seront affectés par les conditions environnementales futures. Ici, nous avons utilisé le laboratoire naturel de Bouraké, dont les caractéristiques ont été décrites dans le chapitre précédent et où les organismes ont été exposés pendant des siècles à des conditions environnementales variables et extrêmes, pour étudier les effets de l'acidification, du réchauffement et de la désoxygénation des océans sur le carbone organique, le recyclage des nutriments et l'activité photosynthétique de l'éponge *R. globostellata*. Des incubations *in-situ* ont été réalisées dans différentes conditions environnementales. Les données suggèrent que les valeurs extrêmes d'acidification et de désoxygénation, seules ou en interaction, n'affectaient pas substantiellement le métabolisme de l'éponge. Cependant, lorsque ces conditions sont combinées à une température élevée (> 31°C), il y a un changement dans le recyclage du carbone organique de l'éponge avec une diminution de la production de débris, bloquant la voie de la «boucle de l'éponge». Ce chapitre est la première étude qui analyse les conséquences *in-situ* de conditions extrêmes sur le métabolisme d'une espèce d'éponge et sa capacité à recycler la matière organique ainsi que sur son activité phototrophique. Les résultats de ce chapitre pourraient mettre en évidence la façon dont les voies trophiques peuvent être affectées par les conditions océaniques changeantes.

Le travail présenté dans ce chapitre a été publié dans la revue *Marine Pollution Bulletin*.



Contents lists available at [ScienceDirect](#)

Marine Pollution Bulletin

journal homepage: [www.elsevier.com/locate/marpolbul](http://www.elsevier.com/locate/marpolbul)



## Sponge organic matter recycling: Reduced detritus production under extreme environmental conditions



Federica Maggioni<sup>a,b,\*</sup>, James J. Bell<sup>c</sup>, Mireille Pujo-Pay<sup>d</sup>, Megan Shaffer<sup>c</sup>, Carlo Cerrano<sup>e</sup>,  
Hugues Lemonnier<sup>a</sup>, Yves Letourneur<sup>a</sup>, Riccardo Rodolfo-Metalpa<sup>a,b</sup>

<sup>a</sup> ENTROPIE, IRD, Université de la Réunion, CNRS, IFREMER, Université de Nouvelle-Calédonie, Nouméa 98800, New Caledonia

<sup>b</sup> Labex ICONA International CO<sub>2</sub> Natural Analogues Network, JSPS, Japan

<sup>c</sup> School of Biological Sciences, Victoria University of Wellington, P.O. Box 600, Wellington, New Zealand

<sup>d</sup> Sorbonne Université, CNRS, Laboratoire d'Océanographie Microbienne, LOMIC, F-66650 Banyuls-sur-Mer, France

<sup>e</sup> Department of Life and Environmental Sciences (DiSVA), Polytechnic University of Marche, Ancona, Italy

DOI: <https://doi.org/10.1016/j.marpolbul.2023.114869>

## Abstract

Sponges are a key component of coral reef ecosystems and play an important role in carbon and nutrient cycles. Many sponges are known to consume dissolved organic carbon and transform this into detritus, which moves through detrital food chains and eventually to higher trophic levels via what is known as the sponge loop. Despite the importance of this loop, little is known about how these cycles will be impacted by future environmental conditions. During two years (2018 and 2020), the organic carbon, nutrient recycling, and photosynthetic activity of the massive HMA, photosymbiotic sponge *Rhabdastrella globostellata* were measured at the natural laboratory of Bouraké in New Caledonia, where the physical and chemical composition of seawater regularly change according to the tide. Main result showed that while sponges experienced acidification and low dissolved oxygen at low tide in both sampling years, a change in organic carbon recycling whereby sponges stopped producing detritus (i.e., the sponge loop) was only found when sponges also experienced higher temperature in 2020. The findings of this chapter provide new insights into how important trophic pathways may be affected by changing ocean conditions.

The work presented in this chapter was published in the journal *Marine Pollution Bulletin*.

### 3.1 Introduction

Exponentially increasing carbon dioxide emissions are causing major changes in the Earth's climate (IPCC, 2021). Ocean acidification (OA), ocean warming (OW) and ocean deoxygenation (OD) will have wide-ranging biological effects on marine organisms (IPCC, 2013). Coral reef survival is predicted to be compromised by climate change (Hughes et al., 2017), with impacts on reef structure and associated communities including biodiversity loss, ecosystem shifts, reduced habitat complexity, and declines in overall reef productivity (i.e., IPCC, 2013; Hoey et al., 2016).

Sponges are an important component of coral reefs across the world, and while corals are predicted to be generally negatively impacted by climate change (i.e., Hughes et al., 2003; Hoegh-Guldberg and Bruno, 2010), some sponges may increase in abundance and in some cases, become functionally dominant on coral reefs (Bell et al. 2013; Bennett, 2017; Bell et al., 2018b). For example, the combined effects of OA and OW have been shown to have no impact on growth, survival, or secondary metabolite biosynthesis in many sponge species (e.g., Pansini et al., 2000; Vicente et al., 2016; Lesser et al., 2016; Bennett et al., 2017). However, some studies have shown that sponges are negatively affected under future climate change scenarios with greater impacts on mortality rates and symbionts (e.g., López-Legentil et al., 2008; Massaro et al., 2012; Fan et al., 2013). Although these earlier studies mainly focused on stress responses and tolerance of sponges to climate change impacts, no studies have investigated to what extent future environmental changes will affect sponge nutrient and organic matter cycling.

The complex association of sponges with microbes, which includes photosynthetic symbionts, have resulted in one of the most diverse and complex holobionts (i.e., the host plus its associated microbes) in the marine environment (Pita et al., 2018). Based on the abundance and diversity of microbes in tissues, sponges can be classified into high microbial abundance (HMA) or low microbial abundance (LMA) ( $10^5$ - $10^6$  bacteria per g of sponge wet weight; Hentschel et al., 2006). While sponges are mainly heterotrophic organisms, many species form relationships with photosynthetic symbionts, where their energy budget depends on associated cyanobacteria or dinoflagellates (Taylor et al., 2008). Sponges play a key role in both carbon recycling and nutrient fluxes (e.g., sink and/or source of nitrogen, phosphate and silicate) in reef ecosystems, and in the movement of carbon between the benthos and higher trophic levels (e.g., Wulff, 2006; Bell, 2008; De Goeij et al., 2008; McMurray et al., 2016). By both converting dissolved organic carbon (DOC) into cellular detritus (i.e., the sponge loop pathway; De Goeij et al., 2013), and by assimilating DOC into biomass (Pawlik et al., 2016; McMurray et al., 2016; 2018), sponges support the coral reef functioning, and support high

productivity and biodiversity in otherwise oligotrophic environments (De Goeij et al., 2013, Rix et al., 2016, Pawlik and McMurray, 2020). Although the importance of these two different organic matter-recycling pathways has been identified, it is unknown how they will be impacted by predicted future changes in environmental conditions or whether sponges can switch between the sponge loop pathway and the production of biomass.

The largest organic resource in the oceans is dissolved organic matter (DOM) (Benner et al., 1992), which is released by primary producers. However, DOM is deemed biologically unavailable to most heterotrophic organisms directly (Carlson et al., 2002), and needs to be recycled in order to be available to higher trophic levels. In the water column, DOM is efficiently recycled by bacterioplankton through the microbial loop (Azam et al., 1983). In the same way, in shallow-water coral reefs, benthic species, particularly corals, release a large amount of DOM in the form of mucus, which can be recycled by sponges (Yahel et al., 2003; De Goeij et al., 2013; Mueller et al., 2014; Rix et al., 2016; 2018; Achlatis et al., 2018; Hoer et al., 2018). The sponge loop was first demonstrated for cryptic sponges, which transform DOM into a source of energy that is more readily available to other benthic reef fauna, particulate organic matter (POM) in the form of detritus (e.g., De Goeij et al., 2013; Alexander et al., 2014; Rix et al., 2017; Archer et al., 2017). This detritus subsequently feeds the detrital food chain. Therefore, sponges not only generate food for their associated detritivores through the production of detritus, but also provide a critical trophic link between corals and sponge-associated detritivores, allowing them access to the energy- and nutrient-rich DOC produced by corals (e.g., Rix et al., 2018). However, not all sponges participate in the sponge loop, nor produce detritus. For such sponges, it seems that DOM is preferentially stored as biomass and eventually transferred to higher trophic levels only via direct predation on sponge tissue (McMurray et al., 2018; Pawlik and McMurray, 2020). While the sponge loop pathway is now well reported for some cryptic encrusting species (e.g., De Goeij et al., 2013; 2017), and in both encrusting and massive sponges living in deep-sea habitats (e.g., Rix et al., 2016; Bart et al., 2021), no studies have reported this pathway in massive Caribbean sponges inhabiting shallow reefs, for which only minor detritus production (i.e., no sponge loop pathway) has been reported in both LMA and HMA sponges (e.g., McMurray et al., 2018, Wooster et al., 2019). For such emergent sponges, it seems that DOM is preferentially stored as biomass and eventually transferred to higher trophic levels only via direct predation on sponge tissue (McMurray et al., 2018; Pawlik and McMurray, 2020). This apparent discrepancy in the detritus production may be due to the different methodologies used to assess the sponge loop. The two most commonly used methods are either: 1) incubations with isotopically enriched DOC, and following its release into the detritus (e.g., De Goeij et al., 2013; Rix et al., 2016;

2017; 2018); and 2) the In/Ex method where sponge recycling is measured by tracking the change in the composition of seawater as it is filtered by the sponges (e.g., McMurray et al., 2018; Wooster et al., 2019). Elements of both methodologies have been criticized (De Goeij et al., 2017), and further comparisons are needed for different sponge morphologies.

Natural laboratories where seawater physical and chemical values deviate from the normal coastal conditions are increasingly being used to study the responses of marine organisms to future climate change (e.g., Hall-Spencer et al., 2008; Camp et al., 2016; 2018; Burt et al., 2020; Maggioni et al., 2021). In this study, I used the semi-enclosed lagoon of Bouraké, New Caledonia (21°56'58.43"S; 165°59'29.46"E; **Appendix B – Figure B3.1**), that is a natural laboratory, characterized by regular tidal fluctuations of pH (8.10–7.23 pHT units), dissolved oxygen (DO, 7.34–1.87 mg L<sup>-1</sup>), and temperature (from +1°C to +3°C). These parameters regularly change from close-to-normal conditions (high tide, HT) to extreme conditions (low tide, LT) (Camp et al., 2017; Maggioni et al., 2021). When averaged (pHT = -0.3 units, T = +2°C, DO = -26 %, compared to nearby control sites), environmental values are close to future climate predictions (RCP 8.5, IPCC, 2014). These fluctuating environmental conditions occur with chronically high concentrations of nutrients, and both dissolved and particulate organic matter, especially during low tide (Maggioni et al., 2021). At Bouraké, despite these extreme environmental conditions, a diverse and abundant benthic community was found, with coral species composition and richness similar to the local control reefs where environmental conditions are at ambient levels (Maggioni et al., 2021). Among sponges, the massive *Rhabdastrella globostellata* (Carter, 1883) is the most abundant species at Bouraké, reaching up to 40 % cover (Maggioni et al., 2021). The massive sponge *R. globostellata* (Carter, 1883) is among the most abundant species at Bouraké, reaching up to 40% cover (Maggioni et al., 2021). This Indo-Pacific high microbial abundance (HMA) species (Moitinho-Silva et al., 2017a) has been demonstrated to efficiently filter bacteria cells and remove DOM from surrounding seawater (Hildebrand et al., 2022), fundamentally altering its composition.

The main aim of this chapter was to assess how the metabolism of *R. globostellata* (i.e., nutrient and organic carbon cycles) is affected by extreme environmental conditions, specifically, the three main drivers of future climate change: acidification, deoxygenation and warming. The effects of extreme conditions on the autotrophic and heterotrophic processes of the sponge holobiont were also assessed. Extreme conditions have been hypothesized to negatively affect the metabolism of sponges and their autotrophic and heterotrophic rates.

## 3.2 Materials and methods

### 3.2.1 The sponge study model *Rhabdastrella globostellata* (Carter, 1883)

Sponge *R. globostellata* is a marine sponge of the family Ancorinidae of Tetractinellida order belonging to the large class of Demospongiae. The sponge is usually about 12-15cm wide, a squat sphere with one or several shallow depressions at the top so that the entire sponge resembles a shallow pot, which gives its name also of yellow pot sponge. The depression is smooth and often has many closely-spaced holes that resemble holes in the cheese. There are bumps on the outside of the 'pot', sometimes forming ridges, its characterized by yellow color in the choanoderm, while the surface is typically dark brown. *R. globostellata* is usually a massive sub globular species that can be cup-shaped in large specimens but a morphotype with a convoluted surface (brain-like) seemed also quite common (Cárdenas et al., 2022). It is found throughout the Indo-Pacific region (World Porifera Database) in shallow-water coral reef environments. It was first described by Henry J. Carter as *Stelletta globostellata* in 1883, named after the *globostellate* shape of its spicules (Latin meaning: "sphere" and *stellātus* = "star-shaped"). Its skeleton consists of silica spicules characterized by triaene, oxyspheraster, oxae, and oxyaster (**Appendix B – Figure B3.2**). In New Caledonia, the distribution of this species is still poorly known (<http://lagplon.ird.nc/>), and thus the difficulty of finding it outside the study site of Bouraké. Thanks to the first chapter results, this species was found as the most massive and abundant throughout the Bouraké lagoon. Then, this sponge has been chosen as a model to understand their strategies to cope with the extreme environment conditions.

### 3.2.2 Experimental design

During both April 2018 and March 2020, *in-situ* dark incubations (lasting 1 h) of *R. globostellata* were performed to assess the effects of environmental conditions on its dark respiration, nutrient and organic matter recycling. In March 2020, these measurements were repeated in addition to light incubations to assess the effect on sponge photosynthetic symbionts (i.e., symbiont photosynthesis and sponge respiration, and photosynthetic efficiency of the symbionts in the sponge pinacoderm). In April 2018, seven incubations, using a set of four dark-shaded chambers, were performed during five consecutive days at different tidal phases, close to both low tide (LT; n = 12) and high tide (HT; n = 16). In March 2020, 15 incubations using a set of six chambers were performed over 14 days. This time we performed incubations exactly at high tide (HT; n = 42) and low tide (LT; n = 48). During the first week of fieldwork, seven out of 15 incubations were done in the dark only (HT = 18, and LT = 24), while the remaining eight incubations measured both photosynthesis and dark respiration during both high tide (n = 24) and low tide (n = 24). In these experiments, respiration rates in the dark

in the morning at high tide were first measured, and subsequently using the same individuals, their photosynthesis in the light were measured. In the afternoon, at low tide the photosynthesis first and then dark respiration were measured. This inverse procedure was adopted because during the afternoon the light availability was already lower ( $337.7 \pm 257.7 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) than during the morning ( $1176.6 \pm 88.4 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), likely affecting any comparison of the photosynthesis measured during HT in the morning (See Appendix B - Table B3.1 for summary of *in-situ* incubations).

In addition to the *in-situ* incubations, in 2020, sponges fragments (HT; n = 9, LT; n = 9) were also incubated *ex-situ* on board the R/V Alis, to assess their photosynthetic responses by pulse-amplitude modulated fluorometry (PAM) (Genty et al., 1989) under both HT and LT conditions.

### 3.2.3 Incubation set up

For each *in-situ* dark incubation or each pair of incubations (light and dark), a fresh independent (i.e., no sponge fragmentation) *R. globostellata* was used, each of similar volume (n = 96,  $232 \pm 73 \text{ cm}^3$ ). Sponges were collected attached to substrate by scuba diving at 2–4 m depth at the station B2 (Appendix B -Figure B3.1). Sediment and epibionts on the surface of the sponges were gently removed underwater to ensure that only the sponge respiration was measured (i.e., the holobiont). Each sponge was placed in a transparent Plexiglas benthic chamber (total volume of 6.4 L), which was hermetically closed and connected to a pump and an YSI 600 multiparameter probe (Biscéré et al., 2015). In the chamber system, the pump allows seawater to recirculate at a water flow of  $2 \text{ L min}^{-1}$ , while the probe recorded the temperature and oxygen concentration every minute, therefore regularly monitoring sponge oxygen consumption (i.e., respiration  $R_{\text{dark}}$ ) and production (i.e., net photosynthesis  $P_n$ , only in 2020). The chambers were dark shaded when measuring the holobiont respiration. During each incubation, which lasted *ca.* 1 h, one chamber with no sponge was used as a blank (i.e., control) to account for the contribution of photosynthetic organisms and microbial metabolism in the water. All the chambers, connecting tubes, and syringes were cleaned at the end of each day using a solution of 10 % HCl for 4 h and further rinsed in deionized water. This incubation protocol was tested in a preliminary experiment to verify that the duration of each incubation was long enough to detect oxygen depletion and avoid low oxygen concentrations in the chamber.

For the *ex-situ* incubations on 14<sup>th</sup> March 2020, nine small fragments (3-4 cm in diameter) of *R. globostellata* were collected during low tide, and another set during high tide, from individual, spatially separated donor sponges. Seawater was collected from Bouraké using 2 x 25 L tanks. Samples were transported on board the R/V in individual hermetic plastic bags containing seawater

and immersed in a cooler. Both sponge fragments and water were maintained in a controlled temperature room settled at the *in-situ* temperature until data collection.

### 3.2.4 Data collection

At the beginning (i.e., 5 minutes after the chambers were closed) and at the end of each dark incubation in 2018 and 2020, 450 mL of seawater was collected from each chamber with a syringe to assess changes in nutrient, organic matter, and physical seawater parameters.

During light incubations, photosynthetically active radiation (PAR, 400-700 nm) was measured using a LI-1500 light sensor logger connected to a 30 m long cable and a spherical quantum sensor, which was positioned close to the chambers. The photosynthesis to respiration ratio ( $P : R_{\text{dark}}$ ) was calculated as:

$$P : R_{\text{dark}} = \frac{P_g \times \text{hours of daylight}}{R_{\text{dark}} \times 24}$$

with  $P_g$  and  $R_{\text{dark}}$  expressed in  $\mu\text{mol O}_2 \text{ cm}^{-2} \text{ h}^{-1}$ . The gross photosynthesis to respiration ratio was calculated based on daily budget of 12 h sunlight (photosynthesis) and 24 h respiration (Wilkinson, 1983).

Consumption (i.e., reduction, negative values) and/or production (i.e., increase, positive values) of nutrients, oxygen and organic matter (i.e., all the parameters measured during the incubations) were calculated as the difference between their concentrations at the beginning and end of the incubations, corrected by the change in concentration occurring in the control chamber, and normalized by the duration of incubation (h), the chamber volume (L), and sponge volume ( $\text{cm}^3$ ). Sponge volume was calculated using a three-dimensional reconstruction technique. Between 80 and 200 photos of each sponge were taken underwater using a waterproof photo-camera (Nikon AW 130) to cover the sponge whole surface. A reference square (3 cm x 3 cm) was placed close to each sponge to scale the model to its real size. The 3D Zephyr Pro software was then used to calculate the volume of each sponge (see **Appendix B – Section B3.1** for method details). In the *ex-situ* incubations, sponge fragments were dark adapted for 30 minutes (Ralph et al., 1999; Biggerstaff et al., 2015), and the effective quantum yield ( $F_v/F_m$ ) and the relative electron transport rate (rETR) were measured using a Diving-PAM, with the RLC curves function.

### 3.2.5 Seawater analyses

In 2018 and 2020 during the dark incubations, seawater samples were collected at the beginning and end of each *in-situ* incubation to calculate the change in seawater physical and chemical parameters

(nutrient and carbon) during the incubations. In 2018, we measured  $\text{pH}_T$  (in total scale), oxygen (DO), temperature, nutrient concentrations (orthosilicic acid  $[\text{Si}(\text{OH})_4]$ , nitrite + nitrate  $[\text{NO}_x]$ , ammonium  $[\text{NH}_4]^+$ , and phosphate  $[\text{PO}_4]^{3-}$ ), bacterial abundance (BA), dissolved organic carbon (DOC), dissolved inorganic carbon (DIC), particulate organic carbon (POC), and particulate organic nitrogen (PON) (see chapitre 2, section 2.2.2.3 for further details of the methods used). In 2020, in addition to the above measurements, we also measured the live particulate organic carbon (LPOC), which is the total amount of bacteria and phytoplankton and is important to better assess the capacity of sponges to recycle organic matter. LPOC was converted into carbon content and used to calculate the consumption/production of carbon discharged by the sponge as detritus (i.e.,  $\text{Detritus} = \text{POC} - \text{LPOC}$ ). In 2020, during the light incubations, we only measured  $\text{pH}_T$ , oxygen, and temperature (see **Appendix B – Section B3.2** for method details).

### 3.2.6 Data analysis

Each chamber was considered as a replicate. Because we performed all incubations at the peak of the tides, and the seawater parameters were fully monitored, we separated all measurements into two tide categories (HT and LT), and for both 2018 and 2020. In order to test for significant differences in: *i*) the environmental conditions at the beginning of the incubations; and *ii*) the change in the chemical parameters during the incubations, due to the sponge metabolism, two-way ANOVA, followed by post hoc Tukey tests (HSD), were performed between high and low tide (HT and LT, respectively), years (2018 and 2020) and their interactions. ANOVAs were performed after verification of normality and homogeneity of the data or residuals. In order to test for significant differences between tidal phases on LPOC, phytoplankton and detritus concentrations collected in 2020, unpaired t-tests were performed on: *i*) the environmental conditions at the beginning of the incubations; and *ii*) the change in the chemical parameters during the incubations. To better summarize and visualize the different responses in sponge metabolism between the two years and tidal phases, a principal Component Analyses (PCA) were performed separately for data collected in 2018 and 2020, and for both HT and LT using the absolute values of the data after normalization. Furthermore, to investigate the effect of the seawater composition on metabolic activities, a PCA using Spearman correlation matrix was performed on data collected in 2020 between the seawater chemical parameters measured at the beginning of the incubations and the absolute values of their changes (i.e., consumption and production) during the incubations. In order to assess differences in photosynthesis ( $P_g$ ), respiration rates ( $R_{\text{dark}}$ ) and their ratios (P:R), as well as differences in photosynthetic efficiency of the photosymbionts using the maximum electron transport rate ( $r\text{ETR}$ ) and the effective quantum yield ( $F_v/F_m$ ) during the dark/light incubations, unpaired t-tests were performed between tides (HT and

LT) on data collected in 2020. Data were graphically presented using the generalized additive models (GAM). All analyses were conducted in RV4.1, using stats, factorminer, and vegan packages (R Core Team, 2019).

### 3.3 Results

#### 3.3.1 Environmental conditions at the start of the dark incubations

Seawater temperature measured at the beginning of the incubations (chambers with sponges + blanks) was significantly different between years, tides and their interactions ( $p < 0.001$ ; **Appendix B - Table B3.2**), while dissolved oxygen and pH were significantly lower at LT ( $p < 0.001$ ; **Appendix B - Table B3.2**), and not significantly different between years (**Table 3.1**; pH  $p = 0.55$ , DO  $p = 0.076$ ; **Appendix B - Table B3.2**). For these parameters, the highest average temperature ( $31.73 \pm 0.71^\circ\text{C}$ ) and lowest pH and DO ( $7.56 \pm 0.03$  pH<sub>T</sub> units and  $3.94 \pm 0.23$  mg O<sub>2</sub> L<sup>-1</sup>, respectively) were found at LT in 2020. HSD post hoc comparisons confirmed that seawater temperature did not differ between tides in 2018; but did differ between tides in 2020; and that temperature in 2020 was higher than in 2018 (**Appendix B - Table B3.2**).

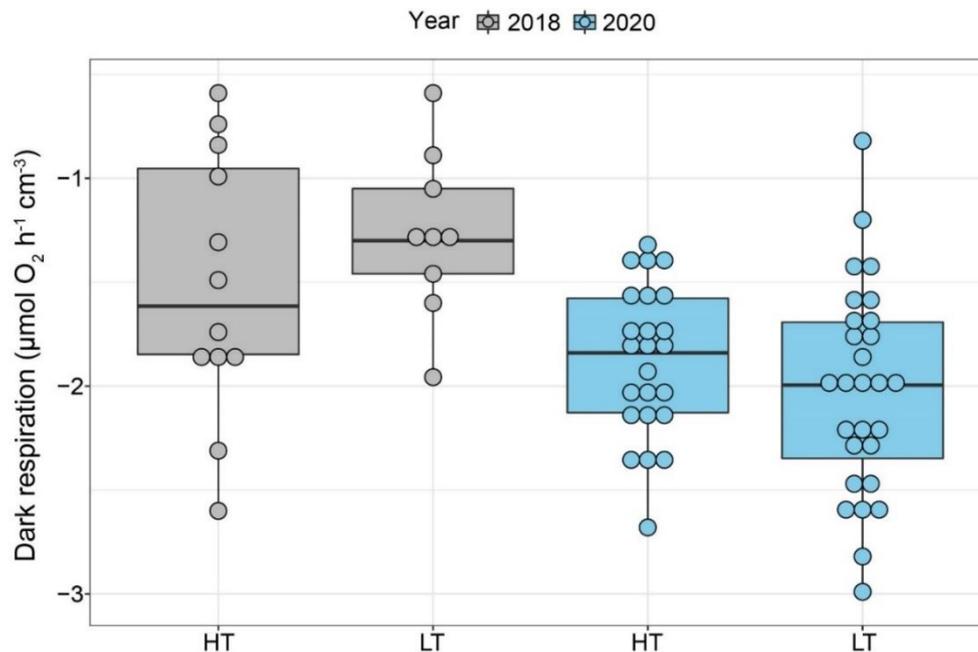
**Table 3.1.** Summary of the environmental parameters measured at the beginning of the incubations in 2018 and in 2020, both at HT and LT. Data are expressed as mean  $\pm$  SD; number of replicates are in brackets; nd means not determined.

	2018		2020	
	HT (n = 16)	LT (n = 12)	HT (n = 18)	LT (n = 24)
<b>Physical parameters</b>				
T (°C)	26.28 $\pm$ 1.35	26.43 $\pm$ 0.74	29.38 $\pm$ 0.13	31.73 $\pm$ 0.71
pH <sub>T</sub>	7.92 $\pm$ 0.18	7.57 $\pm$ 0.09	8.03 $\pm$ 0.01	7.56 $\pm$ 0.03
<b>Chemical parameters</b>				
DIC (μmol C L <sup>-1</sup> )	2158.7 $\pm$ 83.6	2376.5 $\pm$ 116.1	2103.5 $\pm$ 22.00	2647.1 $\pm$ 33.5
DO (mg O <sub>2</sub> L <sup>-1</sup> )	5.54 $\pm$ 1.36	4.26 $\pm$ 0.58	5.66 $\pm$ 0.24	3.94 $\pm$ 0.23
Si(OH) <sub>4</sub> (μmol L <sup>-1</sup> )	11.81 $\pm$ 3.95	19.37 $\pm$ 5.01	9.15 $\pm$ 0.21	21.03 $\pm$ 1.07
No <sub>x</sub> (μmol L <sup>-1</sup> )	1.58 $\pm$ 1.77	1.35 $\pm$ 0.47	0.46 $\pm$ 0.07	0.64 $\pm$ 0.15
PO <sub>4</sub> <sup>3-</sup> (μmol L <sup>-1</sup> )	0.35 $\pm$ 0.11	0.35 $\pm$ 0.10	0.33 $\pm$ 0.02	0.35 $\pm$ 0.10
NH <sub>4</sub> <sup>+</sup> (μmol L <sup>-1</sup> )	0.38 $\pm$ 0.10	0.37 $\pm$ 0.10	0.84 $\pm$ 0.46	0.86 $\pm$ 0.56
DOC (μmol C L <sup>-1</sup> )	93.09 $\pm$ 6.55	129.10 $\pm$ 12.77	130.43 $\pm$ 1.69	219.83 $\pm$ 22.68
POC (μmol C L <sup>-1</sup> )	29.93 $\pm$ 8.44	35.66 $\pm$ 16.13	16.29 $\pm$ 1.42	37.62 $\pm$ 11.80
PON (μmol N L <sup>-1</sup> )	1.94 $\pm$ 0.46	2.27 $\pm$ 1.14	1.59 $\pm$ 0.14	2.62 $\pm$ 0.69
BA (x10 <sup>8</sup> cells L <sup>-1</sup> )	2.52 $\pm$ 4.44	3.91 $\pm$ 1.04	12.3 $\pm$ 1.11	13.7 $\pm$ 5.18
LPOC (μmol C L <sup>-1</sup> )	nd	nd	3.92 $\pm$ 1.90	1.01 $\pm$ 1.39
Phyto (x10 <sup>6</sup> cells L <sup>-1</sup> )	nd	nd	55.5 $\pm$ 6.34	10.3 $\pm$ 4.22
Detritus (μmol C L <sup>-1</sup> )	nd	nd	14.03 $\pm$ 1.64	37.16 $\pm$ 11.75

Chemical parameters, with the exception of  $\text{PO}_4^{3-}$ , significantly differed between years and tides, showing some significant interactions (**Table 3.1; Appendix B - Table B3.2**). Some parameters differed between years, with bacterial abundance (BA) and  $\text{NH}_4^+$  having lower concentrations in 2018 ( $p < 0.001$ ; **Appendix B - Table B3.2**), and  $\text{NO}_x$  higher in 2018 ( $p < 0.001$ ; **Appendix B - Table B3.2**). Most of parameters including DIC,  $\text{Si}(\text{OH})_4$ , DOC, POC and PON showed significant interactions between tides and years ( $p < 0.05$ ; **Appendix B - Table B3.2**). HSD post hoc comparisons showed that all these parameters had higher concentrations during LT, and for both years, reaching the highest concentrations during LT in 2020 (**Table 3.1**). Both phytoplankton and LPOC showed significant differences between tides ( $p < 0.001$ ; **Appendix B - Table B3.2**), with consistently higher concentrations at HT; while detritus, which mostly depends on the POC concentration, was higher at LT ( $p < 0.001$ ; **Appendix B - Table B3.2**).

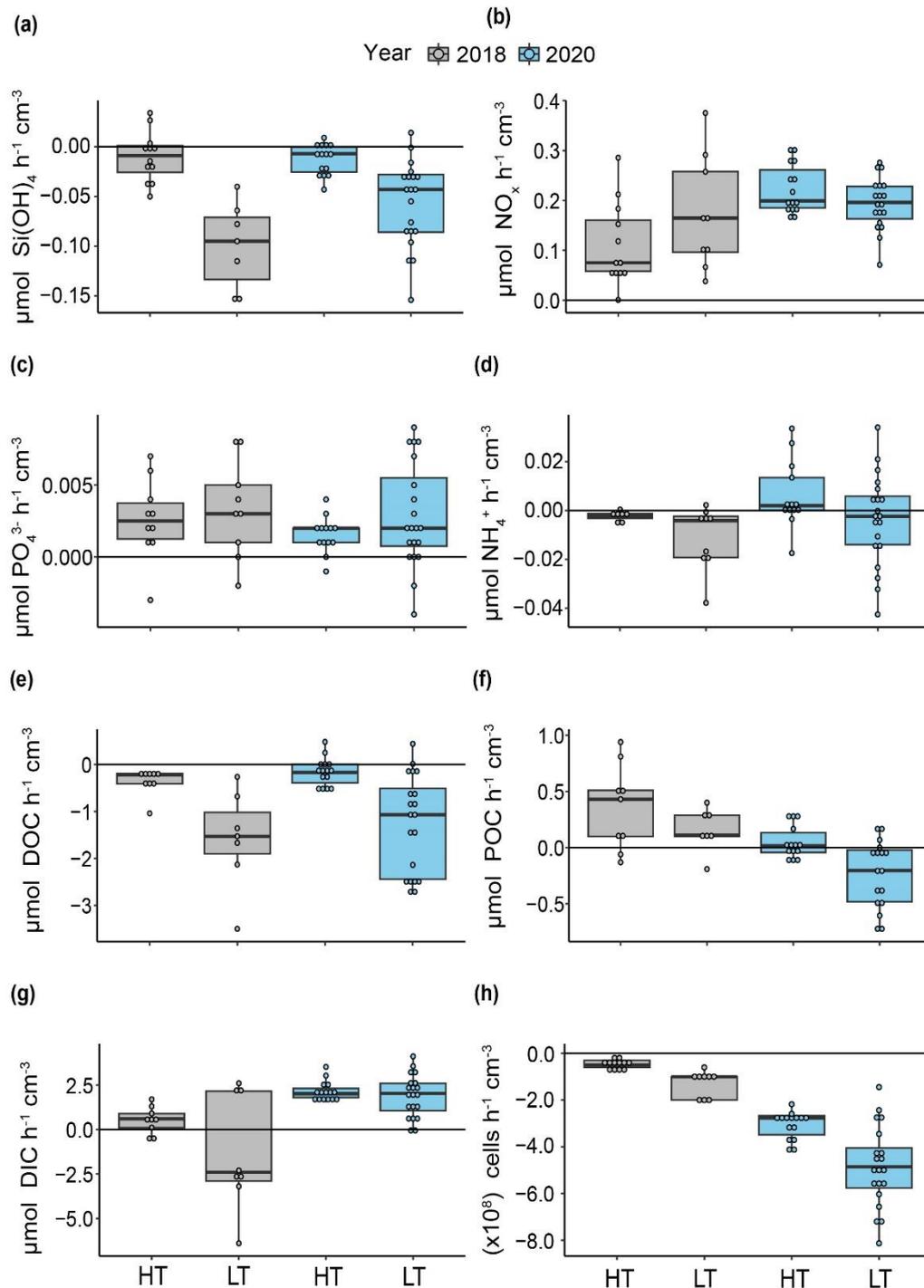
### 3.3.2 Metabolic activities of the sponge holobiont in the dark

In the dark, all sponges depleted dissolved oxygen in both 2018 and 2020 (**Figure 3.1; Appendix B - Table B3.3**). ANOVA showed that oxygen consumption significantly differed between years ( $p < 0.001$ ; **Appendix B - Table B3.4**), but not between tides ( $p = 0.293$ ; **Appendix B - Table B3.4**). Mean oxygen consumption was highest during the LT in 2020, and was the lowest during the LT in 2018.

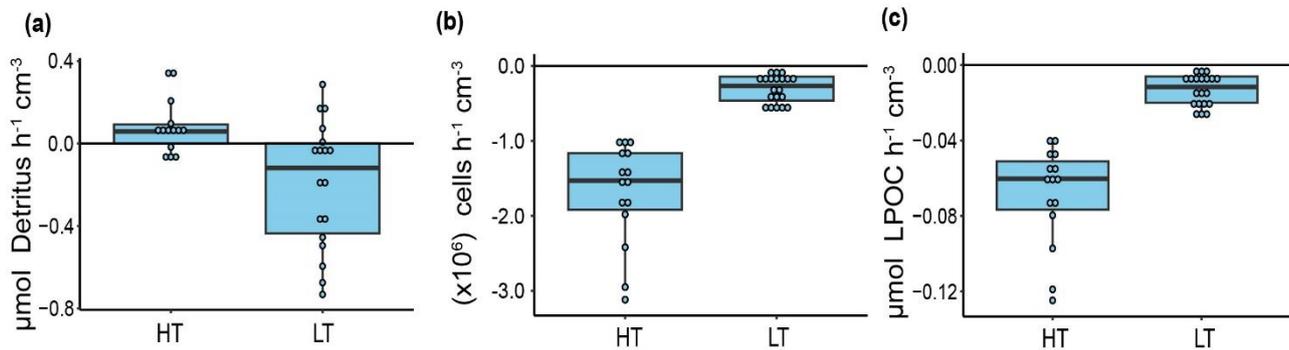


**Figure 3.1.** Dissolved oxygen consumption per sponge volume measured during the incubations in the dark in 2018 (in gray) at both low tide (LT;  $n = 9$ ) and high tide (HT;  $n = 12$ ), and 2020 (in blue) at both high tide (HT;  $n = 24$ ) and low tide (LT;  $n = 28$ ). Boxes are the interquartile range of data (25th and 75th percentiles); the horizontal line is the median, and the whiskers represent the data range (i.e., minimum and maximum).

During the incubations there was a decrease in BA, Si(OH)<sub>4</sub>, NH<sub>4</sub><sup>+</sup>, DOC, LPOC and phytoplankton cells, and an increase in PO<sub>4</sub><sup>3-</sup> and NO<sub>x</sub> (**Figure 3.2; Appendix B - Table B3.3**). Both reductions and increases were found for DIC, POC, and PON (**Appendix B - Table B3.3**). Metabolic parameters were significantly different between years, tide and had significant interactions, without any general common response (**Appendix B - Table B3.4**). DIC and NO<sub>x</sub> only differed significantly between years ( $p < 0.001$ ; **Appendix B - Table B3.4**), while NH<sub>4</sub><sup>+</sup>, Si(OH)<sub>4</sub> and DOC only between tides ( $p < 0.001$ ; **Appendix B - Table B3.4**). BA, POC and PON differed significantly between years and tides ( $p < 0.01$ ; **Appendix B - Table B3.4**), while only Si(OH)<sub>4</sub> and NO<sub>x</sub> showed significant interaction between year and tide ( $p < 0.05$ ; **Appendix B - Table B3.4**). Orthosilicic acid Si(OH)<sub>4</sub> decreased during LT, reaching the highest average consumption in 2018, while NO<sub>x</sub> increased more in 2020 reaching the highest value during HT (**Figure 3.2a, b; Appendix B - Table B3.3**). The highest uptake of DOC was found during LT, while POC was always produced except during LT in 2020 (**Figure 3.2e, f**). Bacteria decreased more during LT in 2020 (**Figure 3.2h**). Detritus, LPOC and phytoplankton were measured only in 2020, where they significantly differed between tides ( $p < 0.05$ ; **Figure 3.3; Appendix B - Table B3.4**). Detritus increased at HT and decreased at LT, while the LPOC and phytoplankton always decreased (i.e., were consumed by the sponge) ( $p < 0.05$ ; **Figure 3.3; Appendix B - Table B3.4**). Phytoplankton was consumed significantly more during HT than LT ( $p < 0.001$ ; **Figure 3.3b; Appendix B - Table B3.4**). Between phytoplankton and bacteria, *Synechococcus* (Syn) was the largest source of carbon at both HT and LT (**Appendix B - Figure B3.4**). Phytoplankton composition showed that at both HT and LT, Syn was the most consumed phytoplankton group (**Appendix B - Table B3.5**).

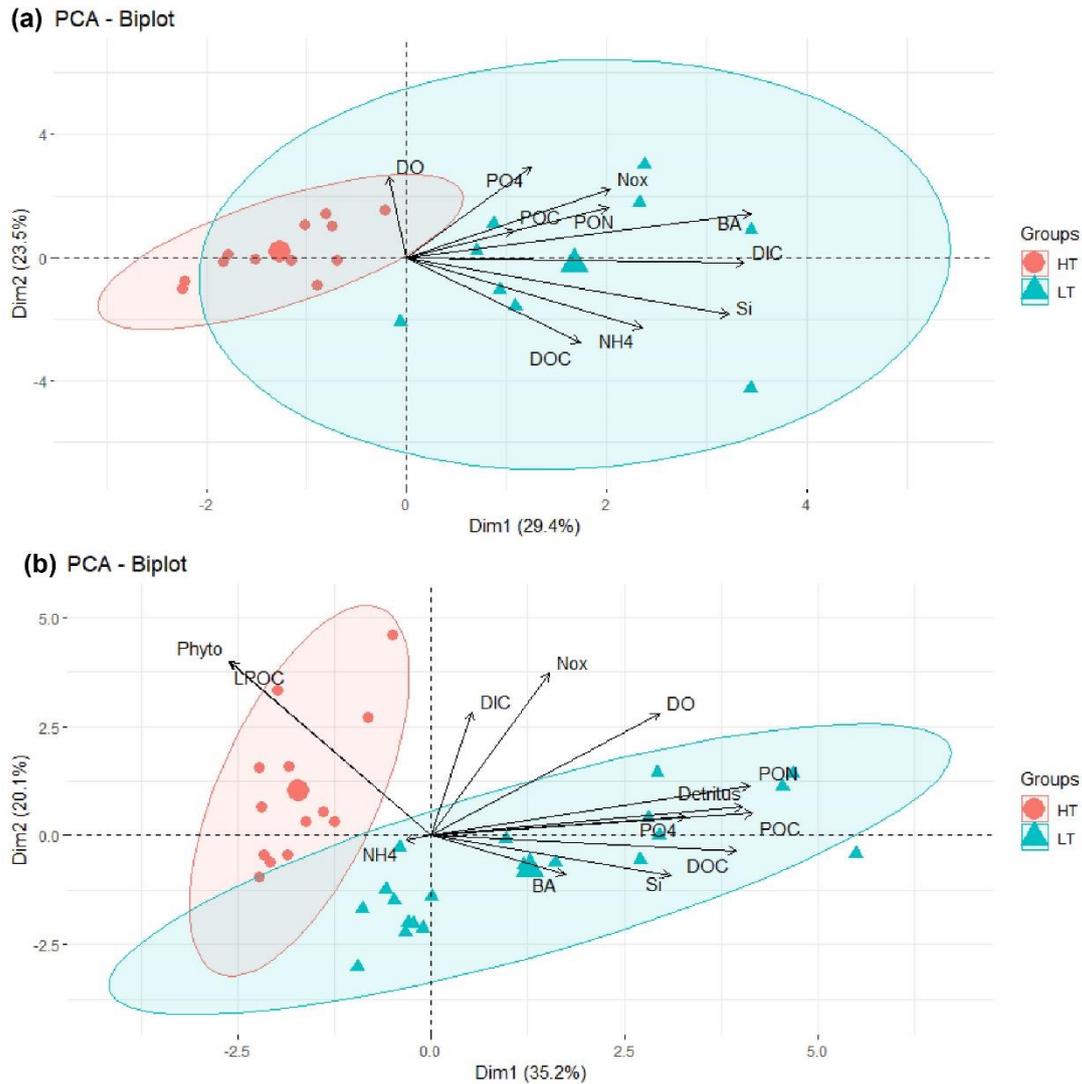


**Figure 3.2.** Sponge nutrients and organic matter consumption/production measured during the incubations during the incubations in the dark at high tide (HT) and low tide (LT) in 2018 and 2020. (a)  $\text{Si(OH)}_4$ , (b)  $\text{NO}_x$ , (c)  $\text{PO}_4^{3-}$ , (d)  $\text{NH}_4^+$ , (e) Dissolved Organic Carbon (DOC), (f) Particulate Organic Carbon (POC), (g) Dissolved Inorganic Carbon (DIC), and (h) Bacteria abundance. Data are the difference between the beginning and the end of the incubations. Boxes represent the interquartile range (25th and 75th percentile); the horizontal line is the median, and the whiskers represent the data range (i.e., minimum and maximum).



**Figure 3.3.** Sponge consumption/production measured during the incubations in the dark at HT and LT in 2020. (a) Detritus (POC-LPOC), (b) phytoplankton abundance, (c) Life Particulate Organic Carbon (LPOC). Data are the difference between the beginning and the end of the incubations. Boxes represent the interquartile range (25th and 75th percentile); the horizontal line is the median, and the whiskers represent the data range (i.e., minimum and maximum).

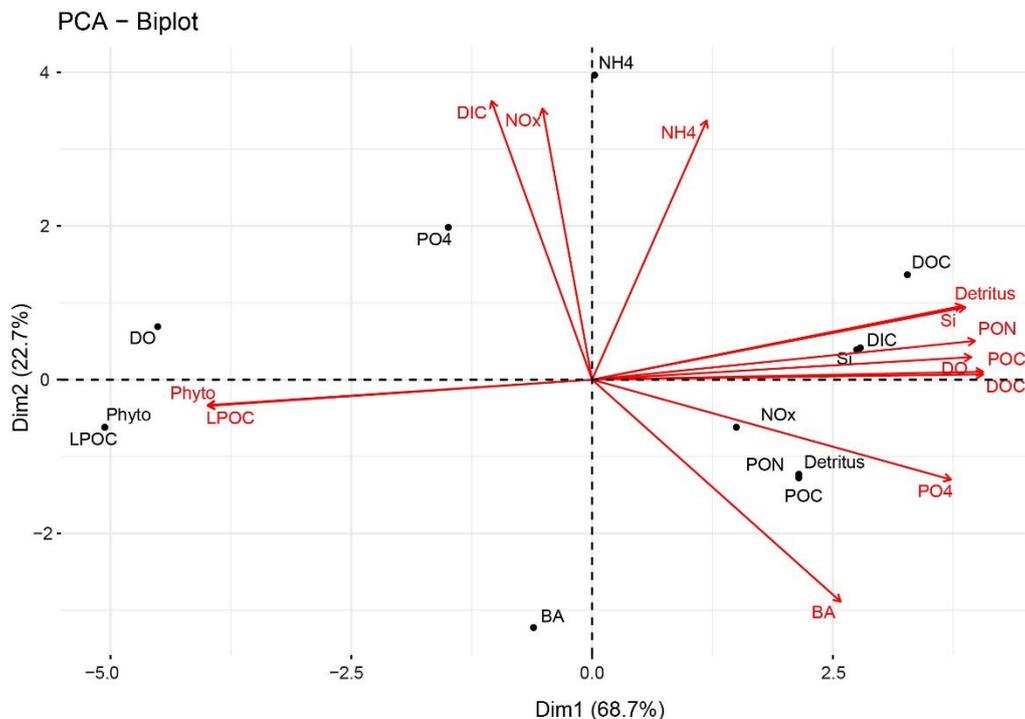
The first two principal components (Dim1 and Dim2) of the PCAs accounted for more than 52% and 55% of the changes in the seawater medium chemistry during the incubations made in 2018 and 2020, respectively (**Figure 3.4a, b**). Samples clustered into two major groups according to the tide for both years. In both years, a clear difference was found for the two tidal phases and most of the parameters we measured contributed to the LT incubation clusters. In 2018, all parameters except DO, showed positive correlations with Dim1 scores, and were densely plotted on the right-hand side of the plot, where samples were clustered into the LT group. In 2020, only phytoplankton and LPOC showed strong negative correlations with the Dim1 scores and were plotted on the left-hand side of the plot, where samples were clustered into the HT group. All the remaining parameters were positively correlated with Dim1 scores and plotted on the right-hand side of the plot where samples were clustered to the LT group. Among them, detritus, PON, DOC and POC showed the strongest positive correlations and appeared to contribute the most to the variation in sponge metabolism during incubations at LT. Detritus, PON, DOC and POC were all more highly concentrated at the beginning of the LT incubations (**Table 3.1**).



**Figure 3.4.** Principal Component Analyses (PCA) on the parameters measured in the incubation medium (a) in 2018, (b) in 2020 at both HT and LT. Each dots in red indicates one single sponge incubation metabolism made during HT, while triangles in blue indicates incubations during LT. Arrows indicate the variable and their length approximates the variance of the variables. The ellipses represent the 95% interval confidence. Large triangles or dots indicate multiple incubations at the same point.

PCA on a Spearman correlation matrix between seawater parameters measured at both the beginning of the incubations in 2020, and absolute value changes during the incubations (i.e., consumption and production) showed clear relationships for some parameters (**Figure 3.5**). The first two principal components (Dim1 and Dim2) accounted for more than 90% of the variation. Most of seawater parameters were positively correlated with Dim1, including DOC, BA, NH<sub>4</sub><sup>+</sup>, and Si(OH)<sub>4</sub>, which were correlated with the sponge nutrient utilization of DOC, Si(OH)<sub>4</sub>, BA and NH<sub>4</sub><sup>+</sup>, suggesting that their initial concentrations in the seawater affected their uptake. Furthermore, significant positive correlations were also found for these parameters (**Appendix B - Figure B3.3**), showing higher consumption at LT when higher concentrations in seawater were observed. In the same way, LPOC

and phytoplankton showing higher consumption at HT when they were more abundant in the seawater (**Figure 3.5; Appendix B - Figure B3.3**). The only exception was  $\text{NH}_4^+$ , which showed positive relationship between the initial concentrations and its consumption, but it was not related to a specific tide.



**Figure 3.5.** Principal Component Analyses (PCA) on Spearman correlation matrix on data collected in 2020 between the seawater chemical parameters measured at the beginning of the incubations, and the absolute values of their changes (i.e., consumption and production) during the incubations. Each dot indicates the sponge metabolism parameters while arrows indicate the variable, and their length approximates the variance of the variables.

### 3.3.3 Sponge holobiont photosynthetic activity

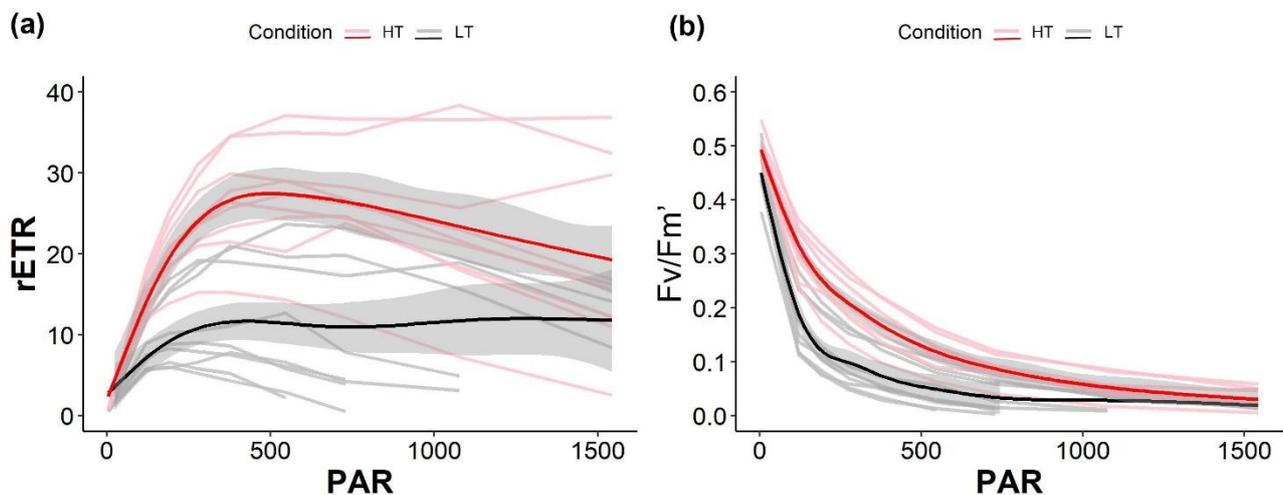
The photosynthetic rates and photosynthetic efficiency were only measured in 2020, the former *in-situ* and the latter *ex-situ*, and at both HT and LT conditions. During the *in-situ* experiment, HT differed from LT conditions in the seawater pH (7.9 vs 7.4  $\text{pH}_T$  units, respectively), DO (6.0 vs 3.4  $\text{mg O}_2 \text{ L}^{-1}$ , respectively) and temperature (29.9 vs 31.6°C, respectively). However, light irradiance differed considerably between HT and LT, and were  $1176.6 \pm 88.4$ , and  $337.7 \pm 257.7 \mu\text{mol m}^{-2} \text{ s}^{-1}$ , respectively. During the *ex-situ* incubations, pH was comparable to the *in-situ* incubations, and temperature was lower than the *in-situ* incubations at 28.9-29.5°C (**Appendix B - Table B3.6**).

Photosynthesis and P:R ratios were significantly higher at HT than LT, but dark respiration did not differ between tides ( $p = 0.3381$ ; **Appendix B - Table B3.7; Figure B3.5**). Gross photosynthesis ( $P_g$ ) was always positive (due to oxygen production), and higher at HT than at LT (**Table 3.2; Appendix**

**B - Figure B3.5;  $p < 0.001$ ; Table B3.7).** P:R ratios at both tide conditions revealed that the oxygen consumption was always higher than its production. The maximum rETR, as well as the initial  $F_v/F_m$  measured during the *ex-situ* incubations in 2020 were significantly different between HT and LT. Both rETR and  $F_v/F_m$  versus irradiance (PAR) showed lower values during LT ( $p < 0.05$ ; **Figure 3.6; Table 3.2; Appendix B - Table B3.7).**

**Table 3.2.** Summary of the gross photosynthesis ( $P_g$ ), respiration ( $R_{dark}$ ), and their ratio (P:R) measured in-situ, and the initial photosynthetic efficiency ( $F_v/F_m$ ) and  $rETR_{max}$  measured ex-situ. For in-situ,  $n = 9$  and  $n = 8$  at HT and LT, respectively; and for ex-situ,  $n = 9$ . Data are normalized and expressed as mean  $\pm$  SD.

	HT	LT	HT	LT	HT	LT
	$R_{dark}$		$P_g$		P:R	
<i>In-situ</i>	$-1.91 \pm 0.19$	$-1.79 \pm 0.29$	$1.25 \pm 0.29$	$0.38 \pm 0.33$	$0.32 \pm 0.07$	$0.10 \pm 0.09$
	$F_v/F_m$		$rETR_{max}$			
<i>Ex-situ</i>	$0.5 \pm 0.04$	$0.5 \pm 0.06$	$27.6 \pm 7.0$	$12.6 \pm 6.5$		



**Figure 3.6.** Generalized additive models (GAM) on (a) relative ETR (rETR) and (b) the effective quantum yield ( $F_v/F_m$ ) versus irradiance (PAR). Data were obtained from dark-adapted sponges during the *ex-situ* incubations in 2020 under high tide (HT;  $n = 9$  red) and low tide (LT;  $n = 9$  black) conditions. The shaded areas indicate the 95% confidence intervals of the GAM model. Individual measurements were also reported.

### 3.4 Discussion

This chapter investigated how the extreme conditions experienced by the massive sponge *R. globostellata* in Bouraké during low tide affect sponge holobiont organic matter and nutrient recycling, and sponge photosynthetic activity. Findings showed different responses between the two years of measurement. In 2018, both at normal (high tide, HT) and extreme (low tide, LT) seawater pH and dissolved oxygen values, our sponge always consumed DOC, and produced POC (i.e., the sponge loop pathway). In contrast, in 2020 the organic matter recycling was altered, because although sponges always consumed DOC, they produced less POC and detritus at high tide, and stopped (i.e., negative values) producing both at low tide. These differences in 2020 occurred at the same seawater pH, and dissolved oxygen values as measured in 2018, but in 2020 the temperature was much higher, being the highest ever recorded at Bouraké. Similarly, photosymbiont photosynthetic activity was negatively affected during low tide in 2020. Results suggests that while the combination of extreme levels of acidification and deoxygenation seem not to affect *R. globostellata* metabolism and the sponge loop, temperature may be a key driver in metabolic shifts, likely when combined with extreme levels of acidification and deoxygenation.

#### 3.4.1 Changes in sponge organic matter recycling

Results of this chapter demonstrate that although the values of acidification and deoxygenation at Bouraké are extreme, the massive sponge *R. globostellata* preferentially produces detritus, which is a prerequisite for the sponge loop pathway. All of the performed incubations under both normal (high tide = HT) and extreme conditions (low tide = LT) confirmed the first step of the sponge loop, i.e., sponge DOC uptake, which is in agreement with the trend reported for many other species, both encrusting and emergent forms (e.g., Yahel et al., 2003; Hoer et al., 2018; McMurray et al., 2018; Wooster et al., 2019), and recently reported for the same study species (Hildebrand et al., 2022). In addition, a positive correlation between DOC consumption and its initial concentration was found (**Appendix B - Figure B3.3**), as also found in previous studies (i.e., Mueller et al., 2014; McMurray et al., 2016; Morganti et al., 2017; McMurray et al., 2018). However, DOC uptake at low tide in 2020 was not higher than in 2018 (**Figure 3.3e**), even though the amount of available organic matter was almost double in 2020 (129.10 vs 219.83  $\mu\text{mol C L}^{-1}$ ). This could suggest that the positive relationship between DOC uptake and DOC availability (as in Archer et al., 2017) only exists up to a certain DOC level, which likely corresponds to the optimum organic matter uptake for the sponge.

Furthermore, all the sponges incubated in 2018 and the ones incubated at HT in 2020 produced both POC and/or detritus (**Figure 3.2f, Figure 3.3a; Appendix B - Table B3.3**), therefore also confirming the second step of the sponge loop, i.e., DOM transformation to both POC and detritus (e.g., De Goeij et al., 2013; Alexander et al., 2014; Rix et al., 2018; Kornder et al., 2022). The reported detritus production (in average  $0.06 \mu\text{mol h}^{-1} \text{cm}^{-3}$ ) was higher compared to the majority of massive sponges in the Caribbean for which only small amounts of detritus production were reported ( $< 0.032 \mu\text{mol h}^{-1} \text{cm}^{-3}$ , from McMurray et al., 2018). While DOM transformation is common in encrusting and deep-sea sponges from the Caribbean and Red Sea, with net detritus production found for 19 out of 22 species (reviewed by de Goeij et al., 2017), to the best of my knowledge, this study is the first demonstrating high detritus production in a massive sponge in shallow-waters from the Pacific Ocean (**Appendix B - Table B3.3**).

In contrast, during the LT (extreme conditions) in 2020 (**Figure 3.2f, Figure 3.3a, Appendix B - Table B3.3**), a negative values of both POC and detritus were found, which means that neither POC nor detritus were produced by the sponges. This result is consistent with recent studies reporting examples of emergent sponges that consumed detritus. For example, the giant barrel sponge *Xestospongia muta* consumed  $0.11 \pm 0.16 \mu\text{mol h}^{-1} \text{cm}^{-3}$  in Key Largo, Florida, and  $0.016 \pm 0.009 \mu\text{mol h}^{-1} \text{cm}^{-3}$  in Belize of detritus (McMurray et al., 2018). The causes of the disruption of detritus production during low tide in 2020, and the consequent interruption of the sponge loop, could be the result of the extreme physical conditions in pH, DO, and temperature. When considering only the condition and results from 2020, we are unable to disentangle the contribution of the single parameters on the sponge loop since pH, temperature, and dissolved oxygen are significantly correlated with the detritus production/consumption (**Appendix B - Figure B3.6**), and all were negative at LT. Based on these results, I conclude that the combination of these extreme parameters may have caused the disruption of the second step of the sponge loop in *R. globostellata* during the LT in 2020.

The regression between POC and the main physical parameters measured at the beginning of the incubation at both 2018 and 2020 was also analysed. Indeed, since POC is mainly composed of detritus, as LPOC comprises a maximum of 24 % of POC (**Table 3.1**), POC production could be a good proxy to assess the second step of the sponge loop, and to compare data between years (**Appendix B - Figure B3.7**). In this case, temperature seem to be the main factors driving the reduction in POC (i.e., detritus) production, clearly showing that while POC was always produced in 2018 at temperature of ca 26–28 °C, it was mostly consumed during the low tide in 2020 at

temperature of ca 31–33 °C ( $p < 0.001$ , **Appendix B - Figure B3.7**). Further laboratory experiments are needed to confirm the effect of temperature on sponge loop pathway.

