Université de la Nouvelle-Calédonie – Ecole doctorale du Pacifique

Habilitation à Diriger des Recherches

Titre

Revising the consensus on the effect of ocean acidification on coral calcification

Révision du consensus sur l'effet de l'acidification des océans sur la calcification des coraux

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Soutenance prévue le 20 Février 2024

Jury :

Yves Letourneur (Professeur, Université de la Nouvelle-Calédonie)	Président
Sophie Cravatte (Directrice de Recherche, UMR Legos, IRD, France)	Rapporteuse
Luisa Mangialaio (Professeure, Ecoseas, Université Côte d'Azur, France)	Rapporteuse
Matthieu Pernice (Directeur, Professeur associé, UTS, Australie)	Rapporteur
Cyril Marchand (Professeur, Université de la Nouvelle-Calédonie)	Examinateur
Nazha Selmaoui-Folcher (Professeure, Université de la Nouvelle-Calédonie)	Examinatrice

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1. CURRICULUM VITAE

Riccardo Rodolfo-Metalpa, born in Genoa, Italy, July 28th 1971. Italian nationality

EDUCATION

Oct 2004/ Oct 2007	**PhD Oceanography** University of Marseille-Aix II (France); Host Institution: Centre Scientifique de Monaco (Monaco). Governmental research fellow: "*Responses of two Mediterranean corals Cladocora caespitosa and Oculina patagonica to environmental and climate change"*. Supervisors: Prof. D. Allemand and Dr. C. Ferrier-Pagès.
July 1993/ July 1998	**Degree in Biological Sciences (B.Sc and M.Sc.)** University of Genoa (Italy); one year M.Sc. at ENEA, Marine Environmental Research Centre (La Spezia, Italy): "*Growth and other aspects of the biology of the coral Cladocora caespitosa (L.) (Cnidaria, Scleractinia): in situ and aquarium observations"*.

EMPLOYMENT HISTORY

Oct 2013/ permanent	IRD - Institut de Recherche pour le Développement (New Caledonia). **Research Scientist** (CR1).
Mar 2013/ Sept 2013	IRD - Institut de Recherche pour le Développement (New Caledonia). **Research fellow** funded by LABEX Corail: "*Changement climatique et production carbonée des algues Corallinacées des récifs coralliens*".
Feb 2011/ June 2012	Marine Biology & Ecology Research Centre, University of Plymouth. **Research fellow** funded by EU FP7 project MedSeA grant no. 265103: "*Mediterranean Sea Acidification under a changing climate*".
Feb 2009/ May 2010	United Nations, International Agency Atomic Energy (AIEA), Monaco. **Research Scientist** funded by Foundation Prince Albert II of Monaco: "*Effect of Ocean Acidification on Mediterranean Biodiversity*". This is a competitive, *ad hominem* fellowship (PI position), and is not equivalent to a postdoctoral position in America or Europe.
Oct 2007/ Dec 2008	Marine Biology & Ecology Research Centre, University of Plymouth. **Research fellow** funded by Leverhulme Trust (PI JM Hall-Spencer): "*Volcanic CO_2 vents and Ocean Acidification*".
Oct 2004/ Oct 2007	Centre Scientifique de Monaco (Monaco). **Research fellow** (PhD) funded by the host Institute: "*Responses of two Mediterranean corals Cladocora caespitosa and Oculina patagonica to environmental and climate change"*.
Apr 2004/ Jul 2004	Centre Scientifique de Monaco (Monaco). **Research associate** "*Biology and physiology of Med corals*".
Nov 2003/ May 2004	Centre Scientifique de Monaco (Monaco). **Research fellow** funded by the EU programme Leonardo da Vinci I: "*Biology and physiology of Mediterranean corals*".
Feb 2002/ Feb 2003	Institute of Marine Biology and Ecology (Piombino, Italy). **Research fellow** funded by the EU project INTEREG III: "*New methodologies for recovery and reutilisation of Posidonia oceanica (L.) drifted ashore and the monitoring of meadow*".
Mars 2000/ Feb 2002	Marine Environmental Research Centre *ENEA* (La Spezia, Italy). **Governmental fellowship**, ENEA and CNR-MURST: "*National plan for the protection of the coast and sea*".

GRANTS & FELLOWS

2024 - Fonds Pacifique: *Partnering for success; Understanding coral symbiosis in the extreme reefs of New Caledonia*, **64K€** (co-PI)

2023 - Agence Nationale de la Recherche (ANR): *Nutrition as a BOOST for corals in the face of marine heat waves* (BOOST) **640K€** (WP leader)

- Fonds Pacifique: *Soaking it up: understanding climate resilience and acclimation in New Caledonian sponges*, **43K€** (co-PI)

2022 - Labex Corail: *Résilience et connectivité des coraux dans les environnements marginaux et extrêmes* (RECONNECTION), **82K€** off 300 K€ (PI)

- Fonds Pacifique: *Adaptation of calcifying algae to climate change*, **32K€** (co-PI)

2021 - Fonds Pacifique: *Adaptation of corals to climate change*, **37K€** (co-PI)

2020 - Oceanographic Cruise R/V Alis SUPERNATURAL 2: *A unique natural laboratory to study corals adaptation to global change*, **200K€** (PI)

- European Research Council (ERC) Cog Grant finalist, Travel grant to Brussels, **7K€**

2019 - Oceanographic Cruise R/V Alis CARiOCA 4th trip : *Acclimatation des coraux à l'acidification des océans autour de résurgences sous-marines de CO_2*, **230K€** (PI)

- European Research Council (ERC) Cog Grant finalist, Travel grant to Brussels, **7K€**

- Fonds Pacifique: *Les coraux vivant dans la mangrove en Nouvelle-Calédonie auraient pu s'adapter à l'acidification et au réchauffement des océans?* (SUPERCORAUX), **18K€** (PI)

- Labex Corail: PhD fellowship project SUPERNATURAL, **44K€** (PhD Director)

- University of New Caledonia PhD fellowship project REEF-ENGINE, **44K€** (Supervisor)

- University of Bretagne-Loire PhD fellowship project ACCLICOST, **44K€** (Supervisor)

- Labex Corail: *Would super corals only be well-fed corals?* (SURF), **20K€** (co-PI)

- Breguet sponsored Oceanographic Cruise on Race for Water Hydrogen vessel: *Accumulation des MIcroplastiques dans les Coraux* (AMICi) (co-PI)

2018 - Oceanographic Cruise R/V Alis CARiOCA 3rd trip : *Acclimatation des coraux à l'acidification des océans autour de résurgences sous-marines de CO_2*, **230K€** (PI)

2017 - Agence Nationale de la Recherche: *Using volcanic CO_2 vents to assess coral reefs ability to acclimatize and adapt to ocean acidification and warming* (*PNG-Vents*), **180K€** (PI)

- Fonds Pacifique: *Identifying the value of New Caledonia's "extreme" corals to manage reefs under climate change* (COLIMATIC), **20K€** (co-PI).

- Oceanographic Cruise R/V Alis CARiOCA 2nd trip : *Acclimatation des coraux à l'acidification des océans autour de résurgences sous-marines de CO_2*, **200K€** (PI)

- Oceanographic Cruise R/V Alis Supernatural 1: *A unique natural laboratory to study corals adaptation to global change*, **96K€** (PI)

2016 - Fonds Pacifique: *Coral reef adaptation to ocean acidification* (AMBITLE*)*, **20K€** (PI).

- Agence Nationale de la Recherche: *Coral reef acclimatization to ocean acidification at CO_2 seeps* (CARiOCA), **354K€** (PI)

- European Research Council (ERC) Cog Grant finalist, Travel grant to Brussels, **7K€**

- Labex Corail: *Nouvelles priorités pour la recherche dans le domaine des changements climatiques sur les coraux constructeurs de récifs* (ENSEMBLE), **12K€** (PI)

- Oceanographic Cruise R/V Alis CARiOCA: *Acclimatation des coraux à l'acidification des océans autour de résurgences sous-marines de CO_2*, **280K€** (PI)

- Labex Corail: *Acclimatation développementale et transgénérationnelle à l'acidification des océans et réchauffement climatique* (ACCLIMACID), **30K€** (collaborator)
- Grand Observatoire du Pacifique du Sud (GOPS): *South Ocean acidification & projected changes of coral reef ecosystems* (ORACLE), **25K€** (PI)

2015 - Labex Corail: *Balance Autotrophie/Hétérotrophie chez les coraux* (COCONUT), **25K€** (co-PI)
- Grand Observatoire du Pacifique du Sud (GOPS): *Coastal seawater pH oscillations: revealing the noise of coral reef communities* (HONOR), **11K€** (PI)

2014 - European Research Council (ERC) Starting Grant finalist, Travel grant to Brussels, **7K€**

2012 - European Research Council (ERC) Starting Grant finalist, Travel grant to Brussels, **7K€**
- 3rd International Symposium on the Ocean in a High-CO_2 World, Travel grant to Monterey, (USA): **1.2k€**)

2011 - Labex Corail: *Conséquences du changement climatique et des apports anthropiques sur les capacités de calcification de deux récifs coralliens* (COMETA), **29K€** (co-PI)

2010 - Post-doc grant from the EU FP7 project MedSeA, 34K€ (PI: J Hall-Spencer)

2009 - Prince Albert II Foundation (Monaco): *Effect of Ocean Acidification on Mediterranean Biodiversity*, **40K€** (PI)
- Society for Experimental Biology Annual Meeting, Travel grant to Glasgow (UK), **0.6K€**
- 1st Mediterranean Symposium on Coralligenous conservation and other calcareous bio-concretions, Travel grant to Tabarka (Tunis), **0.4K€**

2008 - Journal of Experimental Biology, Travel grant to Eilat (Israel), **1.2K€**
- Percy Sladen Memorial Fund, Travel grant to Alicante (Spain), **0.6K€**

2004 - Centre Scientifique de Monaco (CSM), PhD fellowship, **50K€**

2003 - European award Leonardo da Vinci I for training at CSM (Monaco): **2.4K€**

FIELD EXPERIENCES, OCEANOGRAPHIC MISSIONS

I have participated to several oceanographic cruises (SALA 3, MASSFLUX, ARCO, MEDCOR WAGNER-II, PRISTINE, AMICI), being head mission for some others (CARIOCA 1-3, AMBITLE 1-4, SUPERNATURAL 1-2); organised field missions in French Guadeloupe, French Polynesia, Red Sea, Spain, Palau and around 40 missions to study the effect of acidification using CO_2 vents in the Mediterranean Sea (Ischia and Vulcano islands), in the Gulf of California, and at the mangrove extreme system in New Caledonia.
Diving professional certificate (CAH: Classe 1, mention B, and Chef de plongée scientifique, CNRS).

INVITED SPEAKER & SEMINARIES

32 European Research Council ERC. Interview projet ERC CoG "Hope - Hope for coral reefs. Can acclimatization and adaptation follow the pace of climate change?" 22th Sept 2020, Bruxel.

31 European Research Council ERC. Interview projet ERC CoG "Hope - Hope for coral reefs. Can acclimatization and adaptation follow the pace of climate change?" 09th Oct 2019, Bruxel.

30 University of Papua New Guinea UPNG. Coral reef acclimatization to ocean acidification using CO2 seeps of PNG". What can natural analogues tell us about the fate of reefs in the face of climate change? Port Moresby, PNG, 30th August 2019

29 CEN, Conservatoire d'espaces Naturels, "Pourquoi il faut valoriser les « super coraux » de Bouraké". New Caledonia, Kone, 23 February 2018.

28 Workshop organizer. COLIMATIC - Coral associated with extreme conditions: the Bouraké case. (Fonds Pacifique), 30 participantes, Nouméa, 15-16 November 2017.

27 Fourth International Workshop on the Socio-Economic Impacts of Ocean Acidification. French Pacific Islands (Leader). 15-17 October 2017, Monaco.

26 Human impacts on Mediterranean marine ecosystems and the economy. 18-19 October 2017, Monaco.

25 French-Australian Workshop on the protection of coral reefs. Sydney 22 August 2017, Australian Maritime Museum. Assessing impacts of ocean acidification on coral reefs: 15 y of uncertainties.

24 Pacific voices for a global Ocean challenge. Session 3 – Marine and coastal ecosystems: resilience and reduction of climate change impacts. Japan-Pacific ICT Centre – USP, Suva (Fiji) 8-9 June 2017. *Assessing impacts of ocean acidification on coral reefs: 15 y of uncertainties*.

23 Workshop funded by GOPS "Vulnerability and adaptive capacity of the French Polynesian reef lagoons and society in a changing world" 18-23 April 2017, Tahiti.

22 European Research Council ERC. Interview projet ERC CoG "SELECTION - Using volcanic CO_2 vents to assess marine keystone species ability to acclimatize and adapt to ocean acidification" 06th Sept 2016, Bruxel.

21 Our Common future under climate change, Paris 7-10 July, 2015 Parallel Session 1111. *Why coral reefs should care about ocean acidification: general consensus, misconceptions and future research priorities*.

20 Pacific Islands regional ocean acidification workshop, Secretariat of the Pacific Regional Environment Programme (SPREP), Auckland 7-9 October, 2015. *Why coral reefs should care about ocean acidification: general consensus, misconceptions and future research priorities*.

19 Coral reefs face global change (LaBEx CORAIL meeting), Paris 7-9 September, 2015. *Changement climatique et production carbonée des algues corallinacées des récifs coralliens*.

18 Observation systems of climate change in the South Pacific workshop (GOPS & PACE-NET+), Noumèa 11-12 June, 2015

17 European Research Council ERC. Interview projet ERC CoG "Reef Change" – Coral reef response to climate change" 12th Sept 2014, Bruxel.

16 University of Nice Sophie Antipolis (ECOMERS), Nice (France) 22th Mai, 2013. *Résistance des invertébrés a l'acidification des océans*.

15 Centre de Recherche et d'Enseignement de Géosciences de l'Environnement (CEREGE), Aix-en-Provence (France) 25th Mars, 2013. *Resilience des coraux, bryzoaires et gastéropodes a l'acidification océanique: expériences en laboratoire et près des sources volcaniques de CO2 en Méditerranée*.

14 European Research Council ERC. Interview projet ERC CoG "Reef Change" – Coral reef response to climate change" 08th May 2012, Bruxel.

13 3rd International Symposium on the Ocean in a High-CO_2 world, Monterey (USA) 24-27 September, 2012. *Some Mediterranean corals, but also bryozoans, molluscs and gastropods keep calcifying at low carbonate ions concentrations*.

12 European Geophysical Union (EGU) Alexander von Humboldt International Conference; Penang (Malaysia), 20-24 June, 2011. *Corals, bryozoans and gastropods are able to calcify in acidified seawater*.

11 Station Marine d'Endoume, Centre d'Océanologie de Marseille (France) 13th February, 2012. *Coraux, bryozoaires et gastéropodes sont capables de calcifier dans des eaux plus acides*.

10 MedSea annual meeting, Vulcano (Italy) 24-27 May, 2011. *Physiological manipulations at CO_2 vents Vulcano*.

9 Universidad Nacional Autónoma de México (UNAM), Mexico City, 01-07 May, 2011. *Seawater carbonate chemistry of the Wagner and Consag basins (Bay of California), and its suitability for ocean acidification studies.*

8 Marine Biology Station of Piran (Slovenia), 24th November, 2011. *Corals, bryozoans and gastropods are able to calcify in acidified seawater*.

7 International Atomic Energy Agency (IAEA), Monaco 2nd February, 2010. *Effects of Ocean Acidification on Mediterranean shallow and deep-sea corals: Preliminary results and perspectives.*

6 The Society for Experimental Biology (SEB), Glasgow (UK) 1st July, 2009. *Physiological responses of Mediterranean corals to temperature and pH perturbations.*

5 France Embassy in Israel, Tel-Aviv, Wohl Center, Bar Ilan University (Israel) 8th December, 2009. *L'Europe, Israël et l'Union pour la Méditerranée: Les dangers de l'acidification des océans: un projet de recherche scientifique commun.*

4 Aquatic Sciences Meeting (ASLO), Nice (France) 25-30 January 2009. *Response of the Mediterranean coral Cladocora caespitosa to mid- and long-term exposure to elevated pCO_2 and temperature.*

3 International Atomic Energy Agency (IAEA), Monaco 24th Mars, 2009. *The future of scleractinian corals in a warming Mediterranean Sea*.

2 The Mediterranean Science Commission (CIESM), Menton (France) 1-4 October, 2008. *Volcanic carbon dioxide vents reveal ecosystem effects of ocean acidification*.

1 Centro Ricerche Ambiente Marino S. Teresa ENEA, La Spezia (Italy) 03rd July, 2008. *Seasonality, Climate change and ocean acidification on Mediterranean corals*.

STUDENTS and POST DOC SUPERVISION

I have supervised 12 students (mostly during 6-month stages, M.Sc.), 2 post doc and 3 PhD.

2021 Giulia Zini (M.Sc University of Bologna); 6 months research in New Caledonia. *Role of the diffusion boundary layer in the coral metabolic regulations under extreme conditions*.

2020 Cinzia Alessi Maggioni (PhD, University New Caledonia). *Les récifs coralliens associés aux milieux extrêmes ont-ils acquis la capacité de faire face au changement global ?*

2019 Clement Tanvet (PhD, University Brest); *Acclimatation des coraux aux changements globaux : utilisation des signatures isotopiques pour évaluer l'impact de l'acidification des océans sur la calcification*

Federica Maggioni (PhD, University New Caledonia); *Dans quelle mesure les éponges peuvent-elles moduler le réseau alimentaire des écosystèmes récifaux dans un monde en évolution ?*

Rafael Valente (B.Sc. University of New Caledonia); 2 months training in New Caledonia. *Comparing classic and 3D techniques to measure coral colonies surface*.

2018 Federica Maggioni (M.Sc University of Ancona); 6 months research in New Caledonia. *Phisiological responses of sponges living at extreme conditions*.

2017 Thomas Barros (M.Sc University of Montpellier); 6 months research in New Caledonia. *Using CO2 vents to study adaptation of corals to ocean acidification*.

Rian Prasetia (Post doc Project ANR PNG Vents); 12 months research in New Caledonia.

Julie Ripoll (Post doc Project ANR CARiOCA); 16 months research in New Caledonia.

2016 Marco Zampighi (M.Sc University of Ancona); 6 months research in New Caledonia. *Phisiological responses of corals living at extreme conditions*.

2015 Marco Zampighi (M.Sc University of Ancona); 6 months research in New Caledonia. *Phisiological responses of corallinae algae to environmental stress*.
Virginie Nguyen (B.Sc University of New Caledonia); 3 months research in New Caledonia. *Calcification rates of corals and crustose corallinae algae*.
Amaury Durbano (B.Sc University of New Caledonia); 3 months research in New Caledonia. *Calcification rates of corals and crustose corallinae algae*.
2014 Lara Cavalié (B.Sc University of New Caledonia); 3 months research in New Caledonia. *Effects of ocean acidification on corals and crustose corallinae algae*.
2013 Julien Debreuil (Post Doc); 12 months research in New Caledonia. *Effects of ocean acidification on corals and crustose corallinae algae*.
Tom Biscéré (M.Sc Université de Bretagne Occidentale) 6 months research in New Caledonia. *Effets de l'acidification des océans et de concentrations plus élevées en cobalt sur la croissance et l'efficacité photosynthétique de deux espèces de coraux*.
<2013 Four M.Sc: one in Monaco (Adrien Delval, University of Paris VI), two in UK (CR Warner-Holder and Cecilia Baggini, University of Plymouth), one in Italy (Roberto Sicari, University of Palermo).

RADIO and TV COMMUNICATIONS

You tube repository: https://www.youtube.com/channel/UCCOPiCJpW6VVEFSr_mbHoLA
Twitter account: https://twitter.com/RMetalpa
Project CARIOCA (IRD): https://www.youtube.com/watch?v=tfoT6TZfbNw&t=143s
Project CARIOCA (P-Y Cousteau): https://www.youtube.com/watch?v=TcNpQvc0cPw
Project ACCLICOST (BRUToriginal): https://www.youtube.com/watch?v=Psdf2npwYkQ
Project AMICi, Race for Water: https://www.youtube.com/watch?v=EXzyIdIVOfc
Project SUPERNATURAL (IRD): https://www.youtube.com/watch?v=eKbqekbpdps
Project COCONUT (IRD): https://www.youtube.com/watch?v=fpKzaM2JXcE
Journal 20h NC1ere: https://www.youtube.com/watch?v=xLCLLS6ImQI
Journal 20h NC1ere: https://www.youtube.com/watch?v=dW5Og5lG_Yo
Journal 20h NC1ere: https://www.youtube.com/watch?v=jGEP_0nDRLc
Journal 20h NC1ere: https://www.youtube.com/watch?v=nTWhyFs1z_o
Radio NC1ere: https://www.youtube.com/watch?v=kWRe97wUQHo
TV NCTV: https://www.youtube.com/watch?app=desktop&v=DOG-qsvHRPg&t=934s
TV France 2 «13h15 le dimanche» https://www.francetvinfo.fr/replay-magazine/france-2/13h15/13h15-du-dimanche-25-octobre-2020_4131597.html
TV Science et Vie : https://www.facebook.com/ScienceetvieTV/videos/lhomme-au-chevet-des-coraux-extrait-4/636931013495328/
Canal Plus « Un Paradis en péril » : https://www.youtube.com/watch?v=OvdKihHGt44

PUBLICATIONS

71 peer-reviewed publications. Number of citations 6431 (Google scholar), index h=39

2023

71 Maggioni F, Bell JJ, Pujo-Pay M, Shaffer M, Cerrano C, Lemonnier H, Letourneur Y, Rodolfo-Metalpa R. Sponge organic matter recycling: Reduced detritus production under extreme environmental conditions. **Marine Pollution Bulletin** 190:114869.

70 Tanvet C, Camp EF, Sutton J, Houlbrèque F, Thouzeau G, Rodolfo-Metalpa R. Corals adapted to extreme and fluctuating seawater pH increase calcification rates and have unique symbiont communities. **Ecology and Evolution** 13(5): e10099.

69 Schoepf V, Baumann JH, Barshis DJ, Browne NK, Camp EF, Comeau S, Cornwall CE, Guzmán HM, Riegl B, Rodolfo-Metalpa R, Sommer B. Corals at the edge of

environmental limits: A new conceptual framework to re-define marginal and extreme coral communities. **Science of the Total Environment** 163688.

68 Reimer JD, Agostini S, Golbuu Y, Harvey BP, Izumiyama M, Jamodiong EA, Kawai E, Kayanne H, Kurihara H, Ravasi T, Wada S, Rodolfo-Metalpa R. High abundances of zooxanthellate zoantharians (Palythoa and Zoanthus) at multiple natural analogues: potential model anthozoans? **Coral Reefs** 15: 1-9.

2022

67 Bell JJ, Shaffer M, Luter HM, Mana R, Rodolfo-Metalpa R. Phototrophic sponge productivity may not be enhanced in a high CO_2 world. **Global Change Biology** 28(16): 4900-4911.

66 Tanvet C, Benzoni F, Peignon C, Thouzeau G, Rodolfo-Metalpa R. High coral recruitment despite coralline algal loss under extreme environmental conditions. **Frontiers in Marine Science** 9.

65 Jaquemont J, Houlbrèque F, Tanvet C, Rodolfo-Metalpa R. Long-term exposure to an extreme environment induces specie-specific responses in corals' photosynthesis and respiration rates. **Marine Biology** 169(6): 1-5.

64 Meunier V, Bonnet S, Camps M, Benavides M, Dubosc J, Rodolfo-Metalpa R, Houlbrèque F. Ingestion of diazotrophs makes corals more resistant to heat stress. **Biomolecules** 12: 537.

63 Kang J, Nagelkerken I, Rummer JL, Rodolfo-Metalpa R, Munday PL, Ravasi T, Schunter C. Rapid evolution fuels transcriptional plasticity to ocean acidification. **Global Change Biology** 28(9): 3007-3022.

62 Comeau S, Cornwall CE, Shlesinger T, Hoogenboom M, Mana R, McCulloch MT, Rodolfo-Metalpa R. pH variability at volcanic CO_2 seeps regulates coral calcifying fluid chemistry. **Global Change Biology** 28(8): 2751-2763.

2021

61 Geissler L, Meunier V, Rädecker N, Perna G, Rodolfo-Metalpa R, Houlbrèque F, Voolstra, CR. Highly variable and non-complex diazotroph communities in corals from ambient and high CO_2 environments. **Frontiers in Marine Science** 8: 754652.

60 Ponti M, Linares C, Cerrano C, Rodolfo Metalpa R, Hoeksema BW. Biogenic reefs at risk: Facing globally widespread local threats and their interaction with climate change. **Frontiers in Marine Science** 8: 793038.

59 Agostini S, Houlbrèque F, Biscéré T, Harvey BP, Heitzman JM, Takimoto R, Yamazaki W, Milazzo M, Rodolfo-Metalpa R. Greater Mitochondrial Energy Production Provides Resistance to Ocean Acidification in "Winning" Hermatypic Corals. **Frontiers in Marine Science** 7: 600836.

58 Maggioni F, Pujo-Pay M, Aucan J, Cerrano C, Calcinai B, Payri C, Benzoni F, Letourneur Y, 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.

57 Meunier V, Geissler L, Bonnet S, Rädecker N, Perna G, Grosso O, Lambert C, Rodolfo-Metalpa R, Voolstra CR, Houlbrèque F. Microbes support enhanced nitrogen requirements of coral holobionts in a high CO_2 environment. **Molecular Ecology** 30(22): 5888-5899.

2020

56 Horwitz R, Norin T, Watson SA, Pistevos JC, Beldade R, Hacquart S, Gattuso JP, Rodolfo-Metalpa R, Vidal-Dupiol J, Killen SS, Mills SC. Near-future ocean warming and acidification alter foraging behaviour, locomotion, and metabolic rate in a keystone marine mollusc. **Scientific Reports** 10(1): 5461.

55 Bang IC, Bruggemann F, Chen C. et al. Microsatellite records for volume 12, issue 2. **Conservation Genet Resour** 12, 337–351. https://doi.org/10.1007/s12686-020-01148-6

54 Gaubert J, Rodolfo-Metalpa R, Greff S, Thomas OP, Payri CE. Impact of ocean acidification on the metabolome of the brown macroalgae *Lobophora rosacea* from New Caledonia. **Algal Research** 46: 101783.

53 Camp EF, Suggett DJ, Pogoreutz C, Nitschke MR, Houlbreque F, Hume BC, Gardner SG, Zampighi M, Rodolfo-Metalpa R, Voolstra CR. Corals exhibit distinct patterns of microbial reorganisation to thrive in an extreme inshore environment. **Coral Reefs** 3: 1-6.

2019

52 Bambridge T, Chlous F, D'arcy P, Claudet J, Pascal N, Reynaud S, Rodolfo-Metalpa R, Tambutté S, Thomassin A, Virto LR. Society-based solutions to coral reef threats in French pacific territories. **Regional Studies in Marine Science** 29:100667.

51 Biscéré T, Zampighi M, Lorrain A, Jurriaans S, Foggo A, Houlbrèque F. Rodolfo-Metalpa R. High p CO2 promotes coral primary production. **Biology letters** 15(7): 20180777.

50 DeCarlo TM, Harrison HB, Gajdzik L, Alaguarda D, Rodolfo-Metalpa R, D'Olivo J, Liu G, Patalwala D. McCulloch MT. Acclimatization of massive reef-building corals to consecutive heatwaves. **Proceedings of the Royal Society B** 286 (1898): 20190235.

49 Pichler T, Biscéré T, Kinch J, Zampighi M, Houlbrèque F, Rodolfo-Metalpa R. Suitability of the shallow water hydrothermal system at Ambitle Island (Papua New Guinea) to study the effect of high pCO_2 on coral reefs. **Marine Pollution Bulletin** 138: 148-158.

2018

48 Langer G, Sadekov A, Nehrke G, Baggini C, Rodolfo-Metalpa R, Hall-Spencer JM, Cuoco E, Bijma J, Elderfield H. Relationship between mineralogy and minor element partitioning in limpets from an Ischia CO_2 vent site provides new insights into their biomineralization pathway. **Geochimica et Cosmochimica Acta** 236: 218-229.

47 Camp EF, Schoepf V, Mumby PJ, Hardtke LA, Rodolfo-Metalpa R, Smith DJ, Suggett DJ. The future of coral reefs subject to rapid climate change: lessons from natural extreme environments. **Frontiers in Marine Sciences** 5(4). doi: 10.3389/fmars.2018.00004.

46 Tribollet A, Grange JS, Parra H, Rodolfo-Metalpa, R, Carreiro-Silva M. Limited carbonate dissolution by boring microflora at two volcanically acidified temperate sites: Ischia (Italy, Mediterranean Sea) and Faial (Azores, NE Atlantic Ocean). **Global Biogeochemical Cycles**, 32(1): 78-91.

2017

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2. PREAMBULE ON MY RESEARCH ACTIVITIES, PROJECTS AND RESEARCH HISTORY

2.1. Contribution to my field

I have a well-established track record of laboratory and field studies into the effects of climate change on various life stages of key marine species such as algae, seagrasses, foraminiferans, sponges, corals, bryozoans and molluscs.

- In the framework of EU FP7 EPOCA (*European Project on Ocean Acidification*), I performed the first long-term laboratory study on the effect of ocean acidification (OA) on Mediterranean corals and found that one Mediterranean coral was surprisingly resilient to the pH levels projected for 2100 (Rodolfo-Metalpa *et al.* 2010 Biogeosciences).
- I have pioneered the use of submarine CO_2 vents to investigate the responses of benthic communities to lower pH conditions providing the first studies of the effect of OA on ecosystems. This study was published by **Nature** and quickly became a keystone reference with natural CO_2 vents now frequently used to study the effects of OA.
- In 2009 I became a Prince Albert II Foundation independent PI based at the International Agency of Atomic Energy (AIEA, Monaco). My project (http://www.fpa2.com/projet-bassin-mediterraneen-numero-63.html) characterized the effect of climate change on key-calcifying organisms such as corals, bryozoans, mussels and limpets exposed to CO_2 vents. This research (Rodolfo-Metalpa *et al.* 2011 Nature Climate Change) revealed the mechanisms of resistance to OA across a range of taxa.
- I participated in the EU FP7 project MedSeA (*Mediterranean Sea Acidification under a changing climate*) helping to predict the effect on habitats formed by macroalgae, deep-sea corals and vermetid gastropods. Several studies have been published so far, and others are in preparation. Among the most relevant, Milazzo, Rodolfo-Metalpa *et al*. (2014) Scientific Reports is the first study reporting on the detrimental effect of acidification on a key reef-forming vermetid; Rodolfo-Metalpa *et al.* (2014) Global Change Biology assessed the coral ability to acclimatize to future warming; Rodolfo-Metalpa *et al.*(2015) Global Change Biology assessed cold-water corals resistance to acidification; and Garilli, Rodolfo-Metalpa *et al*. (2015) Nature Climate Change explained the physiological mechanism allowing some molluscs to survive to past extinctions caused by ocean acidification.
- In 2013 I was appointed to a permanent position (Research Scientist, first category) at IRD (Institut de Recherche pour le Développement) in New Caledonia. Here, among other research projects, I am using the CO_2 vent system of Papua New Guinea and the extreme sites of Bouraké and Palau as natural laboratories to study coral reef responses to ocean climate change. Thanks to two ANRs projects, and other Labex Corail and AFD grants, I have benefited from the necessary funds to investigate those natural laboratories, establish a high number of international collaborations, and publish several studies on the subject.

2.2. Recognitions from others

My research includes the first experiment on adaptation responses of marine organisms to OA and on the CO_2 dose required that brings about coastal ecosystem tipping points. The Nature paper has been cited >1500 times so far across a range of scientific disciplines and my first Nature Climate Change is expected to take a similar trajectory (>500 citations). Following the publication of these articles, the originality of these results and the uniqueness of the approach used have been reported in several scientific journals, on TV, newspapers and web blogs. The work has been used to help understand the fate of marine organisms at inter-governmental workshops (e.g., **Unesco**, *Surface Ocean CO_2 Variability and Vulnerability Workshop*, Paris April 2007; **IPCC** workshop on *Impacts of Ocean Acidification on Marine Biology and*

Ecosystems, Okinawa January 2011; **CBD** joint expert review on the *Impacts of ocean acidification on marine and coastal biodiversity*, Montreal, October 2011).
During a 5-year postdoc research period, I demonstrated that I could obtain competitive funding: I obtained a grant to carry out an independent project at CO2 vents (Prince Albert Foundations) and other travel and conference awards. My actual permanent position as a research scientist allows me to build, around the acquired expertise, a competitive team and a technically advanced laboratory. For that, during the period 2014 - 2020, I have been able to attract funders to my research projects (as PI) for a total of 2,200K€.

2.3. Research history
My main topic has always been the effect of climate change, with particular attention to OA, on a series of calcifying marine organisms, especially corals. My approach in doing that has been classic in my early studies, i.e., using lab-based experiments, and innovative during the following career since I pioneered the use of natural laboratories where climate change-like conditions naturally characterized the environment. I have published 71 papers so far; 5 before my PhD; and 8 during my PhD. During my post-doctoral career, 41 out of 58 papers I have published were based on natural laboratories simulating future conditions, while 14 used a lab-based approach.

A few early steps of my scientific background may explain the dynamic of my research and the continuity in the main theme that has characterised these last 23 years. In 1997, when I started my M.Sc at the Research Centre ENEA S. Teresa, and during the following four years of research, my supervisors CN Bianchi, A Peirano and C Morri introduced me to the world of scientific research. I performed my first experiment in aquaria, participated in some oceanographic cruises, performed my first coral transplantations in the field, and produced data for the first 5 papers. During my PhD at the Centre Scientifique de Monaco under the supervision of C Ferrier-Pagés and D Allemand, I continued the topic I was studying in Italy during my pre-PhD research period, i.e., the effect of warming on Mediterranean corals. This topic was enlarged to the emerging theme of OA thanks to the collaboration with J-P Gattuso from the laboratory CNRS LOV in Villefranche-sur-mer. From my PhD, I published 8 papers.

During two post-docs with Prof J-H Spencer at the University of Plymouth, which started immediately after my PhD, I was lucky to participate to EPOCA, and MedSeA, the two first European Research projects on OA. This has greatly helped and boosted my career and scientific curiosity. With J-H Spencer, I have pioneered the use of submarine CO_2 vents to investigate the response of temperate benthic communities to low pH conditions, providing the first studies on the effect of OA at the ecosystem scale. Natural CO_2 vents, where continuous emission of near-pure CO_2 from below the seafloor alters the seawater chemistry of the surrounding seawater, provide a powerful experimental tool to study the ability of species to acclimatize/adapt to future acidification levels. Since our first paper in 2008, CO_2 sites have been frequently used as natural analogues to study OA effects on single species and/or entire communities. This approach has strongly characterised my future research during which I have repeatedly used such special sites, and discovered others. For the first time I have described the CO_2 vents of Ischia and Vulcano (Hall-Spencer, Rodolfo-Metalpa et al., 2008 Nature; Boatta et al., 2013 Mar Poll Bull), the Gulf of California deep vents (Prol-Ledesma et al., 2016, Nature Comm), and Ambitle Island (Pichler et al., 2019, Mar Poll Bull). I have worked at the most famous CO_2 vents in Normanby (PNG, Fabricius et al., 2011) and White Island (NZ, Brinkman and Smith, 2014), as well as at the Nikko Bay in Palau (Reimer et al., 2023, Coral Reefs). In addition to CO_2 vents, I discovered a unique site where the three main environmental parameters of climate change, i.e., OA, warming and deoxygenation combined in a single site,

the Bouraké semi-enclosed bay in New Caledonia (Camp et al., 2017, Scientific Reports; Maggioni et al., 2021 Biogeosciences). Most of my research projects from 2017 have been done in Bouraké, and most of my future projects will use this awesome natural laboratory. During this first 26-year-long scientific trip, I have established strong and friendly collaborations with several colleagues working on the same topic. These collaborations were consolidated in the ICONA consortium, "International CO_2 Natural Analogues Network (ICONA)", a core-to-core international collaborative project that aims at using natural analogues to study the effects of OA on coral reef ecosystems. This project has allowed us to work, in 2023 at Nikko Bay (Palau, Micronesia), likely the most emblematic semi-enclosed bay where corals have adapted to 1°C warming and the OA level expected for the future.
The main advantage of using natural laboratories to study the effects of ongoing climate change is that results are ecologically relevant, although some limitations must always be kept in mind. Indeed, most conclusions of impacts of OA (as well as other factor suc as heat-shock stress, deoxygenation, nutrient enrichment, etc) on organisms and consequent extrapolations to the ecosystem level, stem from short-term laboratory experiments on individual organisms. These experiments are certainly informative, as they enable us to identify the effect of one or a few variables, but in isolation they are unable to account for the implications of species' capacity for acclimatization (and adaption) in natural environments. As a consequence, the time exposure to future global change conditions, and the experimental condition itself, become a central issue in improving our understanding of marine organisms' capacity for acclimation to future conditions. Laboratory experiments are also not ecologically realistic since, for instance, they remove the effects of species interactions, natural supply of nutrition, and environmental fluctuations in the main environmental parameters. In addition, the quasi-totality of studies has neglected the role of adaptation since they tested only within-generation responses to global change and during short-term exposure to stress. Exceptions are studies using short-term life-span organisms such as the coccolithophores, copepods and polychaetes, sea urchins and fish or using breeding experiments on sea urchin larvae.

The use of natural labs has certainly helped my recruitment at IRD, UMR ENTROPIE in October 2013 after a brief postdoc period, and fostered the achievement of two ANR grants: CARiOCA - *Coral reef acclimatization to ocean acidification at CO_2 seeps*, and PNG-Vents - *Using volcanic CO_2 vents to assess coral reefs ability to acclimatize and adapt to ocean acidification and warming*. The two consecutive projects allowed my team to perform 7 fieldwork missions in PNG to study corals, sponges and fish around the acidic seawater at CO_2 vents but also allowed me to collaborate with several experts on this topic, therefore forming a network of scientists, all experts on the effect of climate change on coral reefs and associated species. Natural laboratories have been the perfect tool for me to test either specific mechanisms or scale up to habitat-level responses to environmental changes of a series of organisms.

After my PhD in 2007, most of my research has focused on corals (32 out of 58 papers), including shallow water temperate, tropical species, as well as cold water corals, and anemones, but also other taxa (19 out of 58) including bryozoans, coralline algae and molluscs, for which my contribution has been relevant, and forams, coccolithophores, algae, fish, and sponges, which have been investigated thanks to external collaborators with specific expertises. Most of the time I have investigated the effect of OA, sometimes in combination with warming, on calcifying reef species metabolic rates and symbiosis (e.g., photosynthesis, respiration, tissue parameters, etc), but my main interest has always been about their ability to calcify since it is the most obvious and uniform effect across most of the organisms studied in the OA context (Kroeker et al. 2013; IPCC 2015). For that, over my studies, calcification has been investigated

using classic, state-of-the-art techniques such as buoyant, alizarin staining, transplantation etc (39 out of 58), but also using isotopic tracers and geochemical techniques (13 out of 58).

I realise that most of the time my findings have been against the consensus about the effect of OA on metabolism in general, i.e., I have rarely highlighted a dramatic effect of low seawater pH levels on the coral calcification rates despite most studies demonstrating up to 83 % reduction in the coral calcification ability (Langdon et al. 2005). Why that? I decided to elaborate on this question in this HDR manuscript, which is intended to demonstrate that I have worked during my career around a fundamental question, and try to shed light and solve it, which probably is not the case! So, if there is one question I have always tried to answer, it is "Why OA should affect some fundamental metabolic mechanisms, such as calcification, as initially claimed?" Are my divergent results merely the fruit of my desire to stand out from the mainstream? Are they just casual results? or even worse, has the effect of OA been somewhat overstated?

In attempting to answer the question "Why should corals care about ocean acidification?" I will briefly contextualise the danger posed by ocean acidification to corals, trace a synthesized description of the main studies about the effect of OA on coral calcification, revise the main points which have built the consensus about the effect of OA on coral reefs, and to what extent this consensus has been modified from the first studies made around 20 years ago. I will report only studies regarding the effect of OA on calcification.
In this HDR manuscript, I will not enter into the details of the complex chemical and physical mechanisms that govern coral calcification, still to be fully revealed. Indeed, it was not the goal of my research. Then, I will first resume to what extent my studies have contributed to answering the above-mentioned question, and then briefly give some description of what I think have been milestone papers for my research. In doing that, I will describe only studies I have published over the past 20 years focusing on the effect of OA on the calcification ability of reef calcifying species, mostly corals. My intention was not merely to list one paper after another, because although they are listed quite in chronological order, actually they have been incremental in the original scientific question: "Why coral calcification should care about OA?" Those studies have been conducted both using a classic lab-based approach, i.e., maintaining organisms in aquaria under artificial conditions, as well as using natural analogues of future conditions, i.e., natural laboratories where environmental conditions already mimic future scenarios. Since I have been at the forefront of this last approach, and most of my research has been done using those natural labs, I'll give a particular emphasis to such a kind of approach that has deeply marked my career. Finally, I will describe my actual research and future perspective, including the project I presented to the last ERC CoG call in 2021, which remains in my opinion an innovative project; and an invaluable challenge to realise in my career.

3. WHY SHOULD CORALS CARE ABOUT OCEAN ACIDIFICATION: TOWARD A REVISED CONSENSUS?

Ocean acidification (OA) is one of the main threats to marine habitats likely causing changes in biodiversity and ecosystem function within this century. Ocean acidification might affect various physiological parameters at different stages of the animal life history, from their reproduction, through larval phases and adult growth. Calcifying species, such as scleractinian corals which harbour a large part of the world`s ocean biodiversity, apparently will be the most affected by OA since their calcification and dissolution rates seem related to seawater carbonate chemistry. Studies show a decline in the net calcification rates as a result of decreasing pH and carbonate ions concentration, and an increase in the dissolution rate of carbonate skeletons. However, the consensus on the projected hindering of species calcification ability is in contradiction with several past and recent findings showing that some calcifiers do not seem to be affected by OA. These divergent results have clearly shown that our actual knowledge of biological response to OA is extremely limited and that some assumptions we have used so far might be inaccurate to predict species response to OA.
In this report, the consensus of the response of coral calcification rates to acidification, early erroneous assumptions and misconceptions will be revised in the light of recent milestone contributions which impose an adjustment of our current understanding on coral calcification responses face to OA.