Another possible explanation for the change in the detritus production measured during the LT in 2020, is a reduction in sponge pumping rates as demonstrated in previous studies (e.g., Massaro et al., 2012; Stevenson et al., 2020). However, if there was a halt in pumping rates during extreme conditions, sponges would simply stop recycling nutrients and carbon, which was not the case. Although I did not measure this parameter, data showed that even at LT in 2020 sponges continued to recycle DOC, as well as other nutrients (**Figure 3.2**), and respired at the same rates as at HT in the same year, therefore the sponge was not stressed and had a normal basal metabolism (**Figure 3.1; Appendix B - Table B2.3**). However, sponge respiration rates were higher in 2020 than in 2018 (**Appendix B - Table B3.3**), possibly as a mechanisms to cope with the high temperatures (**Table 3.1**).

I also hypothesize that this break in the detritus production at LT in 2020 was due to the lower concentration of LPOC and phytoplankton found at LT (**Table 2.1; Figure 3.5; Appendix B - Figure B3.3**), while both were more abundant and more consumed during HT. However, given LPOC was a very small part of the POC pool, the POC mainly comprised detritus (**Figure 3.2f, Figure 3.3a**), and both POC and detritus were always higher at LT (**Table 3.1**), therefore available for the sponge nutrition. Although 1h incubations were too short to measure the production of ‘waste’ filtering cells, which take place every five to six hours (De Goeij et al., 2013), both at HT and LT in 2018 and HT in 2020 *R. globostellata* produced POC (**Figure 3.2f, Figure 3.3a**) but this stopped at LT in 2020.

The finding that sponges stopped producing POC and detritus during the extreme conditions measured at LT in 2020 means that they reduced cell turnover, which is energetically costly and may represent 75 % of the daily energy (DOC and POC) consumed (Kahn and Leys, 2016). Lower detritus production was also found in mesophotic sponges where it seems that sponges preferentially invest in their growth rather than in cell turnover, when compared with the same species in shallower water (Hadas et al., 2009; De Goeij et al., 2017; Lesser et al., 2020). This hypothesis, which needs to be experimentally validated with others techniques (e.g., NanoSIMS and stable isotope analyses), is in agreement with findings for Caribbean massive sponges where less carbon is released as detritus (e.g., Pawlik et al., 2015; 2016, McMurray et al., 2018). Sokolova et al. (2012) also suggested that when food is not limiting, high growth rates and biomass accumulation can be supported even under stressful conditions. This might partially explain why our study species is so massive and abundant across Bouraké, covering up to 40% of the reef area (Maggioni et al., 2021), but is rare and forms small colonies outside the lagoon.

In conclusion, several hypotheses could explain the change in sponge organic matter recycling, and further studies are needed to understand the processes that occur when carbon is no longer released as detritus. However, based on the environmental conditions measured in Bouraké, the reported observations suggest that high temperature, in combination with acidification and deoxygenation, may be the main factor driving the break.

### 3.4.2 Effect of extreme conditions on sponge holobiont nutrient recycling and feeding

Beyond their role in carbon recycling on coral reefs, sponges likely have a key influence on nutrient biogeochemistry (e.g., De Goeij et al., 2017; Pawlik and McMurray, 2020). Sponges are important sources of dissolved inorganic nitrogen (DIN), such as ammonium, nitrite and nitrate; and also phosphate through their metabolism (Jiménez and Ribes, 2007), and through the inextricable activity of their associated microbial communities. Sponges typically remove ammonium from seawater, as a nitrogen source for chemo- and phototrophic bacteria and an energy source for ammonium oxidizing bacteria and archaea. For instance, both the sponges *Tethya citrina* and *Xestospongia muta* produced  $\text{NO}_x$ , and the latter consumed  $\text{NH}_4^+$  (Fiore et al., 2013; López-Acosta et al., 2019), reflecting the diverse nitrogen metabolic pathways within the sponge holobiont (Archer et al., 2017). In agreement with previous findings, and notwithstanding the extreme conditions, a net production of  $\text{NO}_x$ , especially in 2020, and a consumption of  $\text{NH}_4^+$  were measured (**Figure 3.2b, d; Appendix B - Table B3.3**). Since both aerobic and anaerobic ammonia-oxidizing microbes have been found in sponges (Bayer et al., 2008), these results could be due to both the photosymbionts and anaerobic activity of the associated bacteria. Microbial symbionts may also sequester a significant amount of phosphorus in the form of polyphosphate (Zhang et al., 2015), however a net, and consistent production of  $\text{PO}_4^{3-}$  during incubations was found (**Figure 3.2c; Appendix B - Tables B3.3; B3.4**), which was comparable to the sponge *Tethya citrina* that released  $0.005 \pm 0.004 \mu\text{mol h}^{-1} \text{mL}^{-1}$  of  $\text{PO}_4^{3-}$  (López-Acosta et al., 2019).

Most sponges rely on silicic acid (DSi), such as  $\text{Si}(\text{OH})_4$  to produce their silica skeletons and to grow. *Rhabdastrella globostellata* always consumed  $\text{Si}(\text{OH})_4$ , with higher rates during extreme conditions (low tide) when the DSi concentration in Bouraké was the highest (**Figure 3.2a; Appendix B - Table B3.3**). Average DSi consumption was  $0.10 \pm 0.043$  and  $0.057 \pm 0.043 \mu\text{mol h}^{-1} \text{cm}^{-3}$  during low tide in 2018 and 2020 respectively, which corresponded to a  $\text{Si}(\text{OH})_4$  concentration of  $19.37 \pm 5.01$ , and  $21.03 \pm 1.07 \mu\text{mol h}^{-1} \text{cm}^{-3}$ , respectively. A positive correlation between DSi concentration and uptake was found (**Appendix B - Figure B3.3**), which is in agreement with other studies where consumption of DSi was reported at a similar rate and correlated with DSi availability (Maldonado et al., 2012;

2020; López-Acosta et al., 2019). The high concentration of DSi during low tide in the Bouraké lagoon could be due to the elevated acidification level (minimum  $\text{pH}_T = 7.2$ ), which prevents dissolution of  $\text{Si}(\text{OH})_4$  and increases its concentration in seawater. This chemical condition results in high DSi availability in the Bouraké lagoon, thus facilitating the skeletal growth of *R. globostellata*.

Although the effect of tide and tidally-driven changes in the seawater plankton and bacterial community have yet to be investigated in Bouraké, our measurements in 2020 clearly showed that the abundance of phytoplankton vs bacteria during the tide had a great effect on sponge metabolism (see chapter 2 for further discussion on the Bouraké functioning). Both the live particulate organic carbon (LPOC) and phytoplankton were more concentrated at high tide (**Table 3.1, Appendix B – Table B3.5**), corresponding to the new water from the open lagoon, while bacteria and detritus concentrations were high at low tide, corresponding to the water coming from the mangrove. Differences in the PCA between 2018 and 2020 was likely because in 2020 the incubations were always done at HT and LT and not close to them as in 2018. In agreement with this diurnal variability in the water quality of Bouraké, *R. globostellata* feeds more on LPOC and phytoplankton during the high tide, and inversely, more on detritus and bacteria during the low tide (**Figure 3.2h, Figure 3.3**). This result is in agreement with a recent study showing that *R. globostellata* efficiently filters bacteria cells (Hildebrand et al., 2022), although less than what we found in Bouraké. This difference may be simply due to the different methods used, or more likely because the Bouraké lagoon is eutrophic (**Table 3.1**) when compared to the study site in Guam by Hildebrand et al. (2022). These bacterial cells are likely a food source (Reiswig, 1975; Ribes et al., 1999) for this sponge, although it is well known that sponges are selective filter feeders preferring the relatively rarer but labile resource of phytoplankton compared to the numerical dominant heterotrophic bacteria (Maldonado et al., 2012; McMurray et al., 2016). In contrast, *R. globostellata* in Bouraké seems to change the food source regardless of the tide. For instance, ingestion rates of both the bacteria and phytoplankton groups increased or shifted from one group to another according to their composition based on the tide (**Appendix B - Table B3.5**). Food selection may result from either passive processes, in which the physical properties of the sponge filter leads to differential uptake of picoplankton cell types, or active processes, in which food selection is mediated by sponge behaviour (Jürgens and DeMott, 1995; Maldonado et al., 2010). Therefore, the abundance and metabolic plasticity that make this species highly competitive in Bouraké could be because food is never limited there, and that the passive filtration method is the one preferably used by *R. globostellata*.

### 3.4.3 Effect of extreme conditions on the sponge photosynthetic activity

Sponges form symbiotic relationships with many microbial groups (e.g., Thomas et al., 2016; Moitinho-Silva et al., 2017b), including some photosynthetic symbionts, which provide photosynthetically-derived carbon to their host (Wilkinson, 1983). Some studies have reported that microbial photosynthetic symbionts are not significantly affected when exposed to both OW and OA (Wisshak et al., 2012; Morrow et al., 2015; Luter et al., 2020, Bell et al., 2022). However, earlier studies showed reduced photosynthetic functioning of associated photosymbionts when exposed to high temperature (Cebrian et al., 2011; Webster et al., 2012; Goodwin et al., 2014; Lesser et al., 2016; Beepat et al., 2020). For example, *Cliona orientalis* showed a significant reduction in photochemical efficiency when exposed to a temperature of 27°C (Ramsby et al., 2018b). Consistent with these earlier studies, *in-situ* measurements in this chapter showed a negative effect of the low tide conditions on sponge symbiont photosynthesis, which corresponded to a drop in the symbiont photosynthetic activity on the sponge pinacoderm measured during the *ex-situ* incubation. In fact, significantly lower values were found during low tide for  $P_g$ , P:R,  $F_v/F_m$  and rETR, while no differences in respiration was reported during *in-situ* measurements between tides (**Table 3.2, Appendix B – B3.7; Figure B3.5**). This drop might be due to the extreme conditions experienced by the sponges during the low tide, especially seawater pH and temperature, since it has been already demonstrated that some sponges can be tolerant to reduced levels of oxygen (Micaroni et al., 2022). A similar response was observed in the giant barrel sponge (*Xestospongia muta*), where a decrease in the productivity of cyanobacteria symbionts was observed when sponges were exposed to high temperature and low pH (Lesser et al., 2016). The drop in photosynthesis could be due to the differences in the light levels between incubations (i.e., at low tide in the afternoon the light was less than at high tide in the morning). However, both the photosynthetic efficiency and the relative ETR measured on dark-adapted sponges in the lab confirmed the drop in the sponge symbiont photosynthesis between tides (**Figure 3.6; Appendix B - Table B3.7**). Although long-term measurements should be done on the whole energy budget, it seems that even if the symbiosis of *R. globostellata* in Bouraké were greatly affected by the combination of extreme conditions, the effect may not be that large since sponges can rely on heterotrophy, which may play a greater role in its survival. In fact, *R. globostellata* from Bouraké showed heterotrophy characteristics (P:R ratios < 1.5; **Table 3.2**). To support this hypothesis some studies showed that eutrophic conditions could render bioeroding sponges less dependent on their photosymbionts (i.e., Webb et al., 2017).

### 3.5 Conclusion

In this chapter, the semi-enclosed lagoon of Bouraké as a natural laboratory was used to study for the first time the metabolism and organic carbon recycling in the sponge *R. globostellata* exposed to normal and extreme values in physical and chemical parameters. Results showed that *R. globostellata* can tolerate the combination of low seawater pH and dissolved oxygen during the low tidal phase, and it does not show any metabolic dysfunction in its ability to recycle both the organic matter and nutrients. In contrast, higher temperature coupled with extreme acidification and deoxygenation had a dual effect on the sponge: (i) instead of recycling organic matter into detritus and making it available to higher trophic levels, sponges showed no detritus production; (ii) the photosynthetic activity of the symbionts suffered during periods of thermal stress. On-going laboratory experiments using stable isotope analyses are testing the potential role of temperature as the driver of the break in the detritus production, and the processes that are involved when the detritus production stops. The results of this chapter further shows how important trophic pathways may change under extreme environmental conditions and provides insights into how other sponges may respond to ongoing climate change.



# CHAPTER 4:

## CARBON AND NITROGEN RECYCLING SHIFT IN WARM-ADAPTED SPONGES

---



The experimental set-up at the “Aquarium de Lagon” (Nouméa, New Caledonia), where all *ex-situ* experimental works were undertaken

## Resumé

Dans le chapitre précédent, j'ai pu étudier les réponses métaboliques de l'éponge *R. globostellata* à différentes conditions environnementales. Le principal résultat a montré un arrêt de la production de débris pendant les conditions extrêmes, ce qui nous permet d'émettre l'hypothèse que la température serait l'un des principaux facteurs de ce changement dans le recyclage de la matière organique. Compte tenu des résultats obtenus *in-situ*, ce quatrième chapitre s'attache à comprendre les effets de fortes températures dans le recyclage du carbone par l'éponge. La température de surface de la mer pourrait augmenter possiblement jusqu'à +4°C d'ici la fin du siècle, ce qui aura des conséquences négatives sur les écosystèmes coralliens. Dans les récifs coralliens, les éponges jouent un rôle clé dans les processus écologiques en absorbant et en transformant la DOM en la rendant disponible aux autres niveaux trophiques. Jusqu'à présent, les études ont rapporté des effets contrastés, négatifs ou positifs, de la température dans les processus physiologiques des éponges avec des réponses spécifiques aux espèces. Cependant, il n'existe pas d'étude ayant examiné l'effet de la température sur le cycle du carbone et de l'azote des éponges. Dans ce chapitre, une expérience contrôlée en aquarium a été mise en place pour étudier les effets d'une température élevée (32°C) sur les cycles du carbone et de l'azote chez l'espèce dominante de notre site d'étude, i.e. *R. globostellata*. Les individus ont été collectés dans un environnement à température fluctuante (6.5°C par jour) à Bouraké (Nouvelle-Calédonie), où la température atteint en été 33.2°C, et un site contrôle où la température en été est « stable » à 28°C (île Tupeti, Nouvelle-Calédonie). Du mucus corallien (matière organique utilisable par les éponges) marqué au <sup>13</sup>C et <sup>15</sup>N a été utilisé pour suivre son transfert et son incorporation à la fois dans les tissus des éponges et les débris relégués pendant des expériences d'incubations, durant lesquelles les changements dans la matière organique de l'eau de mer ont également été mesurés. Les résultats principaux de ce chapitre ont montré que l'incorporation de <sup>13</sup>C et <sup>15</sup>N au sein des tissus de l'éponge et dans les débris émis change à 32°C, avec une accumulation plus élevée dans les tissus pour les éponges de Bouraké (adaptées à un environnement extrême). Les résultats montrent que les éponges peuvent s'adapter à des conditions de haute température, possiblement similaires à celles que nous pourrions avoir dans le futur, avec un changement dans l'allocation du carbone et de l'azote entre les tissus et les débris de l'éponge. Enfin, ce travail a mis en évidence que le réchauffement des océans pourrait modifier la manière dont les éponges recyclent le carbone (C) et l'azote (N), donc leur qualité et leur disponibilité dans le récif.

Le travail présenté dans ce chapitre a fait l'objet d'une publication actuellement en cours de révision à la revue *Limnology and Oceanography*.

## Abstract

Ocean warming will affect the functioning of coral reef ecosystems with unknown cascading effects. We previously found that the massive sponge *Rhabdastrella globostellata* inhabiting the semi-enclosed warm, acidic and deoxygenated lagoon of Bouraké changed the carbon (C) recycling when the environmental conditions were extremes. Any perturbation in the ability of sponges to recycle the dissolved organic matter released by the reef, and make it available to higher trophic levels, might have unknown consequences for the reef trophic chain. In this chapter, using  $^{13}\text{C}$ - and  $^{15}\text{N}$ -labeled coral mucus, I experimentally demonstrated that high temperature affected the rates of C and nitrogen (N) incorporated in the sponge tissue and released as detritus. Biogeochemical processes were measured in *R. globostellata* from Bouraké, where temperatures reach  $33.8^{\circ}\text{C}$  and fluctuates by  $6.5^{\circ}\text{C}$  on a daily scale, and from a control reef ( $28^{\circ}\text{C}$ ). Sponges were exposed to constant and variable thermal regimes in an orthogonal experimental design. At the elevated temperature, sponges from Bouraké, which are adapted to elevated and fluctuating temperatures, retained more  $^{13}\text{C}$  and  $^{15}\text{N}$  in the tissue than they released as detritus, while the control had an opposite response. Such a change in C and N allocation at high temperature, with less detritus being redistributed to the surrounding ecosystem, could be a tradeoff used by the Bouraké sponge population to cope with the extreme environmental conditions, likely altering the availability of essential sources of energy with unknown consequences in the reef trophic interactions.

The work presented in this chapter has been the subject of a publication currently under review at the journal *Limnology and Oceanography*.

## 4.1 Introduction

One of the main effects of climate change is the increase in sea surface temperature causing ocean warming (OW). An increase in seawater temperature will have a cascading effect on marine life and ecological processes worldwide. In coral reefs, sponges play a key role by recirculating carbon and nutrients produced by corals, making these sources of energy available to the surrounding trophic chain (e.g., De Goeij et al., 2013; Rix et al. 2017; 2018; Bart et al., 2021). Although many studies have revealed the physiological stress caused by high temperature on sponges (e.g., Bell et al., 2018b), none of these studies have investigated its effect on the sponges ability to recycle carbon and nitrogen.

The negative effects of OW on sponges have been largely demonstrated on the sponge reproduction (e.g., Abdul Wahab et al., 2014), photosynthetic rates (e.g. Bennett et al., 2017), pumping rates (e.g., Massaro et al., 2012), filtration efficiency, choanocyte chamber size and density (Massaro et al., 2012), and change in their associated microbial composition and functioning (Ramsby et al., 2018a). For example, thermal stress causes the reduction in choanocyte chamber size and density, resulting in a reduced ability to generate new choanocyte chambers and normal or even accelerated shedding of choanocytes (De Goeij et al., 2009). Temperatures higher than 31°C increased the expression of heat shock proteins in some species (López-Legentil et al., 2008; Pantile and Webster, 2011; Guzman and Conaco, 2016). Both acute, short- and long-term exposures to high temperature caused mass mortality events (i.e., Cebrian et al., 2011; Di Camillo et al., 2013; Bell et al., 2017), although other studies showed sponges to be more tolerant to OW than other benthic organisms such as corals (Kelmo et al., 2013). Likely, OW effects on sponges' metabolism are species-specific, and depend on whether a particular species is already near or above its thermal optimum. For instance, species living in thermally variable environments cope better with heat stress when compared to species living in more stable environments (e.g., Oliver and Palumbi, 2011; Rivest et al., 2017).

Thanks to their complex association with microbes, sponges can convert the abundant dissolved organic carbon (DOC) in seawater in particulate organic carbon (POC), and release it as detritus (i.e., sponge loop pathway; De Goeij et al., 2013), and/or store it as biomass (e.g., Pawlik et al., 2016; McMurray et al., 2018). In doing that, sponges play a crucial role as ecosystem engineers since they make available carbon (i.e., energy) to other organisms in the reef, and so link different trophic levels (e.g., De Goeij et al., 2013; Hoytema et al., 2023). Therefore, it becomes crucial to better understand to what extent OW will affect the ability of sponge to recycle the organic matter in coral reef ecosystems.

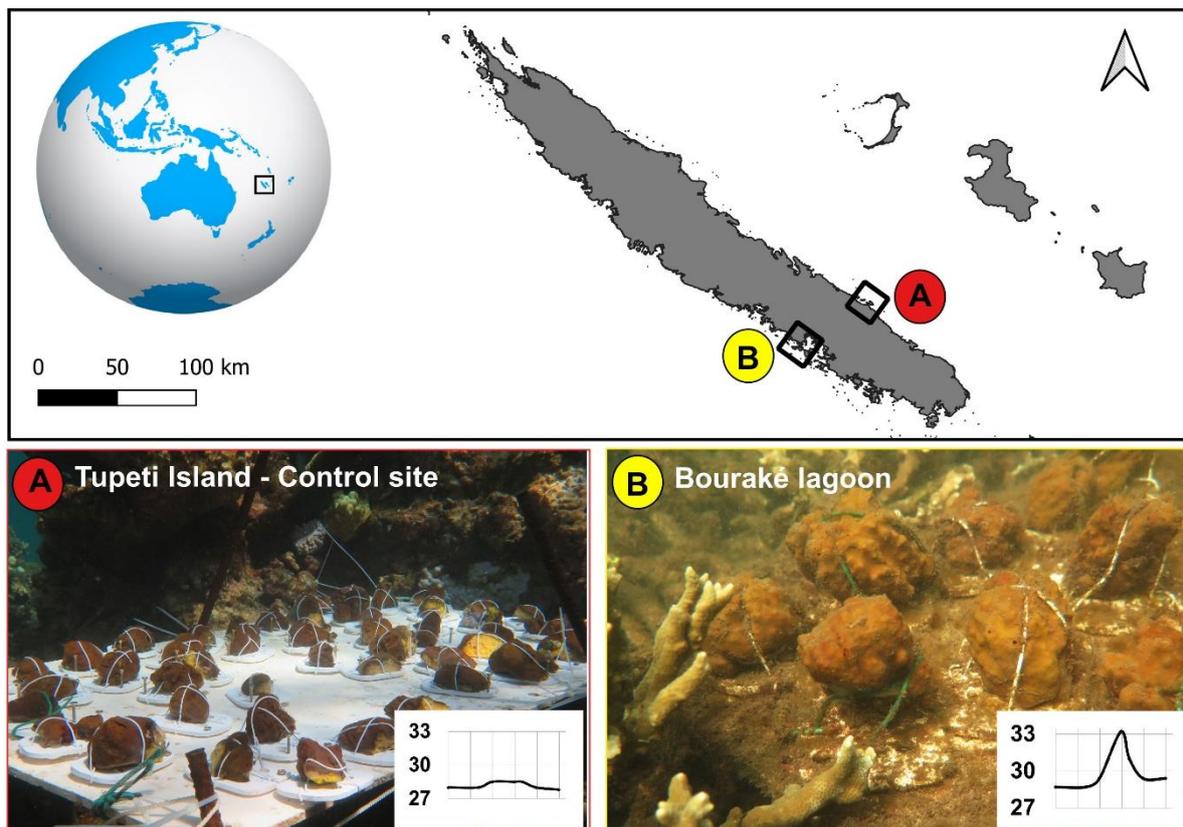
In the previous field-based study (chapter 3) I found that the sponge *Rhabdastrella globostellata* living in the semi-enclosed lagoon of Bouraké has developed mechanisms to cope with the extreme environmental conditions that characterize this special natural laboratory. During the low tide, Bouraké has environmental conditions ( $\text{pH}_T = -0.8$  units; dissolved oxygen = -50%; temperature =  $+2\text{-}3^\circ\text{C}$ ) that could be claimed to be close to, or even worse than conditions predicted under the worst climate scenario. Although the harsh conditions, a healthy coral reef was found (Camp et al., 2017; Maggioni et al., 2021). In Bouraké, the sponge *R. globostellata* forms massive colonies, with up to 40% of coverage, while outside Bouraké it becomes rare and forms only scattered, low-size colonies. By incubating sponges *in-situ* at both high (i.e., normal conditions) and low tide (i.e., extreme conditions), I previously found that at extremist condition tested ( $> 31^\circ\text{C}$ ), the sponge organic matter recycling changed from detritus production to no more detritus production in *R. globostellata* (Maggioni et al., 2023). Because the observations were made *in-situ*, where sponges experience a combination of factors, including warming, acidification and deoxygenation, I was unable to elucidate the driver of the observed change. In this chapter, I tested that *i*) this change was primarily due to the elevated temperature *ii*) carbon (C) and nitrogen (N) were preferentially stored in sponge tissue (e.g., biomass) than recycled as detritus; and *iii*) the observed response was specific of sponges from Bouraké. These hypotheses were tested here by comparing the ability to recycle the organic matter (DOC, POC, PON, detritus consumption and, or production) on warm-adapted *R. globostellata* from Bouraké (variable thermal history between  $28^\circ\text{C}$  and  $33^\circ\text{C}$ ), and non-adapted ones from a control reef (constant around  $28^\circ\text{C}$ ). Enriched  $^{13}\text{C}$  and  $^{15}\text{N}$  incorporated by corals and released as mucus were used to track the C and N incorporations into the sponge tissue and their transfer as detritus.

## 4.2 Materials and methods

### 4.2.1 Samples collection and maintenance

On the 15<sup>th</sup> and 17<sup>th</sup> April 2022, fragments of *R. globostellata* of ca. 5-8 cm long were cut from 50 different mother colonies in Bouraké (west coast of New Caledonia), and at a control reef located in Tupeti Island (Thio, east coast of New Caledonia). While Bouraké is an extreme environment characterized by extreme and fluctuating conditions (Maggioni et al., 2021), Tupeti Island reef is a control site because environmental conditions were assumed to be at the ambient level. At both sites, fragments were collected by scuba diving and immediately fixed on a labelled PVC plate using a wire cable. Plates were then attached to a PVC structure fixed at 2-3 m depth on the reef (**Figure 4.1**). After three weeks of recovery *in-situ*, sponges showed a complete tissue regeneration where the sponge was cut from the mother colony, and did not show signs of tissue necrosis and discoloration. After the recovery period, sponge fragments were collected at both sites and individually transported

in sterile zip plastic bags. Plastic bags were fully filled with seawater and kept in an icebox during transport. At each site, a Hobo pendant temperature logger recorded each 10 min interval the temperature, which was used to set the average temperature in the acclimation tanks (data not showed). In addition, in Bouraké, sixty fragments of 10-20 cm in diameter of the coral *Acropora tenuis* were collected at 2-4 m depth. Sponges and corals were kept in an icebox and transported within 3 h after collection to the “Aquarium des Lagons” in Nouméa (approximately 90 km south from Bouraké, and 144 km from the control site) where the experiment was run.



**Figure 4.1.** Map of New Caledonia (top panel) with the two study sites and pictures of the fragments of *Rhabdastrella globostellata* freshly transplanted at the control site (Tupeti Island, A), and after one month *in-situ* in the Bouraké lagoon (B). Within A and B, a graph reports a typical temperature variation measured during the summer at each study site. Georep New Caledonia database (<https://georep.nc/>, last access: November 2022) and Qgis software were used to build the figures.

#### 4.2.2 Acclimatization and experimental set-up

Two temperature conditions were used during the experimental set-up: 1) typical summer temperature of 28°C; and 2) fluctuating summer temperature ranging daily from 28.5°C to 33.2°C. These two temperature conditions mimic the *in-situ* conditions measured during the summer 2020 when the temperature values were supposed to affect the *R. globostellata*'s metabolism in Bouraké (Maggioni et al., 2023).

Corals were acclimated and maintained in a unique 120 L tank (flow rates at  $2 \text{ L min}^{-1}$ ) receiving *ca.*  $300 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$  from two led light systems (Mitras LX6100, GHL Germany) on a 12:12 h light:dark cycle. Two submersible pumps (ON 1000, EHEIM, Germany) mixed seawater in the corals tank. During the acclimation period (two weeks), temperature in the corals tank was increased by  $1^\circ\text{C}$  each day from the value measured at the sampling sites (*ca.*  $26^\circ\text{C}$ ) until reach  $28^\circ\text{C}$ , and then maintained at  $28^\circ\text{C}$  during the whole experiment.

Sponges were carefully cleaned of any epibionts and distributed into four tanks, two by site (25 sponges each tank of 48 L; flow rates at  $800 \text{ mL min}^{-1}$ ) for acclimatization. Sponges were then gradually acclimated (during one week) to the two temperature conditions of  $28^\circ\text{C}$  and variable from  $28.5^\circ\text{C}$  to  $33.2^\circ\text{C}$ , respectively with one independent tank per site. Each tank was independently supplied with unfiltered and non-stagnant seawater pumped at 3 m depth from the Baie des Citrons (Nouméa, New Caledonia) ensuring at least one complete water renewal every hour. Seawater in the tanks were mixed using a submersible pump (micro-jet MC 320, Aquarium system, USA).

After acclimatization and coral labelling (see below), sponges were randomly divided among the 10 experimental tanks (20 L each) that were positioned close to the coral tank to allow the labelled mucus to be filtered by the sponges. The experimental tanks received an ambient light of *ca.*  $10 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ , as measured using a NKE PAR with LI-193 spherical quantum sensor. Five tanks were setup at constant temperature ( $28^\circ\text{C}$ ) and contained each four sponges from Bouraké and four from Tupeti Island (**Appendix C- Figure C4.1**). Five more tanks were setup at variable temperature ( $28\text{-}33.2^\circ\text{C}$ ). For each temperature treatment, three tanks were positioned below the coral tank and received directly its seawater, which contained labelled corals (see below), but also could receive independent inflows of water. The remaining four tanks (two for each temperature treatment) received “clean” water (i.e., not from the coral tank) and were used as blanks in the labelling calculations.

Temperature was increased using heaters connected to two Apex controller (Neptune System, USA), which were set-up to either maintain a temperature of  $28^\circ\text{C}$  ( $\pm 0.06^\circ\text{C}$ ), or to simulate the natural temperature fluctuation measured in Bouraké. Seawater temperature was continuously measured by the Neptune System, while  $\text{pH}_T$  was measured two times a day in each tank using a portable pH-meter (913, Metrohm) calibrated with TRIS buffer (Dickson lab, batch #T28).

### 4.2.3 Corals labelling

Corals labelling procedure combined methods used by Naumann et al. (2010), and Rix et al. (2018). Corals were enriched with nutritive sources of labelled  $^{13}\text{C}$  inorganic carbon and  $^{15}\text{N}$  nitrogen by the addition of  $^{13}\text{C}\text{-NaHCO}_3$  and  $^{15}\text{N}\text{-NaNO}_3$  label compounds (Cambridge Isotope Laboratories, 99%

$^{13}\text{C}$  and 98%  $^{15}\text{N}$ ) during 5 days. Each morning at 8:00, the inflow to the coral tank was stopped and  $36 \text{ mg L}^{-1} \text{ NaH}^{13}\text{CO}_3$  and  $1 \text{ mg L}^{-1} \text{ Na}^{15}\text{NO}_3$  were added. The effectiveness of this method was previously tested in a trial experiment (**Appendix C - Table C4.1**). Aquaria pumps maintained water circulation and air exchange for the 8h-labelling period after which the seawater flow-through was resumed. During this period the inflows between the corals tank and the experimental tanks, which were positioned below the coral tank, were closed. Mucus from 15 randomly coral colonies were collected at day 0, 1, and 5 to verify the  $^{13}\text{C}$  and  $^{15}\text{N}$  incorporation. Corals were removed, rinsed in label-free seawater and air exposed for mucus production (2 min). The collected mucus was frozen at  $-20^\circ\text{C}$ , for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  determinations.

#### 4.2.4 Transfer and incorporation of coral mucus to sponges

At the end of the five days of corals labelling, seawater from the coral tank containing isotopically-labelled *Acropora tenuis* was allowed to continuously feed the six 20 L experimental tanks containing *R. globostellata*, therefore allowing sponges to filter the labelled coral mucus. The flow through from the coral tank to the six tanks were regulated to allow  $20 \text{ L h}^{-1}$  of water to flow from labelled corals to sponges.

The incorporation of coral mucus ( $^{13}\text{C}$  and  $^{15}\text{N}$ ) in sponges' tissue and then to detritus were assessed on labelled and non-labelled sponges, while organic matter recycling (i.e., DOC, POC, PON and detritus fluxes), were assessed on labelled and non-labelled sponges maintained at the two temperature treatments (constant and variable). Since it was technically difficult to maintain the variation in the temperature during the incubation, sponges from the variable treatment were maintained during incubation at the highest temperature at which sponges are exposed. Therefore, sponges were incubated at two different temperatures according to their temperature treatment: *i*)  $28^\circ\text{C}$  as control summer temperature, and *ii*)  $32^\circ\text{C}$  as the highest temperature to which Bouraké sponges were exposed for the longest time. The latter was also chosen since I previously hypothesized that  $31^\circ\text{C}$  is the threshold value triggering the break in the carbon recycling for this species (Maggioni et al., 2023). Of the 100 samples collected, only 80 healthy individuals that showed actively seawater pumping were used for the experiment. Indeed, 8 fragments collected at the control site showed signs of bleaching and tissue necrosis when we increased the experimental temperature in the tanks. In contrast, sponges from Bouraké did not show evident signs of stress.

The incorporation of mucus into sponges' tissues (6 labelled tanks + 4 non-labelled tanks) was measured on two specimens each site ( $n = 2$ ), each tank ( $n = 10$ ), and from each treatment ( $n = 2$ ). A total of 40 sponges (24 from labelled tanks + 16 from non-labelled tanks) were incubated after four

days, and the remaining were incubated after 8 days during which sponges received labelled seawater from the coral tank. The collected sponges were removed from the experimental tanks and rinsed in labelled-free flowing seawater during 10 min. Each sponge was then transferred into individual 2 L glass beakers filled with fresh labelled-free seawater and incubated for 3 h. Beakers were semi-submerged in a water bath ( $n = 4$ ) containing each one 15-places submersible Variomag stirring plate. Each water bath contained a total of 13 incubation beakers (12 with sponges and one with only water that was used as a blank incubation) for labelled tanks, while 9 incubation beakers (8 with sponges and one as a blank incubation) for non-labelled tanks. Sponges from both the control (Tupeti) and variable temperature conditions (Bouraké) were kept respectively at 28°C and at 32°C during the incubation. The incubation was performed in the quasi-dark to avoid the contribution of photosymbionts into the C and N recycling. Temperature in the water bath was verified by the temperature probes connected to the Apex systems. The same procedure was applied to both labelled and non-labelled sponges.

#### **4.2.5 Organic matter and seawater chemical measurements**

Changes in dissolved organic carbon (DOC), particulate organic carbon (POC), particulate organic nitrogen (PON), detritus, bacteria and phytoplankton were also measured in each beaker as the difference in their contents in the incubation medium between the beginning and the end of each incubation. These changes in organic matter during incubations are considered as a flux resulting from the sponge metabolism. The following measurements were performed on two replicates for each temperature condition at the beginning (water from the inflow alimentering the tanks) and at the end of the incubation (seawater medium on each incubation beaker). Sponges were stored frozen in sterile zip bags at -20°C for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  analyses, and total C and N content determination. All glass bottles, beakers and vials used were pre-combusted, washed with HCl solutions (10%) and rinsed using milliQ water.

For particulate organic matter (PON and POC), 1.8 L of seawater was filtered into pre-combusted (450°C during 4h) GF/F filters. Filters were then dried at 60°C for 24 h before  $^{13}\text{C}$  and  $^{15}\text{N}$  analyses and total C and N content determination.

For DOC, two pre-combusted (4h at 450°C) glass ampoules were filled with water filtered using a glass syringe filtration system (SGETM) throughout two pre-combusted 25-mm GF/F filters. Samples were then acidified with 70% sulfuric acid ( $\text{H}_2\text{SO}_4$ ), sealed, and stored at room temperature for dissolved organic carbon analysis by high-temperature catalytic oxidation (HTCO) (Sugimura and Suzuki, 1988; Cauwet, 1994) on a Shimadzu TOC-L analyzer. Typical analytical precision was  $\pm 0.1$ -

0.5  $\mu\text{M C}$  (SD). Consensus reference materials (<https://hansell-lab.earth.miami.edu/consensus-reference-material/index.html>) were injected every 10 to 20 samples to ensure stable operating conditions.

Total bacteria abundance (BA) and phytoplankton abundance were measured on 1.49 mL of water fixed with 0.165 mL of paraformaldehyde (PFA) in cryovials, first kept 10 min in the dark, and then stored at  $-80^{\circ}\text{C}$ . The different plankton and BA components were detected and counted using BD Accuri<sup>TM</sup> C6 cytometer. In this case, phytoplankton were analyzed for 10 min at high flow rate, while bacterioplankton were analyzed for 5 min at high flow rate (see **Appendix B – Section B3.2** for further details of the methods used). In addition, bacteria and phytoplankton (LPOC) in  $\mu\text{mol C h}^{-1} \text{g}^{-1}$  ingested by sponges was used to evaluate their contribution in the C food sources.

Consumption (i.e., reduction; negative values) and/or production (i.e., increase; positive values) of each parameter measured during the incubations were calculated as the difference between their concentrations at the end and at the beginning of incubations. Then, data were corrected by the change in concentrations occurring in the blank chambers (only water), and normalized by the duration of incubation (h), the tank volume (L), and the sponge dry weight (g). Carbon sources were calculated as the percentage of the carbon (DOC, POC, bacteria and phytoplankton) ingested by the sponges. Note that in this case only consumed parameters (negative values) were considered.

#### 4.2.6 Isotopic analyses

Coral mucus and sponge subsamples were lyophilized, shredded and homogenized by a mortar and pestle for both labeled and unlabeled samples. Subsamples of sponge tissues, coral mucus and decalcified POM filters were then weighed and transferred into tin capsules for bulk  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotope analyses, and total C and N content.

Stable isotope and elemental analyses were conducted using elemental analyzer mass spectrometer Integra CN Sercon at the Mediterranean Institute of Oceanography (Marseille) (Raimbault et al., 2008; Lacoste et al., 2016). The carbon and nitrogen isotopic ratios are expressed in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  notation (Vienna-PeeDee Belemnite limestone for carbon and atmospheric  $\text{N}_2$  for nitrogen) as the deviation from standards in parts per mill, using the following equation:

$$\delta^{13}\text{C} \text{ and } \delta^{15}\text{N} = [(R \text{ sample}/R \text{ standard}) - 1] \times 1000$$

where R sample and R standard are the ratio of  $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$  in the sample and in the standard reference materials, respectively. R standard C = 0.011118 and R standard N = 0.00368 from

Atmospheric N<sub>2</sub>. The precisions of replicate analyses were 0.5‰ and 0.3‰ for  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ , respectively.

The incorporation of excess (i.e., background)  $^{13}\text{C}$  and  $^{15}\text{N}$  is expressed as the specific enrichment, i.e.  $\Delta \delta^{13}\text{C}$  and  $\Delta \delta^{15}\text{N}$  values. This was calculated by subtracting the background  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of the control sponges from the values of the sponges exposed to labeled corals. The fractional abundance (F) of the heavy isotope in the sample ( $^{13}\text{C}/[^{13}\text{C} + ^{12}\text{C}]$  or  $^{15}\text{N}/[^{15}\text{N} + ^{14}\text{N}]$ ) was calculated as  $F_{\text{sample}} = R_{\text{sample}}/(R_{\text{sample}} + 1)$ , and reported as atom percent (atm % =  $F \times 100$ ). The excess fractional abundance of heavy isotope (E) was calculated as the difference between the F sample and F background:  $E = F_{\text{sample}} - F_{\text{background}}$ .

Incorporation rates are the total uptake (I), calculated by multiplying E by the C or N content of the sponge tissue or detritus. To determine the total mucus-derived C ( $^{12}\text{C} + ^{13}\text{C}$ ) and N ( $^{14}\text{N} + ^{15}\text{N}$ ) incorporated, the total uptake (I) was divided by the fractional abundance of the coral mucus supplied to the sponges. To determine the C:N ratios of coral mucus-derived organic matter incorporated into the sponge tissue and detritus, the total amount of coral mucus-derived C incorporated was divided by the total amount of coral mucus-derived N incorporated. The analytical error was less than 0.13 h for carbon and 0.61 h for nitrogen.

The enrichment in the sponge tissue was graphically represented separately for day 4 and day 8, and pooled together for the statistical analysis. The incorporation of  $^{13}\text{C}$  and  $^{15}\text{N}$  into the sponge tissues was normalized by the weight of sponges ( $\text{g}^{-1}$ ) and the number of days ( $\text{day}^{-1}$ ). Similarly, the detritus produced during the 3 h incubations was calculated on a day scale assuming regular daily production in the dark. Incorporation values of both sponges' tissues and detritus are expressed in  $\mu\text{mol C or N g}^{-1} \text{d}^{-1}$ . The specific fluxes of  $^{13}\text{C}$  and  $^{15}\text{N}$  were normalized for the number of days ( $\mu\text{mol C or N d}^{-1}$ ), and expressed as their percentages assimilated in sponge tissue, and excreted as detritus by the sponges.

#### **4.2.7 Statistical analyses**

General linear model GLM analysis with “tank” as fixed factor was performed to test for a potential tank effect (three tanks per treatment). After verification of the absence of any tank effect, data between the replicated tanks were pooled, and the factor tank was excluded from subsequent models. Since we were not interested to test for the effect of time in the incorporation of  $^{13}\text{C}$  and  $^{15}\text{N}$ , but we only tested the rates of their incorporation between tissue and detritus, we pooled the data collected during the two incubations, after 4 and 8 days. Significant differences in the organic matter recycle, enrichment, total flow and incorporation, were tested with two-way ANOVAs between temperature

conditions (two levels, 32°C and 28°C), sponges origin (two levels, control and Bouraké), and their interaction. When the interaction was significant, a Tukey *post hoc* test was performed. ANOVAs were run after verification of normality and homoscedasticity of the data. All analyses were conducted in R V4, using stats, factorminer, and vegan packages (R Core Team, 2019).

## 4.3 Results

### 4.3.1 Environmental parameters

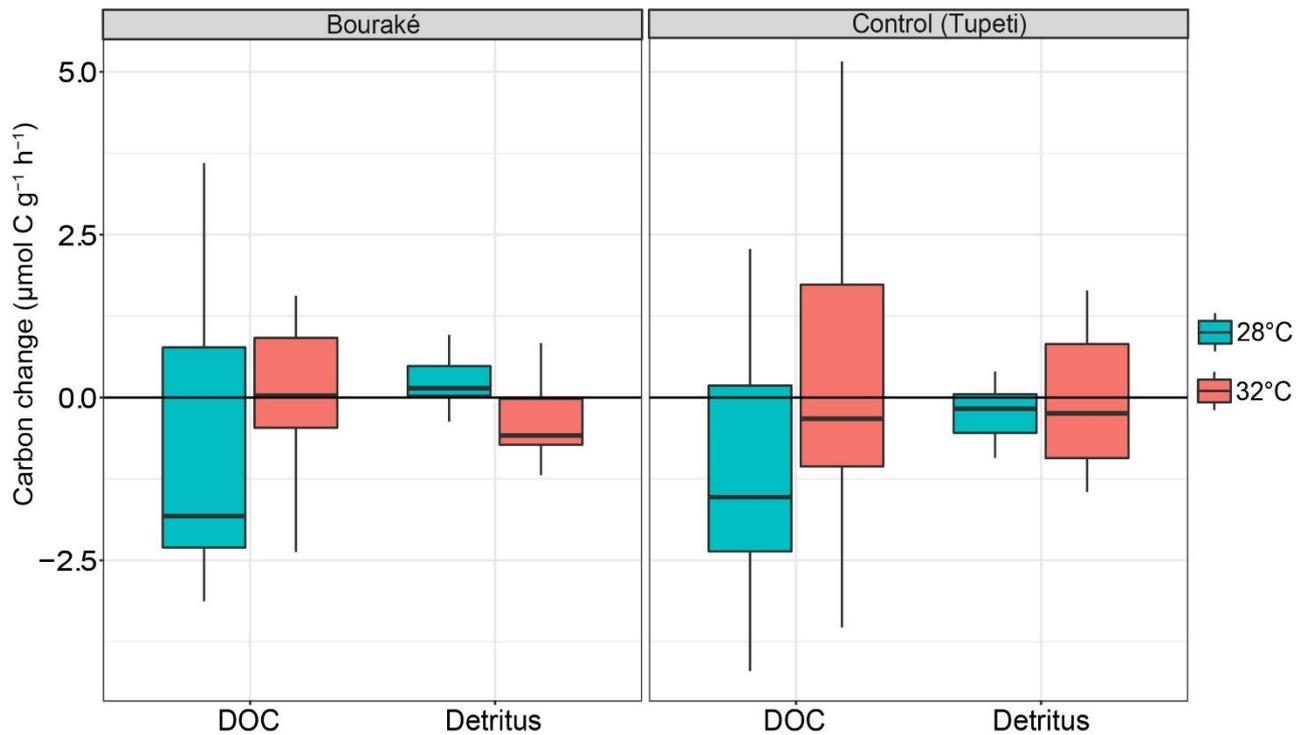
Seawater temperature variation settled, and controlled by the Neptune System, matched with the temperature variability measured *in-situ* during the summer 2020 (**Appendix C - Figure C4.1**). Temperatures varied from 28.5°C to 33.2°C in all the tanks for the variable treatment, and from 27.7°C to 28.3°C in all the tanks for the constant treatment, without graphical differences. Seawater pH<sub>T</sub> were  $8.1 \pm 0.05$  and  $8.0 \pm 0.07$  at the variable and constant treatment, respectively.

### 4.3.2 Carbon and nitrogen consumption and production

Dissolved organic carbon (DOC) was significantly more consumed at 28°C than at 32°C for both control and Bouraké sponges (**Figure 4.2; Table 4.1a**). Although box plots showed some variability in the data (all negative skewness), in general all sponges consumed DOC, with the exception of the control sponges at 32°C, which produced  $0.08 \pm 2.14 \mu\text{mol C g}^{-1} \text{h}^{-1}$  (mean  $\pm$  SD). Control sponges at 28°C consumed the highest amount of DOC (**Appendix C - Table C4.2a**). During incubations, sponges consumed both phytoplankton and bacteria, while produced POC and PON (**Appendix C - Table C4.2a**). Significant differences were found between temperatures for the bacteria and DOC consumption, and for the interaction term between temperature and origin for POC, PON (**Table 4.1a**). A significant interaction between temperatures and origins was also found for detritus with the highest detritus production (i.e., production) and a negative detritus production (i.e., consumption) measured respectively at 28°C and 32°C for Bouraké (**Table 4.1a, Appendix C - Table C4.2a**).

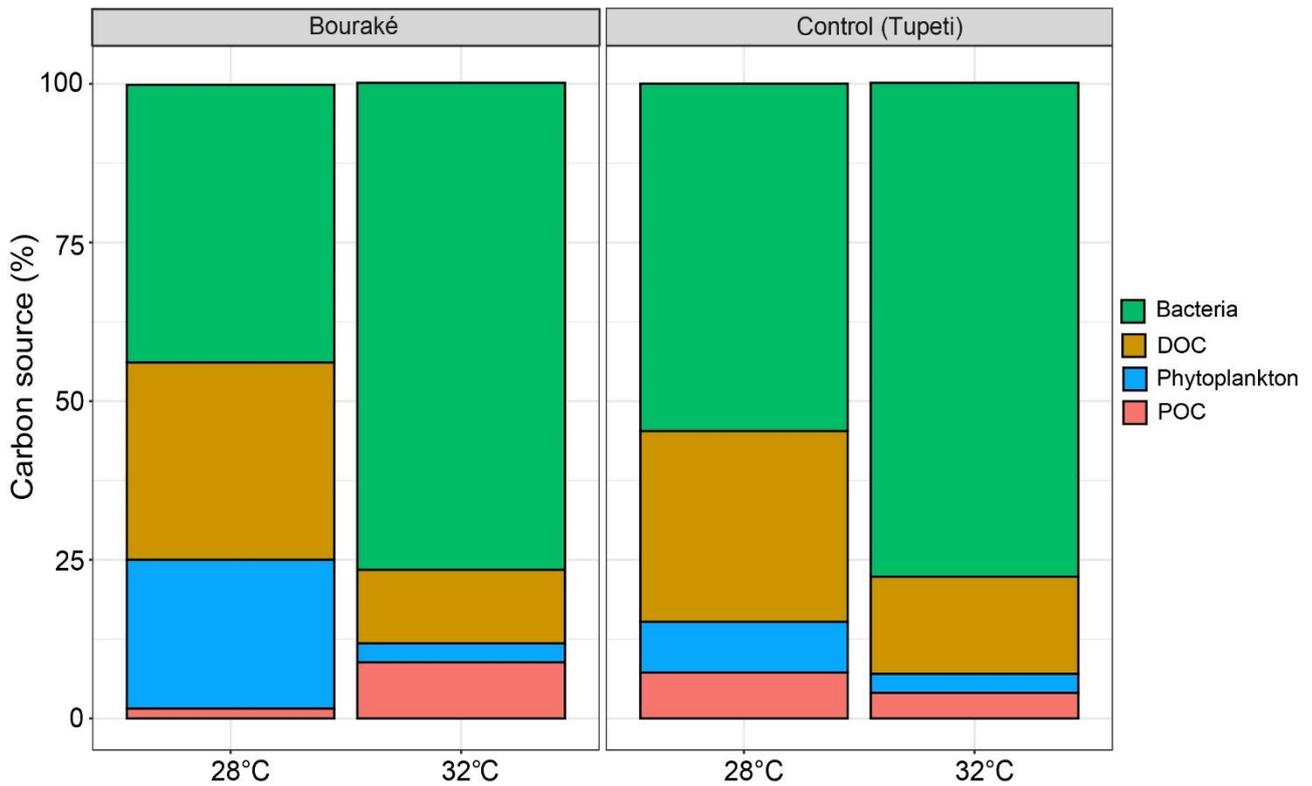
**Table 4.1.** Summary of two-way ANOVAs between temperature conditions (two levels, constant and variable), origin (two levels, control and Bouraké), and their interaction. **a)** the carbon and nitrogen consumption and/or production measured during 3h of incubation; **b)** carbon sources consumed during the incubations (only data showing carbon consumption were included); **c)**  $^{13}\text{C}$  and  $^{15}\text{N}$  enrichments ( $\Delta\delta^{13}\text{C}$  and  $\Delta\delta^{15}\text{N}$ ) measured on both the sponge tissue and detritus at the end of incubations; **d)**  $^{13}\text{C}$  and  $^{15}\text{N}$  incorporation measured on both tissue and detritus; and **e)**  $^{13}\text{C}:^{15}\text{N}$  ratio. When the interaction was significant, a Tukey HSD test was applied and only significant differences were reported. B. is Bouraké and C. the control at 32°C and 28°C.

	Temp F-value ( <i>P</i> )	Origin F-value ( <i>P</i> )	Temp x Origin F-value ( <i>P</i> )	HSD Post hoc ( <i>P</i> )
<b>a) C and N consumption and/or production</b>				
DOC	6.43 (< <b>0.05</b> )	0.006 (0.938)	0.164 (0.687)	
POC	1.865 (0.176)	0.030 (0.8.3)	4.369 (< <b>0.05</b> )	
PON	0.436 (0.511)	0.001 (0.977)	4.635 (< <b>0.05</b> )	
Detritus	2.518 (0.117)	0.003 (0.954)	5.225 (< <b>0.05</b> )	B.32°C < B.28°C (< <b>0.05</b> )
Bacteria	17.60 (< <b>0.001</b> )	1.27 (0.263)	0.364 (0.548)	
Phytoplankton	0.454 (0.502)	3.72 (0.06)	0.428 (0.515)	
<b>b) Carbon sources</b>				
Bacteria	19.46 (< <b>0.001</b> )	2.63 (0.109)	2.45 (0.122)	
DOC	8.305 (< <b>0.01</b> )	0.016 (0.900)	0.614 (0.438)	
POC	8.338 (< <b>0.01</b> )	4.464 (< <b>0.05</b> )	1.954 (1.170)	
Phytoplankton	0.050 (0.824)	2.107 (0.151)	1.266 (0.264)	
<b>c) <math>^{13}\text{C}</math> and <math>^{15}\text{N}</math> enrichment</b>				
$^{13}\text{C}_{\text{tissue}}$	3.841 (0.06)	0.162 (0.689)	5.429 (< <b>0.05</b> )	B.32°C > C.28°C (< <b>0.001</b> ) C.32°C < B.32°C > C.28°C (< <b>0.001</b> )
$^{15}\text{N}_{\text{tissue}}$	8.136 (< <b>0.01</b> )	20.42 (< <b>0.001</b> )	12.14 (< <b>0.001</b> )	B.32°C > B28°C (< <b>0.05</b> ) C.32°C < B.32°C (< <b>0.05</b> )
$^{13}\text{C}_{\text{detritus}}$	1.403 (0.243)	0.234 (0.631)	18.994 (< <b>0.001</b> )	B.32°C > B28°C (< <b>0.05</b> ) C.32°C < B.32°C (< <b>0.05</b> )
$^{15}\text{N}_{\text{detritus}}$	12.85 (< <b>0.001</b> )	0.056 (0.814)	1.558 (0.219)	
<b>d) <math>^{13}\text{C}</math> and <math>^{15}\text{N}</math> incorporation</b>				
$^{13}\text{C}_{\text{tissue}}$	3.22 (0.07)	0.767 (0.386)	14.056 (< <b>0.001</b> )	B.32°C > B.28°C (< <b>0.01</b> ) C.28°C > B.28°C (< <b>0.05</b> )
$^{15}\text{N}_{\text{tissue}}$	8.184 (< <b>0.001</b> )	21.454 (< <b>0.001</b> )	24.546 (< <b>0.001</b> )	B.32°C > B.28°C (< <b>0.001</b> ) C.28°C > B.32°C (< <b>0.001</b> ) C.32°C < B.32°C (< <b>0.001</b> )
$^{13}\text{C}_{\text{detritus}}$	10.13 (< <b>0.01</b> )	0.933 (0.34)	3.194 (0.08)	
$^{15}\text{N}_{\text{detritus}}$	13.51 (< <b>0.001</b> )	1.968 (0.168)	2.061 (0.159)	
<b>e) C:N ratio in sponge tissue and detritus</b>				
C:N <sub>tissue</sub>	1.49 (0.23)	5.71 ( <b>0.05</b> )	0.265 (0.61)	
C:N <sub>detritus</sub>	4.24 ( <b>0.05</b> )	2.36 (0.232)	4.44 ( <b>0.05</b> )	C.32°C > C.28°C (< <b>0.05</b> )



**Figure 4.2.** Sponge dissolved organic carbon (DOC) and detritus consumption/production during the incubations at 28°C and 32°C for both sponges from Bouraké and Control site (Tupeti). Boxes are the interquartile range of data (25th and 75th percentiles); the horizontal line is the median, and the whiskers represent the data range (i.e., minimum and maximum).

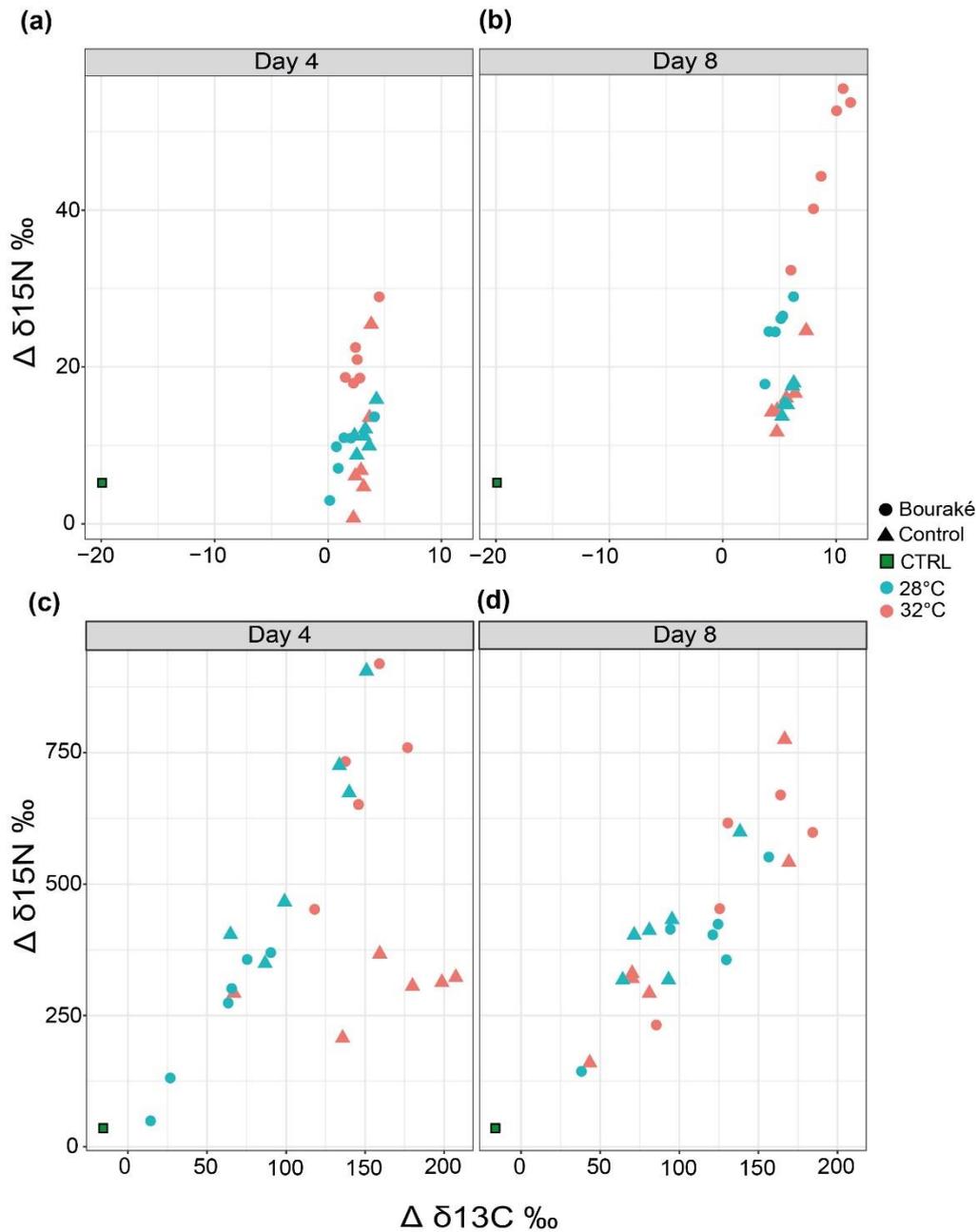
To investigate which carbon source were used by the sponges, only the carbon uptake (negative values) was accounted. Despite DOC was the highest carbon source available (**Appendix C - Table C4.2c, d**), bacteria was the main source of carbon consumed at both temperatures (**Figure 4.3**). Temperature significantly affected carbon source uptake for bacteria, DOC and POC, the latter also showing significant differences between origins. Consumption was the highest at 32°C for bacteria, and at 28°C for DOC and POC (**Table 4.1b; Figure 4.3**), while Bouraké sponges consumed more POC at 32°C and control sponges consumed more POC at 28°C (**Figure 4.3**).