3.1. Building the consensus on the ocean acidification threat

Since the Industrial Revolution, atmospheric CO_2 levels have increased and have nearly doubled since then. The projections for future OA indicate that if CO_2 emissions continue to increase at the current rate, the pH of the surface ocean is estimated to decrease by around 0.4 units by the end of this century compared to pre-industrial levels (Jiang et al. 2023). This rate of acidification has been unprecedented in the past 65 million years, posing significant challenges for marine ecosystems and organisms.
About a third of anthropogenic CO_2 emissions has been absorbed by the oceans, driving the process of OA during which absorbed CO_2 transforms into carbonic acid, increasing the concentrations of H^+, bicarbonate (HCO_3^-), and dissolved carbon dioxide (CO_2), while lowering carbonate (CO_3^{2-}) concentration, the calcium carbonate saturation state (Ω), and seawater pH (Orr et al. 2005). Ω is the ratio of the ion concentration product ($[Ca^{2+}]$ x $[HCO_3^-]$) to the solubility product (K'_{sp}) for the mineral aragonite which compose the coral skeleton.
Coastal regions, where human activities and inputs are concentrated, can experience additional localized pH issues (He & Silliman 2019). Factors such as nutrient runoff from agriculture, sewage discharge, and industrial pollution can exacerbate acidification in these areas. In addition, coastal waters naturally experience pH variability due to various factors, including tidal cycles, temperature changes, freshwater input from rivers, and upwelling events. These natural fluctuations can occur over diurnal (daily) and seasonal time scales. Increased nutrient inputs from agricultural activities and wastewater discharge can stimulate the growth of algae and phytoplankton, leading to excessive organic matter production. In turn, the subsequent decomposition of this organic matter consumes oxygen and releases CO_2, exacerbating localized future acidification.
The notion that the increase in atmospheric pCO_2 forecasted for the 21st century has a negative effect on coral reefs, was born during the late 1990s when a decrease in Ω_{ara}, and therefore CO_3^{2-} was demonstrated to negatively affect coral calcification (Smith and Buddemeier 1992). Gattuso et al. (1999) wrote the first exhaustive review of calcification and photosynthetic mechanisms on corals from the perspective of the relatively new threat to coral reef health. Based on IPCC estimates of pCO_2 increases and the few studies published until 1999 on this

topic, a calcification rate decrease by 10% from 1880 to 1990 was measured and a further decrease by 9 to 30% between 1990 and 2100 was predicted. These estimations were extrapolated from four studies on coralline algae, and three others on coral reef mesocosm (references in Gattuso et al. 1999). Although the Authors supported the hypothesis that calcification is controlled by seawater Ω_{ara} and that experimental evidence suggests a severe drop in calcification rates in the future, they admitted that experimental data were not sufficient to provide an accurate prediction of future scenarios. Further, Kleypas et al. (1999), Gattuso and Buddemeier (2000), and Langdon (2000) used the same dataset on projected changes in the seawater Ω_{ara} to predict a 17-35% decrease in the reef calcification over the next century. These Authors concluded that "*several studies have shown that calcification rates of reef-building corals and coralline algae are depressed by increased levels of* CO_2…", and that "*the decreased saturation state could result in reduced calcification rates*".

Based on these experimental pieces of evidence scientists posted an alarming message for the future of coral reefs face to OA (Hoegh-Guldberg et al. 2007; Erez et al. 2011). Rates of calcification in scleractinian corals will drastically decrease by the end of this century as a result of reduced seawater carbonate concentration due to a doubling of preindustrial levels of atmospheric CO_2 (Kleypas et al. 2006). Furthermore, a 40 to 83% decline in reef calcification (Langdon et al. 2005) and a global shift in coral reefs from net accreting to net dissolving was predicted by the year 2065 (Silverman et al. 2009). For example, a study conducted by Jury and Thomas (2001) examined the effects of reduced pH on the calcification rates of *Porites astreoides*, a common Caribbean coral species. The researchers found that decreasing the pH of seawater leds to a significant decline in coral calcification rates. Cohen and colleagues (2009) found that reduced pH levels reduced the calcification rates of the reef-building coral *Porites astreoides*. Similarly, a study by Albright et al. (2018) demonstrated that lower pH levels decreased calcification rates and altered the skeletal structure of the coral *Porites cylindrica*. The impacts of pH on coral calcification are further compounded by other stressors, such as increasing seawater temperature and nutrient pollution, which can interact synergistically with ocean acidification. However, evidence for a consistent relationship between Ω_{ara} and coral calcification was not always well supported and disagreed with the findings of Lough and Barnes (2000) who did not detect a long-term (1903-1979) decrease in calcification in cores of the long-lived coral *Porites* collected on the Great Barrier Reef. In addition, Atkinson et al. (1999) reported long-term high growth rates for 57 species of coral growing in relatively high-nutrient, very low-pH seawater (ca. 7.6) which indicates that corals can continue to calcify under extremely low CO_3^{2-} concentrations.

After more than 20 years of intense experimental research on the field of OA, it seems that the consensus on the effect of OA on coral reef calcification has persisted until very recently, even though several studies have demonstrated past misconceptions and method biases, and several species have been shown to resist the harmful effects of OA. For example, cold-water corals thrive well and without decreasing their calcification rates near the aragonite saturation horizon (i.e. $\Omega_{ara} = 1$) (references in Rodolfo-Metalpa et al. 2015); some temperate species seem to calcify at normal or even higher rates at acidified seawater (e.g. Rodolfo-Metalpa et al. 2011; Ries et al. 2009); several studies have shown high variable responses of tropical species, including no effect (e.g. Houlbréque et al. 2012; Reynaud et al. 2003).

Is coral calcification impaired by OA? Will coral reefs dissolve as claimed? The increasing knowledge acquired during the last years, especially on the calcification mechanisms, demonstrated that commonly reported predictions on the detrimental effects of OA – reduced calcification and enhanced dissolution – may have to be revised (Roleda et al. 2012).

Here, I revise the consensus on the effect of OA on the coral calcification and dissolution rates in the light of some milestones studies (e.g. Venn et al. 2012, 2011; McCulloch et al. 2012a,

2012b; Comeau et al. 2015a, 2015b, 2014a, 2014b, 2013; Jokiel 2011a, 2011b; Jokiel et al. 2014; Murillo et al. 2014; etc) which have greatly improved our knowledge on coral reef responses to OA, and very recent metanalyses (e.g. Leung et al. 2022; Connell and Leung 2023). Throughout this study, the deleterious effect of warming will be only partly mentioned but it is clear that predicted temperature increases during this century have the potential to greatly affect coral reefs in combination or not with acidification. While the exact effects of OA on the main physiological mechanisms are still subject to debate, there are no doubts that coral reefs are particularly sensitive to temperature increases and that warming will be lethal for most species (e.g. Rodolfo-Metalpa et al. 2011).

3.2. Effect on calcification rates at the organismal scale. Is coral calcification impaired by OA?

One of the main physiological expenditures affected during a period of stress that defines a coral's health status is its somatic growth. Coral calcification is a vital process that plays a crucial role in the growth and survival of coral reefs. It refers to the ability of corals to build their calcium carbonate skeletons, which form the structural foundation of the reef. Maintaining a high rate of coral calcification is crucial for the growth and resilience of coral reefs, providing them with structural integrity and protection from physical disturbances. Calcification is tightly linked with photosynthesis (i.e., light-enhanced calcification; Gattuso et al. 1999), and to the efficiency of certain Symbiodiniaceae to translocate photosynthetic compounds to their hosts (Cooper et al. 2011). Understanding the role of the different carbonate forms (CO_2, HCO_3^-, CO_3^{2-}) on calcification mechanisms, and metabolism in general, is essential to establish informed predictions about future OA impacts on coral reefs. A poor understanding of the calcification mechanisms involved has impaired progress in this debate. Even the fundamental question of which source of inorganic carbon is favoured by corals for their mineralization remains partially unanswered (Allemand et al. 2011). The process of coral calcification involves the uptake of dissolved inorganic carbon (DIC) from the surrounding water, conversion of DIC into bicarbonate ions, and precipitation of calcium carbonate crystals within the coral's tissues (Cohen and McConnaughey 2003). This regulation is critical for the proper functioning of cellular processes involved in calcification, and is strongly related to the coral's ability to maintain a range of internal pH. Evaluating the roles of CO_2, CO_3^{2-}, and HCO_3^- in supplying dissolved inorganic carbon (DIC) for corals calcification poses a significant challenge to progress in this field of investigation, notably in terms of describing calcification mechanisms upon which further studies of OA can be based, and determining the response of corals to OA. To date, several hypotheses have been proposed concerning the origin of the DIC necessary for calcification but they are still matters of controversy. It seems that most of the studies have taken for granted that the formation of $CaCO_3$ would result from the combination of Ca^{2+} and CO_3^{2-} dissolved in seawater. Since calcification and dissolution rates of marine species are related to seawater carbonate chemistry, empirical relationships between Ω_{ara} or $[CO_3^{2-}]$ and $CaCO_3$ deposition have been used to predict future calcification and dissolution rates in response to OA (e.g. Erez et al. 2011; Kleypas et al. 2006; Kleypas et al. 1999). Since the Ca^{2+} concentration is near conservative in seawater, Ω_{ara} is largely determined by $[CO_3^{2-}]$ in seawater, which in turn is one of the building blocks of calcium carbonate (Gattuso and Buddemeier 2000).

Nevertheless, at least three main reactions might explain the formation of $CaCO_3$:

$Ca^{2+} + CO_3^{2-} = CaCO_3$ (1)

$Ca^{2+} + HCO_3^- = CaCO_3 + H^+$ (2)

$Ca^{2+} + CO_2 + H_2O = CaCO_3 + 2H^+$ (3)

It is rare that bicarbonate ions (eq. 2) or carbon dioxide (eq. 3), which can move freely through cell membranes and are available from the photosynthetic process, have been proposed as a substrate for calcification in OA studies. Re-examination of the past literature shows that eq. 1 may practically not contribute to the $CaCO_3$ formation (Allemand et al. 2011) because the $[CO_3^{2-}]/[HCO_3^-]$ ratio at physiological pH (between 7.5 and 9.0 pH units) is extremely low (Venn et al. 2009).

It is known that corals accrete their $CaCO_3$ structures in a fluid-filled medium called subcalicoblastic medium (Allemand et al. 2011) underlying the calicoblastic epithelium which is the calcifying tissue. In the organism's calcifying space, an elevation of more than 1 pH unit compared to ambient pH makes the calcification possible (Allemand et al. 2011; Trotter et al. 2011; Venn et al. 2011). Internal pH is elevated by the activity of the antiport Ca^{2+}/H^+ ATPase, which transfers Ca^{2+} to the site of calcification in exchange of protons. The exchange of H^+ drives the equilibrium toward $CaCO_3$ formation converting HCO_3^- to CO_3^{2-} and thereby increasing the saturation state (Ω) in the calcifying fluid (Cohen and McConnaughey 2003). Using different approaches, Trotter et al. (2011), McCulloch et al. (2012a), and Venn et al. (2011) have nicely shown that some tropical, temperate and deep–sea corals maintain strong gradients of pH between the surrounding seawater and the internal calcifying coral space over a range of seawater pH values, including end-of-century projected values. Therefore, carbonate precipitation in calcified structures does not directly originate from water carbonate but is generated or modulated via several reactions from HCO_3^- and/or CO_2 in the alkaline compartment at calcification rates. The way the two other DIC forms (CO_2 and HCO_3^-) might be delivered to the calcification sites and used to calcify has been extensively discussed (Allemand et al. 2011; Jokiel 2011a, 2011b), and their direct effects on the calcification rates have been proven for some species (e.g. Comeau et al. 2013; Jury et al. 2010; Marubini et al. 2008; Herfort et al. 2008; Schneider and Erez 2006).

In a first attempt to tentatively separate the contribution of the various components of the seawater carbonate system, corals were incubated in seawater where carbonate parameters were manipulated by adding acid, bases or both. Some studies (e.g. Schneider and Erez 2006) showed an enhancement of coral growth after an increase in DIC, suggesting that the ambient DIC concentration of seawater may limit the calcification rates of hermatypic corals. For that, Marubini et al. (2008) separately manipulated the concentration of HCO_3^- and pH to investigate the physiological mechanisms underlying coral calcification. They found that *Stylophora pistillata* grew faster in bicarbonate-enriched seawater independent of pH conditions (pH 7.6 to 8.2) suggesting that calcification was C-limited. Although CO_3^{2-} is the ion species deposited at the calcification site, it seems clear that such deposition at the site of calcification depends on biologically mediated HCO_3^- transport from the external medium. Corals can obtain the carbon for calcification from HCO_3^- in the seawater or from metabolically produced CO_2 (Furla et al. 2000). Carbonic anhydrase located in the cytoplasm of the coral converts HCO_3^-, which is the majority of host intracellular DIC (Venn et al. 2009), into CO_2 and HCO_3^-, which are then used for photosynthesis and calcification, respectively. Manipulating HCO_3^- concentration at constant pH (8.2) on the corals *Acropora* sp. and *Porites porites*, Herfort et al. (2008) confirmed the role of HCO_3^- in stimulating calcification both on corals exposed to light and in the dark, not only by stimulating photosynthesis. However, in this experiment, HCO_3^- and CO_3^{2-} covaried and their effect could not be separate. This was done by Jury et al. (2009) who found that the calcification of the coral *Mandracis mirabilis* not only was unaffected by OA but was enhanced by adding HCO_3^-. Comeau et al. (2013) designed a manipulative experiment to test the roles of HCO_3^- and CO_3^{2-} on the calcification of the coral *Porites rus* and the crustose coralline algae *Hydrolithon onkodes* in the light and dark. The Authors demonstrated that both forms are involved in calcification, and new conceptual models of calcification were proposed.

By measuring skeletal boron geochemistry, Allison et al. (2014) confirmed that for the massive *Porites* spp. HCO_3^- mainly contributes to the DIC pool used during calcification.
Therefore, calcification does not seem to always depend on the concentration of seawater carbonate ions as previously assumed (reviewed in Erez et al. 2011). Roleda et al. (2012) pointed out that although a comprehensive understanding of coral calcification mechanisms was puzzling at the beginning of the OA research era, there was enough knowledge to assert that calcification in corals is biologically modulated and not simply the combination of CO_3^{2-} and Ca^{2+} in a seawater medium. It seems that pioneering OA studies overlooked state-of-the-art knowledge to explain, more simplistically, how the decrease in the CO_3^{2-} and Ω_{ara} resulted in the observed decrease in coral calcification rates.

So why several studies have shown that at the scale of individual organisms, coral calcification rates were affected by high or even moderate decreases in pH, CO_3^{2-} or Ω_{ara}? Is coral calcification impaired by OA?
It is important to note that studies have used a range of physical and chemical conditions which might have resulted in different results, and that the sensitivity of corals to OA is more species-specific than previously assumed. Pandolfi et al. (2011) and Edmunds et al. (2012) reviewed the possible reasons for this high variability among studies and species. In general, they found that the variance in the response to high pCO_2 may be attributed not only to specific physiological mechanisms and differences among coral species (fast vs. slow-growing corals, e.g. Rodolfo-Metalpa et al. 2010), but also to the limitations of experimental methodologies (e.g. manipulation of seawater chemistry), the experimental duration, the experimental setup (water flux, light irradiance, e.g. Comeau et al. 2014b), and the origin of the samples (e.g. samples collected at reef flat where pH variations vary more in than other habitats, Comeau et al. 2014c). A recent meta–analysis exercise on the sensitivity of coral calcification to changes in Ω_{ara} (Chan and Connoly 2013) showed that responses to OA may be less severe than previously suggested. Their analysis suggests that large between-study variability was not explained by different manipulations of seawater chemistry, nor by the coral calcification rates or the culturing irradiance levels. Interestingly, the Authors found that studies measuring calcification via the alkalinity anomaly method (see below) found significantly larger decreases in calcification than studies using buoyant weighing, likely because the latter integrates both light and dark calcification, and it measures the whole coral growth.
To explain the paradigm between the observed reduction in calcification under conditions of increasing OA and the uncertain role of the different DIC components, Jokiel (2011a, 2013) did not investigate the coral calcification process as previous investigators did, therefore trying to understand which components corals require for calcification, but from the end of the process: which wastes corals need to remove to allow calcification. The Author formulated an elegant story, the "Proton flux hypothesis" where coral calcification is regulated by the net efflux rate of H^+ produced during the calcification process (equation 2), from the coral tissue into the surrounding water. Corals are separated from seawater by a boundary layer, called diffusion boundary layer (DBL), the strength of which influences H^+ removal, as well as other coral metabolic waste products. Since the increasing OA causes an increase in the seawater H^+ concentration, the gradient becomes less favourable reducing the flux of protons out of the coral and decreasing its calcification rate. The model reveals that HCO_3^- is the substrate for calcification, obtained either directly from seawater or derived from mitochondrial CO_2 that is converted by carbonic anhydrase into HCO_3^-. The Proton flux hypothesis was further integrated into the "two-compartment proton flux model" (Jokiel 2011b) in which the Author unified existing theories and paradoxes of coral biology in a milestone contribution. Since photosynthetic and calcification processes compete for available inorganic carbon in corals (Gattuso et al. 1999), they developed a morphology which separates the zone of rapid

calcification (ZC) from the zone of rapid photosynthesis (ZP), with ZC situated between the ZP, the diffusion boundary layers (DBL) and the external seawater. Within the DBL, the transport of ions and gases to and from the organism's surface is limited by diffusion, which is, in turn, dependent on the concentration gradient across the DBL. Among the advantages for coral metabolism, this spatial arrangement allows a rapid translocation of the fixed-carbon energy supply from the ZP to the ZC and the efflux of protons out of the coral, therefore regulating calcification and photosynthesis. Furthermore, Jokiel (2013) used data from Comeau et al. (2013) to test the Proton flux hypothesis asserting that CO_3^{2-} and Ω_{ara} have no basic physiological meaning for calcification. Instead, calcification correlates well with the ratio of DIC to proton concentration ([DIC] : [H^+]).
A step forward is the contribution of Hohn and Merico (2015) which improved previous mathematical models for coral calcification suggesting that the combination of transcellular ion transport and paracellular pathway is the most likely mechanism for coral calcification at a reduced energetic cost. Their model indicates that the transcellular pathway would favour the carbon transport across the coral cells via free diffusion of CO_2 over cell membranes, while paracellular diffusion would assure seawater exchange at the calcifying fluid directly. Their conceptual model calculations reveal that the majority of carbon (ca. 98%) enters the calcifying fluid via diffusion of the metabolic CO_2, while only 0.8% is transported actively as bicarbonate. Carbon dioxide which can subsequently react with H_2O to form HCO_3^- and H^+ (catalyzed by the enzyme carbonic anhydrase) could therefore be an alternative inorganic carbon source for calcification in particular taxa.
Hohn and Merico (2015) also evoked a key question regarding the cost of compensating pH differences under OA conditions, therefore in maintaining or not calcification rates (Erez et al. 2011; Pandolfi et al. 2011). This is highly energy consuming (Allemand et al. 2011) with an estimated increase in the metabolic cost of ca. 10% per 0.1 pH unit decrease in seawater pH (McCulloch et al. 2012b). If calcification becomes energetically more costly under elevated pCO_2 due to a decreased Ω_{ara}, then the extra energy needed to maintain calcification might be drawn by increasing feeding rates and/or drawing upon energy reserves. This assumption is particularly important for aposymbiotic corals such as cold-water and deep-sea corals which are uniquely heterotrophic. However, in general, the few studies performed so far did not find decreases in the cold-water coral calcification rates, neither their lipid reserves nor an increase in their respiration rates (reviewed in Rodolfo-Metalpa et al. 2015). McCulloch et al. (2012a) calculated that a decrease in seawater pH from 8.1 to 7.7 units has an extra cost for symbiotic reef-building corals to calcify, which however is insignificant (<1%) when related to the total energy produced by their photosynthesis. Accordingly, Schoepf et al. (2013) found that the energy reserves of four tropical species did not decline with increasing OA during experimental manipulation in aquaria, suggesting that energy reserves do not play a role in sustaining calcification under OA conditions, or that the increased energetic costs of maintaining calcification under OA are relatively insignificant.

3.3. Effect on the reef net community calcification. Will coral reefs dissolve by future OA levels?

Among the few studies used in a first attempt to define the emerging threat of OA on coral reef calcification, some were conducted at the community scale, therefore considering the whole habitat formed by species and substrates (references in Langdon et al. 2010, and Gattuso et al. 1999). These studies used experimental mesocosms where a portion of coral reef was reproduced in large aquaria and the conditions expected in the future were simulated. It is important to note that such a kind of approach has been fundamental to assessing the effect of climate change on coral reefs since it might provide critical information regarding the potential interactive effects of the major biological and environmental parameters in a more realistic and

ecologically relevant setup. Mesocosm experiments integrate a panel of organisms, natural environmental parameters and their variability, as well as food availability and types of substrates to simulate the natural environment. Although its importance, a limited number of studies have been conducted so far at the ecosystem scale using experimental mesocosms (e.g. Comeau et al. 2015a, 2015b; Andersson et al. 2009), likely due to the methodological complexity in reproducing the structure and diversity of benthic communities and in maintaining such systems. While results from experiments conducted on individual corals have shown variable responses ranging from no effect (e.g. Reynaud et al. 2003) to negative (e.g. Erez et al. 2011), and even positive effects (e.g. Rodolfo-Metalpa et al. 2011), in general, studies on the effect of OA on the whole reef community have consistently demonstrated negative effects on the coral net community calcification rates (NCC). For instance, Leclerq et al. (2000) found that the NCC at the Monaco mesocosm was a linear function of Ω_{ara} and that it decreased by 21% in response to acidification. In a long-term study, although individual corals remained healthy and actively calcified, Andersson et al (2009) found a decrease in NCC and net dissolution in response to OA levels expected at the end of this century.
Mesocosm experiments have greatly strengthened the consensus in the OA literature that calcification is inhibited through a reduction in seawater [CO_3^{2-}], and thus Ω_{ara} (Kleypas et al. 2006). Similarly, measurements of the daily natural variability in carbonate chemistry over coral reefs have strengthened the use of Ω_{ara} as one of the main factors controlling the reef NCC, and results have been used to get reliable global-scale predictions. Metabolic and physical processes taking place in a reef, at least for those located in semi-closed lagoons and shallow back reef habitats alter the carbonate chemistry of the surrounding seawater sometimes reaching levels expected in the future. Therefore, the relationship between natural variability in seawater carbonate chemistry and reef NCC can be used to predict future responses to OA (Shaw et al. 2012).
In general, results from natural environments, experimental mesocosms and model prediction have shown that there is a strong positive coupling between seawater Ω_{ara} and the reef NCC (Silverman et al. 2009). Under expected acidification levels, daily NCC will be significantly lowered relative to the rate and deposition of $CaCO_3$ at ambient conditions, and net dissolution at very low Ω_{ara} will be most likely, which means that NCC of many reef systems could become negative because the production of $CaCO_3$ will be exceeded by its dissolution (Langdon 2000; Andersson et al. 2009). Based on the observed relationship between Ω_{ara} and reef calcification, on average, community calcification was predicted to decrease by 60% per unit decrease in Ω_{ara} with values ranging from about 15% to 130% (references in Andersson and Gledhill 2013).

But, if it is true that coral calcification is not always affected by OA as previously thought, why several studies have shown that at the reef community scale coral calcification rates were affected by a decrease in CO_3^{2-} or Ω_{ara}? Will coral reefs dissolve as claimed?
It is important to note that NCC rates have been estimated based on measurements of carbon chemistry in open-flowing water over natural reef communities, mesocosms or flumes. This method assumes that, although a community cannot strictly be described as exhibiting a metabolism per se, it is possible to describe the integrated performance of the ecosystem in terms of the rates at which metabolically relevant chemical species (e.g. DIC, A_T) are modified within the overlying water column. Recent estimates of NCC based on community metabolism from different reef environments yield a broad range of rates, from 22 mmol $CaCO_3$ m^{-2} day^{-1} to 331 mmol $CaCO_3$ m^{-2} day^{-1}. These large discrepancies reflect differences in the reef types, hours of the day, seasons, reef structures and their compositions. The first potential pitfall of this method is that the reef calcification rates are calculated from changes in water-acid base status through the alkalinity anomaly technique (Chisholm and Gattuso 1991) which assumes that for every two moles of total alkalinity (A_T) consumed one mole of $CaCO_3$ is produced. The

reverse reaction occurs during dissolution. Although this technique has been validated for short-term incubations (Chrisholm and Gattuso 1991) and low-size organisms (Langdon et al. 2010), interfering metabolic and acid-base regulation processes cast some doubt on the absolute rates determined. This is especially true when this technique is used to measure the calcification of organisms with large biomasses which excrete significant metabolic wastes, likely affecting A_T values and thereby reef NCC. Other coral reef processes such as organic matter production from photosynthesis, anaerobic diagenesis in sediments and microbial activity, as well as nutrient transformation (references in Murillo et al. 2014 which recently revised the method) might affect A_T. More importantly, when applied to assess the whole reef calcification, this approach integrates the calcification response of all species composing the reef and includes the effect of dissolution of all the biogenic substrates and their bioerosion to the reef NCC. Indeed, at the community scale, it is almost impossible to separate the contribution of dissolution from net calcification, because it is impossible to measure the reef gross calcification. In contrast, at the organism scale the effect of dissolution on the net coral calcification can be measured by the difference of the gross and the net calcification rates. Labeling experiments with the radio-isotope ^{45}Ca in aquaria is the only technique likely to provide measurements of gross calcification, and it has been applied for the first time to test OA effect on the calcification of two temperate corals: *Cladocora caespitosa* and *Balanophyllia europaea* (Rodolfo-Metalpa et al. 2011). While the net calcification rates of *C. caespitosa*, which has skeletal parts that are exposed to the surrounding seawater, significantly decreased at $pH_T < 7.8$ and the coral suffered severe skeletal dissolution, *B. europaea,* which has a skeleton completely covered in tissue, was not affected. By measuring both the net and the gross calcification, it was possible to show that remarkably both corals were able to maintain gross calcification rates at pH levels expected to the end of this century and beyond. These results suggested that coral tissue protects the underlying skeleton from dissolution under OA conditions as recently confirmed for the reef-building coral *Stylophora pistillata* (Tambutté et al. 2015). By measuring the gross calcification of *S. pistillata* held in aquaria at pH_T 7.8, Houlbréque et al. (2012) found no effect of OA. This is in agreement with Cohen and Fine (pers. comm.) which did not find differences in the net and gross calcification rates of nubbins of *S. pistillata* held for 16 months at pH_T 7.6. In another study, Rodolfo-Metalpa et al. (2015) did not find differences in the net, gross calcification and dissolution rates of the cold-water coral *Desmophyllum dianthus* held at pH_T values between 8.1 and 7.7 units. These are only some examples showing that some corals can calcify under OA conditions and that the dissolution of the exposed skeletal structures might be more important than the effect on calcification, according to the protective role played by tissues in preventing dissolution. Although it would be speculative to extrapolate organism-based responses to whole-ecosystem responses, it is evident that skeletal dissolution and bioerosion have an important contribution to the observed decrease in reef NCC subjected to OA conditions. This has been shown by Shamberger et al. (2014), who found relatively high coral cover and diversity on Palau's low-pH bay reef. Furthermore, Barkley et al. (2015) showed that while coral calcification rates at this site were unaffected by OA, coral bioerosion increased 11-fold as pH decreased.

Because reefs are made of diverse habitats with various mineral compositions and benthic communities, calculating NCC from AT variations does not allow distinguishing which reef habitats contribute the most to the final net dissolution (e.g. sediments, corals, macroalgae). This method, for instance, cannot decipher the importance of calcification vs. dissolution rates of the different species and substrates forming a reef. It is likely that the calculated tipping point at which reef carbonate production shifts from net calcification to dissolution (e.g., Andersson et al. 2009; Silverman et al. 2009) is mainly dependent on the contribution of the different reef habitats. Reefs with predominant fractions of sediment, coral pavement (i.e. cemented $CaCO_3$ substratum covered by an assemblage of turf and coralline algae) or macroalgae are important

components of reef systems (e.g. lagoon, backreef, forefront). Under natural conditions, and especially at night, when metabolic respiration drives a decrease in the seawater Ω_{ara}, reef dissolution is indeed most attributable to processes occurring within the carbonate sediments. Under low Ω_{ara} conditions, Andersson et al. (2009) found that most of the dissolution most likely occurred in the thin sediment layer present at the bottom of their mesocosm. Murillo et al. (2014), measuring the NCC of different reef components in flume experiments, suggested that the total alkalinity technique was only valid for coral-dominated environments. When sediments and algae were included, the ratio between A_T and Ca^{2+} deviated from the theoretical 2.0 value, which means that these other substrates largely contributed as sources of A_T. They found that for a mixed community (i.e. algae, sediment and corals) the A_T method will estimate a lower net calcification than for a coral-only community due to A_T recycling. The importance of the sediment fraction in the observed decrease of the reef NCC rate has been further confirmed (Eyre et al. 2014). In other flume experiments, NCC was reduced by 59% and 49% (Comeau et al. 2015a and Comeau et al. 2015b, respectively) under OA conditions. In these studies, the sediment and the reef pavement dissolution explained respectively ca. 50% and 78% of the observed decreases. Using NCC data available from 233 locations including 183 reefs around the globe, Cornwall et al. (2023) investigated in a meta-analysis study the net carbonate production rates and responses of coral reef taxa calcification and bioerosion rates to predicted changes in coral cover driven by climate change by 2050 and 2100. The authors found that future scenarios will cause a net decrease in calcification and an increase in reef erosion. These declines are largely the result of reduced coral cover from bleaching events rather than from the direct impacts of ocean warming or acidification on calcification or bioerosion.

Another fundamental caveat in predicting responses of whole reef ecosystems to a change in the seawater chemistry is that the assumed linear relationship between the NCC and Ω_{ara} is actually altered by the reef photosynthesis and respiration rates, which greatly vary among reefs and all over the daily cycle. Strong relationships between the net reef community production (NCP) and NCC have been found (e.g. Jokiel 2014; Andersson and Gledhill 2013; Cyronak et al. 2013; McMahon et al. 2013) leading to a diel hysteresis pattern in the NCC *versus* Ω_{ara}. Jokiel (2014) measured during daily cycles seawater carbonate chemistry, NCC and NCP in mesocosms containing mixed components of the reef (coral, algae and both the components). Results showed that changes in seawater Ω_{ara} are a consequence of changes in both the reef NCC and NCP rather than a driver of NCC. These results have important implications because if further confirmed, they would invalidate most of the predictive models which assume that the reef NCC is controlled by seawater Ω_{ara}.

3.4. Putting my contribution into the OA debate

What has been surprising during at least the first 15 years of research into the OA debate is that studies showing "different" responses to OA, thereby deviating from the consensus, have timidly increased in number but substantially they did not contribute to changing the message. This is not to say that OA won't affect coral reef calcification, structure integrity and diversity, but the effect will likely be less dramatic than it has been predicated so far. Curiously, during this intense phase of research, which was uniquely focused on the effect of one factor, OA, we all forgot that the future will be the combination of at least two factors, OA and warming, the latter for sure the most dangerous.

My research initially contributed to the consensus but quickly deviated toward a more nuanced effect or no effect at all. Indeed, two first studies (Hall-Spencer et al. 2008; Martin et al. 2008) evaluated the intertidal and seagrasses habitats exposed for centuries to acidified seawater emitted by shallow water CO_2 seeps in the Mediterranean Sea. Our data revealed a shift in communities, with the reduction of important organisms such as sea urchins, and limpets and the absence of scleractinian corals under lowered pH conditions. Coralline algae, which play a

crucial role in seagrass ecosystems, disappeared in acidified conditions, leading to a significant reduction in epiphytic calcium carbonate. These findings underscored the sensitivity of calcareous organisms to elevated pCO_2 levels, and in agreement with most of the studies published from mesocosm and lab-based experiments, highlighted the potential consequences of OA at the ecosystem scale. I might honestly recognise, thanks to my research on other CO_2 seeps in Papua New Guinea, that one of the most common criticisms in using such a natural system to investigate the effect of OA is that the effect is exacerbated by the CO_2 variability. Indeed, we found that the "tipping point" in the pH causing the shift between calcareous to non-calcareous algae around pH 7.83, but it is possible that the shift was caused by the high variability of the pH at the study site with values lower than 7.4. These undersaturated seawater likely caused the observed dissolution of shells and other carbonatic tests. That said, on the one hand, this study was honest in the data presentation and its limits, in the other hand it was the first evidence of the danger that OA might be for a whole ecosystem. For that reason, other CO_2 seeps, almost in the tropic have been further used in the same manner reinforcing the consensus on the effect of OA on coral reefs (see Fabricius et al. 2011, for an example).

However, in Rodolfo-Metalpa et al. (2010), my first study on the effect of OA level predicted to 2100, alone or in combination with a +3°C temperature, did not give the expected results, since we found that both short- and long-term incubation do not fundamentally affect the calcification rate (and the metabolic rates) of the Mediterranean coral *Cladocora caespitosa*. This result was surprising. It introduced a new dimension to the topic by demonstrating that not all organisms respond in the same way to increased pCO_2 levels. Some species, particularly those with slow growth rates, may not exhibit reduced calcification rates. It emphasized that factors like seasonal temperature changes play a significant role in determining the response of organisms to OA, and the impact may not be as widespread as previously thought. In the meantime we finalised this paper, the most famous study by Ries et al (2009) was published, showing similar results on the coral *Oculina arbuscula*, and other calcifiers. One of the biases of my study was that the experimental setup was done to study Mediterranean CCA living at 20-30 m depth, and the temperatures we tested were relative at this depth and not to the upper surface where MHWs threat ecosystems. While *C. caespitosa* lives from 40 m depth to the first 5 m depth, most of them are common in the first 10-15 m and, therefore subjected to higher temperatures (and increasing with the ongoing warming), especially during summer. For that the higher temperature tested in my study, 25.4°C was realistic only for the deep population, and useless to test the combined effect of warming and OA. Although the bias, this study was my first research showing that OA responses are more complex than commonly thought.

In a further study (Rodolfo-Metalpa et al. 2010, Marine Ecology) I highlighted the importance of considering the simultaneous effects of acidification of temperature, this time using an unexpected heat wave during an *in situ* transplantation experiment. Live and dead colonies of the branched bryozoan *Myriapora truncata* were transplanted to normal (pH 8.1), high (mean pH 7.66, minimum value 7.33) and extremely high- CO_2 conditions (mean pH 7.43, minimum value 6.83) at CO_2 vents off Ischia island (Tyrrhenian Sea, Italy). The net calcification rates of live colonies and the dissolution rates of dead colonies were estimated by weighing after 45 days (May-June 2008) and after 128 days (July-October). Data helped to assess if high CO_2 levels affect bryozoan growth and survival differently during moderate and warm water conditions. It seems that *M. truncata* can maintain its calcification rate when exposed to quite low seawater pH but at normal seasonal temperature, but when combined with high temperature, their combination can lead to the mortality of organisms that initially seemed resilient to OA. This suggests that climate change factors, including rising temperatures, can exacerbate the challenges posed by OA and need to be taken into account in predicting future responses.

My previous study using the bryozoan *M. truncata* opened the question about the ability of some species to calcify even at very low pH but, at the same time potentially have their skeleton dissolved because of the lower aragonite (or calcite) saturation state value of seawater. In the previous study, I calculated the gross calcification simply by adding the amount of skeleton being dissolved to the measured net calcification, which is quite inaccurate. In contrast, in Rodolfo-Metalpa et al. (2015, Nature Climate Change) I measured both net and gross calcification on transplanted corals, mussels and limpets. This study explored the protective mechanisms employed by some calcifying organisms to limit skeleton dissolution when exposed to acidic conditions. It showed that certain species when exposed to high CO_2 levels, could calcify and grow even faster than under normal conditions, and suggest that this is a mechanism they use to repair their skeleton. However, as pH levels further drop, the dissolution of shells and skeletons became a risk, underscoring the significance of tissues and organic layers in protecting these structures from corrosive seawater. Our transplantation experiments show each of the species we examined was able to calcify (i.e. gross calcification) and some calcified faster at pH values well below those projected for global surface waters by 2100. Their tolerance to acidification depended on their ability to maintain this protection at elevated levels of CO_2. We showed that although some organisms can up-regulate calcification at lowered carbonate saturation states, they rely on protective organic layers to avoid dissolution. Therefore, projected levels of OA are likely to increase erosion of unprotected biogenic carbonate structures. Worryingly, the coastal calcifiers that we transplanted along natural CO_2 gradients were more vulnerable to the effects of OA when the water was warmest, indicating that OA will likely exacerbate the mass benthic mortality events that have been recorded with increasing frequency in the warming Mediterranean Sea. These corals showed no significant impact on calcification rates under expected pCO_2 levels for 2100. The findings emphasize the importance of considering multiple factors, including temperature and protective mechanisms, when assessing the vulnerability of calcifying organisms and ecosystems to future changes in ocean chemistry.

In another study (Rodolfo-Metalpa et al. 2015, Global Change Biology) we provided insight into the resilience of cold-water corals to OA. There were concerns that cold-water corals are even more vulnerable as they live in areas where aragonite saturation (Ω_{ara}) is lower than in the tropics and is falling rapidly due to CO_2 emissions. However, most of what we know about cold-water coral (CWC) and deep-sea coral (DSC) metabolism was measured during artificial conditions in the lab, since *in situ* data on the physiological response of CWC to environmental variations are logistically hard to get. This certainly has hindered our ability to project their fate in the face of rising CO_2 levels. As a result, it is possible that most of the data published on the effect of OA on those corals could merely be biased by the nutritional diet given during the experiment in aquaria since nothing is known about their diet, and because it has been proven that nutrition affects the performance of organisms ("Fed corals are happy corals": source F. Houlbreque). So, in this experiment, three species of CWC where maintained and artificially fed in aquaria, and their net and gross calcification measured. To test for the effect of these artificial conditions, the only way was to transplant the corals in their natural environment, both at normal and low seawater pH. The only site that was suitable for this kind of experiment was the Ischia CO_2 seeps site where I transplanted one species of them (i.e. *Desmphyllum dianthus*). Since Ischia is an unnatural site for these corals, because it is too shallow, I transplanted the same species (plus two more) also at 300 m depth, where the pH was normal and most importantly the corals could benefit of a natural nutrition. I found that the three coral species had no change in their gross calcification under OA conditions. The species maintained at 300 m had the same calcification rate as its counterpart maintained at low pH in Ischia at very low pH conditions. This study confirmed that one must consider the results obtained in aquaria with caution, especially when the nutrition of the studied organism is unknown. Furthermore, both

aquarium and *in situ* results confirm that acidification does not have a dramatic effect on coral growth.

Further fieldwork observations made at the CO_2 seeps in Vulcano strengthen the hypothesis that some species already know how to counteract future levels of OA. Near the CO_2 seeps at very low pH water, and buried in the sediment where the pH is even lower than in the surrounding seawater, I found shells of two species of tiny gastropods quite dissolved by the undersaturated seawater but still alive. In this study (Rodolfo-Metalpa et al. 2015, Nature Climate Change) we showed that i) two gastropod species adapted to acidified seawater at shallow water CO_2 seeps were smaller than those found in normal pH conditions; ii) they consistently grew less than at the control pH, and iii) they had higher mass-specific energy consumption but significantly lower whole animal metabolic energy demand. These physiological changes allowed the animals to survive, although at a reduced size, and to maintain the calcification mechanism necessary to partially repair shell dissolution. These observations of long-term chronic effects of increased CO_2 levels support the hypothesis that OA contributed to past extinction events and suggest that some species could adapt through dwarfing (i.e., the "Lilliput effect") conferring physiological advantages as the rate of CO_2 emissions continues to increase.
Although most of my studies showed limited effect of OA on some species, i) they were valid only for Mediterranean species; ii) they mixed observations made at unique sites with high pCO_2 variability and during lab-based, unnatural experiment conditions. Therefore my research, although innovative, could not undermine the consensus formed around the tens of amazing studies produced at the magic Normanby Island CO_2 seep, the natural laboratory that Katharina Fabricius discovered and used with professionalism and intelligence for years, in collaboration with top specialists on the effect of OA on corals and other reef organisms. The consensus was that OA would flat coral reefs and only a few winner species would survive. My findings were therefore seen as curious and interesting deviations from the truth.

From 2016 to 2019, in the framework of the project CARIOCA, I visited 3 times the famous CO_2 seeps site studied by K. Fabricius, and 4 times a new CO_2 seep in PNG, Ambitle Island. I have been slow in publishing the data we collected showing i) the higher primary production of corals from these sites (Bisceré et al. 2019; Meunier et al. 2021); ii) the winning association of the corals living at CO2 seeps with particular Diazotroph strains (Geissler et al. 2021) conferring higher thermal resistance to the host (Meunier et al. 2022). Furthemore, data collected in collaboration with M. Hoogenboom and T. Shlesinger (still to be published) demonstrated that OA only partially decreased the coral diversity and abundance and suggested that the low diversity found in Normanby could be site-specific, not a general response of coral reef to OA. While I failed to measure the growth rate of corals transplanted at these sites, using geochemical techniques such as the boron signature in the skeleton of several coral species we showed (Comeau et al. 2022, Global Change Biology) that corals from the CO_2 seeps were always able to maintain higher pH at the calcification rate, therefore to calcify, than corals from the control reef. This ability seems to give an ecological advantage to the coral since casually the species that showed a higher ability to maintain high internal pH were also the most abundant at the CO_2 seeps. In addition, by measuring seawater pH at high frequency, at each site and during each mission, suggested that corals at Normanby are subjected to sudden changes in pH when compared to Ambitle and they were exposed to a very large variability in seawater pH. Although pH averaged similarly, Normanby was more variable than Ambitle, and likely caused some of the effects previously reported.