**Figure 4.3.** Carbon source uptake (%) by the sponge at 28°C and 32°C from Bouraké and Control (Tupeti) sites. Only consumed values (negative values) of bacteria, dissolved organic carbon (DOC), phytoplankton and particulate organic carbon (POC) as sources of carbon were considered.

#### 4.3.3 Sponges and detritus enrichment

After 5 days of incubation with labelled C and N nutritive sources, coral mucus resulted to be enriched on average by  $416.5 \pm 62.5\%$  for  $\delta^{13}\text{C}$  and  $900.6 \pm 185.8\%$  for  $\delta^{15}\text{N}$  (**Appendix C - Tables C4.3**). Isotopic signatures incrementally increased in the sponge tissue between day four and day 8, suggesting gradual accumulations of labelled C and N over time, and finally their transfers as detritus (**Figure 4.4**). Data were pooled between days. Statistical differences were found for the interaction term between temperature and origin for the  $^{13}\text{C}$  accumulated into the sponge tissue and transferred into detritus (**Table 4.1c**). The detritus  $^{15}\text{N}$  enrichment differed between temperatures, while it differed between temperatures, origins, and their interaction in the sponge's tissue. In general, both  $^{13}\text{C}$  and  $^{15}\text{N}$  were highly enriched at 32°C, both in the sponge tissue and into detritus.



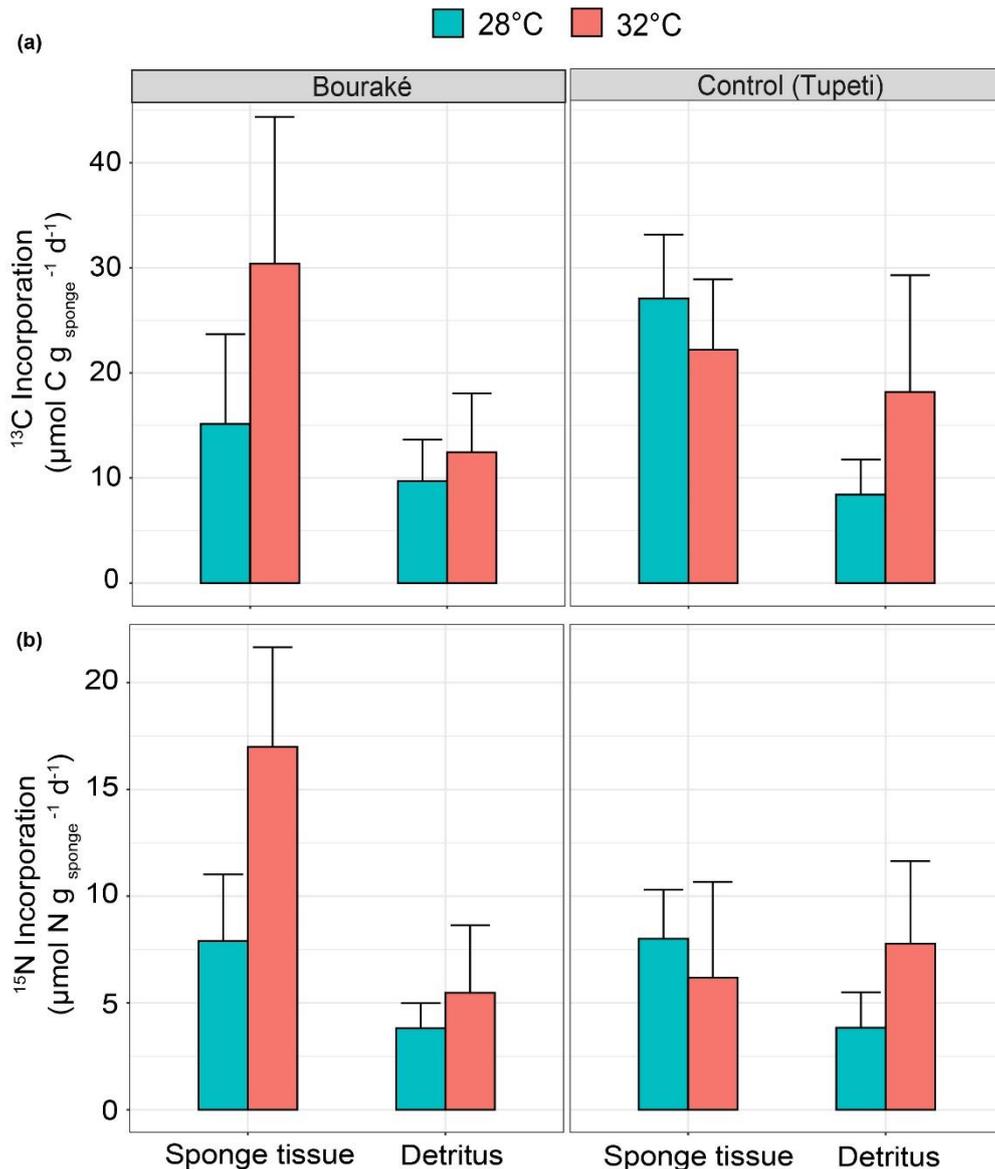
**Figure 4.4.** Stable isotope enrichment of  $^{13}\text{C}$  and  $^{15}\text{N}$  in sponge tissue (a-b) and detritus (c-d) after 4 and 8 days of experiment, at  $28^{\circ}\text{C}$  and  $32^{\circ}\text{C}$ , for both sponges from Bouraké (dots) and Control (triangles). Non-enriched stable isotope signature as also reported as CTRL (square). Data as expressed as isotope tracer enrichment  $\Delta\delta^{13}\text{C}$  (‰) and  $\Delta\delta^{15}\text{N}$  (‰). Note that detritus enrichment was calculated on a day basis.

#### 4.3.4 $^{13}\text{C}$ and $^{15}\text{N}$ total fluxes and incorporation in the sponge tissue and detritus

Enriched  $^{13}\text{C}$  and  $^{15}\text{N}$  total fluxes in sponge tissue were significantly different in the interaction between temperature and origin, while detritus only differed between temperatures (**Appendix C - Table C4.4**). Sponges from Bouraké showed higher  $^{13}\text{C}$  and  $^{15}\text{N}$  total flux at  $32^{\circ}\text{C}$  for both tissue and detritus compared to  $28^{\circ}\text{C}$ , while control sponges showed higher  $^{13}\text{C}$  and  $^{15}\text{N}$  total flux at  $32^{\circ}\text{C}$  only

for detritus compared to 28°C (**Appendix C - Figure C4.2**). Overall, both  $^{13}\text{C}$  and  $^{15}\text{N}$  labelled fluxes of coral mucus were always more accumulated in sponge tissue (ca 57%, and 65%, respectively) than excreted as detritus (ca 42%, and 34%, respectively), with only an exception for control sponges at 32°C, which showed a balance between the total flow excreted (47%  $^{13}\text{C}$ , and 52%  $^{15}\text{N}$ ) vs accumulated (52%  $^{13}\text{C}$ , and 47%  $^{15}\text{N}$ ) (**Appendix C - Figure C4.3**). When carbon and nitrogen fluxes were normalized to the sponge weight, both the statistical significances and the sponge responses stay consistent (**Table 4.1d; Figure 4.5**). Specifically, although significant differences in the interaction of temperatures and origins were found in sponge tissue, detritus showed significant differences only between temperatures with higher incorporation at 32°C for both  $^{13}\text{C}$  and  $^{15}\text{N}$  (**Table 4.1d; Figure 4.5**). Incorporation of  $^{13}\text{C}$  in sponge tissue was higher for the Bouraké sponges at 32°C than at 28°C ( $30.4 \pm 13.9 \mu\text{mol C g}^{-1} \text{d}^{-1}$ , and  $15.1 \pm 8.5 \mu\text{mol C g}^{-1} \text{d}^{-1}$ , respectively), and  $^{13}\text{C}$  in sponge tissue for the Bouraké sponges was lower than control sponges at 28°C (**Table 4.1d; Figure 4.5**). The  $^{15}\text{N}$  also showed higher incorporation ( $16.9 \pm 4.7 \mu\text{mol C g}^{-1} \text{d}^{-1}$ ) for Bouraké sponges at 32°C than at 28°C for both control and Bouraké sponges ( $8.0 \pm 2.3 \mu\text{mol C g}^{-1} \text{d}^{-1}$ , and  $7.9 \pm 3.1 \mu\text{mol C g}^{-1} \text{d}^{-1}$ , respectively). In addition, incorporation of  $^{15}\text{N}$  for Bouraké sponges at 32°C was higher than for control sponges at 32°C (**Table 4.1d; Figure 4.5**).

The C:N ratio in sponge tissues did not significantly differ between temperatures but showed differences between origins, while its ratio in control detritus at 32°C was significantly higher than at 28°C (**Table 4.1e**). The C:N ratio was always double in the sponges tissue than in the detritus (**Appendix C - Figure C4.4**), with a total average of  $4.53 \pm 0.21$  and  $2.74 \pm 1.35$ , for sponge tissue and detritus, respectively (all data pooled).



**Figure 4.5.** Sponge tissue and detritus incorporation of  $^{13}\text{C}$  (a) and  $^{15}\text{N}$  (b) at 28°C and 32°C, for both sponges from Bouraké and control site (Tupeti). Daily incorporation rates (mean  $\pm$  SD) of coral mucus are expressed in  $\mu\text{mol C}$  or  $\text{N g}^{-1} \text{d}^{-1}$ .

## 4.4 Discussion

In the previous chapter I measured *in-situ* the ability of sponge *R. globostellata* living in the lagoon of Bouraké to recycle nutrients and organic matter (Maggioni et al., 2023). Data showed that the massive sponge consumed DOC and produced detritus. However, the process was stopped when sponges underwent very high temperatures (up to 31°C), i.e., the sponge did not produce detritus. While it was possible to obtain these results because the measurements were made *in-situ* and using a sponge species likely adapted to extreme conditions, it was not possible to identify if temperature was the cause of the change in the C and N allocation. Indeed, in experiments conducted in the nature, multi factors can act in combination and synergy. In addition, I only used sponges from Bouraké,

lacking a control population living at “normal” reef conditions. Based on these preliminary findings, in the present chapter *i*) only the effect of temperature was tested by simulating in aquaria the thermal regime experienced at Bouraké and at a control reef; *ii*) enriched stable isotopes were used to follow the allocation of C and N into the sponge’s tissue and/or detritus; *iii*) the responses of sponges living at the extreme reef at Bouraké were compared with a control reef.

Results of this chapter shows that sponges from Bouraké, incorporate more  $^{13}\text{C}$  and  $^{15}\text{N}$  as coral mucus into the tissue than they release as detritus, and that these rates double at the high temperatures of  $32^\circ\text{C}$ . In contrast, the control sponges have an opposite response at elevated temperatures, with less  $^{13}\text{C}$  and  $^{15}\text{N}$  incorporated in the tissue, and more released as detritus. The ability of sponges to uptake the DOC produced by the reef is well known (e.g., Yahel et al., 2003; Hoer et al., 2018; McMurray et al., 2018; Wooster et al., 2019; Hildebrand et al., 2022), as well as the role of the associated microbes in the C and N cycles (e.g., Hoffmann et al., 2009; Feng and Li, 2019). They can be recycled and released as detritus, mainly in encrusting sponges (sponge loop pathway, De Goeij et al., 2013), and/or retained as biomass, mainly in massive sponges (e.g., Pawlik et al., 2016; McMurray et al., 2018). The factors triggering the two distinct pathways have not been fully clarified. In agreement with my previous *in-situ* measurements (Maggioni et al., 2023), sponges from Bouraké incubated at  $28^\circ\text{C}$  recycled DOC and discharged detritus during incubations (i.e., sponge loop pathway) (**Figure 4.2**). In contrast, sponges from the control reef incubated at the same temperature did not produce detritus, as already found for several massive sponges (e.g., Pawlik et al., 2016; McMurray et al., 2018). These findings were corroborated by  $^{13}\text{C}$  and  $^{15}\text{N}$  incorporation in the form of coral mucus, and released as detritus. Indeed, at  $28^\circ\text{C}$  sponges originated from the control reef showed higher accumulation of  $^{13}\text{C}$  in the tissue than sponges from Bouraké (average ratio Tissue T:Detritus D = 3.2 vs 1.6, respectively), while the recycling of N was similar between sponge populations (T:D = 2.11 vs 2.07, respectively) (**Figure 4.5**). These results suggest a different C metabolism that may depend, among other factors, on the environmental characteristics of the sponges’ origin.

Responses were exacerbated at the high temperature showing higher variations in their uptake and incorporation rates. Indeed, at  $32^\circ\text{C}$  the majority of sponges, both Bouraké and control, always continued to uptake DOC (**Figure 4.2**). Most importantly, Bouraké sponges stopped producing detritus, while the control sponges showed high variability with several specimens producing detritus (**Figure 4.2**). The  $^{13}\text{C}$  and  $^{15}\text{N}$  incorporation clearly showed that the differences between the two sponges depend on the sites of origin. While the Bouraké sponges incubated at  $32^\circ\text{C}$  stored more  $^{13}\text{C}$  and  $^{15}\text{N}$  in the tissue than in the detritus, when compared to  $28^\circ\text{C}$  (T:D = 2.4 at  $32^\circ\text{C}$  vs 1.6 at  $28^\circ\text{C}$

for C, and 3.1 vs 2.1 for N), control sponges showed an opposite trend with a tendency to reduce their incorporation in the tissue, and significantly increase incorporation in the detritus (T:D = 1.2 at 32°C vs 3.2 at 28°C for C, and 0.8 vs 2.11 for N) (**Figure 4.5**). These results suggest that sponges adapted to lower temperature (control) could be stressed when exposed to high temperature of 32°C, and they increase cell turnover to maintain tissue homeostasis, resulting in higher cell shedding and tissue degradation (i.e., higher detritus). This was already confirmed during the recovery period of this experiment, because 8 sponges from the control reef died or showed signs of tissue necrosis, while Bouraké sponges did not show any evident sign of stress. Alteration in choanocyte chambers size and density has already been reported on non-adapted sponges exposed to thermal stress (> 31°C) (Massaro et al., 2012). Under stress, sponges continually shed choanocyte cells which undergo a rapid turnover (De Goeij et al., 2009), and consequently produce detritus (Maldonado, 2016). Unfortunately, our short-term experiment did not enable us to follow the fate of sponges in the long term, and see if this stress at 32°C was followed by tissue necrosis and injury or even the sponge death. However, it has been reported that high temperature of more than 30°C induced mortality in sponges (e.g., López-Legentil et al., 2008; Bell et al., 2018b; Ramsby et al., 2018b). Further long-term experiments in aquaria and a reciprocal transplantation of this species between Bouraké and the control reef will help to shed light on its ability to cope with thermal stress.

The Bouraké sponge seems to better cope with a short-term exposure to high temperatures, likely because this population have already experienced chronical exposures to temperatures around 28°C with peaks at 33°C. For example, in summer 2020 sponges experienced temperature higher than 28°C during 40% of the summer season, and species experienced fluctuations in daily temperature of up to 6.5°C (Maggioni et al., 2021). This hypothesis is in agreement with recent studies suggesting that reef organisms living in fluctuating temperature environments are more resistant to temperature stress (e.g., Oliver and Palumbi, 2011; Rivest et al., 2017). Indeed, as we previously found, the respiration rates of *R. globostellata* from Bouraké was not significantly affected by an increase in temperature between 26°C and 32°C, suggesting a degree of adaptation (Maggioni et al., 2023). In agreement, at the high and fluctuating temperatures tested during this experiment, <sup>13</sup>C and <sup>15</sup>N were still more incorporated in the tissues than in the detritus, when compared to 28°C. This response involved a tradeoff causing a detrimental effect on the relative availability of C and N for other trophic levels. I believe that *R. globostellata* have developed some adaptive strategies to save energy in the tissue and avoid distress during the most extreme environmental conditions, fostering its growth instead of recirculating nutrients. This hypothesis is in agreement with a recent study on sponge's transcriptome showing that sub-lethal temperature of 32°C may promote tissue growth and protect sponge cells

against extended stress conditions, therefore increasing the sponge ability to mitigate cellular damage (Guzman and Conaco, 2016).

Although we did not measure microbes associated with *R. globostellata*, it is known that the species has a high-microbial abundance (HMA, Moitinho-Silva et al., 2017a), and that associated bacteria play an important role in the sponge metabolism, especially with regard to N cycling (e.g., Taylor et al., 2007). Variable NO<sub>x</sub> production and NH<sub>4</sub><sup>+</sup> recycling were already measured in *R. globostellata* under different tide conditions (Maggioni et al., 2023). Here, we found that Bouraké sponge at 32°C incorporated more N in the tissues than at 28°C, and likely used it for bacterial metabolic processes that could give them the surplus of energy necessary to support elevated temperatures. Another strategie to cope with high temperatures could be the reduction of the cell turnover, favoring cell proliferation than cell shedding. This could allow sponges to save energy to maintain their health during thermal stress avoiding cells degradation (i.e., detritus production), a protection mechanism that control sponges seem not to have.

One potential factor enabling *R. globostellata* to cope with the thermal stress could be related to its ability to rely on different sources for C, since I found differences in the uptake of food between 28°C and 32°C, and for both Bouraké and control sponges. Food selection may result from either passive processes, in which the physical properties of the sponge filtering lead to differential uptake of picoplankton cell types, or active processes, in which food selection is mediated by sponge behaviour (Jürgens and DeMott, 1995; Maldonado et al., 2010). In general, sponges have been reported to selectively filter phototrophic bacteria over heterotrophic bacteria (Hanson et al., 2009; Topçu et al., 2010; Yahel et al., 2006), but some sponges prefer the relatively rarer but labile phytoplankton when compared to the numerical dominant heterotrophic bacteria (Maldonado et al., 2012; McMurray et al., 2016). *R. globostellata* from Bouraké seems to use passive filtration mechanisms (i.e., not selective) when food is not limiting in the environment (Maggioni et al., 2023). Indeed, *in-situ* incubations showed that the abundance of phytoplankton and bacteria during tides had a great effect on sponge metabolism. Therefore, *R. globostellata* fed more on phytoplankton during the high tide, and inversely, more on detritus and bacteria during the low tide, likely because their respective abundances in seawater differed with the tide. In the present study, blank-normalized ingestion rates measured during incubations in chambers containing the same pool of water (**Appendix C - Table C4.2**), showed that at 28°C, sponges from Bouraké mostly filtered on bacteria and DOC (**Figure 4.3**), in the same way of control sponges, and although the latter showed higher percentages. However, Bouraké sponges consumed more phytoplankton and less POC compared to control sponges, suggesting a different metabolic carbon food source selection depending on the sponge origin. At

32°C, we did not find any difference between the two sites, with bacteria as the main carbon sources filtered. This result confirm a previous study showing that temperature above 31°C also caused a behavioral shift in sponges feeding with higher selective filtration efficiency for phototrophic microbes (Massaro et al., 2012). Similarly, the results of this chapter suggest that high temperatures may change the preferred source of C, possibly toward a more energetic and less cost food source. Future works could include NanoSIMS technique to better understand the utilization of the incorporate C and N in sponge tissues.

Although sponges in both sites accumulated carbon derived coral mucus in their tissues, and released it in the form of detritus, the balance between accumulated and excreted C and N changed according to the thermal regimes and the sponges' origin. Such a change in C and N allocation at high temperature, with less detritus being redistributed to the surrounding ecosystem, could be a tradeoff used by the Bouraké sponge population to cope with the extreme environmental conditions (i.e., high temperatures). This chapter highlighted that ocean warming could change the way by which sponges recycle C and N, altering the quality availability of essential sources of energy in coral reefs.

**CHAPTER 5:**  
GENERAL DISCUSSION

---

This PhD thesis explored the responses of a tropical sponge to extreme environmental conditions (high temperature, low pH, and low dissolved oxygen) in the Bouraké natural laboratory (New Caledonia). There, the average seawater temperature is similar to those forecasted in the context of global ocean changes, and physico-chemical parameters fluctuate with the tide (Maggioni et al., 2021). The seawater pH and dissolved oxygen decrease during falling tide and reach the lowest and extreme values at low tide (7.2 pH and 1.9 mg O<sub>2</sub> L<sup>-1</sup> at Bouraké vs. 7.9 pH and 5.5 mg O<sub>2</sub> L<sup>-1</sup> at control reefs). This thesis aims to fill the gaps in our knowledge of how these conditions affect the recycling of sponge nutrients and organic matter. First, I performed a long-term physical, chemical, and benthic characterization of the Bouraké lagoon (chapter 2), which was used after to study the effects of extreme environmental conditions on sponge metabolism.

*Rhabdastrella globostellata* was chosen as model and incubated *in-situ* using benthic chambers during normal (high tide) and extreme (low tide) conditions (chapter 3). Changes in physical and chemical seawater parameters during the incubations were examined and suggested that temperature may be the main factor affecting the carbon cycle of sponges in Bouraké (stop of detritus production). To confirm this hypothesis, an *ex-situ* aquaria experiment was performed to explore the effect of the high temperature on the ability of *R. globostellata* to recycle nitrogen and carbon (chapter 4). The responses of *R. globostellata* from Bouraké and a control site were analyzed at 28°C and 32°C. The ability of the sponge to recycle nitrogen and carbon was assessed by tracking labeled <sup>13</sup>C and <sup>15</sup>N in both sponge tissue and detritus. Result showed that high temperature drives the shift in C and N allocation in Bouraké sponges.

In this chapter, I will review the main results of my research and show how the responses of Bouraké sponges suggest their ability to cope with extreme conditions. Finally, I will discuss how my results could help to predict sponge responses in future coral reefs and to better understand to what extent their responses will affect the overall reef ecosystem.

### **5.1 *Rhabdastrella globostellata* shows different strategies to cope with extreme environmental conditions**

Sponges influence ecosystem functioning by modifying both biotic and abiotic factors (Bell, 2008). To survive environmental changes, sponges must either acclimate or adapt, like other benthic marine invertebrates (Hoegh-Guldberg, 1999). Acclimatization involves changes in various physiological processes to perform better under new environmental conditions (i.e., acclimatory mechanisms), while adaptation occurs through the selection of individuals better able to cope with the new environmental conditions within the population (Hoegh-Guldberg, 1999).

As described in the third chapter, different environmental conditions (normal and extreme) lead to different responses in the metabolism of *R. globostellata* in Bouraké. The main metabolic difference was found in sponge silicate and carbon cycling. Both silicate and dissolved organic carbon were consumed more during the extreme conditions in the Bouraké lagoon (low tide). During low tide, sponges can benefit from the higher concentrations of orthosilicic acid and organic carbon in the environment to store them as a reserve, and to provide energy and build their skeleton. Some authors have reported that different environmental conditions can lead to a changes in the morphology and composition of spicules (e.g., Bell et al., 2002; Meroz-Fine et al., 2005). For example, the sponge *Tetilla* sp. has a higher proportion of spicules in exposed shallow waters than in still water (Meroz-Fine et al., 2005). A higher proportion of spicules results in a stiffer and more robust sponge shape that has minor deformation, even under high water velocity (Palumbi, 1986). A robust skeleton is likely to facilitate the adaptation of sponges to climate change (Bennett et al., 2017). In addition to the number of spicules, their dimensions (thickness and length) contribute to rigidity and flexibility of the sponge. Sponges living in high energy sites have longer and thicker spicules than low energy sites (Bell et al., 2002). Similarly, in the Bouraké lagoon, sponges may accumulate orthosilicic acid to build numerous long and thick spicules in order to maintain skeletal growth, rigidity, and flexibility to cope with the environmental change and the high current velocity that occurs during rising and falling tides (chapter 2).

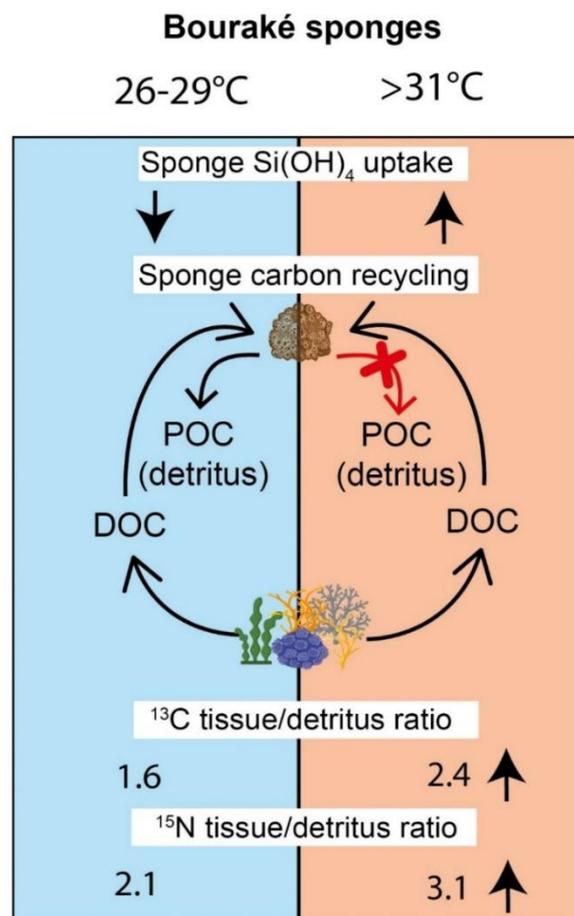
In coral reef ecosystems, encrusting sponges uptake DOM and release it as detritus (e.g., De Goeij et al., 2013; Alexander et al., 2014; Rix et al., 2018), while several massive sponges preferentially store it as biomass (e.g., Pawlik et al., 2016; McMurray et al., 2018; Pawlik and McMurray, 2020). While the Bouraké *R. globostellata* is a massive sponge, I found that it mainly transformed DOM and released it as detritus (i.e., sponge loop pathway). Specifically, my data suggest that *R. globostellata* in Bouraké preferentially uses DOM to maintain cell turnover and rejuvenation, which keeps the sponge healthy. This different strategy from other massive sponges could be due to the large amount of nutrients and organic matter available in the Bouraké lagoon, which allow the sponge to harness this energy to renew its cells, and perhaps even to grow. This hypothesis was also supported by the *ex-situ* experiment (chapter 4) which highlighted different carbon and nitrogen allocations between *R. globostellata* specimens from Bouraké (i.e., detritus production) and the control site (i.e., less detritus production), both exposed to a ‘normal’ temperature of 28°C. My data show that responses are different if sponges are from different sites and may be related to food availability. This result is consistent with those of Alexander et al. (2014) who demonstrated in aquarium that *Mycale microsigmatosa* growing on mangrove roots in Curaçao (Caribbean island) where food is abundant, had better temperatures tolerance and a higher cell proliferation rate than sponges from reference

reefs. This change in strategy seems to be permanent in sponges from Bouraké (i.e., probably resulting from an adaptive response) because they have maintained the *in-situ* response (i.e., detritus production) also when environmental conditions and food availability were different (i.e., in aquarium), also highlighting possible different ecological traits between the two sponge population.

At higher temperature conditions, the sponges from the control site produced more detritus and showed higher accumulation of  $^{13}\text{C}$  and  $^{15}\text{N}$  in the detritus, suggesting that the sponges underwent stress, which caused a severe cell degradation. Conversely, *R. globostellata* from Bouraké accumulates more  $^{13}\text{C}$  and  $^{15}\text{N}$  in the tissues than in the detritus when the temperature exceeds  $31^{\circ}\text{C}$ , suggesting that the sponge has developed strategies to resist extreme conditions. These differences in strategy could be related to sponge thermal history. Sponges living in fluctuating temperature environments could be more tolerant to temperature stresses (e.g., Oliver and Palumbi, 2011; Rivest et al., 2017). Bouraké lagoon frequently exceeds  $31^{\circ}\text{C}$  in summers, and if we assume this has occurred for centuries, this specific metabolism could be the result of an adaptive response of *R. globostellata* to recurring thermal stress to maintain their state of health. In this case, carbon and nitrogen are accumulated more in the sponge tissues at  $32^{\circ}\text{C}$  than at  $28^{\circ}\text{C}$ . Although it is clear that sponges store carbon and nitrogen in their tissues, I have not identified the mechanism used for this. Carbon and nitrogen stored in sponge tissues are probably used in different metabolic processes and provide additional energy for sponge health and growth. This change in energy allocation can be the result of an adaptive mechanism that allows *R. globostellata* to perform better under stressful temperatures and, thus, thrive in the Bouraké lagoon (**Figure 5.1**).

During the extreme conditions in 2020 (i.e., when the change in strategy was observed *in-situ*), *R. globostellata* took up more amount of carbon energy available for metabolic processes. An approximation of the carbon balance in sponges could be defined using the I:R ratio where I is the carbon ingested (DOC + POC) and R is the carbon used for respiration. Since the sponge showed no significant difference in respiration rates between the two environmental conditions (normal and extreme), we can assume that the sponge does not need to use more carbon energy for respiration to withstand extreme conditions (chapter 3). Respiration rates in sponges are considered a proxy for understanding sponge fitness and stress response in organisms (Coma et al., 2002). My results suggest that there is no stress response in *R. globostellata* under extreme conditions, which means that the metabolic physiology of the sponges allows them to respond to environmental changes (e.g., Coma et al., 2002; Morley et al., 2016; Bell et al., 2017). Higher respiration rates generally means that more energy is needed to maintain the sponge's basal metabolism and life processes (Clarke, 1991). The high availability of food in the Bouraké lagoon (chapter 2) may help the sponges to maintain their

basal metabolic rate and enhance carbon storage under stressful conditions. *R. globostellata* may then compensate for metabolic losses (including higher respiration rates) and ensure an optimal allocation of excess energy for metabolic processes such as growth, as reported for other organisms (Kozłowski, 1992; Perrin and Sibly, 1993). This hypothesis is supported by other studies showing that when food is not limiting, high growth rates and biomass accumulation can be supported even under stressful conditions (Sokolova et al., 2012). These responses could allow the sponge to keep their basal metabolism and thrive well also under the extreme condition in Bouraké, suggesting a possible level of adaptation of the sponge to these conditions. This hypothesis is reinforced by the fact that *R. globostellata* increased its respiration rate following the temperature increase between 2018 and 2020 (+4°C) (regardless of the condition) without affecting its survival. It seems able to reach a new basal metabolic "equilibrium" under changing environmental conditions.



**Figure 5.1.** Synthetic scheme of the main strategies used by the Bouraké sponge *R. globostellata* to cope (i.e., adaptive response) with a change in temperature.

The higher accumulation of carbon and nitrogen in *R. globostellata* from Bouraké also suggests a greater exchange of carbon and nitrogen between the host cells and the symbiont bacteria. Microbes associated with sponges are assumed to play an important role in mediating the sponge uptake of DOM (Weisz et al., 2008). The sponge symbionts may play an important role in its adaptation. Recent studies showed that the microbiome of OA-tolerant sponge species had increased their functional potential for energy-efficient carbon and nitrogen metabolism (Botté et al., 2019), contributing to the ecological adaptability and plasticity of the holobiont (Bang et al., 2018). Similarly, the bacterial community associated with *R. globostellata* could contribute to carbon and nitrogen assimilation (extra energy) and could play an important role in their survival and adaptation mechanisms to the extreme environmental conditions of Bouraké. Although this bacterial contribution needs to be further demonstrated, in my thesis carbon and nitrogen seem to be mainly stored in the sponge biomass at 32°C, suggesting a major involvement of symbionts at high temperatures. Among the associated microbial community, the photosynthetic microbes could have different response to OA and OW, ranging from no impact (Bell et al., 2022) to a decrease in both steady state and maximum quantum yields of their PSII (photosystem II) fluorescence (e.g., Lesser et al., 2016). By measuring the photosynthetic activity in *R. globostellata* under normal and extreme conditions, my results revealed that photosymbionts were negatively affected (i.e., less oxygen production and reduction in photosynthetic activity) by extreme conditions, suggesting that they may have little contribution in the sponge acclimation responses to extreme conditions. Heterotrophic sponges have been reported to be more tolerant to temperature anomalies than phototrophic sponges (Bennett, 2017). The studied species *R. globostellata* resulted mainly heterotrophic (P:R ratios < 1.5, following Wilkinson, 1983), suggesting that the heterotrophic processes, and the heterotrophic symbionts, may play an important role in the sponge adaptation to extreme conditions. Similarly, recent studies have shown that an increase in heterotrophic bacterial abundance can promote greater sponge productivity (e.g., Perea-Blázquez et al., 2012) and that eutrophic conditions could make bio-eroding sponges less dependent on their photosymbionts (Webb et al., 2017).

My work showed that sponges have metabolic plasticity to cope with different environmental conditions. Although I have only studied the responses of one sponge species at the local spatial level, this study demonstrates that *R. globostellata* can cope with extreme conditions in an advantageous way. In summary, the success and adaptation of *R. globostellata* in Bouraké, despite the extreme physico-chemical environmental conditions, could be linked to *i*) the fluctuating environment, which provides the sponges with regular phases of extreme conditions and optimal conditions, likely allowing them to recover from stress, and *ii*) the wide range of heterotrophic inputs, nutrients, carbon, and nitrogen sources that can help sponges cope with extreme conditions through additional energy.

If we consider that sponges living in the Bouraké lagoon have been exposed to these environmental conditions for decades, more likely centuries, these responses could be the result of adaptive mechanism take in place by the sponge to thrive under extreme conditions such as high temperature.

## **5.2 Bouraké sponges: a model of adaptation for future coral reefs?**

My results show that *R. globostellata* has developed strategies to survive and thrive in the extreme environment of Bourake. However, it is questionable whether these results are appropriate for predicting how organisms will respond to future conditions elsewhere.

Although we cannot predict whether the natural fluctuations expected for other coastal habitats will be comparable to what has been measured in the Bouraké lagoon, the models predict larger fluctuations in physico-chemical water parameters. Seasonal pH amplitude could increase by 80%, extreme values of ocean acidity will be more frequent, and pH variability will increase further at sites where variability is already high today (Kwiatkowski and Orr, 2018; Burger et al., 2020). The situation could be similar also for seawater temperature. At Bouraké, the mean values of the environmental parameters ( $\text{pH}_T = -0.3$  units,  $\text{DO} = -26\%$ , and  $T = +3^\circ\text{C}$ , in contrast to current values) nevertheless approach the values predicted by the models under the IPCC scenario ( $\text{pH}_T = -0.3$  units,  $\text{DO} = -4\%$ , and  $T = +3^\circ\text{C}$ ), suggesting that this site can be used to study the adaptive responses of organisms to future conditions.

In contrast, at Bouraké, changes in physical parameters are driven by the tide, exposing organisms to normal or extreme conditions every *ca.* 6 h. The response mechanisms observed in this study were thus the result of this fluctuation, which of course allow the sponge to limited periods of quasi-normal conditions. Over time, the tide could have exposed the sponges to the stressful conditions long enough to induce adaptation but short enough to avoid sponge mortality as reported in some coral studies (e.g., Oliver and Palumbi, 2011; Castillo et al., 2012; Palumbi et al., 2014; DeCarlo et al., 2019). Although this could be true for temperatures, pH and DO inside the Bouraké lagoon rarely reach normal values exposing organisms to these extreme values for decades, even centuries, which has probably allow long-term selection of resistant organisms and resulted in local adaptation. It is questionable whether the same mechanisms will be able to take place in different environments where organisms are likely to be exposed to stressful conditions more rapidly and more regularly. However, recent studies have shown that rapid adaptation and plasticity in certain physiological traits may be heritable, helping organisms (e.g., corals) to cope with rapid environmental change as well (e.g., Torda et al., 2017; Putnam, 2021).

Overall, the interpretation of my results about future climate change responses should be taken carefully, as several other environmental factors, besides physical parameters, such as nutrients and organic matter concentrations, varied at the site and are likely to influence the organisms' adaptive responses. Coastal lagoons, such as Bouraké, are highly productive ecosystems that serve as nursery grounds for many marine species (Anthony et al., 2009) and receive terrigenous runoffs from rainfall, sewage, and/or agricultural fertilizer (Taylor et al., 1995). In Bouraké, this organic matter input is amplified by the surrounding mangrove, considered one of the world's most productive ecosystems (e.g., Alongi et al., 2004; Dittmar et al., 2006; Bouillon et al., 2008). The benthic communities of Bouraké have access to a wide range of heterotrophic inputs, nutrients, carbon, and nitrogen sources. Although these inputs can become toxic if they are too concentrated, they could also help organisms cope with extreme conditions.

No natural laboratory is perfect for studying the impact of future climatic conditions, but the lagoon of Bouraké offers a realistic combination of multiple factors that will affect future coral reefs. Despite the small spatial scale offered by the Bouraké natural laboratory, my results provide essential insights into the potential mechanisms of sponge acclimation and adaptation to different environmental conditions. The temperature in coral reefs is expected to increase by 3°C in the future, a condition met in the lagoon of Bouraké during low tide. My results experimentally demonstrate that *i*) above a certain threshold (> 31°C tested here) temperature induced a change in organic matter allocation in *R. globostellata*; and *ii*) the observed responses were unique to Bouraké sponges, probably owing to their presumed ability to cope with extreme temperature variations (**Figure 5.1**). This suggests that while the Bouraké sponges could maintain their normal metabolic and allocate more resources to the tissue, the control sponges probably experienced metabolic stress and cell degradation.

Assuming that contemporary sponges will adapt as they did sponges in Bouraké, this study suggests that future coral reefs are likely to be dominated by larger sponges that invest more energy in their individual growth. The change in C and N allocation may have consequences in the amount of detritus available for the other trophic levels in the ecosystem. Less detritus will be incorporated by sponges-associated detritivores, and less detritus will reach different trophic levels. Carbon and nitrogen are mainly stored in the sponge tissues. Sponge predators would be advantaged because a high sponge biomass would be available for them. However, only a limited number of species of fish, turtles, and nudibranch species are able to cope with sponge secondary metabolites in their diet. Consequently, I believe that the potential use of sponge biomass by predators remains a (very) minor organic matter pathway. The biomass accumulated by sponges may re-enter the system when the temperature conditions change (i.e., detritus production) or when the sponge dies, thus following a

necrophagous/detritivorous trophic pathway. Although adaptation of all reef organisms and their interactions will need to be considered and that these results remains hypothetical and restricted to a specific case, my work has highlighted that ocean warming could change how sponges recycle C and N, and thus the food quality and availability in the reef.

Several personal observations noticed that the reefs in Bouraké have changed over the past years, with significant negative impacts on corals (i.e., bleaching and mortality), while the sponge abundance and their massiveness remain high. Stressful conditions (rainfall, cyclones, La Niña/El Niño events and temperature rise) appear to have more effect on other organisms than on sponges, which could have less competition for space and continue to take full advantage of high nutrient levels for growth under non-optimal conditions. These observations agree with my results showing that *R. globostellata* could exhibits adaptive mechanisms that allow it to compete successfully in this extreme environment.

In conclusion, this research has highlighted the importance of studying how temperatures can affect the ecological role of sponges in coral reefs, as temperature may be one of the major climate change consequences driving changes in the metabolism of most sponge species. Changes in sponge metabolism, such as different allocation strategies of C and N between sponge tissue and detritus, as well as the ability of sponges to recycle organic matter, could have significant effects on the entire reef. Effects on trophic interactions could lead to a shift towards communities dominated by sponges and other organisms that can adapt to and take advantage by high temperatures.

### **5.3 Future perspectives**

Sponges play an important ecological role by recycling carbon and nitrogen in coral reef ecosystems (e.g., Maldonado et al., 2012; De Goeij et al., 2013). Both pathways of carbon recycling in sponges (sponge loop and biomass storage) need to be fully assessed to get a better idea of the carbon cycle (and organic matter in general) in massive and encrusting sponges, as well as the contribution of microbial symbionts in these processes. More than 8,500 sponge species have been described (van Soest et al., 2012), and there is no information on carbon and nutrient cycles for most of them. Although I studied only *R. globostellata*, my research provides an initial and original insight into understanding the effects of extreme conditions, mainly OW, on the sponge nutrient and carbon cycles. Further work should consider more sponge species, both encrusting and massive, and their nutrient and carbon cycles must be assessed under various environmental conditions, including extremes ones. Future studies should prioritize cross-transplanting experiments using other natural laboratories to explore how sponge's nutrient and carbon cycles could change under future conditions.

*Ex-situ* experiments maintaining sponges at elevated temperatures for more extended periods should be performed to understand the level of sponge temperature tolerance and provide better insight into how the sponge carbon and nitrogen cycles might be affected under future temperatures.

While I did not investigate which metabolic processes the sponges used to accumulate carbon, my research showed that under extreme conditions ( $T > 31^{\circ}\text{C}$ ) carbon is mainly stored in the sponge tissues rather than released as detritus. Future studies using specific techniques to label and follow carbon transfer in sponges (e.g., NanoSIMS) will help to better understand the processes that occur when carbon and nitrogen are no longer released as detritus. Such studies can shed light on the strategies used by sponges to adapt to high temperatures. This innovative approach, should anyway be coupled with classic sponge biomass measurements (i.e., buoyant), pumping rates, and food quality.

Finally, because sponges are holobionts, the responses of the associated microbial community must be considered. Some studies have indicated that changes in the sponge microbiome could be the key to sponge adaptation to new environmental conditions (e.g., Webster and Reusch, 2017; Pita et al., 2018). The role of the microbiome on sponge organic matter recycling still needs to be better understood, particularly its contribution to adaptation under extreme conditions, including OW. I showed several evidence of adaptation in my research, but complementary analyses are needed to demonstrate it fully. Future studies on lipidic contents, heat shock proteins, and transcriptomic could be key to understand if the responses of *R. globostellata* from Bouraké are the result of adaptation or a physiological response under stressed conditions.

## **5.4 General conclusion**

Environmental changes, as expected under future climate scenarios, could strongly affect marine species in the ocean. Sponges have different and species-specific responses to climate change and environmental variations. Studies so far have mainly focused on the effects of climate change on sponge physiological traits rather than their ecological role, such as carbon and nitrogen cycles in coral reefs.

My thesis provided 1) the first long-term physico-chemical characterization and assessment of the Bouraké Lagoon benthic community, which is now considered a natural laboratory for studying the adaptation of organisms to extreme conditions; 2) the first insights into the effects of extreme conditions on *R. globostellata* metabolism, showing a significant change in the carbon cycle to cope and thrive in extreme environments; 3) information about the effects of temperature on *R. globostellata* carbon and nitrogen recycling, showing that sponges may be able to tolerate short-term

high temperature exposure (+3°C), and that sponges subjected to high temperatures for decades, even centuries (Bouraké), showed an adaptive response that involve a greater accumulation of carbon and nitrogen in the tissues than in the detritus. Whether these are mechanisms that other common sponges could use to cope with climate change in the future remains to be understood.

In a world where climate change is accelerating, and many of the effects of these changes have yet to be studied on organisms, my thesis provides an example of how a tropical sponge can cope with extreme acidification, warming, and deoxygenation conditions. I showed that the studied sponge could adapt to different environmental conditions taking maximum advantage for their health and suggesting that survival of sponges, and hopefully other taxa, to future environmental conditions, may be possible. However, the possible adaptation to high temperatures may result in a change in metabolic responses and thus a change in trophic interactions in coral reefs.



---

**REFERENCES**

---

- Abdul Wahab, M. A., de Nys, R., Webster, N. and Whalan, S.: Phenology of sexual reproduction in the common coral reef sponge, *Carteriospongia foliascens*, *Coral Reefs*, doi:10.1007/s00338-013-1119-9, 2014.
- Achlatis, M., Van Der Zande, R. M., Schönberg, C. H. L., Fang, J. K. H., Hoegh-Guldberg, O. and Dove, S.: Sponge bioerosion on changing reefs: Ocean warming poses physiological constraints to the success of a photosymbiotic excavating sponge, *Scientific Reports*, doi:10.1038/s41598-017-10947-1, 2017.
- Achlatis, M., Pernice, M., Green, K., Guagliardo, P., Kilburn, M. R., Hoegh-Guldberg, O. and Dove, S.: Single-cell measurement of ammonium and bicarbonate uptake within a photosymbiotic bioeroding sponge, *ISME Journal*, doi:10.1038/s41396-017-0044-2, 2018.
- Agostini, S., Harvey, B. P., Wada, S., Kon, K., Milazzo, M., Inaba, K. and Hall-Spencer, J. M.: Ocean acidification drives community shifts towards simplified non-calcified habitats in a subtropical–temperate transition zone, *Scientific Reports*, doi:10.1038/s41598-018-29251-7, 2018.
- Aiuppa, A., Hall-Spencer, J. M., Milazzo, M., Turco, G., Caliro, S. and Di Napoli, R.: Volcanic CO<sub>2</sub> seep geochemistry and use in understanding ocean acidification, *Biogeochemistry*, doi:10.1007/s10533-020-00737-9, 2021.
- Alderdice, R., Suggett, D. J., Cárdenas, A., Hughes, D. J., Kühl, M., Pernice, M. and Voolstra, C. R.: Divergent expression of hypoxia response systems under deoxygenation in reef-forming corals aligns with bleaching susceptibility, *Global Change Biology*, doi:10.1111/gcb.15436, 2021.
- Alexander, B. E., Liebrand, K., Osinga, R., Van Der Geest, H. G., Admiraal, W., Cleutjens, J. P. M., Schutte, B., Verheyen, F., Ribes, M., Van Loon, E. and De Goeij, J. M.: Cell turnover and detritus production in marine sponges from tropical and temperate benthic ecosystems, *PLoS ONE*, doi:10.1371/journal.pone.0109486, 2014.
- Alongi, D. M., Sasekumar, A., Chong, V. C., Pfitzner, J., Trott, L. A., Tirendi, F., Dixon, P. and Brunskill, G. J.: Sediment accumulation and organic material flux in a managed mangrove ecosystem: Estimates of land-ocean-atmosphere exchange in peninsular Malaysia, in *Marine Geology*, 2004.
- Anthony, A., Atwood, J., August, P., Byron, C., Cobb, S., Foster, C., Fry, C., Gold, A., Hagos, K., Heffner, L., Kellogg, D. Q., Lellis-Dibble, K., Opaluch, J. J., Oviatt, C., Pfeiffer-Herbert, A., Rohr, N., Smith, L., Smythe, T., Swift, J. and Vinhateiro, N.: Coastal lagoons and climate change: Ecological and social ramifications in U.S. Atlantic and Gulf coast ecosystems, *Ecology and Society*, doi:10.5751/ES-02719-140108, 2009.
- Anthony, K. R. N. and Fabricius, K. E.: Shifting roles of heterotrophy and autotrophy in coral energetics under varying turbidity, *Journal of Experimental Marine Biology and Ecology*, doi:10.1016/S0022-0981(00)00237-9, 2000.
- Archer, S. K., Stevens, J. L., Rossi, R. E., Matterson, K. O. and Layman, C. A.: Abiotic conditions drive significant variability in nutrient processing by a common Caribbean sponge, *Ircinia felix*, *Limnology and Oceanography*, doi:10.1002/lno.10533, 2017.
- Aronson, R. B., Precht, W. F., Toscano, M. A. and Koltes, K. H.: The 1998 bleaching event and its aftermath on a coral reef in Belize, *Marine Biology*, doi:10.1007/s00227-002-0842-5, 2002.
- Atkinson, M. J. and Cuet, P.: Possible effects of ocean acidification on coral reef biogeochemistry: Topics for research, *Marine Ecology Progress Series*, doi:10.3354/meps07867, 2008.

- Atkinson, M. J., Carlson, B. and Crow, G. L.: Coral growth in high-nutrient, low-pH seawater: a case study of corals cultured at the Waikiki Aquarium, Honolulu, Hawaii, *Coral Reefs*, doi:10.1007/BF00334344, 1995.
- Azam, F., Fenchel, T., Field, J., Gray, J., Meyer-Reil, L. and Thingstad, F.: The Ecological Role of Water-Column Microbes in the Sea, *Marine Ecology Progress Series*, doi:10.3354/meps010257, 1983.
- Bang, C., Dagan, T., Deines, P., Dubilier, N., Duschl, W. J., Fraune, S., Hentschel, U., Hirt, H., Hülter, N., Lachnit, T., Picazo, D., Pita, L., Pogoreutz, C., Rådecker, N., Saad, M. M., Schmitz, R. A., Schulenburg, H., Voolstra, C. R., Weiland-Bräuer, N., Ziegler, M. and Bosch, T. C. G.: Metaorganisms in extreme environments: do microbes play a role in organismal adaptation?, *Zoology*, doi:10.1016/j.zool.2018.02.004, 2018.
- Barkley, H. C., Cohen, A. L., Golbuu, Y., Starczak, V. R., DeCarlo, T. M. and Shamberger, K. E. F.: Changes in coral reef communities across a natural gradient in seawater pH, *Science Advances*, doi:10.1126/sciadv.1500328, 2015.
- Barkley, H. C., Cohen, A. L., McCorkle, D. C. and Golbuu, Y.: Mechanisms and thresholds for pH tolerance in Palau corals, *Journal of Experimental Marine Biology and Ecology*, doi:10.1016/j.jembe.2017.01.003, 2017.
- Barshis, D. J., Ladner, J. T., Oliver, T. A., Seneca, F. O., Traylor-Knowles, N. and Palumbi, S. R.: Genomic basis for coral resilience to climate change, *Proceedings of the National Academy of Sciences of the United States of America*, doi:10.1073/pnas.1210224110, 2013.
- Bart, M. C., Hudspith, M., Rapp, H. T., Verdonschot, P. F. M. and de Goeij, J. M.: A Deep-Sea Sponge Loop? Sponges Transfer Dissolved and Particulate Organic Carbon and Nitrogen to Associated Fauna, *Frontiers in Marine Science*, doi:10.3389/fmars.2021.604879, 2021.
- Bates, T. E. M. and Bell, J. J.: Responses of two temperate sponge species to ocean acidification, *New Zealand Journal of Marine and Freshwater Research*, doi:10.1080/00288330.2017.1369132, 2018.
- Bayer, K., Schmitt, S. and Hentschel, U.: Physiology, phylogeny and in situ evidence for bacterial and archaeal nitrifiers in the marine sponge *Aplysina aerophoba*, *Environmental Microbiology*, doi:10.1111/j.1462-2920.2008.01582.x, 2008.
- Becking, L. E., Cleary, D. F. R. and De Voogd, N. J.: Sponge species composition, abundance, and cover in marine lakes and coastal mangroves in Berau, Indonesia, *Marine Ecology Progress Series*, doi:10.3354/meps10155, 2013.
- Beepat, S. S., Davy, S. K., Woods, L. and Bell, J. J.: Short-term responses of tropical lagoon sponges to elevated temperature and nitrate, *Marine Environmental Research*, doi:10.1016/j.marenvres.2020.104922, 2020.
- Bell, J. J.: The functional roles of marine sponges, *Estuarine, Coastal and Shelf Science*, doi:10.1016/j.ecss.2008.05.002, 2008.
- Bell, J. J., Barnes, D. K. A. and Turner, J. R.: The importance of micro and macro morphological variation in the adaptation of a sublittoral demosponge to current extremes, *Marine Biology*, doi:10.1007/s002270100665, 2002.
- Bell, J. J., Davy, S. K., Jones, T., Taylor, M. W. and Webster, N.: Could some coral reefs become sponge reefs as our climate changes?, *Global change biology*, doi:10.1111/gcb.12212, 2013.
- Bell, J. J., Biggerstaff, A., Bates, T., Bennett, H., Marlow, J., McGrath, E. and Shaffer, M.: Sponge

- monitoring Moving beyond diversity and abundance measures, *Ecological Indicators*, 78, 470–488, doi:10.1016/j.ecolind.2017.03.001, 2017.
- Bell, J. J., Rovellini, A., Davy, S. K., Taylor, M. W., Fulton, E. A., Dunn, M. R., Bennett, H. M., Kandler, N. M., Luter, H. M. and Webster, N. S.: Climate change alterations to ecosystem dominance: how might sponge-dominated reefs function?, *Ecology*, doi:10.1002/ecy.2446, 2018a.
- Bell, J. J., Bennett, H. M., Rovellini, A. and Webster, N. S.: Sponges to be winners under near-future climate scenarios, *BioScience*, doi:10.1093/biosci/biy142, 2018b.
- Bell, J. J., Shaffer, M., Luter, H. M., Mana, R. and Rodolfo-Metalpa, R.: Phototrophic sponge productivity may not be enhanced in a high CO<sub>2</sub> world, *Global Change Biology*, (May), 1–12, doi:10.1111/gcb.16235, 2022.
- Bellworthy, J. and Fine, M.: Warming resistant corals from the Gulf of Aqaba live close to their cold-water bleaching threshold, , (1843), doi:10.7717/peerj.11100, 2021.
- Benner, R., Pakulski, J. D., McCarthy, M., Hedges, J. I. and Hatcher, P. G.: Bulk chemical characteristics of dissolved organic matter in the ocean, *Science*, doi:10.1126/science.255.5051.1561, 1992.
- Bennett, H. M.: Climate change and tropical sponges: The effect of elevated pCO<sub>2</sub> and temperature on the sponge holobiont, , 1–14, 2017.
- Bennett, H. M., Altenrath, C., Woods, L., Davy, S. K., Webster, N. S. and Bell, J. J.: Interactive effects of temperature and pCO<sub>2</sub> on sponges: from the cradle to the grave, *Global Change Biology*, doi:10.1111/gcb.13474, 2017.
- Benzoni, F., Houlbrèque, F., André, L. V. and Payri, C.: Plan d'action rapide et adaptatif en cas de blanchissement corallien : Le cas de la Nouvelle-Calédonie, épisode 2016, synthèse, *Sciences de la Mer. Biologie Marine. Rapports Scientifiques et Techniques* ; 6, (December), 90, 2017.
- Bertolino, M., Oprandi, A., Santini, C., Castellano, M., Pansini, M., Boyer, M. and Bavestrello, G.: Hydrothermal waters enriched in silica promote the development of a sponge community in North Sulawesi (Indonesia), *European Zoological Journal*, doi:10.1080/11250003.2016.1278475, 2017.
- Biggerstaff, A., Smith, D. J., Jompa, J. and Bell, J. J.: Photoacclimation supports environmental tolerance of a sponge to turbid low-light conditions, *Coral Reefs*, doi:10.1007/s00338-015-1340-9, 2015.
- Biscéré, T., Rodolfo-Metalpa, R., Lorrain, A., Chauvaud, L., Thébault, J., Clavier, J. and Houlbrèque, F.: Responses of two scleractinian corals to cobalt pollution and ocean acidification, *PLoS ONE*, doi:10.1371/journal.pone.0122898, 2015.
- Bongiorni, L., Shafir, S., Angel, D. and Rinkevich, B.: Survival, growth and gonad development of two hermatypic corals subjected to in situ fish-farm nutrient enrichment, *Marine Ecology Progress Series*, doi:10.3354/meps253137, 2003.
- Borges, A. V., Djenidi, S., Lacroix, G., Théate, J., Delille, B. and Frankignoulle, M.: Atmospheric CO<sub>2</sub> flux from mangrove surrounding waters, *Geophysical Research Letters*, doi:10.1029/2003GL017143, 2003.
- Botté, E. S., Nielsen, S., Abdul Wahab, M. A., Webster, J., Robbins, S., Thomas, T. and Webster, N. S.: Changes in the metabolic potential of the sponge microbiome under ocean acidification, *Nature Communications*, doi:10.1038/s41467-019-12156-y, 2019.
- Bouillon, S., Middelburg, J. J., Dehairs, F., Borges, A. V., Abril, G., Flindt, M. R., Ulomi, S. and

- Kristensen, E.: Importance of intertidal sediment processes and porewater exchange on the water column biogeochemistry in a pristine mangrove creek (Ras Dege, Tanzania), *Biogeosciences*, doi:10.5194/bg-4-311-2007, 2007.
- Bouillon, S., Connolly, R. M. and Lee, S. Y.: Organic matter exchange and cycling in mangrove ecosystems: Recent insights from stable isotope studies, *Journal of Sea Research*, doi:10.1016/j.seares.2007.05.001, 2008.
- Bouillon, S., Yambélé, A., Gillikin, D. P., Teodoru, C., Darchambeau, F., Lambert, T. and Borges, A. V.: Contrasting biogeochemical characteristics of the Oubangui River and tributaries (Congo River basin), *Scientific Reports*, doi:10.1038/srep05402, 2014.
- Boyd, P. W., Cornwall, C. E., Davison, A., Doney, S. C., Fourquez, M., Hurd, C. L., Lima, I. D. and McMinn, A.: Biological responses to environmental heterogeneity under future ocean conditions, *Global change biology*, doi:10.1111/gcb.13287, 2016.
- Briand, M. J., Bonnet, X., Goiran, C., Guillou, G. and Letourneur, Y.: Major sources of organic matter in a complex coral reef lagoon: Identification from isotopic signatures ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ), *PLoS ONE*, doi:10.1371/journal.pone.0131555, 2015.
- Burger, F. A., John, J. G. and Frölicher, T. L.: Increase in ocean acidity variability and extremes under increasing atmospheric  $\text{CO}_2$ , *Biogeosciences*, doi:10.5194/bg-17-4633-2020, 2020.
- Burt, J. A., Camp, E. F., Enochs, I. C., Johansen, J. L., Morgan, K. M., Riegl, B. and Hoey, A. S.: Insights from extreme coral reefs in a changing world, *Coral Reefs*, doi:10.1007/s00338-020-01966-y, 2020.
- Byrne, M. and Przeslawski, R.: Multistressor impacts of warming and acidification of the ocean on marine invertebrates' life histories, *Integrative and Comparative Biology*, doi:10.1093/icb/ict049, 2013.
- Calcinai, B., Bastari, A., Makapedua, D. M. and Cerrano, C.: Mangrove sponges from Bangka Island (North Sulawesi, Indonesia) with the description of a new species, *Journal of the Marine Biological Association of the United Kingdom*, doi:10.1017/S0025315416000710, 2017.
- Caldeira, K. and Wickett, M. E.: Anthropogenic carbon and ocean pH, *Nature*, doi:10.1038/425365a, 2003.
- Call, M., Maher, D. T., Santos, I. R., Ruiz-Halpern, S., Mangion, P., Sanders, C. J., Erler, D. V., Oakes, J. M., Rosentreter, J., Murray, R. and Eyre, B. D.: Spatial and temporal variability of carbon dioxide and methane fluxes over semi-diurnal and spring-neap-spring timescales in a mangrove creek, *Geochimica et Cosmochimica Acta*, doi:10.1016/j.gca.2014.11.023, 2015.
- Di Camillo, C. G., Bartolucci, I., Cerrano, C. and Bavestrello, G.: Sponge disease in the Adriatic Sea, *Marine Ecology*, doi:10.1111/j.1439-0485.2012.00525.x, 2013.
- Camp, E. F., Smith, D. J., Evenhuis, C., Enochs, I., Manzello, D., Woodcock, S. and Suggett, D. J.: Acclimatization to high-variance habitats does not enhance physiological tolerance of two key Caribbean corals to future temperature and pH, *Proceedings of the Royal Society B: Biological Sciences*, doi:10.1098/rspb.2016.0442, 2016a.
- Camp, E. F., Suggett, D. J., Gendron, G., Jompa, J., Manfrino, C. and Smith, D. J.: Mangrove and Seagrass Beds Provide Different Biogeochemical Services for Corals Threatened by Climate Change, *Frontiers in Marine Science*, doi:10.3389/fmars.2016.00052, 2016b.
- Camp, E. F., Nitschke, M. R., Rodolfo-Metalpa, R., Houlbreque, F., Gardner, S. G., Smith, D. J., Zampighi, M. and Suggett, D. J.: Reef-building corals thrive within hot-acidified and deoxygenated

- waters, *Scientific Reports*, doi:10.1038/s41598-017-02383-y, 2017.
- Camp, E. F., Schoepf, V., Mumby, P. J. and Suggett, D. J.: Editorial: The Future of Coral Reefs Subject to Rapid Climate Change: Lessons From Natural Extreme Environments, *Frontiers in Marine Science*, doi:10.3389/fmars.2018.00433, 2018.
- Camp, E. F., Edmondson, J., Doheny, A., Rumney, J., Grima, A. J., Huete, A. and Suggett, D. J.: Mangrove lagoons of the Great Barrier Reef support coral populations persisting under extreme environmental conditions, *Marine Ecology Progress Series*, doi:10.3354/meps13073, 2019.
- Campbell, L., Nolla, H. A. and Vault, D.: The importance of *Prochlorococcus* to community structure in the central North Pacific Ocean, *Limnology and Oceanography*, doi:10.4319/lo.1994.39.4.0954, 1994.
- Canadell, J. G., Le Quéré, C., Raupach, M. R., Field, C. B., Buitenhuis, E. T., Ciais, P., Conway, T. J., Gillett, N. P., Houghton, R. A. and Marland, G.: Contributions to accelerating atmospheric CO<sub>2</sub> growth from economic activity, carbon intensity, and efficiency of natural sinks, *Proceedings of the National Academy of Sciences of the United States of America*, doi:10.1073/pnas.0702737104, 2007.
- Cao, L. and Zhang, H.: The role of biological rates in the simulated warming effect on oceanic CO<sub>2</sub> uptake, *Journal of Geophysical Research: Biogeosciences*, doi:10.1002/2016JG003756, 2017.
- Cárdenas, P. and Rapp, H. T.: Disrupted spiculogenesis in deep-water Geodiidae (Porifera, Demospongiae) growing in shallow waters, *Invertebrate Biology*, doi:10.1111/ivb.12027, 2013.
- Cárdenas, P., Gamage, J., Hettiarachchi, C. M. and Gunasekera, S.: Good Practices in Sponge Natural Product Studies: Revising Vouchers with Isomalabaricane Triterpenes, *Marine Drugs*, doi:10.3390/md20030190, 2022.
- Carlson, C. A., Giovannoni, S. J., Hansell, D. A., Goldberg, S. J., Parsons, R., Otero, M. P., Vergin, K. and Wheeler, B. R.: Effect of nutrient amendments on bacterioplankton production, community structure, and DOC utilization in the northwestern Sargasso Sea, *Aquatic Microbial Ecology*, doi:10.3354/ame030019, 2002.
- Carstensen, J. and Duarte, C. M.: Drivers of pH Variability in Coastal Ecosystems, *Environmental Science and Technology*, doi:10.1021/acs.est.8b03655, 2019.
- Castillo, K. D., Ries, J. B., Weiss, J. M. and Lima, F. P.: Decline of forereef corals in response to recent warming linked to history of thermal exposure, *Nature Climate Change*, doi:10.1038/nclimate1577, 2012.
- Cathalot, C., Van Oevelen, D., Cox, T. J. S., Kutti, T., Lavaleye, M., Duineveld, G. and Meysman, F. J. R.: Cold-water coral reefs and adjacent sponge grounds: Hotspots of benthic respiration and organic carbon cycling in the deep sea, *Frontiers in Marine Science*, doi:10.3389/fmars.2015.00037, 2015.
- Cauwet, G.: HTCO method for dissolved organic carbon analysis in seawater: influence of catalyst on blank estimation, *Marine Chemistry*, doi:10.1016/0304-4203(94)90013-2, 1994.
- Cebrian, E. and Uriz, M. J.: Grazing on fleshy seaweeds by sea urchins facilitates sponge *Cliona viridis* growth, *Marine Ecology Progress Series*, doi:10.3354/meps323083, 2006.
- Cebrian, E., Uriz, M. J., Garrabou, J. and Ballesteros, E.: Sponge mass mortalities in a warming mediterranean sea: Are cyanobacteria-harboring species worse off?, *PLoS ONE*, doi:10.1371/journal.pone.0020211, 2011.
- Cerrano, C., Bavestrello, G., Bianchi, C. N., Cattaneo-Vietti, R., Bava, S., Morganti, C., Morri, C.,

- Picco, P., Sara, G., Schiaparelli, S., Siccardi, A. and Sponga, F.: A catastrophic mass-mortality episode of gorgonians and other organisms in the Ligurian Sea (North-western Mediterranean), summer 1999, *Ecology Letters*, doi:10.1046/j.1461-0248.2000.00152.x, 2000.
- Chapin, F. S., Walker, B. H., Hobbs, R. J., Hooper, D. U., Lawton, J. H., Sala, O. E. and Tilman, D.: Biotic control over the functioning of ecosystems, *Science*, doi:10.1126/science.277.5325.500, 1997.
- Cheshire, A. C. and Wilkinson, C. R.: Modelling the photosynthetic production by sponges on Davies Reef, Great Barrier Reef, *Marine Biology*, doi:10.1007/BF01320226, 1991.
- Chevaldonné, P., Pérez, T., Crouzet, J. M., Bay-Nouailhat, W., Bay-Nouailhat, A., Fourt, M., Almón, B., Pérez, J., Aguilar, R. and Vacelet, J.: Unexpected records of “deep-sea” carnivorous sponges *Asbestopluma hypogea* in the shallow NE Atlantic shed light on new conservation issues, *Marine Ecology*, doi:10.1111/maec.12155, 2015.
- Chu, J. W. F., Maldonado, M., Yahel, G. and Leys, S. P.: Glass sponge reefs as a silicon sink, *Marine Ecology Progress Series*, doi:10.3354/meps09381, 2011.
- Clarke, A.: What is cold adaptation and how should we measure it?, *Integrative and Comparative Biology*, doi:10.1093/icb/31.1.81, 1991.
- Codiga: Unified Tidal Analysis and Prediction Using the UTide Matlab Functions, , (September), 59, doi:10.13140/RG.2.1.3761.2008, 2011.
- Coles, S. L. and Jokiel, P. L.: Effects of temperature on photosynthesis and respiration in hermatypic corals, *Marine Biology*, doi:10.1007/BF00402313, 1977.
- Colman, A. S.: Sponge symbionts and the marine P cycle, *Proceedings of the National Academy of Sciences of the United States of America*, doi:10.1073/pnas.1502763112, 2015.
- Coma, R., Ribes, M., Gili, J. M. and Zabala, M.: Seasonality of in situ respiration rate in three temperate benthic suspension feeders, *Limnology and Oceanography*, doi:10.4319/lo.2002.47.1.0324, 2002.
- Comeau, S., Edmunds, P. J., Spindel, N. B. and Carpenter, R. C.: The responses of eight coral reef calcifiers to increasing partial pressure of CO<sub>2</sub> do not exhibit a tipping point, *Limnology and Oceanography*, doi:10.4319/lo.2013.58.1.0388, 2013.
- Comeau, S., Cornwall, C. E., Pupier, C. A., DeCarlo, T. M., Alessi, C., Trehern, R. and McCulloch, M. T.: Flow-driven micro-scale pH variability affects the physiology of corals and coralline algae under ocean acidification, *Scientific Reports*, doi:10.1038/s41598-019-49044-w, 2019.
- Cornwall, C. E., Boyd, P. W., McGraw, C. M., Hepburn, C. D., Pilditch, C. A., Morris, J. N., Smith, A. M. and Hurd, C. L.: Diffusion boundary layers ameliorate the negative effects of ocean acidification on the temperate coralline macroalga *Arthrocardia corymbosa*, *PLoS ONE*, doi:10.1371/journal.pone.0097235, 2014.
- Cornwall, C. E., Comeau, S., DeCarlo, T. M., Moore, B., D’Alexis, Q. and McCulloch, M. T.: Resistance of corals and coralline algae to ocean acidification: Physiological control of calcification under natural pH variability, *Proceedings of the Royal Society B: Biological Sciences*, doi:10.1098/rspb.2018.1168, 2018.
- Cornwall, C. E., Comeau, S., DeCarlo, T. M., Larcombe, E., Moore, B., Giltrow, K., Puerzer, F., D’Alexis, Q. and McCulloch, M. T.: A coralline alga gains tolerance to ocean acidification over multiple generations of exposure, *Nature Climate Change*, doi:10.1038/s41558-019-0681-8, 2020.
- Craig, P., Birkeland, C. and Belliveau, S.: High temperatures tolerated by a diverse assemblage of

- shallow-water corals in American Samoa, *Coral Reefs*, doi:10.1007/s003380100159, 2001.
- Crook, E. D., Cohen, A. L., Rebolledo-Vieyra, M., Hernandez, L. and Paytan, A.: Reduced calcification and lack of acclimatization by coral colonies growing in areas of persistent natural acidification, *Proceedings of the National Academy of Sciences of the United States of America*, doi:10.1073/pnas.1301589110, 2013.
- Crossland, C., Hatcher, B., Atkinson, M. and Smith, S.: Dissolved nutrients of a high-latitude coral reef, Houtman Abrolhos Islands, Western Australia, *Marine Ecology Progress Series*, doi:10.3354/meps014159, 1984.
- D'Angelo, C. and Wiedenmann, J.: Impacts of nutrient enrichment on coral reefs: New perspectives and implications for coastal management and reef survival, *Current Opinion in Environmental Sustainability*, doi:10.1016/j.cosust.2013.11.029, 2014.
- Dandan, S. S., Falter, J. L., Lowe, R. J. and McCulloch, M. T.: Resilience of coral calcification to extreme temperature variations in the Kimberley region, northwest Australia, *Coral Reefs*, doi:10.1007/s00338-015-1335-6, 2015.
- Davis, G. E.: A century of natural change in coral distribution at the Dry Tortugas: a comparison of reef maps from 1881 and 1976., *Bulletin of Marine Science*, doi:10.1016/0198-0254(82)90301-6, 1982.
- DeCarlo, T. M., Harrison, H. B., Gajdzik, L., Alaguarda, D., Rodolfo-Metalpa, R., D'Olivo, J., Liu, G., Patalwala, D. and McCulloch, M. T.: Acclimatization of massive reef-building corals to consecutive heatwaves, *Proceedings of the Royal Society B: Biological Sciences*, doi:10.1098/rspb.2019.0235, 2019.
- Diaz, M. C.: Mangrove and coral reef sponge faunas: Untold stories about shallow water Porifera in the Caribbean, *Hydrobiologia*, doi:10.1007/s10750-011-0952-5, 2012.
- Diaz, M. C. and Rützler, K.: Sponges: An essential component of Caribbean coral reefs, in *Bulletin of Marine Science*., 2001.
- Diaz, M. C. and Rützler, K.: Biodiversity and Abundance of Sponges in Caribbean Mangrove: Indicators of Environmental Quality, *Smithsonian Contributions to the Marine Sciences*, doi:10.5479/si.01960768.38.151, 2009.
- Dinerstein, E., Vynne, C., Sala, E., Joshi, A. R., Fernando, S., Lovejoy, T. E., Mayorga, J., Olson, D., Asner, G. P., Baillie, J. E. M., Burgess, N. D., Burkart, K., Noss, R. F., Zhang, Y. P., Baccini, A., Birch, T., Hahn, N., Joppa, L. N. and Wikramanayake, E.: A Global Deal for Nature: Guiding principles, milestones, and targets, *Science Advances*, doi:10.1126/sciadv.aaw2869, 2019.
- Dittmar, T., Hertkorn, N., Kattner, G. and Lara, R. J.: Mangroves, a major source of dissolved organic carbon to the oceans, *Global Biogeochemical Cycles*, doi:10.1029/2005GB002570, 2006.
- Donato, D. C., Kauffman, J. B., Mackenzie, R. A., Ainsworth, A. and Pflieger, A. Z.: Whole-island carbon stocks in the tropical Pacific: Implications for mangrove conservation and upland restoration, *Journal of Environmental Management*, doi:10.1016/j.jenvman.2011.12.004, 2012.
- Dubuc, A., Baker, R., Marchand, C., Waltham, N. J. and Sheaves, M.: Hypoxia in mangroves: Occurrence and impact on valuable tropical fish habitat, *Biogeosciences*, doi:10.5194/bg-16-3959-2019, 2019a.
- Dubuc, A., Waltham, N. J., Baker, R., Marchand, C. and Sheaves, M.: Patterns of fish utilisation in a tropical Indo-Pacific mangrove-coral seascape, New Caledonia, *PLoS ONE*, doi:10.1371/journal.pone.0207168, 2019b.

- Ducklow, H. W., Kirchman, D. L., Quinby, H. L., Carlson, C. A. and Dam, H. G.: Stocks and dynamics of bacterioplankton carbon during the spring bloom in the eastern North Atlantic Ocean, *Deep-Sea Research Part II*, doi:10.1016/0967-0645(93)90016-G, 1993.
- Duckworth, A. R. and Peterson, B. J.: Effects of seawater temperature and pH on the boring rates of the sponge *Cliona celata* in scallop shells, *Marine Biology*, doi:10.1007/s00227-012-2053-z, 2013.
- Duckworth, A. R., West, L., Vansach, T., Stubler, A. and Hardt, M.: Effects of water temperature and pH on growth and metabolite biosynthesis of coral reef sponges, *Marine Ecology Progress Series*, doi:10.3354/meps09853, 2012.
- Dufault, A. M., Cumbo, V. R., Fan, T. Y. and Edmunds, P. J.: Effects of diurnally oscillating pCO<sub>2</sub> on the calcification and survival of coral recruits, *Proceedings of the Royal Society B: Biological Sciences*, doi:10.1098/rspb.2011.2545, 2012.
- Eberl, G.: A new vision of immunity: Homeostasis of the superorganism, *Mucosal Immunology*, doi:10.1038/mi.2010.20, 2010.
- Engel, S. and Pawlik, J. R.: Interactions among Florida sponges. II. Mangrove habitats, *Marine Ecology Progress Series*, doi:10.3354/meps303145, 2005.
- Enochs, I. C., Manzello, D. P., Donham, E. M., Kolodziej, G., Okano, R., Johnston, L., Young, C., Iguel, J., Edwards, C. B., Fox, M. D., Valentino, L., Johnson, S., Benavente, D., Clark, S. J., Carlton, R., Burton, T., Eynaud, Y. and Price, N. N.: Shift from coral to macroalgae dominance on a volcanically acidified reef, *Nature Climate Change*, doi:10.1038/nclimate2758, 2015.
- Enochs, I. C., Manzello, D. P., Tribollet, A., Valentino, L., Kolodziej, G., Donham, E. M., Fitchett, M. D., Carlton, R. and Price, N. N.: Elevated Colonization of Microborers at a Volcanically Acidified Coral Reef, *PLoS ONE*, doi:10.1371/journal.pone.0159818, 2016.
- Ezzat, L., Maguer, J. F., Grover, R. and Ferrier-Pagès, C.: Limited phosphorus availability is the Achilles heel of tropical reef corals in a warming ocean, *Scientific Reports*, doi:10.1038/srep31768, 2016a.
- Ezzat, L., Towle, E., Irisson, J. O., Langdon, C. and Ferrier-Pagès, C.: The relationship between heterotrophic feeding and inorganic nutrient availability in the scleractinian coral *T. reniformis* under a short-term temperature increase, *Limnology and Oceanography*, doi:10.1002/lno.10200, 2016b.
- Ezzat, L., Maguer, J. F., Grover, R., Rottier, C., Tremblay, P. and Ferrier-Pagès, C.: Nutrient starvation impairs the trophic plasticity of reef-building corals under ocean warming, *Functional Ecology*, doi:10.1111/1365-2435.13285, 2019.
- Fabricius, K. E., Langdon, C., Uthicke, S., Humphrey, C., Noonan, S., De'ath, G., Okazaki, R., Muehllehner, N., Glas, M. S. and Lough, J. M.: Losers and winners in coral reefs acclimatized to elevated carbon dioxide concentrations, *Nature Climate Change*, doi:10.1038/nclimate1122, 2011.
- Fabricius, K. E., De'ath, G., Noonan, S. and Uthicke, S.: Ecological effects of ocean acidification and habitat complexity on reef-associated macroinvertebrate communities, *Proceedings of the Royal Society B: Biological Sciences*, doi:10.1098/rspb.2013.2479, 2013.
- Fabricius, K. E., Kluibenschedl, A., Harrington, L., Noonan, S. and De'Ath, G.: In situ changes of tropical crustose coralline algae along carbon dioxide gradients, *Scientific Reports*, doi:10.1038/srep09537, 2015.
- Fabry, V. J., Seibel, B. A., Feely, R. A. and Orr, J. C.: Impacts of ocean acidification on marine fauna and ecosystem processes, in *ICES Journal of Marine Science.*, 2008.

- Fan, L., Liu, M., Simister, R., Webster, N. S. and Thomas, T.: Marine microbial symbiosis heats up: The phylogenetic and functional response of a sponge holobiont to thermal stress, *ISME Journal*, doi:10.1038/ismej.2012.165, 2013.
- Fang, J. K. H., Mello-Athayde, M. A., Schönberg, C. H. L., Kline, D. I., Hoegh-Guldberg, O. and Dove, S.: Sponge biomass and bioerosion rates increase under ocean warming and acidification, *Global Change Biology*, doi:10.1111/gcb.12334, 2013.
- Fantazzini, P., Mengoli, S., Pasquini, L., Bortolotti, V., Brizi, L., Mariani, M., Di Giosia, M., Fermani, S., Capaccioni, B., Caroselli, E., Prada, F., Zaccanti, F., Levy, O., Dubinsky, Z., Kaandorp, J. A., Konglerd, P., Hammel, J. U., Dauphin, Y., Cuif, J. P., Weaver, J. C., Fabricius, K. E., Wagermaier, W., Fratzl, P., Falini, G. and Goffredo, S.: Gains and losses of coral skeletal porosity changes with ocean acidification acclimation, *Nature Communications*, doi:10.1038/ncomms8785, 2015.
- Feely, R. A., Sabine, C. L., Hernandez-Ayon, J. M., Ianson, D. and Hales, B.: Evidence for upwelling of corrosive “acidified” water onto the continental shelf, *Science*, doi:10.1126/science.1155676, 2008.
- Fenchel, T.: The microbial loop - 25 years later, *Journal of Experimental Marine Biology and Ecology*, doi:10.1016/j.jembe.2008.07.013, 2008.
- Feng, G. and Li, Z.: Carbon and Nitrogen Metabolism of Sponge Microbiome, in *Symbiotic Microbiomes of Coral Reefs Sponges and Corals.*, 2019.
- Ferrier-Pagès, C., Gattuso, J. P. and Jaubert, J.: Effect of small variations in salinity on the rates of photosynthesis and respiration of the zooxanthellate coral *Stylophora pistillata*, *Marine Ecology Progress Series*, doi:10.3354/meps181309, 1999.
- Fey, P., Parravicini, V., Bănar, D., Dierking, J., Galzin, R., Lebreton, B., Meziane, T., Polunin, N. V. C., Zubia, M. and Letourneur, Y.: Multi-trophic markers illuminate the understanding of the functioning of a remote, low coral cover Marquesan coral reef food web, *Scientific Reports*, doi:10.1038/s41598-021-00348-w, 2021.
- Fiore, C. L., Baker, D. M. and Lesser, M. P.: Nitrogen Biogeochemistry in the Caribbean Sponge, *Xestospongia muta*: A Source or Sink of Dissolved Inorganic Nitrogen?, *PLoS ONE*, doi:10.1371/journal.pone.0072961, 2013.
- Fitt, W. K., Brown, B. E., Warner, M. E. and Dunne, R. P.: Coral bleaching: Interpretation of thermal tolerance limits and thermal thresholds in tropical corals, *Coral Reefs*, 20(1), 51–65, doi:10.1007/s003380100146, 2001.
- Flórez, L. V., Biedermann, P. H. W., Engl, T. and Kaltenpoth, M.: Defensive symbioses of animals with prokaryotic and eukaryotic microorganisms, *Natural Product Reports*, doi:10.1039/c5np00010f, 2015.
- Furnas, M., Mitchell, A. W. and Skuza, M.: Nitrogen and phosphorus budgets for the Central Great Barrier Reef., 1995.
- Gattuso, J. P. and Hansson, L.: *Ocean acidification*, Oxford university press, 2011.
- Genty, B., Briantais, J. M. and Baker, N. R.: The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence, *Biochimica et Biophysica Acta - General Subjects*, doi:10.1016/S0304-4165(89)80016-9, 1989.
- Gleeson, J., Santos, I. R., Maher, D. T. and Golsby-Smith, L.: Groundwater-surface water exchange in a mangrove tidal creek: Evidence from natural geochemical tracers and implications for nutrient budgets, *Marine Chemistry*, doi:10.1016/j.marchem.2013.02.001, 2013.

- De Goeij, J. M., Van Den Berg, H., Van Oostveen, M. M., Epping, E. H. G. and Van Duyl, F. C.: Major bulk dissolved organic carbon (DOC) removal by encrusting coral reef cavity sponges, *Marine Ecology Progress Series*, doi:10.3354/meps07403, 2008.
- De Goeij, J. M., De Kluijver, A., Van Duyl, F. C., Vacelet, J., Wijffels, R. H., De Goeij, A. F. R. M., Cleutjens, J. P. M. and Schutte, B.: Cell kinetics of the marine sponge *Halisarca caerulea* reveal rapid cell turnover and shedding, *Journal of Experimental Biology*, doi:10.1242/jeb.034561, 2009.
- De Goeij, J. M., Van Oevelen, D., Vermeij, M. J. A., Osinga, R., Middelburg, J. J., De Goeij, A. F. P. M. and Admiraal, W.: Surviving in a marine desert: The sponge loop retains resources within coral reefs, *Science*, doi:10.1126/science.1241981, 2013.
- De Goeij, J. M., Lesser, M. P. and Pawlik, J. R.: Nutrient fluxes and ecological functions of coral reef sponges in a changing ocean, in *Climate Change, Ocean Acidification and Sponges: Impacts Across Multiple Levels of Organization.*, 2017.
- Golbuu, Y., Gouezo, M., Kurihara, H., Rehm, L. and Wolanski, E.: Long-term isolation and local adaptation in Palau's Nikko Bay help corals thrive in acidic waters, *Coral Reefs*, doi:10.1007/s00338-016-1457-5, 2016.
- Goodwin, C., Rodolfo-Metalpa, R., Picton, B. and Hall-Spencer, J. M.: Effects of ocean acidification on sponge communities, *Marine Ecology*, doi:10.1111/maec.12093, 2014.
- Granek, E. F., Compton, J. E. and Phillips, D. L.: Mangrove-exported nutrient incorporation by sessile coral reef invertebrates, *Ecosystems*, doi:10.1007/s10021-009-9235-7, 2009.
- Grover, R., Maguer, J. F., Allemand, D. and Ferrier-Pagès, C.: Nitrate uptake in the scleractinian coral *Stylophora pistillata*, *Limnology and Oceanography*, doi:10.4319/lo.2003.48.6.2266, 2003.
- Guerra-Castro, E., Young, P., Pérez-Vázquez, A., Carteron, S. and Alvizu, A.: Spatial variability and human disturbance of sponge assemblages associated with mangrove roots in the southern Caribbean, *Marine and Freshwater Research*, doi:10.1071/MF10267, 2011.
- Guzman, C. and Conaco, C.: Gene expression dynamics accompanying the sponge thermal stress response, *PLoS ONE*, doi:10.1371/journal.pone.0165368, 2016.
- Haas, A. F., Smith, J. E., Thompson, M. and Deheyn, D. D.: Effects of reduced dissolved oxygen concentrations on physiology and fluorescence of hermatypic corals and benthic algae, *PeerJ*, doi:10.7717/peerj.235, 2014.
- Hadas, E., Shpigel, M. and Ilan, M.: Particulate organic matter as a food source for a coral reef sponge, *Journal of Experimental Biology*, doi:10.1242/jeb.027953, 2009.
- Hall-Spencer, J. M. and Harvey, B. P.: Ocean acidification impacts on coastal ecosystem services due to habitat degradation, *Emerging Topics in Life Sciences*, doi:10.1042/ETLS20180117, 2019.
- Hall-Spencer, J. M., Rodolfo-Metalpa, R., Martin, S., Ransome, E., Fine, M., Turner, S. M., Rowley, S. J., Tedesco, D. and Buia, M. C.: Volcanic carbon dioxide vents show ecosystem effects of ocean acidification, *Nature*, doi:10.1038/nature07051, 2008.
- Hall-Spencer, J. M., Belfiore, G., Tomatsuri, M., Porzio, L., Harvey, B. P., Agostini, S. and Kon, K.: Decreased Diversity and Abundance of Marine Invertebrates at CO<sub>2</sub> Seeps in Warm-Temperate Japan, *Zoological science*, doi:10.2108/zs210061, 2022.
- Hansen, J., Sato, M., Ruedy, R., Lo, K., Lea, D. W. and Medina-Elizade, M.: Global temperature change, *National Academy of Sciences*, doi:10.1073/pnas.0606291103, 2006.
- Hanson, C. E., McLaughlin, M. J., Hyndes, G. A. and Strzelecki, J.: Selective uptake of prokaryotic

- picoplankton by a marine sponge (*Callyspongia* sp.) within an oligotrophic coastal system, *Estuarine, Coastal and Shelf Science*, doi:10.1016/j.ecss.2009.05.019, 2009.
- Heinze, C., Meyer, S., Goris, N., Anderson, L., Steinfeldt, R., Chang, N., Le Quéré, C. and Bakker, D. C. E.: The ocean carbon sink - Impacts, vulnerabilities and challenges, *Earth System Dynamics*, doi:10.5194/esd-6-327-2015, 2015.
- Hemminga, M. A. and Mateo, M. A.: Stable carbon isotopes in seagrasses: Variability in ratios and use in ecological studies, *Marine Ecology Progress Series*, doi:10.3354/meps140285, 1996.
- Hentschel, U., Usher, K. M. and Taylor, M. W.: *Marine sponges as microbial fermenters.*, 2006.
- Hildebrand, T., Osterholz, H., Bunse, C., Grotheer, H., Dittmar, T. and Schupp, P. J.: Transformation of dissolved organic matter by two Indo-Pacific sponges, *Limnology and Oceanography*, 2483–2496, doi:10.1002/lno.12214, 2022.
- Hill, J. and Wilkinson, C.: *Methods for ecological monitoring of coral reefs - A Resource for Managers*, Australian Institute of Marine Science, 2004.
- Hill, M. S.: Spongivory on Caribbean reefs releases corals from competition with sponges, *Oecologia*, doi:10.1007/s004420050642, 1998.
- Hill, M. S. H. and A. L.: *Encyclopedia of inland waters.*, 2009.
- Hoegh-Guldberg, O.: Climate change, coral bleaching and the future of the world's coral reefs, *Marine and Freshwater Research*, doi:10.1071/MF99078, 1999.
- Hoegh-Guldberg, O. and Bruno, J. F.: The impact of climate change on the world's marine ecosystems, *Science*, doi:10.1126/science.1189930, 2010.
- Hoegh-Guldberg, O. and Fine, M.: Low temperatures cause coral bleaching, *Coral Reefs*, doi:10.1007/s00338-004-0401-2, 2004.
- Hoegh-Guldberg, O. and Smith, G. J.: The effect of sudden changes in temperature, light and salinity on the population density and export of zooxanthellae from the reef corals *Stylophora pistillata* Esper and *Seriatopora hystrix* Dana, *Journal of Experimental Marine Biology and Ecology*, doi:10.1016/0022-0981(89)90109-3, 1989.
- Hoegh-Guldberg, O., Fine, M., Skirving, W., Johnstone, R., Dove, S. and Strong, A.: Coral bleaching following wintry weather, *Limnology and Oceanography*, doi:10.4319/lno.2005.50.1.0265, 2005.
- Hoer, D. R., Gibson, P. J., Tommerdahl, J. P., Lindquist, N. L. and Martens, C. S.: Consumption of dissolved organic carbon by Caribbean reef sponges, *Limnology and Oceanography*, doi:10.1002/lno.10634, 2018.
- Hoey, A. S., Howells, E., Johansen, J. L., Hobbs, J. P. A., Messmer, V., McCowan, D. M., Wilson, S. K. and Pratchett, M. S.: Recent advances in understanding the effects of climate change on coral reefs, *Diversity*, doi:10.3390/d8020012, 2016.
- Hoffmann, F., Radax, R., Wobken, D., Holtappels, M., Lavik, G., Rapp, H. T., Schläppy, M. L., Schleper, C. and Kuypers, M. M. M.: Complex nitrogen cycling in the sponge *Geodia barretti*, *Environmental Microbiology*, doi:10.1111/j.1462-2920.2009.01944.x, 2009.
- Holmes, R. M., Aminot, A., Kérouel, R., Hooker, B. A. and Peterson, B. J.: A simple and precise method for measuring ammonium in marine and freshwater ecosystems, *Canadian Journal of Fisheries and Aquatic Sciences*, doi:10.1139/f99-128, 1999.
- Hooper, J. N. A. and Van Soest, R. W. M.: *Systema Porifera. A Guide to the Classification of*

Sponges, in *Systema Porifera.*, 2002.

Houlbrèque, F., Tambutté, E., Richard, C. and Ferrier-Pagès, C.: Importance of a micro-diet for scleractinian corals, *Marine Ecology Progress Series*, doi:10.3354/meps282151, 2004.

Hoytema, N. Van, Goeij, J. M. De, Kornder, N. A., El, Y. and Oevelen, D. Van: A carbon cycling model shows strong control of seasonality and importance of sponges on the functioning of a northern Red Sea coral reef, *Coral Reefs*, (0123456789), doi:10.1007/s00338-022-02339-3, 2023.

Hughes, D. J., Alderdice, R., Cooney, C., Kühl, M., Pernice, M., Voolstra, C. R. and Suggett, D. J.: Coral reef survival under accelerating ocean deoxygenation, *Nature Climate Change*, doi:10.1038/s41558-020-0737-9, 2020.

Hughes, T. P., Baird, A. H., Bellwood, D. R., Card, M., Connolly, S. R., Folke, C., Grosberg, R., Hoegh-Guldberg, O., Jackson, J. B. C., Kleypas, J., Lough, J. M., Marshall, P., Nyström, M., Palumbi, S. R., Pandolfi, J. M., Rosen, B. and Roughgarden, J.: Climate change, human impacts, and the resilience of coral reefs, *Science*, doi:10.1126/science.1085046, 2003.

Hughes, T. P., Kerry, J. T., Álvarez-Noriega, M., Álvarez-Romero, J. G., Anderson, K. D., Baird, A. H., Babcock, R. C., Beger, M., Bellwood, D. R., Berkelmans, R., Bridge, T. C., Butler, I. R., Byrne, M., Cantin, N. E., Comeau, S., Connolly, S. R., Cumming, G. S., Dalton, S. J., Diaz-Pulido, G., Eakin, C. M., Figueira, W. F., Gilmour, J. P., Harrison, H. B., Heron, S. F., Hoey, A. S., Hobbs, J. P. A., Hoogenboom, M. O., Kennedy, E. V., Kuo, C. Y., Lough, J. M., Lowe, R. J., Liu, G., McCulloch, M. T., Malcolm, H. A., McWilliam, M. J., Pandolfi, J. M., Pears, R. J., Pratchett, M. S., Schoepf, V., Simpson, T., Skirving, W. J., Sommer, B., Torda, G., Wachenfeld, D. R., Willis, B. L. and Wilson, S. K.: Global warming and recurrent mass bleaching of corals, *Nature*, doi:10.1038/nature21707, 2017.

Hurd, C. L.: Slow-flow habitats as refugia for coastal calcifiers from ocean acidification, *Journal of Phycology*, doi:10.1111/jpy.12307, 2015.

Iglesias-Prieto, R., Galindo-Martínez, C. T., Enríquez, S. and Carricart-Ganivet, J. P.: Attributing reductions in coral calcification to the saturation state of aragonite, comments on the effects of persistent natural acidification, *Proceedings of the National Academy of Sciences of the United States of America*, doi:10.1073/pnas.1318521111, 2014.

Inoue, S., Kayanne, H., Yamamoto, S. and Kurihara, H.: Spatial community shift from hard to soft corals in acidified water, *Nature Climate Change*, doi:10.1038/nclimate1855, 2013.

IPCC: *Climate Change 2007 - The Physical Science Basis: Working Group I Contribution to the Fourth Assessment Report of the IPCC*, Science, doi:volume, 2007.

IPCC: *Climate Change 2014 Synthesis Report - IPCC.*, 2014.

IPCC: *Climate Change 2021—The Physical Science Basis*, Chemistry International, doi:10.1515/ci-2021-0407, 2021.

IPCC Working Group 1, I., Stocker, T. F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S. K., Boschung, J., Nauels, A., Xia, Y., Bex, V. and Midgley, P. M.: *IPCC, 2013: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*, IPCC, 2013.

Jacquemont, J., Houlbrèque, F., Tanvet, C. and Rodolfo-Metalpa, R.: Long-term exposure to an extreme environment induces species-specific responses in corals' photosynthesis and respiration rates, *Marine Biology*, 169(6), 1–15, doi:10.1007/s00227-022-04063-6, 2022.

Jacquet, S., Delesalle, B., Torréton, J. P. and Blanchot, J.: Response of phytoplankton communities

- to increased anthropogenic influences (southwestern lagoon, New Caledonia), *Marine Ecology Progress Series*, doi:10.3354/meps320065, 2006.
- Jiménez, E. and Ribes, M.: Sponges as a source of dissolved inorganic nitrogen: Nitrification mediated by temperate sponges, *Limnology and Oceanography*, doi:10.4319/lo.2007.52.3.0948, 2007.
- Jokiel, P. L., Hunter, C. L., Taguchi, S. and Watarai, L.: Ecological impact of a fresh-water “reef kill” in Kaneohe Bay, Oahu, Hawaii, *Coral Reefs*, doi:10.1007/BF00334477, 1993.
- Jürgens, K. and DeMott, W. R.: Behavioral flexibility in prey selection by bacterivorous nanoflagellates, *Limnology and Oceanography*, doi:10.4319/lo.1995.40.8.1503, 1995.
- Justić, D., Rabalais, N. N., Turner, R. E. and Dortch, Q.: Changes in nutrient structure of river-dominated coastal waters: Stoichiometric nutrient balance and its consequences, *Estuarine, Coastal and Shelf Science*, doi:10.1016/S0272-7714(05)80014-9, 1995.
- Kahn, A. S. and Leys, S. P.: The role of cell replacement in benthic-pelagic coupling by suspension feeders, *Royal Society Open Science*, doi:10.1098/rsos.160484, 2016.
- Kapsenberg, L. and Cyronak, T.: Ocean acidification refugia in variable environments, *Global Change Biology*, doi:10.1111/gcb.14730, 2019.
- Kelmo, F., Bell, J. J. and Attrill, M. J.: Tolerance of Sponge Assemblages to Temperature Anomalies: Resilience and Proliferation of Sponges following the 1997-8 El-Niño Southern Oscillation, *PLoS ONE*, doi:10.1371/journal.pone.0076441, 2013.
- Kelmo, F., Bell, J. J., Moraes, S. S., Gomes, R. D. C. T., Mariano-Neto, E. and Attrill, M. J.: Differential responses of emergent intertidal coral reef fauna to a large-scale el-niño southern oscillation event: Sponge and coral resilience, *PLoS ONE*, doi:10.1371/journal.pone.0093209, 2014.
- Kemp, D. W., Oakley, C. A., Thornhill, D. J., Newcomb, L. A., Schmidt, G. W. and Fitt, W. K.: Catastrophic mortality on inshore coral reefs of the Florida Keys due to severe low-temperature stress, *Global Change Biology*, doi:10.1111/j.1365-2486.2011.02487.x, 2011.
- Komornicki, J.: Etude de l'ichtyofaune de la mangrove de Bouraké ( Nouvelle Calédonie ) Etude de l'ichtyofaune de la mangrove de Bouraké ( Nouvelle Calédonie ), *Memoires de stage*, 1–20, 1988.
- Kornder, N. A., Esser, Y., Stoupin, D., Leys, S. P., Mueller, B., Vermeij, M. J. A., Huisman, J. and de Goeij, J. M.: Sponges sneeze mucus to shed particulate waste from their seawater inlet pores, *Current Biology*, 32(17), 3855-3861.e3, doi:10.1016/j.cub.2022.07.017, 2022.
- Koropatnick, T. A., Engle, J. T., Apicella, M. A., Stabb, E. V., Goldman, W. E. and McFall-Ngai, M. J.: Microbial factor-mediated development in a host-bacterial mutualism, *Science*, doi:10.1126/science.1102218, 2004.
- Kozłowski, J.: Optimal allocation of resources to growth and reproduction: Implications for age and size at maturity, *Trends in Ecology and Evolution*, doi:10.1016/0169-5347(92)90192-E, 1992.
- Kroeker, K. J., Micheli, F., Gambi, M. C. and Martz, T. R.: Divergent ecosystem responses within a benthic marine community to ocean acidification, *Proceedings of the National Academy of Sciences*, doi:10.1073/pnas.1107789108, 2011.
- Kroeker, K. J., Kordas, R. L., Crim, R., Hendriks, I. E., Ramajo, L., Singh, G. S., Duarte, C. M. and Gattuso, J. P.: Impacts of ocean acidification on marine organisms: Quantifying sensitivities and interaction with warming, *Global Change Biology*, doi:10.1111/gcb.12179, 2013.
- Kurihara, H., Suhara, Y., Mimura, I. and Golbuu, Y.: Potential Acclimatization and Adaptive

- Responses of Adult and Trans-Generation Coral Larvae From a Naturally Acidified Habitat, *Frontiers in Marine Science*, doi:10.3389/fmars.2020.581160, 2020.
- Kurihara, H., Watanabe, A., Tsugi, A., Mimura, I., Hongo, C., Kawai, T., Reimer, J. D., Kimoto, K., Gouezo, M. and Golbuu, Y.: Potential local adaptation of corals at acidified and warmed Nikko Bay, Palau, *Scientific Reports*, doi:10.1038/s41598-021-90614-8, 2021.
- Kwiatkowski, L. and Orr, J. C.: Diverging seasonal extremes for ocean acidification during the twenty-first century, *Nature Climate Change*, doi:10.1038/s41558-017-0054-0, 2018.
- Lacoste, É., Raimbault, P., Harmelin-Vivien, M. and Gaertner-Mazouni, N.: Trophic relationships between the farmed pearl oyster *Pinctada margaritifera* and its epibionts revealed by stable isotopes and feeding experiments, *Aquaculture Environment Interactions*, doi:10.3354/aei00157, 2016.
- Laffoley, D. and Baxter, J.: Global evidence of ocean deoxygenation. [online] Available from: <https://policycommons.net/artifacts/1372493/ocean-deoxygenation/1986686/>, 2019.
- Langdon, C. and Atkinson, M. J.: Effect of elevated pCO<sub>2</sub> on photosynthesis and calcification of corals and interactions with seasonal change in temperature/ irradiance and nutrient enrichment, *Journal of Geophysical Research C: Oceans*, doi:10.1029/2004JC002576, 2005.
- Leopold, A., Marchand, C., Deborde, J. and Allenbach, M.: Water Biogeochemistry of a Mangrove-Dominated Estuary Under a Semi-Arid Climate (New Caledonia), *Estuaries and Coasts*, doi:10.1007/s12237-016-0179-9, 2017.
- Lesser, M. P. and Slattery, M.: Ecology of caribbean sponges: Are top-down or bottom-up processes more important?, *PLoS ONE*, doi:10.1371/journal.pone.0079799, 2013.
- Lesser, M. P., Fiore, C., Slattery, M. and Zaneveld, J.: Climate change stressors destabilize the microbiome of the Caribbean barrel sponge, *Xestospongia muta*, *Journal of Experimental Marine Biology and Ecology*, doi:10.1016/j.jembe.2015.11.004, 2016.
- Lesser, M. P., Mueller, B., Pankey, M. S., Macartney, K. J., Slattery, M. and de Goeij, J. M.: Depth-dependent detritus production in the sponge, *Halisarca caerulea*, *Limnology and Oceanography*, doi:10.1002/lno.11384, 2020.
- Letourneur, Y., Lison de Loma, T., Richard, P., Harmelin-Vivien, M. L., Cresson, P., Banaru, D., Fontaine, M. F., Gref, T. and Planes, S.: Identifying carbon sources and trophic position of coral reef fishes using diet and stable isotope ( $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ ) analyses in two contrasted bays in Moorea, French Polynesia, *Coral Reefs*, doi:10.1007/s00338-013-1073-6, 2013.
- Letourneur, Y., Briand, M. J. and Guillou, G.: Pathways of organic matter in an estuarine mangrove trophic network assessed by carbon and nitrogen stable isotopes, *Journal of the Marine Biological Association of the United Kingdom*, doi:10.1017/S0025315417001412, 2018.
- Leung, J. Y. S., Zhang, S. and Connell, S. D.: Is Ocean Acidification Really a Threat to Marine Calcifiers? A Systematic Review and Meta-Analysis of 980+ Studies Spanning Two Decades, *Small*, 18(35), doi:10.1002/sml.202107407, 2022.
- Levas, S., Grottoli, A. G., Warner, M. E., Cai, W. J., Bauer, J., Schoepf, V., Baumann, J. H., Matsui, Y., Gearing, C., Melman, T. F., Hoadley, K. D., Pettay, D. T., Hu, X., Li, Q., Xu, H. and Wang, Y.: Organic carbon fluxes mediated by corals at elevated pCO<sub>2</sub> and temperature, *Marine Ecology Progress Series*, doi:10.3354/meps11072, 2015.
- Li, C. W., Chen, J. Y. and Hua, T. E.: Precambrian sponges with cellular structures, *Science*, doi:10.1126/science.279.5352.879, 1998.

- López-Acosta, M., Leynaert, A., Chavaud, L., Amice, E., Bihannic, I., Le Bec, T. and Maldonado, M.: In situ determination of Si, N, and P utilization by the demosponge *Tethya citrina*: A benthic-chamber approach, *PLoS ONE*, doi:10.1371/journal.pone.0218787, 2019.
- López-Legentil, S., Song, B., McMurray, S. E. and Pawlik, J. R.: Bleaching and stress in coral reef ecosystems: hsp70 expression by the giant barrel sponge *Xestospongia muta*, *Molecular Ecology*, doi:10.1111/j.1365-294X.2008.03667.x, 2008.
- Luter, H. M., Andersen, M., Versteegen, E., Laffy, P., Uthicke, S., Bell, J. J. and Webster, N. S.: Cross-generational effects of climate change on the microbiome of a photosynthetic sponge, *Environmental Microbiology*, doi:10.1111/1462-2920.15222, 2020.
- Maggioni, F.: The mangrove system of Bouraké (New Caledonia) as a natural laboratory to study the effect of ocean acidification. Distribution of benthic assemblages and porifera metabolism., UNIVPM Thesis Deposit., 2018.
- Maggioni, F., Pujo-Pay, M., Aucan, J., Cerrano, C., Calcinai, B., Payri, C., Benzoni, F., Letourneur, Y. and Rodolfo-Metalpa, R.: The Bouraké semi-enclosed lagoon (New Caledonia)- A natural laboratory to study the lifelong adaptation of a coral reef ecosystem to extreme environmental conditions, *Biogeosciences*, 18(18), 5117–5140, doi:10.5194/bg-18-5117-2021, 2021.
- Maggioni, F., Bell, J. J., Pujo-Pay, M., Shaffer, M., Cerrano, C., Lemonnier, H., Letourneur, Y. and Rodolfo-Metalpa, R.: Sponge organic matter recycling: Reduced detritus production under extreme environmental conditions, *Marine Pollution Bulletin*, 190, 114869, doi:10.1016/J.MARPOLBUL.2023.114869, 2023.
- Maldonado, M.: Sponge waste that fuels marine oligotrophic food webs: a re-assessment of its origin and nature, *Marine Ecology*, doi:10.1111/maec.12256, 2016.
- Maldonado, M., Carmona, M. C., Uriz, M. J. and Cruzado, A.: Decline in Mesozoic reef-building sponges explained by silicon limitation, *Nature*, doi:10.1038/44560, 1999.
- Maldonado, M., Carmona, M. C., Velásquez, Z., Puig, A., Cruzado, A., López, A. and Young, C. M.: Siliceous sponges as a silicon sink: An overlooked aspect of benthopelagic coupling in the marine silicon cycle, *Limnology and Oceanography*, doi:10.4319/lo.2005.50.3.0799, 2005.
- Maldonado, M., Zhang, X., Cao, X., Xue, L., Cao, H. and Zhang, W.: Selective feeding by sponges on pathogenic microbes: A reassessment of potential for abatement of microbial pollution, *Marine Ecology Progress Series*, doi:10.3354/meps08411, 2010.
- Maldonado, M., Ribes, M. and van Duyl, F. C.: Nutrient Fluxes Through Sponges. Biology, Budgets, and Ecological Implications, in *Advances in Marine Biology.*, 2012.
- Maldonado, M., Acosta, M. L., Beazley, L., Kenchington, E., Koutsouveli, V. and Riesgo, A.: Cooperation between passive and active silicon transporters clarifies the ecophysiology and evolution of biosilicification in sponges, *Science Advances*, doi:10.1126/sciadv.aba9322, 2020.
- Manucharyan, G. E., Brierley, C. M. and Fedorov, A. V.: Climate impacts of intermittent upper ocean mixing induced by tropical cyclones, *Journal of Geophysical Research: Oceans*, doi:10.1029/2011JC007295, 2011.
- Manzello, D. and Lirman, D.: The photosynthetic resilience of *Porites furcata* to salinity disturbance, in *Coral Reefs.*, 2003.
- Manzello, D. P.: Ocean acidification hot spots: Spatiotemporal dynamics of the seawater CO<sub>2</sub> system of eastern Pacific coral reefs, *Limnology and Oceanography*, doi:10.4319/lo.2010.55.1.0239, 2010.

- Manzello, D. P., Kleypas, J. A., Budd, D. A., Eakin, C. M., Glynn, P. W. and Langdon, C.: Poorly cemented coral reefs of the eastern tropical Pacific: Possible insights into reef development in a high-CO<sub>2</sub> world, *Proceedings of the National Academy of Sciences of the United States of America*, doi:10.1073/pnas.0712167105, 2008.
- Manzello, D. P., Enochs, I. C., Bruckner, A., Renaud, P. G., Kolodziej, G., Budd, D. A., Carlton, R. and Glynn, P. W.: Galápagos coral reef persistence after ENSO warming across an acidification gradient, *Geophysical Research Letters*, doi:10.1002/2014GL062501, 2014.
- Massaro, A. J., Weisz, J. B., Hill, M. S. and Webster, N. S.: Behavioral and morphological changes caused by thermal stress in the Great Barrier Reef sponge *Rhopaloeides odorabile*, *Journal of Experimental Marine Biology and Ecology*, doi:10.1016/j.jembe.2012.02.008, 2012.
- Mayfield, A. B. and Gates, R. D.: Osmoregulation in anthozoan-dinoflagellate symbiosis, *Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology*, doi:10.1016/j.cbpa.2006.12.042, 2007.
- McMurray, S. E., Henkel, T. P. and Pawlik, J. R.: Demographics of increasing populations of the giant barrel sponge *Xestospongia muta* in the Florida Keys, *Ecology*, doi:10.1890/08-2060.1, 2010.
- McMurray, S. E., Johnson, Z. I., Hunt, D. E., Pawlik, J. R. and Finelli, C. M.: Selective feeding by the giant barrel sponge enhances foraging efficiency, *Limnology and Oceanography*, doi:10.1002/lno.10287, 2016.
- McMurray, S. E., Stubler, A. D., Erwin, P. M., Finelli, C. M. and Pawlik, J. R.: A test of the sponge-loop hypothesis for emergent Caribbean reef sponges, *Marine Ecology Progress Series*, doi:10.3354/meps12466, 2018.
- Meroz-Fine, E., Shefer, S. and Ilan, M.: Changes in morphology and physiology of an East Mediterranean sponge in different habitats, *Marine Biology*, doi:10.1007/s00227-004-1532-2, 2005.
- Micaroni, V., Strano, F., McAllen, R., Woods, L., Turner, J., Harman, L. and Bell, J. J.: Adaptive strategies of sponges to deoxygenated oceans, *Global Change Biology*, doi:10.1111/gcb.16013, 2022.
- Milazzo, M., Rodolfo-Metalpa, R., Chan, V. B. S., Fine, M., Alessi, C., Thiagarajan, V., Hall-Spencer, J. M. and Chemello, R.: Ocean acidification impairs vermetid reef recruitment, *Scientific Reports*, doi:10.1038/srep04189, 2014.
- Mohamed, N. M., Saito, K., Tal, Y. and Hill, R. T.: Diversity of aerobic and anaerobic ammonia-oxidizing bacteria in marine sponges, *ISME Journal*, doi:10.1038/ismej.2009.84, 2010.
- Moitinho-Silva, L., Steinert, G., Nielsen, S., Hardoim, C. C. P., Wu, Y. C., McCormack, G. P., López-Legentil, S., Marchant, R., Webster, N., Thomas, T. and Hentschel, U.: Predicting the HMA-LMA status in marine sponges by machine learning, *Frontiers in Microbiology*, doi:10.3389/fmicb.2017.00752, 2017a.
- Moitinho-Silva, L., Nielsen, S., Amir, A., Gonzalez, A., Ackermann, G. L., Cerrano, C., Astudillo-Garcia, C., Easson, C., Sipkema, D., Liu, F., Steinert, G., Kotoulas, G., McCormack, G. P., Feng, G., Bell, J. J., Vicente, J., Björk, J. R., Montoya, J. M., Olson, J. B., Reveillaud, J., Steindler, L., Pineda, M. C., Marra, M. V., Ilan, M., Taylor, M. W., Polymenakou, P., Erwin, P. M., Schupp, P. J., Simister, R. L., Knight, R., Thacker, R. W., Costa, R., Hill, R. T., Lopez-Legentil, S., Dailianis, T., Ravasi, T., Hentschel, U., Li, Z., Webster, N. S. and Thomas, T.: The sponge microbiome project, *GigaScience*, doi:10.1093/gigascience/gix077, 2017b.
- Morel, A., Yu-Hwan Ahn, Partensky, F., Vaultot, D. and Claustre, H.: *Prochlorococcus* and *Synechococcus*: a comparative study of their optical properties in relation to their size and

- pigmentation, *Journal of Marine Research*, doi:10.1357/0022240933223963, 1993.
- Morganti, T., Coma, R., Yahel, G. and Ribes, M.: Trophic niche separation that facilitates co-existence of high and low microbial abundance sponges is revealed by in situ study of carbon and nitrogen fluxes, *Limnology and Oceanography*, doi:10.1002/lno.10546, 2017.
- Morley, S. A., Berman, J., Barnes, D. K. A., Carbonell, C. de J., Downey, R. V. and Peck, L. S.: Extreme phenotypic plasticity in metabolic physiology of Antarctic demosponges, *Frontiers in Ecology and Evolution*, doi:10.3389/fevo.2015.00157, 2016.
- Morrow, K. M., Bourne, D. G., Humphrey, C., Botté, E. S., Laffy, P., Zaneveld, J., Uthicke, S., Fabricius, K. E. and Webster, N. S.: Natural volcanic CO<sub>2</sub> seeps reveal future trajectories for host-microbial associations in corals and sponges, *ISME Journal*, doi:10.1038/ismej.2014.188, 2015.
- Mueller, B., De Goeij, J. M., Vermeij, M. J. A., Mulders, Y., Van Der Ent, E., Ribes, M. and Van Duyl, F. C.: Natural diet of coral-excavating sponges consists mainly of dissolved organic carbon (DOC), *PLoS ONE*, doi:10.1371/journal.pone.0090152, 2014.
- Mumby, P. J. and Steneck, R. S.: Paradigm lost: Dynamic nutrients and missing detritus on coral reefs, *BioScience*, doi:10.1093/biosci/biy055, 2018.
- Muscantine, L. and Porter, J. W.: Reef Corals: Mutualistic Symbioses Adapted to Nutrient-Poor Environments, *BioScience*, doi:10.2307/1297526, 1977.
- Muthiga, N. A. and Szmant, A. M.: The effects of salinity stress on the rates of aerobic respiration and photosynthesis in the hermatypic coral *Siderastrea siderea*, *The Biological Bulletin*, doi:10.2307/1541699, 1987.
- Nagelkerken, I., Blaber, S. J. M., Bouillon, S., Green, P., Haywood, M., Kirton, L. G., Meynecke, J. O., Pawlik, J., Penrose, H. M., Sasekumar, A. and Somerfield, P. J.: The habitat function of mangroves for terrestrial and marine fauna: A review, *Aquatic Botany*, doi:10.1016/j.aquabot.2007.12.007, 2008.
- Naumann, M. S., Haas, A., Struck, U., Mayr, C., el-Zibdah, M. and Wild, C.: Organic matter release by dominant hermatypic corals of the Northern Red Sea, *Coral Reefs*, doi:10.1007/s00338-010-0612-7, 2010.
- Nelson, H. R. and Altieri, A. H.: Oxygen: the universal currency on coral reefs, *Coral Reefs*, doi:10.1007/s00338-019-01765-0, 2019.
- Nicholson, J. K., Holmes, E., Kinross, J., Burcelin, R., Gibson, G., Jia, W. and Pettersson, S.: Host-gut microbiota metabolic interactions, *Science*, doi:10.1126/science.1223813, 2012.
- Le Nohaïc, M., Ross, C. L., Cornwall, C. E., Comeau, S., Lowe, R., McCulloch, M. T. and Schoepf, V.: Marine heatwave causes unprecedented regional mass bleaching of thermally resistant corals in northwestern Australia, *Scientific Reports*, doi:10.1038/s41598-017-14794-y, 2017.
- Oliver, T. A. and Palumbi, S. R.: Do fluctuating temperature environments elevate coral thermal tolerance?, *Coral Reefs*, doi:10.1007/s00338-011-0721-y, 2011.
- Palumbi, S. R.: How body plans limit acclimation: responses of a demosponge to wave force., *Ecology*, doi:10.2307/1938520, 1986.
- Palumbi, S. R., Barshis, D. J., Traylor-Knowles, N. and Bay, R. A.: Mechanisms of reef coral resistance to future climate change, *Science*, doi:10.1126/science.1251336, 2014.
- Pansini, M., Morri, C. and Bianchi, C. N.: The sponge community of a subtidal area with hydrothermal vents: Milos Island, Aegean Sea, *Estuarine, Coastal and Shelf Science*,

doi:10.1006/ecss.2000.0674, 2000.

Pantile, R. and Webster, N.: Strict thermal threshold identified by quantitative PCR in the sponge *Rhopaloeides odorabile*, *Marine Ecology Progress Series*, doi:10.3354/meps09128, 2011.

Pawlik, J. R.: Coral reef sponges: Do predatory fishes affect their distribution?, *Limnology and Oceanography*, doi:10.4319/lo.1998.43.6.1396, 1998.

Pawlik, J. R. and McMurray, S. E.: The Emerging Ecological and Biogeochemical Importance of Sponges on Coral Reefs, *Annual Review of Marine Science*, doi:10.1146/annurev-marine-010419-010807, 2020.

Pawlik, J. R., McMurray, S. E. and Henkel, T. P.: Abiotic factors control sponge ecology in Florida mangroves, *Marine Ecology Progress Series*, doi:10.3354/meps339093, 2007.

Pawlik, J. R., Loh, T. L., McMurray, S. E. and Finelli, C. M.: Sponge Communities on Caribbean Coral Reefs Are Structured by Factors That Are Top-Down, Not Bottom-Up, *PLoS ONE*, doi:10.1371/journal.pone.0062573, 2013.

Pawlik, J. R., McMurray, S. E., Erwin, P. and Zea, S.: A review of evidence for food limitation of sponges on Caribbean reefs, *Marine Ecology Progress Series*, doi:10.3354/meps11093, 2015.

Pawlik, J. R., Burkepille, D. E. and Thurber, R. V.: A Vicious Circle? Altered Carbon and Nutrient Cycling May Explain the Low Resilience of Caribbean Coral Reefs, *BioScience*, doi:10.1093/biosci/biw047, 2016.

Paytan, A., Crook, E. D., Cohen, A. L., Martz, T. R., Takashita, Y., Rebolledo-Vieyra, M. and Hernandez, L.: Reply to Iglesias-Prieto et al.: Combined field and laboratory approaches for the study of coral calcification, *Proceedings of the National Academy of Sciences of the United States of America*, doi:10.1073/pnas.1319572111, 2014.

Perea-Blázquez, A., Davy, S. K. and Bell, J. J.: Estimates of particulate organic carbon flowing from the pelagic environment to the benthos through sponge assemblages, *PLoS ONE*, doi:10.1371/journal.pone.0029569, 2012.

Pernice, M., Meibom, A., Van Den Heuvel, A., Kopp, C., Domart-Coulon, I., Hoegh-Guldberg, O. and Dove, S.: A single-cell view of ammonium assimilation in coral-dinoflagellate symbiosis, *ISME Journal*, doi:10.1038/ismej.2011.196, 2012.

Perrin, N. and Sibly, R. M.: Dynamic models of energy allocation and investment, *Annual Review of Ecology and Systematics*, doi:10.1146/annurev.es.24.110193.002115, 1993.

Philp, R. B.: Effects of pH and oxidant stressors (hydrogen peroxide and bleach) on calcium-induced aggregation of cells of the marine sponge *Microciona prolifera*, *Comparative Biochemistry and Physiology - C Pharmacology Toxicology and Endocrinology*, doi:10.1016/S0742-8413(97)00155-2, 1997.