In New Caledonia, thanks to the discovery of Bouraké, where the geomorphology of the site causes seawater to vary in pH, oxygen, temperature and other environmental and chemical parameters, I have conducted several experiments to test the effect of OA in combination with other factors on the coral calcification, and not only. The most surprising evidence at such a site is that the healthy corals living there seem to grow well at pH which regularly varies from normal to 7.3 (in total scale)! This posed the question of how they can continue to calcify at pH and saturation state for aragonite so low. In the first study, performed with colleagues from UTS (Australia; E. Camp and D. Suggett) we first characterized the environmental conditions during 2 weeks and the first measurements of the physiological response of three species of corals. Using the alkalinity technique (short incubations) we found that corals from Bouraké calcified, although less than the reference ones, and respired more, likely as a consequence of the higher heterotrophic ability compensating the higher cost of calcification. After that, I performed a reciprocal transplantation of four species between 2 stations in Bouraké and 2 outside, as a reference (unpublished) and found, using the buoyant weight technique (i.e., difference in the coral weight in the long term) that i) corals from Bouraké always grew more than their counterparts from the reference site; ii) corals from the reference sites grew better when inside in Bouraké; iii) corals from Bouraké suffered at the reference sites. These preliminary (1-year experiment) data suggested that both the Bouraké population has adapted to the environmental conditions, and that the environmental condition itself can favour their growth. To test for the hypothesis that the higher heterotrophic condition of the Bouraké lagon caused the observed higher growth rate, therefore better investigating the potential of adaptation of these corals, and also to test the effect of seawater pH variability, previously suggested to increase the corals resilience, with my PhD Clement Tanvet, we performed a 100 days experiment in aquaria testing uniquely the effect of pH (constant and variable) on 3 coral species from both Bouraké and Reference. All corals were fed in the same manner. This study confirmed my previous data on transplanted corals and added a new crucial piece to the puzzle. Indeed, i) it excludes that heterotrophy is the main factor causing the high growth in Bouraké since both populations received the same level of nutritional diet in aquaria; ii) it sheds light on the ability of corals from Bouraké to calcify at a reference site (i.e. at normal pH). Indeed, while in my first transplantation I measured a decrease in calcification of corals when transplanted from Bouraké to the reference, the same corals maintained in aquaria at normal pH (reference conditions) grew even faster; iii) it confirms that Bouraké corals have in some ways adapted to the extreme conditions since they always maintained higher growth, irrespecting the seawater pH; iv) it confirms (after preliminary results by Camp et al. 2020) that one of the mechanisms of adaptation was their diverse Symbiodiniaceae hosted in their tissue. These results were further confirmed during the PhD of Cinzia Alessi who performed a transplantation of 2 coral species from 2 reference sites in Bouraké.

If during both *in situ* observations and lab-based experiments I found variable and sometimes limited effects of OA on adult corals, one of the bottlenecks of the reef status is their ability to reproduce, settle and continuously form persistent reefs. Healthy and functional coral reef communities directly depend on their ability to release larvae, and to their recruitment success that, in turn, depends on the abundance of their preferred settling substrate i.e., crustose coralline algae (i.e., CCA). Ocean acidification has been predicted to greatly affect different stages of this natural and complex mechanism. My first study in this research field was on the ability of the coral *Pocillopora damicornis* from Bouraké to reproduce, settle and grow in the laboratory under acidified conditions (Rodolfo-Metalpa et al., unpublished). This story has been rejected by the same reviewers three times, in three different journals. I left it in a corner but it seems we have been quite unlucky. Indeed, the major concern was that we compared offspring performances of colonies from two different sites, Bouraké and one control reef, for which we do not know the whole peak planulation timing and, therefore the optimal larval phenotypes

versus suboptimal larvae. The reviewer concluded with this encouraging sentence “Further the conclusions of this manuscript come from comparing apples to oranges at the two different sites given the well-documented impact of larval release times on larval phenotype”. Well, *Pocillopora damicornis* release larvae from October to Mai, and clearly at different rates from site to site, colony to colony and year to year. It will take years for me to have a valid dataset on the peak planulation timing for the two studied sites. We aimed to assess if life-long preconditioning to a contrasting fluctuating environment can change the offspring tolerance to OA and warming during 28 days of exposure to manipulated pCO_2 (500 and 1,500 ppm) and temperature (26 and 29°C) in a full-factorial mesocosm experiment. At the end of the incubation, we measured the offspring post-settlement survival, lateral growth and budding of new polyps. Likely, this study said something the reviewer did not like, that is the lack of differences between the two populations, one adapted to extreme conditions, and one from a normal fringing reef. Our measurements confirmed the negative effect of high pCO_2 on the lateral growth and budding rates of recruits, and we found no evidence that individuals from a more variable environment gain in fitness performances and tolerance to future-like conditions. The skeletal microstructure of recruits from both environments was basically unaffected, although the elevated pCO_2 tested. We insisted on the observation that reproductive output was more important in corals from Bourake because colonies produced enormously more planulae than colonies from the reference. But we were right as we demonstrated in the study made by my PhD student Clement Tanvet on the effect of the Bouraké extreme conditions on CCA coverage settled on artificial ceramic tiles, and coral recruitment rates (Tanvet et al. 2021). In this study, we assessed the effects of extreme and fluctuating environmental conditions at Bouraké on the abundance of CCA, algal turf, and coral recruits. To address this objective, we obtained CCA-covered tiles from a forereef and moved them to Bouraké and two fringing coastal reef sites with distinct environmental conditions. Based on the reported effect of OA on CCA cover and coral recruitment rates, we hypothesized that in Bouraké both will be heavily affected by the extreme conditions. While we confirmed previous findings on the sensitivity of CCA to OA, since they decreased in coverage in Bouraké, surprisingly, we found that coral recruitment rates in Bouraké were higher than at both the two fringing reefs. Our findings contradicted results from several OA laboratory-based experiments and reinforced the idea that coral recruitment is possible even under extreme conditions and that, particularly for the Bouraké site, corals may have adapted to such variable and extreme environmental conditions. Finally, my PhD student Cinzia Alessi measured during thre years the spawning timing and fecundity of *Acropora tenuis*, *Montipora digitata*, and *M. stellata* from Bouraké and three reference sites located at variable distances from Bouraké (Alessi et al. 2023 in prep.). Interestingly, no reduction in the number of eggs per fecund polyp or proportion of fecund polyps was observed in Bouraké compared with the reference sites, in any species. However, *A. tenuis* and *M. digitata* had smaller eggs in Bouraké compared to reference sites, with possible repercussions on the development of early life-history stages and coral fitness. Cinzia also measured less coral recruits on ceramic tiles settled in Bouraké during the years 2020-2022, which is in contrast to our previous findings in Tanvet et al. (2021) during the year 2017-2019. Likely, this difference was due to the effect of the three consecutive years of La Nina in New Caledonia and the consequent massive coral mortality of corals in Bouraké, but this is another story…..

3.5. Conclusions and perspectives

OA might affect coral calcification, mainly on species that cannot regulate their internal pH and facilitate the deposition of $CaCO_3$ as some others do. OA might have an effect on sediments and reef skeletons causing their dissolution, and this will be more important if corals will die and the reef will degrade in the future. OA might affect other taxa than corals, some will take advantage of the high CO_2 levels, such as macroalgae, maybe sponges; others will be strongly affected such as plankton species. OA might have an indirect effect on the abiotic parameters that in turn regulate the complex exchanges between corals metabolism and the surrounding environment. OA might interact with other environmental parameters and combine toward a synergistic negative effect. The most prominent example is its combination with high temperature, which itself seems to be the causative factor of the next coral mass extinction.

Having said that, in light of my research and some recent analyses of the scientific output on this topic, I think that the effect of OA on calcification will be marginal on the survival of corals in the face of ongoing global warming.

An attentive reader will certainly have noticed that this memorandum, which intends to answer the question "Why should corals care about ocean acidification?" is already old. I started writing about these considerations back in 2016. Year after year I added articles without ever finding the courage to publish what should be a food for thought paper, perhaps out of fear, perhaps out of opportunism, since I have been working on this for almost 20 years.

My research has been centred on the effect of OA on corals and other organisms, especially corals, especially concerning their ability to calcify. Of course, I have studied other organisms, of course not only calcification, of course using both aquaria and natural laboratory setups, but in the end my main research has been transplanting corals and measuring their calcification. I started this approach when the theme was very hot and the expectations were dramatic since the OA issue was expected to devastate corals. Fortunately, the effect has been demonstrated to be less important than previously announced and my studies have partially contributed to improving this research field. It is worth mentioning my research demonstrating the protective role of coral tissue (and periostracum on bivalves) in preventing skeleton dissolution. Another result I consider very important was the measurement of the gross calcification of corals (therefore excluding any contribution of skeletal dissolution) I did use the ^{45}Ca technique at IAEA. For the first time, it was demonstrated that corals can calcify at the same rates under OA conditions. But what has fundamentally pushed my understanding of the effect of OA on corals was to see that actually, corals can thrive in extreme environments such as at CO_2 seeps and other natural analogues such as Bouraké and Nikko Bay. The chance I had to use these special sites has certainly improved my knowledge of the resilience of various organisms to future conditions and suggested to us the potential mechanisms used by some species to face such extreme environments. Natural analogues are imperfect systems to test for the effect of future, even worse environmental conditions on resident, likely adapted organisms. Although the potential limitations, such as the variability of the abiotic parameters, the confounding effects of other unexpected parameters, the difficulty of replicating the sites, etc, natural laboratories are the best way to improve our ability to better test for coral resilience and adaptation to climate change. By using these natural laboratories, I furnished pieces of evidence that adaptation is

likely possible. What I could not demonstrate is if the corals that were able to survive simply acclimatized or adapted to the harsh conditions. In both cases, it becomes crucial to better understand if these mechanisms are rapid or take several generations to guarantee the coral's survival under future conditions. This becomes central to give some hope to the survival of coral species and whole reef ecosystems in a rapid climate change, and to cope with the rapid on-going change. My ERC project, which was not funded, addresses this issue. The aquarium facilities I am building in Nouville will be a great tool to boost this project, despite the lack of a consistent research fund. I'll do my best for that.

The project will focus on species that occur both inside (suboptimal environment) and outside (reference environment) the natural analogue of Bouraké. My ambition is to target at least five coral species that have developed and grown in these contrasting conditions throughout their entire life cycle, possibly over several generations (which is the big ambition of this project). A multi-disciplinary experimental approach will be used that quantifies variations in physiology, genetic makeup and functional traits (i) among species, (ii) within species using colonies collected inside and outside the Bouraké system to assess long-term acclimatization and/or adaptation; and (iii) among clones and genotypes using individuals reciprocally transplanted between environments. Reciprocal transplant experiments will be carried out using clonal fragments of multiple genotypes for each species to assess their level of acclimatization vs. genetic adaptation. This project will be the first to experiment with corals that have been exposed chronically, throughout their life, to suboptimal conditions (temperature, acidification, and deoxygenation) in a natural environment. Long-term transplantation of adult colonies and their recruits will be performed in situ during transgenerational experiments, providing robust evaluations of corals' capacity for phenotypic buffering and propensity for adaptation.

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4. PREVIOUS RESEARCH

4.1. Response of the temperate coral *Cladocora caespitosa* to mid- and long-term exposure to pCO_2 and temperature levels projected for the year 2100

Biogeosciences, 7, 289–300, 2010
www.biogeosciences.net/7/289/2010/

Biogeosciences

Response of the temperate coral *Cladocora caespitosa* to mid- and long-term exposure to pCO_2 and temperature levels projected for the year 2100 AD

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This study started in 2006, during the last year of PhD but was finally published in 2010. This detail is important to correctly place the study in the growing OA literature as it was one of the first long-term experiments in aquaria testing for the combined effect of OA and OW across seasons, the first on Mediterranean corals. The study was performed in collaboration with Sophie Martin and Jean-Pierre Gattuso in the framework of the first EU project on OA, EPOCA, led by J-P Gattuso. This study and this collaboration have terribly boosted my career.

Introduction to the topic

At the beginning of the century, the scientific community started realizing that atmospheric CO_2 partial pressure (pCO_2) already increased by 32% between 1880 and 2005 (280 vs. 379 µatm), and a further doubling was expected by the end of this century (IPCC 2007). This finding was the fundamental recognition that anthropogenic CO_2 emitted to the atmosphere and absorbed by the oceans is causing the decrease in pH, CO_3^{2-} concentration and the related $CaCO_3$ saturation state (Ω) of seawater, a phenomenon called ocean acidification (OA). Rising atmospheric pCO_2 is also causing an increase in global sea temperature with an expected additional average warming of ca. 3°C (Mc Neil and Matear 2007).

Although limited in 2006, some studies already showed that coral calcification rate is largely controlled by the degree of saturation of seawater for aragonite (Ω_{ara}; see review by Kleypas et al. 2006), which varies with latitude (Orr et al. 2005). As a result, coral calcification is expected to decline dramatically in the future, raising widespread concerns about the future of our oceans in a high-CO_2 world (e.g. Hoegh-Guldberg et al. 2007). Several studies on the effect of OA on fast-growing tropical corals show that calcification could decline by 0 to 56% under a doubling of $pCO2$ alone (Kleypas et al. 2006) or in combination with a +3°C increase in temperature (Reynaud et al. 2003, Anthony et al. 2008). In contrast, rates of photosynthesis are either not affected (e.g. Langdon et al. 2003, Reynaud et al. 2003, Schneider and Erez 2006, Marubini et al. 2008) or slightly increased (e.g. Langdon and Atkinson 2005) at the level of pCO_2 expected in 2100. The only study that investigated the effect of high pCO_2 on a Mediterranean coral, *Oculina patagonica* (Fine and Tchernov 2007) revealed a complete dissolution of the skeleton at pH 7.4, a value lower than the one expected in 2100.

The experiment

We investigated in aquaria the effect of mid- and long-term exposure in an orthogonal experimental design with normal and elevated temperatures (T and T+3°C) and pCO_2 (400 µatm and 700 µatm) on calcification and photosynthesis of the Mediterranean zooxanthellate coral *Cladocora caespitosa* living at 20-30 m depth. Colonies were subject to (1) mid-term perturbations (1 month) in summer and winter conditions of irradiance and temperature, and (2) a long-term perturbation (1 year), mimicking the seasonal temperature changes (from 13.4°C to 21.7°C) and irradiance.

Main results and discussion

While temperature is a critical environmental parameter controlling the physiology and calcification of *C. caespitosa*, an increase in CO_2 partial pressure, within the values expected by the end of 2100, did not significantly affect either their photosynthetic performance or the calcification rates. In this study, we confirmed previous findings on the effect of temperature on the calcification rates of *C. caespitosa* (Rodolfo-Metalpa et al. 2008b, c) and other temperate corals (e.g. Jacques et al. 1993, Howe and Marshall 2002). Rates of photosynthesis and respiration were much lower in winter than in summer. Such reduction of photosynthetic activity could explain the difference in calcification rates between the two seasons. A 3°C increase in temperature during this winter period stimulated the coral metabolism, enhancing both photosynthesis and respiration. This stimulation was higher in summer and caused a significant increase in the calcification rates in agreement with the stimulating effect of temperature reported for both tropical (e.g. Clausen and Roth 1975) and temperate corals (Jacques et al. 1983, Howe and Marshall 2002).

At 22°C, a 3°C increase in temperature did not affect photosynthesis, symbiont biomass and calcification rates. The highest temperature slightly increased, albeit not significantly, the respiration rate.

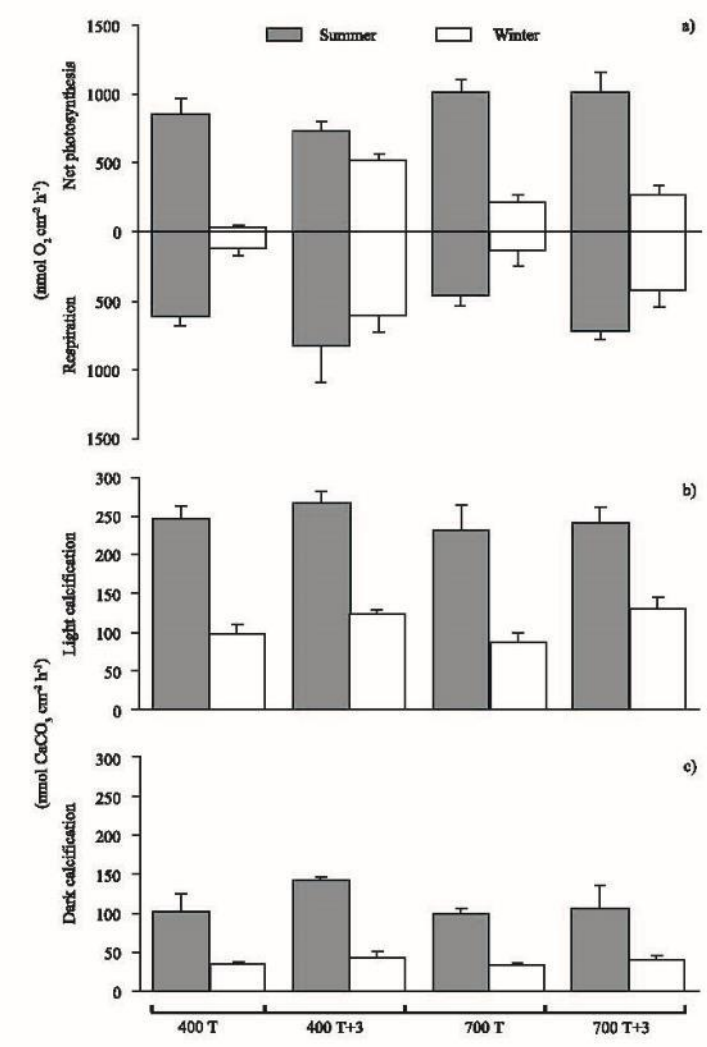
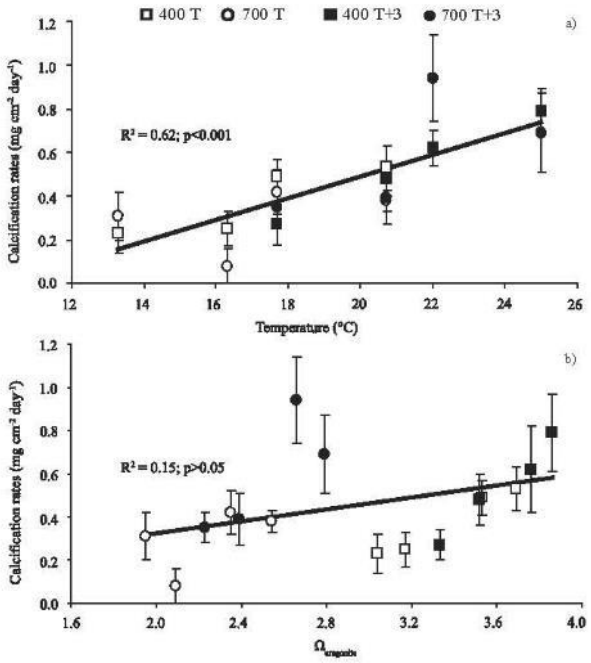


Fig. 4.1.1. Net photosynthesis and respiration (a), and calcification rates measured in the dark and at the culture irradiance level (b) during the mid-term experiments (summer and winter) on *Cladocora caespitosa* exposed to the combined effect of normal (400 µatm) and elevated (700 µatm) pCO2, and normal (T) and elevated (T +3°C) temperatures. Data are mean±s.e.m.

These results suggest that the coral metabolism was at its upper-temperature limit. Photosynthesis does not seem to be greatly affected by OA in symbiotic corals (e.g. Langdon et al. 2003, Reynaud et al. 2003, Schneider and Erez 2006), likely because corals do not rely on dissolved CO_2 for photosynthesis (Gattuso et al. 1999).

Laboratory and mesocosm experiments have shown a common trend of decreased calcification rates with increased pCO_2 in reef-building corals (e.g. Gattuso et al. 1999, Langdon et al. 2005, Kleypas et al. 2006). It has been suggested that below a value of $\Omega_{ara} = 3.3$, corresponding to a pCO_2 level of around 480 µatm, calcification rates will approach zero (Hoegh-Guldberg et al. 2007; Silverman et al. 2009). In contrast, the temperate coral *C. arbuscula* maintained similar calcification rates for Ω_{ara} ranging from 1.95 to 3.86 (pCO_2 values of 709-475 µatm), suggesting that this species may be resistant to an increase in pCO_2 in the range predicted for the end of the century. Similarly, Ries et al. (2009) reported that the calcification rate of the temperate coral *Oculina arbuscula* is unaffected by an increase of pCO_2 up to 840 µatm ($\Omega_{ara} = 1.8$). A drastic decrease in calcification was only found at a pCO_2 of 2240 µatm, corresponding to a Ω_{ara} of 0.8. The low sensitivity of these two temperate corals to an increase in pCO_2 is at odds with the consensus on the negative relationship between pCO_2 and calcification of tropical corals (14 to 30% decrease by 2100; Gattuso et al. 1999, Kleypas et al. 1999).

Except for Ries et al. (2009) and the present study, all experiments carried out on corals used tropical, fast-growing species which grow up to 5 times faster than their tropical counterparts (Rodolfo-Metalpa et al. 2006). Fast-growing corals likely need higher Ω_{ara} than the slow-growing *C. caespitosa* or other temperate or cold species. It is conceivable that the requirement for carbonate ions of slow-growing corals is low and that the concentration of carbonate ions does not become limiting, even under high pCO_2 concentrations (ca. 700 µatm).



Although the negative relationship between calcification and pCO_2 is widely accepted for tropical corals and other calcifying organisms, temperate species may be less sensitive to an increase in seawater pCO_2. For the Mediterranean zooxanthellate coral *C. caespitosa*, the temperature is by far the main factor controlling the rate of calcification, even at the lower Ω_{ara} values predicted for the end of the century for surface seawater in most regions.

Fig. 4.1.2. Relationships between the mean calcification rates of *Cladocora caespitosa* measured during the long-term experiment and: (a) temperature regimes (range 13.3–25°C); (b) Ωaragonite. Open square 400T; closed square 400T +3; open circle 700T; closed circle 700T +3. The coefficient of the regression is reported as R2. Data are mean±s.e.m. (n=7).

4.2. Using volcanic CO_2 seeps to study the effect of OA in a natural setting

Our first description of the organisms inhabiting the CO_2 seeps in Ischia (Hall-Spencer et al. 2008) was the first that proposed this kind of approach. These naturally occurring sites, where CO2-rich fluids seep into the ocean from subterranean sources, offer a unique and powerful platform to investigate the impact of elevated CO_2 levels on marine life, and to advance our understanding of ocean acidification's ecological consequences. Among the advantages, CO_2 seeps provide an ecologically realistic setting (i) where marine organisms have been exposed for long-term (ii) CO_2 levels and pH reductions that closely mimic those projected for future oceans. This extended observation period enables us to assess the chronic and cumulative impacts of OA on marine ecosystems, offering insights that shorter-term experiments cannot provide. (iii) CO_2 seep environments encompass a wide range of marine habitats, including coral reefs, kelp forests, and seagrass meadows, therefore allowing us to investigate how OA affects various ecosystems and their resident species, leading to more comprehensive insights. In addition (iv), because of the presence of multispecies, and natural abiotic and biotic conditions, responses are naturally driven not only by the OA parameters but also by the interaction of the natural environment and species, which facilitates a more holistic ecosystem-level assessment. Finally, CO_2 seeps not only simulate future conditions but also provide an opportunity to observe natural variations in CO_2 levels and pH due to tides, currents, and seasonal changes. This variation allows researchers to explore how different temporal patterns of acidification affect marine life.

4.2.1. The CO_2 seeps site of Ischia Island (Gulf of Naples, Italy)

Ischia is a volcanic Island in front of the Gulf of Naples, where the most famous Vesuvio volcano is still active. The area is quite active and Ischia in particular has been subjected to several earthquakes, the last in 2017. In 2007, Jason Hall-Spencer formed a team including me, and two students from the University of Plymouth. During 3 weeks of intense work, we started reporting the first data on the effect of OA on the local benthic ecosystem. The fieldwork was possible only thanks to the collaboration with the Stazione Zoologica Anton Dohrn and local scientists with whom this collaborative study was performed. Following this first study, we have continued to use this special site performing several transplantations, opening to new international collaborations and finally producing several studies, some of them published in high-impact factor journals. This has contributed to making the site and the local scientific station worldwide known where to perform experiments and test hypotheses on the effect of OA at different scales, from bacteria to the whole ecosystem.

Fig. 4.2.1. Castello Aragonese (on the left), and CO_2 seeps in a *Posidonia oceanica* meadow at 2 m depth south, Ischia, in June 2009 (From Hall-Spencer & Rodolfo-Metalpa 2012).

An area of CO_2 seeps occurs in shallow water (<10 m depth) around Castello Aragonese (40° 43.84' N, 13° 57.08' E) off Ischia island (Gulf of Naples, Italy) where the local marine flora and fauna has been the subject of detailed long-term studies by researchers at the Stazione Zoologica Anton Dohrn (reviewed by Cigliano et al. 2010). The vent gas comprises 90 – 95 % CO2, 3 – 6% N2, 0.6 – 0.8 % O2, 0.2 – 0.8 % CH4 and 0.08 – 0.1 % Ar (no sulphur). These vents are especially suitable for OA studies since they lack the toxic compounds (e.g. sulphur and arsenic) that characterise the majority of marine vents (Tunnicliffe et al. 2009; Couto et al. 2010; Karlen et al. 2010). In addition, the vents are at the same salinity (38 psu) and have the same total alkalinity (2.5 mEq kg^{-1}) and seasonal temperature fluctuations (13-28°C) as the surrounding surface waters off Ischia. The acidified areas encompass intertidal rock, infralittoral rock, infralittoral sediment, cave-dwelling communities and seagrass meadows allowing investigations into the consequences of acidification on a range of different coastal habitats. Two vent sites have been monitored (north and south Castello) since April 2007 to assess gradients in seawater pH, dissolved inorganic carbon, and the saturation states of calcite and aragonite.

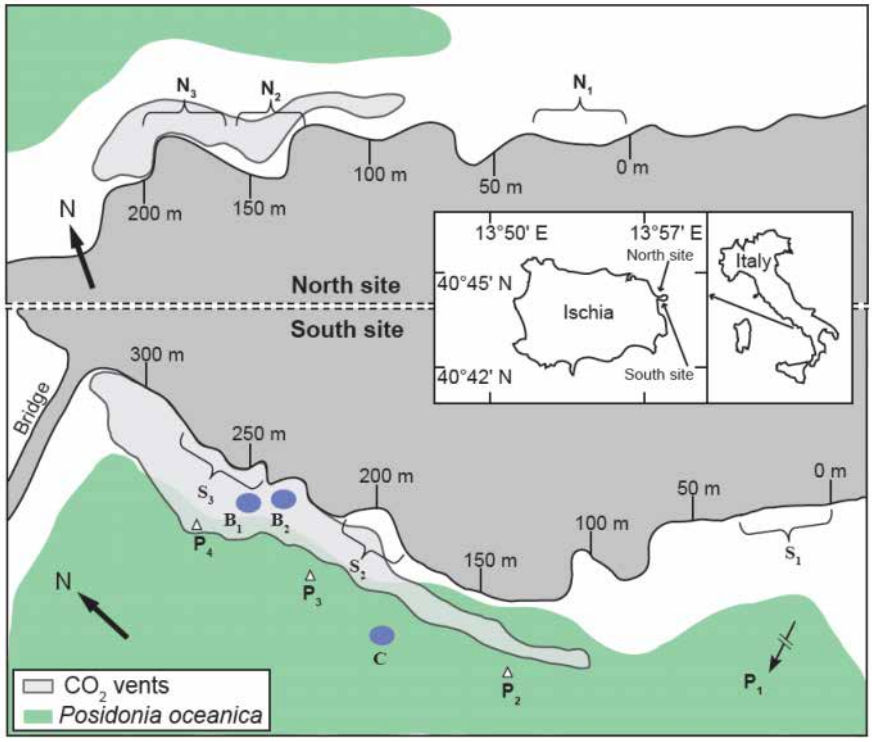


Fig. 4.2.2. Map of CO_2 vents sites north and south of Castello Aragonese, off Ischia Island, Italy. Water chemistry has been monitored in rocky shore zones N1-N3 and S1-S3 as well as at transplant stations C, B1 and B2 and Posidonia monitoring stations P1-P4 by Hall-Spencer et al. (2008), Martin et al. (2008), Rodolfo-Metalpa et al. (2010) and Cigliano et al. (2010).

From 2008, in the framework of consecutive postdocs at Plymouth University, The Prince Albert Foundation and the International Atomic Energy Agency, I participated in around 15 studies using this site. A first assessment of the biological effects of the Ischia vents quantified the abundance of dominant macroorganisms in intertidal and subtidal zones within and adjacent to the acidified waters (Hall-Spencer et al. 2008). Subsequently, the carbonate content and biodiversity of seagrass epibionts were evaluated along a pCO_2 gradient (Martin et al. 2008) as well and invertebrate recruitment was investigated during spring larval settlement using artificial collectors (Cigliano et al. 2010). A series of calcifiers were transplanted such as the corals *Cladocora caespitosa* and *Balanophyllia europaea*, the molluscs *Mytilus galloprovincialis* and *Patella caerulea* and the bryozoans *Myriapora truncata* and *Calpensia mirabilis* (Rodolfo-Metalpa et al. 2010; Rodolfo-Metalpa et al. 2011), as well as cold water corals (Rodolfo-Metalpa et al. 2015). The coral microbiome was assessed (Meron et al. 2012), as well as the photosynthetic and enzymatic capacity of sea anemones (Suggett et al 2012), the

geochemistry and shell structure of bivalves (Hahn et al. 2012), limpets (Langer et al. 2014, 2018), bryozoans (Rodolfo-Metalpa et al. 2010; Lombardi et al. 2011), corals (Trotter et al. 2011), boring microflora on coral skeletons (Tribollet et al. 2018) and sponge distribution (Goodwin et al. 2013).

4.2.2. The CO_2 seeps site of Vulcano Island (Sicily, Italy)

Volcano Island specifically Levante Bay has been actively investigated by Italian volcanologists since a very active volcanic area. Shallow submarine gas vents in Levante Bay, Vulcano Island (Italy), emit around 3.6 t CO_2 per day providing a natural laboratory for the study of biogeochemical processes related to seabed CO_2 leaks and ocean acidification. While this site was used first by Prof Marco Milazzo to study the effect of OA on benthic organisms, we joined his team in 2011 within the framework of the European OA project MedSea (P Ziveri PI). At Levante Bay, we found intense submerged seeps along the beach in Levante Bay, where dispersed underwater leaks cover a 130 x 35 m area at less than 1 m depth. This huge degassing acidifies most of the bay along a gradient of intensity.

We first characterised (Boatta et al. 2016) the most prominent geochemical parameters of the waters of the whole Levante Bay area (Fig. 1) and we provided a thorough assessment of the spatio-temporal variability of pH/CO_2 of the northern shore where our OA studies have begun, as this coastline is situated well away from the main vents but has acidified waters that form a shallow pCO_2 gradient along ca. 200 m of rocky habitats.

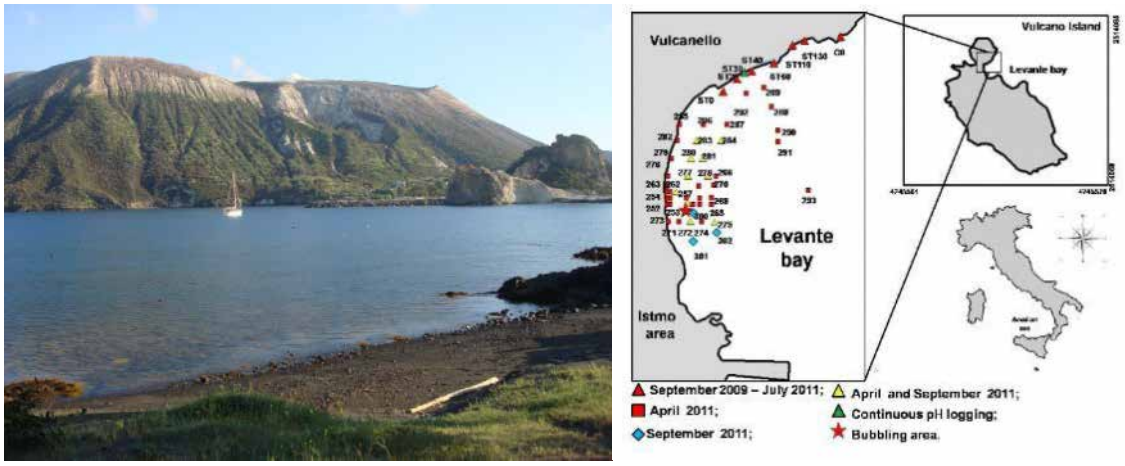


Fig. 4.2.2. The Levante Bay (Vulcano Island, Eolian archipelago) and the stations used to assess the bay pH and seawater chemical variation along the coast (Boatta et al. 2016).

In collaboration with my friend Marco Milazzo, I performed two common garden transplantations, one using the Med corals *Cladocora caespitosa*, and *Astroides calycularis* (still to be published), and the second one (Milazzo et al. 2014) using cores of the reef-building gastropod *Dendropoma petraeum*, which forms the troittoirs in the intertidal zone. Other studies focused on metabolic and enzymatic activities in sea anemones (Ventura et al. 2016), coccolithophore diversity (Ziveri et al. 2014), fish reproduction (Milazzo et al. 2016), and gastropods calcification (Garilli, Rodolfo-Metalpa et al. 2015).

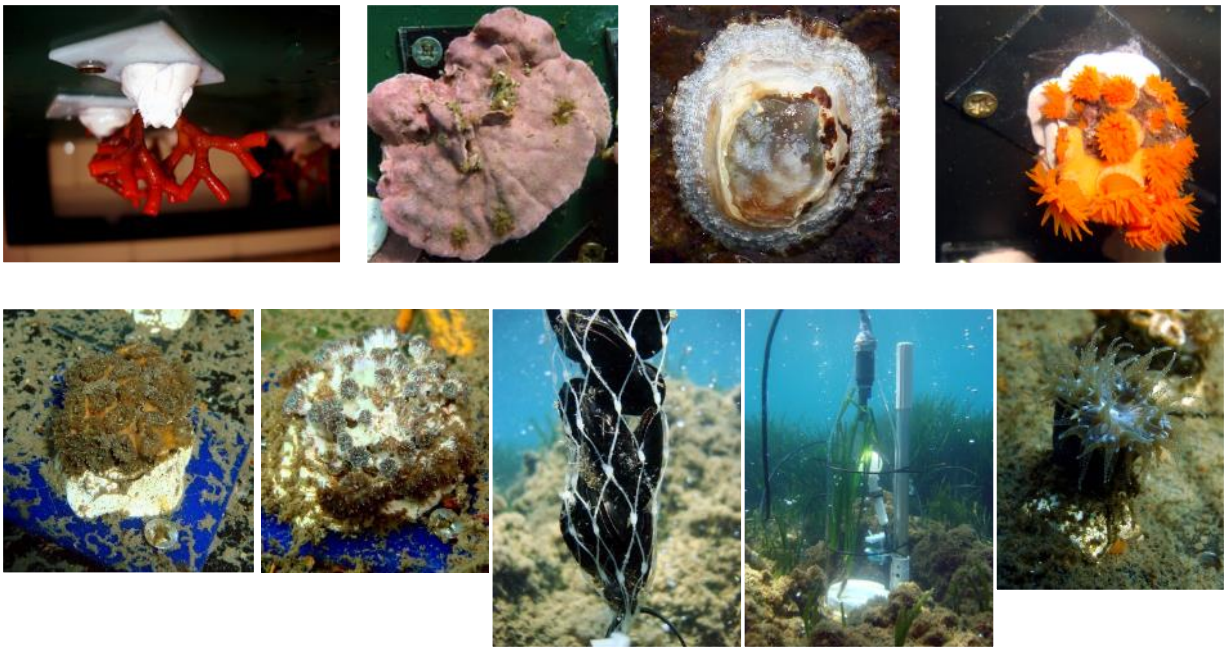


Fig. 4.2.3. Some of the species that were transplanted and measured at Ischia and Vulcano CO_2 seeps.

4.3. Volcanic carbon dioxide vents reveal ecosystem effects of ocean acidification

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LETTERS

Volcanic carbon dioxide vents show ecosystem effects of ocean acidification

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This study was the first I published as a postdoc. It is a milestone in this topic and has offered the scientific community a new approach to studying OA and revealing the effect of OA at the ecosystem level. It describes for the first time the CO_2 seeps in Ischia, which has rapidly become the centre of the universe for such a kind of research study attracting a lot of scientists, and media. The work has been used to help understand the fate of marine organisms at inter-governmental workshops (e.g. Unesco, Surface Ocean CO_2 Variability and Vulnerability Workshop, Paris April 2007; IPCC workshop on Impacts of Ocean Acidification on Marine Biology and Ecosystems, Okinawa January 2011; CBD joint expert review on the Impacts of ocean acidification on marine and coastal biodiversity, Montreal, October 2011, etc). This study has pioneered the use of CO_2 seeps and has reinforced the need to study OA in nature, not only in artificial systems, thus including interaction between species, natural nutrition and introducing the concept of adaptation, which had never before been addressed in the field of OA research.

Our understanding of how increased ocean acidity may affect marine ecosystems is currently very limited since almost all studies have been in vitro, short-term, rapid perturbation experiments on isolated elements of the ecosystem. Here we show the effects of acidification on benthic ecosystems at shallow coastal sites where volcanic CO_2 vents lower the pH of the water column. Along gradients of normal pH (8.1-8.2) to lowered pH (mean 7.8-7.9, min 7.4-7.5), typical rocky shore communities with abundant calcareous organisms shifted to communities lacking scleractinian corals with significant reductions in sea urchin and coralline algal abundance. This is the first ecosystem-scale validation of predictions that these important groups of organisms are susceptible to elevated levels of pCO_2. Seagrass production was highest in an area at a mean pH of 7.6 (1827 µatm pCO_2) where coralline algal biomass was significantly reduced and gastropod shells were dissolving due to periods of carbonate sub-saturation. The vent sites revealed a suite of organisms that are resilient to naturally high levels of pCO_2 and that OA may benefit highly invasive non-native algal species. Our results provide the first *in situ* insights into how shallow-water marine communities might change when susceptible organisms are removed due to ocean acidification.

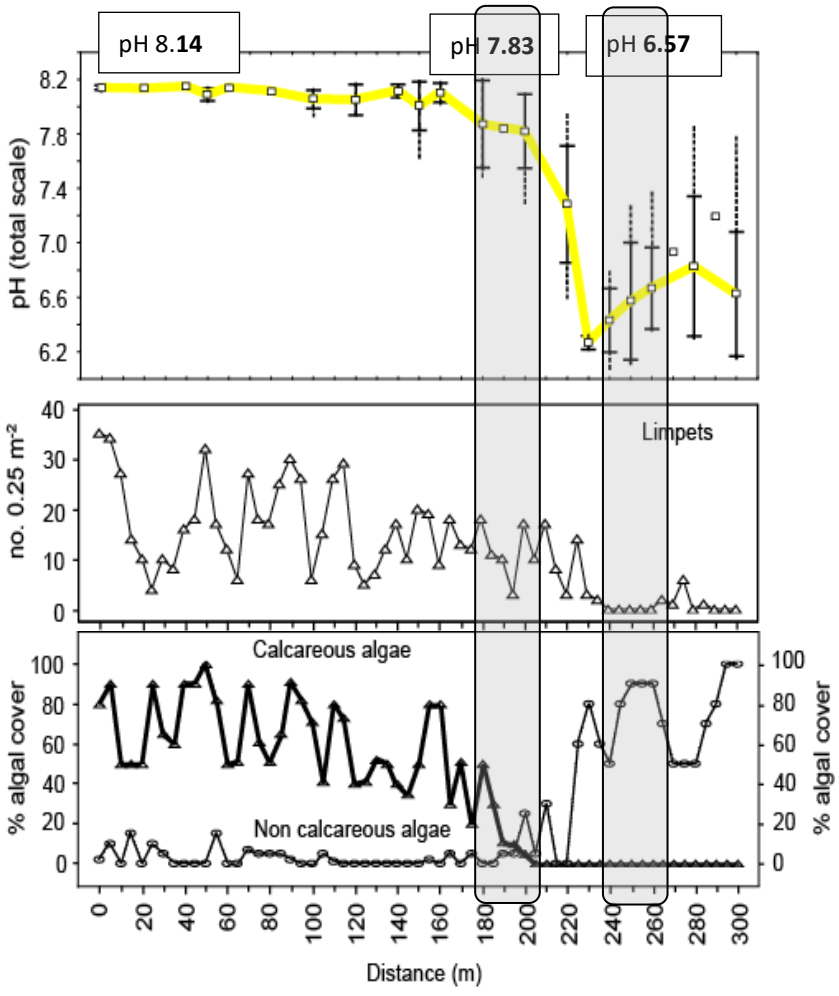

Fig. 4.3.1. Variation in pH, abundance of limpets and cover of algae at CO_2 vents south of Castello d'Aragonese. Data are from stations S1–S3, from 18 April to 9 May 2007. The percentage cover of calcareous (triangles) and noncalcareous algae (circles) is shown.

4.4. Effects of ocean acidification and high temperatures on the bryozoan *Myriapora truncata* at natural CO_2 vents

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an evolutionary perspective

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ORIGINAL ARTICLE

Effects of ocean acidification and high temperatures on the bryozoan *Myriapora truncata* at natural CO_2 vents

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Introduction to the study

Several papers started demonstrating that although OA decrease the calcification rates of ecologically important organisms such as coralline algae (Kuffner et al. 2008; Martin et al. 2008), foraminiferans (e.g. Moy et al. 2009), corals (e.g. Silverman et al. 2009), echinoderms (e.g. Michaelidis et al. 2005) and molluscs (Gazeau et al. 2007), with rates of calcification have been predicted to fall by up to 60% within this century, depending on the physiology of the species and their mineralogy (Kleypas et al. 2006). However, responses were highly species-specific (Ries et al. 2009) and sometimes differences and inconsistencies between studies were due, among other confusing factors (light, temperature, pH manipulation, seawater current velocity, foods, etc), to the different mineralogy of the organisms, and the skeleton exposition.
Shells can dissolve when exposed to seawater with low carbonate saturation states such as in estuaries (Marshall et al. 2008), in upwelling areas (Feely et al. 2008) and around CO_2 vents (Hall-Spencer et al. 2008). Shells and/or skeletons made of high Mg-calcite are highly susceptible to dissolution as carbonate saturation states fall, followed by aragonitic skeletons and finally low Mg-calcite skeletons.

The experiment

Here, we investigated rates of calcification and dissolution of the robust, branched bryozoan *M. truncata* (Pallas 1766).
Although 15% of the species of bryozoan are aragonitic and 17% are bimineralic (Smith et al. 2006), *M. truncata* is typical of most Bryozoa in that it has a calcitic skeleton. Our study aimed to investigate the effects of four-month in situ exposure to different pH conditions on the calcification and dissolution of *M. truncata* using in situ transplantation experiments at natural volcanic CO_2 vent sites. We test the hypothesis that temperature affects the degree to which ocean acidification affects net calcification and dissolution in this bryozoan. Live colonies and dead ones were transplanted to normal (pH 8.1), high (mean pH 7.66, minimum value 7.33) and extremely high-CO_2 conditions (mean pH 7.43, minimum value 6.83) at CO_2 vents off Ischia island (Tyrrhenian Sea, Italy). The net calcification rates of live colonies and the dissolution rates of dead colonies were estimated by weighing after 45 days (May-June 2008) and after 128 days (July-October) to examine the hypothesis that high CO_2 levels affect bryozoan growth and survival differently during moderate and warm water conditions.