Pichler, T., Biscéré, T., Kinch, J., Zampighi, M., Houlbrèque, F. and Rodolfo-Metalpa, R.: Suitability of the shallow water hydrothermal system at Ambitle Island (Papua New Guinea) to study the effect of high pCO<sub>2</sub> on coral reefs, *Marine Pollution Bulletin*, doi:10.1016/j.marpolbul.2018.11.003, 2019.

Pierrot, D., Lewis, E. and Wallace, D. W. R.: MS Excel program developed for CO<sub>2</sub> system calculations, in ORNL/CDIAC-105a. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, US Department of Energy, Oak Ridge, Tennessee., 2006.

Piniak, G. A. and Brown, E. K.: Temporal variability in chlorophyll fluorescence of back-reef corals in Ofu, American Samoa, *Biological Bulletin*, doi:10.1086/BBLv216n1p55, 2009.

- Pita, L., Rix, L., Slaby, B. M., Franke, A. and Hentschel, U.: The sponge holobiont in a changing ocean: from microbes to ecosystems, *Microbiome*, doi:10.1186/s40168-018-0428-1, 2018.
- Pons, L. N., Calcinai, B. and Gates, R. D.: Who's there? – First morphological and DNA barcoding catalogue of the shallow Hawai'ian sponge fauna, *PLoS ONE*, 12(12), doi:10.1371/journal.pone.0189357, 2017.
- Porter, J. W., Battey, J. F. and Smith, G. J.: Perturbation and change in coral reef communities, *Proceedings of the National Academy of Sciences*, doi:10.1073/pnas.79.5.1678, 1982.
- Pörtner, H. O.: Ecosystem effects of ocean acidification in times of ocean warming: A physiologist's view, *Marine Ecology Progress Series*, doi:10.3354/meps07768, 2008.
- Pörtner, H. O. and Farrell, A. P.: Ecology: Physiology and climate change, *Science*, doi:10.1126/science.1163156, 2008.
- Pratchett, M. S., Wilson, S. K. and Baird, A. H.: Declines in the abundance of *Chaetodon* butterflyfishes following extensive coral depletion, *Journal of Fish Biology*, doi:10.1111/j.1095-8649.2006.01161.x, 2006.
- Prince, E. D. and Goodyear, C. P.: Hypoxia-based habitat compression of tropical pelagic fishes, *Fisheries Oceanography*, doi:10.1111/j.1365-2419.2005.00393.x, 2006.
- Putnam, H. M.: Avenues of reef-building coral acclimatization in response to rapid environmental change, *Journal of Experimental Biology*, doi:10.1242/jeb.239319, 2021.
- Le Quéré, C., Takahashi, T., Buitenhuis, E. T., Rödenbeck, C. and Sutherland, S. C.: Impact of climate change and variability on the global oceanic sink of CO<sub>2</sub>, *Global Biogeochemical Cycles*, doi:10.1029/2009GB003599, 2010.
- R Core Team: R: A language and environment for statistical computing., 2018.
- R Core Team: R.studio Team, R Development Core Team., 2019.
- Raimbault, P., Garcia, N. and Cerutti, F.: Distribution of inorganic and organic nutrients in the South Pacific Ocean – evidence for long-term accumulation of organic matter in nitrogen-depleted waters, *Biogeosciences*, doi:10.5194/bg-5-281-2008, 2008.
- Ralph, P. J., Gademann, R., Larkum, A. W. D. and Schreiber, U.: In situ underwater measurements of photosynthetic activity of coral zooxanthellae and other reef-dwelling dinoflagellate endosymbionts, *Marine Ecology Progress Series*, doi:10.3354/meps180139, 1999.
- Ramsby, B. D., Hoogenboom, M. O., Whalan, S. and Webster, N. S.: Elevated seawater temperature disrupts the microbiome of an ecologically important bioeroding sponge, *Molecular Ecology*, doi:10.1111/mec.14544, 2018a.
- Ramsby, B. D., Hoogenboom, M. O., Smith, H. A., Whalan, S. and Webster, N. S.: The bioeroding sponge *Cliona orientalis* will not tolerate future projected ocean warming, *Scientific Reports*, doi:10.1038/s41598-018-26535-w, 2018b.
- Raven, John, Caldeira, K., Elderfield, H., Hoegh-Guldberg, O., Liss, P., Riebesell, U., Shepherd, J., Turley, C. and Watson, A.: Ocean acidification due to increasing atmospheric carbon dioxide, *The Royal Society*, 2005.
- Raven, J. A., Wollenweber, B. and Handley, L. L.: A comparison of ammonium and nitrate as nitrogen sources for photolithotrophs, *New Phytologist*, doi:10.1111/j.1469-8137.1992.tb01088.x, 1992.

- Reiswig, H. M.: Particle feeding in natural populations of three marine Demosponges, *The Biological Bulletin*, doi:10.2307/1540270, 1971.
- Reiswig, H. M.: Bacteria as food for temperate-water marine sponges, *Canadian Journal of Zoology*, doi:10.1139/z75-072, 1975.
- Ribes, M., Coma, R. and Gili, J. M.: Heterotrophic feeding by gorgonian corals with symbiotic zooxanthella, *Limnology and Oceanography*, doi:10.4319/lo.1998.43.6.1170, 1998.
- Ribes, M., Coma, R. and Gili, J. M.: Natural diet and grazing rate of the temperate sponge *Dysidea avara* (Demospongiae, Dendroceratida) throughout an annual cycle, *Marine Ecology Progress Series*, doi:10.3354/meps176179, 1999.
- Riisgard, H. U., Thomassen, S., Jakobsen, H., Weeks, J. M. and Larsen, P. S.: Suspension feeding in marine sponges *Halichondria panicea* and *Haliclona urceolus*: effects of temperature on filtration rate and energy cost of pumping, *Marine Ecology Progress Series*, doi:10.3354/meps096177, 1993.
- Rivest, E. B., Comeau, S. and Cornwall, C. E.: The Role of Natural Variability in Shaping the Response of Coral Reef Organisms to Climate Change, *Current Climate Change Reports*, doi:10.1007/s40641-017-0082-x, 2017.
- Rix, L., De Goeij, J. M., Mueller, C. E., Struck, U., Middelburg, J. J., Van Duyl, F. C., Al-Horani, F. A., Wild, C., Naumann, M. S. and Van Oevelen, D.: Coral mucus fuels the sponge loop in warm-and cold-water coral reef ecosystems, *Scientific Reports*, doi:10.1038/srep18715, 2016.
- Rix, L., de Goeij, J. M., van Oevelen, D., Struck, U., Al-Horani, F. A., Wild, C. and Naumann, M. S.: Differential recycling of coral and algal dissolved organic matter via the sponge loop, *Functional Ecology*, doi:10.1111/1365-2435.12758, 2017.
- Rix, L., De Goeij, J. M., Van Oevelen, D., Struck, U., Al-Horani, F. A., Wild, C. and Naumann, M. S.: Reef sponges facilitate the transfer of coral-derived organic matter to their associated fauna via the sponge loop, *Marine Ecology Progress Series*, doi:10.3354/meps12443, 2018.
- Rodolfo-Metalpa, R., Houlbrèque, F., Tambutté, É., Boisson, F., Baggini, C., Patti, F. P., Jeffree, R., Fine, M., Foggo, A., Gattuso, J. P. and Hall-Spencer, J. M.: Coral and mollusc resistance to ocean acidification adversely affected by warming, *Nature Climate Change*, doi:10.1038/nclimate1200, 2011.
- Rogers, C. and Herlan, J.: Life on the edge: corals in mangroves and climate change, *Proceedings of the 12th International Coral*, 2012.
- Rogers, C. S.: A unique coral community in the mangroves of Hurricane Hole, St. John, US Virgin Islands, *Diversity*, doi:10.3390/d9030029, 2017.
- Rosset, S., D'Angelo, C. and Wiedenmann, J.: Ultrastructural biomarkers in symbiotic algae reflect the availability of dissolved inorganic nutrients and particulate food to the reef coral holobiont, *Frontiers in Marine Science*, doi:10.3389/fmars.2015.000103, 2015.
- Rosset, S., Wiedenmann, J., Reed, A. J. and D'Angelo, C.: Phosphate deficiency promotes coral bleaching and is reflected by the ultrastructure of symbiotic dinoflagellates, *Marine Pollution Bulletin*, doi:10.1016/j.marpolbul.2017.02.044, 2017.
- Rützler, K.: Sponges in coral reefs, in *Coral reefs: research methods.*, 1978.
- Rützler, K.: Low-Tide Exposure of Sponges in a Caribbean Mangrove Community, *Marine Ecology*, doi:10.1111/j.1439-0485.1995.tb00402.x, 1995.
- Rützler, K.: Impact of Crustose Clionid Sponges on Caribbean Reef Corals, *Acta geológica hispánica*,

2002.

Safaie, A., Silbiger, N. J., McClanahan, T. R., Pawlak, G., Barshis, D. J., Hench, J. L., Rogers, J. S., Williams, G. J. and Davis, K. A.: High frequency temperature variability reduces the risk of coral bleaching, *Nature Communications*, doi:10.1038/s41467-018-04074-2, 2018.

Saxby, T., Dennison, W. C. and Hoegh-Guldberg, O.: Photosynthetic responses of the coral *Montipora digitata* to cold temperature stress, *Marine Ecology Progress Series*, doi:10.3354/meps248085, 2003.

Schoepf, V., Stat, M., Falter, J. L. and McCulloch, M. T.: Limits to the thermal tolerance of corals adapted to a highly fluctuating, naturally extreme temperature environment, *Scientific Reports*, doi:10.1038/srep17639, 2015.

Schoepf, V., Cornwall, C. E., Pfeifer, S. M., Carrion, S. A., Alessi, C., Comeau, S. and McCulloch, M. T.: Impacts of coral bleaching on pH and oxygen gradients across the coral concentration boundary layer: a microsensor study, *Coral Reefs*, doi:10.1007/s00338-018-1726-6, 2018.

Schoepf, V., Jung, M. U., McCulloch, M. T., White, N. E., Stat, M. and Thomas, L.: Thermally Variable, Macrotidal Reef Habitats Promote Rapid Recovery From Mass Coral Bleaching, *Frontiers in Marine Science*, doi:10.3389/fmars.2020.00245, 2020.

Schönberg, C. H. L.: No taxonomy needed: Sponge functional morphologies inform about environmental conditions, *Ecological Indicators*, doi:10.1016/j.ecolind.2021.107806, 2021.

Seibel, B. A.: Critical oxygen levels and metabolic suppression in oceanic oxygen minimum zones, *Journal of Experimental Biology*, doi:10.1242/jeb.049171, 2011.

Shaffer, G., Olsen, S. M. and Pedersen, J. O. P.: Long-term ocean oxygen depletion in response to carbon dioxide emissions from fossil fuels, *Nature Geoscience*, doi:10.1038/ngeo420, 2009.

Shamberger, K. E. F., Cohen, A. L., Golbuu, Y., McCorkle, D. C., Lentz, S. J. and Barkley, H. C.: Diverse coral communities in naturally acidified waters of a Western Pacific reef, *Geophysical Research Letters*, doi:10.1002/2013GL058489, 2014.

Shamberger, K. E. F., Lentz, S. J. and Cohen, A. L.: Low and variable ecosystem calcification in a coral reef lagoon under natural acidification, *Limnology and Oceanography*, doi:10.1002/lno.10662, 2018.

Sitnikova, T., Kiyashko, S., Bukshuk, N., Zemskaya, T., Khlystov, O. and Moore, M. V.: Stable isotope signatures and distribution of deepwater sponges in Lake Baikal, *Hydrobiologia*, doi:10.1007/s10750-016-2674-1, 2016.

Soares, M. de O.: Marginal reef paradox: A possible refuge from environmental changes?, *Ocean and Coastal Management*, doi:10.1016/j.ocecoaman.2019.105063, 2020.

van Soest, R. W. M., Boury-Esnault, N., Vacelet, J., Dohrmann, M., Erpenbeck, D., de Voogd, N. J., Santodomingo, N., Vanhoorne, B., Kelly, M. and Hooper, J. N. A.: Global diversity of sponges (Porifera), *PLoS ONE*, doi:10.1371/journal.pone.0035105, 2012.

Sokolova, I. M., Frederich, M., Bagwe, R., Lannig, G. and Sukhotin, A. A.: Energy homeostasis as an integrative tool for assessing limits of environmental stress tolerance in aquatic invertebrates, *Marine Environmental Research*, doi:10.1016/j.marenvres.2012.04.003, 2012.

Somero, G.: Temporal patterning of thermal acclimation: from behavior to membrane biophysics, *Journal of Experimental Biology*, doi:10.1242/jeb.109843, 2015.

Southwell, M. W., Weisz, J. B., Martens, C. S. and Lindquist, N.: In situ fluxes of dissolved inorganic

- nitrogen from the sponge community on Conch Reef, Key Largo, Florida, *Limnology and Oceanography*, doi:10.4319/lo.2008.53.3.0986, 2008.
- Stevenson, A., Archer, S. K., Schultz, J. A., Dunham, A., Marliave, J. B., Martone, P. and Harley, C. D. G.: Warming and acidification threaten glass sponge *Aphrocallistes vastus* pumping and reef formation, *Scientific Reports*, 10(1), 1–11, doi:10.1038/s41598-020-65220-9, 2020.
- Stewart, H. A., Kline, D. I., Chapman, L. J., Altieri, A. H., Kline, D. I., Chapman, L. J., Altieri, A. H. and C Peters, D. P.: Caribbean mangrove forests act as coral refugia by reducing light stress and increasing coral richness, , doi:10.1002/ecs2.3413, 2021.
- Stewart, H. A., Wright, J. L., Carrigan, M., Altieri, A. H., Kline, D. I. and Araújo, R. J.: Novel coexisting mangrove-coral habitats: Extensive coral communities located deep within mangrove canopies of Panama, a global classification system and predicted distributions, *PLoS ONE*, 17(6 June), 1–28, doi:10.1371/journal.pone.0269181, 2022.
- Strahl, J., Francis, D. S., Doyle, J., Humphrey, C. and Fabricius, K. E.: Biochemical responses to ocean acidification contrast between tropical corals with high and low abundances at volcanic carbon dioxide seeps, *ICES Journal of Marine Science*, doi:10.1093/icesjms/fsv194, 2016.
- Stramma, L., Prince, E. D., Schmidtko, S., Luo, J., Hoolihan, J. P., Visbeck, M., Wallace, D. W. R., Brandt, P. and Körtzinger, A.: Expansion of oxygen minimum zones may reduce available habitat for tropical pelagic fishes, *Nature Climate Change*, doi:10.1038/nclimate1304, 2012.
- Stubler, A. D., Furman, B. T. and Peterson, B. J.: Sponge erosion under acidification and warming scenarios: Differential impacts on living and dead coral, *Global Change Biology*, doi:10.1111/gcb.13002, 2015.
- Sugimura, Y. and Suzuki, Y.: A high-temperature catalytic oxidation method for the determination of non-volatile dissolved organic carbon in seawater by direct injection of a liquid sample, *Marine Chemistry*, doi:10.1016/0304-4203(88)90043-6, 1988.
- Sultana, R., Casareto, B. E., Sohrin, R., Suzuki, T., Alam, M. S., Fujimura, H. and Suzuki, Y.: Response of Subtropical Coastal Sediment Systems of Okinawa, Japan, to Experimental Warming and High pCO<sub>2</sub>, *Frontiers in Marine Science*, doi:10.3389/fmars.2016.00100, 2016.
- Sunday, J. M., Fabricius, K. E., Kroeker, K. J., Anderson, K. M., Brown, N. E., Barry, J. P., Connell, S. D., Dupont, S., Gaylord, B., Hall-Spencer, J. M., Klinger, T., Milazzo, M., Munday, P. L., Russell, B. D., Sanford, E., Thiyagarajan, V., Vaughan, M. L. H., Widdicombe, S. and Harley, C. D. G.: Ocean acidification can mediate biodiversity shifts by changing biogenic habitat, *Nature Climate Change*, doi:10.1038/nclimate3161, 2017.
- Sutherland, K. P., Porter, J. W. and Torres, C.: Disease and immunity in Caribbean and Indo-Pacific zooxanthellate corals, *Marine Ecology Progress Series*, doi:10.3354/meps266273, 2004.
- Szmant, A. M.: Nutrient enrichment on coral reefs: Is it a major cause of coral reef decline?, *Estuaries*, doi:10.1007/BF02804903, 2002.
- Takahashi, T., Sutherland, S. C., Chipman, D. W., Goddard, J. G. and Ho, C.: Climatological distributions of pH, pCO<sub>2</sub>, total CO<sub>2</sub>, alkalinity, and CaCO<sub>3</sub> saturation in the global surface ocean, and temporal changes at selected locations, *Marine Chemistry*, doi:10.1016/j.marchem.2014.06.004, 2014.
- Tanvet, C., Benzoni, F., Peignon, C., Thouzeau, G. and Rodolfo-Metalpa, R.: High Coral Recruitment Despite Coralline Algal Loss Under Extreme Environmental Conditions, *Frontiers in Marine Science*, 9(June), 1–15, doi:10.3389/fmars.2022.837877, 2022.

- Taylor, D., Nixon, S., Granger, S. and Buckley, B.: Nutrient limitation and the eutrophication of coastal lagoons, *Marine Ecology Progress Series*, doi:10.3354/meps127235, 1995.
- Taylor, F. J. R., Hoppenrath, M. and Saldarriaga, J. F.: Dinoflagellate diversity and distribution, *Biodiversity and Conservation*, doi:10.1007/s10531-007-9258-3, 2008.
- Taylor, M. W., Radax, R., Steger, D. and Wagner, M.: Sponge-Associated Microorganisms: Evolution, Ecology, and Biotechnological Potential, *Microbiology and Molecular Biology Reviews*, doi:10.1128/MMBR.00040-06, 2007.
- Thollot, P.: Importance des mangroves pour la faune ichthyologique des récifs coralliens de Nouvelle-Calédonie, *Cybium*, 1992.
- Thollot, P., Kulbicki, M. and Harmelin-Vivien, M.: Réseaux trophiques et fonctionnement trophodynamique de l'ichtyofaune des mangroves de Nouvelle-Calédonie, *Comptes Rendus de l'Académie des Sciences - Serie III*, doi:10.1016/S0764-4469(00)88531-5, 1999.
- Thomas, T., Moitinho-Silva, L., Lurgi, M., Björk, J. R., Easson, C., Astudillo-García, C., Olson, J. B., Erwin, P. M., López-Legentil, S., Luter, H., Chaves-Fonnegra, A., Costa, R., Schupp, P. J., Steindler, L., Erpenbeck, D., Gilbert, J., Knight, R., Ackermann, G., Victor Lopez, J., Taylor, M. W., Thacker, R. W., Montoya, J. M., Hentschel, U. and Webster, N. S.: Diversity, structure and convergent evolution of the global sponge microbiome, *Nature Communications*, doi:10.1038/ncomms11870, 2016.
- Topçu, N. E., Pérez, T., Grégori, G. and Harmelin-Vivien, M.: In situ investigation of *Spongia officinalis* (Demospongiae) particle feeding: Coupling flow cytometry and stable isotope analysis, *Journal of Experimental Marine Biology and Ecology*, doi:10.1016/j.jembe.2010.03.017, 2010.
- Torda, G., Donelson, J. M., Aranda, M., Barshis, D. J., Bay, L., Berumen, M. L., Bourne, D. G., Cantin, N., Foret, S., Matz, M., Miller, D. J., Moya, A., Putnam, H. M., Ravasi, T., Van Oppen, M. J. H., Thurber, R. V., Vidal-Dupiol, J., Voolstra, C. R., Watson, S. A., Whitelaw, E., Willis, B. L. and Munday, P. L.: Rapid adaptive responses to climate change in corals, *Nature Climate Change*, doi:10.1038/nclimate3374, 2017.
- Vargas, C. A., Lagos, N. A., Lardies, M. A., Duarte, C., Manríquez, P. H., Aguilera, V. M., Broitman, B., Widdicombe, S. and Dupont, S.: Species-specific responses to ocean acidification should account for local adaptation and adaptive plasticity, *Nature Ecology and Evolution*, doi:10.1038/s41559-017-0084, 2017.
- Varillon D., Fiat S., Magron F., Allenbach M., Hoibian T., de Ramon N'Yeurt A., Ganachaud A., Aucan J., Pelletier B., H. R.: ReefTEMPS : The Pacific Island coastal ocean observation network, doi:https://doi.org/10.17882/55128, 2021.
- Veron, J. E. N. and Wallace, C. C.: *Scleractinia of Eastern Australia – Part V. Family Acroporidae*, Australian Institute of Marine Science, Monograph, Series 6: 1–485, 1984.
- Veron, J. E. N.: *Corals of the world*, Institute of Marine Science. Townsville, 11, Corals of the World, 2000.
- Vicente, J., Silbiger, N. J., Beckley, B. A., Raczkowski, C. W. and Hill, R. T.: Impact of high pCO<sub>2</sub> and warmer temperatures on the process of silica biomineralization in the sponge *Mycale grandis*, *ICES Journal of Marine Science*, doi:10.1093/icesjms/fsv235, 2016.
- Vicente, V. P.: Response of sponges with autotrophic endosymbionts during the coral-bleaching episode in Puerto Rico, *Coral Reefs*, doi:10.1007/BF00265011, 1990.
- Vizzini, S., Di Leonardo, R., Costa, V., Tramati, C. D., Luzzu, F. and Mazzola, A.: Trace element

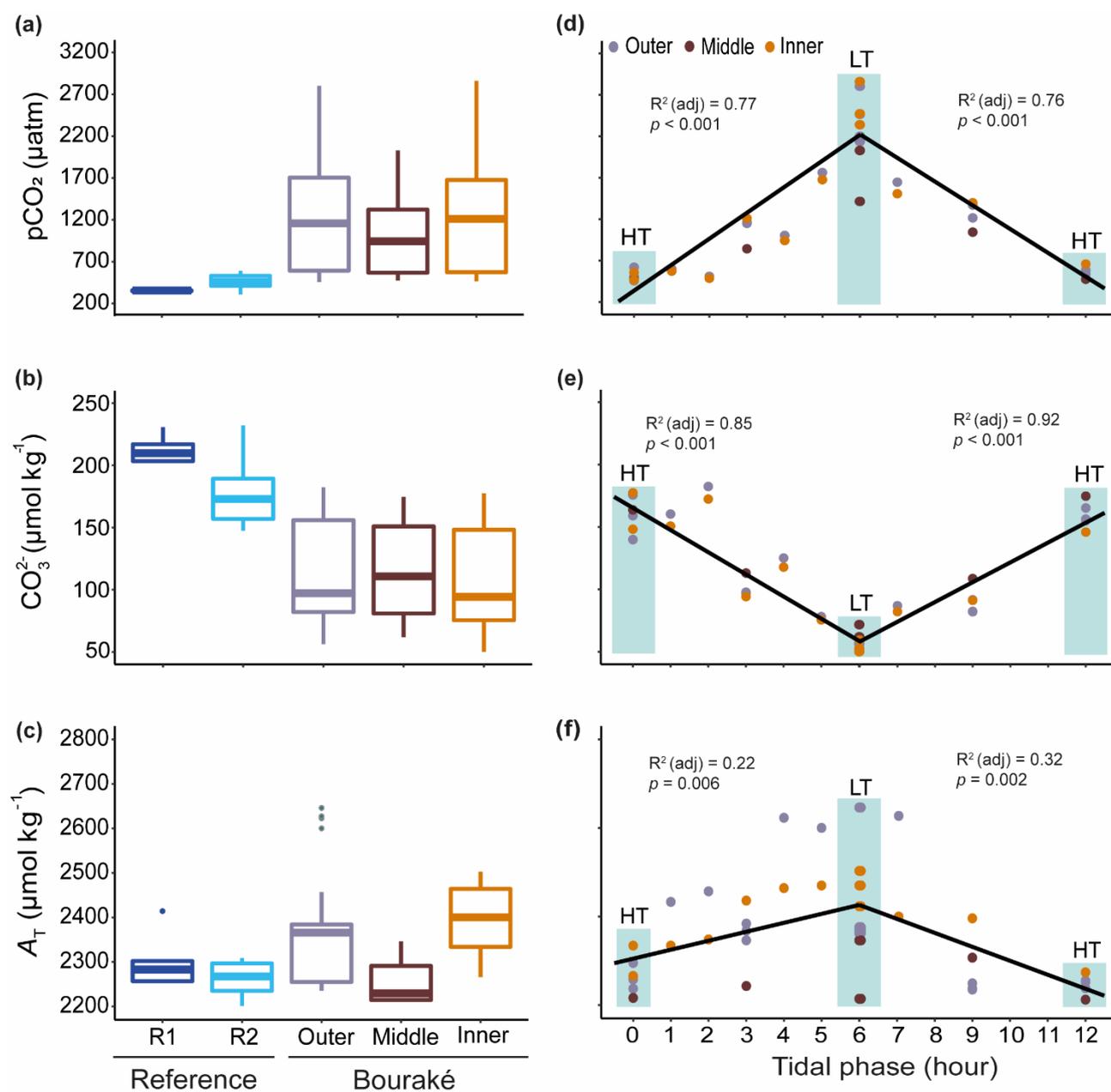
- bias in the use of CO<sub>2</sub> vents as analogues for low pH environments: Implications for contamination levels in acidified oceans, *Estuarine, Coastal and Shelf Science*, doi:10.1016/j.ecss.2013.09.015, 2013.
- De Vos, L., Boury-esnault, N., Donadey, C. and Vacelet, J.: Atlas of sponge morphology., 1991.
- Van De Waal, D. B., Verspagen, J. M. H., Lüring, M., Van Donk, E., Visser, P. M. and Huisman, J.: The ecological stoichiometry of toxins produced by harmful cyanobacteria: An experimental test of the carbon-nutrient balance hypothesis, *Ecology Letters*, doi:10.1111/j.1461-0248.2009.01383.x, 2009.
- Wall, C. B., Ricci, C. A., Wen, A. D., Ledbetter, B. E., Delania, E., Mydlarz, L. D., Gates, R. D. and Putnam, H. M.: Shifting Baselines: Physiological legacies contribute to the response of reef coral to frequent heat waves, *bioRxiv*, 1–47, doi:https://doi.org/10.1101/2020.04.23.056457, 2020.
- Wallace, C. C.: Staghorn corals of the world: a revision of the coral genus *Acropora* (Scleractinia; Astrocoeniina; Acroporidae) worldwide, with emphasis on morphology, phylogeny and biogeography, *Choice Reviews Online*, CSIRO, Collingwood, 1999.
- Ward, J. H.: Hierarchical Grouping to Optimize an Objective Function, *Journal of the American Statistical Association*, doi:10.1080/01621459.1963.10500845, 1963.
- Warner, M. E., Fitt, W. K. and Schmidt, G. W.: The effects of elevated temperature on the photosynthetic efficiency of zooxanthellae in hospite from four different species of reef coral: A novel approach, *Plant, Cell and Environment*, doi:10.1111/j.1365-3040.1996.tb00251.x, 1996.
- Webb, A. E., van Heuven, S. M. A. C., de Bakker, D. M., van Duyl, F. C., Reichart, G.-J. and de Nooijer, L. J.: Combined Effects of Experimental Acidification and Eutrophication on Reef Sponge Bioerosion Rates, *Frontiers in Marine Science*, doi:10.3389/fmars.2017.00311, 2017.
- Webster, N. S. and Reusch, T. B. H.: Microbial contributions to the persistence of coral reefs, *ISME Journal*, doi:10.1038/ismej.2017.66, 2017.
- Webster, N. S., Cobb, R. E. and Negri, A. P.: Temperature thresholds for bacterial symbiosis with a sponge, *ISME Journal*, doi:10.1038/ismej.2008.42, 2008.
- Webster, N. S., Luter, H. M., Soo, R. M., Botté, E. S., Simister, R. L., Abdo, D. and Whalan, S.: Same, same but different: Symbiotic bacterial associations in GBR sponges, *Frontiers in Microbiology*, doi:10.3389/fmicb.2012.00444, 2012.
- Weisz, J. B., Hentschel, U., Lindquist, N. and Martens, C. S.: Linking abundance and diversity of sponge-associated microbial communities to metabolic differences in host sponges, *Marine Biology*, doi:10.1007/s00227-007-0708-y, 2007.
- Weisz, J. B., Lindquist, N. and Martens, C. S.: Do associated microbial abundances impact marine demosponge pumping rates and tissue densities?, *Oecologia*, doi:10.1007/s00442-007-0910-0, 2008.
- Wiedenmann, J., D'Angelo, C., Smith, E. G., Hunt, A. N., Legiret, F. E., Postle, A. D. and Achterberg, E. P.: Nutrient enrichment can increase the susceptibility of reef corals to bleaching, *Nature Climate Change*, doi:10.1038/nclimate1661, 2013.
- Wild, C., Huettel, M., Klueter, A., Kremb, S. G., Rasheed, M. Y. M. and Jørgensen, B. B.: Coral mucus functions as an energy carrier and particle trap in the reef ecosystem, *Nature*, doi:10.1038/nature02344, 2004.
- Wilkinson, C. R.: Net primary productivity in coral reef sponges, *Science*, doi:10.1126/science.219.4583.410, 1983.

- Winton, M., Griffies, S. M., Samuels, B. L., Sarmiento, J. L. and Licher, T. L. F.: Connecting changing ocean circulation with changing climate, *Journal of Climate*, doi:10.1175/JCLI-D-12-00296.1, 2013.
- Wisshak, M., Schönberg, C. H. L., Form, A. and Freiwald, A.: Ocean Acidification Accelerates Reef Bioerosion, *PLoS ONE*, doi:10.1371/journal.pone.0045124, 2012.
- Wisshak, M., Schönberg, C. H. L., Form, A. and Freiwald, A.: Sponge bioerosion accelerated by ocean acidification across species and latitudes?, *Helgoland Marine Research*, doi:10.1007/s10152-014-0385-4, 2014.
- Van Woosik, R., Houk, P., Isechal, A. L., Idechong, J. W., Victor, S. and Golbuu, Y.: Climate-change refugia in the sheltered bays of Palau: Analogs of future reefs, *Ecology and Evolution*, doi:10.1002/ece3.363, 2012.
- Wooster, M. K., McMurray, S. E., Pawlik, J. R., Morán, X. A. G. and Berumen, M. L.: Feeding and respiration by giant barrel sponges across a gradient of food abundance in the Red Sea, *Limnology and Oceanography*, doi:10.1002/lno.11151, 2019.
- Wulff, J.: Assessing and monitoring coral reef sponges: Why and how?, in *Bulletin of Marine Science*, 2001.
- Wulff, J. L.: Sponge feeding by Caribbean angelfishes, trunkfishes, and filefishes, *Sponges in time and space*, 1994.
- Wulff, J. L.: Sponge predators may determine differences in sponge fauna between two sets of mangrove cays, *Belize Barrier Reef, Atoll Research Bulletin*, doi:10.5479/si.00775630.477., 2000.
- Wulff, J. L.: Trade-offs in resistance to competitors and predators, and their effects on the diversity of tropical marine sponges, *Journal of Animal Ecology*, doi:10.1111/j.1365-2656.2005.00925.x, 2005.
- Wulff, J. L.: Ecological interactions of marine sponges, *Canadian Journal of Zoology*, doi:10.1139/z06-019, 2006.
- Yahel, G., Sharp, J. H., Marie, D., We, A., Yahel, R., Ayalon, I., Ohevia, M., Wyeth, C., Munkes, B., Motro, R., Eckstein, S., Brandes, G., Shif, Y., Weil, D., Inditzky, A., Cohen, N., Hazan, E., Cohen, T., Gutman, L., Meallem, I., Savidge, K. B., Andrews, R., Canfield, D. E. and Rinker, K.: In situ feeding and element removal in the symbiont-bearing sponge *Theonella swinhoei*: Bulk DOC is the major source for carbon., 2003.
- Yahel, G., Eerkes-Medrano, D. I. and Leys, S. P.: Size independent selective filtration of ultraplankton by hexactinellid glass sponges, *Aquatic Microbial Ecology*, doi:10.3354/ame045181, 2006.
- Yates, K. K., Rogers, C. S., Herlan, J. J., Brooks, G. R., Smiley, N. A. and Larson, R. A.: Diverse coral communities in mangrove habitats suggest a novel refuge from climate change, *Biogeosciences*, doi:10.5194/bg-11-4321-2014, 2014.
- Zhang, F., Blasiak, L. C., Karolin, J. O., Powell, R. J., Geddes, C. D. and Hill, R. T.: Phosphorus sequestration in the form of polyphosphate by microbial symbionts in marine sponges, *Proceedings of the National Academy of Sciences*, doi:10.1073/pnas.1423768112, 2015.
- Zubkov, M. V., Sleigh, M. A., Tarran, G. A., Burkill, P. H. and Leakey, R. J. G.: Picoplanktonic community structure on an Atlantic transect from 50°N to 50°S, *Deep-Sea Research Part I: Oceanographic Research Papers*, doi:10.1016/S0967-0637(98)00015-6, 1998.

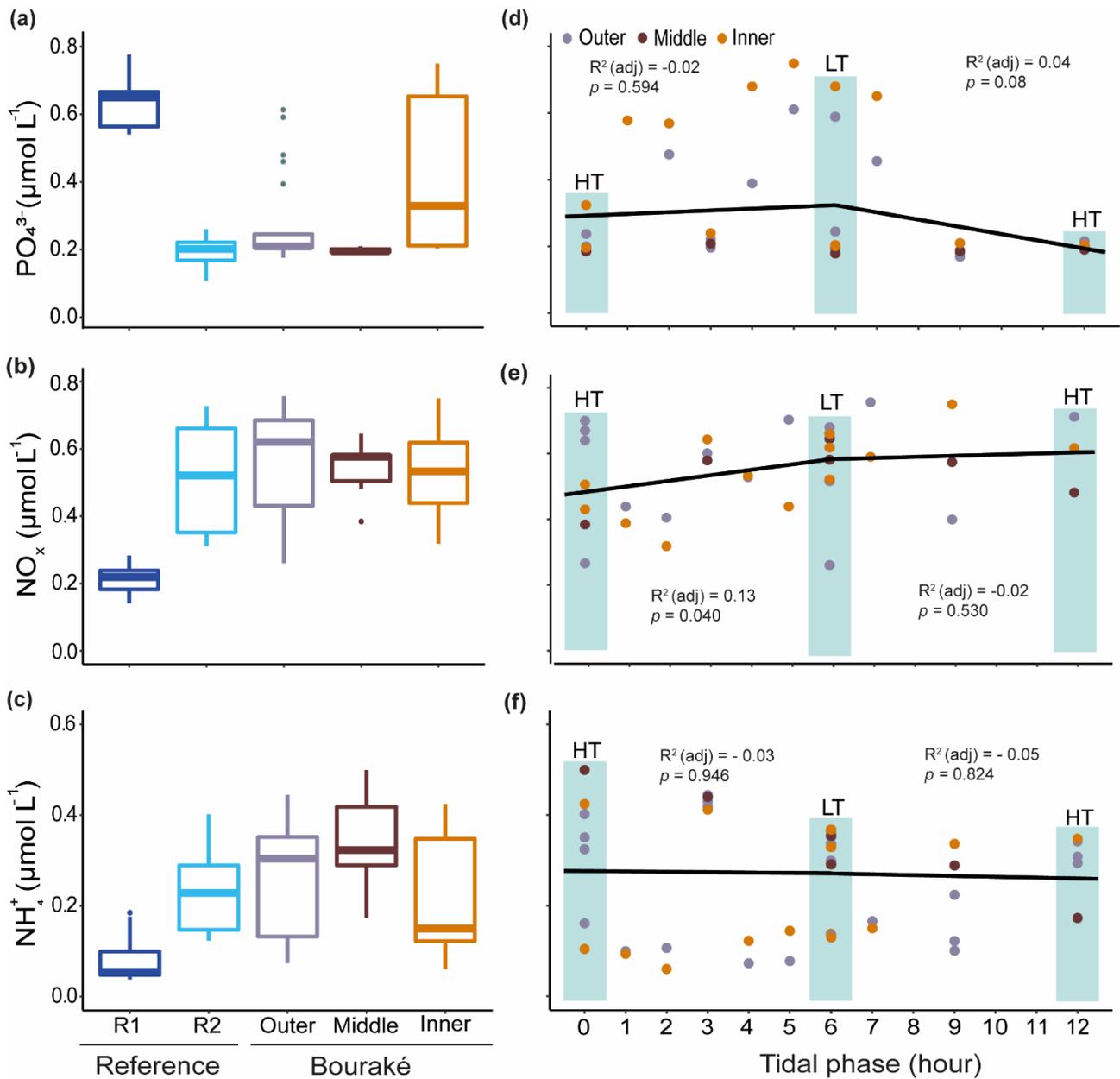
## APPENDICES

---

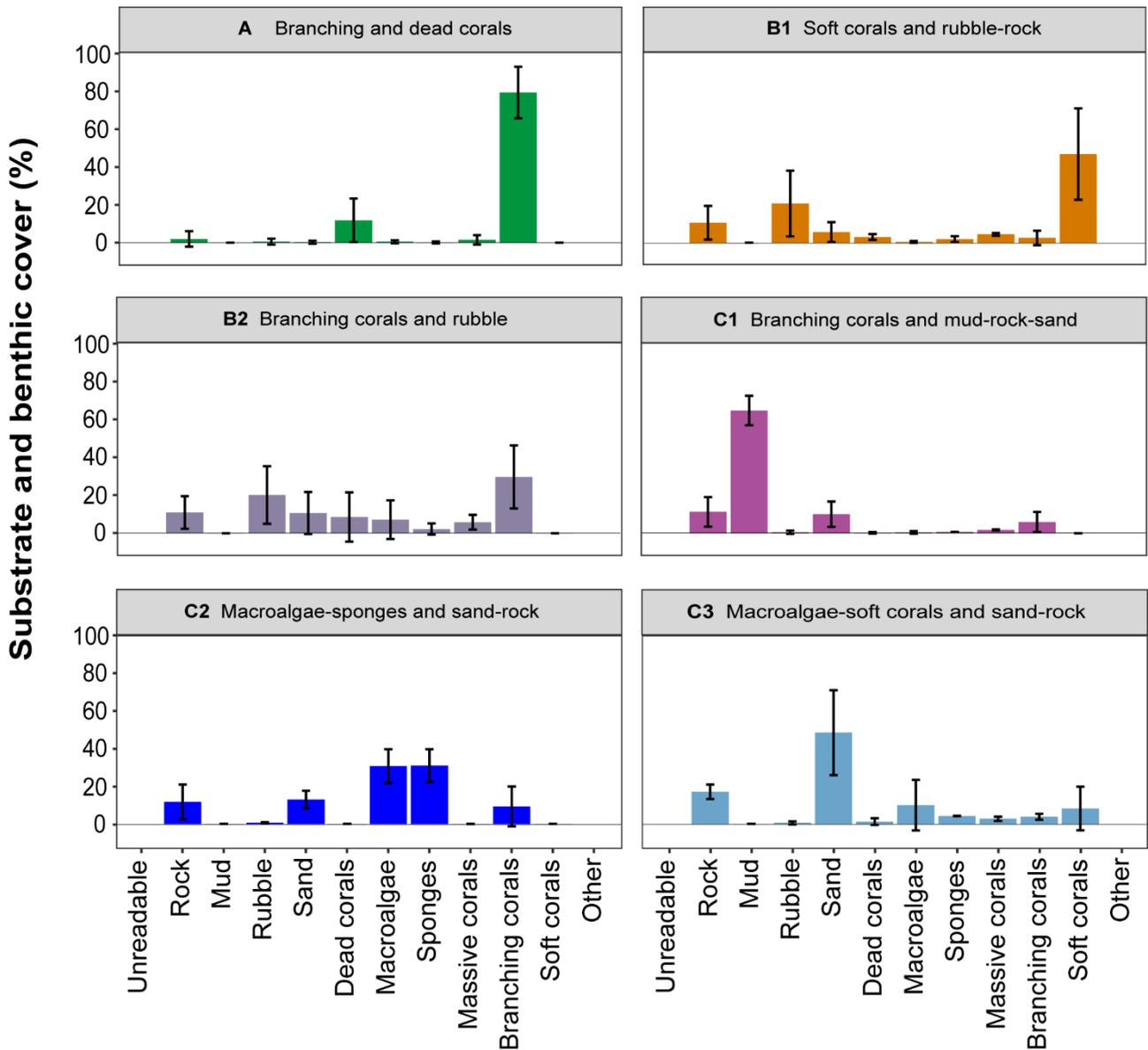
## APPENDIX A



**Figure A2.1.** Seawater carbonate chemistry measured ( $A_T$ ) and calculated ( $p\text{CO}_2$  and  $\text{CO}_3^{2-}$ ) during diel cycles in 2017 and 2019 (pooled data, see also Figure 8) at R1, R2 and the Bouraké reefs (outer, middle and inner) (a-c). Changes are illustrated across a 12-h tidal phase in Bouraké (d-f). Boxes ( $n = 6-14$ ) represent the interquartile range (25<sup>th</sup> and 75<sup>th</sup> percentile), the horizontal line is the median, and the whiskers represent the data range (i.e., minimum and maximum).



**Figure A2.2.** Phosphate, nitrogen oxide and ammonium parameters measured during diel cycles in 2017 and 2019 (pooled data, see also Figure 9) at R1, R2 and the Bouraké reefs (outer, middle and inner) (a-d). Change in the carbonate concentrations illustrated across a 12-h tidal phase in Bouraké (d-f). Boxes ( $n = 6-14$ ) represent the interquartile range (25<sup>th</sup> and 75<sup>th</sup> percentile), the horizontal line is the median, and the whiskers represent the data range (i.e., minimum and maximum).



**Figure A2.3.** Benthic community and bottom substrate characterisation in the Bouraké lagoon. Biotic and abiotic descriptors are averaged by transects ( $n = 30$ ) within each cluster. Bars represent the standard error of the mean. Clusters as defined in Figure 2.10a.

**Table A2.1.** Summary of all oceanographic sensors deployed from 2016 to 2020 at the reference (St R1 and R2) and Bouraké reefs (St A-U, B1 and B2). Sampling periods were divided between long-, medium- and short-term monitoring.

<b>Long-term monitoring</b>								
<b>HOBO - Temperature</b>								
	<i>From</i>	<i>To</i>						
St R1	01/01/2019	27/04/2020						
St R2	19/09/2017	31/10/2017						
	01/11/2018	05/03/2018						
	02/10/2018	27/04/2020						
St B1	02/10/2018	27/04/2020						
St B2	19/09/2017	31/10/2017						
	01/11/2018	05/03/2018						
	02/10/2018	27/04/2020						
<b>Medium-term monitoring</b>								
	<b>SeaFet - pH</b>		<b>YSI - Dissolved oxygen</b>					
	<i>From</i>	<i>To</i>	<i>From</i>	<i>To</i>				
St R1	19/03/2019	31/03/2019	19/03/2019	04/04/2019				
			15/07/2019	18/07/2019				
St R2	08/02/2016	12/02/2016	08/02/2016	12/02/2016				
			22/03/2016	31/03/2016				
			05/04/2017	06/04/2017	13/04/2017	15/04/2017		
			05/04/2018	15/04/2018				
			02/10/2018	04/10/2018	02/10/2018	16/10/2018		
St B1	19/03/2019	04/04/2019	19/03/2019	30/03/2019				
			15/07/2019	18/07/2019				
			19/03/2019	03/04/2019				
St B2	09/02/2016	12/02/2016	09/02/2016	12/02/2016				
			05/03/2018	08/03/2018				
			02/10/2018	17/10/2018				
			19/03/2019	23/03/2019	16/03/2019	23/03/2019		
			10/04/2019	25/04/2019	10/04/2019	25/04/2019		
					15/07/2019	18/07/2019		
<b>Short-term monitoring</b>								
	<b>SeaFet - pH</b>		<b>YSI - Dissolved oxygen</b>		<b>YSI - Salinity</b>			
	<i>From</i>	<i>To</i>	<i>From</i>	<i>To</i>	<i>From</i>	<i>To</i>		
St R1					15/07/2019	18/07/2019		
St R2					29/11/2020	02/12/2020		
					15/07/2019	18/07/2019		
St A			06/03/2018	07/03/2018	29/11/2020	04/12/2020		
	03/04/2018	04/04/2018	03/04/2018	04/04/2018				
St B			06/03/2018	07/03/2018				
	03/04/2018	04/04/2018	03/04/2018	05/04/2018				
St C			06/03/2018	07/03/2018				
	03/04/2018	04/04/2018	03/04/2018	05/04/2018				
St D			06/03/2018	07/03/2018				

(continue Table A2.1)

<b>Short-term monitoring</b>	<b>SeaFet - pH</b>		<b>YSI - Dissolved oxygen</b>		<b>YSI - Salinity</b>	
St E			06/03/2018	07/03/2018		
St F			22/03/2016	31/03/2016		
			06/03/2018	07/03/2018		
			10/04/2019	24/04/2019		
St G			06/03/2018	07/03/2018		
St H			05/03/2018	06/03/2018		
St I			05/03/2018	06/03/2018		
St L			05/03/2018	06/03/2018		
St M			05/03/2018	06/03/2018		
St N			05/03/2018	06/03/2018		
St O			07/03/2018	08/03/2018		
St P			07/03/2018	08/03/2018		
St Q			07/03/2018	08/03/2018		
St R	24/04/2018	25/04/2018				
			06/03/2018	07/03/2018		
			24/04/2018	25/04/2018		
St S	25/04/2018	26/04/2018				
St T	06/04/2017	12/04/2017			29/11/2020	04/12/2020
St U	07/04/2017	11/04/2017				
St B1	19/03/2019	04/04/2019			15/07/2019	18/07/2019
St B2	19/03/2019	23/03/2019			15/07/2019	18/07/2019
					29/11/2020	04/12/2020

**Table A2.2.** Summary of all seawater sampling made during the tidal cycles in winter 2017 and summer 2019. NC refers to New Caledonia time zone (+11 GMT).

*Tidal cycle in June 2017*

<b>Station</b>	<b>Zone</b>	<b>Data</b>	<b>Sampling time</b>
R2	Reference	31/05/2017	14:30; 17:20
		01/06/2017	07:30; 11:30; 15:20
		02/06/2017	09:30
B01	Outer	31/05/2017	14:30; 17:20
		01/06/2017	07:30; 11:30; 15:20
		02/06/2017	09:30
B02	Outer	31/05/2017	14:30; 17:20
		01/06/2017	07:30; 11:30; 15:20
		02/06/2017	09:30
B03	Outer	31/05/2017	14:30; 17:20
		01/06/2017	07:30; 11:30; 15:20
		02/06/2017	09:30
B04	Middle	31/05/2017	14:30; 17:20
		01/06/2017	07:30; 11:30; 15:20
		02/06/2017	09:30
B05	Inner	31/05/2017	14:30; 17:20
		01/06/2017	07:30; 11:30; 15:20
		02/06/2017	09:30

*Tidal cycle in July 2019*

<b>Station</b>	<b>Zone</b>	<b>Data</b>	<b>Sampling time</b>
R1	Reference	17/07/2019	08:00; 09:00; 10:00; 11:00; 12:00; 13:00; 14:00; 15:00
R2	Reference	18/07/2019	08:00; 09:00; 10:00; 11:00; 12:00; 13:00; 14:00; 15:00
B1	Outer	16/07/2019	08:10; 09:00; 10:00; 11:45; 13:00; 14:30; 15:40
B2	Inner	16/07/2019	08:40; 09:20; 10:00; 12:00; 13:20; 14:50; 15:30

**Table A2.3.** Summary of multiple linear regressions analyses applied to the seawater carbonate chemistry and the chemical parameters during falling tide (0-6) and rising tide (6-12). Data was collected in the semi-enclosed lagoon of Bouraké during diel cycles in 2017 and 2019.

Parameter	(df)	F	p	R <sup>2</sup>	R <sup>2</sup> (adj)
<i>Falling tide (from high to low tide - phase 0-6)</i>					
pH <sub>T</sub>	(1, 30)	204.2	< <b>0.001</b>	0.87	0.87
pCO <sub>2</sub>	(1, 30)	106.9	< <b>0.001</b>	0.78	0.77
CO <sub>3</sub> <sup>2-</sup>	(1, 30)	175.2	< <b>0.001</b>	0.85	0.85
Ω <sub>aragonite</sub>	(1, 30)	177.2	< <b>0.001</b>	0.85	0.85
DIC	(1, 30)	23.46	< <b>0.001</b>	0.44	0.42
A <sub>T</sub>	(1, 30)	8.86	<b>0.006</b>	0.23	0.22
SiOH <sub>4</sub>	(1, 30)	31.71	< <b>0.001</b>	0.51	0.50
NO <sub>x</sub>	(1, 30)	4.60	<b>0.040</b>	0.13	0.10
NH <sub>4</sub> <sup>+</sup>	(1, 29)	0.005	0.946	0.00	-0.03
PO <sub>4</sub> <sup>3-</sup>	(1, 29)	0.29	0.594	0.00	-0.02
DOC	(1, 18)	16.33	< <b>0.001</b>	0.48	0.45
POC	(1, 30)	1.16	0.289	0.04	0.01
PON	(1, 30)	0.09	0.762	0.03	-0.03
<i>Rising tide (from low to high tide - phase 6-12)</i>					
pH <sub>T</sub>	(1, 22)	177.5	< <b>0.001</b>	0.89	0.88
pCO <sub>2</sub>	(1, 22)	72.23	< <b>0.001</b>	0.77	0.76
CO <sub>3</sub> <sup>2-</sup>	(1, 22)	257.6	< <b>0.001</b>	0.92	0.92
Ω <sub>aragonite</sub>	(1, 22)	265.1	< <b>0.001</b>	0.92	0.91
DIC	(1, 22)	22.63	< <b>0.001</b>	0.51	0.48
A <sub>T</sub>	(1, 22)	12.04	<b>0.002</b>	0.35	0.32
SiOH <sub>4</sub>	(1, 22)	29.63	< <b>0.001</b>	0.57	0.55
NO <sub>x</sub>	(1, 20)	0.41	0.530	0.02	-0.02
NH <sub>4</sub> <sup>+</sup>	(1, 21)	0.05	0.824	0.02	-0.05
PO <sub>4</sub> <sup>3-</sup>	(1, 22)	1.84	0.189	0.08	0.04
DOC	(1, 18)	8.47	<b>0.009</b>	0.32	0.28
POC	(1, 22)	2.85	0.105	0.11	0.07
PON	(1, 22)	2.31	0.142	0.09	0.05

**Table A2.4.** *Percent cover of biotic descriptors by transect in the Bouraké mangrove lagoon.*

<b>Transect</b>	<b>Macroalgae</b>	<b>Sponges</b>	<b>Massive corals</b>	<b>Branching corals</b>	<b>Soft corals</b>
<b>T1</b>	0.00	1.97	3.97	0.67	61.81
<b>T2</b>	19.79	4.36	3.63	2.75	16.71
<b>T3</b>	1.23	0.99	1.69	21.37	0.00
<b>T4</b>	0.99	3.59	5.36	0.10	19.41
<b>T5</b>	0.31	0.43	4.32	7.14	62.50
<b>T6</b>	41.72	22.78	0.00	21.19	0.00
<b>T7</b>	24.75	31.20	0.00	7.20	0.00
<b>T8</b>	27.19	40.48	0.07	0.00	0.00
<b>T9</b>	0.00	0.75	1.63	2.27	0.00
<b>T10</b>	0.99	0.72	2.00	10.00	0.00
<b>T11</b>	0.48	4.19	1.93	4.95	0.00
<b>T12</b>	0.09	0.17	12.71	29.61	0.00
<b>T13</b>	0.11	0.00	2.23	85.73	0.00
<b>T14</b>	0.00	0.00	0.22	88.59	0.00
<b>T15</b>	0.00	0.00	0.88	89.78	0.00
<b>T16</b>	1.13	0.00	7.14	63.10	0.00
<b>T17</b>	8.85	2.96	7.74	10.47	0.00
<b>T18</b>	1.15	2.35	5.25	30.92	0.00
<b>T19</b>	2.21	0.00	0.38	59.73	0.00
<b>T20</b>	2.27	8.78	7.75	48.66	0.00
<b>T21</b>	29.70	0.00	6.00	15.48	0.00
<b>T22</b>	0.12	0.00	0.00	85.74	0.00
<b>T23</b>	0.44	1.30	0.00	95.57	0.00
<b>T24</b>	8.63	0.92	0.95	57.07	0.00

**Table A2.5.** List of macroalgal and sponges species identified in the Bouraké lagoon, and corals identified from both Bouraké and R2.**MACROALGAE**

Family	Genus	Species	Site
Caulerpaceae	<i>Caulerpa</i>	<i>chemnitzia</i> (J.V. Lamouroux, 1809)	B
Caulerpaceae	<i>Caulerpa</i>	<i>racemose</i> (J. Agardh, 1873)	B
Caulerpaceae	<i>Caulerpa</i>	<i>serrulata</i> (J. Agardh, 1837)	B
Caulerpaceae	<i>Caulerpa</i>	<i>verticillata</i> (J. Agardh, 1847)	B
Halimedaceae	<i>Halimeda</i>	<i>distorta</i> (Hillis-Colinvaux, 1968)	B
Halimedaceae	<i>Halimeda</i>	<i>cylindracea</i> (Decaisne, 1842)	B
Halimedaceae	<i>Halimeda</i>	<i>discoidea</i> (Decaisne, 1842)	B
Siphonocladaceae	<i>Dictyosphaeria</i>	<i>versluysii</i> (Weber-van Bosse, 1905)	B
Siphonocladaceae	<i>Dictyosphaeria</i>	<i>cavernosa</i> ((Forsskål) Børgesen, 1932)	B
Lithophyllaceae	<i>Lithophyllum</i>	sp.	B
Lithothamniaceae	<i>Lithothamnion</i>	sp.	B
Hydrolithaceae	<i>Hydrolithon</i>	<i>reinboldii</i> (Foslie, 1909)	B
Dictyotaceae	<i>Dictyota</i>	sp.	B
Dictyotaceae	<i>Dictyota</i>	<i>friabilis</i> (Setchell, 1926)	B
Dictyotaceae	<i>Dictyota</i>	<i>bartayresiana</i> (J.V. Lamouroux, 1809)	B
Dictyotaceae	<i>Dictyota</i>	<i>ceylanica</i> (Kützing, 1859)	B
Dictyotaceae	<i>Styopodium</i>	sp.	B
Dictyotaceae	<i>Lobophora</i>	<i>rosacea</i> (Vieira, Payri & De Clerck, 2014)	B
Dictyotaceae	<i>Padina</i>	<i>australis</i> (Hauck, 1887)	B
Dictyotaceae	<i>Padina</i>	<i>okinawaensis</i> (Ni-Ni-Win, Arai & Kawai, 2010)	B
Sargassaceae	<i>Hormophysa</i>	<i>cuneiformis</i> ((J.F.Gmelin) P.C.Silva, 1987)	B
Sargassaceae	<i>Sargassum</i>	sp.	B
Sargassaceae	<i>Sargassum</i>	<i>spinuligerum var crispatum</i> (Sonder, 1845)	B
Sargassaceae	<i>Sargassum</i>	cf <i>flabelliforme</i>	B
Sargassaceae	<i>Sargassum</i>	cf. <i>ilicifolium</i> (C.Agardh 1820)	B
Sargassaceae	<i>Turbinaria</i>	<i>conoides</i> (J. Agardh) Kützing, 1860)	B
Cystoclonisaceae	<i>Hypnea</i>	sp.	B
Galaxauraceae	<i>Dichotomaria</i>	sp.	B

**CORALS (Scleractinians)**

Family	Genus	Species	Site
Acroporidae	<i>Acropora</i>	<i>aculeus</i> (Dana, 1846)	B
Acroporidae	<i>Acropora</i>	cf. <i>acuminata</i> (Verrill, 1864)	R2
Acroporidae	<i>Acropora</i>	<i>aspera</i> (Dana, 1846)	B-R2
Acroporidae	<i>Acropora</i>	<i>austera</i> (Dana, 1846)	R2
Acroporidae	<i>Acropora</i>	<i>carduus</i> (Dana, 1846)	B
Acroporidae	<i>Acropora</i>	<i>cerealis</i> (Dana, 1846)	B
Acroporidae	<i>Acropora</i>	<i>divaricata</i> (Dana, 1846)	B
Acroporidae	<i>Acropora</i>	<i>echinata</i> (Dana, 1846)	B
Acroporidae	<i>Acropora</i>	<i>florida</i> (Dana, 1846)	R2
Acroporidae	<i>Acropora</i>	<i>gemmifera</i> (Brook, 1892)	R2

(continue Table A2.5)