Main results and discussion

In the first observation period seawater temperatures ranged from 19°C to 24ºC; dead *M. truncata* colonies dissolved at high-CO_2 levels (pH 7.66) whereas live specimens maintained the same net calcification rate as those growing at normal pH. In extremely high-CO_2 conditions (mean pH 7.43), the live bryozoans calcified significantly less than those at normal pH. Therefore, established colonies of *M. truncata* seem well able to withstand the levels of ocean acidification predicted in the next two hundred years, possibly because the soft tissues protect the skeleton from an external decrease in pH. However, during the second period of observation, a prolonged period of high seawater temperatures (25-28ºC) halted calcification both in controls and at high CO_2 and all transplants died when high temperatures were combined with extremely high-CO_2 levels.

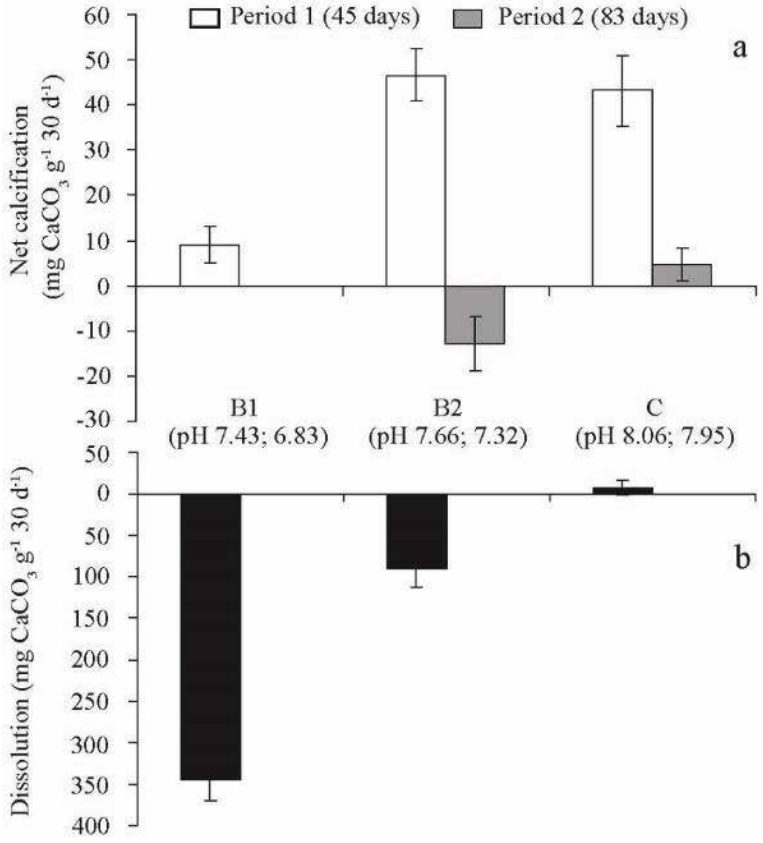

Fig. 4.4.1. Net calcification (a) measured during Period 1 and Period 2, and dissolution rates (b) measured during Period 1 respectively on live and dead transplanted colonies of *M. truncata* at extremely low (B1), low (B2) and normal pH (C) near and outside CO_2 vents at Ischia. Data are mean ± s.e. (n = 8 and 5). The mean and minimum pH measured during the whole experiment are reported in brackets.

Regarding the ability to calcify even under lo (B2) and extreme pH levels (B1), the results were not surprising since we expected to find no effect at pH 7.66 and a low growth rate at pH 7.43. However, from our data, it was not clear if this decrease was due to either the loss in the ability to calcify or the increase in the dissolution of the skeleton. Indeed, the buoyant weight technique (Davies et al. 1994) here used is the sum of both and measures the net calcification. When this work was performed, the measurement of gross calcification was difficult to perform because it needed the use of radioactive calcium. So, in this study, the gross calcification was calculated for the B2 samples by adding $CaCO_3$ dissolution to the net calcification rates measured on dead and live fragments. We found that the calcification was three times higher than the net calcification rates measured under normal conditions. An increase in calcification under acidified conditions has recently been reported for several species (Wood et al. 2008; Findlay et al. 2009; Ries et al. 2009). However, before firm conclusions are made about the ability of *M. truncata* to increase its calcification rate under high CO_2 conditions, more experiments are necessary using accurate calcification methods such as ^{45}Ca uptake (Tambutté et al. 1995).

With regard to skeleton dissolution, during this initial 45-day period, dead *M. truncata* skeletons did not dissolve and were heavily colonized by epibionts at normal pH, therefore increasing their weight, while they were dissolving both in B2 (90 mg g^{-1} 30 day^{-1}) and at a very high rate in B1 (344 mg g^{-1} 30 day^{-1}). Dissolution at B1 and B2 occurred even though these treatments normally experienced saturated $\Omega_{calcite}$ levels. This is likely due to both periods of carbonate undersaturation which occur at the site when the sea state is particularly calm (Hall-Spencer *et al.* 2008) and during the night.
Concerning colony survival, although adult *M. truncata* colonies were resilient to acidified conditions in the cooler part of our study (Period 1), at the end of Period 2, all B1 specimens had died, while fragments in B2 and C, although still living, showed near-zero calcification rates. Indeed, from 16 May 2008 to 26 June 2008 (Period 1) the water around the transplants warmed steadily from 19 to 24°C and the bryozoans grew well; then, during Period 2, it remained abnormally high at 25-28°C for three months. The mortality of all samples maintained 128 days under severe hypercapnia was presumably due to the synergistic effect of elevated seawater temperature and prolonged exposure to low pH levels. Although *M. truncata* was resilient to short-term exposure to high levels of ocean acidification at normal temperatures, our field transplants showed that its ability to calcify at higher temperatures was compromised, adding it to the growing list of species now threatened by global warming.

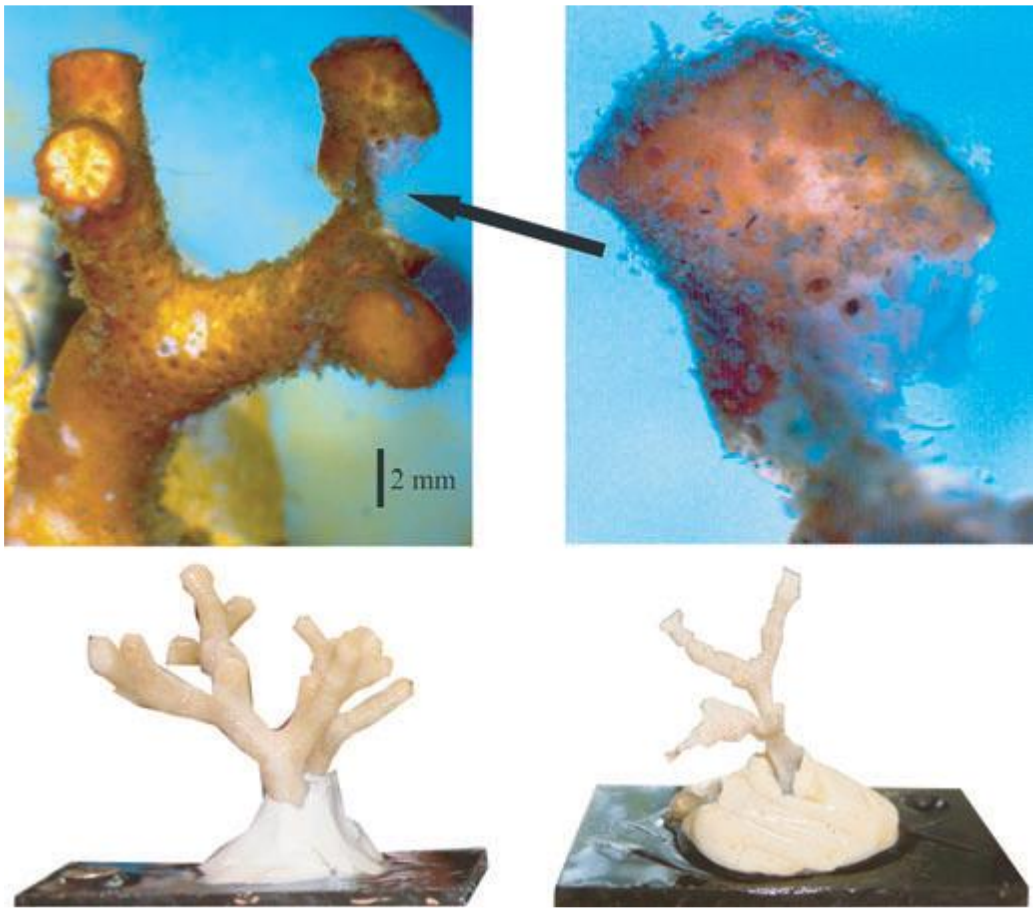

Fig. 4.4.2. *Myriapora truncata* maintained 128 days at mean pH 7.43 in B1 (a, b) showing the breakage of the zooidal soft tissues and the dissolution of the skeleton; dead fragments after 45 days at mean pH of 8.06, and 7.43 (d) in C and B1, respectively.

4.5. Coral and mollusc resistance to ocean acidification adversely affected by warming


LETTERS
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nature climate change

Coral and mollusc resistance to ocean acidification adversely affected by warming

R. Rodolfo-Metalpa[1,2*], F. Houlbrèque[1†], É. Tambutté[3], F. Boisson[1], C. Baggini[2], F. P. Patti[4], R. Jeffree[1†], M. Fine[5,6], A. Foggo[2], J-P. Gattuso[7,8] and J. M. Hall-Spencer[2]

This study has been possible thanks to the collaboration with E. Tambutté (Centre Scientifique de Monaco) and Fanny Houlbreque, who helped me with the ^{45}Ca technique I performed at the IAEA centre (Monaco), and again to E Tambutté for all the SEM he performed. The study was done in the framework of the project funded by the Prince Albert Foundation (F Boisson PI). This is my best paper!

Here, we show that corals and molluscs transplanted along gradients of carbonate saturation state at Mediterranean CO_2 vents can calcify and grow at even faster than normal rates when exposed to the high CO_2 levels projected for the next 300 years. Calcifiers remain at risk, however, due to the dissolution of exposed shells and skeletons that occurs as pH levels fall. Our results show that tissues and external organic layers play a major role in protecting shells and skeletons from corrosive seawater, limiting dissolution and allowing organisms to calcify. Our combined field and laboratory results demonstrate that the adverse effects of global warming are exacerbated when high temperatures coincide with acidification.

Introduction to the study

The impact of acidification on the ability of individual species to calcify has remained elusive, however, as measuring net calcification (NC) fails to disentangle the relative contributions of gross calcification (GC) and dissolution rates on growth. Previous workers have not determined the impact of acidification on the ability of individual species to calcify since NC was measured [i.e. (GC) minus dissolution] which fails to disentangle the relative contributions of GC and dissolution rates. A decrease in NC could result from a decrease in GC, an increase in dissolution rates, or both. Recent studies indicate inconsistencies in the use of carbonate saturation state to predict marine calcification since some species can maintain, or even increase, their NC under low pH conditions. It is thought that this species-specific sensitivity is due to the presence and composition of external organic layers, and an ability to elevate pH and $[CO_3^{2-}]$ at sites of calcification. For example, measurements of the boron isotopic composition in *Cladocora caespitosa* held in aquaria at pH_T 7.8 and from CO_2 vents showed that this Mediterranean coral was able to maintain a higher internal pH and hence ΔpH relative to ambient seawater pH17, therefore allowing calcification in undersaturated seawater. Calcification is known to occur between the tissue and the shell or skeleton where extrapallial fluid (in molluscs) and extracellular calcifying fluid (in corals) pH is 0.5 to > 1 unit higher than in ambient seawater.

The experiment

We used a series of transplantation experiments along natural pCO_2 gradients at volcanic vents off Ischia (Tyrrhenian Sea, Italy) to investigate whether the projected reduction in calcification in key marine habitats by 2100 and beyond is a result of the suppression of GC, carbonate dissolution or both. We compared (1) a mollusc that has a periostracum covering the outside of

the shell (*Mytilus galloprovincialis*) with one that lacks a periostracum (*Patella caerulea*) and (2) the zooxanthellate coral *Balanophyllia europaea*, which has a skeleton completely covered in tissue with *Cladocora caespitosa* which has skeletal parts that are exposed to the surrounding seawater (Fig. 1). Shell and coral dissolution rates and coral NC rates were measured by buoyant weighing samples whilst GC rates of all four species were measured in aquaria with the radiotracer ^{45}Ca after incubation *in situ*.

Main results and discussion (on corals only)

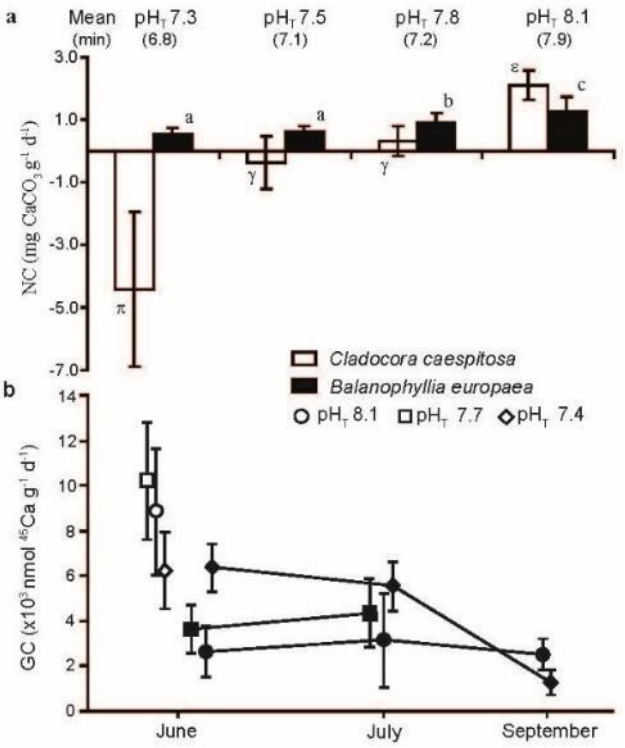


Fig. 4.5.1. Calcification rates of corals transplanted along a CO_2 gradient off Ischia. **a,** net calcification (NC) measured after three months in 2008. Different symbols (for *C. caespitosa*) and letters (for *B. europaea*) are significantly different ($P < 0.05$). Data are means ± s.d. (n = 12 for *C. caespitosa*; n = 14 for *B. europaea*). **b**, gross calcification (GC) measured in aquaria with ^{45}Ca labelled seawater at pH_T 7.4, 7.7 and 8.1 on samples transplanted up to 7 months in 2009. *C. caespitosa* GC was only measured in June 2009 after three months. Data are means ± s.d. (n = 16 for *C. caespitosa*; n = 10 for *B. europaea*).

<u>*Gross vs net calcification.*</u> As expected from other coral studies, NC after three months decreased significantly with decreasing pH in our coral transplants (*C. caespitosa*: $F_{3,44} = 50$; $P < 0.001$; *B. europaea*: $F_{3,52} = 17$; $P < 0.001$). *C. caespitosa* had significantly slower linear growth rates as pH decreased ($F_{2,65} = 62$; $P < 0.001$; mean ± s.d.: 0.19 ± 0.03, 0.32 ± 0.05, and 0.34 ± 0.07 mm month^{-1}, at mean pH_T 7.5, 7.8 and 8.1 respectively). At mean pH_T 7.3 all the transplanted colonies had dissolved after *ca*. five months so no measurements were possible. In *C. caespitosa*, NC rates became negative at mean $pH_T < 7.5$ (mean $\Omega_a = 1.69$, minimum $\Omega_a = 0.40$) but remained positive for *B. europaea* even at mean pH_T 7.3 (mean $\Omega_a = 1.13$, minimum $\Omega_a = 0.33$).

Labelling with ^{45}Ca showed that, remarkably, both coral species were able to maintain GC at pH levels projected for 2100 and beyond. Gross calcification in *C. caespitosa* was similar at pH_T 8.1 and 7.7 and decreased by 30% at pH_T 7.4 ($F_{2,45} = 10$; $P < 0.001$), whereas GC in *B. europaea* significantly increased with decreasing pH in June and July ($F_{2,54} = 26$; $P < 0.001$). This raises the possibility, as in other corals that *Balanophyllia* may rely on HCO_3^- for calcification.

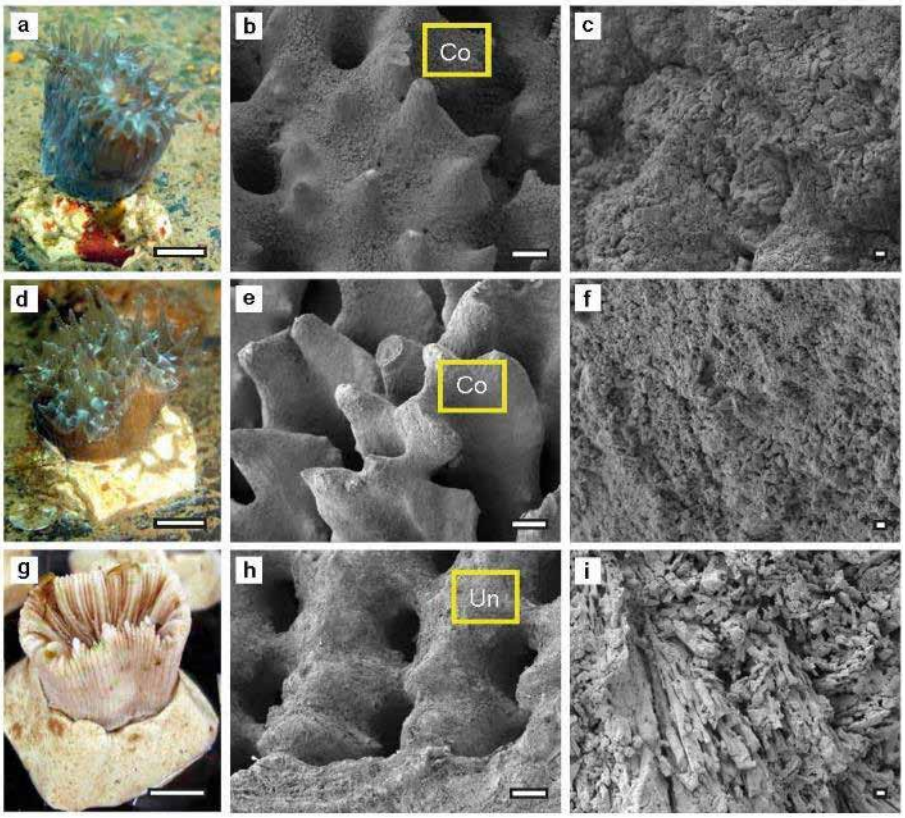


Fig. 4.5.2. Underwater and SEM images of *Balanophyllia europaea* transplanted along a CO_2 gradient off Ischia. **A-c**, live coral after seven months at mean pH_T 8.1 and (**d-f**) at mean pH_T 7.3. **g-i**, dead coral after three months at mean pH_T 7.3. Details of the outer corallite wall showing normal skeleton when covered (Co) in tissue (**b, e**) and (**h**) dissolved skeleton when uncovered (Un) in tissue. Enlargements (yellow boxes on **b, e, h**) show organized (**c, f**) and dissolved (**i**) bundles of aragonite crystals. Scale bars = 1 cm (a, d, g), 100 μm (b, e, h) and 1 μm (c, f, i).

Skeleton dissolution. The dissolution of live samples transplanted at the vents was species-specific. While *C. caespitosa*, which has large parts of its skeleton exposed, showed evident marks of dissolution, *B. europaea* has a skeleton that is completely covered in tissue and was unaffected. The morphology of the exposed skeletons of live corals, as well as dead samples of both corals, maintained at mean pH_T 7.3 for three months, showed disordered aragonite crystals. In contrast, the skeleton covered in tissue had organized bundles of fine aragonite crystals as in samples maintained at normal pH. Just as periostracum protected mussel shells, coral tissues protected the skeletons from corrosive ($\Omega_{ara} < 1$) seawater. This protective role may explain why some corals increase tissue thickness when seawater pH is lowered.

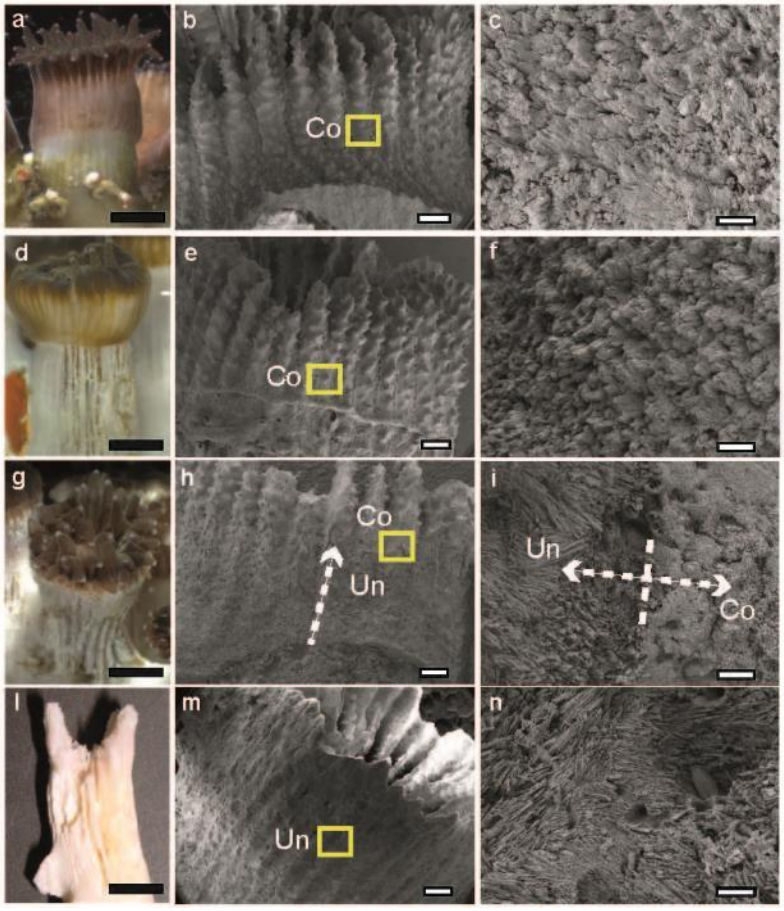


Fig. 4.5.3. Single corallites of *Cladocora caespitosa* transplanted along a CO_2 gradient. **A-c**, live corals after 7 months at mean pH_T 8.1; after three (**d-f**) and 7 months (**g-i**) at mean pH_T 7.3. **l-n**, dead samples after three months at mean pH_T 7.3. Details of the external calyx skeleton organisation (**b, e, h, m**). Enlargements (yellow boxes on **b, e, h, n**) of aragonite crystals showing normal (**c, f**) and dissolved (**i, n**) aragonite. The polyp of this species only covers the apical part of each corallite, leaving the skeleton exposed to the surrounding seawater. Corals transplanted to mean pH_T 7.3 (**g**) showed the exposed skeleton highly damaged and a gradual polyp retraction into the calyx (as indicated by the arrow), likely due to the effect of acidified seawater on the border between tissue and skeleton. This response increased the surface of exposed coral skeleton (Un = uncovered), which eventually dissolved, while parts still covered in tissue (Co = covered) maintained normal skeletal morphology. Scale bars are 0.3 cm (a, d, g, l), 100 µm (b, f, h, m) and 10 µm (c, f, i, n).

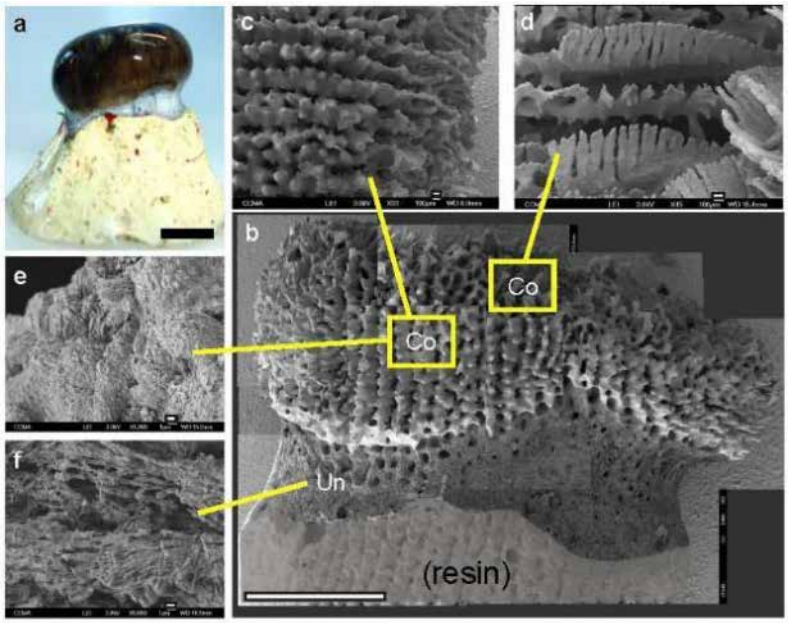
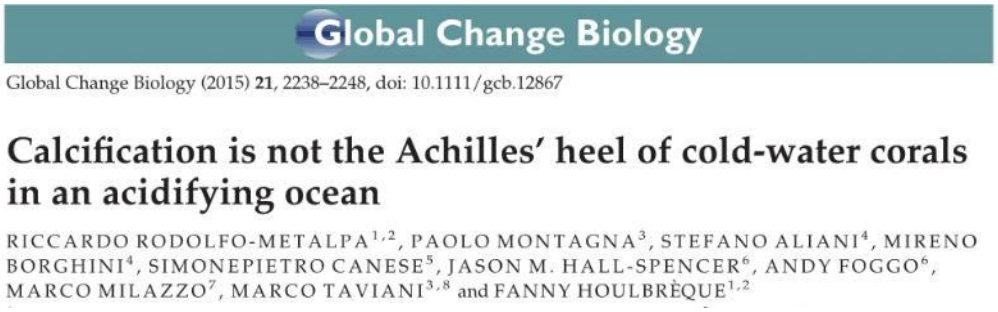


Fig. 4.5.4. *Balanophyllia europaea* transplanted along a CO_2 gradient. **A**, after three months at mean pH_T 7.2 the polyp retracted, likely due to the interaction with algae which fixed on the resin, therefore exposing part of the external calyx wall during the incubation. **B**, whole corallite showing dissolved uncovered (Un) and normal skeleton (Co). **c-f**, enlargements (yellow boxes in b) of the external wall (**c**, **e**) and of the calyx rim (**d**) showing normal skeleton organisation when the skeleton was covered in tissue (Co). **f**, enlargement of the external wall showing the dissolved skeleton. Scale bars are 0.5 cm (a, b), 100 µm (c, d) and 1 µm (e, f).

4.6. Calcification is not the Achilles' heel of cold-water corals in an acidifying ocean



Before starting the PhD I participated in some cruises on board Italian oceanographic vessels, in collaboration with the ENEA and the CNR. Thanks to these collaborations, my position at the IAEA in Monaco, and the research activities made in Ischia, I had the unique opportunity to collect some CWC, measure in the IAEA lab their gross calcification with isotopic technique, and finally transplant them for months both at the CO_2 seep of Ischia and at 350 m depth on a mooring line. This experiment was the first of its kind and revealed that CWC calcification was not fundamentally affected by OA.

Introduction to the study

Research into the effects of acidification on CWCs in aquaria has provided conflicting results. Form & Riebesell (2012) found a significant decrease in the net calcification of *Lophelia pertusa* during a one-week exposure to high CO_2 levels but found that this reef-forming coral was able to acclimate in the long-term (6 months), increasing its calcification rate in seawater that was undersaturated concerning aragonite compared to saturated conditions. Work on other CWCs revealed that their calcification was not affected by acidification during the first 182 and 240 days of incubation (Movilla et al., 2014a; Carreiro-Silva et al., 2014 respectively). On the other hand, *Dsmophyllum dianthus* had a 70% reduction in skeletal growth rate after 314 days whereas *Dendrophyllia cornigera* showed no differences between treatments (Movilla et al., 2014a). Other studies have shown that calcification rates of *L. pertusa* and *Madrepora oculata* (which also forms deep-water reefs) were unaffected by future projected pCO_2 levels in short- (hours-days; Maier et al., 2012) and long-term (weeks-months; Maier et al., 2013a,b) experiments. McCulloch et al. (2012b) showed that CWCs can calcify at or close to the aragonite saturation horizon by elevating their internal pH, thus buffering external changes in seawater pH. These studies imply that shoaling of the aragonite saturation horizon may not cause the dramatic declines in coral calcification rates that were first feared (Guinotte et al., 2006; Jackson et al., 2014). However, to what extent undersaturated seawater might affect reef integrity by increasing the dissolution of exposed coral skeletons has not yet been investigated and all studies showing that they maintain calcification rates at high pCO_2 levels have been carried out in aquaria where feeding may have artificially boosted their energy reserves, likely altering responses to acidification.

The experiment

In this study, we first measured the effects of acidification on the net and gross calcification rates as well as the respiration rates of three CWC species cultured in aquaria under present and future Representative Concentration Pathway (RCP) 8.5 pCO2 scenario (IPCC, 2014). In addition, we quantified skeletal dissolution rates of D. dianthus maintained in aquaria during two-month incubations at present and high pCO_2 levels. Finally, we compared the calcification rate of *D. dianthus* fed in aquaria in aragonite-saturated conditions with those of corals

transplanted into undersaturated (CO_2 seeps of Ischia) and saturated conditions (at 350 m depth) off Italy.
Caryophyllia smithii, Desmophyllum dianthus and *Dendrophyllia cornigera* were collected during three cruises in the Med Sea and transported to the IAEA laboratory where they were maintained in the dark at a seawater temperature of 13°C and fed twice per week with frozen krill or freshly hatched *Artemia nauplii*. Two sets of corals were acclimated for one month either to ambient pH and pCO_2 (pH_T = 8.07; pCO_2 = 319 µatm), or at levels projected by the end of the century (pH_T = 7.70; pCO_2 = 1058 µatm), and maintained for further two months. Their net and gross calcification rates were measured.
A set of the most abundant *Desmophyllum dianthus* was suspended at 350 m depth on an instrumented mooring cable in the Corsica channel. Corals were weighed and attached to individual plates, which were put into an open cage fixed to the mooring cable. In Ischia, they were transplanted during the coldest month onto a rocky seabed at CO_2 seep sites previously studied by Rodolfo-Metalpa *et al*. (2011): 1) station B1 at 3 m depth with a mean pH of 7.43 ± 0.31; 2) and station C at 5 m depth with a mean pH of 8.06 ± 0.07. At both sites corals were positioned calyx upwards inside an open cage made with two PVC plates (60 x 90 cm), mounted using bolts and attached to 30 kg concrete blocks. Corals were maintained for 258 days at 350 m depth and for 43 days at 3 m depth.

Fig. 4.6.1. Transplantations of *D. dianthus* suspended at 350 m depth on a mooring off Corsica.

Main results and discussion
We found that gross and net calcification rates of *Desmophyllum dianthus*, *Caryophyllia smithii* and *Dendrophyllia cornigera*, as well as dissolution rates of exposed skeleton and respiration rates of living *D. dianthus*, did not significantly change when exposed to high seawater pCO_2 (pH_T = 7.70; pCO_2 = 1058 µatm, Ω_{ara} = 1.29). We tested this observation further by transplanting *D. dianthus* to 350 m depth at ambient seawater conditions (pH_T = 8.02; pCO_2 = 448 µatm; Ω_{ara} = 2.58) and into undersaturated seawater (pH_T = 7.35; pCO_2 = 2879 µatm; Ω_{ara} = 0.76) near CO_2 seeps and confirmed that net calcification rates were not affected by the differences in seawater carbonate chemistry. This is likely linked to their ability to maintain a high Ωara level and pH at the calcification site. It is well known that to build their skeletons, corals pump protons out of the extracellular calcifying medium to increase internal pH and favour calcification. This is highly energy consuming (Allemand *et al*. 2011) with an estimated metabolic extra cost of ca. 10% per 0.1 pH unit decrease in seawater pH (McCulloch *et al*. 2012b). To meet this energy demand, corals can increase feeding rates and/or draw upon energy reserves. In our aquaria experiment, corals were fed twice a week, which is lower than several

previous studies on CWC, and likely increased their net calcification rates when compared to samples grown *in situ*.

Studies testing the resilience of CWC to ocean acidification used arbitrary feeding rates from no artificial feeding (Maier *et al*. 2012) to five times per week with *Artemia* nauplii and/or frozen Cyclops, Mysidacea, minced mussels, fish flakes (Tsounis *et al*. 2010; Orejas *et al*. 2011; Naumann *et al*. 2011; 2013a,b; Carreiro-Silva *et al*. 2014; Movilla *et al*. 2014a). Sometimes this diet was used for years before the experiment was carried out (e.g. Movilla *et al*. 2014a). According to our findings, corals acclimated to such optimal feeding rates might have their metabolism and nutritional habits completely changed, as well as their energy distribution. For instance, in the presence of artificial food in aquaria CWC tend to be permanently expanded, which is not the case in the field. Corals shown to calcify at reduced seawater Ω_{ara} (Maier *et al*. 2013a; Movilla *et al*. 2014a; Carreiro-Silva *et al*. 2014) might only have been able to meet the extra energy demands as they were acclimated to abundant food conditions.

To test whether the OA responses of artificially fed corals differed from naturally fed corals we transplanted them to field conditions with normal and elevated pCO_2 where they were seen feeding naturally. In both cases, the corals calcified at rates that were 44% lower than corals kept at similar temperatures and pH in aquaria but artificially fed. Corals that we held in aquaria calcified faster than those in the field, likely due to greater food availability, but they were able to calcify at predicted levels of increased pCO_2 both in the field and in the laboratory. In conclusion, both corals in aquaria and the field, artificially and naturally fed respectively, showed the same response to acidification: calcification rates were always unaffected by seawater carbonate chemistry.

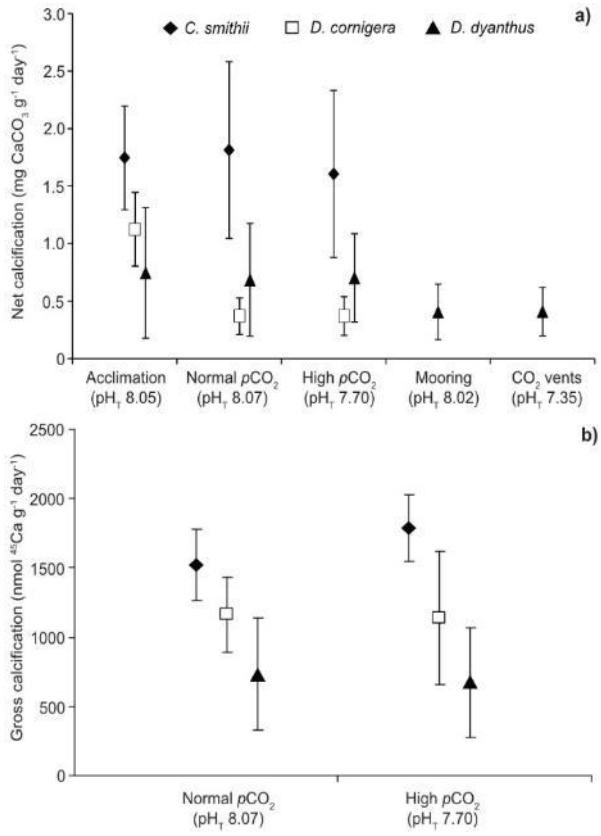
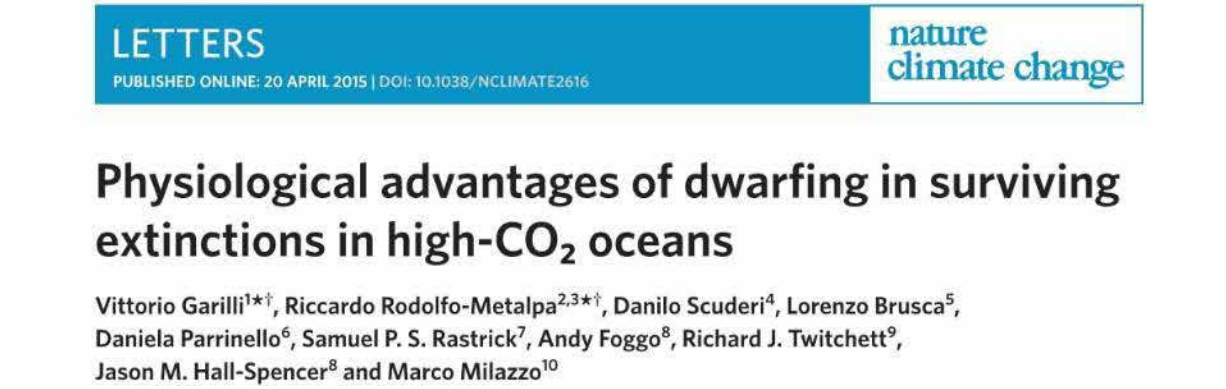


Fig. 4.6.2. (a) Net and (b) gross calcification rates of the three Mediterranean cold-water corals held in aquaria during a one-month acclimation period at normal pCO_2 levels followed by three months at normal and increased pCO_2 levels. Net calcification was also measured for *Desmophyllum dianthus* transplanted to 350 m depth (Mooring) and at CO_2 seeps. Data are mean ± SD. Replicates per treatment are: (a) Acclimation, n = 14, 14 and 46; Normal and High pCO_2, 8, 8, 40 for *Caryophyllia smithii*, *Dendrophyllia cornigera* and *D. dianthus*, respectively; Mooring, 22 and CO_2 seeps 11 *D. dianthus*. (b) Normal and High pCO_2, n = 8 each species.

4.7. Physiological advantages of dwarfing in surviving extinctions in high CO_2 oceans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nature climate change

Physiological advantages of dwarfing in surviving extinctions in high-CO_2 oceans

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Among the different model organisms I have used to test for the effect of OA on the ability of organisms to calcify and face acid stress, this study is at the top of the list. Indeed, during my fieldwork at the CO_2 seeps in Vulcano, I observed, buried in the sediment at very low pH water (and in the sediment where the pH is even lower than in the surrounding seawater), shells of two species of tiny gastropods quite dissolved by the undersaturated seawater but still alive. Thanks to the collaboration with a malacologist, Vittorio Garilli, we compared their size to other populations around Sicily and found that their sizes were reduced. Further measures for their calcification at the IAEA lab and their shell structure and periostracum composition at the Centre Scientifique de Monaco, allow us to demonstrate that these species (i) despite the very low pH calcified, although at lower levels; (ii) had a periostracum that protected the inner shell from dissolution (as we previously found for Mytilus galloprovincialis in Ischia (Rodolfo-Metalpa et al. 2011); (iii) adapted to such conditions by a mechanism called Lilliput effect.
Here, we show that two gastropod species adapted to acidified seawater at shallow water CO_2 seeps were smaller than those found in normal pH conditions and had higher mass-specific energy consumption but significantly lower whole animal metabolic energy demand. These physiological changes allowed the animals to maintain calcification and to partially repair shell dissolution. These observations of long-term chronic effects of increased CO_2 levels forewarn of changes we can expect in marine ecosystems as CO_2 emissions continue to rise unchecked and support the hypothesis that ocean acidification contributed to past extinction events. The ability to adapt through dwarfing can confer physiological advantages as the rate of CO_2 emissions continues to increase.

Introduction to the study
In the immediate aftermath of the mass extinction events, many of the survivors were smaller than before (e.g. brachiopods, gastropods, bivalves and shelled cephalopods); a phenomenon termed the 'Lilliput effect'. After the most severe Late Permian extinction, gastropod species remained relatively small for millions of years. One hypothesis is that this dwarfing was an adaptation to ocean acidification to mitigate against the increased energy cost of carbonate secretion. To test for this hypothesis, we studied two nassariid gastropods Nassarius corniculus and *Cyclope neritea*, which were abundant on coarse sand and gravel at ca 100 m from the main seeps in Vulcano. These species are widespread in coastal lagoons and salt marshes in the Mediterranean as well as at shallow water hydrothermal seeps (e.g. off Milos and Pantelleria). We know that populations of *C. neritea* and *N. corniculus* had developed at the CO_2 seeps since their shells had paucispiral protoconches so these snails lack a planktotrophic larval stage. Seawater off Vulcano has been acidified since the late Pleistocene and a dwarf population of *N. corniculus* has been present for at least 30 years providing an opportunity to study the chronic effects of ocean acidification on gastropods submitted to high CO_2 levels over multiple generations.

The experiment

Here, we compared gastropods living in naturally acidified shallow-water conditions near CO_2 seeps off Vulcano with those at sites with ambient seawater pH to test for their ability to cope with acidification and, potentially, adapt. Shell morphology, dissolution and repair were examined using scanning electron microscopy. Animals collected in September and November 2011 were incubated in aquaria at CO_2 levels similar to those measured at reference and CO_2 seep sites and their gross calcification rates were measured using ^{45}Ca at both seawater pH levels. Rates of metabolic oxygen uptake of individuals from CO_2 seeps and a reference site were determined by Stop-Flow respirometry at pH 6.5 or pH 8.1 respectively within 24 hours of collection.

Main results and discussion

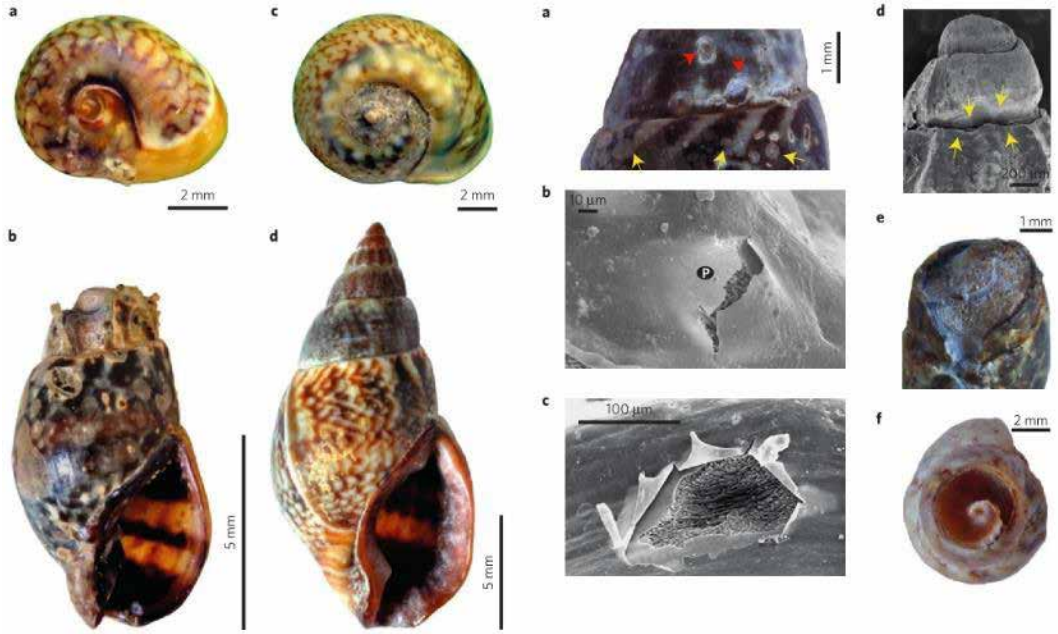

Fig. 4.7.1. On the left side. Shells of *Cyclope neritea* (a, c) and *Nassarius corniculus* (b, d). Samples living at CO_2 seeps (a, b) showing shell dissolution and apex truncation when compared to shells collected at reference site C1, ambient pH (c, d). Bare scales are two mm (a, b) and five mm (c, d). On the right side. Shell dissolution, apical truncation and repairing calcification process in *Nassarius corniculus* living at CO_2 seeps. A. Dissolution first affects shell periostracum (yellow arrows) and then shell mineralised layers (red arrows). B-c. Scanning electronic microscopy images showing shell periostracum (P) fractures (b), followed by shell dissolution (c). d. Juvenile specimen preserving the paucispiral protoconch, and showing dissolution of the sutural area (arrows) as first step toward shell apical truncation. E. Adult specimen from CO_2 seeps showing a dramatic effect of dissolution. Although it lacks shell apex, the animal was able to repair its shell closing the internal cavity. F. Shell cross section of a sample from reference site C1 artificially broken to show the normal empty space between the outer part of the shell and the columella.

By comparing the size of individuals from the seeps and the reference sites, we found that they were significantly smaller at CO_2 seeps, showing 1.26 & 1.37 mean (log) volume ratios between ambient and acidified conditions. These shell volume shifts, which approximate to biomass shifts, provide a means of directly comparing experimental and fossil data. In his global study of clade-level size change in gastropods Payne (2005) recorded a shell volume shift of 1.45 mean (log) volume between the Late Permian and Early Triassic, which is of the same order as that recorded by our experimental data.

In *N. corniculus*, shell integrity was affected by the corrosive seawater at CO_2 seeps; pockmarks were present on the teleoconch in almost all samples, corresponding to zones where the periostracum was affected by swelling and breaking. Deterioration of the periostracum is the first step before mineralized shell layers undergo dissolution as previously shown in mussels at shallow and deep CO_2 seeps. For *C. neritea* the early shell whorls were also corroded at the CO_2 seeps, with loss of the protoconch and pitting on the first teleoconch whorl.
Gross calcification rates in *N. corniculus* collected at CO_2 seeps (pH_T 7.2) were generally lower than samples from the reference site (pH_T 8.0) whereas they were consistently greater in *C. neritea* from CO_2 seeps than those living in normal conditions. Nassarius corniculus from high-CO_2 environments consistently maintained significantly lower calcification rates than those at reference sites even when they were removed from elevated CO_2 and cultured at pH_T 8.0 showing adaptation to acidified environments rather than plasticity of calcification in response to pH conditions. Our findings add to a growing body of evidence that calcification responses to acidification are both species-specific, which is unsurprising since there can be a great deal of biological control over calcification, and adaptive rather than plastic.

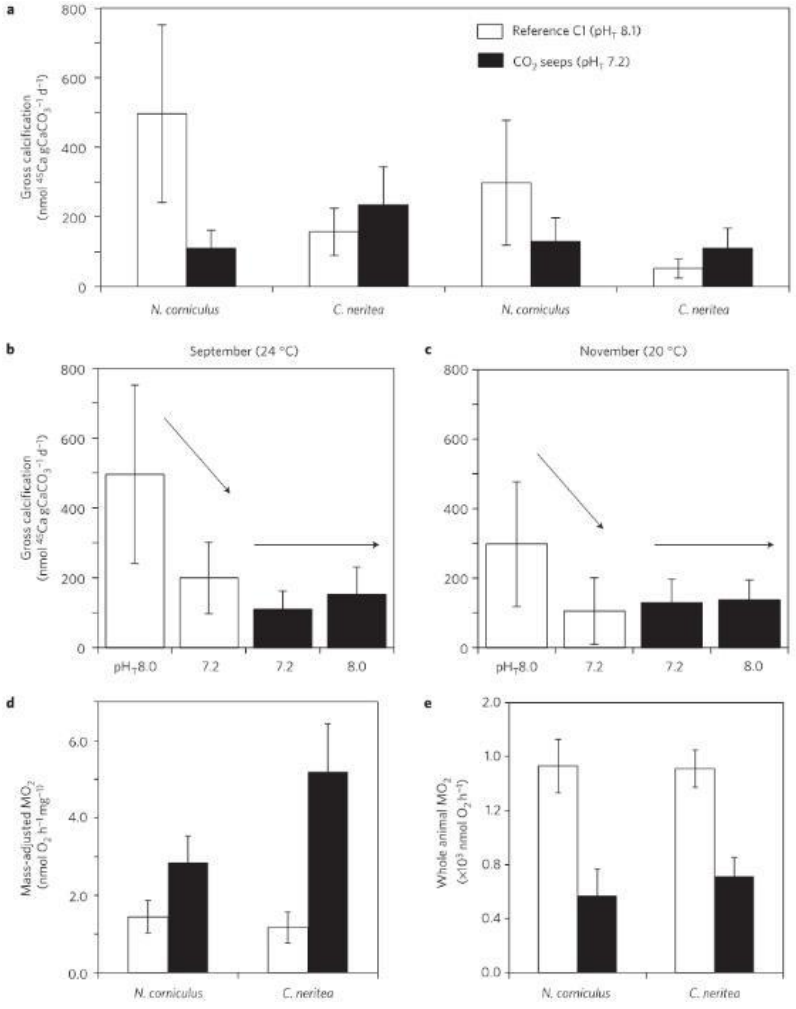

Fig. 4.7.2. Gross calcification (GC) and metabolic oxygen consumption (MO_2) in *Nassarius corniculus* and *Cyclope neritea* across normal and acidified sites. A. GC in September and November at pH measured at collection sites. b-c. GC of *N. corniculus* in September (a) and November (b) to measure the response of samples collected within the seeps and at Reference site and incubated at crossed pH treatments. Arrows show the GC change on samples incubated at pH of collection (CO_2 seeps or Reference C1 sites) and the pH of the incubation (pH_T 8.0 and 7.2). Oxygen uptake as an index of MO_2 expressed as body mass adjusted MO_2 (d) and whole animal MO_2 (e).

4.8. Papua New Guinea CO_2 seeps, Normanby and Ambitle Islands

In 2011, Fabricius and collaborators published the first of a series of studies showing the effect of ocean acidification on a coral reef in PNG. This study has been a milestone research since the Authors demonstrated that the effect of a shallow water CO_2 seep, lowering the surrounding seawater pH at the level expected for 2100, was deleterious for the reef. There, branching corals were absent and only massive *Porites* dominated the site. In addition, their growth was reduced confirming model predictions that ocean acidification, together with temperature stress, will probably lead to severely reduced diversity, structural complexity and resilience of Indo-Pacific coral reefs within this century. To support these conclusions, the Authors used the Normanby CO_2 seeps and two more seep sites, the latter actually not at the same ecological value as Normaby because very limited in space and quite sulphuric. Overall, this study has accelerated several studies and collaborations in this field and motivated a decade of research to find out how future reefs will be affected by acidification because according to what was found in Normanby, they certainly will be.
In 2016, within the framework of the ANR-funded project CARIOCA 'Coral Reefs Acclimatization to Ocean Acidification", I performed 3 expeditions and studied the Normanby seeps. To do that, I used a local diving boat, the Chertan and invited Prof K. Fabricius (AIMS, Australia) for the first mission. In addition, I found in the literature a promising site, in the East Britain province, previously studied from a geochemistry point of view by the team of Prof T. Pichler (University of Bremen, Germany) because the CO_2 vents were hot and rich in arsenic. In parallel to Normanby, I investigated this site 4 times, the first in collaboration with T. Pichler.

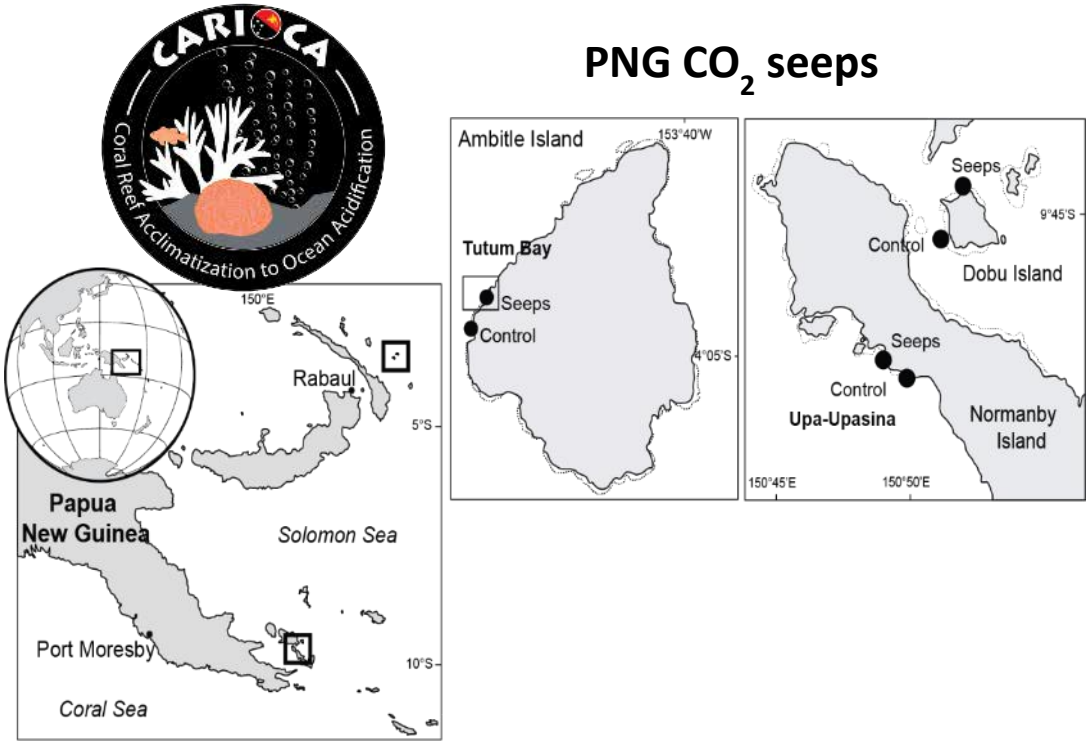

Fig. 4.8.1. Localisation of the two volcanic sites were the CARIOCA project studied the effect of CO_2 seep emissions on coral reefs.

4.8.1. CO_2 seep of Normanby Island (Upa-Upasina reef)

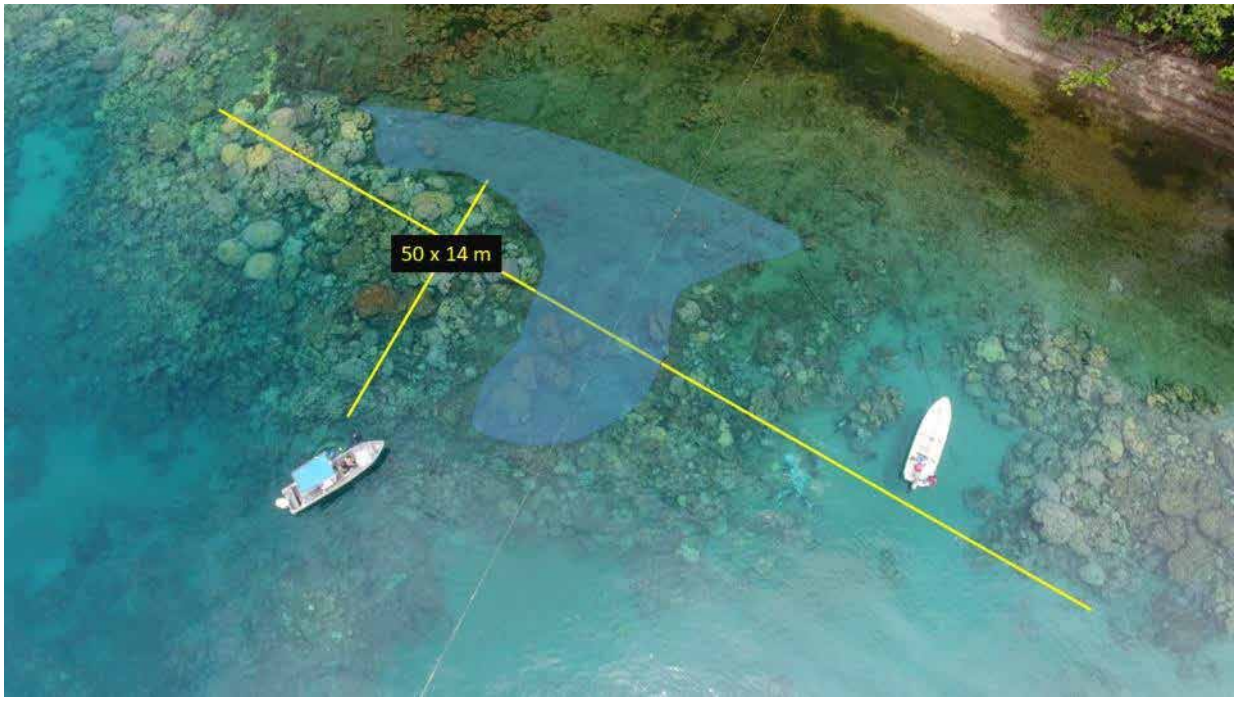

Fig. 4.8.1.2. Aerial picture of the Upa-Upasina reef. The shadow area is where the 2-3 vents with important gas emissions were found. Sparse seeps were also found in shallow water closer to the beach. The two yellow lines indicate the area where the seawater pH varied according to the dominant current and weather conditions.

This site has been used by our Australian colleagues from AIMS, JCU and several of the most renewed scientists working on the OA topic from different countries. A quick research on Google Scholar using "Normanby CO2 seeps", and "Ocean acidification + CO_2 seeps" as keywords for the research has given more than 40 studies, most of them published in very high IF journals, all performed at the Normanby CO_2 seep site. This is a non-exhaustive list of contributions, describing the changes in habitat and community composition, to the effect on fish, forams, bacteria, etc from molecular to the behaviour level. However, only some focused on corals and metabolic changes, including photosynthesis and calcification. Among them, is the first study, by Fabricius et al (2011), where the authors described for the first time the site. They found that the low pH, which average was as expected for the end of the century, caused the reduction in coral diversity, recruitment and abundance of structurally complex framework builders, and shifts in competitive interactions between taxa. However, coral cover remained constant between pH 8.1 and ~7.8, because massive *Porites* corals established dominance over structural corals, despite low rates of calcification. Reef development ceased below pH 7.7. This lack of reef complexity and altered settlement substrata was suggested to become a bottleneck for corals coral communities' establishment under ocean acidification (Fabricius et al. 2014; Sunday et al. 2017) directly, due to the difficulty for recruits to set (Fabricius et al. 2017; Noonan et al. 2018), and indirectly by reducing the habitat used by zooplankton, which is part of the diet of corals, fish and other reef species, therefore affecting their ecological success (Smith et al. 2016). At the molecular level, while Kenkel et al. (2018) showed that corals have undergone long-term acclimatization to natural variation in pCO_2, with only 61 genes differentially expressed in response to pCO_2 environment, a recent study by Leiva et al. (2023) demonstrated on the same data coral adaptation at the high CO_2 conditions. The authors demonstrated that adaptation to ocean acidification involved the three main compartments of the coral holobiont. They identified 441 coral host candidate adaptive genes involved in

calcification, response to acidification, and symbiosis; population genetic differentiation in dinoflagellate photosymbionts; and consistent transcriptional microbiome activity despite microbial community shifts. Corals from natural analogues to future ocean conditions harbour beneficial genetic variants with far-reaching rapid adaptation potential. The most complete studies on the physiology of some coral species at the CO_2 seep were performed by Strahl et al (2015; 2015) on two abundant species *Porites* sp. and *Pocillopora damicornis* and two more rare species *Acropora millepora* and *Seriatopora hystrix* at the seep site. They found that the net photosynthesis rates increased considerably in *Porites* spp. and *A. millepora* and slightly in *P. damicornis* at increased pCO_2, but remained unaltered in *S. hystrix*. Rates of light calcification declined in *S. hystrix* at high pCO_2 but were unaffected by pCO_2 in the other three coral taxa. Dark calcification rates remained unchanged in massive Porites and P. damicornis, but were drastically reduced at high pCO_2 in *A. millepora* and *S. hystrix*. However, skeletal densities were similar at both seep and control sites in all coral taxa investigated. Overall the Authors recognised that pCO_2-tolerant corals were characterized by an increased ability to acclimatize to ocean acidification, e.g. by maintaining net calcification. Thus, robust corals, such as *Porites* spp. and pCO_2, are more likely to persist for longer in a future high pCO_2 world than those unable to acclimatize. What the Author did not highlight is that the two less robust species, which are rarer at the seeps, are also the species more sensitive to heat stress and that have been decimated at the site during repeated bleaching events (pers. observation).

Fig. 4.8.1.3. Reef dominated by massive corals *Porites* spp. in Upa-Upasina reef and sparse CO_2 seeps. Signs of severe colony mortality were evident.

4.8.2. CO_2 seep of Ambitle Island (Tutum bay)

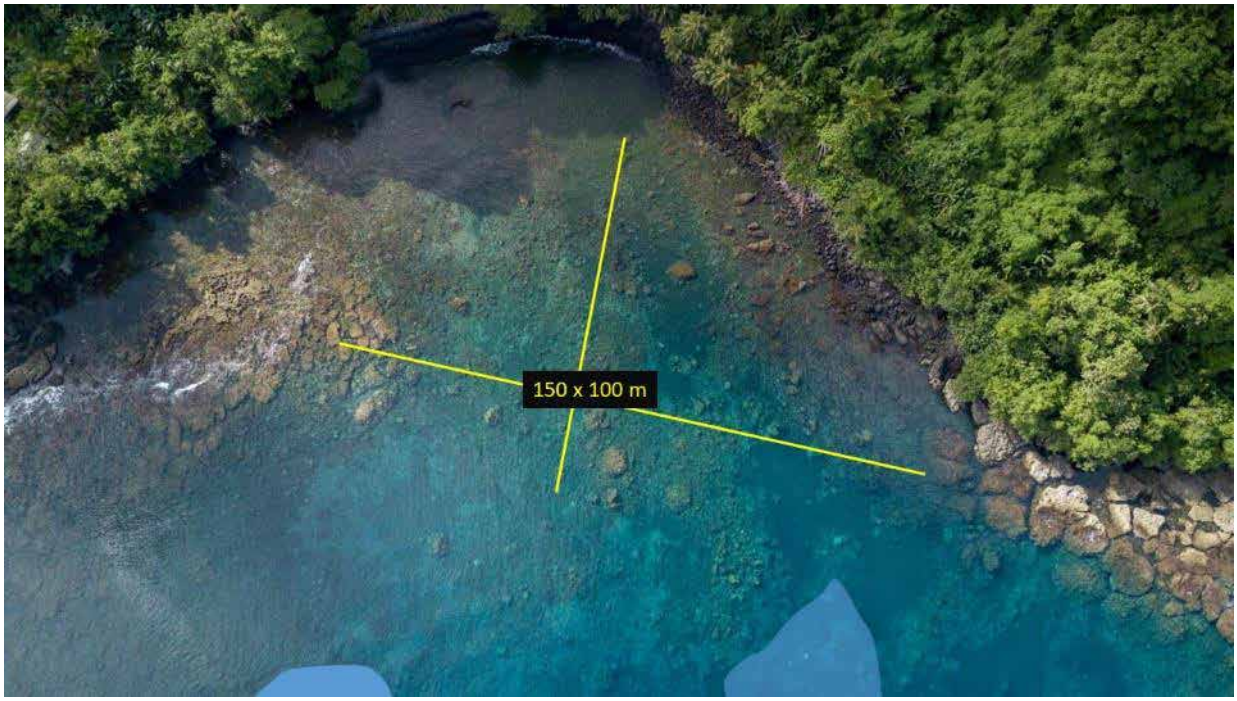

Fig. 4.8.2.1. Aerial picture of the Tutum Bay reef. The shadow area is where the 3-4 cratera with important gas emissions were found. Sparse seeps were also found everywhere in the bay. The two yellow lines indicate the area where the seawater pH was lower than the pH measured at the adjacent reference reef.

I added this site in the ANR Carioca, originally based only on the site of Normanby, because I read a paper on the journal Marine Chemistry (Pichler et al. 1999) where some amazing pictures showed massive degassing at 8 m depth in a coral reef. Prof Pichler discovered tis site likely in 2016 and performed there several expeditions and published very important contributions. Prof Pichler and colleagues paid lower attention to the effect of high CO_2 on the reef, likely because it was too early for this topic, but as a geochemist, they fully described the emitted fluids, their origin and how they change in the seawater column (Pichler & Dix 1996). For this site already exists a complete chemical description of the hydrothermal fluids, sediments pore waters including trace elements contents as well as a general census of some key-habitat species, including archaea and bacteria (e.g., Akerman et al. 2011; Karlen et al. 2010; Meyer-Dombard et al. 2012). These studies showed that the concentrations of Si, Mn, Cs, Fe, Zn, and particularly As were much higher in the hydrothermal fluid and that they were slightly elevated in Tutum Bay seawater when compared to "normal" seawater (Pichler et al. 1999a; Price and Pichler 2005; Price et al. 2013b). While metal pollution, however is not specific to coral reefs around CO_2 vents as many reefs worldwide (*e.g.* Costa Rica, Panama, Red Sea, Thailand, New Caledonia) can have high metal levels (Ali et al. 2011; Biscéré et al. 2017, 2015; Fujita et al. 2014; Guzmán and Jiménez 1992; Moreton et al. 2009; Tanaka et al. 2013; Whitall et al. 2014), and many experimental studies have emphasized that some of them can play key roles in the functioning of corals (Biscéré et al. 2018, 2017; Ferrier-Pagès et al. 2001), Arsenic could become toxic and therefore be a great bias in using this site as natural lab. With this in mind we performed the first expedition in Ambitle in collaboration with Prof T. Pichler with the aim at providing a full description of the CO_2 variation in the Bay, and more importantly the concentration of As on the bottom cora reef level. During this first cruise, and 3 more we reciprocally transplanted (control and high CO_2 sites) three coral species during 7 months, collected cores *Porites*, skeletons of a series of coral species, perfomed on board incubations

and field incubations for the determination of their metabolic rates, transplanted corals near a hot source, re-transplanted fragments of 7 species between sites, did experiments on the nutritional needed of corals exposed to high pCO_2, measured diversity, etc etc. Some of these data have been published, other are waiting for collaborators, and other unfortunately will never be published.

Overall, this site is in my opinion the best CO_2 seep available today, unfortunately is in a very remote geographical position. Indeed, without a good research vessel it is impossible to perform any research there. It is the best because we clearly showed that there is no effect of arsenic and that other potential harmful elements and gasses emitted by seeps were not bioavailable at the bottom seabed, and either dispersed or fixed in the surrounding seawater column (Pichler et al. 1999). We measured several time the variability of the seawater carbonate chemistry in the bay showing that although seawater pH varied, its variability was tiny when compared to the variability measured at the Normanby site. This clearly affected the ability of corals to keep elevated calcification pH values and, as a consequence their ecological success (Comeau et al 2021). This site was surprisingly rich and diverse with around one hundred of coral species (Shlessinger, unpubl data). This findings was in contrast to what studies reported from the most famous Normanby site and questioned the validity of some previous findings that established some corals as the winnners vs other loosers. I mentioned that I performed two transplantations between the control and the seep sites. Unfortunately, I have been unlucky on that, and for two reasons. The first transplanting was a real success and all corals survived to the 7 months. It was planned to measure their calcification using the alkalinity technique, which measures the amount of carbonate used to calcify during the incubations we performed *in situ* inside semi-autonomous respirometric chambers; in addition, their transcriptomic, and the change induced by the new environment had to be measured by my colleagues, partners of the ANR. Neither the alkalinity method nor the collaboration with my colleagues worked and these two important pieces of the puzzle remain missing to date. During a second transplantation, I measured the coral growth using the buoyant weight tecnique, and I did it on the land, at the local tribu (a real experience!), using a balance and a battery. Here again, I was unlucky since the corals transplanted at the control were lost, probably during a previous storm. Surprisingly, corals at the CO_2 seeps survived and grew a lot, but I do not have the data to compare these results to a reference condition. More data and findings are still to be published from the expeditions performed at Tutum Bay.

Fig. 4.8.2.2. Photomosaic showing the transplatented samples of *Porites* at the beginning and after 7 month at CO_2 seeps in Ambitle; the 'cathedral', i.e., the transplantation we did to study the combined effect of warming (note the tube trapping a hot emission) and OA on transplanted corals; some working on land with the special help from local people; the 6 respirometric chambers we used to measure the coral respiration; the second transplant setting and a nice vent eroding a *Porites* colony.

Fig. 4.8.2.3. One of the large CO_2 degassing (vent) and sparse seeps observed in Tutum Bay at ca. 8-10 m depth.

Fig. 4.8.2.4. Some examples of the reef diversity found in Tutum Bay.

4.9. pH variability at volcanic CO_2 seeps regulates coral calcifying fluid chemistry

RESEARCH ARTICLE

Global Change Biology WILEY

pH variability at volcanic CO_2 seeps regulates coral calcifying fluid chemistry

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This study apparently deviates from my field of research on coral physiology since it applies geochemical techniques on the skeletal composition of scleractinian corals living at the CO_2 seeps in PNG to investigate their ability to calcify at low pH conditions. In reality, although I continue to lack this expertise, I have always looked to collaborate with specialists in that exciting field of research. Indeed, I have been co-author of 9 studies analysing lithium isotope compositions ($^{7}Li/^{6}Li$), Li/Ca, Li/Mg, $^{88}Sr/^{86}Sr$, B/Ca ratios of shallow-water and deep-sea corals (Rollion-Bard et al. 2009; Trotter et al. 2011; Montagna et al. 2014; Fruchter et al. 2016), carbon and oxygen-isotope analyses on mussels (Hahn et al., 2012), shell structural limpets (Langer et al. 2014, 2018). Geochemical techniques definitely allow investigating calcification processes at a fine scale and the outcome from using such techniques has greatly improved our knowledge on the effect of OA on the ability of corals (and not only to calcify under past and future environmental conditions. For that, I recently supervised the PhD of Clement Tanvet that coupled geochemistry to the physiology of corals from Bouraké. In the Comeau et al paper I have been lucky to collaborate with a panel of great experts of different fields, from which I learned a lot. This study indeed links boron skeletal composition measurements of 7 coral species living in and out of two CO_2 seeps sites in PNG with their distribution and abundance, and the variability of the main environmental parameters. By combining geochemical, ecological, and chemical approaches, our study demonstrates that even under seawater pH lower than that predicted by the end of the century because of climate change, a variety of corals that exert strong control on their calcifying fluid might still be able to calcify, grow, and persist.

Introduction to the study

To form their skeleton, corals can modify the chemical conditions of the calcifying fluid to facilitate the mineralization process. Similarly, increasing seawater DIC under OA elevates DICcf (Comeau et al. 2018). While this increase in DICcf could partially alleviate the negative effects of decreasing pHcf (Cornwall et al. 2018; Schoepf et al. 2017) large uncertainties exist in the literature about the magnitude and physiological controls of these effects. In addition, a large range of coral responses to treatments with different levels of pH variability has been reported in laboratory experiments, ranging from no measurable impacts (Camp et al. 2016) to positive offsets against OA (Comeau et al. 2014b). This range of impacts could arise due to species-specific responses to pH variability, but also because of differences among studies in the frequency and magnitude of pH fluctuations used in the experiments. Studies that attempt to shed light on the reasons coral calcification rates varied under OA conditions in experiments are therefore needed. But the reason that pushed toward this research comes from my observation and comparison of two different CO_2 seeps in PNG, the Normanby site, where only a few coral species resist OA conditions, as reported by Fabricius and colleagues, and the Ambitle site, where we observed a rich and diverse reef (Pichler et al. 2019). In Normanby, the deleterious effects of low pH on the physiology, abundance, and diversity of calcareous organisms were reported (Fabricius et al. 2011, 2017) causing the reduction of species diversity

and evenness compared to control sites. The site indeed is dominated by massive *Porites* spp. likely due to its capacity to maintain elevated pHcf under a large range of seawater pH, as demonstrated both *in situ* (Wall et al. 2016; 2019a) and *ex situ* (Comeau et al., 2019). This could be a general mechanism of certain coral species under low pH that favours their presence in naturally acidified sites (Wall et al. 2019a; 2019b). The main question behind this project was: "Were Fabricius and colleagues really in the right when using the Normanby site they demonstrated that OA has a dramatic effect on corals?". This study does not respond to this question but contributes to clarifying it, and many results remain to be published.
In this study, we aimed firstly to understand whether changes in mean seawater pH and the magnitude of variability in seawater pH affect the control of coral calcifying fluid in the field for multiple species. Secondly, we aimed to explore whether the capacity of species to better regulate calcifying fluid (CF) chemistry is correlated to species relative abundances at sites with different pH and DIC conditions. To that end, we utilised two natural CO_2 seep locations with distinct CO_2 variability, relatively limited in Ambitle, and large in Normanby. Using physiological measurements and field observations of coral species abundances, we tested three complementary hypotheses: 1) corals growing in acidified sites can maintain chemical conditions optimal for calcification in their calcifying fluid (i.e., pHcf homeostasis), 2) the most abundant corals in acidified sites are the ones with the best control on their calcifying fluid, and 3) seawater pH variability will alter both ecological outcomes and corals' CF chemistry.

Main results and discussion

Seawater pH variability. Ambient mean pH values were 8.01 and 7.96 in the control sites, and 7.64 and 7.51 in the seeps sites in Ambitle and Normanby, respectively. Seawater pH variability was considerably larger at the Normanby seep site where the pH dropped down as low as 6.64 pH units. During the entire time frame of pH logging, corals were exposed to low pH conditions (i.e., pH ranging between 7.6–7.8) for 60% of the time at Ambitle, and for 31% of the time at Normanby. Very low pH values (i.e., pH < 7.6) were less frequent in Ambitle than in Normanby (24% and 43% of the time, respectively). Similarly, high pH values (> 7.8) were less frequent in Ambitle than in Normanby (16% and 26% of the time, respectively). Despite similar mean pH at both CO_2 seeps, corals in Normanby experienced much larger variations in seawater pH.

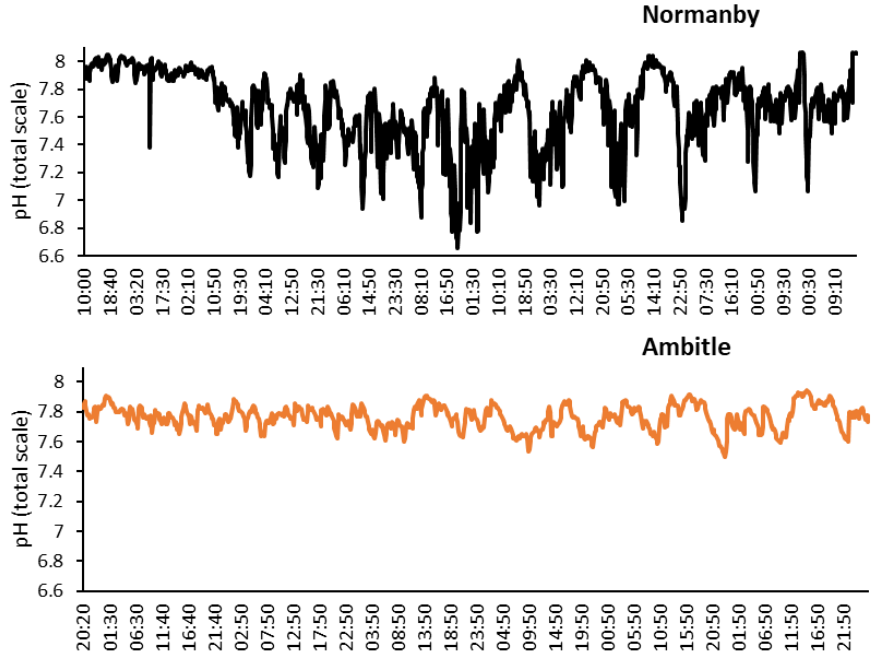

Fig. 4.9.1. Seawater pH measured at the Normanby and Ambitle reef over 6 days.

This includes frequent records of pH as low as 6.6, but also values close to ambient conditions (pH 8.0) very frequently. pH in Normanby occasionally varied by as much as one pH unit in less than one hour. This large variability in pH is likely driven by the shallow topography of the Upa-Upasina reef, where the depth varies between ~1–4 m, which makes seawater pH extremely dependent on water mixing caused by local wind conditions (Fabricius et al. 2011). In contrast, Pichler et al. (2019) showed that in Tutum Bay (Ambitle Island) the main seep and other associated sparse seeps change the seawater carbonate chemistry of the whole bay (1-8 m deep). In Tutum Bay, pH variability is mostly driven by tides, with lower pH associated with low tides.

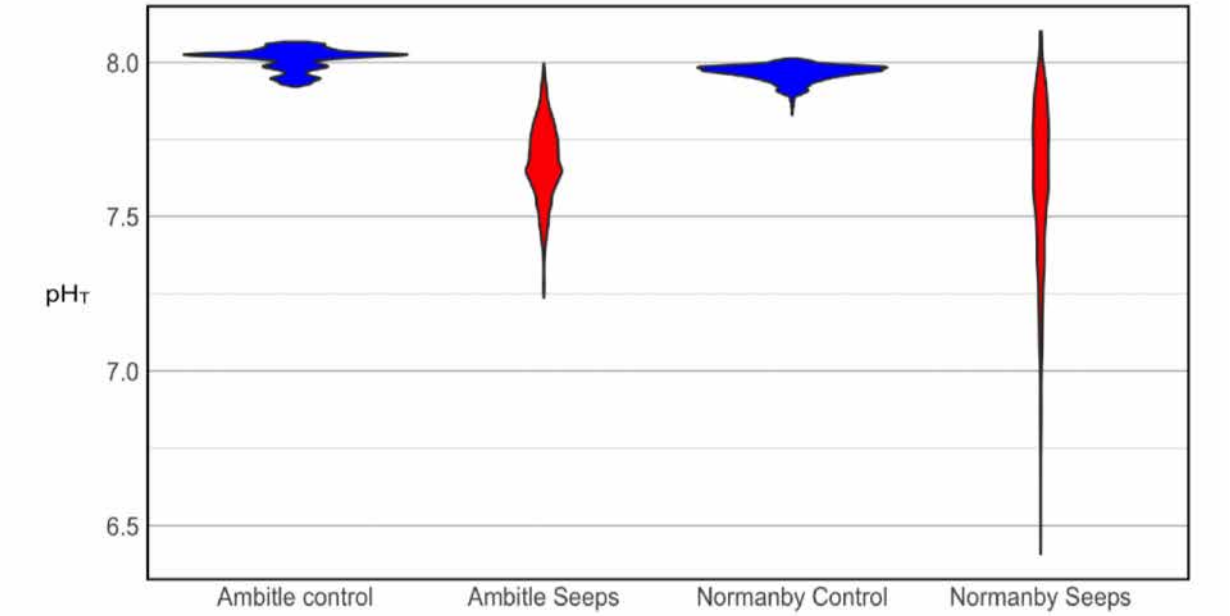

Fig. 4.9.2. Violin plot showing the *in situ* pH (n > 15,000 for each site) measured using autonomous pH sensors SeaFET at both Ambitle and Normanby seeps and respective control sites during fieldwork in September 2016 and May 2017.

pH of coral calcifying fluid. Across the 14 coral species studied in Normanby average pHcf was higher in the control site (8.43 ± 0.02, mean ± SE, n = 42) than in the seeps site (8.39 ± 0.02, mean ± SE, n = 42, t-test, $p < 0.05$). The highest pHcf was observed in *Galaxea fascicularis* at the control site (8.57 ± 0.01, n = 3), while the lowest was measured in *Favites halicora* at the seeps site (8.27 ± 0.01). pH_{cf} was significantly lower at the seep site compared with the control site in five species (t-test, $p < 0.05$ for all five), with delta pHcf the lowest in *Acropora samoensis* (-0.15).
In Ambitle, for the 8 species pooled together, pH_{cf} was on average significantly higher (t-test, $p < 0.05$) in the control site (8.46 ± 0.02, n = 24) than in the seeps site (8.35 ± 0.02, n = 24). The highest pHcf was measured in *Acropora tenuis* at the control site (8.54 ± 0.04), while the lowest was found in *Echinopora lamellosa* at the seeps site (8.23 ± 0.03). pH_{cf} was higher in the control site compared with the seeps site in all species (t-test, $p < 0.05$ for all) but one, *Montipora foliosa*, which showed no differences. As a result, delta pH_{cf} which is equal to pHcf seeps – pHcf ambient varied between -0.19 in *Echinopora lamellosa* and -0.02 in *Montipora foliosa*. We suggest that the disparity between responses of pHcf in the different locations is most likely due to the larger range and variability in pH at Normanby.

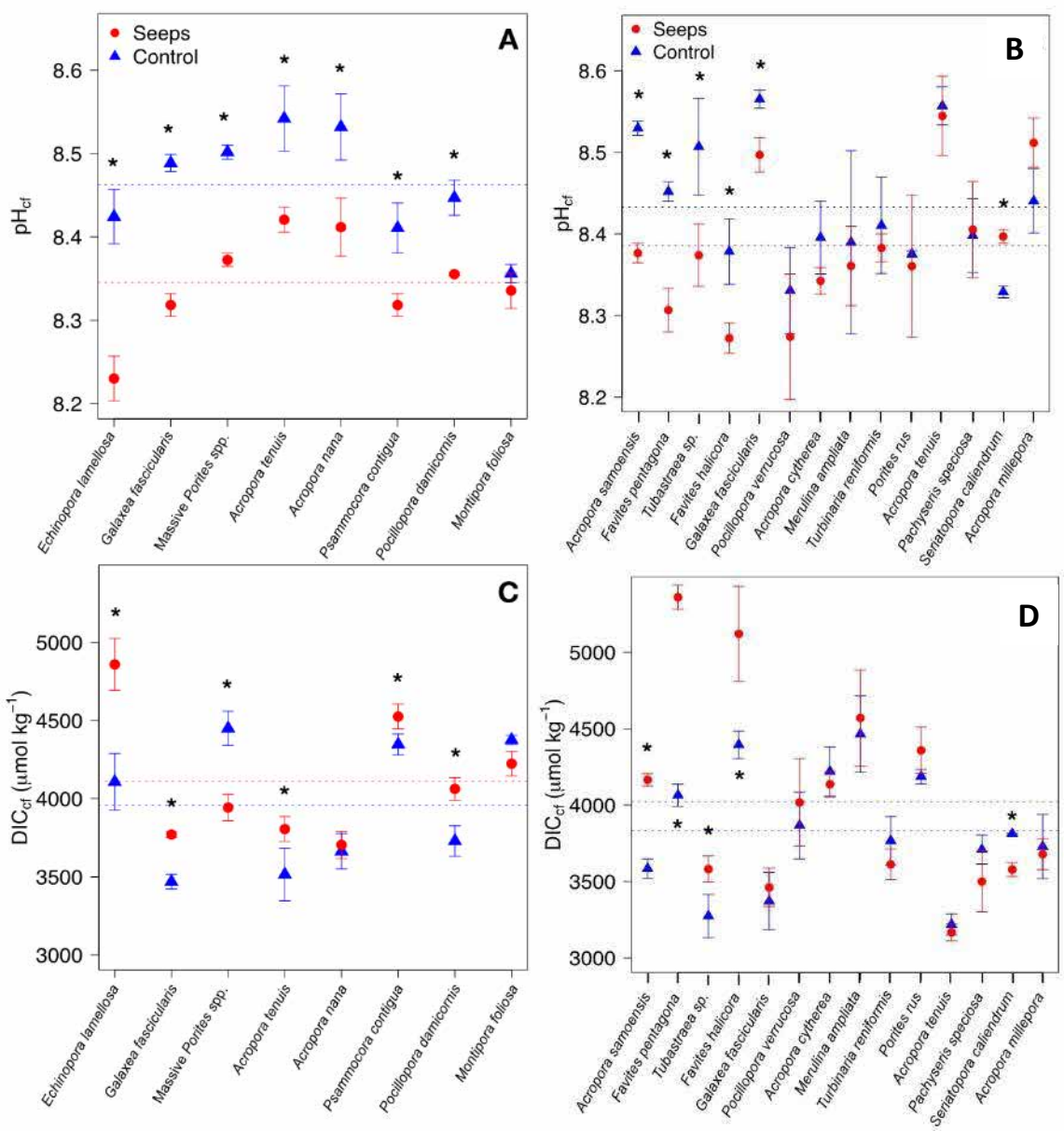

Fig. 4.9.3. Calcifying fluid carbonate chemistry estimates of 8 coral species from the control and seeps sites in Ambitle Island (A, C), and of 14 coral species from the control and seeps sites in Normanby Island (B, D). A, B) pH of the calcifying fluid (pH_{cf}); C, D) Dissolved inorganic carbon at the site of calcification (DIC_{cf}). Blue and red colours indicating the control and seeps data, respectively. Dashed lines represent the pooled pH_{cf} and DIC_{cf} mean across all species in each site. Asterisks indicating species in which significant differences were found. All data presented as mean ± SE, with n = 3.

Coral abundance and calcifying fluid chemistry. In Normanby, most of the studied species were either similarly abundant (absolute abundance) in both sites or more abundant at the control site than at the seeps site. By contrast, this pattern was not observed in Ambitle, where three species were more abundant at the control site than at the seeps site (*G. fascicularis*, *P. damicornis*, and massive *Porites* spp.) but two other species were more abundant at the seeps site than at the control site (*A. nana* and *M. foliosa*). Although being relatively rare in Ambitle, two more species had opposing abundances: *A. tenuis* was more abundant at the seeps site than at the control site (0.22 ± 0.9 and 0.06 ± 0.2 mean abundance per transect, respectively) while *E. lamellose* was more abundant at the control site than at the seeps site (1 ± 1.1 and 0.05 ± 0.2 mean abundance per transect, respectively).