**CORALS (Scleractinians)**

<b>Family</b>	<b>Genus</b>	<b>Species</b>	<b>Site</b>
Acroporidae	<i>Acropora</i>	<i>grandis</i> (Brook, 1892)	R2
Acroporidae	<i>Acropora</i>	<i>horrida</i> (Dana, 1846)	B-R2
Acroporidae	<i>Acropora</i>	<i>humilis</i> (Dana, 1846)	B-R2
Acroporidae	<i>Acropora</i>	<i>kirstyae</i> (Veron & Wallace, 1984)	B-R2
Acroporidae	<i>Acropora</i>	<i>latistella</i> (Brook, 1892)	R2
Acroporidae	<i>Acropora</i>	cf. <i>longycyathus</i> (Milne Edwards, 1860)	R2
Acroporidae	<i>Acropora</i>	<i>microphthalma</i> (Verrill, 1869)	B-R2
Acroporidae	<i>Acropora</i>	<i>millepora</i> (Ehrenberg, 1834)	R2
Acroporidae	<i>Acropora</i>	<i>muricata</i> (Linnaeus, 1758)	B-R2
Acroporidae	<i>Acropora</i>	<i>nana</i> (Studer, 1879)	B
Acroporidae	<i>Acropora</i>	<i>polystoma</i> (Brook, 1891)	R2
Acroporidae	<i>Acropora</i>	<i>pulchra</i> (Brook, 1891)	B-R2
Acroporidae	<i>Acropora</i>	<i>robusta</i> (Dana, 1846)	R2
Acroporidae	<i>Acropora</i>	<i>retusa</i> (Dana, 1846)	R2
Acroporidae	<i>Acropora</i>	<i>samoensis</i> (Brook, 1891)	B-R2
Acroporidae	<i>Acropora</i>	cf. <i>secale</i> (Studer, 1878)	R2
Acroporidae	<i>Acropora</i>	<i>tenuis</i> (Dana, 1846)	B-R2
Acroporidae	<i>Acropora</i>	<i>valida</i> (Dana, 1846)	B-R2
Acroporidae	<i>Acropora</i>	<i>vaughani</i> (Wells, 1954)	B-R2
Acroporidae	<i>Anacropora</i>	<i>forbesi</i> (Ridley, 1884)	B
Acroporidae	<i>Anacropora</i>	<i>matthai</i> (Pillai, 1973)	B-R2
Acroporidae	<i>Anacropora</i>	<i>puertogalerae</i> Nemenzo, 1964	R2
Acroporidae	<i>Isopora</i>	<i>palifera</i> (Lamarck, 1816)	R2
Acroporidae	<i>Montipora</i>	<i>aequituberculata</i> Bernard, 1897	R2
Acroporidae	<i>Montipora</i>	<i>cactus</i> (Bernard, 1897)	B
Acroporidae	<i>Montipora</i>	<i>digitata</i> (Dana, 1846)	B-R2
Acroporidae	<i>Montipora</i>	<i>efflorescens</i> Bernard, 1897	R2
Acroporidae	<i>Montipora</i>	<i>hispida</i> (Dana, 1846)	B-R2
Acroporidae	<i>Montipora</i>	<i>mollis</i> Bernard, 1897	R2
Acroporidae	<i>Montipora</i>	cf. <i>nodosa</i> (Dana, 1846)	R2
Acroporidae	<i>Montipora</i>	<i>stellata</i> (Bernard, 1897)	B-R2
Agariciidae	<i>Pavona</i>	<i>cactus</i> (Forskål, 1775)	B-R2
Agariciidae	<i>Pavona</i>	<i>clavus</i> Dana, 1846	R2
Agariciidae	<i>Pavona</i>	<i>decussata</i> (Dana, 1846)	B-R2
Caryophylliidae	<i>Polycyathus</i>	<i>fulvus</i> (Wijsman-Best, 1970)	B
Dendrophylliidae	<i>Turbinaria</i>	<i>mesenterina</i> (Lamarck, 1816)	B
Dendrophylliidae	<i>Turbinaria</i>	<i>stellulata</i> (Lamarck, 1816)	R2
Dendrophylliidae	<i>Tubastraea</i>	<i>coccinea</i> (Lesson, 1830)	B
Dendrophylliidae	<i>Tubastraea</i>	<i>micranthus</i> (Ehrenberg, 1834)	B
Euphylliidae	<i>Galaxea</i>	<i>fascicularis</i> (Linnaeus, 1767)	B-R2
Fungiidae	<i>Leptastrea</i>	<i>purpurea</i> (Dana, 1846)	B
Fungiidae	<i>Heliofungia</i>	<i>actiniformis</i> (Quoy & Gaimard, 1833)	B

(continue Table A2.5)

**CORALS (Scleractinians)**

<b>Family</b>	<b>Genus</b>	<b>Species</b>	<b>Site</b>
Fungiidae	<i>Halomitra</i>	<i>pileus</i> (Linnaeus, 1758)	B
Fungiidae	<i>Fungia</i>	<i>fungites</i> (Linnaeus, 1758)	B
Fungiidae	<i>Ctenactis</i>	<i>echinata</i> (Pallas, 1766)	B
Fungiidae	<i>Cantharellus</i>	<i>noumeae</i> (Hoeksema & Best, 1984)	B
Fungiidae	<i>Sandalolitha</i>	<i>dentata</i> (Quelch, 1884)	B
Incertae sedis	<i>Pachyseris</i>	<i>rugosa</i> (Lamarck, 1801)	B-R2
Incertae sedis	<i>Pachyseris</i>	<i>speciosa</i> (Dana, 1846)	B-R2
Lobophylliidae	<i>Echinophyllia</i>	<i>aspera</i> (Ellis & Solander, 1786)	B
Lobophylliidae	<i>Lobophyllia</i>	cf. <i>hemprichi</i> (Ehrenberg, 1834)	B
Lobophylliidae	<i>Lobophyllia</i>	<i>corymbosa</i> (Forskål, 1775)	R2
Merulinidae	<i>Coelastrea</i>	<i>aspera</i> (Verrill, 1866)	B-R2
Merulinidae	<i>Cyphastrea</i>	<i>serailia</i> (Forskål, 1775)	R2
Merulinidae	<i>Cyphastrea</i>	sp.	B
Merulinidae	<i>Dipsastrea</i>	<i>pallida</i> (Dana, 1846)	B
Merulinidae	<i>Dipsastrea</i>	cf. <i>lizardensis</i> (Veron, Pichon, & Wijsman-Best, 1977)	B
Merulinidae	<i>Echinopora</i>	<i>lamellosa</i> (Esper, 1795)	B-R2
Merulinidae	<i>Echinopora</i>	<i>horrida</i> Dana, 1846	R2
Merulinidae	<i>Favites</i>	<i>abdita</i> (Ellis & Solander, 1786)	B
Merulinidae	<i>Favites</i>	<i>melicerum</i> (Ehrenberg, 1834)	B
Merulinidae	<i>Goniastrea</i>	<i>favulus</i> (Dana, 1846)	B-R2
Merulinidae	<i>Goniastrea</i>	<i>pectinata</i> (Ehrenberg, 1834)	R2
Merulinidae	<i>Merulina</i>	<i>scabricula</i> (Dana, 1846)	B
Merulinidae	<i>Merulina</i>	<i>ampliata</i> (Ellis & Solander, 1786)	B
Merulinidae	<i>Pectinia</i>	<i>lactuca</i> (Pallas, 1766)	R2
Merulinidae	<i>Pectinia</i>	<i>paeonia</i> (Dana, 1846)	B-R2
Merulinidae	<i>Platygyra</i>	<i>sinensis</i> (Milne Edwards & Haime, 1849)	B
Merulinidae	<i>Platygyra</i>	<i>daedalea</i> (Ellis & Solander, 1786)	B
Merulinidae	<i>Trachyphyllia</i>	<i>geoffroyi</i> (Audouin, 1826)	B
Merulinidae	<i>Hydnophora</i>	<i>rigida</i> (Dana, 1846)	B
Pocilloporidae	<i>Pocillopora</i>	<i>damicornis</i> (Linnaeus, 1758)	B-R2
Pocilloporidae	<i>Pocillopora</i>	<i>verrucosa</i> (Ellis & Solander, 1786)	R2
Pocilloporidae	<i>Stylophora</i>	<i>pistillata</i> Esper, 1797	R2
Psammocoridae	<i>Psammocora</i>	<i>contigua</i> (Esper, 1794)	B-R2
Poritidae	<i>Goniopora</i>	cf. <i>minor</i> (Crossland, 1952)	B
Poritidae	<i>Porites</i>	sp.	B
Poritidae	<i>Porites</i>	cf. <i>annae</i> (Crossland, 1952)	B
Poritidae	<i>Porites</i>	<i>lutea</i> (Edwards & Haime, 1851)	B-R2
Poritidae	<i>Porites</i>	<i>lobata</i> (Dana, 1846)	B-R2
Poritidae	<i>Porites</i>	<i>cylindrica</i> (Dana, 1846)	B-R2
Poritidae	<i>Porites</i>	<i>rus</i> (Forskål, 1775)	R2
Siderastreidae	<i>Pseudosiderastrea</i>	<i>tayamai</i> (Yabe & Sugiyama, 1935)	B

**CORALS (non-scleractinians)**

<b>Family</b>	<b>Genus</b>	<b>Species</b>	<b>Site</b>
Alcyoniidae	<i>Sinularia</i>	sp.	B

(continue Table A2.5)

**SPONGES**

<b>Family</b>	<b>Genus</b>	<b>Species</b>	<b>Site</b>
Ancorinidae	<i>Rhabdastrella</i>	<i>globostellata</i> (Carter, 1883)	B
Callyspongiidae	<i>Callyspongia</i> ( <i>Cladochalina</i> )	<i>diffusa</i> (Ridley, 1884)	B
Chalinidae	<i>Haliclona</i> ( <i>Gellius</i> )	<i>cymaeformis</i> (Esper, 1806)	B
Clionidae	<i>Sphaciospongia</i>	<i>vagabunda</i> (Ridley, 1884)	B
Clionidae	<i>Cliona</i>	<i>orientalis</i> (Thiele, 1900)	B
Clionidae	<i>Cliona</i>	sp.	B
Halichondriidae	<i>Amorphinopsis</i>	<i>fenestrata</i> (Ridley, 1884)	B
Halichondriidae	<i>Topsentia</i>	sp.	B
Niphatidae	<i>Gelloides</i>	<i>carnosa</i> Dendy, 1889	B
Spongiidae	<i>Hippospongia</i>	sp.	B
Tedaniidae	<i>Tedania</i> ( <i>Tedania</i> )	cf. <i>klausii</i> (Wulff, 2006)	B

**Table A2.6.** Summary of sponge spicules and fibres dimensions for the 11 species collected and identified from the Bouraké lagoon. All sponge spicules (*Oxeas*, *Orthotriaenes*, *Microscleres*, *Sigmas*, *Tylostyles*, *Spirasters*, *Amphiasters*, and *Onychaetes*) with range, length and width in  $\mu\text{m}$ , and sponge skeleton fibres diameter (primary, secondary and tertiary). Ectosomal (E) and choanosomal (C).

**a) Spicules**

<i>Rhabdastrella globostellata</i>	<b>Oxeas</b> 400-870 x 10-30	<b>Orthotriaenes</b> 410-750 x 10-20 (rhabdome) 70-200 x 10-30 (clads)	<b>Microscleres</b> 15-40 (Oxyspheraster, Oxyaster lenght)	
<i>Callyspongia (Cladochalina) diffusa</i>	<b>Oxeas</b> 95-115 x 5-10	<b>Primary fibers</b> 30-80 (E) 100-200 (C)	<b>Secondary fibers</b> 30-80 (E) ~ 40 (C)	<b>Tertiary fibers</b> 10-20 (C)
<i>Haliclona (Gellius) cymaeformis</i>	<b>Oxeas</b> 110-150 x 2.5	<b>Sigmas</b> 17-20		
<i>Sphaciospongia vagabunda</i>	<b>Tylostyles</b> 410-730 x 10-20	<b>Spirasters</b> 7.5-25		
<i>Cliona orientalis</i>	<b>Tylostyles</b> 315-435 x 15-20	<b>Spirasters</b> 22.9 $\pm$ 2.7 (rare)		
<i>Cliona</i> sp.	<b>Tylostyles</b> 395.4 $\pm$ 25.6	<b>Spirasters</b> 19.5 $\pm$ 3.2	<b>Amphiasters</b> 11.5 $\pm$ 1.3	
<i>Amorphinopsis fenestrata</i>	<b>Oxeas</b> 480-770 x 5-21			
<i>Topsentia</i> sp.	<b>Oxeas</b> 480-770 x 5-21			
<i>Gelliodes carnosa</i>	<b>Oxeas</b> 135-150 x 5-10	<b>Sigmas</b> 22-25		
<i>Hippospongia</i> sp.	<b>Primary fibers</b> 83.5 $\pm$ 14.6	<b>Secondary fibers</b> 26 $\pm$ 7.8		
<i>Tedania (Tedania)</i> sp.	<b>Styles</b> 250-280 x 7.5 (C)	<b>Tylostyles</b> 170-232 x 5 (E)	<b>Large Onychaetes</b> 160-200	<b>Small Onychaetes</b> 50-125

## APPENDIX B

### Section B3.1. – *Method article submitted to Limnology and Oceanography Methods journal*

#### Testing the performance of 3D photogrammetry to calculate underwater sponge volume

Maggioni Federica<sup>1</sup>, Ravache Andreas<sup>1</sup>, Pecorari Federica<sup>2</sup>, Rodolfo-Metalpa Riccardo<sup>1</sup>

<sup>1</sup> ENTROPIE, IRD, Université de la Réunion, CNRS, IFREMER, Université de Nouvelle-Calédonie, Nouméa 98800, New Caledonia

<sup>2</sup> Air Craft Studio Design, Modena 41123, Italy.

#### Abstract

Sponges play a key role in coral reefs ecology and their study is of growing interest to the scientific community. The study of these organisms often involves calculating their volume and dry weight, which is usually done by removing them from their environment. New non-intrusive methods are emerging to allow these measurements in situ, and it is now necessary to verify their validity compared to traditional methods. Here, we tested the performance of the 3D software Zephyr Pro® to calculate the volume and surface area of 58 sponges by photogrammetry. The results were related to those obtained by traditional methods of displaced volume. The volume calculated by 3D photogrammetry was also related to dry weight and 3D surface measurements. A log-logarithmic regression predicted with high accuracy the volume of sponges calculated by water displacement from the photogrammetry results. Considering the water displacement method as the best estimate of the actual sponge volume, an adjustment factor is proposed here to obtain comparable values from the photogrammetry data, without the need to sample the individuals. However, the higher correlation between photogrammetric measurements and dry weight of individuals compared to the traditional method suggests that 3D photogrammetry could in some cases be used without adjustment factors.

#### Introduction

Sponges are among the oldest extant metazoans on Earth (Müller et al., 1999). They are increasingly recognized as key ecosystem engineers in many aquatic habitats, playing important roles in ecological processes, such as habitat supply and nutrient cycling (Bell, 2008, Maldonado et al., 2012; de Goeij et al., 2017). Interest in their ecological roles and their associate microbiome has grown rapidly, revealing their important role in the ecology of coral reefs. Quantitative estimate of the total mass of an organism measured as volume or mass (live, dead, dry or ash-free weight) has been considered fundamental in benthic ecology, in particular for understanding energy flow, cycling of organic

matter, and carbonate production in aquatic ecosystems (Golikov and Scarlato, 1973; Pomeroy, 2001).

One of the most accurate methods to measure organisms' biomass is by 'ash-free dry weight', which involves drying and incineration (Edgar, 1990). Other techniques for accurately calculating volume, and indirectly biomass, imply measurement of water displaced after placing the specimen in a cylinder filled with water (Schiller, 1993). However, this method was first evaluated for objects and then applied on corals, which have a "full" skeleton and not with the presence of multiple channels as in massive sponges. Furthermore, all these sampling methods involve removing the sponge from its natural environment and the sacrifice of the organism for a single measurement, which is highly intrusive. Although the organism after its measurement can be replace back into the water, the detachment and manipulation of benthic animals (i.e., corals, sponges) and the air exposure of aquatic species could alter the physiological condition of the organisms and lead to death. Therefore, there is a critical need to find non-intrusive sampling and survey methods while accurately measuring the biomass, surface and volume occupied by sponges.

Only a few non-invasive, *in situ* methods are available today to estimate surface and volume of benthic organisms. For instance, the 2D photography (Rahav et al., 1991, Holmes et al., 2008, Naumann et al., 2009), where the object is photographed from its top and the 2D images are processed to calculate their surface. Another accurate but more time-consuming method is to calculate parts of the object as a different geometrical shape (e.g., cylinders, spheres, prism etc.) (Naumann et al., 2009). However, these methods are not practical for large and complex three-dimensional structures. This problem led to the development of photogrammetry, the reconstruction of three-dimensional models from photography (Bythell et al., 2001). Recent methods in photogrammetry and 3D reconstructions offers potential alternative methods to calculate surface and volume in non-intrusive manner. The 3D model of an object can be obtained using laser scanner or photogrammetry. The laser scanner method can be very accurate but requires expensive equipment and remains difficult to apply *in situ* (Holmes 2008). The photogrammetry method is low cost but requires expensive software and computer power. Until now, 3D modelling has mainly been used in terrestrial studies (e.g., architectural buildings) and is not yet widespread in marine research. Although several studies have applied 3D modeling to measure the surface area and volume of corals (e.g., Bythell et al., 2001; Cocito et al. 2003; Courtney et al. 2007; Fisher et al. 2007; Holmes 2008; Holmes et al. 2008; Veal et al. 2010; Lavy et al., 2015; Gutierrez-Heredia et al., 2016; Reichert et al., 2016; Urbina-barreto et al., 2021), this approach has been scarcely used to calculate sponges volume and in general only two methods (3D modelling and water displacement) were compared (e.g., Lavy et al., 2015).

This study aims to increased poor knowledge on 3D reconstruction to calculate the volume of sponges by testing the validity of 3D methods compared to the traditional ones. To do that, we compared the volume of sponges calculated with 3D methods ( $V_{[MOD3D]}$ ) using 3D photogrammetry (3D Zephyr Pro software, ) to water displacement ( $V_{[WATER]}$ ) and to dry weight, and surface ( $S_{[MOD3D]}$ ).

## Methods

### ***In situ* photography acquisition**

Image recording was carried out using a digital video camera Nikon AW130 used directly underwater to take picture of 58 individual of *Rhabdastrella globostellata* sponges in the Bouraké semi-enclosed lagoon (New Caledonia, South Pacific). The convergent axis capture method has been applied thanks to the possibility to move around the sponges. In brief, sponges were keep in the center of the frame, avoiding direct light sources that may cast shadows and hide surface areas. At least 80 pictures were taken, ensuring overlap between each photo (70-80%), and that each part of the sponge should appear in at least three separate views taken from different locations. The calibration of the sponge in the software is made possible by adding a square (3cm x 3cm) next to the sponge.

### **3D model reconstruction**

For each sponge, 80-150 images, depending on sponge complexity, were uploaded to 3D Zephyr Pro software which auto calibrate all the pictures. Four steps composed the 3D reconstruction in 3DF Zephyr: **1)** Structure from motion, **2)** Dense point cloud generation **3)** Mesh generation, and **4)** Textured mesh generation. The first step, automatically extract internal and external camera parameters, in this case the key points density was selected as “medium” to extracts up to 7500 key points per image. The second step, extract a dense point cloud by matching the image pixels, here resolution is set to 50% images. The third step is about the surface generation, in other hand, the mesh extraction from the dense point cloud. The final step is to generate the texture of the sponge where color balancing is automatically computed. Basically, the standard reconstruction settings have been applied and 3D reconstruction of sponges was performed. Before measuring the volume and surface of the sponges, the final 3D reconstruction sponge was scaled using the control point function and distances between points. Here, points corresponding to the corners of the square, placed next to the sponge during the photos, were selected to set the real distance and then scale the entire sponge with the real distances.

### **3D methods: volume and surface measurements**

Volume ( $V_{[MOD3D]}$ ) and surface ( $S_{[MOD3D]}$ ) measurements are rapidly computed by the software and expressed in  $\text{cm}^3$  and  $\text{cm}^2$ , respectively. Before calculating volume of the mesh, the parts of the sponge mesh that were not perfectly reconstructed were automatically filled in in order to obtain a

whole mesh. The correct reconstruction and coverage of the holes by the software was checked manually before proceeding with the volume and surface measurements.

### **Traditional methods: volume by water displacement and dry weight**

Following the *in situ* 3D measurement, sponge samples were collected and transported to the laboratory for a water displacement volume calculation ( $V_{[WATER]}$ ). Volume was determined by measuring the volume before and after the sponge was kept suspended in the water (Scherle 1970). After water displacement measurements, sponges emptied of water were placed in oven at 100°C for 48h and their dry weight were measured using a precision balance.

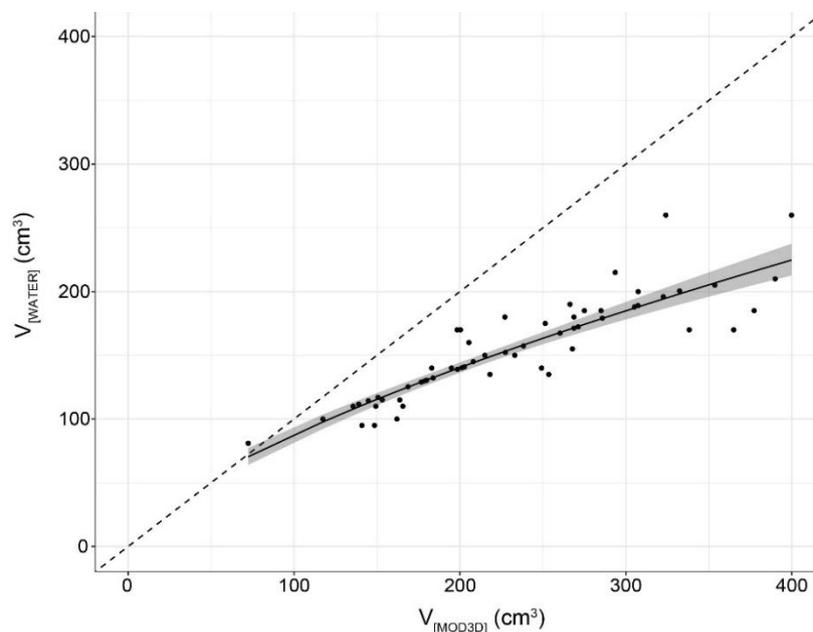
### **Statistical analysis**

Local Polynomial Regressions (LOESS) were first fitted to visualize the relationship between  $V_{[WATER]}$  and  $V_{[MOD3D]}$ ,  $V_{[WATER]}$  and Dry weight,  $V_{[WATER]}$  and Surface,  $V_{[MOD3D]}$  and Dry weight, and  $V_{[MOD3D]}$  and  $S_{[MOD3D]}$  without assumptions on the linearity or the distribution of the data. Since the use of a linear model between  $V_{[WATER]}$  and  $V_{[MOD3D]}$  did not meet the application conditions, a log-log linear regression was used to depict the relation between  $V_{[WATER]}$  and  $V_{[MOD3D]}$ . Other relations between variable were studied by regular linear regression after verifying the model assumptions (linearity, homoscedasticity, independence and normality). The mean R-squared and mean Root Mean Square Error (RMSE) was calculated for each model by a 5-fold cross validation procedure. The R-squared indicates the percentage of the variance in the dependent variable explained by the independent variable. A higher R-squared suggest a stronger relationship between the model and the variable. The RMSE indicates the average distance between the predicted values from the model and the actual values in the dataset. Then, a lower RMSE indicates a better fit of the model. The equation of the log-log regression between  $V_{[WATER]}$  and  $V_{[MOD3D]}$  was then used as an adjustment factor to calculate the adjusted volume of the sponge  $V_{[ADJ]}$  calculated by photogrammetry. Relative difference between  $V_{[MOD3D]}$  and  $V_{[WATER]}$  (%) was calculated as:  $[(V_{[MOD3D]} - V_{[WATER]}) / (V_{[WATER]})] * 100$  and represented graphically in bar plots. To avoid differences between underestimates and overestimates affecting the mean error, the difference between  $V_{[MOD3D]}$  and measured value  $V_{[WATER]}$  was also calculated as the percentage of difference considering the absolute values  $|V_{[ADJ]}|$ . A t-test was performed to compare the estimation between  $V_{[WATER]}$  (%) of  $V_{[MOD3D]}$  and  $|V_{[ADJ]}|$ . All statistical analyses were conducted using R software (R Core Team), and parametric statistical tests were performed after normal distribution was verified.

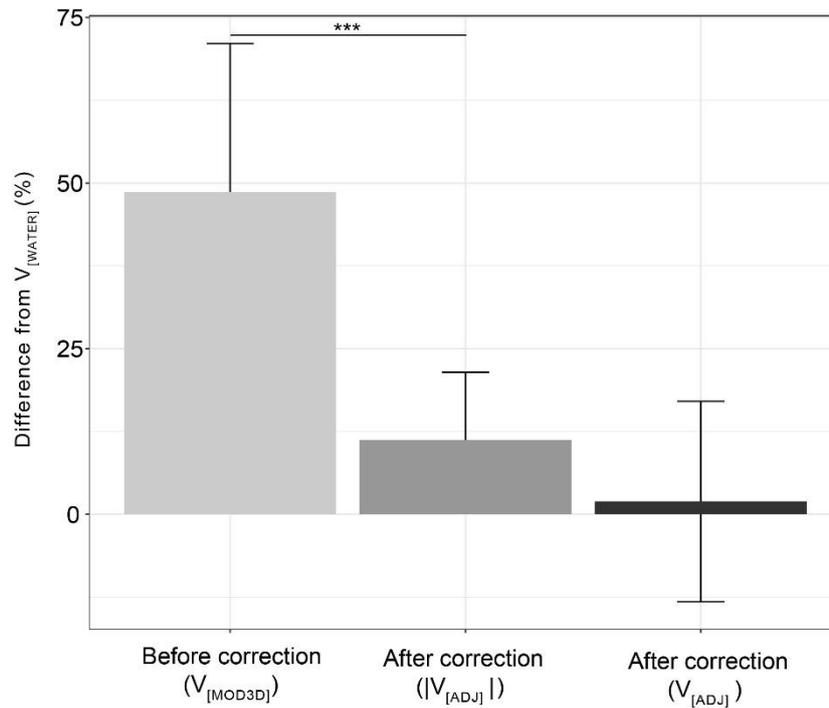
## Results

### Volume measurement methods comparison

The LOESS smooth regression between the different metrics suggested a good fit of the linear regression models and of the log-log linear regression between  $V_{[MOD3D]}$  and  $V_{[WATER]}$ . A significant log-log linear relationship ( $P < 0.001$ , mean  $R^2 = 0.86$ ,  $RMSE = 0.10$ ) was found between  $V_{[MOD3D]}$  and  $V_{[WATER]}$  (**Fig. B3.1.1**). According to the slope of regression plots, 3D modelling measurements overestimated the volume of the sponges compared to the water displacement method (slope  $< 1$ ). The equation of the modeled relation between the two variables is  $V_{[WATER]} = \exp(1.344026 + 0.6794931 * \log(V_{[MOD3D]}))$ . Applying this equation as an adjustment factor,  $|V_{[ADJ]}|$  resulted in significant better estimates of sponge volume (**Fig. B3.1.2**). The  $V_{[MOD3D]}$  showed an estimation that differs, on average, by 48.7% from  $V_{[WATER]}$ , while after adjustment factor application  $|V_{[ADJ]}|$  showed an estimation that differs, on average, by 11.2% (**Fig. B3.1.2**). The most accurate volumetric 3D estimation of sponge samples resulted in an underestimation of -1.3% of the volumetric measurement by water displacement. Considering both positive and negative differences between  $V_{[MOD3D]}$  and  $V_{[WATER]}$ , without absolute values ( $V_{[ADJ]}$ ), data showed a general overestimation of the 3D modelling of 1.9% ( $\pm 2.0$  SE) (**Fig. B3.1.2**).



**Fig. B3.1.1.** Log-log linear regression plot of volume estimated by 3D models  $V_{[MOD3D]}$  and water displacement method  $V_{[WATER]}$  after logarithmic transformation.  $V_{[WATER]}$  are overestimated by 3D models (slope  $< 1$ ).

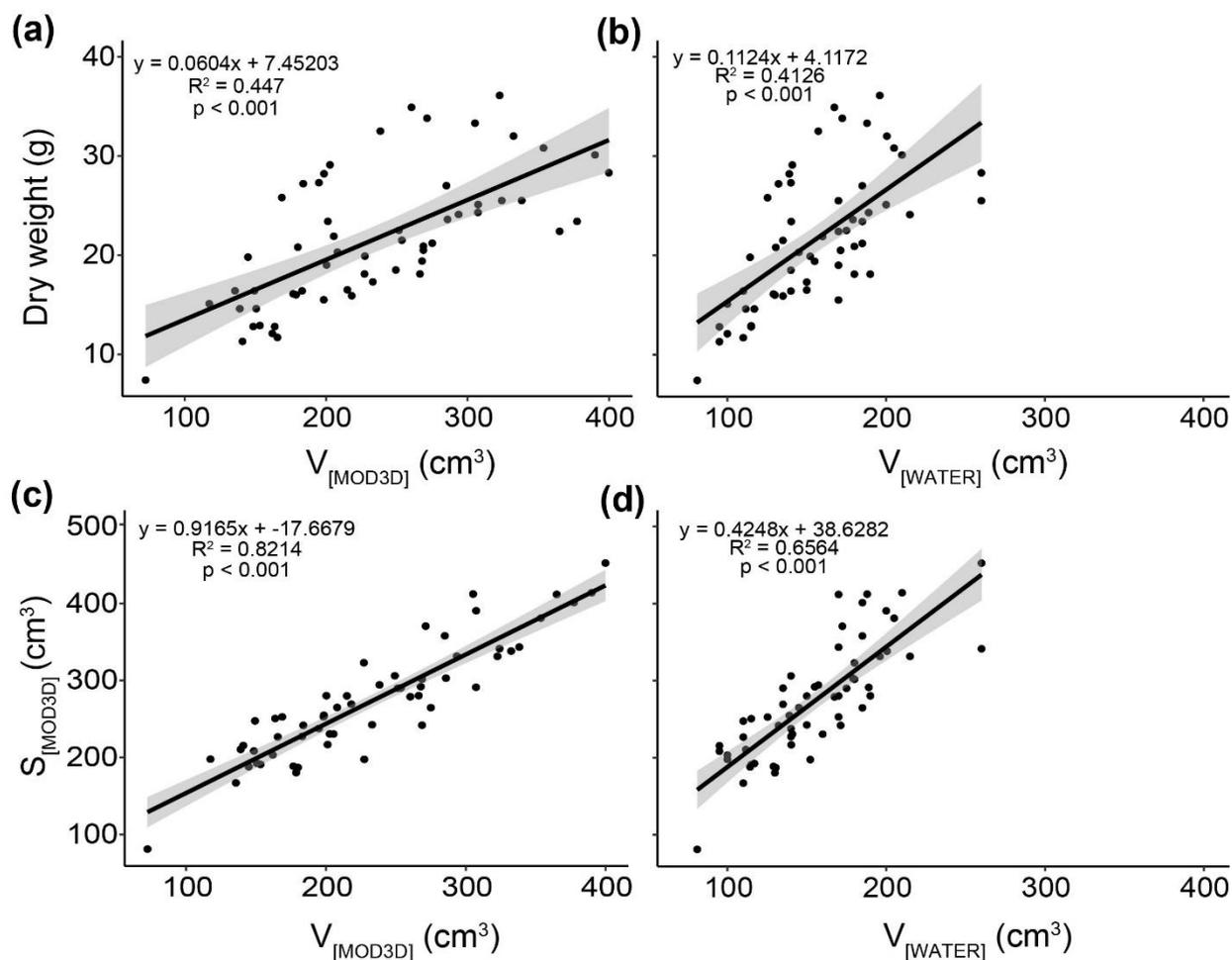


**Fig. B3.1.2.** Bar plot of per cent of difference from  $V_{[WATER]}$  of volume before correction  $V_{[MOD3D]}$ , volume after correction with adjustment factor (obtained in Fig. 1) of both absolute values ( $|V_{[ADJ]}|$ ) and non-absolute values ( $V_{[ADJ]}$ ). The adjustment factor application results in significant better estimate of volume (t-test,  $p < 0.001$ ).

### Sponge volume measurements comparison

A significant linear correlation was found between each explanatory variables (dry weight, volume, and surface) and the descriptive variables ( $V_{[MOD3D]}$  or  $V_{[WATER]}$ ) (**Fig. B3.1.3, Table B3.1.1**).

The R-squared and RMSE calculated for each regression using a 5-fold cross validation indicated a better fit and better predictive capabilities while using the volume of 3D photogrammetry to predict both dry weight and surface of the sponges. R-squared indicated a high proportion of the surface variance explained by  $V_{[MOD3D]}$  ( $R^2 = 0.8214$ ) and in a lesser extent by  $V_{[WATER]}$  ( $R^2 = 0.6564$ ). However, variance in dry weight explained by these variables was lower, with a  $R^2$  of 0.487 and 0.442 for  $V_{[MOD3D]}$  and  $V_{[WATER]}$ , respectively.



**Fig. B3.1.3. Linear regression plots** between dry weight and (a)  $V_{[MOD3D]}$ , (b)  $V_{[WATER]}$ ; and between  $S_{[MOD3D]}$  and (c)  $V_{[MOD3D]}$ , (d)  $V_{[WATER]}$ . Slope < 1: the model is overestimating the measurement, slope > 1: the model is underestimating the measurements.

**Table B3.1.1. Linear regression results.** Comparison between  $V_{[MOD3D]}$  and  $V_{[WATER]}$  to dry weight, surface and volume of sponges. Average  $R^2$  calculated on each fold corresponding to 4/5 of the data set ( $R^2$  cv), Root mean square error (RMSE), Interval of Confidence (CI) and  $p$ -value are expressed.

Variable to express	Volume [MOD3D]				Volume [WATER]			
	$R^2$ (cv)	RMSE	CI	$p$ -value	$R^2$ (cv)	RMSE	CI	$p$ -value
Dry weight	0.487	5.002	[0.043; 0.078]	< 0.001	0.442	5.065	[0.077; 0.147]	< 0.001
Surface	0.822	31.181	[0.788; 1.011]	< 0.001	0.668	43.778	[1.261; 1.857]	< 0.001

## Discussion

Nowadays there are no accurate methods by which to measure sponge volume *in situ* other than detaching the sponge and measuring its volume by water displacement (Rutzler, 1972). In an attempt to provide metrics to evaluate recent non-invasive volume calculation methods, we compared the results obtained by the traditional method of water displacement with the 3D photogrammetry

method. A formula was proposed in order to correct the value calculated by the latter method to obtain values closer to those obtained by the traditional method, in the range of the volume values tested.

The 3D model showed a good estimation of the volume of the massive sponge. However an overestimation of volume calculated with 3D method compared to that measured with the water displacement was reported, with greater difference between the two methods the larger the sponges are. This overestimation could be given by some limitations of the 3D method, which considered sponges as filled objects, while the species *R. globostellata* presents a series of channels inside that are not occupied by tissue. It suggests that this method would be more appropriate to calculate the volume of solid organisms without channels such as corals. Furthermore, photography in the water can be the cause of errors and failures due to constant movement, turbidity, light intensity changes and limited time linked with diving restrictions (Bowens 2008). Further errors could be caused by the subjectivity of the operator who takes the photos and the sponge species which is taken into account and 3D models are limited to the portions of the target organisms that can be photographed and it's difficult to use for organisms with complex morphologies. For example, a study on the comparison of several methods to calculate the surface of three coral morphologies showed that wax dipping was the most accurate method, while photogrammetric reconstruction was found to be the most inaccurate method for massive corals and incapable to reconstruct branching coral morphologies (Vael et al., 2010). However, this technology develops very quickly, improving the whole 3D reproductions and results. The formula proposed in this study allows to reduce the 3D overestimation and give a better volume estimation through the use of an adjustment factor ( $\text{Adjusted } V_{[\text{MOD3D}]} = 0.68 * (V_{[\text{WATER}]} + 1.34)$ ). The same overestimation of 3D methods was previously reported by other studies (i.e., Lavy et al., 2015; Gutierrez-Heredia et al., 2016), but no significant differences were found between volume before and after correction with adjustment factor (Lavy et al., 2015). However, a small number of samples were used compared to our study.

Although we suggest to use an adjustment factor, also an underestimation of volume by water displacement was already reported because the perforated nature of the sponge body (Morganti et al., 2019). This leaves one to think that the accuracy of the latter method depends greatly on the morphology and characteristics of the sponge being studied. Since the biggest setbacks of volume calculate with water displacement are awkward, destructive and can only be applied to small removed colonies or fragments (Holmes 2008; Naumann et al., 2009), we also tested the direct capacity of ( $V_{[\text{MOD3D}]}$ ) to predict the sponges dry weight and surface. In fact, recent studies highlighted the efficiency of some 3D method and photogrammetry for the calculation of sponge volume and growth rate (Jokiel et al., 1991; Pawlink et al., 2008; Lavy et al., 2015, Olinger et al., 2019; Prado et al.,

2021). The results of the present study suggests that 3D methods better predict the dry weight and surface (also calculated with 3D methods) of sponges compared to water displacement. Despite some limitations, the advantage of the 3D photogrammetry software are that organisms remaining intact and healthy as previously shown in other studies (Bythell, Pan and Lee 2001, Cocito et al., 2003, Courtney et al., 2007). Furthermore, the 3D photogrammetry is very useful, being both easy and cost effective compared for example to 3D scanning methods, which the purchase price for the equipment is still relatively higher. The advantage and efficiency of 3D techniques are already well reported for corals, which despite their complex structure, this technique showed satisfactory results (e.g., Bythell et al., 2001; Cocito et al. 2003; Courtney et al. 2007; Fisher et al. 2007; Holmes 2008; Holmes et al. 2008; Veal et al. 2010; Figueira et al., 2015; Burns et al., 2016; Urbina-Barreto et al.2021). In addition, many potential applications in the field are possible with the 3D reconstruction method proposed: these, related to surface area, include ecophysiological studies by measuring tissue biomass, long-term monitoring of partial mortality due to predation, bleaching and other disease episodes, symbiont density, filtering surface and other ecologically important processes. The procedure is also applicable to morphometric measurements such as height, branch length and spacing, cavity width, distance among satellite colonies, and to volume measurements, useful to estimate biomass, growth rate estimation, carbonate standing stock and carbonate production.

To the best of our knowledge, this is the first study that used such a large number of samples to compare both traditional techniques of sponge dry weight and sponge volume by displacement with the 3D Zephyr pro software. Although an adjustment factor is suggested when we compared  $V_{[MOD3D]}$  to  $V_{[WATER]}$ , the direct  $V_{[MOD3D]}$  showed a better correlation with dry weight (i.e., biomass calculation), allowing for a wide range of applications than traditional methods due to its less intrusive applications. This study demonstrated that the 3D photogrammetry reconstruction is an alternative tool to calculate volume of sponges and a realistic method for normalize data keeping the sponge in their natural environment, providing new opportunities for ecological and evolutionary research on sponges. Further studied considering different species with different morphologies are needed to generalize our results in the use of the 3D Zephyr pro software.

## References

- Bowens, A. (ed) (2008) Chapter 2: Photography. Underwater Archaeology: The NAS Guide to Principles and Practice, 2nd edn. Wiley-Blackwell Publishing Ltd, Hoboken, New Jersey, USA.
- Bell, J. J.: The functional roles of marine sponges, *Estuarine, Coastal and Shelf Science*, doi:10.1016/j.ecss.2008.05.002, 2008.
- Burns, J. H. R., Delparte, D., Kapon, L., Belt, M., Gates, R. D. and Takabayashi, M.: Assessing the impact of acute disturbances on the structure and composition of a coral community using innovative 3D reconstruction techniques, *Methods in Oceanography*, doi:10.1016/j.mio.2016.04.001, 2016.
- Bythell, J., Pan, P. and Lee, J.: Three-dimensional morphometric measurements of reef corals using underwater photogrammetry techniques, *Coral Reefs*, doi:10.1007/s003380100157, 2001.

- Cocito, S., Sgorbini, S., Peirano, A. and Valle, M.: 3-D reconstruction of biological objects using underwater video technique and image processing, *Journal of Experimental Marine Biology and Ecology*, doi:10.1016/S0022-0981(03)00369-1, 2003.
- Courtney, L. A., Fisher, W. S., Raimondo, S., Oliver, L. M. and Davis, W. P.: Estimating 3-dimensional colony surface area of field corals, *Journal of Experimental Marine Biology and Ecology*, doi:10.1016/j.jembe.2007.06.021, 2007.
- Edgar, G. J.: The use of the size structure of benthic macrofaunal communities to estimate faunal biomass and secondary production, *Journal of Experimental Marine Biology and Ecology*, doi:10.1016/0022-0981(90)90185-F, 1990.
- Figueira, W., Ferrari, R., Weatherby, E., Porter, A., Hawes, S. and Byrne, M.: Accuracy and precision of habitat structural complexity metrics derived from underwater photogrammetry, *Remote Sensing*, doi:10.3390/rs71215859, 2015.
- Fisher, W. S., Davis, W. P., Quarles, R. L., Patrick, J., Campbell, J. G., Harris, P. S., Hemmer, B. L. and Parsons, M.: Characterizing coral condition using estimates of three-dimensional colony surface area, *Environmental Monitoring and Assessment*, doi:10.1007/s10661-006-9527-8, 2007.
- De Goeij, J. M., Lesser, M. P. and Pawlik, J. R.: Nutrient fluxes and ecological functions of coral reef sponges in a changing ocean, in *Climate Change, Ocean Acidification and Sponges: Impacts Across Multiple Levels of Organization.*, 2017.
- Golikov, A. N. and Scarlato, O. A.: Comparative characteristics of some ecosystems of the upper regions of the shelf in tropical, temperate and Arctic waters, *Helgoländer Wissenschaftliche Meeresuntersuchungen*, doi:10.1007/BF01609513, 1973.
- Gutierrez-Heredia, L., Benzoni, F., Murphy, E. and Reynaud, E. G.: End to End Digitisation and Analysis of Three-Dimensional Coral Models, from Communities to Corallites, *PLoS ONE*, doi:10.1371/journal.pone.0149641, 2016.
- Holmes, G.: Estimating three-dimensional surface areas on coral reefs, *Journal of Experimental Marine Biology and Ecology*, doi:10.1016/j.jembe.2008.07.045, 2008.
- Holmes, G., Ortiz, J., Kaniewska, P. and Johnstone, R.: Using three-dimensional surface area to compare the growth of two Pocilloporid coral species, *Marine Biology*, doi:10.1007/s00227-008-1040-x, 2008.
- Lavy, A., Eyal, G., Neal, B., Keren, R., Loya, Y. and Ilan, M.: A quick, easy and non-intrusive method for underwater volume and surface area evaluation of benthic organisms by 3D computer modelling, *Methods in Ecology and Evolution*, doi:10.1111/2041-210X.12331, 2015.
- Maldonado, M., Ribes, M. and van Duyl, F. C.: Nutrient Fluxes Through Sponges. Biology, Budgets, and Ecological Implications, in *Advances in Marine Biology.*, 2012.
- Morganti, T. M., Ribes, M., Yahel, G. and Coma, R.: Size Is the Major Determinant of Pumping Rates in Marine Sponges, *Frontiers in Physiology*, doi:10.3389/fphys.2019.01474, 2019.
- Müller, W. E. G., Kruse, M., Blumbach, B., Skorokhod, A. and Müller, I. M.: Gene structure and function of tyrosine kinases in the marine sponge *Geodia cydonium*: Autapomorphic characters of Metazoa, *Gene*, doi:10.1016/S0378-1119(99)00226-7, 1999.
- Naumann, M. S., Niggel, W., Laforsch, C., Glaser, C. and Wild, C.: Coral surface area quantification-evaluation of established techniques by comparison with computer tomography, *Coral Reefs*, doi:10.1007/s00338-008-0459-3, 2009.
- Olinger, L. K., Scott, A. R., McMurray, S. E. and Pawlik, J. R.: Growth estimates of Caribbean reef sponges on a shipwreck using 3D photogrammetry, *Scientific Reports*, doi:10.1038/s41598-019-54681-2, 2019.
- Pawlik, J. R., Henkel, T. P., McMurray, S. E., López-Legentil, S., Loh, T. L. and Rohde, S.: Patterns of sponge recruitment and growth on a shipwreck corroborate chemical defense resource trade-off, *Marine Ecology Progress Series*, doi:10.3354/meps07615, 2008.
- Pomeroy, L. R.: Caught in the food web: Complexity made simple?, *Scientia Marina*, doi:10.3989/scimar.2001.65s231, 2001.
- Prado, E., Cristobo, J., Rodríguez-Basalo, A., Ríos, P., Rodríguez-Cabello, C. and Sánchez, F.: In situ Growth Rate Assessment of the Hexactinellid Sponge *Asconema setubalense* Using 3D Photogrammetric Reconstruction, *Frontiers in Marine Science*, doi:10.3389/fmars.2021.612613, 2021.
- R Core Team: R.studio Team, R Development Core Team., 2019.
- Rahav, O., Ben-Zion, M., Achituv, Y. and Dubinsky, Z.: A photographic, computerized method for in situ growth measurements in reef-building cnidarians, *Coral Reefs*, doi:10.1007/BF00290422, 1991.
- Reichert, J., Schellenberg, J., Schubert, P. and Wilke, T.: 3D scanning as a highly precise, reproducible, and minimally invasive method for surface area and volume measurements of scleractinian corals, *Limnology and Oceanography: Methods*, doi:10.1002/lom3.10109, 2016.
- Rutzler, K.: Principles of sponge distribution in indo-pacific coral reefs: results of the Austrian indo-westpacific expedition 1959/60, in *Proceedings of the First International Symposium on Corals and Coral Reefs.*, 1972.
- Schiller, C.: Ecology of the Symbiotic Coral *Cladocora caespitosa* (L.) Faviidae, Scleractinia in the Bay of Piran (Adriatic Sea): I. Distribution and Biometry, *Marine Ecology*, doi:10.1111/j.1439-0485.1993.tb00480.x, 1993.
- Urbina-Barreto, I., Chiroleu, F., Pinel, R., Fréchon, L., Mahamadaly, V., Elise, S., Kulbicki, M., Quod, J. P., Dutrieux, E., Garnier, R., Henrich Bruggemann, J., Penin, L. and Adjeroud, M.: Quantifying the shelter capacity of coral reefs using photogrammetric 3D modeling: From colonies to reefscape, *Ecological Indicators*, doi:10.1016/j.ecolind.2020.107151, 2021.
- Veal, C. J., Holmes, G., Nunez, M., Hoegh-Guldberg, O. and Osborn, J.: A comparative study of methods for surface area and three dimensional shape measurement of coral skeletons, *Limnology and Oceanography: Methods*, doi:10.4319/lom.2010.8.241, 2010.

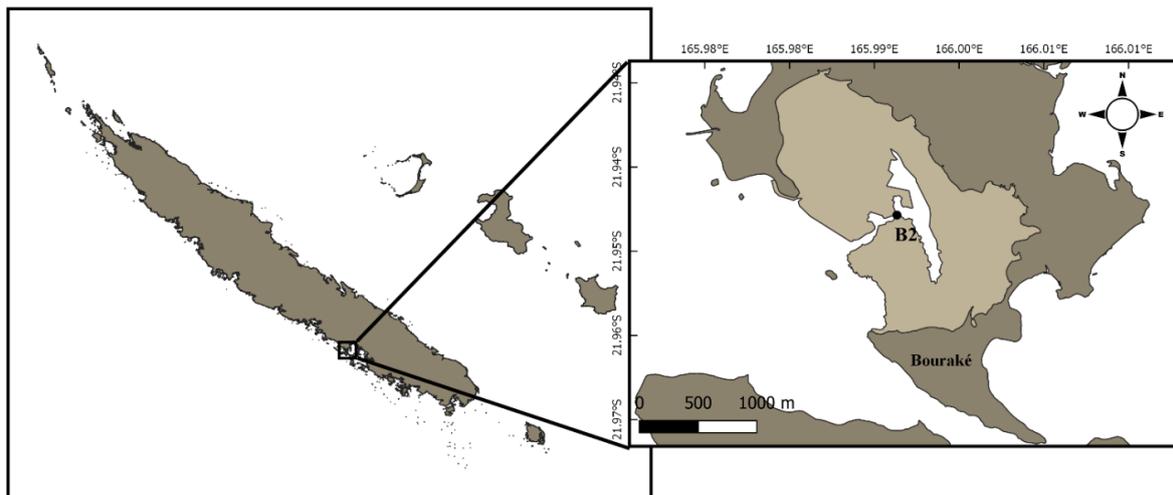
### Section B3.2 - Seawater analyses

Seawater pH and temperature were measured immediately on board the R/V using a portable pH-meter (913, Metrohm) calibrated with TRIS buffer (Dickson lab, batch #T28).

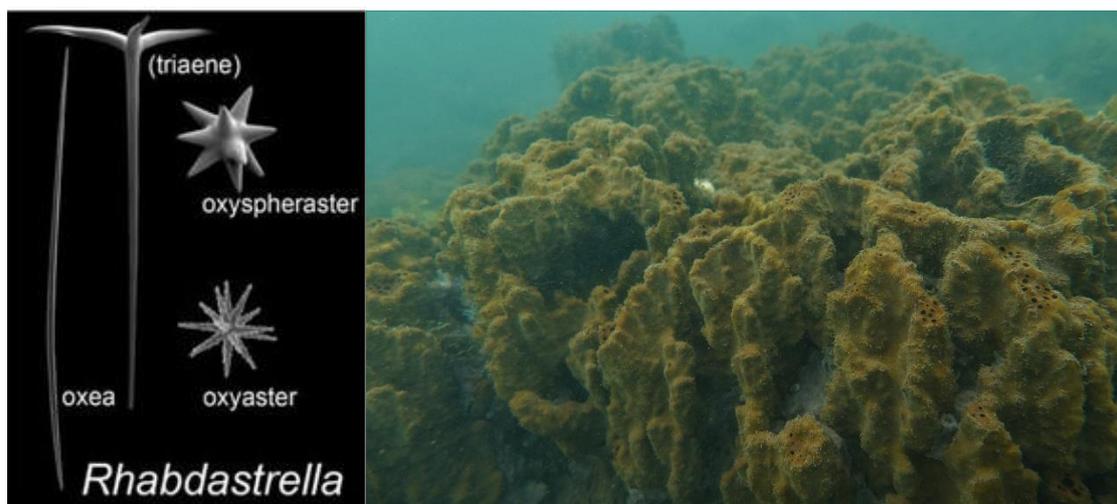
One litre of unfiltered seawater was collected for POC and PON analysis in a borosilicate glass bottle and stored on ice during sampling. For that, at the beginning of the incubations, seawater was collected near the chamber using a one-L borosilicate bottle, while at the end of the incubation, each Plexiglas chamber, including empty chambers, was separated from the system, isolated (i.e., no exchange with the surrounding seawater) and transported to the dinghy. There, the chambers were carefully moved in order to collect the detritus deposited during the incubation, and 1 L of seawater was collected as above. In the laboratory, particulate matter was collected on pre-combusted (4 h at 450°C) Whatman<sup>TM</sup> GF/F filters using a Nalgene® vacuum system. The filters were dried at 60°C in an oven for 24 h and stored in airtight glass vials at 4°C in the dark until analysis on a CHN Perkin Elmer 2400.

Total bacteria abundance (BA) and phytoplankton abundance were measured in 1.49 mL of water fixed with 0.165 mL of paraformaldehyde (PFA) in cryovials kept 10 min in the dark and quickly stored at -80° C. In 2018, only BA was measured, while in 2020 we also differentiated the different plankton and BA components, allowing us to measure the LPOC, which was considered as the sum of phytoplankton (Prochlorococcus spp. [Pro], Synechococcus spp. [Syn], photosynthetic picoeukaryote [Pico], nanoeukaryote [NanoEuk]), and bacterioplankton (high-nucleic acid bacteria [HNA] and low-nucleic acid bacteria [LNA]), all converted into their respective carbon contents. They were counted using BD Accuri<sup>TM</sup> C6 cytometer. For that, cells were excited with 488 nm laser and green fluorescence (530 nm), orange fluorescence (575 nm), and red fluorescence (695 nm) emissions were measured. Phytoplankton were analysed for 15 min at high flow rate and classified based on their characteristic flow cytometry signatures, while bacterioplankton were analysed for 5 min at high flow rate and quantified by staining samples with Sybr Green-I. Carbon content of each type of phytoplankton and bacterioplankton was estimated using standard cell conversions: 53 fg C cell<sup>-1</sup> for Proc (Morel et al., 1993), 470 fg C cell<sup>-1</sup> for Syn (Campbell et al., 1994), 1496 fg C cell<sup>-1</sup> for NanoEuk and Pico (Zubkov et al., 1998), and 20 fg C cell<sup>-1</sup> for bacteria (Ducklow et al., 1993). Finally, detrital carbon production/consumption during the *in situ* dark sponge incubations was calculated as the portion of total POC not accounted for the live POC (LPOC) (i.e., Detritus = POC - LPOC) (Ribes et al., 1999; Hadas et al., 2009; McMurray et al., 2016; 2018). Bacteria and phytoplankton ingestion rates were calculated according to the equations of Ribes et al. (1998) and Houlbrèque et al. (2004), which take into account the growth of the prey during the incubations.

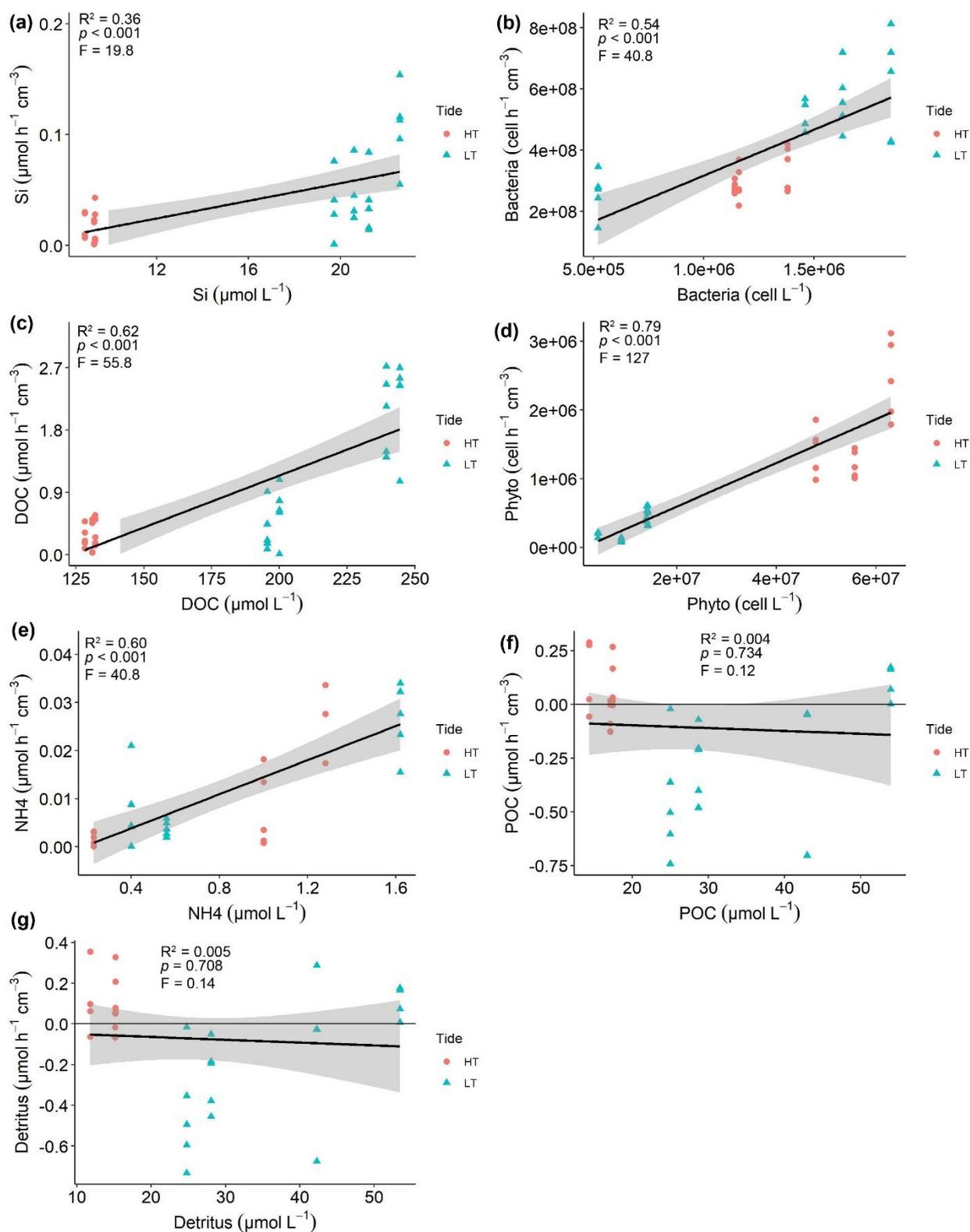
All glass bottles and vials used were pre-combusted, washed with HCl solutions (10%) and rinsed using milliQ water before use.



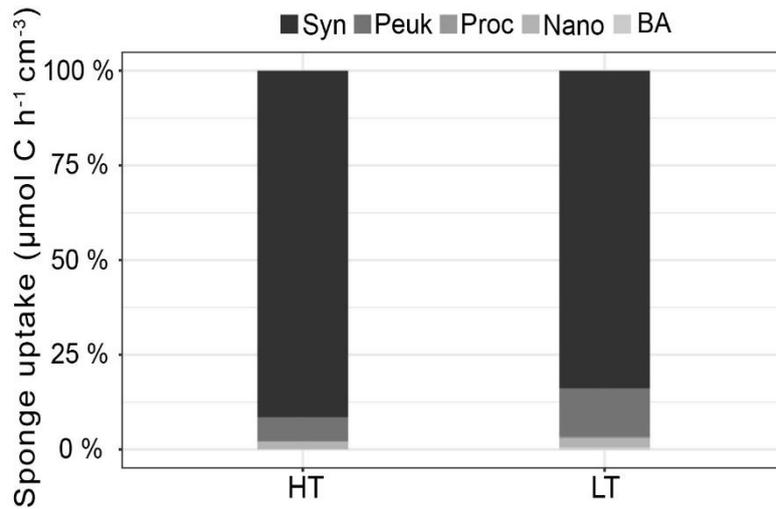
**Figure B3.1.** Map of New Caledonia (South Pacific) and zoom (right panel) on the study site (B2) where the *in situ* experiment was performed. Georep New Caledonia database (<https://georep.nc/>) and QGIS software were used to build the figure.



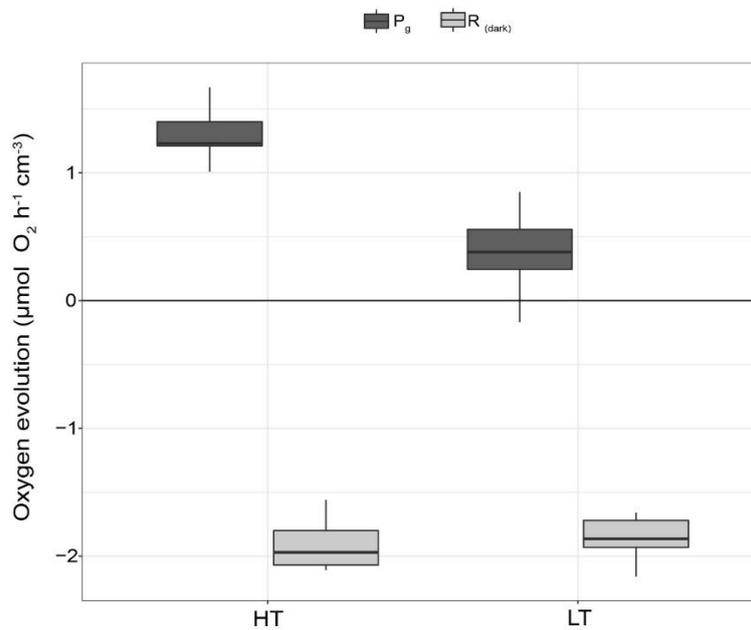
**Figure B3.2.** *Rhabdastrella globostellata* silica spicules (left), and picture of the sponge species at the Bouraké study site (© Federica Maggioni).