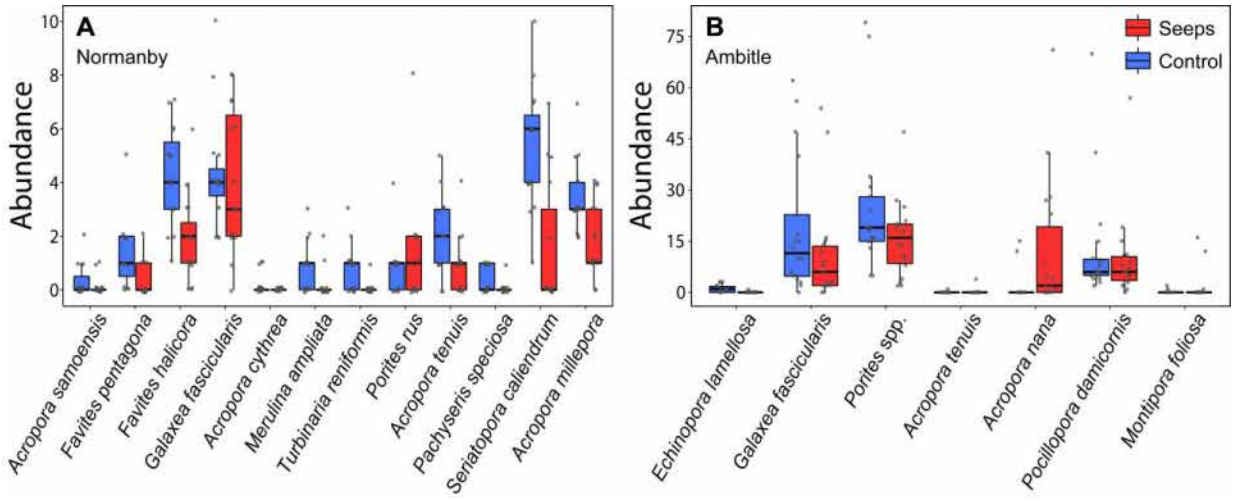

Fig. 4.9.4. Abundance of the studied species in A) Normanby Island; and, B) Ambitle Island. The abundance is presented as the number of colonies per belt transect with points indicating individual belt transect data (n = 15 at each site in Normanby, and n = 18 at each site in Ambitle). To aid the visualization of panel B, in Ambitle, three outlying data points were excluded. Two of these points were values > 100 for *G. fascicularis* in the control and the third was a value > 80 for *Porites* spp. in the control. Blue and red colours indicating the control and seeps data, respectively. Center lines of the box plots indicate the medians, boxes indicate the lower and upper quartiles, and whiskers indicate 1.5x interquartile range.

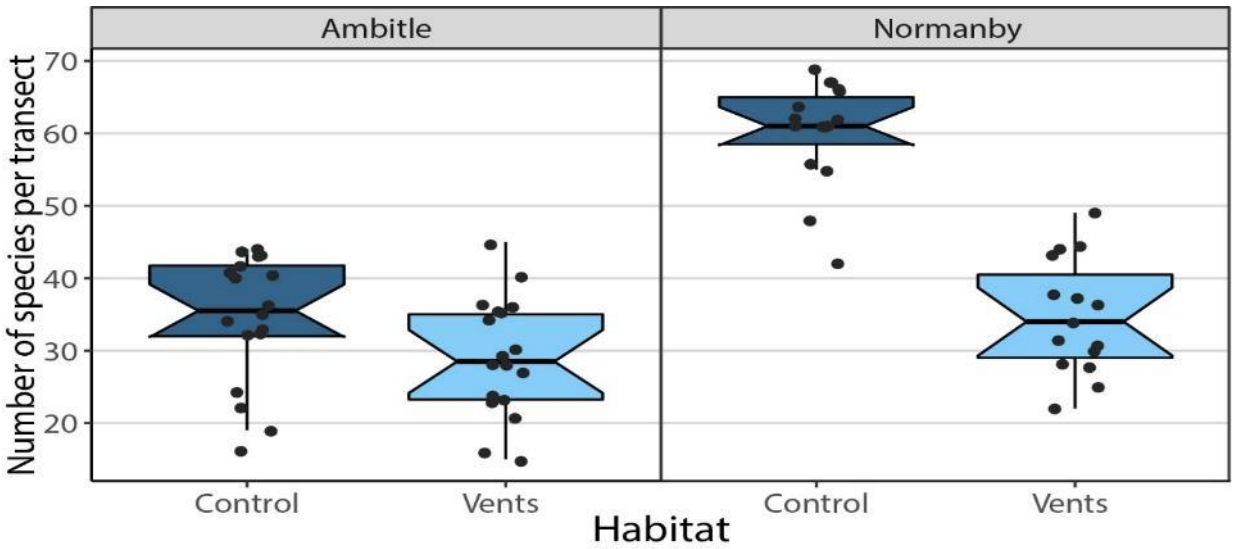

Fig. 4.9.5. Violin plot of the number of species per transect found in Ambitle and Normanby in May 2017 September 2016, respectively.

Overall, our results support the idea that species-specific coral physiology controls responses to OA *in situ* (as observed with seaweed inorganic carbon use previously; Cornwall et al. 2017) with no or minor relations to coral phylogeny and morphological traits. Moreover, our findings suggest that coral control of carbonate chemistry in the calcifying fluid might influence their ecological success under OA. This manifested in Ambitle, where pH variability is low and where corals with the highest control on their calcifying fluid pH generally had a higher change in relative abundance between the CO_2 seeps and control sites. However, these traits only provide partial information and further research at a more extensive set of sites is now required. In contrast, our study also shows that large pH variability, such as the one found in Normanby, could mask the link between species' physiological traits and ecological success, highlighting the importance of characterizing environmental conditions *in situ* at high temporal resolution.

4.10. The semi-enclosed lagoon of Bouraké

Fig. 4.10.1. A picture of the semi-enclosed lagoon of Bouraké, surrounded by a rich mangrove forest, taken by M. Nitschke during the landing of the plane at the nearby airport.

In December 2015 David Suggett (University of Technology of Sydney) I worked with in Ischia, asked me if in New Caledonia there was a mangrove area inhabited by corals, which means that the mangrove should not be in a river and estuary area. Thanks to the precious suggestion of the team of specialists in mangrove forests, led at IRD by Dr Cyril Marchand, I visited the Bouraké mangrove and performed the first observations and measurements. At this site, 150 km from Nouméa, lagoon waters flow inside the system with the rising tide, circulate and then exit at the falling tide according to a semi-diurnal cycle. The depth of the system varies from a few centimetres to more than 6 m. The channel, which is more than 80 m wide, penetrates the mangrove and creates large pools over a total area of more than 60,000 m^2 (without considering the mangrove area). The site is very rich in corals that form true fringing reefs at several points of the system, mostly at the end of the terraces that extend from the mangrove toward the channel. The most dense and compact corals live at the border of these terraces, likely because the terraces are always exposed to the air during the low tide. At each tide, new lagoon water enters through the channel into the vast inner basin of the mangroves. During this journey, the water chemistry changes, due to metabolic reactions in the sediment, coral reefs and mangrove habitats, and it mixes with the already present water which is more acidic, hot and deoxygenated.

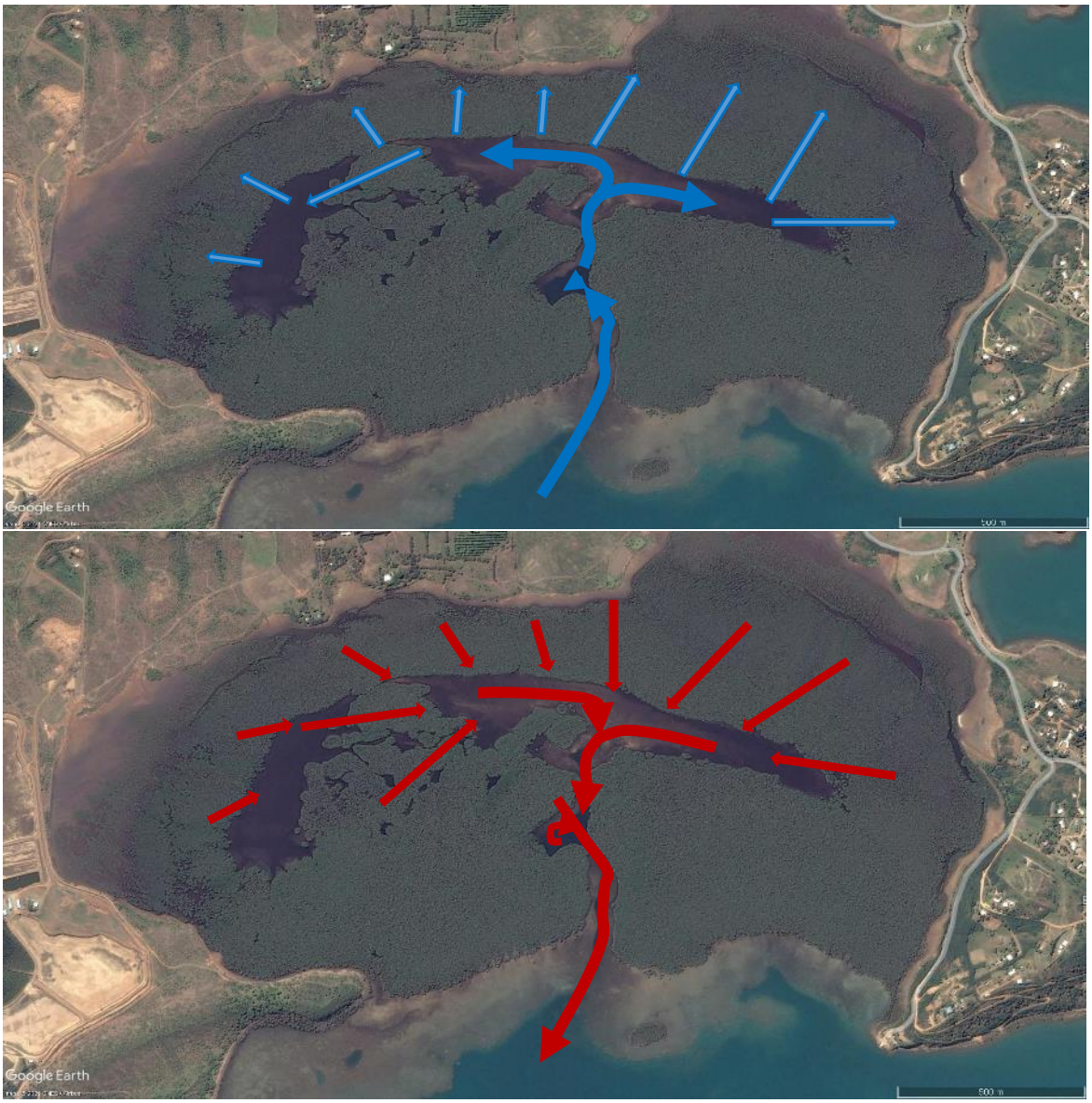

Fig. 4.10.2. Satellite picture of the mangrove area of Bouraké. The blue arrow indicates the direction of the rising tide that brings new seawater from the external lagoon, through the channel and the back reservoir, and finally the mangrove forest. On the contrary, the red arrow indicates the opposite displacement of the hot, acid and deoxygenated seawater from the mangrove toward the external lagoon.

The first measurements of the daily pH fluctuations, during a 24-hour cycle, revealed the value of this unique site for studying the capacity of corals to acclimatize and adapt to extreme conditions. Even at high tide, the water in the system never returns to "normal" values. For example, the maximum pH values are around 7.9 (the normal pH of the ocean is currently 8.05 and is predicted to decrease to 7.7-7.8 in 2100). Only during very high tides does the pH value become 8.0. At falling tide, seawater becomes more acidic and oxygen-depleted as the system begins to drain. The large volumes of water that were inside the mangrove forest enter the system and are then discharged into the lagoon. At low tide, near corals, the pH reaches the extreme value of 7.3 and O_2 has a concentration of 2 mg L^{-1} (the normal concentration of O_2 at the coast is 4-6 mg L^{-1}).

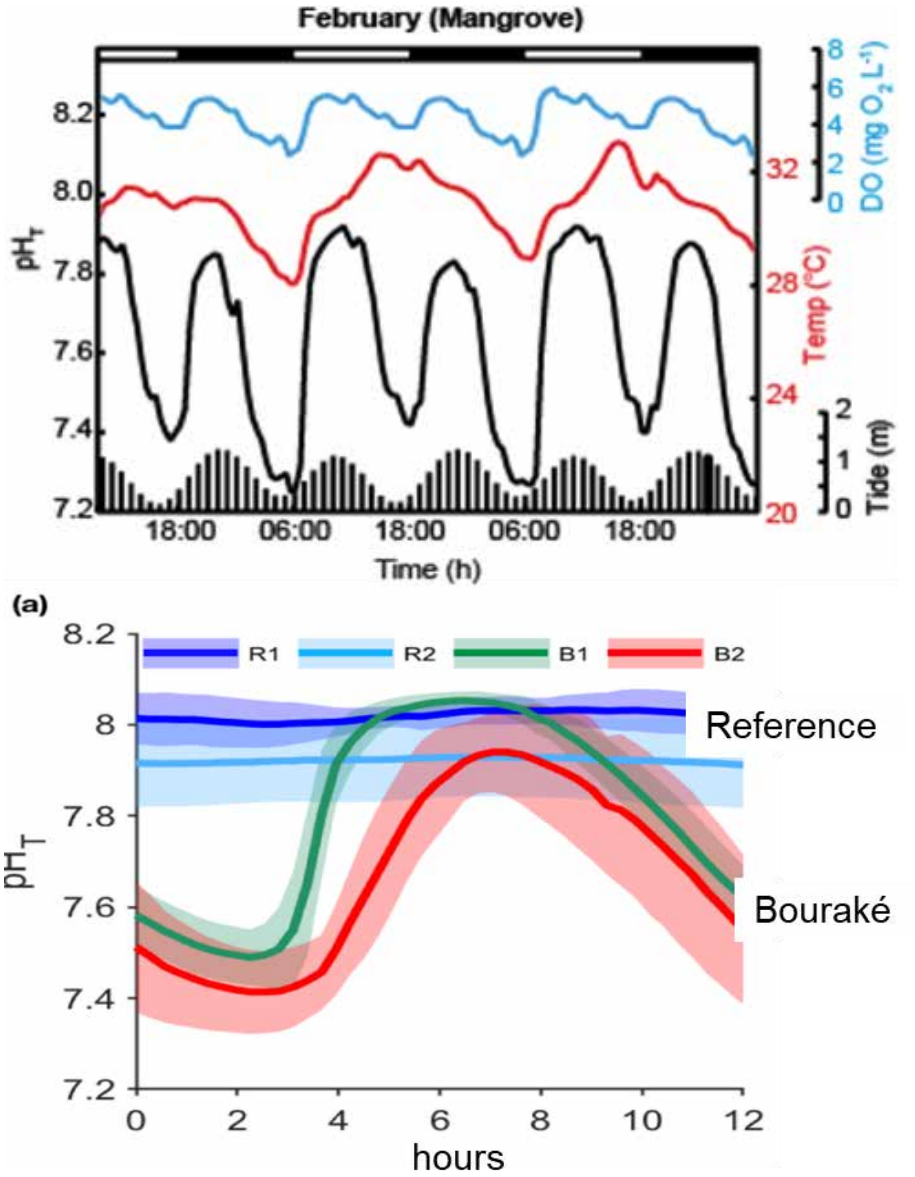

Fig. 4.10.3. The graph on the top shows the seawater pH_T, temperature, oxygen changes and tide measured in February 2017 during a 3-day cycle in Bouraké. On the bottom, seawater pH_T variations were recorded at the reference (Sts R1 and R2) and Bouraké (Sts B1 and B2) reefs. Data were overlaid at a single tidal phase (12 h). Data are 22, 72, 31, and 72 semidiurnal tidal cycles.

In addition, seawater temperature also is higher than outside the system because it stagne in the mangrove forest at a very low depth, therefore it is exposed during winter to the atmospheric cold temperature (down to 13°C in the area), and during summer to high temperature due to solar insolation (up to 36°C in the air). This causes the water to be 1°C colder in winter, and 0.5-3°C warmer in summer than outside the system. These changes happen quite suddenly and cause the coral to be exposed to real MHWs all the time with changes in temperature of up to 6°C especially in summer. All these parameters exhibit detectable fluctuations over a 24-hour cycle, which is extremely important in assessing the amount of stress that corals experience over time. This makes this site much more interesting than other natural laboratories in which the duration and intensity of stress (e.g., volcanic vents) are not consistent in time or space.

In a first attempt to describe this site and give the first idea of the ability of corals living there to face the extreme conditions, the team composed of Emma Camp, David Suggett and Matthew

Nitschke worked during two fieldwork periods. From these researches, we published three papers (Camp et al. 2017; 2019 and 2020) that described the seawater environmental conditions; produced a list of coral species, and gave the first analyses of the bacterial community living in symbiosis with the corals. In Camp et al. (2017) we also measured high respiration and increased heterotrophy of corals from Bouraké and interpreted it as an adaptive response of corals to the harsh conditions which was favoured by the autotrophic conditions of seawater (i.e., organic input from the mangrove). However, we did not know at the time of this first fieldwork, in 2016, that seawater temperatures were abnormally high and that corals were under stress, therefore physiological responses partially altered. These precursor papers substantially anticipated further years of research dedicated to reinforcing the environmental measurements with long-term and higher frequency data and diel cycles in the chemical parameters of seawater. All the data collected were analysed in a compelling assessment of this unique natural laboratory (Maggioni et al. 2021). In this study, we also performed a whole dataset of coral, macroalgae and sponge taxonomical list throughout Bouraké, and found 66 species of corals, which was similar to the control reefs but intriguingly more abundant for some species such as *Acropora* (12 species) and *Montipora* (2 species). The site was not dominated by *Porites* as the CO_2 seeps in Normanby. A 3D model of the water circulation completed the study and it was realized using data collected simultaneously by 3 ADCP during 15 days and crossing a cyclon event, although these data have not been yet valorized in a paper. Data showed that the current can be either very strong (up to 1.5 Knt) or almost absent, according to the area considered. The first simulations showed that the water mass moves in and out of the system up to seven times before disappearing. These data are of great value when considering the larval dispersion during spawning, or measuring the DBL of corals at different flow conditions.

Starting from December 2015, I have visited hundreds of times Bouraké, organized fieldwork for whole teams from different institutions and countries, collaborated with tens of people, supervised 3PhD, 1 postdoc, 5 M.Sc, got funds for a series of projects, presented the site for an ambitious ERC Cog project, and finally published a handful of papers from the data obtained from this site. But the most important thing in my eyes is the fact that the site has gained a certain notoriety because it is unique in the world.

Fig. 4.10.3. Aerial picture of the entrance of the Bouraké semi-enclosed system during low tide.

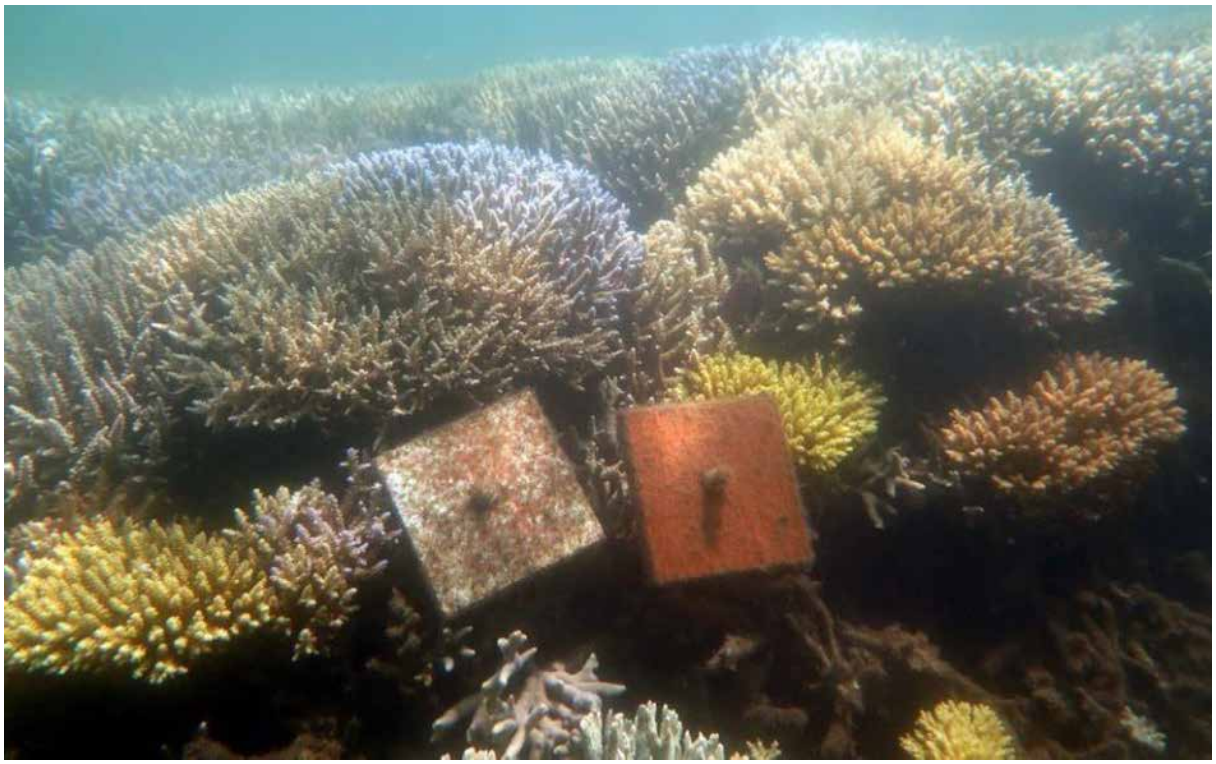

Fig. 4.10.4. The reef dominated by *Acropora* spp. in Bouraké with two terracotta tiles positioned on the reef to assess coral recruits, one having CCA and one deprived.

4.11. High coral recruitment despite coralline algal loss under extreme environmental conditions

This study reports the effect of such extreme environmental conditions of the Bouraké semi-enclosed lagoon on hard coral recruitment and CCA originally settled at a forereef on artificial substrates that were transplanted over two years in two fringing reefs and the Bouraké lagoon. Our data emphasize the negative effects of the extreme conditions in our study sites on the CCA, which decreased in cover by ca. 80% and lost in the competition with turf algae, which, in turn, increased up to 162% at the end of the two years. Conversely, hard coral recruitment remained high at Bouraké throughout the study, three-fold higher than at two sites located outside Bouraké where environmental conditions were typical for coastal fringing reefs. Our findings show that while such extreme, climate change conditions have a direct and adverse effect on CCA abundance, and despite a certain persistence, coral larvae settlement was not affected. Based on previous findings from Bouraké, and the present observations, both coral recruits and adults seem to be unaffected despite the extreme environmental conditions. This study supports previous research illustrating how extreme natural and variable environments may reveal unexpected and positive insights into the processes underlying coral acclimatization and adaptation to global change.

Introduction to the study

One of the bottlenecks of the reef status is their ability to reproduce, settle and continuously form persistent reefs. Ocean acidification has been predicted to greatly affect this natural and complex mechanism. Scleractinian corals begin their life as pelagic larvae eventually settling to the reef, growing and establishing as adult, long-lasting colonies (Harrison and Wallace 1990). Healthy and functional coral reef communities directly depend on coral recruitment success that, in turn, depends on the abundance of their preferred settling substrate i.e., crustose coralline algae (CCA, family Corallinaceae, Lithophyllaceae, and Porolithaceae) (Heyward and Negri 1999). Indeed, CCA are known as framework binders and act as a preferential settlement substrate for coral larvae (Morse et al. 1988; Heyward and Negri 1999; Harrington et al. 2004; Nelson 2009; Price 2010). Several lab-based experiments addressing the effects of OA on CCA have demonstrated reduced calcification and growth rates (Kuffner et al. 2008; Comeau et al. 2013), increased dissolution (Anthony et al. 2008; Diaz-Pulido et al. 2012), and reduced recruitment (Kuffner et al. 2008; Bradassi et al. 2013). Field studies have shown reduced recruitment (Fabricius et al. 2011, 2015) or impaired capacity to attract coral larval settlement (Doropoulos et al. 2012). However, it has been recently shown that lab-based versus field experiments tend to conclude that OA has a stronger impact on CCA (Page et al. 2022). Indeed, CCA and corals persist at CO_2 seep sites (Kamenos et al. 2016; Enochs et al. 2020; but see also Fabricius et al. 2011 for an opposite finding) and at low pH environments in Palau (Barkley et

al. 2015). For coral recruits, OA has been shown to cause skeletal malformations (Foster et al. 2016), and a decrease in the availability of settlement cues (Albright et al. 2010; Albright and Langdon 2011). Thus, in general, OA could affect coral recruitment by dissolving CCA or changing their microbial biofilm compounds, hence decreasing the availability of a supposedly ideal substrate for coral recruits (Webster et al. 2013) and finally affecting the recruit survival (Doropoulos et al. 2012; Doropoulos and Diaz-Pulido 2013). However, OA is only one of the ongoing threats to coral reefs, and its combination with both ocean warming (OW) and deoxygenation (OD) will likely cause CCA bleaching and mortality (Diaz-Pulido et al. 2012; Cornwall et al. 2019; Hughes et al. 2020), and impairment of coral larvae settlement (Jorissen and Nugues 2021).

Here, we assessed the effects of extreme and fluctuating environmental conditions (i.e., low pH, O_2, and high temperature) on the abundance of CCA, algal turf, and coral recruits. To address this objective, we obtained CCA-covered tiles from a forereef and moved them to three fringing coastal reef sites with distinct environmental conditions. We investigated the effect of the environmental conditions typical of coastal fringing reefs on the CCA cover twice during the two years experiment and we quantified and identified the coral family recruited on the tiles at the end. Based on the reported effect of OA on CCA cover and coral recruitment rates, we hypothesized that in Bouraké both will be heavily affected by the extreme conditions.

Our findings reinforce the idea that coral recruitment is possible even under extreme conditions and that, particularly for the Bouraké site, corals may have adapted to such variable and extreme environmental conditions.

Main results and discussion

After 7 months of conditioning at a forereef, i.e., just before they were moved and randomly shared between the three study stations (T0), the top sides of the tiles were predominantly covered in CCA (median % cover of 69.21%, all tiles pooled), while algal turf was rare (median per cent cover of 11.95%). Two main CCA genera were recognized on the tiles on a simple visual check regarding the conceptacle's size and disposal: *Porolithon* and *Neogoniolithon*. After two months (T2) at the three stations, the CCA median per cent cover was significantly different among stations and significantly decreased at B3 from 69.21% to 41.4%. CCA cover significantly increased at the two stations R2 and M1, respectively 64.4% and 81.3%. In contrast, turf cover was significantly different between stations, reaching the highest values in B3 (20.4%), and only 6.4% and 9.7% at R2 and M1, respectively. After two years of deployment (T24/26), CCA median %cover on the top sides of the tiles was lower than the one at T2 at all stations: this decrease was dramatic at B3 (from 41.4% to 8.4%) and M1 (from 81.3% to 1.5%), but to a much lower extent at R2 (from 64.4% to 49.3%).

CCA cover on the top sides of the tiles remarkably declined at Bouraké after only 2 months of immersion, while remaining stable or slightly higher at the two other reef stations. This suggests that ca. 40% of CCA settled during the tiles pre-deployment, and dissolved during a short-term exposure, therefore further indicating a strong negative effect of the Bouraké natural environmental conditions on CCA. At the same time, turf algae cover was significantly higher at Bouraké than at other reef stations. CCA cover on the top sides of the tiles remarkably declined at Bouraké after only 2 months of immersion, while remaining stable or slightly higher at the two other reef stations.

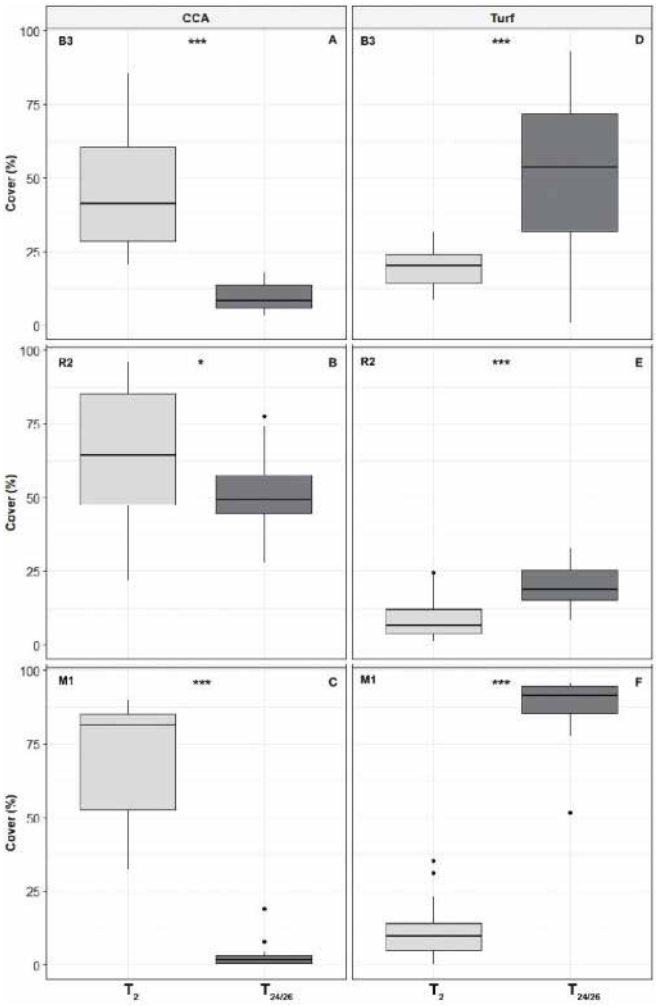

Fig. 4.11.1. Change in crustose coralline algae (CCA) (A, B, and C) and Turf percent cover (D, E, and F) on top sides of the tiles between the beginning (two months after the time of deployment, T2) and the end (after ca two years of deployment, T24/26) of collector deployment at study stations B3, R2 and M1 (number of tiles n = 15, 20 and 19, respectively). Data are median values ± 25th and 75th percentiles (box), minimum and maximum values (whiskers), and outliers (dots). Stars represent statistical significance.

Coral recruitment significantly differed among stations and sides after two years of tile immersion. It was found to be ca. 3-fold higher at B3 than R2 and M1 with 18.4, 4.6, and 5.5 recruits per tile (all tiles pooled per station), respectively. Regarding where the larvae preferentially settled on the tile, the number of recruits was always higher on the bottom than on the top, and scarce on the lateral sides.

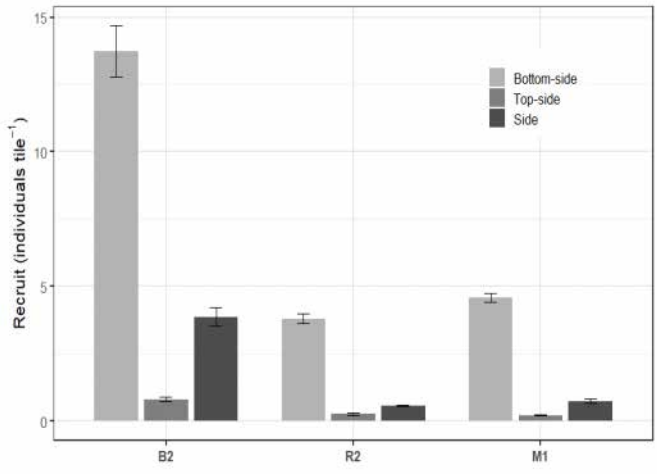

Fig. 4.11.2. Number of coral recruits (per tile) found on the different sides of the tiles immersed during ca. two years at Bouraké (B3) and stations R2 and M1. Data are means ± SD (number of tiles n = 15, 20, and 19, for B3, R2, and M1, respectively). Data from the lateral sides were pooled. Stars represent statistical significance.

Acroporidae was the dominant family of coral recruits settled in our experiment, with similar relative abundances between stations (65.58%, 73.91%, and 67.33%, at B3, R2, and M1, respectively). Pocilloporidae was the second most abundant family, with recruits who equally settled at all three stations in terms of relative abundance (14.85%, 17.39%, and 16.83% at B3, R2, and M1, respectively). Only a few *Poritidae* were found at B3 and M1 (17.39%, and 6.52%), and none at M1 which exhibit more unknown genera.

Coral recruitment rates in Bouraké were higher than outside, at both the two fringing reefs. While our findings appear to contradict results from several OA laboratory-based experiments (e.g., Doropoulos et al. 2012), they are in agreement with some field experiments (Kamenos et al. 2016; Enochs et al. 2020) showing in general, a lesser impact of OA (Page et al. 2022).

4.12. Corals adapted to extreme and fluctuating seawater pH increase calcification rates and have unique symbiont communities

RESEARCH ARTICLE

Ecology and Evolution WILEY

Corals adapted to extreme and fluctuating seawater pH increase calcification rates and have unique symbiont communities

Clément Tanvet[1,2,3] | Emma F. Camp[4] | Jill Sutton[2] | Fanny Houlbrèque[1,3] | Gérard Thouzeau[2] | Riccardo Rodolfo-Metalpa[1,3]

Introduction to the study

Although data from our first study (Camp et al. 2017) showed that corals from Bouraké had lower calcification rates, I have transplanted corals several times in Bouraké and found no effect or even increased performance in corals.

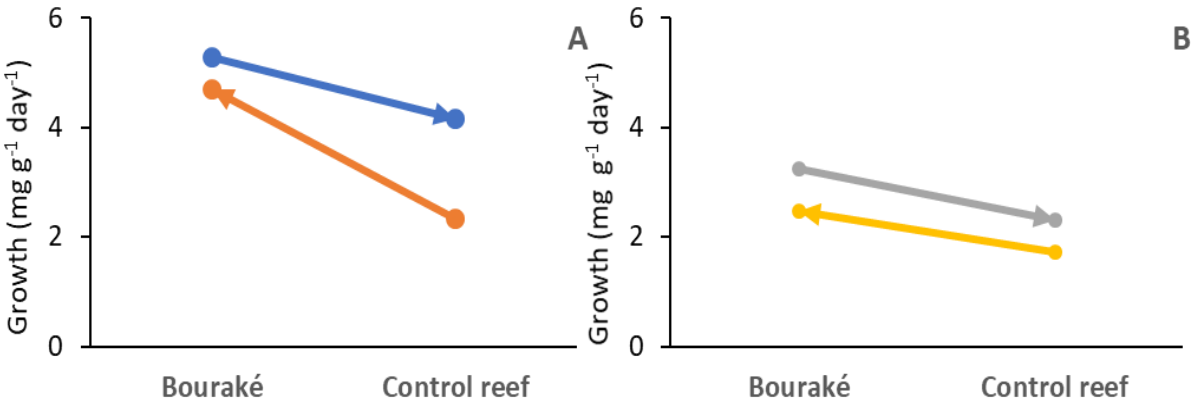

Fig. 4.12.1. Growth rates measured by buoyant weight technique on colonies of the coral *Pocillopora damicornis* reciprocally transplanted between Bouraké and a nearby control reef for 12 months. The arrows indicate the direction of the cross-transplantation (Rodolfo-Metalpa, unpublished data).

For instance, the growth rate of the coral *Pocillopora damicornis* (and two more species) showed that colonies from Bouraké always grew more than control colonies and that these latter increased their growth when moved to Bouraké, which is opposite to what happened to the Bouraké colonies transplanted to the control. These unpublished results suggest that i) although Bouraké has extreme conditions, for some reason its environment favours the growth of this coral species since the control colonies increase their growth rates; ii) the colonies that have lived there all their life have adapted and can perform better even when moved to the control environment.

It seems that chronic environmental changes could make corals more resilient to future changes in pH and/or temperature (Comeau et al. 2022; Enochs et al. 2020; Schoepf et al. 2020). Bouraké and the population of corals living there offer a great opportunity to test several hypotheses from the role of fluctuating environmental parameters to the role of heterotrophic inputs, likely making corals more resistant to face OA, and finally to the host adaptative capacity in the short- and long-term.

In this study, Tanvet et al conducted a 100-day OA experiment in aquaria using three coral species (*Acropora tenuis*, *Montipora digitata* and *Porites* sp.) from the highly variable Bouraké and a less variable adjacent reference reef to assess: i) whether corals adapted to ambient or

fluctuating pH conditions alter their metabolic rates and calcification under different levels of OA (i.e., 7.54, 7.76, 8.11 and 7.56-8.07), and ii) whether Symbiodiniaceae communities are distinct between habitats and treatments at the end of the experiment. In addition, we considered both static pHNBS (7.54 ± 0.08; 7.76 ± 0.07; 8.11 ± 0.05) and variable (ranging from 7.56 ± 0.07 to 8.07 ± 0.07) pH_{NBS} conditions to assess the role that fluctuating pH may play in the success of Bouraké corals, and whether corals adapted to stable pH can acclimate when exposed during 100 days to variable pH. We hypothesized that corals from Bouraké exhibit enhanced physiological traits and a distinct Symbiodiniaceae community, compared with corals from the less variable reef, and that the natural diel fluctuations in seawater pH promote their resilience to OA.

Main results and discussion

Growth rate. In agreement with the consensus on the effect of OA on coral calcification (Gattuso et al. 1999), recently revised by Leung et al. (2022), we found that calcification rates of both *A. tenuis* and *Porites* sp. reference corals were significantly decreased when exposed to future and extreme pH levels. The mean growth rates of the three corals originating from the reference and maintained at control pH, generally decreased when maintained at future, extreme and also variable pH. This decrease was more evident in the two branching corals than in *Porites* sp. In contrast, the mean growth rates of the three corals originating from Bouraké and maintained at future, extreme and variable pH i) were always higher than their counterpart originating from the reference and maintained at the same pH condition; ii) were almost always (with one exception, *A. tenuis* at variable pH) higher than their counterpart originating from the reference and maintained at the control pH condition; iii) were almost always higher or did not change (with one exception, *M. digitata* at variable pH) when maintained at the control pH condition.
These unexpected findings suggest that Bouraké corals have adapted to OA because they have been exposed to extreme conditions throughout their lives, and regain even higher rates of calcification than the same coral counterparts adapted to open-water pH conditions, once at actual open-water pH (i.e., ca. 8.10).
Our data do not consistently suggest that Bouraké corals have acquired a particular mechanism that accounts for better physiological plasticity to cope with low pH conditions, thereby maintaining higher calcification rates. Indeed, we did not find a clear effect of coral origin with a systematic increase in photosynthetic rates, higher contents of Symbiodiniaceae, chlorophylls, and protein in Bouraké corals.

Effect of variable pH. One of our hypotheses to explain the success of Bouraké corals was the potential positive effect that diurnal pH fluctuations could have on coral metabolism, as previously found for corals in GBR mangrove lagoons (Camp et al. 2019), St Vincent and the Grenadines CO_2 vents (Enochs et al. 2020), and for others incubated in mesocosms (Brown et al. 2022; Dufault et al. 2012). Our results show that short-term exposure (i.e. acclimation of reference corals during our experiment) to fluctuating pH has a negative effect on coral calcification as all coral species from the reference decreased their growth when incubated at variable pH. In contrast, Bouraké corals, which are acclimatized and/or adapted to local conditions when exposed to variable pH calcified from 7.6% (*A. tenuis*) to 116.2% (*M. digitata*) more than their counterpart from the reference site. Furthermore, Bouraké corals incubated at future and extreme pH maintained these higher growth rates and even increased them when grown under control conditions, confirming their resistance to OA. Although the duration of our experiment was longer than most OA experiments (i.e., 77% lasted 1-11 weeks; Brown et al. 2022; Ziegler et al. 2021), we acknowledge that the duration of variable pH exposure experienced by corals from the reference site during the experiment was too short to compare

with that experienced by corals at Bouraké. Corals exposed to a variable environment throughout their life are physiologically more plastic than corals adapted to stable environments (Kenkel & Matz 2017); such plasticity is probably time-dependent. Future experiments should take into account the length of exposure to variable pH (as well as other environmental parameters) that the corals experienced before the collection.

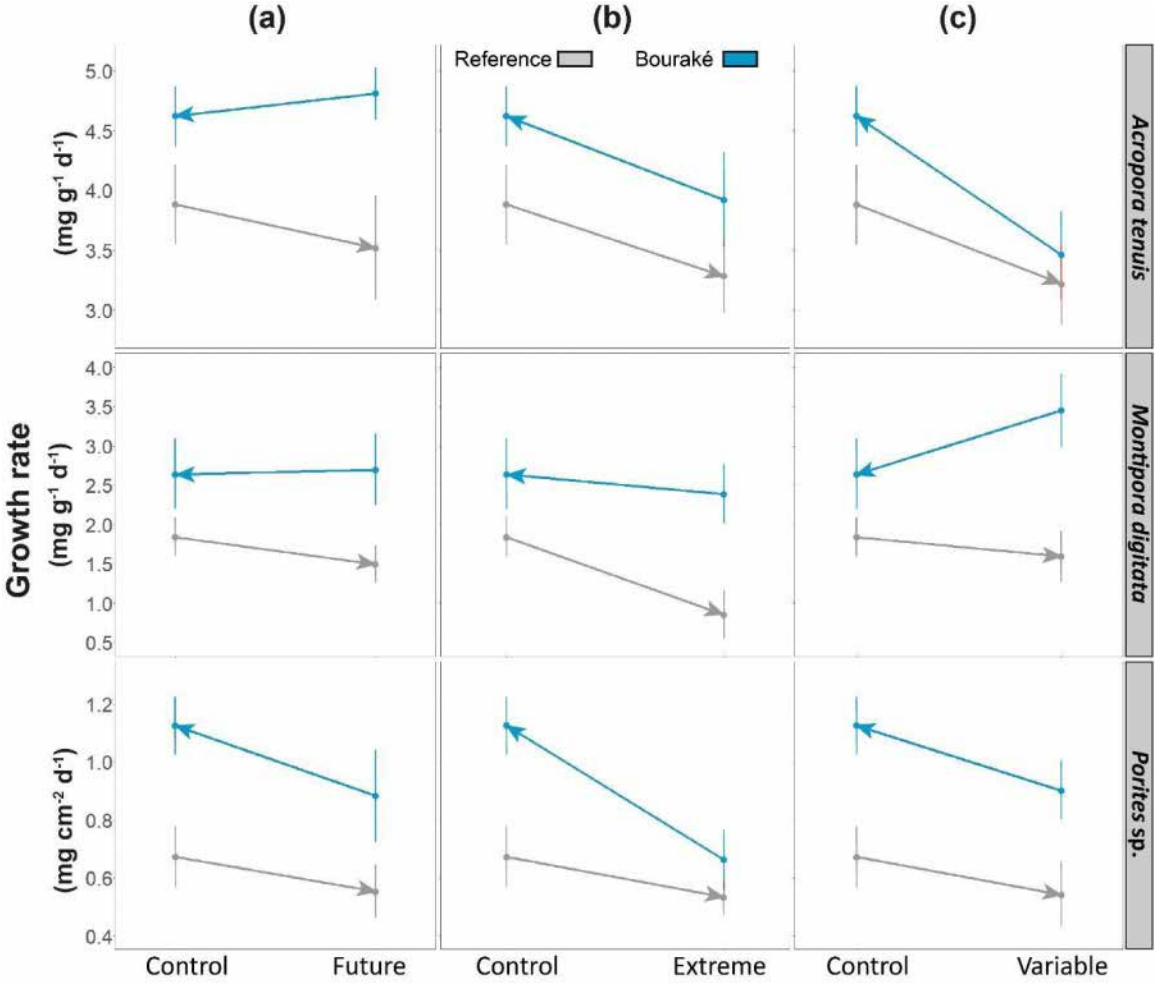

Fig. 4.12.2. Reaction norm of the growth rates of corals from Bouraké and Reference site maintained during 100 days at four pH conditions (Control (8.11), Future (7.76), Extreme (7.54), and Variable (7.56-8.07)). The arrow indicates the direction of the change from the origin to the pH condition. Data represented as dots are means of the three replicate tanks ± SE (overlapping SEs are in red) (n = 13-16, depending on species and pH condition).