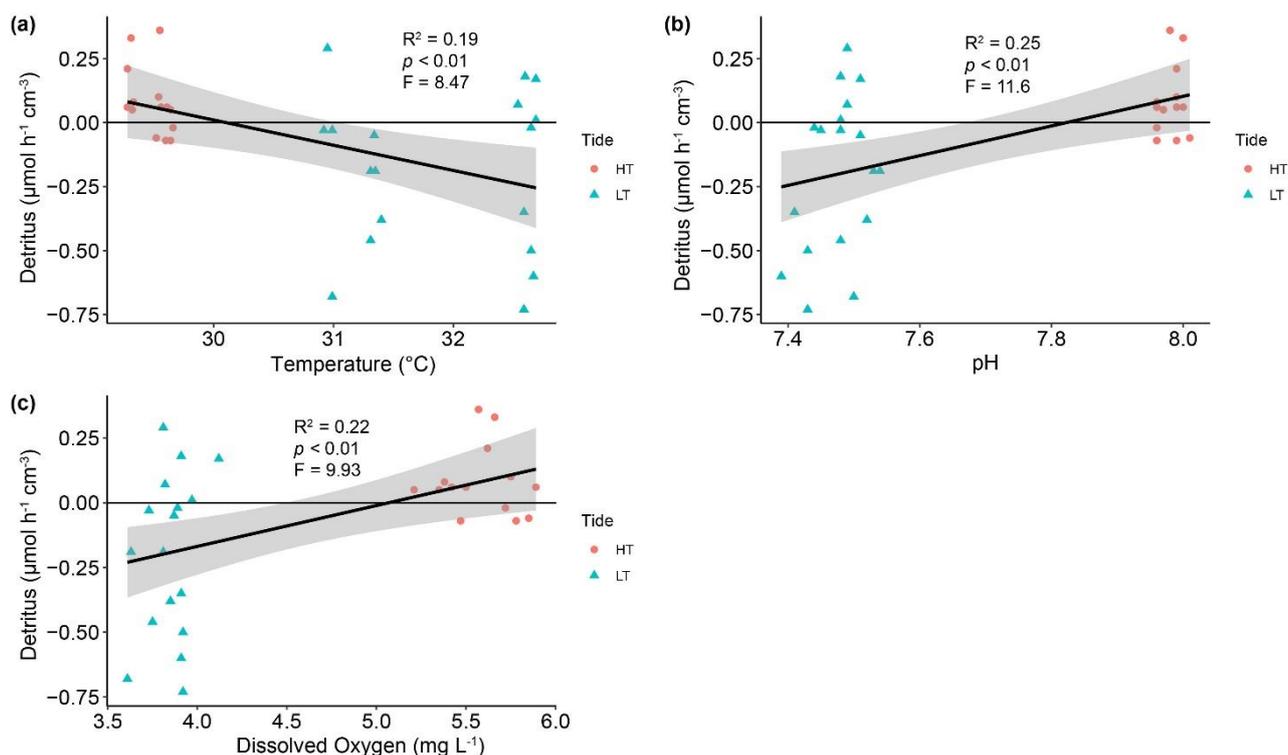
**Figure B3.3.** Linear regressions (black line) of data collected during the incubations in the dark in 2020, at both HT (red dots) and LT (blue triangles), between the absolute values of nutrient reduction/increase (y-axis), and their relative initial concentrations (x-axis) of (a)  $\text{Si}(\text{OH})_4$ , (b) Bacteria, (c) Dissolved organic carbon, (d) Phytoplankton, (e)  $\text{NH}_4^+$ , (f) Particulate organic carbon, and (g) Detritus. The shaded areas indicate the 95% confidence intervals.



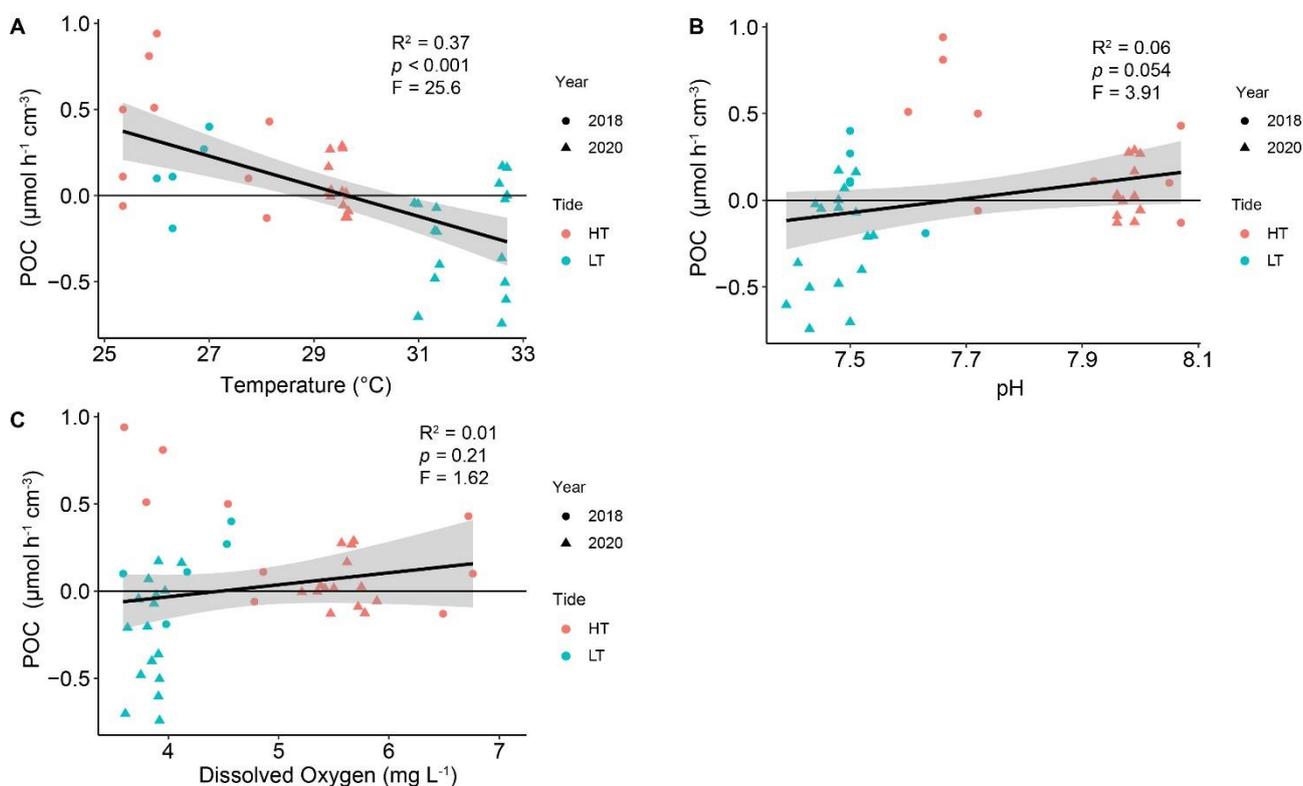
**Figure B3.4.** Sponge carbon uptake (LPOC) during the incubations in 2020, at both HT and LT by phytoplankton components (Syn, Peuk, Proc, Nano) and bacteria (BA).



**Figure B3.5.** Gross photosynthesis ( $P_g$ ) and respiration ( $R_{dark}$ ), of the sponge holobiont measured in situ, at HT and LT in 2020. Boxes represent the interquartile range (25th and 75th percentile); the horizontal line is the median, and the whiskers represent the data range (i.e., minimum and maximum).



**Figure B3.6.** Linear regressions (black line) between detritus and physical parameters: **a)** Temperature, **b)** pH, and **c)** Dissolved Oxygen (DO) in 2020 during both high tide (HT; red dots) and low tide (LT; blue triangles). The shaded areas indicate the 95% confidence intervals.



**Figure B3.7.** Linear regressions (black line) between POC and physical parameters: **a)** Temperature, **b)** pH and **c)** Dissolved Oxygen (DO) at both years (2018 dots, and 2020 triangles), and tides (HT in red, and LT in blue). The shaded areas indicate the 95% confidence intervals.

**Table B3.1.** Summary of all in situ sponge incubations performed in 2018 and 2020. The number of chambers and the blank (+ 1) was specified. Incubations during high tide (HT) and low tide (LT), as well as in dark (D) or light (L) conditions was reported.

Year	n° incubation	n° chambers each incubation	n° incubation by conditions
2018	7	3 + 1	4 HT (D); 3 LT (D)
2020	7	5 + 1	3 HT (D); 4 LT (D)
2020	8	5 + 1	2 HT (D); 2 HT (L); 2 LT (D); 2 LT (L)

**Table B3.2.** Summary of the statistical analysis on the environmental parameters measured at the beginning of the incubations in the dark in 2018 at both high tide (HT; n=16) and low tide (LT; n=12) and, in 2020 at both high tide (HT; n=18) and low tide (LT; n=24). Two-way ANOVA was used between years, tides and their interaction, and the F- and p-value (in brackets) were reported. Tukey's HSD test was applied on their interaction term when significant. For data measured only in 2020, unpaired t-test (t) between tides was used.

	Year F-value (P)	Tide F-value (P)	Year x Tide F-value (P)	t (P)	HSD Post hoc (P)
T	332.24 (< 0.001)	38.15 (< 0.001)	28.97 (< 0.001)		LT <sub>2018</sub> = HT <sub>2018</sub> < HT <sub>2020</sub> ; HT <sub>2020</sub> < LT <sub>2020</sub> (< 0.05)
pH <sub>T</sub>	0.36 (0.55)	307.55 (< 0.001)	7.60 (< 0.01)		LT <sub>2018</sub> = LT <sub>2020</sub> < HT <sub>2018</sub> ; HT <sub>2018</sub> < HT <sub>2020</sub> (< 0.05)
DIC	31.24 (< 0.001)	278.30 (< 0.001)	98.18 (< 0.001)		LT <sub>2020</sub> > HT <sub>2018</sub> > HT <sub>2020</sub> ; LT <sub>2018</sub> > HT <sub>2018</sub> (< 0.05)
DO	3.25 (0.076)	78.74 (< 0.001)	1.54 (0.218)		
Si(OH) <sub>4</sub>	1.44 (0.235)	192.75 (< 0.001)	9.41 (< 0.05)		HT <sub>2020</sub> < HT <sub>2018</sub> ; HT <sub>2020</sub> < LT <sub>2020</sub> = LT <sub>2018</sub> (< 0.05)
NO <sub>x</sub>	18.75 (< 0.001)	0.006 (0.941)	0.95 (0.332)		
PO <sub>4</sub> <sup>3-</sup>	0.42 (0.518)	0.312 (0.578)	0.34 (0.561)		
NH <sub>4</sub> <sup>+</sup>	20.14 (< 0.001)	0.006 (0.939)	0.03 (0.856)		
DOC	162.6 (< 0.001)	199.5 (< 0.001)	41.12 (< 0.001)		HT <sub>2018</sub> < LT <sub>2018</sub> = HT <sub>2020</sub> ; LT <sub>2020</sub> > HT <sub>2020</sub> (< 0.05)
POC	2.28 (0.136)	31.89 (< 0.001)	8.33 (< 0.01)		HT <sub>2018</sub> > HT <sub>2020</sub> < LT <sub>2020</sub> ; LT <sub>2018</sub> > HT <sub>2020</sub> (< 0.05)
PON	0.21 (0.646)	20.74 (< 0.001)	4.25 (< 0.05)		LT <sub>2020</sub> > HT <sub>2018</sub> > HT <sub>2020</sub> ; HT <sub>2020</sub> < LT <sub>2018</sub> (< 0.05)
BA	170.43 (< 0.001)	3.405 (0.0694)	0.00 (0.989)		
LPOC				5.34 (< 0.001)	
Phytoplankton				26.21 (< 0.001)	
Detritus				-9.52 (< 0.001)	

**Table B3.3.** Summary of the parameters measured in the incubation medium as the difference between the end and the beginning of the incubations in the dark, in 2018 and in 2020 at both at high tide (HT) and low tide (LT). Negative values are considered as consumption while positive as production. Data are expressed as mean  $\pm$  SD; number of replicates is in brackets; nd means not determined. Replicates for DO in 2020 are  $n = 24$  and  $n = 28$  for HT and LT, respectively.

	2018		2020	
	HT (n = 12)	LT (n = 9)	HT (n = 15)	LT (n = 20)
DIC ( $\mu\text{mol C h}^{-1} \text{cm}^{-3}$ )	0.52 $\pm$ 0.74	-1.28 $\pm$ 3.25	2.16 $\pm$ 0.56	1.90 $\pm$ 1.1
DO ( $\mu\text{mol O}_2 \text{h}^{-1} \text{cm}^{-3}$ )	-1.51 $\pm$ 0.63	-1.27 $\pm$ 0.40	-1.88 $\pm$ 0.36	-2.02 $\pm$ 0.50
Si(OH) <sub>4</sub> ( $\mu\text{mol h}^{-1} \text{cm}^{-3}$ )	-0.01 $\pm$ 0.025	-0.10 $\pm$ 0.043	-0.013 $\pm$ 0.015	-0.057 $\pm$ 0.043
No <sub>x</sub> ( $\mu\text{mol h}^{-1} \text{cm}^{-3}$ )	0.11 $\pm$ 0.08	0.17 $\pm$ 0.11	0.22 $\pm$ 0.05	0.19 $\pm$ 0.052
PO <sub>4</sub> <sup>3-</sup> ( $\mu\text{mol h}^{-1} \text{cm}^{-3}$ )	0.003 $\pm$ 0.003	0.003 $\pm$ 0.003	0.002 $\pm$ 0.001	0.003 $\pm$ 0.004
NH <sub>4</sub> <sup>+</sup> ( $\mu\text{mol h}^{-1} \text{cm}^{-3}$ )	-0.002 $\pm$ 0.002	-0.011 $\pm$ 0.013	0.006 $\pm$ 0.014	-0.004 $\pm$ 0.019
DOC ( $\mu\text{mol C h}^{-1} \text{cm}^{-3}$ )	-0.41 $\pm$ 0.37	-1.65 $\pm$ 1.28	-0.16 $\pm$ 0.29	-1.27 $\pm$ 1.04
POC ( $\mu\text{mol C h}^{-1} \text{cm}^{-3}$ )	0.36 $\pm$ 0.38	0.14 $\pm$ 0.22	0.034 $\pm$ 0.15	-0.23 $\pm$ 0.30
PON ( $\mu\text{mol N h}^{-1} \text{cm}^{-3}$ )	0.02 $\pm$ 0.03	0.003 $\pm$ 0.05	-0.0015 $\pm$ 0.013	-0.021 $\pm$ 0.02
BA ( $\times 10^7$ cells $\text{h}^{-1} \text{cm}^{-3}$ )	-4.58 $\pm$ 1.83	-12.89 $\pm$ 5.49	-30.6 $\pm$ 5.94	-48.5 $\pm$ 17.3
LPOC ( $\mu\text{mol C h}^{-1} \text{cm}^{-3}$ )	nd	nd	-0.069 $\pm$ 0.026	-0.013 $\pm$ 0.008
Phyto ( $\times 10^5$ cells $\text{h}^{-1} \text{cm}^{-3}$ )	nd	nd	-16.9 $\pm$ 6.75	-3.10 $\pm$ 1.82
Detritus ( $\mu\text{mol C h}^{-1} \text{cm}^{-3}$ )	nd	nd	0.06 $\pm$ 0.11	-0.16 $\pm$ 0.34

**Table B3.4.** Summary of the statistical analysis on sponge metabolism measured during the incubations in the dark, in 2018, at both high tide (HT;  $n=12$ ) and low tide (LT;  $n=9$ ) and, in 2020 at both high tide (HT;  $n=15$ ) and low tide (LT;  $n=20$ ). Two-way ANOVA was used between years, tides and their interaction, and the *F*- and *p*-value (in brackets) were reported. Tukey's HSD test was applied on their interaction term when significant. For data measured only in 2020 (LPOC, phytoplankton and detritus) unpaired *t*-test (*t*) between tides was used.

	Year	Tide	Year x Tide		HSD Post hoc ( <i>P</i> )
	F-value ( <i>P</i> )	F-value ( <i>P</i> )	F-value ( <i>P</i> )	<i>t</i> ( <i>P</i> )	
DIC	26.270 (< 0.001)	3.196 (0.08)	2.93 (0.093)		
DO	19.604 (< 0.001)	1.251 (0.293)	2.428 (0.124)		
Si(OH) <sub>4</sub>	0.254 (0.617)	38.195 (< 0.001)	5.31 (< 0.05)		LT <sub>2020</sub> < LT <sub>2018</sub> ; HT <sub>2020</sub> = HT <sub>2018</sub> > LT <sub>2018</sub> (< 0.001)
NO <sub>x</sub>	11.275 (< 0.01)	0.101 (0.751)	5.30 (< 0.05)		HT <sub>2018</sub> < LT <sub>2020</sub> ; HT <sub>2020</sub> > HT <sub>2018</sub> (< 0.001)
PO <sub>4</sub> <sup>3-</sup>	0.517 (0.476)	1.717 (0.191)	0.11 (0.741)		
NH <sub>4</sub> <sup>+</sup>	2.805 (0.1008)	4.958 (< 0.05)	0.11 (0.917)		
DOC	0.623 (0.434)	21.181 (< 0.001)	0.04 (0.837)		
POC	19.98 (< 0.001)	10.59 (< 0.01)	0.13 (0.722)		
PON	7.364 (< 0.01)	4.932 (< 0.05)	0.029 (0.866)		
BA	109.98 (< 0.001)	22.09 (< 0.001)	2.41 (0.127)		
LPOC				-7.8606 (< 0.001)	
Phytoplankton				-7.732 (< 0.001)	
Detritus				3.3924 (< 0.05)	

**Table B3.5.** Summary of the bacteria and phytoplankton compositions measured during incubations in the dark in 2020, at both at high tide (HT) and low tide (LT); A) in the seawater medium at the beginning of the incubations, and B) their ingestion rate by the sponge during the incubations. Data are expressed as mean  $\pm$  SD; number of replicates is in brackets. Percentages (%) were calculated on the sum of bacteria and phytoplankton.

	<b>HT</b> (n = 18)	%	<b>LT</b> (n = 24)	%
<b>a) Initial composition</b>				
Total BA (x10 <sup>8</sup> cells L <sup>-1</sup> )	12.3 $\pm$ 1.09	95.7	13.6 $\pm$ 5.17	99.3
Syn (x10 <sup>6</sup> cells L <sup>-1</sup> )	52.0 $\pm$ 6.84	4.06	9.10 $\pm$ 3.64	0.66
Proc (x10 <sup>5</sup> cells L <sup>-1</sup> )	17.9 $\pm$ 4.41	0.14	3.88 $\pm$ 1.62	0.03
Peuk (x10 <sup>5</sup> cells L <sup>-1</sup> )	12.8 $\pm$ 1.98	0.10	6.04 $\pm$ 3.51	0.04
Nano (x10 <sup>4</sup> cells L <sup>-1</sup> )	43.4 $\pm$ 6.83	0.03	21.7 $\pm$ 9.71	0.02
<b>b) Ingestion rate</b>				
Total BA (x10 <sup>6</sup> cells h <sup>-1</sup> cm <sup>-3</sup> )	6.14 $\pm$ 3.13	96.9	27.6 $\pm$ 13.1	99.8
Syn (x10 <sup>4</sup> cells h <sup>-1</sup> cm <sup>-3</sup> )	18.0 $\pm$ 21.4	2.8	2.79 $\pm$ 2.70	0.10
Proc (x10 <sup>3</sup> cells h <sup>-1</sup> cm <sup>-3</sup> )	10.7 $\pm$ 6.58	0.17	4.17 $\pm$ 5.49	0.02
Peuk (x10 <sup>3</sup> cells h <sup>-1</sup> cm <sup>-3</sup> )	5.89 $\pm$ 2.80	0.09	9.80 $\pm$ 8.57	0.04
Nano (x10 <sup>3</sup> cells h <sup>-1</sup> cm <sup>-3</sup> )	2.22 $\pm$ 0.80	0.04	2.24 $\pm$ 2.47	0.01

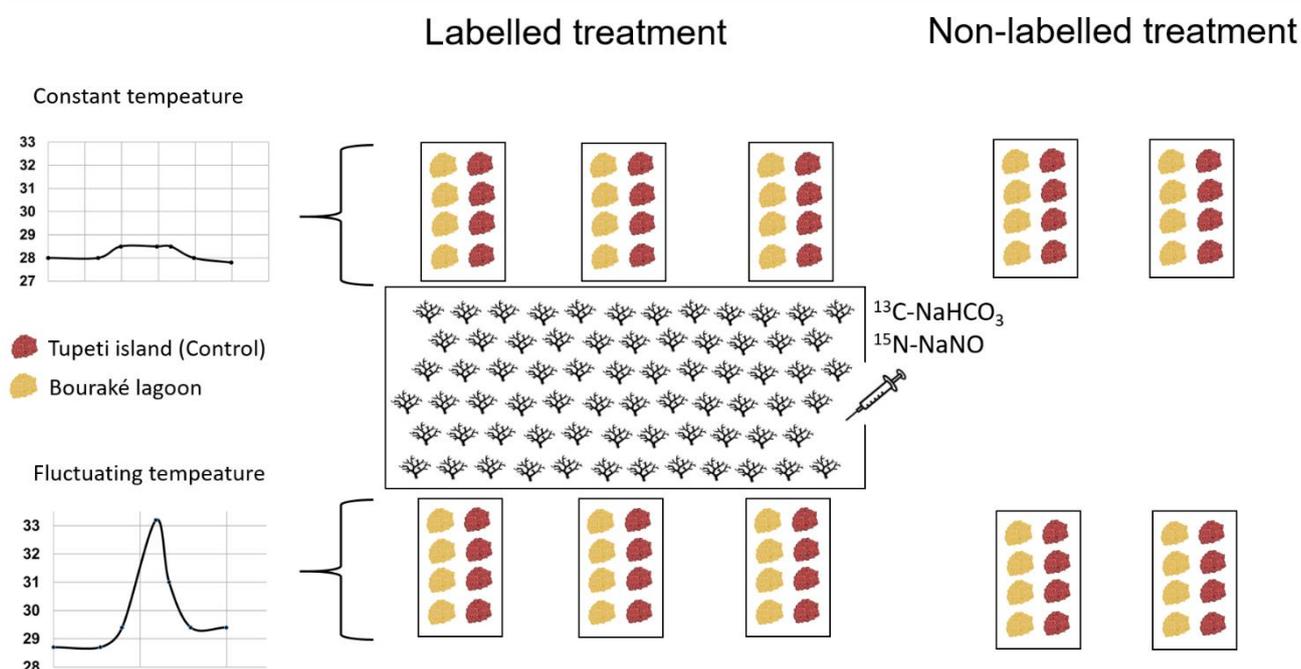
**Table B3.6.** Seawater temperature,  $pH_T$  (total scale), and dissolved oxygen  $DO$  ( $mg\ O_2\ L^{-1}$ ) measured at the beginning of the incubations for dark and light incubations, and for both ex-situ and in situ in 2020. Data are mean ( $\pm$  SD).

	<i>Ex-situ</i>		<i>In-situ</i>			
	<b>HT</b>	<b>LT</b>	<b>HT</b>		<b>LT</b>	
	Dark	Dark	Dark	Light	Dark	Light
<b>T</b> (°C)	29.5 (0.2)	28.9 (0.2)	30.1 (0.01)	29.9 (0.3)	31.6 (0.2)	31.6 (0.7)
<b>pH<sub>T</sub></b>	7.9 (0.1)	7.4 (0.2)	7.9 (0.1)	8.0 (0.1)	7.4 (0.1)	7.5 (0.1)
<b>DO</b>	3.3 (0.8)	2.7 (0.7)	5.8 (1.4)	6.0 (0.9)	3.4 (1.1)	3.9 (0.8)

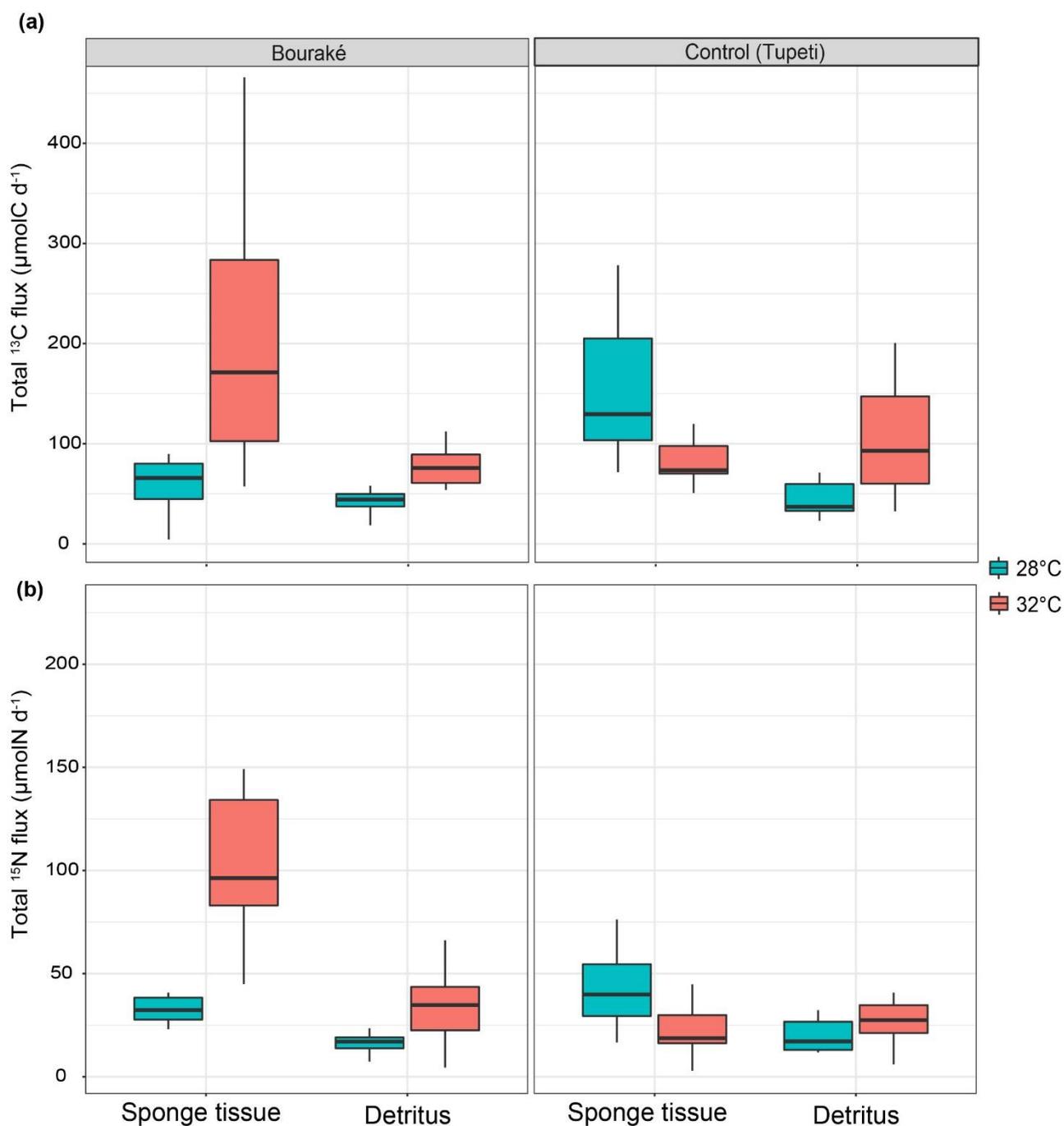
**Table B3.7.** Summary of statistical data for dark and light incubations made in situ and PAM measurements made ex-situ in 2020. Respiration in the dark ( $R_{dark}$ ), gross photosynthesis ( $P_g$ ), photosynthesis to respiration ratio (P:R), maximum electron transport rate ( $rETR_{max}$ ) and the initial effective quantum yield ( $F_v/F_m$ ) were compared between LT and HT using unpaired t-test analysis.

<i>In-situ</i>	<b>t</b>	<b>df</b>	<b>P-value</b>
$R_{dark}$	0.9986	11.726	0.3381
$P_g$	-5.755	14.05	< <b>0.001</b>
P:R	5.8016	12.538	< <b>0.001</b>
<i>Ex-situ</i>			
$rETR_{max}$	-4.6991	15.99	< <b>0.001</b>
$F_v/F_m$	-2.604	12.016	< <b>0.05</b>

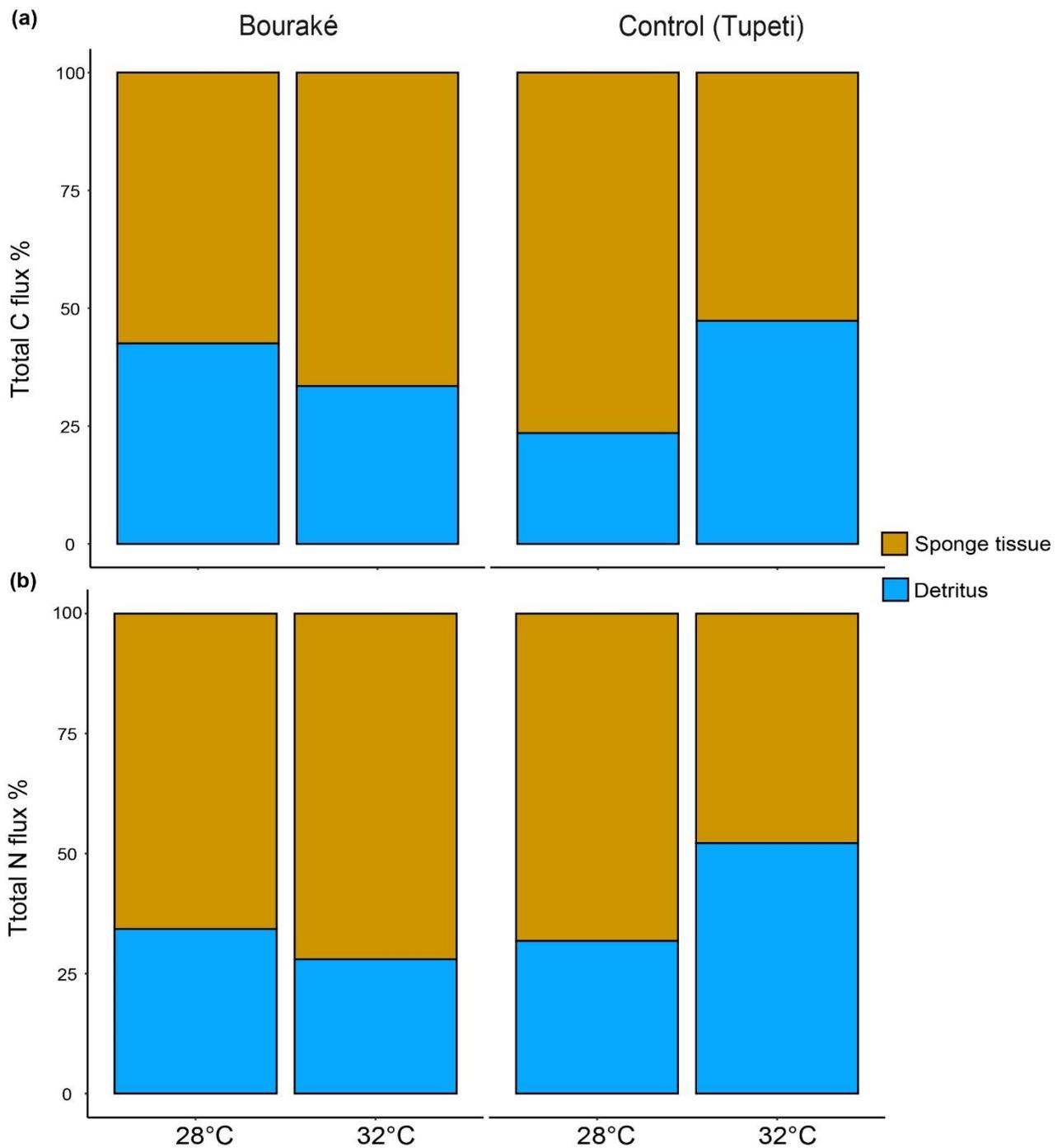
## APPENDIX C



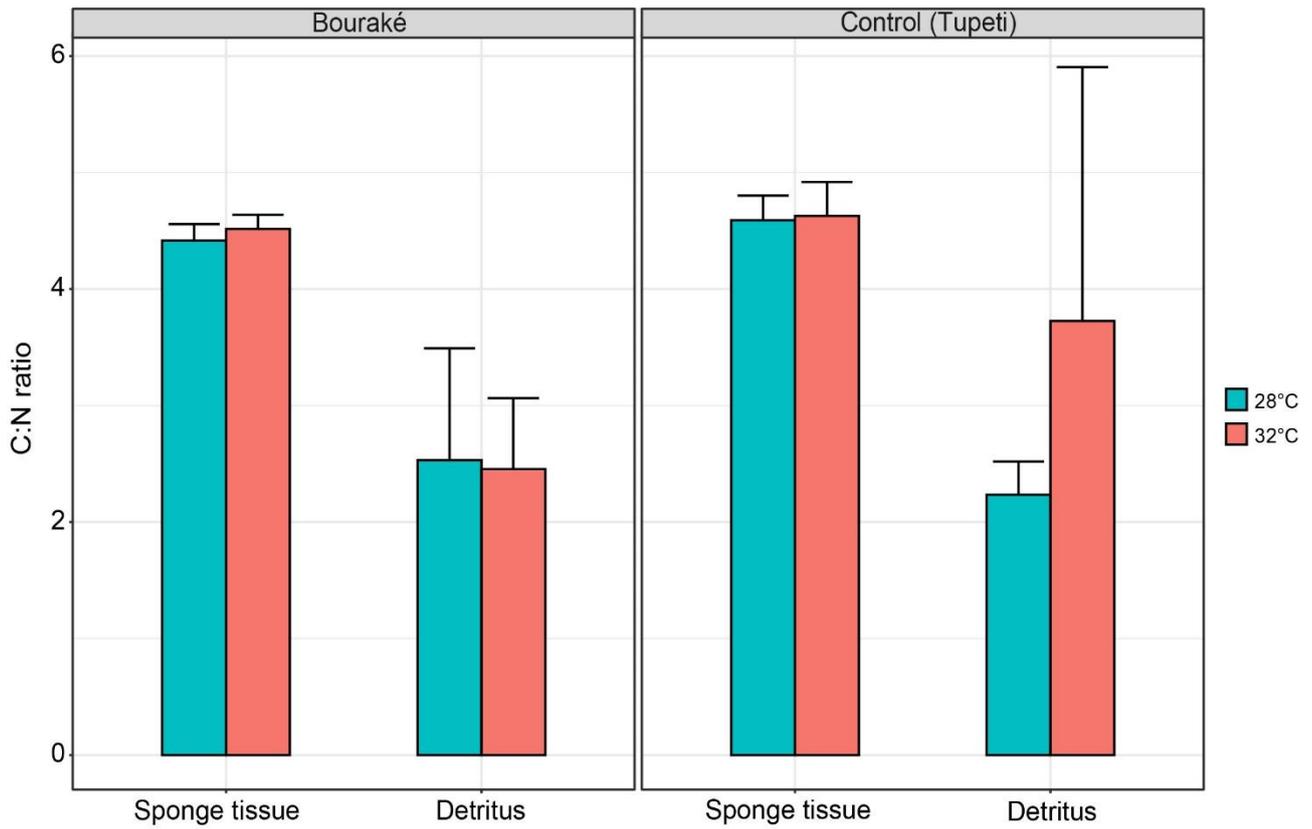
**Figure C4.1.** Schematic representation of the experimental set-up.  $^{13}\text{C}$  and  $^{15}\text{N}$  were added during 5 days labelling in the coral tank (120L), and 6 flow through were open at the end of the labelled period. Flow through allowed the labelled mucus to be naturally transferred to the 6 experimental tanks (20L) where 8 sponges (4 per site of origin) were maintained. Four more tanks contained an equal number of sponges but they were alimented by separates seawater flow throughs and non-labelled. For both labelled and non-labelled treatments half tanks were maintained at constant temperature, while the remaining at variable temperature.



**Figure C4.2.** Total fluxes of  $^{13}\text{C}$  (a) and  $^{15}\text{N}$  (b) enrichment measured in sponge tissue and in detritus at 28°C and 32°C, for both sponges from Bouraké and control (Tupeti). Data were normalized by days and expressed in  $\mu\text{mol C}$  or  $\text{N d}^{-1}$ . Boxes are the interquartile range of data (25th and 75th percentiles); the horizontal line is the median, and the whiskers represent the data range (i.e., minimum and maximum).



**Figure C4.3.** Sponge weight normalized total fluxes (%) enrichments for  $^{13}\text{C}$  (a) and  $^{15}\text{N}$  (b) measured in sponge tissue and excreted in detritus at 28°C and 32°C, for sponges from both Bouraké and control sites (Tupeti). Percentages were calculated on data normalized by the sponge weight and per days ( $\mu\text{mol C or N g}^{-1} \text{d}^{-1}$ ).



**Figure C4.4.** Carbon vs nitrogen ratios in the sponge tissue and detritus after incubations at 28°C and 32°C, for both sponges from Bouraké and Control sites (Tupeti).

**Table C4.1.** Trial experiment showing the average enrichment  $\Delta\delta^{13}\text{C}\%$  and  $\Delta\delta^{15}\text{N}\%$  in *Acropora* sp. coral mucus, *R. globostellata* tissue and detritus.

	$\Delta\delta^{13}\text{C}\%$	$\Delta\delta^{15}\text{N}\%$
<b>Coral mucus day 5 (n = 4)</b>	198 ± 102	897 ± 112
<b>Sponge tissue (n = 6)</b>	1.6 ± 1.5	4.3 ± 3.4
<b>Sponge detritus (n = 6)</b>	57 ± 37	107 ± 133

**Table C4.2.** Main carbon and nitrogen components measured, during incubations, from the medium at 28°C and 32°C, and for both incubations of sponges from Bouraké (B) and Control (C). **a)** Consumption (negative) and/or production (positive) of dissolved organic carbon (DOC), particulate organic carbon (POC), particulate organic nitrogen (PON), bacteria, phytoplankton and detritus; **b)** Sponge cells consumption rates of bacteria and main phytoplankton groups; **c)** Carbon and nitrogen sources in the seawater medium at the beginning of incubations. The relative % of available carbon was calculated on DOC, POC, bacteria and phytoplankton; **d)** Cells concentrations available for the sponge at the beginning of incubations. Data are expressed as mean ± SD.

Temperature	28°C		32°C	
Origin	C	B	C	B
<b>a) Consumption and production during incubations</b> ( $\mu\text{mol C or N g}^{-1} \text{h}^{-1}$ )				
DOC	-1.133 ± 1.67	-1.00 ± 1.97	0.08 ± 2.14	-0.11 ± 1.32
POC	0.06 ± 1.20	0.71 ± 1.42	0.25 ± 1.67	-0.34 ± 0.57
Bacteria	-0.05 ± 0.15	-0.04 ± 0.09	-0.16 ± 0.09	0.12 ± 0.04
Phytoplankton	-0.11 ± 0.06	-0.09 ± 0.06	-0.13 ± 0.06	-0.09 ± 0.04
PON	0.01 ± 0.28	0.15 ± 0.36	0.11 ± 0.33	-0.05 ± 0.14
Detritus	0.07 ± 1.20	0.72 ± 1.42	0.26 ± 1.67	-0.47 ± 0.76
<b>b) Consumption of cells during incubations</b> ( $\times 10^6 \text{ cells g}^{-1} \text{h}^{-1}$ )				
Total bacteria	25.2 ± 10.8	23.8 ± 7.97	38.0 ± 18.1	27.9 ± 9.15
Syn	1.30 ± 0.61	1.17 ± 0.70	1.57 ± 0.71	1.19 ± 0.57
Pro	4.30 ± 1.99	1.47 ± 6.70	4.16 ± 1.68	3.30 ± 1.28
Peuk	6.20 ± 3.31	4.03 ± 5.45	7.53 ± 2.88	5.94 ± 1.92
Nan	1.61 ± 1.26	0.67 ± 2.17	1.39 ± 0.90	1.20 ± 1.00
<b>c) Carbon and nitrogen at the beginning of incubations</b> ( $\mu\text{mol C or N L}^{-1}$ ) (C %)				
DOC	85.39 ± 7.73 (89.6%)		86.97 ± 6.34 (86.7%)	
POC	8.69 ± 0.80 (9.2%)		11.21 ± 1.18 (11.9%)	
Total bacteria	0.52 ± 0.14 (0.5%)		0.66 ± 0.11 (0.1%)	
Phytoplankton	0.66 ± 0.27 (0.7%)		0.78 ± 0.29 (0.8%)	
PON	1.65 ± 0.19		2.43 ± 0.47	
<b>d) Bacteria and phytoplankton contents at the beginning of incubations</b> ( $\times 10^x \text{ cells L}^{-1}$ )				
Total bacteria ( $\times 10^8$ )	3.12 ± 0.81		4.19 ± 0.69	
Syn ( $\times 10^7$ )	1.60 ± 0.67		1.81 ± 0.70	
Proc ( $\times 10^5$ )	5.82 ± 1.94		5.16 ± 1.58	
Peuk ( $\times 10^5$ )	8.17 ± 1.23		8.81 ± 1.58	
Nano ( $\times 10^5$ )	2.46 ± 1.08		3.18 ± 1.49	

**Table C4.3.** Average enrichment of  $\delta^{13}\text{C}\text{‰}$  and  $\delta^{15}\text{N}\text{‰}$  in *Acropora sp. coral mucus*.

<b>Coral mucus (n = 15)</b>	<b><math>\delta^{13}\text{C}\text{‰}</math></b>	<b><math>\delta^{15}\text{N}\text{‰}</math></b>
Day 0	-18.3 ± 2.8	21.09 ± 15
Day 1	197.7 ± 170	862.9 ± 980
Day 5	416.5 ± 63	900.6 ± 186

**Table C4.4.** Summary of two-way ANOVAs between temperature conditions (two levels, 28°C and 32°C), origin (two levels, control and Bouraké), and their interaction of  $^{13}\text{C}$  and  $^{15}\text{N}$  total flux ( $\mu\text{mol C}$  or  $\text{N d}^{-1}$ ). When the interaction was significant, a Tukey HSD test was applied. B. is Bouraké and C the control at 32°C and 28°C.

	<b>Temp</b> F-value ( <i>P</i> )	<b>Origin</b> F-value ( <i>P</i> )	<b>Temp x Origin</b> F-value ( <i>P</i> )	<b>HSD Post hoc (<i>P</i>)</b>
$^{13}\text{C}_{\text{tissue}}$	2.110 (0.154)	0.076 (0.784)	18.502 (<0.001)	B.32°C > B.28°C (<0.001) C.28°C > B.28°C (<0.05) C32°C < B.32°C (<0.05)
$^{15}\text{N}_{\text{tissue}}$	8.18 (<0.01)	21.45 (<0.001)	24.55 (<0.001)	B.32°C > B.28°C (<0.001) C.28°C < B.32°C (<0.05) C.32°C < B.32°C (<0.05)
$^{13}\text{C}_{\text{detritus}}$	10.13 (<0.01)	0.93 (0.34)	3.19 (0.08)	
$^{15}\text{N}_{\text{detritus}}$	13.51 (<0.001)	1.968 (0.168)	2.06 (0.16)	

## FRENCH SUMMARY - RESUME

### Chapitre 1 : introduction générale

Les émissions de gaz à effet de serre provenant des activités humaines (dioxyde de carbone, méthane, etc.) ont un impact sur l'environnement et les écosystèmes (Chapin et al., 1997 ; Dinerstein et al., 2019). On estime que sur les quelques 350 milliards de tonnes de CO<sub>2</sub> qui ont déjà été émises dans l'atmosphère depuis la révolution industrielle, 25% ont déjà été absorbées par l'océan (Canadell et al., 2007 ; Le Quéré et al., 2010 ; Heinze et al., 2015 ; Cao et Zhang, 2017). Le CO<sub>2</sub> absorbé dans l'océan provoque une augmentation de la pression partielle de CO<sub>2</sub> ( $p\text{CO}_2$ ) et forme de l'acide carbonique (H<sub>2</sub>CO<sub>3</sub>) qui se dissocie ensuite en ions hydrogène (H<sup>+</sup>) et bicarbonate (HCO<sub>3</sub><sup>-</sup>) et, à un taux plus faible, en ions carbonate (CO<sub>3</sub><sup>2-</sup>). L'augmentation de H<sup>+</sup> affecte la chimie de l'eau en provoquant une acidification de l'eau de mer (Raven et al., 2005) et une diminution du pH de l'océans (Pörtner et Farrell, 2008). Ce phénomène est appelé acidification de l'océan (OA) (i.e., Langdon et Atkinson, 2005). L'augmentation du CO<sub>2</sub> atmosphérique a également entraîné une hausse des températures à la surface du globe d'environ 0,2°C par décennie au cours des 30 dernières années (Hansen et al., 2006). L'océan absorbe une quantité importante de cet excès d'énergie et le Groupe d'Experts Intergouvernemental sur l'Évolution du Climat (GIEC) prévoit que le réchauffement des océans (OW) résultant de l'augmentation du CO<sub>2</sub> atmosphérique entraînera une augmentation supplémentaire des températures de surface de l'océan de 1,1°C à 4,0°C d'ici la fin du siècle (GIEC, 2014). Le réchauffement de l'océan influence également les processus océaniques tels que le mélange des eaux et la dynamique des courants ce qui contribue à l'appauvrissement en oxygène de l'eau de mer (Winton et al., 2013). Ce phénomène est appelé désoxygénation de l'océan (DO).

Ces trois principaux effets du changement climatique (OA, OW et DO) ont des répercussions sur les structures et les communautés récifales (e.g., Fabricius et al., 2013 ; Sunday et al., 2017 ; Agostini et al., 2018). Par exemple, l'OA entraîne des modifications physiologiques et métaboliques des organismes, et dans la dynamique des populations et écosystèmes (Fabry et al., 2008 ; Pörtner, 2008). L'OW entraîne des changements dans la répartition des espèces, des mortalités massives, des réductions de la productivité des océans, et des perturbations de la dynamique des réseaux alimentaires (e.g., Pörtner et Farrell, 2008 ; Hoegh-Guldberg and Bruno, 2010; Byrne et Przeslawski, 2013). Enfin, le DO a principalement un impact sur les processus écologiques fondamentaux en provoquant des modifications dans la physiologie des organismes (Seibel, 2011 ; Somero, 2015).

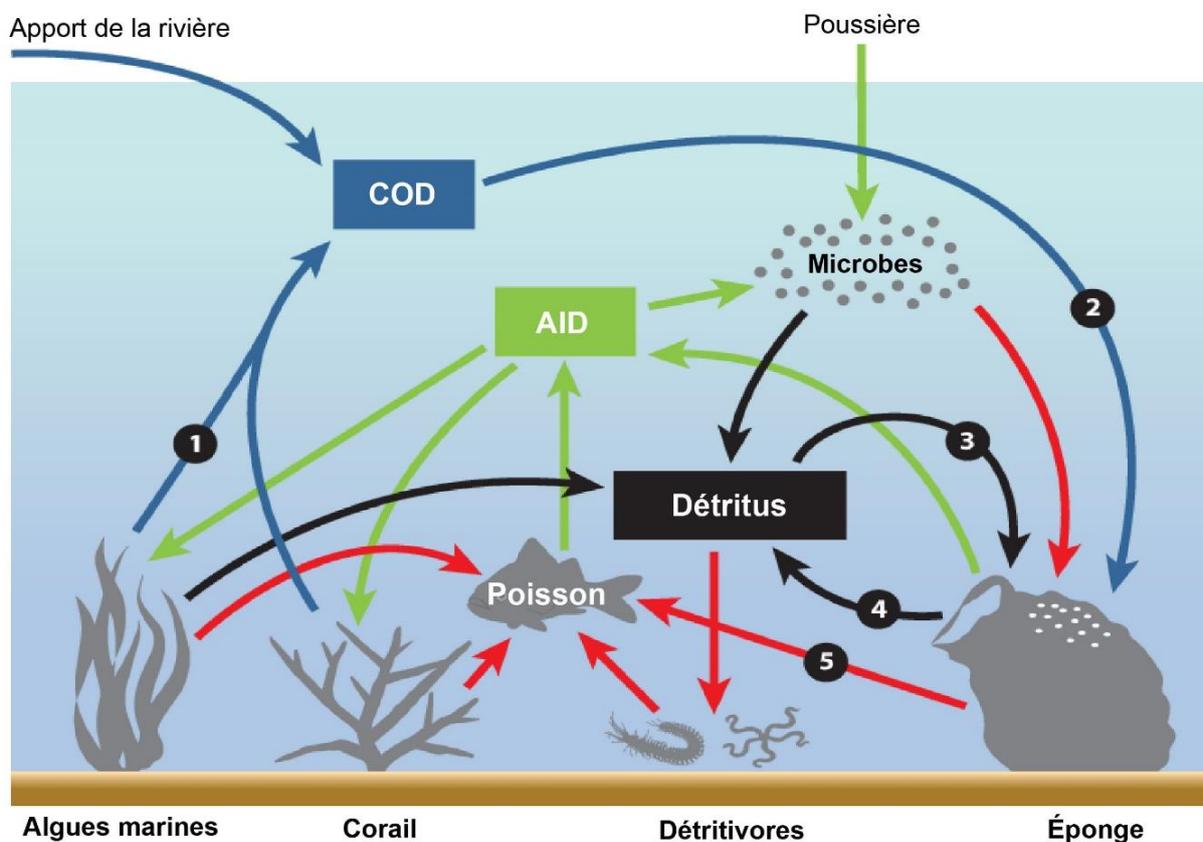
Dans les récifs coralliens, les éponges sont les plus anciens organismes pluricellulaires de la Terre et les plus anciens métazoaires vivants sur notre planète (Li et al., 1998). Elles sont les représentants

exclusifs du phylum Porifera qui est divisé en quatre classes : les Calcarea, les Hexantinellida, les Homoscleromorpha et les Demospongiae ; cette dernière classe regroupe à elle seule 83% de toutes les éponges connues actuellement (van Soest et al., 2012). Les éponges ont un niveau d'organisation simple. Elles sont dépourvues d'organes mais possèdent des cellules spécialisées assurant diverses fonctions vitales. L'architecture d'une éponge s'organise autour du système aquifère constitué par un réseau de canaux et de chambres où circule l'eau de mer. Il permet l'alimentation de l'éponge (par pinocytose/phagocytose), l'excrétion et les échanges O<sub>2</sub>/CO<sub>2</sub> (par simple diffusion). Le squelette de l'éponge est constitué de matériaux organiques et inorganiques. Le matériau organique est composé de microfibrilles de collagène, que l'on appelle par exemple spongine chez les démosponges. Les matériaux inorganiques augmentent la rigidité et sont composés de silice (SiO<sub>2</sub>) ou de carbonate de calcium (CaCO<sub>3</sub>).

Les éponges illustrent parfaitement la notion d'holobionte, étant donné la diversité exceptionnelle des communautés microbiennes qu'elles abritent (environ 40 000 unités taxonomiques opérationnelles, ou UTO, recensées) (Thomas et al., 2016 ; Moitinho-Silva et al., 2017a). Les holobiontes peuvent être classés en deux types nutritionnels, les phototrophes et les hétérotrophes sur la base de l'association avec des symbiotes phototrophes ou hétérotrophes, respectivement (Cheshire et Wilkinson, 1991). Sur la base de l'abondance et de la diversité des microbes dans leurs tissus, les éponges peuvent être aussi classées « à forte abondance microbienne » (HMA) ou « à faible abondance microbienne » (LMA). Les éponges sont des filtreurs essentiels à la santé des systèmes marins en reliant les nutriments de la colonne d'eau aux communautés benthiques. Leur large distribution, leur biomasse parfois dominante, et leurs rôles fonctionnels font que leur contribution au fonctionnement des écosystème est importante et très probablement plus élevée qu'on ne le pensait auparavant (Bell, 2008).

Les éponges jouent un rôle écologique important dans l'environnement marin et contribuent à des processus biogéochimiques complexes, tels que le cycle du carbone, le cycle de l'azote, le cycle des silicates et le cycle du phosphore (Diaz et Rützler, 2001 ; Wulff, 2001 ; Bell, 2008 ; Maldonado et al., 2012). Dans l'écosystème corallien, le plus grand réservoir de matière organique se présente sous la forme de matière organique dissoute (MOD). Cependant, la MOD reste largement inaccessible à la plupart de la faune récifale (Benner et al., 1992). Dans les eaux tropicales peu profondes, une voie de recyclage de la MOD est appelée "boucle de l'éponge" (« sponge loop » en anglais) (De Goeij et al., 2013). La matière organique dissoute libérée par les producteurs primaires est convertie par les éponges en matière organique particulaire (MOP) sous forme de débris, la rendant ainsi plus facilement accessible à la plupart des organismes détritivores de la faune associée aux éponges (e.g.,

De Goeij et al., 2013 ; Alexander et al., 2014 ; Rix et al., 2017 ; Archer et al., 2017). Cependant, aucune production de débris n'a été trouvée pour plusieurs éponges massives pour lesquelles une autre voie du recyclage de la matière organique a été suggérée. Dans cette voie alternative, la MOD assimilée par les éponges serait transférée à d'autres niveaux trophiques *via* la prédation directe sur les tissus des éponges, et/ou *via* l'utilisation de la biomasse de l'éponge par des nécrophages/détritivores à la mort de l'éponge (e.g., Pawlik et al., 2016 ; McMurray et al., 2018 ; Pawlik et McMurray, 2020). Par ces deux voies, les éponges contribuent aux processus marins descendants (Pawlik et al., 2013), comme la prédation ou la nécrophagie, et ascendants, comme le contrôle de la disponibilité de la matière organique dans les récifs coralliens (Lesser and Slattery, 2013) (**Figure R1**).



**Figure R1.** Cycle du carbone organique dissous (COD, bleu), de l'azote inorganique dissous et des nutriments (AID, vert), et des détritivores (noir) dans les environnements des Caraïbes (d'après Pawlik et McMurray, 2020). La consommation des organismes composants est indiquée en rouge. Cette figure résume les composants importants de la boucle des éponges (De Goeij et al., 2013) et la prédation directe sur les tissus des éponges (Pawlik et al., 2016; Mumby et Steneck, 2018). Des preuves de la production par les algues marines de COD labile (1), qui constitue une composante majeure du régime alimentaire des éponges (2), ainsi que des détritivores (3). Alors que l'hypothèse de la boucle des éponges proposait la transformation du COD en détritivores par les éponges cryptiques (4), cela ne se produit pas pour les éponges émergentes, qui renvoient le COD à l'écosystème en tant que nourriture pour les spongivores (5) (d'après Pawlik et McMurray, 2020).

Malgré le rôle fondamental des éponges dans les récifs coralliens, la plupart des études sont axées sur la réponse au stress ou à la tolérance aux conditions environnementales futures (e.g., Bennet et al. 2017 ; Bell et al., 2018 ; 2022) plutôt que sur les processus écologiques, tels que le cycle du carbone et des nutriments. Certaines études ont identifié les éponges comme étant plus tolérantes à l'OW et à l'OA que plusieurs autres groupes benthiques, et elles bénéficieront probablement de l'évolution des conditions océaniques grâce à une productivité accrue et à une concurrence spatiale réduite (Bell et al., 2013 ; 2018). En revanche, des diminutions de l'abondance des éponges ont également été signalées. En fonction des caractéristiques environnementales locales, des réponses spécifiques des différentes espèces et de l'environnement dans lequel elles vivent, les éponges pourraient être avantagées ou désavantagées au sein des récifs coralliens soumis aux changements globaux.

La plupart des études reposent sur la reproduction de ces conditions futures dans des environnements contrôlés, ce qui donne une vision biaisée de la réponse des organismes aux multiples effets du changement climatique. Les expériences *in vitro* ont été largement utilisées pour donner un aperçu de la manière dont les organismes des récifs répondront potentiellement aux climats futurs. Travailler en aquarium permet de standardiser les expériences, d'étudier un seul facteur à la fois, et de modifier plusieurs paramètres dans des conditions contrôlées dans le temps. Toutefois, ces expériences sont limitées dans leur capacité à reproduire les interactions écologiques et biogéochimiques complexes du système corallien. En outre, ces expériences prennent mal en compte certains facteurs limitants comme les nutriments, les courants et l'irradiation. Certaines expériences (e.g., avec plusieurs paramètres) sont également difficiles à simuler, et la plupart sont trop brèves pour permettre l'acclimatation complète des organismes (Atkinson and Cuet, 2008).

La découverte de « laboratoires naturels », où les organismes vivent dans les conditions proches de ce que pourraient être les conditions futures, a permis d'étudier les effets du changement climatique *in situ*, incluant la variabilité naturelle. Par conséquent, ces sites naturels sont devenus des outils importants pour étudier la réponse réelle des organismes exposés aux conditions futures tout au long de leur vie, et pour obtenir des réponses réalistes aux conditions du changement climatique (Camp et al., 2018). Les laboratoires naturels tels que les suintements volcaniques de CO<sub>2</sub> en eaux peu profondes, les zones de remontée d'eau à faible pH, les habitats de mangrove et les baies semi-fermées sont des systèmes d'étude où l'eau de mer environnante est soumise à une augmentation localisée ou généralisée de la pCO<sub>2</sub> et/ou de la température, et dans certains cas à une diminution de l'oxygène dissous (Camp et al., 2018). Parmi les différents exemples de laboratoires naturels, la baie semi-fermée de Bouraké (Nouvelle-Calédonie) est un site caractérisé par des variations journalières de température, du pH et de l'oxygène qui sont guidés par la marée. Ces variations atteignent des valeurs

extrêmes, cette à dire une eau de mer plus chaude ( $> 33,0^{\circ}\text{C}$ ), acidifiée ( $< 7,3 \text{ pH}_T$ ) et désoxygénée ( $< 2,3 \text{ mg L}^{-1}$ ) par rapport à la lagune externe pendant la marée basse (Camp et al., 2017). Malgré ces conditions extrêmes, Bouraké abrite un récif en bonne santé, riche et diversifié.

L'objectif de cette thèse est de combler les lacunes concernant les effets des conditions environnementales extrêmes sur les réponses écologiques des éponges et de donner un aperçu sur les futures conséquences possibles sur les récifs coralliens. Pour ce faire, j'ai utilisé le laboratoire naturel de Bouraké où les éponges sont particulièrement abondantes et où les conditions environnementales sont proches ou même pires que celles prévues pour le futur. Après une caractérisation physico-chimique à long terme de la lagune de Bouraké, j'ai effectué une série d'incubations *in situ* avec *Rhabdastrella globostellata* (l'éponge la plus abondante trouvée à Bouraké) pendant des conditions environnementales normales (marée haute) et à des conditions environnementales extrêmes (marée basse), qui se produisent naturellement sur ce site. De plus, pour séparer l'effet du réchauffement des autres facteurs sur le recyclage du carbone et de l'azote de l'éponge, j'ai réalisé une expérience *ex situ* avec du  $^{13}\text{C}$  et du  $^{15}\text{N}$  marqués à l'Aquarium des lagons (Nouméa, Nouvelle-Calédonie). Les objectifs spécifiques de cette thèse sont divisés en trois chapitres principaux :

### 1. *Caractérisation physico-chimique à long terme et évaluation de la distribution des communautés benthiques dans le laboratoire naturel de Bouraké*

Dans ce chapitre (Chapitre 2), j'ai étudié les paramètres physico-chimiques, la distribution des communautés benthiques ainsi la signature isotopique d'organismes benthiques à Bouraké. Sur la base des données collectées de 2016 à 2020, j'ai comparé la température ( $T^{\circ}\text{C}$ ), le  $\text{pH}_T$ , l'oxygène dissous (OD), les concentrations en nutriments et en matière organique de Bouraké avec ceux de deux sites de référence. J'ai utilisé des transects photo-quadrat pour évaluer la distribution des principaux taxons benthiques vivant dans la lagune de Bouraké. Enfin, j'ai mesuré les signatures isotopiques stables de  $\delta^{13}\text{C}$  et  $\delta^{15}\text{N}$  des éponges et des macroalgues pour mettre en évidence les différences de composition de la source de carbone et d'azote entre la zone la plus externe et la plus interne de la lagune. Une partie de ce travail a débuté lors de mon stage de Master (Maggioni, 2018). Ce chapitre fournit une description complète du site d'étude et a permis d'identifier Bouraké comme un laboratoire naturel particulièrement original pour étudier les effets des conditions extrêmes sur les organismes marins (Maggioni et al., 2021).

## 2. Effets des conditions environnementales extrêmes sur les nutriments et le recyclage de la matière organique de *R. globostellata* ainsi que sur son activité photosynthétique

Dans ce chapitre (chapitre 3), j'ai réalisé 70 incubations *in situ* en utilisant des chambres respirométriques benthiques pour évaluer les réponses métaboliques (i.e., le recyclage des nutriments et de la matière organique) et l'activité photosynthétique de *R. globostellata* pendant la saison estivale. Les incubations ont été réalisées à marée haute et à marée basse, représentant respectivement des conditions environnementales "normales" et extrêmes. Les changements dans les contenus en nutriments et en matière organique de l'eau de mer au début et à la fin des incubations ont été utilisés pour évaluer leur production et/ou leur consommation dans le métabolisme des éponges. Ce chapitre fournit une première information sur la réponse métabolique de l'éponge *R. globostellata* soumis à des conditions environnementales différentes (Maggioni et al., 2023).

## 3. Effets de la température sur le recyclage du carbone et de l'azote de *R. globostellata*

Dans ce chapitre (chapitre 4), j'ai réalisé une expérience *ex situ* basée sur les résultats obtenus dans le chapitre 3 qui a montré que la température estivale, lorsqu'elle est  $> 31^{\circ}\text{C}$ , semble être le facteur principal affectant le cycle du carbone des éponges. Pour étudier spécifiquement l'effet de la température sur le recyclage du carbone et de l'azote de l'éponge, j'ai suivi l'incorporation de  $^{13}\text{C}$  et  $^{15}\text{N}$  marqués dans le tissu de l'éponge et les détritiques produits par l'éponge à  $28^{\circ}\text{C}$  et à  $32^{\circ}\text{C}$ . La découverte en 2021 de l'éponge *R. globostellata* en dehors de Bouraké m'a permis de comparer une population "normale", soumise à des conditions de température « normales », avec les éponges à Bouraké soumises à des conditions de température fluctuantes qui atteignent la valeur extrême de  $33^{\circ}\text{C}$ . Ce chapitre fournit une première évaluation des conséquences de la haute température sur le recyclage du carbone (C) et de l'azote (N) dans l'éponge *R. globostellata*, avec des différences dans les réponses entre les éponges adaptées aux hautes températures (Bouraké) et celles qui ne le sont pas (site contrôle) (Maggioni et al., *en cours de révision à Limnology and Oceanography*).

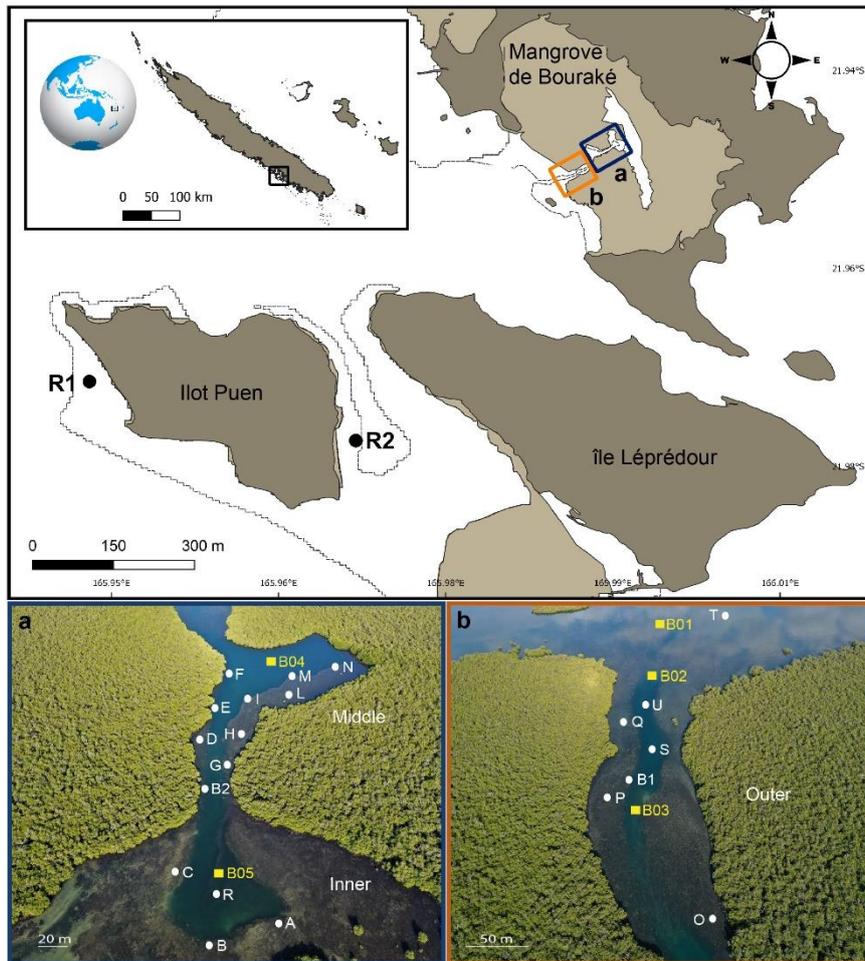
Dans le dernier chapitre (chapitre 5), j'ai résumé les résultats clés de mes recherches, en présentant la lagune de Bouraké comme un laboratoire naturel pour étudier les réponses des organismes à des conditions extrêmes et en discutant comment l'éponge *R. globostellata* pouvait s'adapter, *via* divers processus physiologiques et écologiques, à ces conditions et spécifiquement à une température élevée. Je me suis ensuite concentrée sur les effets de ces conditions sur les principaux processus écologiques des éponges, tels que le recyclage des nutriments et de la matière organique. J'ai discuté des principales stratégies qui pourraient permettre à l'éponge *R. globostellata* de faire face et de prospérer dans de telles conditions environnementales. Enfin, j'ai utilisé mes résultats pour mieux comprendre

les processus écologiques des éponges dans une perspective globale, en évaluant les perspectives et les conséquences futures sur leur rôle et donc sur les interactions trophiques dans les futurs récifs coralliens.

## **Chapitre 2 : la lagune semi-fermée de Bouraké (Nouvelle-Calédonie) - un laboratoire naturel pour étudier l'adaptation d'un écosystème corallien à des conditions environnementales extrêmes**

Selon les données et les prévisions actuelles, les récifs coralliens pourraient être très fortement impactés, voire possiblement disparaître d'ici la fin du siècle, si les émissions de CO<sub>2</sub> restent inchangées. Toutefois, la découverte de récifs riches et diversifiés dans des environnements où règnent des conditions extrêmes suggèrent que certains coraux seraient capables de se développer dans une eau de mer chaude, à forte pression partielle de CO<sub>2</sub> ( $p\text{CO}_2$ ) et désoxygénée. Les cheminées volcaniques sous-marines de CO<sub>2</sub>, les remontées d'eaux de faible pH, les lagons semi-fermés et les estuaires de mangrove sont des sites où les valeurs de certains paramètres environnementaux, importants pour la vie dans les océans, sont proches ou même dépassent celles prévues pour l'année 2100. Bien qu'ils ne reproduisent pas parfaitement les conditions futures, ces « laboratoires naturels » offrent des possibilités uniques pour explorer les mécanismes que les espèces récifales pourraient utiliser pour survivre et s'adapter au changement climatique. Pour y parvenir, il est essentiel de caractériser l'environnement dans son ensemble et suivre les facteurs environnementaux qui pourraient varier dans le futur pour voir s'ils évoluent comme le prévoient les modèles prédictifs car cela conditionnera la réponse de l'écosystème.

Ce chapitre se concentre sur la lagune semi-fermée de Bouraké (Nouvelle-Calédonie, océan Pacifique Sud-Ouest) où un écosystème récifal sain prospère dans une eau chaude, à pH réduit et appauvrie en O<sub>2</sub>. L'étude a été réalisée avec des données collectées entre 2016 et 2020. Une approche multi-échelle a été utilisée pour caractériser les principaux paramètres physico-chimiques, cartographier la composition des communautés benthiques et fournir des informations préliminaires sur la caractérisation isotopique des organismes sessiles et des poissons en analysant leurs valeurs de  $\delta^{13}\text{C}$  et de  $\delta^{15}\text{N}$ . La lagune a été subdivisée en trois zones principales : un récif extérieur, un récif intermédiaire et un récif intérieur. Les principaux paramètres environnementaux chimiques (Alcalinité totale, Si(OH)<sub>4</sub>, NO<sub>x</sub>, PO<sub>4</sub><sup>3-</sup>, NH<sub>4</sub><sup>+</sup>, COD, CID, COP, NOP) et physiques (Température, pH<sub>T</sub>, OD, salinité) de l'eau de mer ont été mesurés sur le site d'étude et sur deux récifs de référence adjacents : R1 un récif frangeant typique, et R2 une baie semi-fermée peu profonde et relativement grande (**Figure R2**).



**Figure R2.** Carte du site d'étude (panneau supérieur) montrant la lagune de mangrove semi-fermée de Bouraké et les récifs de référence (R1 et R2). Les photos **a** et **b** (panneaux du bas) sont des photos aériennes (prises à 130 m au-dessus de la lagune de Bouraké) **a** des récifs intérieur et intermédiaire et **b** du récif extérieur. Les points blancs et les carrés jaunes indiquent les stations d'échantillonnage utilisées pour le suivi environnemental physique et chimique, respectivement. La base de données Georep Nouvelle-Calédonie (<https://georep.nc/>) et le logiciel QGIS ont été utilisés pour construire la figure (panneau supérieur).

Les données ont révélé que la plupart des paramètres physiques et chimiques sont régulés par la marée, qu'ils fluctuent fortement 3 à 4 fois par jour et qu'ils sont entièrement prévisibles. Le pH de l'eau de mer et l'oxygène dissous diminuent à marée descendante et atteignent des valeurs extrêmement faibles à marée basse (7,2 pH<sub>T</sub> et 1,9 mg O<sub>2</sub> L<sup>-1</sup> à Bouraké, contre 7,9 pH<sub>T</sub> et 5,5 mg O<sub>2</sub> L<sup>-1</sup> sur les récifs de référence). L'oxygène dissous, la température et le pH fluctuent en fonction de la marée avec des amplitudes jusqu'à 4,91 mg O<sub>2</sub> L<sup>-1</sup>, 6,50°C et 0,69 pH<sub>T</sub> en un seul jour. De plus, la concentration de la plupart des paramètres chimiques est jusqu'à cinq fois plus élevée dans la lagune de Bouraké que sur les sites de référence, en particulier le carbone organique, le carbone inorganique et l'azote, mais aussi pour certains nutriments, notamment les silicates. De manière surprenante, malgré les conditions environnementales extrêmes et la composition chimique altérée de l'eau de mer à Bouraké, les résultats révèlent une communauté benthique diversifiée, caractérisée par une

couverture élevée de macroalgues (28 espèces), d'éponges (11 espèces) et de coraux (66 espèces). De façon générale, les différents organismes ont montré une signature plus élevée en  $\delta^{13}\text{C}$  et plus faible en  $\delta^{15}\text{N}$  dans la partie intérieure de la lagune de Bouraké que dans la partie extérieure.

Les résultats de ce chapitre fournissent une caractérisation complète de la lagune de Bouraké au niveau chimique, physique et benthique. La variabilité environnementale et la concentration des nutriments pourraient contribuer à la survie des organismes dans des conditions environnementales aussi extrêmes. Ce chapitre confirme que ce site peut être utilisé comme laboratoire naturel afin de mieux étudier la réponse adaptative des organismes à la multitude des facteurs de stress environnemental.

### **Chapitre 3 : recyclage de la matière organique des éponges : production réduite de détritux dans des conditions environnementales extrêmes**

La survie des récifs coralliens pourrait être compromise par le changement climatique, avec des impacts sur la structure des récifs et les communautés associées allant de la perte de biodiversité, aux changements d'écosystème, à la réduction de la complexité de l'habitat et au déclin de la productivité globale des récifs. Dans les récifs coralliens, les éponges jouent un rôle clé dans le recyclage du carbone, les flux de nutriments (par exemple, puits et/ou source d'azote, de phosphate et de silicate), et dans le transfert du carbone vers les autres niveaux trophiques. En convertissant le carbone organique dissous (COD) en détritux cellulaires (i.e., la « boucle de l'éponge »), et/ou en assimilant le COD en biomasse, les éponges soutiennent le fonctionnement des récifs coralliens, ainsi qu'une productivité et une biodiversité élevées dans des environnements habituellement oligotrophes. Bien que l'importance de ces deux voies de recyclage de la matière organique ait été identifiées, on ne sait pas comment elles seront impactées par les changements des conditions environnementales dans le futur.

Les laboratoires naturels où les valeurs physiques et chimiques de l'eau de mer s'écartent des conditions côtières normales sont de plus en plus utilisés pour étudier les réactions des organismes marins aux futurs changements climatiques. Le chapitre précédent a mis en évidence que le site de Bouraké (Nouvelle-Calédonie) était caractérisé par des fluctuations importantes du pH (7,23-8,10 units), de la température (17,5-33,8°C) et de l'oxygène (1,87-7,24 mg L<sup>-1</sup>), qui atteignent des valeurs extrêmes à chaque marée basse. Malgré ces conditions, la mangrove abrite 66 espèces de coraux, 11 espèces d'éponges et 28 espèces de macroalgues. Parmi les différentes éponges qui peuplent Bouraké, l'espèce dominante, *Rhabdsastrella globostellata*, est particulièrement massive et abondante et a été choisie comme modèle d'étude. Ce troisième chapitre met en évidence la manière dont le métabolisme de l'éponge évolue en fonction des variations des conditions environnementales dans la lagune.

Des incubations avec des chambres benthiques ont été réalisées *in situ* dans différentes conditions environnementales, à savoir pendant la marée haute (conditions normales) et pendant la marée basse (conditions extrêmes) pendant l'été 2018 et l'été 2020. J'ai réalisé des incubations à l'obscurité pour évaluer la réponse métabolique de l'éponge, et des incubations à la lumière puis à l'obscurité pour évaluer l'effet des différentes conditions sur les symbiotes photosynthétiques. Pour les incubations à l'obscurité, 450 ml d'eau de mer ont été prélevés dans chaque chambre au début et à la fin de l'expérience pour évaluer les changements des paramètres physiques, de la concentration des nutriments et de la concentration de la matière organique. Dans les incubations réalisées à la lumière puis à l'obscurité, les mesures d'oxygène (au début et à la fin) et de l'activité photosynthétique des symbiotes ont été réalisées pour évaluer les différentes consommations d'oxygène et donc l'effet des conditions environnementales sur les symbiotes.

Les résultats principaux de ce chapitre ont montré que la respiration des éponges ne diffère pas significativement entre les conditions de marée haute et basse. Par contre, les conditions extrêmes ont un effet négatif sur les symbiotes photosynthétiques de l'éponge. Cependant, lorsque l'acidification et la désoxygénation sont combinées à une température élevée ( $> 31^{\circ}\text{C}$  en 2020), il y a un changement dans le recyclage du carbone organique, avec un blocage de la boucle de l'éponge et donc une diminution de la production de détritus.

Cette étude a permis d'analyser pour la première fois en milieu naturel les conséquences de conditions extrêmes sur le métabolisme d'une éponge (capacité à recycler la matière organique et activité phototrophique). Ce chapitre montre comment l'éponge *R. globostellata* peut répondre à des conditions extrêmes. Ces changements dans le recyclage du carbone chez une éponge adaptée à des conditions extrêmes mettent en évidence la façon dont les voies trophiques pourraient être affectées par les conditions océaniques changeantes. Par ailleurs, cela permet d'émettre l'hypothèse que la température est très vraisemblablement l'un des principaux facteurs conditionnant ce changement dans le recyclage de la matière organique.

## Chapitre 4 : les hautes températures modifient le cycle du carbone et de l'azote des éponges

La température de surface de la mer pourrait augmenter possiblement jusqu'à +4°C d'ici la fin du siècle, ce qui aura des conséquences négatives sur les écosystèmes coralliens. Dans les récifs coralliens, les éponges jouent un rôle clé dans les processus écologiques en absorbant et en transformant la MOD pour la rendre disponible aux autres niveaux trophiques. Jusqu'à présent, les études ont rapporté des effets contradictoires sur les processus physiologiques des éponges avec des réponses spécifiques aux espèces. Cependant, il n'existe pas d'étude ayant examiné l'effet de la température sur le cycle du carbone et de l'azote des éponges. Grâce aux résultats du chapitre précédent, j'ai pu étudier les réponses métaboliques de l'éponge *R. globostellata* à différentes conditions environnementales. Le principal résultat a montré un arrêt de la production de détritits pendant les conditions extrêmes, ce qui permet d'émettre l'hypothèse que la température est l'un des principaux facteurs de ce changement dans le recyclage de la matière organique. Compte tenu du résultat obtenu *in situ*, ce quatrième chapitre s'attache à comprendre les effets de la haute température sur le recyclage du carbone et de l'azote par l'éponge.

Dans ce chapitre, une expérience contrôlée en aquarium a été mise en place pour étudier les effets d'une température élevée (32°C) sur les cycles du carbone et de l'azote chez l'espèce dominante de notre site d'étude, *R. globostellata*. Les individus ont été collectés dans un environnement à température fluctuante (variation de 6,5°C par jour) à Bouraké (Nouvelle-Calédonie), où la température atteint en été 33,2°C en été, et un site contrôle où la température en été est « stabilisée » à 28°C (île Tupeti, Nouvelle-Calédonie). Du mucus corallien marqué au  $^{13}\text{C}$  et  $^{15}\text{N}$  a été utilisé pour suivre son transfert et son incorporation à la fois dans les tissus des éponges et les détritits relâchés pendant des expériences d'incubations. En plus, les changements en matière organique dans l'eau de mer ont également été mesurés pendant les incubations.

Cette étude montre que *R. globostella*, à Bouraké et du site contrôle, incorporent (dans les tissus) et excrètent (sous forme de détritits) le  $^{13}\text{C}$  et  $^{15}\text{N}$  assimilés. De plus, les éponges de Bouraké, incorporent toujours plus de  $^{13}\text{C}$  et  $^{15}\text{N}$  dans les tissus qu'elles ne les libèrent sous forme de détritits, avec un taux d'incorporation qui double aux températures élevées (32°C). En revanche, les éponges du site témoin ont une réponse opposée aux températures élevées, avec moins de  $^{13}\text{C}$  et  $^{15}\text{N}$  incorporés dans les tissus, et plus excrétés dans les détritits. Ces résultats suggèrent que si les éponges de Bouraké ont pu maintenir leur catabolisme normal et allouer plus de ressources aux tissus, les éponges du site contrôle ont probablement subi un stress thermique qui aurait provoqué une importante dégradation cellulaire, génératrice de détritits.

Bien que les éponges des deux sites aient accumulé le carbone (C) et l'azote (N) dérivé du mucus corallien dans leurs tissus, et l'aient rejeté sous forme de détritits, l'équilibre entre la portion accumulée (dans les tissus) et excrétés (détritits) présente des différences. Cet équilibre semble changer en fonction du site d'origine de l'éponge et donc des caractéristiques thermiques auxquelles elle est soumise, *sensu* de leur adaptation au stress thermique. Cette étude montre pour la première fois que le changement dans l'allocation de la matière organique chez *R. globostellata* peut être déclenchée par une hausse de température quand les éponges sont adaptées. Si on émet l'hypothèse que les éponges contemporaines ont suffisamment de temps pour s'adapter au changement climatique on peut suggérer qu'elles pourront survivre à l'augmentation de la température en changeant l'allocation du carbone et de l'azote qui serait surtout dirigée dans les tissus de l'éponge. Ce changement, avec moins de C et de N présents dans les détritits, pourrait modifier leur qualité et leur disponibilité pour les autres organismes du récif.

## Chapitre 5 : discussion générale

Cette thèse de doctorat a exploré les réponses d'une éponge tropicale à des conditions environnementales extrêmes (température élevée, faible pH et faible teneur en oxygène dissous) dans le laboratoire naturel de Bouraké (Nouvelle-Calédonie). La température moyenne de l'eau de mer y est similaire à celles prévues dans le contexte des changements océaniques globaux, et les paramètres physiques fluctuent avec la marée (Maggioni et al., 2021). Le pH de l'eau de mer et l'oxygène dissous diminuent à marée descendante et atteignent les valeurs extrêmement faibles à marée basse (7,2 pH et 1,9 mg O<sub>2</sub> L<sup>-1</sup> à Bouraké contre 7,9 pH et 5,5 mg O<sub>2</sub> L<sup>-1</sup> sur les récifs témoins). Cette thèse vise à comprendre comment ces conditions affectent la manière dont les éponges recyclent les nutriments et la matière organique.

Après avoir inventorié les communautés benthiques, j'ai effectué une caractérisation pluriannuelle des paramètres physiques et chimiques de la lagune de Bouraké (chapitre 2) qui a ensuite été utilisée pour étudier les effets des conditions environnementales extrêmes sur le métabolisme des éponges. *Rhabdastrella globostellata* a été choisie comme modèle et à d'abord été incubée *in situ* dans des chambres benthiques pendant des conditions normales (marée haute) et extrêmes (marée basse) (chapitre 3). Les changements des paramètres physiques et chimiques de l'eau de mer pendant les incubations ont été examinés et ont suggéré que la température pourrait être le principal facteur affectant le cycle du carbone des éponges de Bouraké (arrêt de la production de détritits). Pour confirmer cette hypothèse, une expérience en aquarium *ex situ* a été réalisée pour étudier plus précisément l'effet d'une température élevée sur la capacité de *R. globostellata* à recycler l'azote et le carbone (chapitre 4). Les réponses de *R. globostellata* de Bouraké et d'un site témoin ont été analysées

à 28°C et 32°C. La capacité de l'éponge à recycler l'azote et le carbone a été évaluée en suivant le  $^{13}\text{C}$  et le  $^{15}\text{N}$  marqués dans les tissus de l'éponge et la production de détrit. Les résultats montrent qu'une température élevée entraîne un changement dans l'allocation du carbone et de l'azote chez les éponges de Bouraké.

Dans cette partie, je passerai en revue les principaux résultats de mes recherches et montrerai comment les réponses des éponges des Bouraké suggèrent sa capacité à s'adapter à des conditions extrêmes. Enfin, je discuterai de la façon dont mes résultats peuvent être utilisées pour tenter de prédire les réponses des éponges dans les récifs coralliens du futur et évaluer leur implication sur l'ensemble de l'écosystème récifal.

### ***Rhabdastrella globostellata* possède différentes stratégies pour faire face à des conditions environnementales extrêmes**

Les éponges influencent le fonctionnement des écosystèmes en modifiant les facteurs biotiques et abiotiques (Bell, 2008). Pour survivre aux changements environnementaux, les éponges doivent s'acclimater ou s'adapter, comme d'autres invertébrés marins benthiques (Hoegh-Guldberg, 1999). L'acclimatation implique des changements dans divers processus physiologiques afin d'être plus performant dans les nouvelles conditions environnementales (i.e., les mécanismes d'acclimatation), tandis que l'adaptation se produit par la sélection d'individus qui sont mieux à même de faire face aux nouvelles conditions environnementales au sein de la population (Hoegh-Guldberg, 1999).

Comme je l'ai décrit dans le troisième chapitre, les différentes conditions environnementales (normales et extrêmes) entraînent des réponses différentes dans le métabolisme de *R. globostellata* à Bouraké. Les principaux changements concernant les cycles du silicate et du carbone de l'éponge. L'acide orthosilicique et le carbone organique dissous ont été plus consommés pendant les conditions extrêmes (marée basse). Pendant la marée basse, les éponges de Bouraké pourraient bénéficier des concentrations plus élevées d'acide orthosilicique et de carbone organique dissous dans l'environnement pour les stocker comme réserve d'énergie et construire leur squelette. Il a déjà été montré qu'un changement de conditions environnementales provoquaient des changements dans la morphologie et la composition des spicules (par exemple, Bell et al., 2002 ; Meroz-Fine et al., 2005). Par exemple, l'éponge *Tetilla* sp. a une plus grande proportion de spicules dans les eaux peu profondes exposées, alors que la même éponge vivant dans des eaux calmes avait un contenu de spicules plus faible (Meroz-Fine et al., 2005). Un plus grand nombre de spicules rend d'éponge plus rigide et limite sa déformation même dans de fort courants (Palumbi, 1986). Outre le nombre de spicules, leurs dimensions (épaisseur et longueur) contribuent à la rigidité et à la flexibilité de l'éponge. Par exemple,

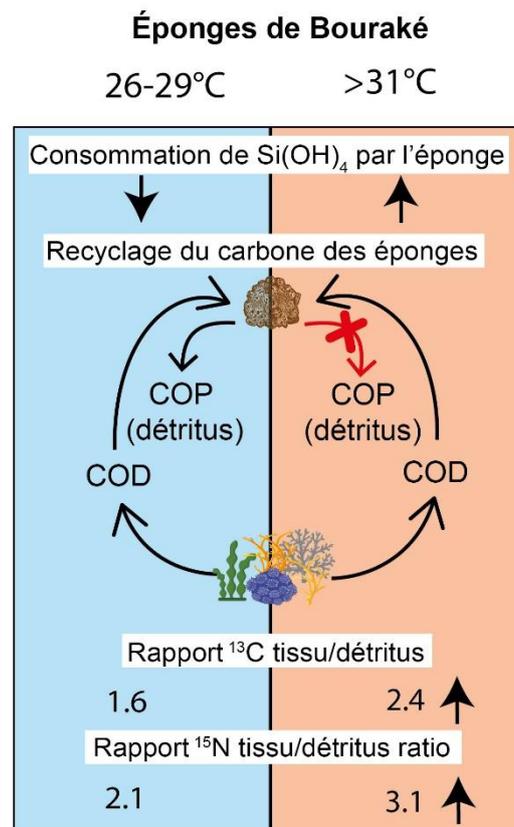
les éponges vivant dans des sites à haute énergie ont des spicules plus longs et plus épais comparativement à celles vivant dans des sites à faible énergie (Bell et al., 2002). De même, nous pouvons émettre l'hypothèse qu'à Bouraké, les éponges accumulent de l'acide orthosilicique pour construire un grand nombre de spicules, plus longs et plus épais, afin de maintenir la croissance du squelette, sa rigidité et sa flexibilité pour faire face aux conditions extrêmes et aux forts courants qui se produisent lors des marées montantes et descendantes (chapitre 2).

Dans les écosystèmes coralliens, les éponges encroûtantes (e.g., De Goeij et al., 2013 ; Rix et al., 2018) absorbent la MOD et la libèrent principalement sous forme de détritits, tandis que les éponges massives la stockent principalement sous forme de biomasse (par exemple, Pawlik et al., 2016 ; McMurray et al., 2018). Bien que *R. globostellata* de Bouraké soit une éponge massive, j'ai constaté qu'elle transformait principalement la MOD et la libérait sous forme de détritits (i.e., boucle de l'éponge, ou « sponge loop » en anglaise). A Bouraké, *R. globostellata* utiliserait préférentiellement la MOD pour maintenir un bon niveau de renouvellement cellulaire, ce qui la maintiendrait en bonne santé. Cette différence par rapport aux autres éponges massives pourrait être due à la grande quantité de nutriments et de matière organique disponible à Bouraké, qui permettraient à l'éponge de croître malgré des conditions environnementales extrêmes. Cette hypothèse est soutenue par l'expérience *ex situ* (chapitre 4) qui a montré que les éponges de Bouraké avaient un métabolisme différent (i.e., une production de détritits) des éponges du site témoin (i.e., une moindre production de détritits) à une température normale.

À 28°C, les éponges des deux sites ont alloué différemment le carbone et l'azote alors qu'elles étaient placées dans les mêmes conditions en aquarium. Mes données révèlent que les réponses sont différentes si les éponges proviennent de sites différents et quelles pourraient être liées à la disponibilité de la nourriture. Ce résultat est cohérent avec ceux d'Alexander et al. (2014) qui ont démontré que *Mycale microsigmatosa* provenant d'une mangrove à Curaçao (Caraïbes) où la nourriture est abondante, avait une meilleure tolérance à la température et un taux de prolifération cellulaire plus élevé que les éponges des récifs de référence lorsqu'elles étaient déplacées en aquarium. Ce changement de stratégie semble être permanent chez les éponges de Bouraké (i.e., résultant d'une réponse adaptative) car elles ont maintenu cette réponse *in situ* (i.e., la production de détritits) même lorsque les conditions environnementales et la disponibilité de la nourriture étaient différentes (i.e., en aquarium).

Quand la température dépasse 31°C, les éponges du site témoin ont produit plus de détritits et ont montré une plus grande accumulation de  $^{13}\text{C}$  et  $^{15}\text{N}$  dans les détritits. Ce résultat pourrait être une conséquence du stress thermique qui aurait provoqué une forte dégradation cellulaire. Inversement,

*R. globostellata* de Bouraké accumule plus de  $^{13}\text{C}$  et  $^{15}\text{N}$  dans les tissus que dans les détritits lorsque la température dépasse  $31^\circ\text{C}$ , ce qui suggère que l'éponge a développé des stratégies pour s'adapter aux conditions extrêmes. Ces différences pourraient être liées à leur histoire thermique. Les éponges vivant dans des environnements à température fluctuante sont probablement plus tolérantes aux températures stressantes (e.g., Oliver et Palumbi, 2011 ; Rivest et al., 2017). Mes résultats vont dans le sens de cette hypothèse et permettent d'expliquer les réponses mises en place par les éponges de Bouraké au fil du temps pour maintenir leur état de santé sous des températures élevées. Dans ce cas, le carbone et l'azote sont davantage accumulés dans les tissus de l'éponge à  $32^\circ\text{C}$  qu'à  $28^\circ\text{C}$ . Bien que je n'aie pas identifié quel(s) mécanisme(s) est(sont) utilisé(s), il semble clair que les éponges stockent du carbone et de l'azote dans leurs tissus. Le carbone et l'azote ainsi stockés pourraient être utilisés dans différents processus métaboliques et probablement fournir de l'énergie supplémentaire pour assurer la santé et la croissance des éponges. Ce changement dans l'allocation de l'énergie peut permettre à *R. globostellata* d'être avantagée dans des conditions de température élevée, et donc de bien se développer dans la lagune de Bouraké grâce à une adaptation à ces conditions (**Figure R3**).



**Figure R3.** Schéma synthétique les principales stratégies utilisées par l'éponge *R. globostallata* à Bouraké pour parvenir à survivre (i.e., réponse adaptative) dans des conditions extrêmes et à des températures élevées.

Une approximation du bilan carbone des éponges peut être réalisé en calculant le rapport I:R où I est le carbone ingéré (COD + COP) et R est le carbone utilisé pour la respiration. A Bouraké, *R. globostellata* a absorbé plus de COD et de COP en conditions extrêmes, ce qui suggère qu'elle a plus d'énergie issue du carbone disponible pour ses processus métaboliques. Nous avons montré que le taux de respiration (en 2018 et 2020) ne variait pas significativement lors du changement de condition (normale vs. extrême) ce qui suggère que l'éponge n'affecte pas plus d'énergie à la respiration en conditions extrêmes (chapitre 3). Le taux de respiration des éponges est considéré comme un bon indicateur pour comprendre leur condition physique et leur niveau de stress (Coma et al., 2002). Mes résultats suggèrent qu'il n'y a pas de réponse au stress chez l'éponge dans des conditions extrêmes, ce qui signifie que la physiologie métabolique permet à cette espèce de répondre aux changements environnementaux (e.g., Coma et al., 2002 ; Morley et al., 2016 ; Bell et al., 2017). En outre, des taux de respiration plus élevés font généralement référence à un besoin d'énergie plus important pour maintenir le métabolisme de base et les processus vitaux (Clarke, 1991). La grande disponibilité de nutriments à Bouraké (chapitre 2) peut aider les éponges à maintenir leur métabolisme basal dans les conditions théoriquement stressantes. *R. globostellata* pourrait donc être capable de compenser les pertes métaboliques (incluant les pertes par respiration) par ses gains d'énergie provenant de l'environnement et ainsi assurer une allocation optimale de l'énergie excédentaire à des processus métaboliques tels que la croissance, comme cela a été rapporté pour d'autres organismes (Kozłowski, 1992 ; Perrin et Sibly, 1993). Cette hypothèse est également soutenue par d'autres études qui montrent que lorsque la nourriture n'est pas limitante, des taux de croissance élevés et l'accumulation de biomasse peuvent être soutenus même dans des conditions stressantes (Sokolova et al., 2012). Ces réponses pourraient permettre à l'éponge de conserver son métabolisme basal pour bien se développer même dans des conditions extrêmes, suggérant son adaptation à ces conditions. Cette hypothèse est renforcée par le fait que *R. globostellata* a augmenté son taux de respiration suite à l'augmentation des températures entre 2018 et 2020 (+3°C) sans que cela n'affecte sa survie, suggérant sa capacité à atteindre un nouvel " équilibre " métabolique basal en cas d'évolution des conditions du milieu.

Le fait que *R. globostellata* à Bouraké accumule plus de carbone et d'azote peut également suggérer qu'il y a un échange plus important de carbone et d'azote entre les cellules hôtes et les bactéries symbiotes. Les microbes associés aux éponges sont supposés jouer un rôle important dans la médiation de l'absorption de la MOD par les éponges (Weisz et al., 2008). Cette hypothèse suggère que les symbiotes des éponges pourraient jouer un rôle important dans leur adaptation. Des études récentes ont montré que le microbiome des espèces d'éponges tolérantes à l'OA a augmenté leur potentiel fonctionnel pour le métabolisme énergétiquement efficace du carbone et de l'azote (Botté et al., 2019), contribuant à l'adaptabilité écologique et à la plasticité de l'holobionte (Bang et al., 2018).

De même, la communauté bactérienne associée à *R. globostellata* pourrait contribuer à l'assimilation du carbone et de l'azote (énergie supplémentaire) et pourrait avoir un rôle important dans leurs mécanismes de survie et d'adaptation pour faire face aux conditions environnementales extrêmes de Bouraké. Bien que cela n'ait pas été clairement démontré dans ma thèse, le carbone et l'azote semblent être principalement stockés dans la biomasse des éponges à 32°C, suggérant une implication majeure des symbiotes lors de températures élevées. Parmi les microbes associés, les photosynthétiques peuvent réagir différemment à l'OA et à l'OW, allant d'un impact nul de OA (Bell et al., 2022) à une diminution de l'état d'équilibre et des rendements quantiques maximaux de la fluorescence de leur photosystème II (e.g., Lesser et al., 2016).

En mesurant l'activité photosynthétique de *R. globostellata* dans des conditions normales et extrêmes, mes résultats ont révélé que les photosymbiotes sont affectés par les conditions extrêmes, ce qui suggère que ces symbiotes sont peu impliqués dans les réponses de l'éponge pour faire face aux conditions extrêmes. À l'inverse, les éponges hétérotrophes ont été signalées comme étant plus tolérantes aux anomalies de température que les éponges phototrophes (Bennett, 2017). Dans cette étude, *R. globostellata* est principalement hétérotrophe (rapports P:R < 1,5, selon la définition de Wilkinson, 1983), ce qui suggère que les processus hétérotrophes de cette espèce pourraient principalement contribuer à faire face aux conditions extrêmes, et que les symbiotes hétérotrophes pourraient jouer un rôle important dans l'adaptation des éponges. De même, des études récentes ont montré qu'une augmentation de l'abondance des bactéries hétérotrophes soutiendrait une plus grande productivité des éponges (e.g., Perea-Blázquez et al., 2012), et que des conditions eutrophes (i.e., comme celles de Bouraké), pourraient rendre les éponges moins dépendantes de leurs photosymbiotes (Webb et al., 2017).

Dans mon travail, j'ai montré que les éponges ont une capacité métabolique pour faire face à différentes conditions environnementales. Bien que je n'aie étudié les réponses que d'une seule espèce d'éponge à une échelle spatiale locale (Bouraké), cette étude a clairement montré que cette éponge pouvait faire face à des conditions de températures extrêmes et élevées de manière avantageuse. En résumé, le succès de cette éponge à Bouraké, malgré les conditions physiques extrêmes, pourrait être lié à *i*) l'environnement fluctuant, qui offre aux éponges des phases de conditions optimales pour récupérer entre des phases de conditions extrêmes, et *ii*) la large gamme d'apports hétérotrophes, de nutriments, de sources de carbone et d'azote qui pourraient aider les éponges à faire face aux conditions extrêmes grâce à un gain d'énergie supplémentaire provenant de l'environnement. Si l'on considère que les éponges vivant à Bouraké sont soumises à ces conditions environnementales depuis des décennies ou des siècles, ces réponses sont les résultats d'un mécanisme adaptatif mis en place

par l'éponge pour bien se développer dans des conditions extrêmes et notamment une température élevée.

## **Les éponges de Bouraké, un modèle d'adaptation pour les futurs récifs coralliens?**

Mes résultats montrent que *R. globostellata* a développé des stratégies adaptatives pour survivre et se développer dans l'environnement extrême de Bouraké. On peut néanmoins se demander si ces résultats peuvent être utilisés pour prédire comment d'autres organismes récifaux pourraient répondre aux conditions futures.

Bien que nous ne puissions pas prédire si les fluctuations naturelles attendues pour les autres habitats côtiers seront comparables à ce qui a été mesuré dans la lagune de Bouraké, les modèles prévoient des fluctuations importantes des paramètres physico-chimiques de l'eau. L'amplitude saisonnière du pH pourrait augmenter de 80%, les valeurs extrêmes de l'acidité des océans seront plus fréquentes, et la variabilité du pH augmentera encore plus sur des sites où la variabilité est déjà importante aujourd'hui (Kwiatkowski et Orr, 2018 ; Burger et al., 2020). L'évolution pourrait être similaire pour la température de l'eau de mer. A Bouraké, les valeurs moyennes des paramètres environnementaux ( $\text{pH}_T = -0,3$  unités,  $\text{DO} = -20\%$ , et  $T = +3^\circ\text{C}$ ) s'approchent néanmoins des valeurs prédites par les modèles selon le scénario du GIEC (RCP 8.5:  $\text{pH}_T = -0,3$  unités,  $\text{DO} = -4\%$ , et  $T = +3^\circ\text{C}$ ) ce qui laisse penser que ce site peut être utilisé pour étudier les réponses adaptatives des organismes aux conditions futures.

Par contre, à Bouraké, les changements des paramètres physiques sont déterminés par la marée, qui fait passer les organismes des conditions normales aux conditions extrêmes toutes les 6 heures. Les mécanismes de réponse observés pourraient donc être le résultat de cette fluctuation qui, malgré son caractère récurrent, limite dans le temps leur exposition aux conditions stressantes. Au fil du temps, la marée pourrait avoir exposé les éponges aux conditions stressantes suffisamment longtemps pour induire une adaptation tout en évitant une exposition trop longue à court terme qui aurait pu causer des effets délétères, voire leur mortalité comme ça a été rapporté dans certaines études sur les coraux (e.g., Oliver et Palumbi, 2011 ; Castillo et al., 2012 ; Palumbi et al., 2014 ; DeCarlo et al., 2019). Ce processus, répété pendant des décennies, voire des siècles, à ces valeurs extrêmes, a pu permettre une sélection à long terme des organismes résistants et aboutir à la situation actuelle (i.e., des organismes adaptés). On peut se demander si les mêmes mécanismes pourront se mettre en place dans des environnements différents où les organismes risquent d'être exposés plus rapidement, plus régulièrement et plus longtemps à des conditions stressantes. Toutefois, des études récentes ont

montré que l'adaptation rapide et la plasticité de certains traits physiologiques pourraient être héréditaires, ce qui aiderait les organismes à faire face également à un changement environnemental rapide (Torda et al., 2017 ; Putnam, 2021).

La généralisation de mes résultats et leur utilisation pour prédire le potentiel adaptatif des organismes récifaux dans d'autres sites reste toutefois hasardeuse, car plusieurs autres facteurs environnementaux tels que la concentration en nutriments et la concentration en matière organique, varient à Bouraké et sont susceptibles d'influencer les réponses adaptatives des organismes. Les lagunes côtières, telles que Bouraké, sont des écosystèmes hautement productifs qui servent de zones de reproduction pour de nombreuses espèces marines (Anthony et al., 2009), et qui reçoivent des apports terrigènes provenant des pluies, des eaux usées et/ou des engrais agricoles (Taylor et al., 1995). Cet apport de matière organique à Bouraké peut également être amplifié par le fait que la lagune soit entourée par l'un des systèmes les plus productifs au monde, la mangrove (e.g., Alongi et al., 2004 ; Dittmar et al., 2006 ; Bouillon et al., 2008). Les communautés benthiques de Bouraké pourraient avoir accès à un large éventail d'intrants hétérotrophes, de nutriments, de sources de carbone et d'azote. Bien que ces apports puissent devenir toxiques s'ils sont trop concentrés, ils peuvent également aider les organismes à faire face à des conditions extrêmes.

Bien qu'il n'existe pas de "laboratoire naturel" idéal où étudier les réponses des organismes aux conditions futures, Bouraké offre une combinaison réaliste de multiples facteurs qui affecteront très probablement les récifs coralliens dans le futur. Mes résultats fournissent des indications importantes sur les potentiels mécanismes d'adaptation des éponges à des conditions environnementales extrêmes, malgré les spécificités du site. La température dans les récifs coralliens devrait augmenter de 3°C dans le futur. Mes résultats ont démontré expérimentalement que *i*) qu'au-delà d'un certain seuil (> 31°C testé ici) la température induisait un changement dans l'allocation de la matière organique chez *R. globostellat* ; et *ii*) les réponses n'étaient observées que chez les éponges de Bouraké, car elles sont probablement capables de faire face aux variations extrêmes de température (**Figure R3**). Cela suggère que si les éponges de Bouraké ont pu maintenir leur métabolisme normal et allouer plus de ressources aux tissus, les éponges du site contrôle ont probablement subi un stress métabolique qui a conduit à une dégradation des cellules.

Si nous émettons l'hypothèse que les éponges contemporaines pourraient avoir suffisamment de temps face au changement climatique pour s'adapter comme elles l'ont fait à Bouraké, cette étude suggère que les futurs récifs coralliens seraient probablement dominés par des éponges plus grandes qui investissent plus d'énergie dans leur croissance individuelle. Le changement d'allocation du carbone et de l'azote peut avoir des conséquences sur la quantité de débris disponible pour les autres

niveaux trophiques de l'écosystème. Moins de détritits seront incorporés par les détritivores au sein de l'écosystème corallien et par conséquent, moins de détritits atteindront les autres niveaux trophiques par cette voie. Le carbone et l'azote sont principalement stockés dans les tissus des éponges, et les prédateurs des éponges seront théoriquement avantagés car ils disposeront de plus de biomasse d'éponges avec plus de carbone et d'azote. Cependant, les prédateurs d'éponges sont limités à quelques espèces de poissons, de tortues et de nudibranches (toutes ces espèces sont généralement peu abondantes), et toutes ne peuvent pas tolérer les métabolites secondaires produits par les éponges comme moyen de défense. Cette utilisation potentielle de la biomasse des éponges par les prédateurs reste donc très probablement une voie (très) mineure du cheminement de la matière organique. La biomasse accumulée peut réintégrer le système lorsque les conditions de température changent (i.e., production de détritits) ou à la mort de l'éponge, alimentant alors une voie nécrophage/détritivore au sein du réseau trophique. Bien que cela reste hypothétique et limité à un cas spécifique, ce travail a mis en évidence que le réchauffement de l'océan pourrait changer la façon dont les éponges recyclent le carbone et l'azote, donc leur qualité et leur disponibilité dans le récif.

Un certain nombre d'observations en cours rapportent que le récif de Bouraké a changé ces dernières années, principalement avec un impact négatif majeur sur les coraux (i.e. blanchiment et mortalité observés), alors que l'abondance des éponges et leur massivité restent toujours élevées. Il semble que les conditions stressantes (précipitations, cyclones, événements ENSO et/ou augmentation de la température) ont un effet plus important sur les autres organismes que sur les éponges, qui continuent à profiter pleinement des niveaux élevés de nutriments pour leur croissance individuelle dans des conditions non optimales. Ceci est en accord avec mes résultats montrant que *R. globostellata* présente des mécanismes d'acclimatation et d'adaptation qui lui permettent de rivaliser efficacement dans cet environnement extrême.

En conclusion, cette recherche a souligné l'importance d'étudier comment les températures peuvent affecter le rôle écologique des éponges dans les récifs coralliens, car la hausse de la température est l'une des conséquences majeures du changement climatique, entraînant des changements dans le métabolisme de la plupart des éponges (organismes *sensu lato*). Ces changements dans le métabolisme des éponges, tels que la répartition du carbone et de l'azote entre les tissus des éponges et les détritits, ainsi que la capacité des éponges à recycler la matière organique, pourraient avoir des effets potentiellement importants sur l'ensemble du récif corallien. L'effet sur les interactions trophiques pourrait conduire à un changement possible vers des communautés dominées par des organismes avantagés par les températures élevées.

## Perspectives futures

Les éponges jouent un rôle écologique important en recyclant le carbone et l'azote dans les écosystèmes des récifs coralliens (e.g., Maldonado et al., 2012 ; De Goeij et al., 2013). Les deux voies (« boucle de l'éponge » et stockage de biomasse) sur le recyclage du carbone des éponges doivent être pleinement évaluées pour avoir une meilleure compréhension du cycle du carbone (et de la matière organique en général) dans les éponges massives et encroûtantes et également évaluer la contribution des symbiotes microbiens dans ces processus. Il y a plus de 8 500 espèces d'éponges décrites (Van Soest et al., 2012), et pour la plupart d'entre elles, il n'y a aucune information concernant le cycle du carbone et des nutriments.

Bien que mon étude soit focalisée sur *R. globostellata*, elle fournit un aperçu original de la compréhension des effets des conditions extrêmes, et principalement de l'OW, sur le cycle des nutriments et du carbone. Des travaux ultérieurs devraient considérer plus d'espèces d'éponges, encroûtantes et massives, et leurs cycles de nutriments et de carbone doivent être évalués dans diverses conditions environnementales, y compris dans les conditions extrêmes. De plus, des expériences futures de transplantation croisée et en utilisant d'autres laboratoires naturels devraient être considérées comme une priorité pour explorer comment le cycle des nutriments et du carbone des éponges changerait dans des conditions futures, afin d'avoir une réponse plus générale. De plus, des expériences *ex situ* maintenant les éponges à des températures contrastées pendant de plus longues périodes, à la fois dans des sites contrôles et des « laboratoires naturels », pourraient être réalisées pour comprendre le degré de tolérance à la température chez les éponges et donner un meilleur aperçu de la façon dont le cycle du carbone et de l'azote pourrait être affecté dans le cas de futures augmentations de température.

En outre, bien que cette thèse n'ait pas permis d'étudier les processus métaboliques permettant l'accumulation de carbone dans les éponges, mes recherches suggèrent que dans des conditions extrêmes ( $T > 31^{\circ}\text{C}$ ), le carbone est principalement stocké dans les tissus des éponges et n'est pas libéré sous forme de détrit. Les études futures devront utiliser des techniques plus spécifiques pour marquer et suivre le carbone dans les éponges, comme par exemple le NanoSIMS qui sera un outil utile pour évaluer où le carbone est alloué et utilisé dans les tissus de l'éponge. Cela donnera une meilleure compréhension de la stratégie utilisée pour s'adapter aux hautes températures. En outre, les mesures de la biomasse des éponges, les taux de pompage et la qualité de la nourriture doivent être évalués afin de mieux comprendre les mécanismes utilisés pour faire face aux conditions extrêmes et futures.

Enfin, les éponges étant par définition des holobiontes, les réponses des microbes associés à l'éponge doivent être prises en compte. Certaines études ont signalé que les changements dans le microbiome pourraient être la clé de l'adaptation des éponges à de nouvelles conditions environnementales (e.g., Webster et Reusch, 2017 ; Pita et al., 2018). Le rôle du microbiome de cette espèce et d'autres espèces doit être mieux compris, en particulier sur le recyclage de la matière organique des éponges, et sa contribution à l'adaptation dans des conditions extrêmes. Bien que dans mes recherches, j'ai mis en évidence plusieurs résultats suggérant l'existence de mécanismes d'adaptation, il reste difficile de la démontrer pleinement sans analyses complémentaires. Ainsi, des travaux futurs sur le contenu en lipides, les protéines de choc thermique, et la transcriptomique pourraient être la clé pour comprendre si les réponses trouvées pour *R. globostellata* à Bouraké sont le résultat d'une adaptation ou d'une réponse physiologique dans des conditions de stress.

## Conclusion générale

Les changements environnementaux, tels que prévus dans les scénarios climatiques futurs, pourraient fortement affecter les espèces marines. Parmi elles, les éponges ont des réponses différentes et spécifiques au changement climatique et aux variations environnementales. Jusqu'à présent, les études se sont principalement concentrées sur les effets des changements climatiques sur les caractéristiques physiologiques des éponges plutôt que sur leur rôle écologique, comme par exemple dans le cycle du carbone et de l'azote dans les récifs coralliens.

Ma thèse a fourni 1) la première caractérisation physico-chimique à long terme et l'évaluation de la communauté benthique dans la lagune de Bouraké, qui est maintenant considérée comme un site particulièrement original pour étudier l'adaptation des organismes aux conditions extrêmes; 2) les premiers aperçus des effets des conditions extrêmes sur le métabolisme de *R. globostellata* (l'espèce dominante de ce site), montrant un changement principal dans le cycle du carbone pour faire face et prospérer dans des environnements extrêmes; 3) les premières informations sur les effets de la température sur le recyclage du carbone et de l'azote de *R. globostellata*, montrant que les éponges peuvent être capables de tolérer une exposition répétée à des températures élevées à court terme (+3°C). Les éponges soumises aux hautes températures pendant des décennies / siècles (Bouraké) montreraient une réponse adaptative qui impliquerait en une plus grande accumulation du carbone et d'azote dans les tissus que dans les détrit.

Dans un monde où le changement climatique s'accélère et où les effets de ces changements sur les organismes ne sont pas encore toujours compris, ma thèse démontre comment une éponge tropicale pourrait faire face à l'acidification, au réchauffement et à la désoxygénation. J'ai montré que *R. globostellata* pouvait s'adapter à différentes conditions environnementales en tirant le meilleur parti

de son bien-être individuel et en suggérant que l'adaptation des éponges, et espérons-le d'autres taxons, aux conditions environnementales futures pouvait être possible. Cette adaptation aux hautes températures peut toutefois entraîner un changement des réponses métaboliques et donc une modification des interactions trophiques au sein des organismes coralliens.



---

**ITALIAN SUMMARY - SINTESI**

---

Le emissioni antropogeniche di anidride carbonica e di altri gas a effetto serra sono aumentate in modo esponenziale dalla rivoluzione industriale, causando cambiamenti significativi nel clima della Terra. I tre principali effetti del cambiamento climatico sugli ecosistemi marini sono il riscaldamento, l'acidificazione e la diminuzione dell'ossigeno. Mentre la maggior parte della ricerca ecologica e fisiologica è rivolta ai coralli, gli effetti sulle spugne e il loro ruolo ecologico devono ancora essere approfonditi. Le spugne svolgono funzioni ecologiche essenziali, tra cui il contributo al ciclo dei nutrienti e del carbonio. Possono assorbire il carbonio organico disciolto e trasformarlo e rilasciarlo in carbonio organico particolato (cioè detrito), che può essere utilizzato dai livelli trofici superiori. Capire come le future condizioni climatiche potrebbero alterare il ruolo ecologico delle spugne è fondamentale per comprendere meglio il futuro delle barriere coralline. Questa tesi fornisce le prime informazioni su come i nutrienti delle spugne e il riciclo della materia organica potrebbero cambiare in condizioni ambientali estreme (alta temperatura, basso pH e basso ossigeno disciolto).

In primo luogo (capitolo 2), ho eseguito una caratterizzazione fisica, chimica e bentonica a lungo termine della laguna di Bouraké (Nuova Caledonia). I dati raccolti dal 2016 al 2020 hanno mostrato che questo sito è caratterizzato da una variazione giornaliera, guidata dalle maree, e dai parametri chimici e fisici, che raggiungono valori estremi durante la bassa marea (33°C, 7,2 pH<sub>T</sub>; 1,9 mg O<sub>2</sub> L<sup>-1</sup>). Nonostante queste condizioni ambientali estreme, è stata trovata una comunità bentonica ricca e sana con 66 specie di coralli, 28 specie di macroalghe e 11 specie di spugne. I miei risultati dimostrano che la laguna di Bouraké è un sito unico che può essere utilizzato come laboratorio naturale per studiare gli effetti delle condizioni ambientali estreme sugli organismi.

Dopo la caratterizzazione del sito di studio, ho eseguito 118 incubazioni *in situ* nella laguna di Bouraké, utilizzando camere bentoniche respirometriche, per studiare gli effetti di condizioni estreme sul ciclo del carbonio e dei nutrienti delle spugne e sull'attività fotosintetica dei loro simbionti associati (capitolo 3). La spugna *Rhabdastrella globostellata* (Carter, 1883) è stata scelta come specie modello, poiché è la specie più abbondante trovata a Bouraké, ed è stata incubata *in-situ* sia in condizioni normali (alta marea) che estreme (bassa marea). Le risposte della spugna olobionte (cioè la spugna e i suoi batteri associati) sono state esaminate attraverso i cambiamenti dei parametri fisici e chimici dell'acqua di mare tra l'inizio e la fine delle incubazioni. I risultati di questo capitolo hanno mostrato che le spugne hanno smesso di produrre detrito (cioè il ciclo delle spugne, "sponge loop" in inglese) in condizioni estreme. I miei risultati suggeriscono inoltre che l'alta temperatura (> 31°C)

potrebbe essere la causa principale di questa interruzione del riciclo del carbonio organico delle spugne, soprattutto se combinata con livelli estremi di acidificazione e deossigenazione.

Dai risultati degli esperimenti *in situ*, si è ipotizzato che la temperatura sia il fattore principale che influenza il ciclo del carbonio delle spugne. Esperimenti in acquario *ex-situ* sono stati effettuati per esplorare la risposta di riciclo dell'azoto e del carbonio di *R. globostellata* esposta ad alte temperature (capitolo 4). Esempari di *R. globostellata* provenienti dalla laguna di Bouraké e da un sito di controllo sono stati esposti a 28°C e a 32°C. Le risposte sono state valutate attraverso il rilevamento di <sup>13</sup>C e <sup>15</sup>N marcati nel tessuto delle spugne e la produzione di detrito. I risultati mostrano che le spugne di Bouraké incorporano nei tessuti più <sup>13</sup>C e <sup>15</sup>N, assimilati come muco di corallo, che rilasciati come detrito. Questi tassi raddoppiano quando sono esposte a 32°C rispetto a 28°C. Al contrario, le spugne del sito di controllo incorporano meno <sup>13</sup>C e <sup>15</sup>N nei loro tessuti e ne rilasciano di più nel detrito. Il cambiamento nell'allocazione del carbonio e dell'azoto, con un maggiore accumulo nei tessuti delle spugne degli esemplari di Bouraké potrebbe essere un adattamento alle condizioni ambientali estreme del sito.

In un mondo in cui i cambiamenti climatici stanno accelerando e molti degli effetti di questi cambiamenti sugli organismi non sono ancora stati studiati, la mia ricerca dimostra come la spugna *R. globostellata* risponda a diverse condizioni ambientali e come il suo metabolismo cambia per prosperare in ambienti estremi. Ho fornito la prima prova che l'esposizione a temperature elevate (+3°C) provoca un cambiamento nell'allocazione e nell'utilizzo del carbonio e dell'azoto tra il tessuto della spugna e il detrito, fornendo anche una visione del possibile cambiamento nella disponibilità e nella qualità delle fonti di cibo in una futura barriera corallina. Infine, ho dimostrato che le spugne sono in grado di sviluppare dei meccanismi di adattamento alle diverse condizioni ambientali, sfruttandoli al meglio e lasciando la speranza che l'adattamento delle spugne, e auspicabilmente di altri taxa, alle future condizioni ambientali sia possibile.