By assessing the holobiont physiological responses and the Symbiodiniaceae profiles of three coral species from both Bouraké and a reference reef to a large range in pH, we found that corals from Bouraké always exhibited higher growth rates and had a specific and more consistent ITS2 majority sequence than corals from the reference reef both under low and variable pH conditions. Our data suggest that seawater pH level, whether stable or variable, at future or extreme levels, does not affect the Symbiodiniaceae major ITS2 type profiles of corals (at least over 100 days), since at the end of the experiment we found no significant effect among treatments. Microbiome stability (both for symbionts and/or bacteria) was linked to greater physiological resilience to OA (Ge et al. 2021; Grottoli et al. 2018; Quigley et al. 2017, 2019; Ros et al. 2021), suggesting that consistent major ITS2 type profiles for Bouraké corals under pH treatments could facilitate their success; however, further work will be needed to verify this hypothesis.

5. FUTURE RESEARCH"Hope for coral reefs. Can acclimatization and adaptation follow the pace of climate change?"

5.1. Abstract

Coral reefs shelter one of the largest biodiversity on earth and provide US$36 Billion per year of ecosystem services. According to current experimental evidence, they could disappear or be drastically decimated within the coming decades if CO2 emissions remain unabated. Yet, I recently discovered a remarkable site, which the only natural analogue of future climatic conditions exhibiting the three main parameters that drive climate change in the oceans: lower pH (<7.8) and oxygen (-20 to 30%), and warmer temperatures (+ 0.5 to 3°C). While these conditions are generally recognized as unfavourable to corals, there, a rich and abundant coral reef has developed. This novel natural analogue brings new hope for the future of coral reefs and provides a unique natural laboratory to explore how corals could keep pace with climate change.

HOPE crosses traditional interdisciplinary boundaries between evolutionary biology, physiology, ecology, and genetics to study the mechanisms by which coral reefs may acclimatize or adapt to suboptimal conditions. Ambitious and unprecedented experiments will be carried out in situ where phenotypic responses will be measured for a diverse array of life-history traits in several species. Within-generation, parental effects and transgenerational plasticity responses to suboptimal conditions will be assessed using specifically designed reciprocal transplantations on adult and juvenile corals. Complementary laboratory experiments and analyses will provide solid evidence of the underlying evolutionary mechanisms that corals might use to face climate change. Results will also identify the species that are more likely to resist in the future, which is particularly important for coral reef conservation and assisted evolution projects that aim to enhance the resilience of corals. Overall, HOPE will provide critical and ground-breaking results that can fundamentally change our understanding of how climate change will impact coral reefs in the future.

5.2. State-of-the-art and objectives

Atmospheric carbon dioxide (CO_2) has steadily increased over the industrial period [1] leading to ocean warming (OW) and ocean acidification (OA). Current projections of average global warming by the end of the 21st century range from the most optimistic 1.8ºC to the most pessimistic 4.0ºC temperature increase, in combination with a 0.2 to 0.3 unit drop in ocean pH [2]. In their latest report, the IPCC panel of scientists raise the alarm about the consequences of climate change [3] and, based on current experimental evidence, significant impacts on key marine organisms and ecosystem services are predicted for the coming decades [e.g., 4-5]. Specifically, 70 to 90 % of coral reefs (and associated species) are projected to decline with a global warming of 1.5°C, whereas virtually all reef species (>99 %) would be lost with a 2ºC warming.

Although the effects of OA are still debated, there is no doubt that it will negatively impact a range of marine organisms [6, 7]. Projected impacts on reef structures and communities include reduced growth, weaker skeletons, increased bioerosion, dissolution of carbonate substrates, as well as other functional traits [8], with consequent biodiversity loss [9]. In contrast, studies also revealed that responses are species-specific and that some taxa may be able to cope with, or even thrive, under projected acidification conditions, at least for some functional traits [e.g., 10-18]. Responses to OW depend on species thermal tolerances and their thermal history. In general, metabolic rates increase with temperature, sometimes with an apparent benefit for some traits such as growth [19]. However, above a thermal tolerance threshold, growth, body size, behavior, reproductive success and a suite of metabolic functions are hindered [20]. For instance, tropical coral reefs are extremely vulnerable to OW and have undergone repeated massive bleaching events during the last decades [5, 21, 22].

One of the limitations of our current knowledge of the impacts of OA and OW on marine organisms is that most studies are based on deterministic models or short-term laboratory experiments of individual organisms [e.g., 4, 23, 24]. These experiments identify the effects of one or a few variables in isolation,

but they are unable to account for the capacity of species to acclimatize in natural environments. Laboratory experiments are also not ecologically realistic since, for instance, they do not consider the effects of species interactions, natural provision of nutritive supplies, and natural fluctuations in the main environmental parameters [25].
But the most significant gap in the current knowledge is that most studies have neglected the role of adaptation and only within-generation responses to global change have been tested during short-term exposures to stress [reviewed in 26]. Exceptions include experiments using short-lived organisms such as coccolithophores, copepods and polychaetes, coralline algae, sea urchins and fish [e.g., 27-34], or breeding experiments involving sea urchin larvae [35, 36]. For example, Pespeni et al. [35] demonstrated the genetic changes and rapid evolutionary capacity of sea urchin larvae collected from coastal areas exposed to upwelling-driven acidification and reared in aquaria under low pH conditions. Unlike previous laboratory experiments [e.g., 37], larval development and morphology showed little response to high pCO_2 levels, probably because they were already able to tolerate these conditions. Transgenerational studies showed that in some mollusks [38-40], sea urchins [41] and coral reef fishes [42-45], the exposure of adults to OA can promote positive carryover effects facilitating the acclimatization and survival of larvae and juveniles under acidified conditions. While adult metabolic performances of the coral *Pocillopora damicornis* were impacted when exposed to a combination of OW and OA, their larvae exhibited metabolic acclimation when subsequently re-exposed [46, 47]. However, as reported for other taxa [reviewed in 48], the duration and timing of parental precondition may induce a transgenerational response in the offspring, resulting in positive but also null or negative adaptive responses [49, 50, 51]. Evidence of natural intra- and trans-generational plasticity (TGP) to increasing temperature was also shown for some corals (e.g., decreasing bleaching effects [52-58]) and reef fish species (e.g., maintaining performance at high temperatures [59, 60]).
Distinguishing whether a species can acclimatize to changes in its environment via phenotypic plasticity or rapid genetic adaptation is challenging since it requires multi-generational experiments, which are difficult to set up, particularly for species with long generation time such as corals. In addition, these approaches have only been possible under artificial laboratory conditions. While TGP has been studied in the laboratory on short-lived organisms, few studies have addressed this important challenge on corals and only for early life stages [46, 47, 51, 61]. No study has tracked performances on later stages of the same offspring, used adult parent colonies that experienced the experimental conditions during their entire life, or performed experiments in the wild. In general, evolutionary aspects of OA and climate change have been largely overlooked. Current projections ignore the capacity of species for phenotypic buffering and their adaptive potential [62-65].

The overall goal of this project is to explore the likelihood and mechanisms by which coral reefs may withstand environmental stressors like climate change. I hypothesize that non-genetic inherited traits can contribute to long term population adaptive responses and help corals to persist under climate change conditions. To reach this goal, I will assess phenotypic responses for a diverse array of life-history traits in selected coral species, together with their genetic diversity and gene expression. Within-generation, parental effects and TGP response (F0-F2) to suboptimal conditions will be assessed during *in situ* long-term reciprocal transplant experiments using a natural analogue of future conditions as the main laboratory. This will allow for realistic and ecologically relevant projections. Combining designed experiments at the natural analogue with laboratory experiments, is the best approach to provide robust evidence of the evolutionary mechanisms coral reefs might use to face climate change. I expect ground-breaking results that can fundamentally change our understanding of how climate change will affect coral reefs and the underlying biological mechanisms involved.

5.3. Distinguishing acclimatization from adaptation. Organismal acclimatization (i.e., the phenotypic plasticity organisms express during their entire life to maximize their fitness in response to multiple environmental factors under natural conditions [64]), can be measured during short- and long-term experiments. Plasticity often consists of three main categories: developmental and reversible, both occurring within a single generation (i.e., within-generation plasticity), and transgenerational plasticity (TGP) which is driven by the interaction of parents and a new generation with their environment. While it is widely accepted that phenotypic plasticity can increase the survival of organisms, there is no general agreement on whether plasticity can lead to rapid evolution under specific conditions [66]). Because

acclimatization requires energy, the crucial role of phenotypic plasticity may not be sustained over the long term [65]. Under such circumstances, phenotypic plasticity may be subject to natural selection resulting in evolution through genetic assimilation or phenotypic accommodation [67]. Adaptation (i.e., genetic changes through natural selection of heritable genotypes that express a phenotype enhancing fitness in a new environment [64]) acts over multiple generations at the population level, while phenotypic plasticity acts on individuals within a generation and is generally not heritable [67]. The evolution in the environment through random occurring natural selection is generally considered to be a slow process, requiring hundreds or even thousands of generations. However, recent evidence demonstrated that evolutionary changes in nature are possible over only a few generations [68]. Adaptive transgenerational (heritable) plasticity may buffer a population against such evolutionary change by allowing existing genotypes to maintain their fitness while coping with climate change [69]. In evolutionary ecology, discriminating between acclimatization and adaptation is essential in determining a species capacity for trans-generational response to a rapid increase in anthropogenic disturbances. Reaction norms (i.e., the pattern of phenotypic expression of a single genotype across a range of environments) provide an excellent means of evaluating the phenotypic plasticity [67, 70] of clones and/or genotypically distinct individuals as a function of the environment. Technically, this can be achieved by transplanting multiple individuals from genetically distinct populations both within and between their respective habitats (i.e., 'reciprocal transplant' [71]). Although these approaches have been used for studying adaptation in terrestrial animals and plants [e.g., 71, 72], they have rarely been applied to marine ecosystems [e.g., 73, 74].

5.4. Objectives, originality and innovative nature of the project. The originality of my project arises from my recent discovery of an exceptional habitat at Bouraké, in New Caledonia, where environmental conditions (temperature, pH and oxygen) are below the optimum known for corals (i.e., "suboptimal" conditions), and similar to those forecasted for the future ("natural analogue"). While these conditions are generally recognized as unfavorable to corals, a rich and abundant coral reef has developed. With this project I want to understand how corals can thrive in this hostile environment and derive projections of the fate of corals under similar future conditions worldwide.

As a first step, we will characterize the genetic makeup of corals inside and outside Bouraké using RAD sequencing. Neutral markers will be used to describe population genetic patterns, including population connectivity inside and outside Bouraké, while loci under selection will be used to test and describe the genetic adaptation mechanisms. Significant genomic differences among stations with high levels of connectivity would indicate that strong environmental filtering occurs with each generation, and demonstrate sufficient standing genetic variation in natural populations to cope with environmental differences between stations. Low levels of connectivity between genetically different populations would suggest that adaptation takes place slowly, over several generations. The absence of genomic differences between populations would indicate that non-genetic acclimatization is sufficient for corals to inhabit different environments. Then, reciprocal transplant and transgenerational experiments on corals inside and outside Bouraké will make it possible to reveal the physiological and molecular mechanisms that corals use to cope with environmental changes within and between generations.

To test whether the specific traits that allow corals to survive are heritable over several generations, I will conduct transgenerational experiments and perform reciprocal transplantations of F1 recruits born from adult corals (F0) that have spent their whole life in suboptimal conditions in the wild. The experiment was designed to include an ambitious temporal effect and distinguish TGP from parental effects. For that, the duration of exposure of parents and eggs to climate change-like conditions before spawning will vary.

In the short-term, the project will provide robust data about within-generation responses of adult corals, as well as parental and transgenerational carryover effects throughout the larvae and subsequent early life stages. In the long-term, it will identify responses in the later stages of juveniles, in adult colonies of the F1 generation (> 2 to -3 years old), and possibly in an F2 generation. The project represents a significant step forward in addressing global change challenges. It will perform, for the first time, *in situ* experiments on several coral species, to decipher their abilities to acclimatize and adapt to future conditions. This work will help to fundamentally improve our understanding of the fate of coral reefs within an evolutionary context.

HOPE is structured around three primary aims that explore the following questions:

Aim 1. Assessing genetic diversity and connectivity. Are corals living in the suboptimal environment of Bouraké genetically selected, and/or genetically connected to the neighboring lagoon fringing reefs?

Aim 2. Assessing the role of plasticity. Is plasticity a sustainable response? Is plasticity heritable in the broad sense? Does plasticity have a genomic basis?

Aim 3. Assessing the contribution of plasticity to adaptation. Are parental and transgenerational effects used by parent colonies reflected in the traits of the offspring? Do they persist into adulthood, at least for the F1 generation?

5.5. Natural analogues to study the effects of climate change. One way of demonstrating how species acclimatize or adapt to future environmental conditions, such as warming, acidification, or deoxygenation, is to provide evidence from species that already live in "suboptimal" conditions. Natural analogues for future conditions are study sites where at least one or more environmental parameters naturally mimic climate change-like conditions over a large area of the ecosystem, therefore providing an opportunity to simultaneously investigate changes in species responses and their capacity to acclimatize and eventually adapt.

Thanks to my scientific background, my network, my research team and our recent findings, I am particularly well-positioned to develop novel *in situ* approaches that can provide ground-breaking discoveries about the effects of climate change on marine ecosystems. During my career, I have pioneered the study of submarine CO_2 vents to understand how species acclimatize and/or adapt to OA, providing the first studies on the effect of OA at the scale of ecosystems (24 publications using CO_2 vents in the Mediterranean Sea [e.g., 13, 75]). During the last three years, my team has investigated coral reef responses to OA using three CO_2 vents in Papua New Guinea (PNG). At these unique sites (see https://youtu.be/xBQjV7ZbHw4 and https://youtu.be/SAa0vsOChn8), continuous emissions of near-pure CO_2 from below the seafloor alter the chemistry of the surrounding seawater, providing an ideal experimental environment to study the ability of species to acclimatize or adapt to future acidification levels [76]. In the framework of two French ANR projects, I carried out seven expeditions and found unusual metabolic and molecular plasticity in coral species (Biscere et al [77], Rippol et al in prep. [78]). Using cross-transplantation of resistant and sensitive species, I found that these corals were able to grow and survive in high pCO_2 conditions (Rodolfo-Metalpa et al in prep. [79]). Interestingly, while previous studies [76] found only a limited number of branching corals at the Normanby CO_2 vent in PNG when compared to massive corals, I found a very rich and diverse reef at another CO_2 vent (Ambitle Island, [80]). At this site, despite the low seawater pH, I did not find any substantial loss of biodiversity or dominance of massive corals as initially expected [Shlessinger et al in prep. 81]. These ground-breaking findings opened new questions about the real capacity of corals to acclimatize to OA and highlight once again the necessity to repeat experiments using more realistic conditions.

In parallel to the PNG project, I discovered a novel natural analogue in New Caledonia [82]. The Bouraké system is a semi-enclosed lagoon with no freshwater input (Fig. 5.5.1. A-B). A channel of more than 80 m wide and 6 m deep penetrates a dense mangrove forest and creates large pools over an estimated area of more than 60,000 m^2. The first measurements of the diel pH, temperature and oxygen fluctuations (Fig. 5.5.1. C-D) revealed the value of this site for studying the capacity of corals to acclimatize and/or adapt to suboptimal conditions.

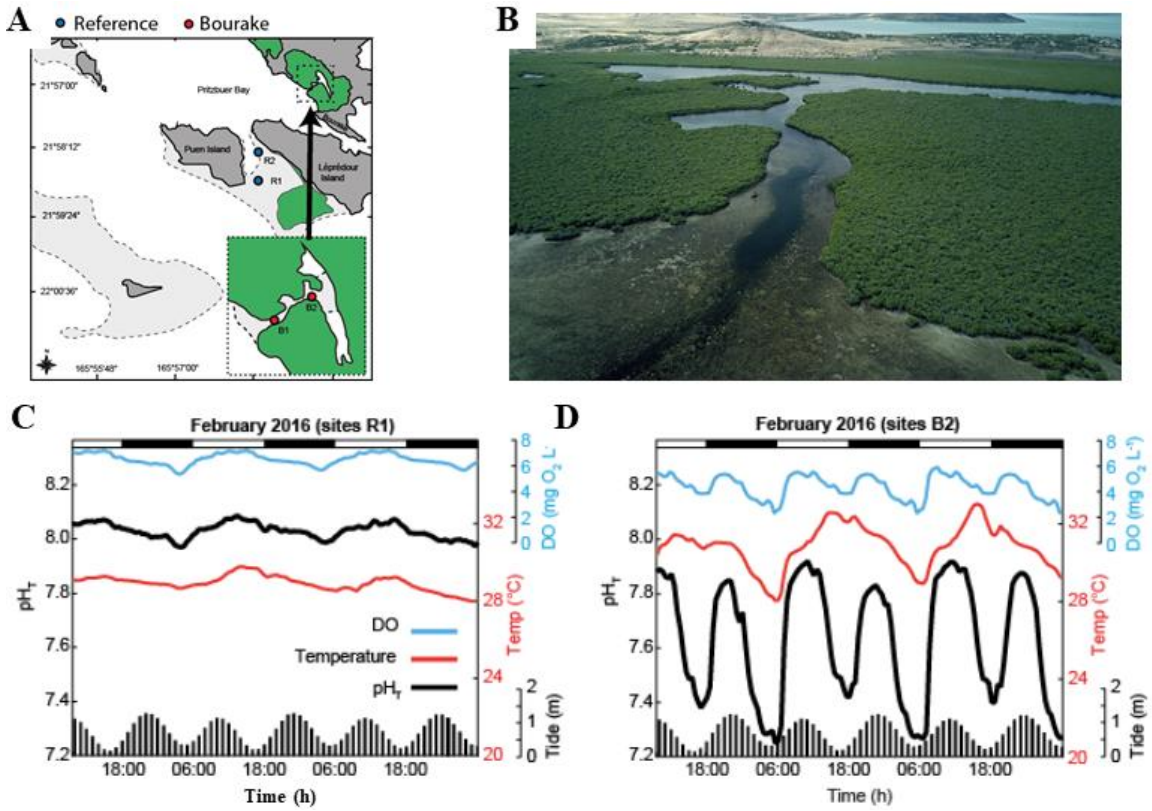

Fig. 5.5.1. Map showing study sites and stations (**A**); aerial photograph of the semi-enclosed lagoon of Bouraké (**B**); physicochemical parameters of seawater at control (**C**) and suboptimal stations (**D**) in February 2016.

At each high tide, new water from the lagoon enters through the channel into the vast inner basin of the mangrove. During this journey, the water chemistry changes due to metabolic reactions in the sediment, coral reefs and mangrove habitats, and mixes with more acidic, hot and deoxygenated seawater. Even at high tide, the water rarely returns to "normal" with maximum pH_T (in total scale) values are around 7.9 (currently, the typical average pH_T of the ocean is 8.05). At low tide, seawater becomes more acidic and oxygen-depleted as the system begins to drain. The large volume of water that was inside the mangrove forest enters the system and is discharged into the lagoon. At low tide, pH values reach a minimum of 7.3 and O_2 a concentration of 2 mg L^{-1} (the average concentration of O_2 at the coast is 4 to 6 mg L^{-1}) around corals. Both parameters exhibit predictable fluctuations according to the semi-diurnal tidal cycle (1.2 ± 0.3 m), which is extremely important when assessing the amount of stress that corals experience over time. As a result, corals are exposed 45% of the time to a pH_T between 7.9 and 7.7, and 53% of the time to $pH_T < 7.6$ (see [82]).

Sea surface temperatures (SSTs) are also of particular interest. During the first survey in 2016 [82], when SSTs were particularly hot in the Pacific, including in New Caledonia, I found that, inside the Bouraké system, seawater temperatures were 2 to 3°C higher than in the lagoon outside the system (Fig. 5.5.1. D). The seawater temperature was then monitored inside and outside the system from September 2017 to May 2018, including an unusually cold summer. I found that the average (±sd) temperature difference was 0.57 ± 0.58°C with a peak of 1.46°C. This revealed that the coral reefs inside the system are not only exposed to extremely high temperatures during anomalous thermal events, but they also live at temperatures 0.5 to 1°C higher than other fringing reefs outside the system for most of the year. Although these values slightly differ from those expected by the end of the century, they already correspond to those forecasted for the next 20 to 30 years. Besides, corals in the Bouraké system experience significant and chronic oxygen fluctuations towards lower concentrations. As oceans are warming, projections indicate that the upper oceanic layers will become more oxygen-depleted. In combination with coastal eutrophication, deoxygenation has been put forward as the third most important factor potentially affecting marine life in the future, after pH and temperature (the "deadly trio" *sensu* [83]). We logged these three environmental parameters since February 2016 using

autonomous multiparametric probes deployed at the study stations. Tides and the unique geomorphology of the site appear to be responsible for the suboptimal conditions. Our preliminary studies also indicate that this unique site has not changed for at least the last 80 to100 years, and most likely longer. This data, therefore, suggests that corals of the Bouraké lagoon have most likely experienced the current suboptimal conditions for several generations.

To this day, and to the best of my knowledge, the discovery of such a study site is unprecedented. The Bouraké system is the only natural analogue with suboptimal values of the three most ecologically relevant parameters, which are driven by climate change in the oceans: (i) a significant drop in seawater pH with extreme and regular variations below 7.8, (ii) temperatures 0.5 to 3° C warmer, and (iii) 20 to 30% less oxygen than usual.

5.6. Preliminary experiments demonstrating the value of the Bouraké system as a natural analogue. Since 2017, I have been leading a consortium of scientists to provide preliminary data and prove the exceptional value of this unique site for studying the effects of climate change on coral reef ecosystems. This research has already produced ground-breaking results, while other promising experiments, still in progress, have been instrumental in testing the feasibility of the proposed project.

We first developed a hydrodynamic model (MARS 3D) and forced it with high-frequency data to simulate the effect of the tide on pH, oxygen and temperature (Rodolfo-Metalpa et al in prep. [84]). This model will help to investigate changes in key environmental parameters as well as nutrients and organic compounds across the system, and their effect on coral species distribution and abundance. In May 2017, I then led the first research cruise in collaboration with ten scientists from different disciplines. During two weeks, onboard the research vessel *Alis,* we recorded species distribution of the three main macrobenthos taxa (macroalgae, sponges and corals), as well as precise measurements of seawater parameters at both suboptimal and reference sites (or "inside" and "outside" the system, hereafter). Despite considerable differences in the main environmental parameters, both sites were equally rich and diverse, and the corals diversity and abundance were very similar between sites (52 and 58 species were found inside and outside the system, respectively).

As the first evidence of acclimatization, we measured a change in corals' gross productivity to respiration ratios, which suggests heterotrophic plasticity as a response to local conditions [82]. Interestingly, we found different microbiomes (both eukaryotic and prokaryotic) between the two types of environment (collaboration with C. Voolstra, KAUST; Camp et al. [85]).

We also investigated the reproductive ability of the brooder coral *Pocillopora damicornis* (Dec. 2017-May 2018) using specimens collected both inside and outside the system and maintained in aquaria under controlled pH and temperature conditions (Prasetia et al submitted [86]). We found that preconditioned parent colonies produced offspring that were not particularly high in fitness and metabolic plasticity. Although recruits from the suboptimal site displayed lateral growth and budding rates that were 2 and 1.2 times higher, respectively, than recruits at the reference site, these differences were not statistically significant. However, unlike coral recruits at the reference site, the skeletal microstructure of the recruits at the suboptimal site was not affected by high pCO_2 when combined to high temperature. With this experiment, we also obtained an F1 generation of corals fully acclimatized to suboptimal conditions, which we both transplanted at suboptimal and reference stations where they are currently growing, although they suffered from high mortality rates. The experiment was repeated during the latest coral spawning in New Caledonia (17th & 18th of Nov. 2019) using the broadcast spawner *Acropora tenuis*. We collected gametes from stations inside the Bouraké system, and larvae successfully settled on ceramic plugs in tanks, a method I plan to use during the project. Recruits will be transplanted at the study sites in early 2020 and monitored throughout their growth.

Finally, I tested the hypothesis that the resistance of corals living inside the Bouraké system is due to heterotrophic plasticity [82]. I performed reciprocal transplantation between the suboptimal and reference sites using fragments of the coral *Psammocora contigua*. As there are higher heterotrophic inputs in the Bouraké system, which could explain the higher resistance of corals to suboptimal conditions, I expect the hypothesis to be confirmed if the transplanted corals grow better inside the system, regardless of their origin, thus proving their potential for heterotrophic plasticity. In contrast, if

corals from outside the system do not acclimatize (no heterotrophic plasticity, e.g., no change in calcification rates), this would indicate that the phenotype of the corals inside the system is genetically fixed. Preliminary results, eight months after the start of the experiment, show that fragments of *P. contigua* from the suboptimal environment grow better than those from the reference sites, independently of the transplanting site. The fact that calcification rates of coral fragments from the reference did not decrease after transplantation to the suboptimal sites, raises questions. Although these are preliminary results, they indicate that *P. contigua* has calcified more because it is adapted to the suboptimal conditions, rather than due to an increase in its heterotrophic capacity.

5.7. Scientific and technical programs, project organization.

5.7.1. Study sites. The project will use the unique Bouraké system as a study site where environmental conditions (temperature, pH and oxygen) are "suboptimal" and mimic conditions forecasted for the future and beyond. Seawater carbonate chemistry, oxygen and temperature are spatially uniform throughout the whole system, i.e., there is no substantial difference between the entrance (near the lagoon) and the inner part (near the mangrove forest) during the same tide. Two replicate stations were selected inside the system and two reference stations were selected outside Bouraké (Fig. 1A-B). Due to the complexity of the experimental design, as well as the number of target species and measurements, replicating stations is an high-risk/high-gain objective. Reference stations were carefully selected according to the following criteria: i) be relatively distant from the site of Bouraké but not too far so that working at both study sites on the same day is possible; ii) be as similar as possible to the site of Bouraké in terms of habitat geomorphology, hydrodynamics and main environmental parameters, except for pH, temperature and oxygen, which need to be in the normal/average range; iii) host the same coral species I found in Bouraké. The selected stations are abundant fringing reefs typical of coastal semi-enclosed shallow water bays.

5.7.2. Environmental parameters. Comparing species and population responses to contrasting environmental conditions requires knowledge of fine-scale spatial and temporal variations in seawater physicochemical parameters at the study sites. The data collection, carried out by my team over the past two years, will be continued to provide a consistent and longer-term sequence of environmental data. This data will be instrumental during the project, particularly when analyzing physiological measurements. For at least 15 days each month, SeaFET autonomous pH sensors and YSI multi-parameter probes will be deployed to routinely log temperature, salinity, dissolved oxygen, and pH variations. Diel irradiance will be measured on each fieldwork day using Li-Cor quantum sensors. A substantial effort will be devoted to characterizing the chemistry of the water column, which may change with the tide. Repeated diel cycle measurements (two consecutive days each two months) will assess variations in the seawater carbonate chemistry (including DIC[1], A_T), total suspended organic matter (DOC, NP, POC), nutrients, prokaryote and virus diversity, chlorophyll content, metals and other trace elements, seawater $\delta^{13}C$ and $\delta^{15}N$ signatures. My team is experienced with managing such data and has the required competencies to analyze environmental parameters and descriptors. However, most analyses have to be processed overseas, which can cause significant delays in the project. Being able to process samples locally would allow us to interpret our results quickly and adjust our experimental design accordingly. This critical task requires a significant investment in terms of instruments, consumables and lab assistance (see budget).

5.7.3. Study species. Our pilot studies recorded 65 coral species in the Bouraké system, of which about 30 also occur at the two reference stations. For this project, we selected five of these species for their different life-history strategies and traits, and the availability of relevant molecular reference data: *Acropora tenuis, Pocillopora damicornis, Montipora digitata, Platygyra daedalea* and *Galaxea fascicularis.*

[1]DIC: dissolved inorganic matter; A_T: Total alkalinity; DOC: dissolved organic matter; NP: nitrogen particulate; POC: particulate organic carbon.

Species	Genome	Reproduction	Morphology	Thermal tolerance	*Symbiodiniacea* transmission
Acropora tenuis	published	hermaphroditic spawner	corymbose	medium	horizontal
Pocillopora damicornis	published	brooder	corymbose	low	vertical
Montipora digitata	ready	hermaphroditic spawner	digitate	high	vertical
Platygyra daedalea	ready	hermaphroditic spawner	massive	high	horizontal
Galaxea fascicularis	ready	hermaphroditic spawner	phaceloid	high	vertical

5.8. Experimental designs.

5.8.1. Aim 1 – Assessing genetic diversity and connectivity.

Our first goal is to understand whether corals naturally growing at the site of Bouraké are genetically different from those that live at the reference site and whether their populations are connected via gamete or larval dispersal. To this end, during the first field trip, we will tag and sample 20 colonies of each of our study species at each station (20 colonies x 5 species x 4 stations = 400 colonies). Samples will be snap-frozen in liquid nitrogen and preserved in DMSO for RAD sequencing, gene expression studies and microbiome analyses. RADseq data will be used to identify clonemates among our tagged colonies so that downstream analyses can be adjusted accordingly. RADseq data will then be mapped on the reference genomes and neutral markers will be separated from selected markers. Neutral SNP markers will be analyzed in the non-equilibrium population genomic model *Moments* [87] to assess the magnitude and direction of connectivity among populations. The model-free *StairwayPlot* method [88] will then reconstruct historical demographic patterns. Genetic distances will also be calculated based on selected loci to determine whether corals are genetically adapted to their respective environments. In tandem, the microbial community of corals will be described using ITS2 and 16s metabarcoding.

5.8.1.1. Expected outcome: The population genomic patterns described in this component will indicate the mechanisms of adaptation to the suboptimal environment of the Bouraké lagoon. If there are significant genomic differences among stations with high levels of connectivity, strong environmental filtering must occur on every new pulse of recruitment. This would indicate that there is high enough standing genetic variation in the natural populations of the reference stations to create genotypes suitable for highly contrasting environments. This result would suggest that coral populations around the world could possess the inherent capacity for instantaneous adaptation, following strong selective sweeps, e.g., the 2015-17 bleaching events. On the other hand, low levels of connectivity among populations that are significantly different in their allele frequencies on selected loci would suggest that adaptation happens slowly, over several generations. In turn, this would indicate that coral populations worldwide will require decades to adapt to climate change. And finally, if we find no significant genomic differences among populations of the suboptimal lagoon habitat and the reference stations, we can assume that other, non-genetic mechanisms dominate the acclimatization of corals (see Aim 2 and 3). Acclimatization via the microbiome will be evaluated using the ITS2 and 16S metabarcoding data. Results of the transcriptome analyses will reveal if gene expression levels are different for putative adaptive loci even in the absence of allelic differences, indicative of epigenetic modifications.
The results of Aim 1 will set the basis for downstream analyses.

5.8.1.2. Risks mitigation: Results from this task are relevant for the whole project outcomes since it will enable to select clonemates and genetically different colonies to be used during the project. However, this task has relatively low risks since RAD sequencing is routinely used, and I selected coral species for which the genome is becoming to be well known. In addition, several expressions of interest in participating in this project have been manifested by some of the most experts in the field such as G. Torda (JCU, Australia), F. Benzoni (KAUST, Saudi Arabia) and C. Voolstra (University of Konstanz, Germany) offering, to some extent, to partially cover the cost of the molecular analyses if necessary.

Another difficulty that could arise is the confusion between cryptic species during underwater sampling. This would result in fewer samples per species than expected, at least for *Pocillopora damicornis* which can be confused in the field with *P. acuta,* as both species can be found in sympatry at the site scale in New Caledonia. If this happens, additional sampling will be organized.

5.8.2. Aim 2 – Assessing the role of plasticity.

A long-term reciprocal transplantation experiment, involving fragments from a subset of the colonies tagged in Aim 1 was designed to investigate the corals' phenotypic plasticity. This experiment will evaluate if the phenotypic plasticity is genetically determined or not by investigating whether trait differences between individuals from different environments disappear after transplantation. By comparing the phenotypic responses, the diversity of the associated *Symbiodiniacea* and bacteria, and gene expression levels in transplanted fragments, I will be able to assess the role of plasticity in acclimatization and answer the following questions: Is plasticity a long-term response? Is plasticity heritable in the broad sense? Does plasticity have a genomic basis?

To understand the mechanisms of within-generation plasticity, I will measure several physiological traits because not all traits are expected to have the same plasticity levels. Using semi-autonomous Plexiglas chambers *in situ*, I will measure several physiological traits at both the Bouraké and reference stations, and for each of the selected species, including photosynthesis, respiration, calcification, and carbon excretion as well as host trophic status, proteins, lipids, ATP, mitochondrial activity, chlorophyll contents and *Symbiodiniacea* shifts. Changes in traits will be compared using reaction norms, and the adaptive potential of each functional trait will be approximated using a quantitative genetic approach and calculating broad sense heritability (H^2), i.e., the proportion of trait phenotypic variation of the traits that has a genetic basis. This approach does not distinguish the contribution of physiological plasticity from epigenetic or genetic components of variation in the stress response. Indeed, because corals are modular organisms, several clonal fragments can be obtained from a single colony, therefore providing an opportunity to study the response of clones with limited biological variations to environmental conditions.

Corals live in close association with unicellular *Symbiodiniacea* species, which have a central role in the success of tropical reef-builders. They also live in association with a range of bacteria, fungi, and viruses, which have been shown to influence coral immunity, nitrogen fixation, nutrient cycling and can affect the overall fitness of the holobiont (i.e., host and microbiome together). Therefore, it is very likely that the response of corals to climate change strongly depends on these associations. Studies have shown that the response of the microbiome to a change in the environment is more pronounced than for the coral host [89-90]. In contrast to the host, both *Symbiodiniacea* and microbes have short generation times which facilitates rapid responses to altered conditions, likely contributing to the acclimatization and adaptive capacity of their coral hosts. It is, therefore, important to assess qualitative and quantitative measurements of both the host and microbiome separately to properly evaluate the adaptive response of the holobiont. Subsamples of the transplanted corals will be analyzed before transplanting colonies (from their station of origin, tagged and collected during Aim 1) and at the end of the experiment (at least one year long) for metagenomics and transcriptomics.

5.8.2.1. Reciprocal transplant experiment: To tell apart phenotypic from adaptive responses using a quantitative genomic approach, I will conduct reciprocal transplant experiments using coral nubbins from (i) the same colonies, i.e., belonging to the same clone, and (ii) colonies of multiple genotypes from both the suboptimal and reference stations. Similar experiments have compared within-genotype and between-genotype variability and enabled the quantification of heritability in the broad sense or the proportion of variation attributable to genetic factors [74, 91-93]. Nubbins will be sampled to assess metabolic and physiological changes in the short (months) and long terms (> one year). These measurements will be repeated at regular time intervals and at different seasons to account for natural environmental fluctuations, with particular attention to the warm season (Dec. to Apr.), which is a long period when higher SST could significantly impact corals. Indeed, I measured up to 33°C inside the Bouraké system in 2016, which was 3°C warmer than at the reference site, where SST was already 2°C above the average temperature for this period of the year in New Caledonia. As previously mentioned, I found that coral bleaching rates inside the Bouraké system were lower than elsewhere, suggesting the

corals' ability to cope with the combination of higher temperature, lower pH and oxygen. Overall, the experiment will: (i) assess the response of potentially adapted corals to suboptimal conditions; (ii) assess the potential mechanisms of resistance that colonies, which live in reference conditions (reference stations), use to cope with a rapid change to suboptimal conditions, as well as the resilience of colonies that live in suboptimal conditions once transferred to reference conditions (i.e., plasticity *vs*. adaptation); (iii) test if the cost of maintaining such responses is viable in the long-term (>12 months); (iv) assess the heritability of climate change tolerance for a series of functional traits; and (v) characterize the genomic basis of plasticity and adaptation in both coral hosts and microbiome.

5.8.2.2. Sampling, handling, and experimental design: The duration of the transplant experiment is 12 months at minima using the five selected species. For each species, at each station (x2) and for each site (x2), 10 genetically distinct and large coral colonies will be selected among those identified in Aim 1. From each colony, 20 nubbins/clones (i.e., clonemates within the same colony) about 5 cm-long will be collected (Fig. 2A). They will be prepared and transplanted at four stations (including two replicates) following a full reciprocal transplant experiment (Fig. 2B).

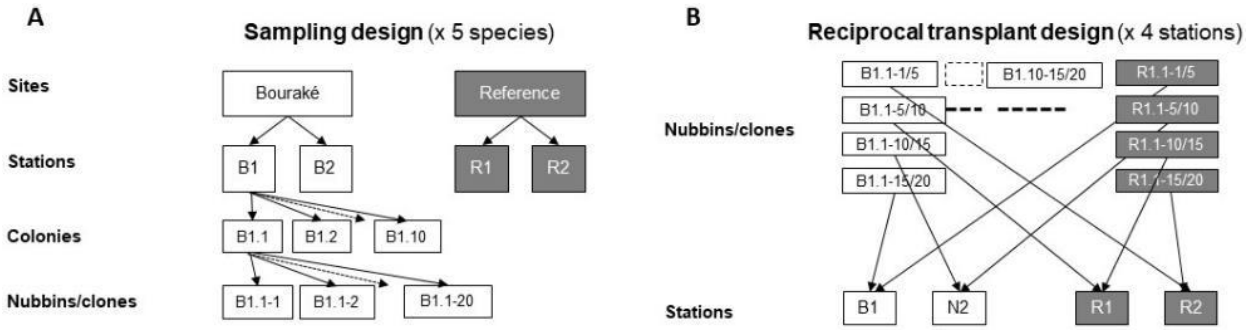

Fig. 5.8.2.2. (**A**) Sampling design for each of the five coral species and (**B**) an example of how nubbins/clones from 10 colonies will be reciprocally transplanted between the 4 stations (both at Bouraké and reference sites). Please, note: the design is oversimplified for graphic convenience; only one level of nubbin distribution is depicted for clarity, e.g., on panel B only clones B1.1 are shown.

Five nubbins from each of the 10 colonies, and for each of the five species will be cross-transplanted at each of the four stations. In total, at each station, I will transplant 1,000 nubbins for a total of 5,000 transplants (see also the Risk mitigation section for a backup experimental design). This experimental design make it possible to apply all treatments to all genotypes, to compare the results within and between each colony, and to assess broad sense H^2. All fragments will be weighed before and after being individually glued onto tagged plates (see [13, 14]) and attached to non-invasive structures, which will be anchored to a hard substrate.

5.8.2.3. Physiological traits: At the beginning of the transplantation, and every two months, photosynthetic efficiency (F_v/F_m) and electron transport rate (ETR) will be measured *in situ* on the transplanted corals using a Diving-Pam. The measurement frequency will be increased during the warm summer period depending on the amount of the hot spots or in case of bleaching.
Metabolic rates will be measured directly *in situ,* to avoid handling stress, during two seasons: summer (February) and winter (August). For each species and station, five genetically distinct nubbins will be incubated using underwater semi-autonomous Plexiglas chambers. Technically, each nubbins will be placed in one of the 10 experimental respirometry chambers (1-2 L) run simultaneously. A reference chamber– with no coral will account for seawater microbial activity. Each chamber will be hermetically sealed and connected to a YSI 6920 multi-parameter probe recording dissolved oxygen (to assess photosynthetic and respiration rates in the light and dark, respectively), temperature and salinity at 1 min intervals. Seawater will be recirculated between the chamber and the probe at a water flow of 2 L min^{-1} using an adjustable submersible pump (see [94, 95] for details). At the beginning and at the end of each of incubation, usually lasting 1h, seawater samples will be collected in each chamber using 250

mL syringes to measure pH, A_T, nutrient depletion and total organic carbon (for carbon excretion: DIC, DOC, POC, Np and bacterial concentration). Metabolic rates will be calculated as the difference between parameters measured at the beginning and at the end of the incubation, for the highest daily irradiance (maximum photosynthetic rate) and in the dark (respiration rate). Photosynthetically active radiation (PAR, 400-700 nm) will be measured using a LI-1500 light sensor logger connected to a spherical quantum sensor. These measurements will help to quantify corals' heterotrophic and autotrophic rates. Carbon and nitrogen isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) will be determined in *Symbiodiniacea*, host tissue, as well as in the different components of the coral food sources (plankton and particulate organic matter in seawater and sediments). The levels of $\delta^{13}C$ and $\delta^{15}N$ will be analyzed in each sample using an automated nitrogen-carbon analyzer (Flash EA 1112) coupled with an isotope ratio mass spectrometer (IRMS, Delta V Advantage with an interface Conflo IV) located at the University of La Rochelle (collaboration with Dr. Anne Lorrain, IRD-LEMAR). Samples will then be gradually collected, carefully transported to the field laboratory located 15 min away from the study area, and gently cleaned. They will be maintained in thermostatically controlled tanks filled with seawater collected at the sampling stations and regularly replaced. The weight of each nubbin will be measured using the buoyant weight technique and their calcification rates calculated. The time necessary to clean and process all samples from one station is estimated to two-three days. Nubbins will then be returned to their station of origin. Metabolic rates will be normalized by skeleton weight. Univariate data will be analyzed in R using G/LM/ER, or GLS, with correction for autocorrelation to deal with the repeated measures. At the end of the experiment, and after all the measurements have been performed, subsamples of each nubbin will be collected, treated and preserved (-20°C; -80°C) for later analysis of their protein, ATP, lipids, and chlorophyll contents, *Symbiodiniacea* densities and diversity, trophic status, and transcriptomic, while the rest of the coral samples will be maintained alive at each station for further long-term assessment and future projects. All coral parameters will be standardized either by skeleton weight, chlorophyll or protein contents.

5.8.2.4. Symbiodinium and microbial changes: *Symbiodiniacea* diversity will be identified using pyrosequencing of the nuclear ribosomal DNA internal transcribed spacer 2 (ITS2), a technique that allows the best sensitive and quantitative method for *Symbiodiniacea* genotyping. Tag-encoded FLX amplicons will be sequenced using Illumina MiSeq pyrosequencing (Integrated Microbiome Resource (CGEB IMR), Halifax, NS B3H 4R2, Canada) producing 2 x 300 tbp-long reads. Bioinformatics processing and sequence analyses will be performed using QIIME and MOTHUR pipelines, as well as the SymPortal [96]. The annotation will be performed using several reference sequences per type that are represented in our reference library and GenBank. Haplotype diversity and intra-genomic variation will be assessed, as well as quantification of relative abundances of symbiont types. Pairwise comparisons of haplotypes (i.e., within type reference sequence variants), parametric pairwise testing of haplotype abundance and haplotype networks will be build using R packages.
The diversity of bacteria will be assessed using the same nucleic acid extract. The V1-V3 hypervariable regions of 16S rRNA genes (specific of prokaryotes) will be PCR amplified using primers 27F-519R [97, 98], which are mostly specific for bacteria. Sequencing and bioinformatics processing will be the same as above. Species assignment will be performed using SILVA v.128 amended with our own coral sequence database for optimal taxonomic identifications. Direct multivariate statistical analysis (such as CCA, RDA) will also be performed to evaluate the link between prokaryotic diversity and the other variables measured in this project.
The transcriptome of each genotype will be developed at the beginning (Aim 1) and the end of the transplant experiment for comparison. If significant changes in gene expression levels are found, the relevant loci will be assessed using target-capture bisulfite sequencing to determine if changes in gene body or promoter methylation underpin the observed transcriptomic changes.

5.8.2.5. Risk mitigation: The experiment has the ambition of assessing the largest coral transplantation experiment ever performed so far at a natural analogue, measuring a series of functional traits *in situ* for at least five coral species, and coupling physiological with molecular responses to suboptimal conditions. This ambitious project presents limited risks since the methods have already been successfully tested during the CARIOCA project at CO_2 seeps in PNG (2016-2019). Both molecular and physiological techniques are state of the art and, although the large number of samples is very

challenging, my team has enough experience to achieve this work successfully. Some data interpretation, such as the analysis of microbial diversity, will be carried out by a senior and expert post-doc as well as through our established collaborations. For *in situ* incubations, the experimental design will need to be restricted to two stations (B1 and R1), three replicate clones for each of the five genetically distinct colonies. I plan ca. 960 incubations (3 clones x 5 colonies x 4 treatments x 4 stations x 4 species), which is particularly ambitious but feasible over 20 working days when using a set of 10 chambers (plus one reference). In case of technical problems, or due to difficulties for the measurement of maximum photosynthetic rates during days with low insolation, the number of measures will be adapted.
The availability of a fieldwork laboratory close to the study area is a significant asset. Both the Bouraké and reference site are sheltered areas with no risk of difficulties due to waves or extreme weather events, which could cause sample loss and handling issues or injuries. A preliminary experiment, carried out on the coral *Psammocora contigua*, confirmed the feasibility of the project and the protocol used, particularly handling and transplantation, is tolerated by the corals. However, more fragile species may react differently.
I recognize that our sampling plan is open to criticism, particularly due to the number of replicates and species, which can be considered high-risk. However, this will also be the largest coral transplant experiment ever carried out at a natural analogue mimicking future environmental conditions, and as such, it represents a promise of high knowledge gain. With this in mind, I am aware that natural mortality or unscheduled events could decrease the power of our analyses. The primary phase of fieldwork will be critical to decide whether increasing the number of replicates (distinct colonies and clone nubbins) is feasible or not. The appropriate experimental design will be settled during Aim 1. As the selection of coral species is particularly critical for this ambitious experiment, I made sure to choose species that are abundant at both stations and represent as many life-history strategies, genera and morphologies as possible; they are suitable for transplantation, resist handling, and recover well from breakage. The number of clone replicates (n = 20) is realistic if large colonies are targeted to minimize the impact of repeated sampling. In case of substantial mortality, we will repeat the experiment with a different, more robust set of species.

5.8.3. Aim 3 – Assessing the contribution of plasticity to adaptation.

As climate change is likely to occur on timescales that span multiple generations of corals, parental effects and/or TGP could be a viable mechanism for corals to adapt to new environmental conditions. Aim 3 is designed to test if the duration and timing of exposure of a coral to climate change-like conditions can influence the performances of the following generation. This component, therefore, intends to establish if plasticity is adaptive and to assess which mechanism is involved between phenotypic acclimatization, epigenetic inheritance, parental effect and TGP. Parental effects have been demonstrated in the offspring of several short-lived marine benthic species when mature adults are exposed to specific environments during their reproductive period, whereas TGP is observed in the offspring when parents develop in a new environment from early life stages [92, 93]. Discriminating between the two mechanisms is difficult, particularly in corals, because the main difference relies on the timing of parental exposition (and therefore of eggs and larvae), which depends on the experimental design, as well as the reproduction mode and life-span of the organisms considered. The duration and timing of parental exposition are, therefore, critical to distinguish TGP from parental effects. Corals inside the Bouraké system have settled and grown in suboptimal conditions during their entire life, and are consequently fully acclimatized. This is why my approach is particularly innovative compared to previous studies: it uses colonies fully acclimatized in a natural analogue system. Previous studies have mainly assessed the effects of OA or OW in isolation, and only four studies have been published that evaluate the effect of high pCO_2 in combination with high temperatures on the performances of coral offspring [46, 47, 51, 61]. In these studies, adult colonies of the brooder *P. damicornis* were exposed to experimental conditions for a maximum to two months before larval release, and their performances were measured during a period of 1 to 6 months.
My experiment is designed to distinguish TGP from parental effects by exposing entire colonies from a reference environment to the suboptimal conditions of the natural analogue of Bouraké. I will set up classic reciprocal transplant experiments at two dates: 11 months and 2 days before the following

predicted spawning period (i.e., 3-4 days after the full moon in November-December) (Fig. 3). As a consequence, eggs, sperms and larvae/planulae will be exposed (inside their parental colonies) to suboptimal and/or reference conditions during different periods: never (mother colonies from the reference stations), two days, 11 months and during their entire development (mother colonies from the suboptimal stations). The TGP of one generation can be determined from its developmental plasticity because the duration of the offspring exposition to the environmental conditions of their parents will be different. Larvae will be settled in aquaria and, once the recruits are of sufficient size (at least 4 to 6 months-old) they will be transplanted *in situ* at the Bouraké and reference stations following a full orthogonal experimental design (Fig. 3). My ambition is to follow the performance of these recruits after 6 months, 12 months and beyond, and to assess their functional and fitness-related traits, as well as gene expression change from the larvae to the adult stage. The F1 colonies will be kept until sexual maturity (generally 2 to 3 years), and the experiment repeated to obtain an F2 generation.

5.8.3.1. Experimental design for larvae and recruits: This experiment will use four hermaphroditic spawner species (Table 1), genetically identified in Aim 1, by set of two over two years. Each treatment will use six adult colonies (Fig. 3); i.e., six colonies will not be moved ("never") at both the Bouraké and reference stations, six colonies will be cross-transplanted from the reference to Bouraké in December (11 months before the spawning period), and six colonies will be collected two days before the spawning period from the reference stations. Two days before the expected spawning day, all colonies will be transferred to the *Aquarium des lagons* in Nouméa where the experiment will take place. Each tank will reproduce a similar environmental conditions as measured *in situ* at each stations. In the evening of the spawning day, which usually is three to five days after the full moon of November (i.e., synchronous to the GBR spawning event) each set of colonies (4 treatments x 6 colonies x 2 species) will be maintained in individual large tanks with static seawater.

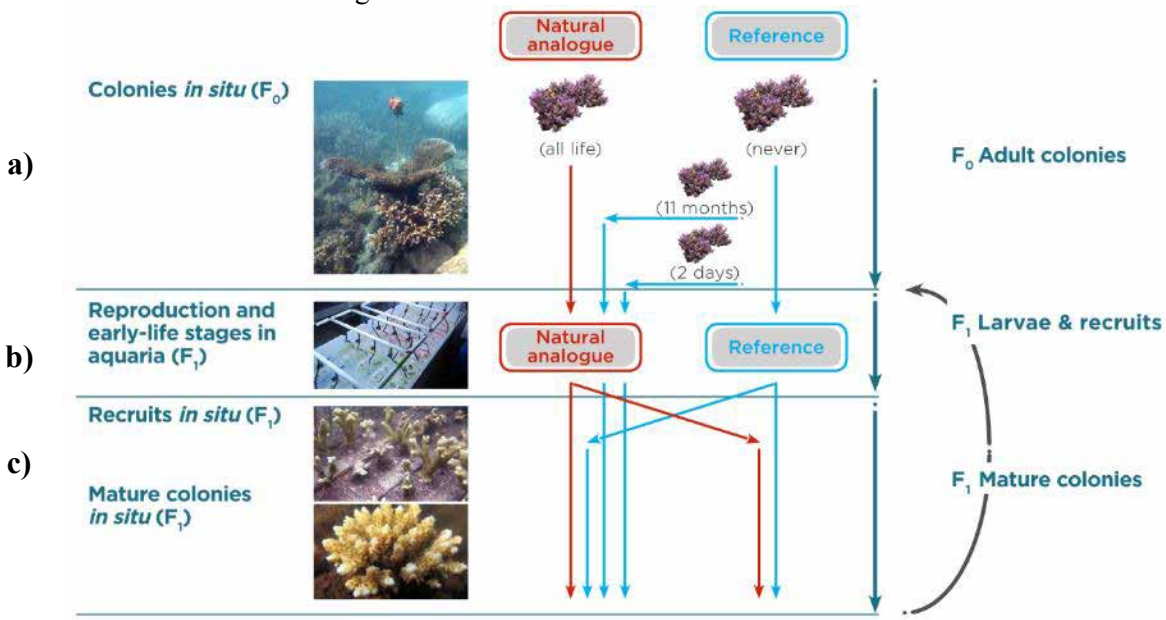

Fig. 5.8.3.1. Experimental design for Aim 3. From the top: **a)** colonies are selected at both the natural analogue and the reference stations, and **b)** colonies from the reference stations are transplanted (11 months and 2 days before spawning) to the natural analogue. All colonies are collected the day before spawning and maintained in tanks in conditions similar to those experienced *in situ*. After coral spawning, cross-fertilization, and metamorphosis, larvae are allowed to settle and grow during 4 to 6 months. **c)** recruits are then cross-transplanted *in situ*. Once mature, colonies are collected again, and the experiment repeated with the new generation.

Gametes will be cross-fertilized within each treatment and the resulting lineage will be tracked. Larvae will be collected and maintained in eight 20 L conic tanks explicitly designed for larvae maintenance (details in Prasetia et al. subm. [86]). Seawater will be filtered (50 µm), renewed and maintained at the same temperature and pH than previously experienced by parental colonies. For each treatment, larvae

respiration rates will be measured in 8 mL glass vials (n=10 per each set of colonies) equipped with an O_2 sensor spot for optical measurement of dissolved oxygen concentration using a Fibox 4. Oxygen consumption (i.e., dark respiration) will be measured for 30 min in darkness. The larvae size will be measured and subsamples preserved for later assessment of gene expression as well as protein and lipid contents.
Larvae will then be allowed to settle on ceramic and aragonite plugs (Aquasonics, n=40 for each set of colonies), previously deployed *in situ* for at least 7 months to allow for biofilm development. Once the primary polyps are observed, plugs (n=10 per each set of colonies per species) with one or more recruits will be photographed under a graduate stereomicroscope and recruits lateral growth will be monitored for 5 to 8 months. Settlement and survival rates will be recorded during the first two weeks. According to the number of recruits available, some will be sacrificed to compare skeleton integrity using scanning electron microscopy (SEM), and to measure lipid, protein and ATP contents.
At this point, all the plugs with recruits (F1) will be labeled and transplanted to the station where their parent colonies were sampled, following a full-orthogonal experimental design (Fig. 3). Plugs with recruits for each set of colonies/treatment will be equally transplanted between the Bouraké and reference stations. This will be achieved by attaching several plugs from the same treatment to PVC plates protected with plastic nets to avoid fish grazing and recruit disturbance. This stage is critical because, in the wild, young coral recruits suffer high levels of mortality due to several factors such as competition with algae and encrusting invertebrates or smothering by sediments [99]. In a preliminary TGP experiment on the offspring of the coral *P. damicornis* [86], I observed high mortality rates at both the suboptimal and reference stations, likely due to the absence of protective nets and high sedimentation rates during the month following transplantation. Although all care will be taken to avoid excessive loss, any mortality in transplanted corals will be interpreted as natural selection. After six months, photosynthetic efficiency will be measured *in situ* using a non-intrusive Diving-Pam with a 3 mm optical probe. Plugs will then be collected, carefully cleaned, photographed and the lateral growth rate of recruits measured. This will be repeated every six months together with the measurement of a series of functional traits (see Aim 1) and fitness-related traits (e.g., survival, fecundity). Changes in fitness values, the correlation between fitness and the reaction norm slope, adaptive plasticity strategies, and intergenerational inheritance of best traits, as well as transcriptomics, will be compared between generations. If significant changes in gene expression levels are found, the relevant loci will be assessed using target-capture bisulfite sequencing to determine if changes in gene body or promoter methylation underpin the observed transcriptomic differences; and if epigenetic marks were inherited from one generation to the next. Changes in microbiome across generations will be assessed by the microbial profiling of the recruits (see Aim 2). The contribution of physiological and genetic changes will be assessed in a Quantitative Trait Locus analysis framework, and using boosted regression trees.

5.8.3.2. Risk mitigation: Aim 3 is very ambitious, and several delicate stages can impact the success of the experiment. However, this section of the project can provide unprecedented results as very limited information is currently available on the transgenerational plasticity of coral species. Our preliminary experiments using the brooder coral *Pocillopora damicornis* and the broadcast spawner *Acropora tenuis* were encouraging, suggesting that our protocol is efficient. Among the limitations, I estimated that six is the minimum number of colonies per treatment to use in such an experimental design, and this is already challenging. In addition, it would be ideal, but logistically impossible, starting with the 4 species at the same time. Because corals are long-lived organisms, it will not be possible to go beyond an F2 generation, at least during the duration of the HOPE project. Reaching an F2 generation before the end of the project will be an incredible opportunity and will, at the same time, greatly facilitate access to new funds to continue the project.
A possible risk is that only a few sexually mature corals may be found at the study stations, as sexual reproduction in corals does not occur every year for all colonies. This can only be anticipated one week before spawning by searching for bundles on coral fragments and selecting colonies accordingly. But, of course, that will not be possible for colonies transplanted 11 months before. To prevent, or at least mitigate, the risk due to the absence of parent colonies, I will transplant more than six colonies per station but will use only six in the final crosses. In the worst scenario, I will use the brooder coral *P. damicornis*, which reproduction period extends to May making it possible a 'rescue' experiment starting from December. I have been working with this easy to reproduce species, and if I have to use it, the

"temporal effect" will be reduced from 11 to 5 months. Our pilot studies indicated that the transportation of colonies between the study stations and the aquarium does not impact the reproductive activity. If transportation was to affect other species, colonies could be kept in large tanks near the study for the duration of the spawning event, or eggs and sperm could be collected directly *in situ* using underwater mesh tents. I will test this in a pilot study during the spawning in 2020 using the R/V *Alis* (cruise planned 28 Nov. to 08 Dec.).

The Gantt chart below illustrates the schedule of the project. Black boxes indicated the whole period of the project devoted to the specific Aim. Grey boxes indicate the provisional period necessary for each task.

	202X				**202x+1**				**202x+2**				**202x+3**				**202x+4**			
Aim 1- Coral identification																				
Sampling & labeling																				
Coral colony genotyping																				
Aim 2- Within-generation plasticity																				
Corals transplantation (RT)																				
Physiology																				
Symbiodinium and microbiome genotyping																				
Gene expression																				
Aim 3- Multi-generation plasticity (TGP)																				
Reproduction and offspring																				
Physiology on offspring, juveniles & adult corals																				

5.9. Feasibility and mitigation strategies. I have been studying natural analogues for more than ten years, and have built strong collaborations with EU research partners, as well as with other research groups studying the effects of climate change on marine ecosystems. Based on my experience, I do not foresee any major obstacles to implement the proposed research and build the dataset. I have an in-depth knowledge of working on the effects of OA at the scale of ecosystems [e.g., 75], particularly on species traits [e.g., 14, 100]. My main interest is calcification response [e.g., 12-14], body size metrics [e.g., 101] and, more recently, I became interested in incorporating a larger selection of traits and using –*omics* approaches (e.g., CARiOCA project). My growing confidence on this topic is demonstrated through the popularity of my studies, which are increasingly cited.
The project is very ambitious in terms of objectives and experimental design, but from my extensive experience of working with natural analogues, I am confident that it is feasible in the framework of this grant, which will provide enough time and funds. For each experiment and aim, I estimated the risks and anticipated alternatives. I also carried out preliminary experiments on the coral *P. contigua* (transplantations), *A. tenuis* and *P. damicornis* type α (reproduction and settlement), which demonstrated the feasibility of our protocols. Technically, our experimental design is innovative and dense, but both molecular and physiological techniques are state of the art and, although there is a large number of samples to process, which is challenging, my team will have enough experience to achieve this work successfully. Besides, I will select the best students and post-doctoral candidates with relevant expertise in genetics, microbiome analysis, and coral reproduction. Given that the project plans a significant amount of laboratory work, I will also appoint a research assistant.

5.10. Expected results of the project: high-risk/high-gain research. HOPE represents a significant step forward in addressing the global change challenge, and understanding the evolutionary response of coral reefs. Previous studies have been performed in the laboratory using short-lived species only. Only a few studies have been applied to corals, but their outcomes remained limited due to the constraints of working in a laboratory set-up. Studies at CO_2 vents and other natural analogues have only been able to draw conclusions about the effect of acidification.

My approach will use a novel natural analogue where seawater pH, temperature and oxygen concentrations are suboptimal for corals and mimic conditions forecasted for the future. The validity of this unique site lies in that the extreme environmental conditions at the natural analogue are: i) entirely predictable according to tide; ii) variations are similar from one day to the other; ii) the site geomorphology has not changed during at least the last century, indicating that the environmental conditions experienced by the corals have been similar for a very long time. Although this system is not perfect, like any other of the known natural analogues, it offers unique advantages for exploring the evolutionary mechanisms that corals might develop in a changing world. Using my proposal offers to explore a new model system using an unprecedented strategy. This project has the potential to deliver ground-breaking results that will boost our understanding of how coral reefs respond to the combined effects of several suboptimal environmental parameters, in combination and in nature. My approach is the next logical step forward in this field of science, using existing methodologies combined into ambitious experimental designs. The technical risk is relatively high, particularly due to the high number of species, samples and replicates, but whether we can complete the full experimental design or have to use alternatives, the knowledge gain will be huge. For the first time, *in situ* experiments will be performed on several coral species, and over the long-term, to decipher their ability to acclimatize and adapt to future environmental conditions. I expect ground-breaking results that can fundamentally change our understanding of how climate change will affect coral reefs and the underlying biological mechanisms involved. My project will deliver unprecedented data on the physiological response of coral species to climate change and their ability to "evolve" in this context. By revealing that corals from the natural analogue of Bouraké are already resistant to climate change, and the mechanisms they have used to do that, HOPE will contribute critical knowledge to future projects, particularly those aiming at using assisted evolution to improve coral reef resilience [112]. And finally, after five years, the project will conclude with an unprecedented F2 generation of coral colonies grown in the wild under climate change-like conditions, offering incredible opportunities to leverage a synergy grant.

5.11. Size and nature of the research team and host institute.

My project offers an innovative way of tackling fundamental knowledge of the evolutionary trajectories that coral reefs might develop to face climate change. Thanks to its high degree of interdisciplinarity, the project will explore the physiological responses of different species through the measurements of genes, proteins, and metabolic variability. This comprehensive scientific approach calls for a team able to address various fields of research, techniques and philosophical methods. The team will be composed of a core of high-class and motivated members, including postdocs and PhDs mentored by expert scientists. My vast network of collaborations with internationally renowned experts will be called upon to support and possibly collaborate if necessary.

Principal investigator: I have a permanent research scientist position at the French Institute for Research and Development (IRD) in the research group ENTROPIE ("Tropical Marine Ecology of the Pacific and Indian Oceans", http://umr-entropie.ird.nc/index.php/home), which is based in New Caledonia. Since 2015, thanks to several grants, I have established a team (https://rodolfo-metalpa-lab.com) to study the effects of climate change on coral physiology. I am particularly interested in the responses of a range of reef-forming species to extreme conditions such as experienced at CO_2 vents, in mangroves, and along turbid coastal reefs. I believe that natural analogues are the best model to improve our knowledge of this research topic. Although most of my research is in the field, I am investing funds in an aquarium laboratory hosted at the *Aquarium des Lagons* in Nouméa. There, several facilities and gear make it possible to maintain corals at finely controlled water temperatures and pCO_2, and run experiments to test the corals' physiological mechanisms of response to environmental stresses. As the group leader, I manage the group's budget, write scientific reports, and organize and develop further collaborations and projects. I am responsible for fieldwork, developing methods and the required technological tools needed, as well as organizing the necessary collaborations within our institute, as well as nationally and internationally when needed. During the project, I will play an active role in preparing and submitting manuscripts for publication in high-profile journals, while training graduate students and postdoctoral researchers to independently write papers and grant proposals. I will ensure that group members are provided with opportunities to learn the skills necessary for the project through scheduled lab meetings, seminars, and courses. All members devoted to fieldwork will be HSE professional divers. Undergraduate students will help with routine measurements of the seawater

carbonate chemistry, protein, chlorophyll, etc. The project will have an extensive media coverage (included in the budget) with the creation of a website, publications on social platforms, and documentaries that will be produced by the IRD Image department. Our communication manager will organize press releases and interviews with national and international newspapers and TV.

The team: At IRD in New Caledonia, I perform my research projects in collaboration with one staff scientist, Dr. Fanny Houlbreque, who contributes her extensive experience of coral physiology and biochemistry, and one research assistant, who will be dedicated to molecular analyses at the IRD "Plateforme du vivant". Two students will start their PhD project on the Bouraké system. Dr. Hélène Magalon and Cecile Fauvelot are experts on coral phylogeny and population genetics; they will join the team for the duration of Aim 1. One senior postdoc, with relevant expertise, will be appointed and he/she will work for three years on the host and microbiome molecular analyses and data interpretation. Fanny Houlbreque will be committed to transplantation experiments, assessing species physiological responses, and collecting environmental parameters. She will be assisted by the IRD professional divers, and one PhD for three years. One research assistant, with laboratory skills in biochemical and chemical measurements (DIC, nutrients etc) will be appointed for 4 years. One junior post-doc will be appointed for three years to focus on colony transplantation and transgenerational experiments. All participants, a total of 10 persons, will help during coral spawning events.

Host institute: The host is the Institute for Research and Development (IRD: https://en.ird.fr/). The IRD is a French public interdisciplinary organization covering a large panel of scientific fields, from terrestrial to marine ecology as well as human, social and economic sciences. One of its goals is to assess the effects of climate change on ecosystems and populations living in southern countries. The IRD has established representations in Africa, the Mediterranean, Latin America, Asia and the French tropical overseas territories including New Caledonia, which is an EU associated country. This large geographical presence is an asset for scientists to develop transcontinental collaborations, research projects, and fieldwork worldwide. In New Caledonia, the main objective of the IRD team ENTROPIE is to better understand Indo-Pacific marine ecosystems in the context of global change. It has access to several facilities and gear for field research including boats (three diving boats and one 30m R/V), diving services, and cutting-edge laboratories for chemical, molecular, microbiological and physiological measurements.

5.12. References

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6. TRADUCTION EN FRANÇAIS DU PARAGRAPHE 2.3 AU PARAGRAPHE 3.5

2.3. Historique de la recherche

Mon sujet principal a toujours été l'effet du changement climatique, avec une attention particulière sur l'acidification des océans (AO), sur une série d'organismes marins calcifiants, en particulier les coraux. Mon approche à cet égard a été classique dans mes premières études, c'est-à-dire en utilisant des expériences en laboratoire, et innovante au cours de la carrière suivante puisque probablement j'ai été le premier à utiliser des laboratoires naturels où des conditions proches à celles prevues à l'avenir, caractérisaient naturellement l'environnement. J'ai publié 71 articles à ce jour : 5 avant mon doctorat et 8 pendant mon doctorat. Au cours de ma carrière post-doctorale, 41 des 58 articles que j'ai publiés étaient effectués dans des laboratoires naturels simulant des conditions futures, tandis que 14 utilisaient une approche en laboratoire.

Quelques étapes préliminaires de mon parcours scientifique peuvent expliquer la dynamique de mes recherches et la continuité du thème principal qui a caractérisé ces 23 dernières années. En 1997, lorsque j'ai commencé mon master au Centre de recherche ENEA S. Teresa, et pendant les quatre années de recherche qui ont suivies, mes superviseurs C.N. Bianchi, A. Peirano et C. Morri m'ont fait découvrir le monde de la recherche scientifique. J'ai réalisé ma première expérience en aquarium, participé à quelques croisières océanographiques, effectué mes premières transplantations de coraux sur le terrain et produit des données pour les cinq premiers articles. Pendant mon doctorat au Centre Scientifique de Monaco sous la supervision de C. Ferrier-Pagès et D. Allemand, j'ai continué sur le sujet que j'étudiais en Italie pendant ma période de recherche pré-doctorale, c'est-à-dire l'effet du réchauffement sur les coraux méditerranéens. Ce sujet a été élargi au thème émergent de l'AO grâce à la collaboration avec J-P Gattuso du laboratoire CNRS LOV à Villefranche-sur-mer. Pendant ma thèse de doctorat, j'ai publié 8 articles.

Au cours de mes deux années de post-doctorat avec le professeur J-H Spencer de l'université de Plymouth, qui ont commencé immédiatement après mon doctorat, j'ai eu la chance de participer à EPOCA et MedSeA, les deux premiers projets de recherche européens sur l'AO. Cela a beaucoup aidé et stimulé ma carrière et ma curiosité scientifique. Avec J-H Spencer, j'ai été le premier à utiliser des resurgences de CO_2 sous-marins pour étudier la réponse des communautés benthiques des zones tempérées à des conditions de faible pH, fournissant ainsi les premières études sur l'effet de l'AO à l'échelle de l'écosystème. Les resurgences naturels de CO_2, où l'émission volcanique de CO_2 modifie la chimie de l'eau de mer environnante, constituent un outil expérimental puissant pour étudier la capacité des espèces à s'acclimater ou à s'adapter aux futurs niveaux d'acidification. Depuis notre premier article en 2008, les sites de CO_2 ont été fréquemment utilisés comme analogues naturels pour étudier les effets de l'AO sur des espèces et/ou des communautés entières. Cette approche a fortement caractérisé mes recherches futures, au cours desquelles j'ai utilisé à plusieurs reprises ces sites spéciaux et en ai découvert d'autres. J'ai décrit pour la première fois les resurgences de CO_2 d'Ischia et de Vulcano (Hall-Spencer, Rodolfo-Metalpa et al. 2008 *Natur*e ; Boatta et al. 2013 *Mar Poll Bull*), les resurgences profonds du Golfe de Californie (Prol-Ledesma et al. 2016, *Nature Comm*), et l'île d'Ambitle (Pichler et al. 2019, *Mar Poll Bull*). J'ai travaillé sur les plus célèbres resurgences de CO_2 à Normanby (PNG, Fabricius et al. 2011) et White Island (NZ, Brinkman et Smith, 2014), ainsi qu'à Nikko Bay à Palau (Reimer et al. 2023, *Coral Reefs*). En plus des resurgences de CO_2, j'ai découvert un site unique où les trois principaux paramètres environnementaux du changement climatique, à savoir l'AO, le réchauffement et la désoxygénation, se combinent en

un seul site, la baie semi-fermée de Bouraké en Nouvelle-Calédonie (Camp et al. 2017, *Scientific Reports* ; Maggioni et al. 2021). La plupart de mes projets de recherche de 2017 ont été réalisés à Bouraké, et la plupart de mes projets futurs utiliseront ce formidable laboratoire naturel.
Au cours de ce premier voyage scientifique de 26 ans, j'ai établi des collaborations fortes et amicales avec plusieurs collègues travaillant sur le même sujet. Ces collaborations ont été consolidées au sein du consortium ICONA, "International CO_2 Natural Analogues Network (ICONA)", un projet de collaboration internationale qui vise à utiliser des analogues naturels pour étudier les effets de l'AO sur les écosystèmes des récifs coralliens. Ce projet nous a permis de travailler, en 2023, à Nikko Bay (Palau, Micronésie), probablement la baie semi-fermée la plus emblématique où les coraux se sont adaptés à un réchauffement de 1°C et au niveau d'AO prévu pour l'avenir.
Le principal avantage de l'utilisation de laboratoires naturels pour étudier les effets du changement climatique en cours est que les résultats sont écologiquement pertinents, bien qu'il faille toujours garder à l'esprit certaines limites. En effet, la plupart des conclusions relatives à l'impact de l'AO (ainsi que d'autres facteurs tels que le stress dû au choc thermique, la désoxygénation, l'enrichissement en nutriments, etc.) sur les organismes et les extrapolations qui en découlent au niveau de l'écosystème, proviennent d'expériences de laboratoire à court terme sur des organismes individuels. Ces expériences sont certainement instructives, car elles nous permettent d'identifier l'effet d'une ou de quelques variables, mais isolément, elles sont incapables de rendre compte des implications de la capacité d'acclimatation des espèces. Par conséquent, le temps d'exposition aux futures conditions de changement global et les conditions expérimentales elles-mêmes deviennent une question centrale pour améliorer notre compréhension de la capacité d'acclimatation des organismes marins aux conditions futures. Les expériences en laboratoire ne sont pas non plus réalistes d'un point de vue écologique car, par exemple, elles ne tiennent pas compte des effets des interactions entre les espèces, de l'apport naturel de nutriments et des fluctuations des principaux paramètres environnementaux. En outre, la quasi-totalité des études ont négligé le rôle de l'adaptation puisqu'elles n'ont testé que les réponses au sein d'une même génération au changement global et lors d'une exposition à court terme au stress. Les études utilisant des organismes à courte durée de vie, tels que les coccolithophores, les copépodes et les polychètes, les oursins et les poissons, ou utilisant des expériences de reproduction sur des larves d'oursins, constituent des exceptions.

L'utilisation de laboratoires naturels a certainement contribué à mon recrutement à l'IRD, UMR ENTROPIE en octobre 2013 après une brève période de post-doctorat, et a favorisé l'obtention de deux subventions de l'ANR : CARiOCA - *Acclimatation des récifs coralliens à l'acidification des océans au niveau des suintements de CO_2*, et PNG-Vents - *Utilisation des resurgences volcaniques de CO_2 pour évaluer la capacité des récifs coralliens à s'acclimater et à s'adapter à l'acidification et au réchauffement des océans*. Ces deux projets consécutifs ont permis à mon équipe d'effectuer 7 missions de terrain en Papouasie N.lle Guinée pour étudier les coraux, les éponges et les poissons vivants dans l'eau de mer acidifiée au sein des resurgences de CO_2. Elles m'ont également permises de collaborer avec plusieurs experts sur ce sujet, formant ainsi un réseau de scientifiques, tous experts de l'effet du changement climatique sur les récifs coralliens et les espèces associées. Les laboratoires naturels ont été pour moi l'outil idéal pour tester des mécanismes spécifiques ou des réponses à l'échelle de l'habitat aux changements environnementaux d'une série d'organismes.

Après mon doctorat en 2007, la plupart de mes recherches se sont concentrées sur les coraux (32 articles sur 58), y compris les espèces tempérées et tropicales d'eaux peu profondes, ainsi que les coraux d'eau froide et les anémones, mais aussi sur d'autres taxons (19 sur 58), y compris

les bryozoaires, les algues corallines et les mollusques, pour lesquels ma contribution a été importante, et les foraminifères, les coccolithophores, les algues, les poissons et les éponges, qui ont été étudiés grâce à des collaborateurs externes possédant des expertises spécifiques. La plupart du temps, j'ai étudié l'effet de l'AO, parfois en combinaison avec le réchauffement, sur les taux métaboliques et la symbiose des espèces récifales calcifiantes (par exemple, la photosynthèse, la respiration, les paramètres des tissus, etc.), mais mon intérêt principal a toujours porté sur leur capacité à calcifier car il s'agit de l'effet le plus évident et le plus uniforme sur la plupart des organismes étudiés dans le contexte de l'AO (Kroeker et al. 2013 ; IPCC 2015). Pour cela, au cours de mes études, la calcification a été étudiée en utilisant des techniques classiques et de pointe telles que la pesée dans l'eau, la coloration à l'alizarine, la transplantation, etc. (39 sur 58), mais aussi en utilisant des traceurs isotopiques et des techniques géochimiques (13 sur 58).

Je me rends compte que la plupart du temps, mes résultats vont à l'encontre du consensus concernant l'effet de l'AO sur le métabolisme en général, c'est-à-dire que j'ai rarement mis en évidence un effet dramatique des faibles niveaux de pH de l'eau de mer sur les taux de calcification corallienne, bien que la plupart des études démontrent une réduction allant jusqu'à 83 % de la capacité de calcification corallienne (Langdon et al. 2005). Pourquoi ? J'ai décidé de développer cette question dans ce manuscrit d'HDR, qui vise à démontrer que j'ai travaillé tout au long de ma carrière autour d'une question fondamentale, et que j'ai essayé de l'éclairer et de la résoudre, ce qui n'est probablement pas le cas ! Ainsi, s'il est une question à laquelle j'ai toujours tenté de répondre, c'est bien "Pourquoi l'AO devrait-elle affecter certains mécanismes métaboliques fondamentaux, tels que la calcification, comme on le prétendait initialement ?" Mes résultats divergents sont-ils simplement le fruit de mon désir de me démarquer du courant dominant ? Ou pire encore, l'effet de l'AO a-t-il été quelque peu exagéré ?

Pour tenter de répondre à la question "Pourquoi les coraux devraient-ils se préoccuper de l'acidification des océans ?", je vais brièvement replacer dans son contexte l'acidification des océans. Je vais brièvement contextualiser le danger que représente l'acidification des océans pour les coraux, faire une description synthétique des principales études concernant l'effet de l'AO sur la calcification corallienne, revoir les principaux points qui ont permis d'établir le consensus sur l'effet de l'AO sur les récifs coralliens, et dans quelle mesure ce consensus a été modifié par rapport aux premières études réalisées il y a une vingtaine d'années. Je ne rapporterai que les études concernant l'effet de l'AO sur la calcification.
Dans ce manuscrit d'HDR, je n'entrerai pas dans les détails des mécanismes chimiques et physiques complexes qui régissent la calcification des coraux et qui n'ont pas encore été entièrement révélés. En effet, ce n'était pas le but de mes recherches. Je terminerai par résumer dans quelle mesure mes études ont contribué à répondre à la question susmentionnée, puis je décrirai brièvement les articles qui, selon moi, ont fait date dans ma recherche. Ce faisant, je ne décrirai que les études que j'ai publiées au cours des 20 dernières années et qui portaient sur l'effet de l'AO sur la capacité de calcification des espèces calcifiantes des récifs, principalement des coraux. Mon intention n'était pas simplement d'énumérer les publications les uns après les autres, car bien qu'ils soient énumérés dans un ordre chronologique, ils ont en fait été progressifs par rapport à la question scientifique initiale : "Pourquoi la calcification corallienne devrait-elle se préoccuper de l'AO ?" Ces études ont été menées à la fois en utilisant une approche classique en laboratoire, c'est-à-dire en maintenant des organismes dans des aquariums dans des conditions artificielles, et en utilisant des analogues naturels de conditions futures, c'est-à-dire des laboratoires naturels où les conditions environnementales imitent déjà les scénarios futurs. Étant donné que j'ai été à l'avant-garde de cette dernière approche et que la plupart de mes recherches ont été menées dans ces laboratoires naturels, je mettrai particulièrement l'accent sur

ce type d'approche qui a profondément marqué ma carrière. Enfin, je décrirai mes recherches actuelles et mes perspectives d'avenir, y compris le projet que j'ai présenté au dernier appel CoG de l'ERC en 2021, qui reste à mon avis un projet innovant et un défi inestimable à relever dans ma carrière.

3. POURQUOI LES CORAUX DOIVENT-ILS SE PRÉOCCUPER DE L'ACIDIFICATION DES OCÉANS : VERS UN REVISION DU CONSENSUS ?

L'acidification des océans est l'une des principales menaces pesant sur les habitats marins, susceptible de provoquer des changements dans la biodiversité et la fonction des écosystèmes au cours de ce siècle. L'acidification des océans pourrait affecter divers paramètres physiologiques à différents stades du cycle de vie des animaux, de la reproduction à la croissance des adultes en passant par les phases larvaires. Les espèces calcifiantes, telles que les coraux scléractiniaires qui abritent une grande partie de la biodiversité océanique mondiale, seront apparemment les plus touchées par l'AO puisque leurs taux de calcification et de dissolution semblent liés à la chimie des carbonates de l'eau de mer. Des études montrent une diminution des taux nets de calcification en raison de la baisse du pH et de la concentration en ions carbonate, ainsi qu'une augmentation du taux de dissolution des squelettes carbonatés. Toutefois, le consensus sur la projection d'une réduction de la capacité de calcification des espèces est en contradiction avec plusieurs découvertes passées et récentes montrant que certains calcificateurs ne semblent pas être affectés par l'AO. Ces résultats divergents ont clairement montré que nos connaissances actuelles sur la réponse biologique à l'AO sont extrêmement limitées et que certaines hypothèses que nous avons utilisées jusqu'à présent pourraient être inexactes pour prédire la réponse des espèces à l'AO.
Dans ce rapport, le consensus sur la réponse des taux de calcification corallienne à l'acidification, les premières hypothèses erronées et les idées fausses seront révisées à la lumière des contributions récentes qui imposent un ajustement de notre compréhension actuelle des réponses de la calcification corallienne face à l'AO.

3.1. Établir un consensus sur la menace que représente l'acidification des océans

Depuis la révolution industrielle, les niveaux de CO_2 dans l'atmosphère ont augmenté et ont presque doublé depuis. Les projections concernant l'avenir de l'AO indiquent que si les émissions de CO_2 continuent d'augmenter au rythme actuel, le pH de l'océan de surface devrait diminuer d'environ 0,4 unité d'ici la fin du siècle par rapport aux niveaux préindustriels (Jiang et al. 2023). Ce taux d'acidification est sans précédent depuis 65 millions d'années et pose des problèmes importants pour les écosystèmes et les organismes marins.
Environ un tiers des émissions anthropiques de CO_2 a été absorbé par les océans, entraînant le processus d'AO au cours duquel le CO_2 absorbé se transforme en acide carbonique, augmentant les concentrations de H^+, de bicarbonate (HCO_3^-) et de dioxyde de carbone dissous (CO_2), tout en diminuant la concentration de carbonate (CO_3^{2-}), l'état de saturation du carbonate de calcium (Ω) et le pH de l'eau de mer (Orr et al. 2005). Ω est le rapport entre le produit de la concentration d'ions ($[Ca^{2+}] \times [HCO_3^-]$) et le produit de solubilité (K'_{sp}) pour l'aragonite minérale qui compose le squelette corallien.
Les régions côtières, où les activités et les apports humains sont concentrés, peuvent connaître d'autres problèmes localisés de pH (He & Silliman 2019). Des facteurs tels que le ruissellement des nutriments provenant de l'agriculture, les rejets d'eaux usées et la pollution industrielle peuvent exacerber l'acidification dans ces zones. En outre, les eaux côtières connaissent naturellement une variabilité du pH due à divers facteurs, notamment le cycle des marées, les changements de température, l'apport d'eau douce par les rivières et les remontées d'eau

profonde. Ces fluctuations naturelles peuvent se produire sur des échelles de temps diurnes (quotidiennes) et saisonnières. L'augmentation des apports en nutriments provenant des activités agricoles et des rejets d'eaux usées peut stimuler la croissance des algues et du phytoplancton, entraînant une production excessive de matière organique. La décomposition ultérieure de cette matière organique consomme de l'oxygène et libère du CO_2, ce qui exacerbe l'acidification future localisée.

L'idée que l'augmentation de la pCO_2 atmosphérique prévue pour le 21eme siècle a un effet négatif sur les récifs coralliens est née à la fin des années 1990, lorsqu'il a été démontré qu'une diminution de l'Ω_{ara}, et donc du CO_3^{2-}, avait un effet négatif sur la calcification corallienne (Smith et Buddemeier 1992). Gattuso et al. (1999) ont réalisé la première étude exhaustive des mécanismes de calcification et de photosynthèse des coraux dans la perspective de la menace relativement nouvelle qui pèse sur la santé des récifs coralliens. Sur la base des estimations du GIEC concernant les augmentations de pCO_2 et des quelques études publiées jusqu'en 1999 sur ce sujet, une diminution du taux de calcification de 10 % entre 1880 et 1990 a été mesurée et une nouvelle diminution de 9 à 30 % entre 1990 et 2100 a été prédite. Ces estimations ont été extrapolées à partir de quatre études sur les algues coralliennes et de trois autres sur des mésocosmes de récifs coralliens (références dans Gattuso et al. 1999). Bien que les auteurs soutiennent l'hypothèse selon laquelle la calcification est contrôlée par l'Ω_{ara} de l'eau de mer et que les preuves expérimentales suggèrent une chute sévère des taux de calcification à l'avenir, ils admettent que les données expérimentales ne sont pas suffisantes pour fournir une prédiction précise des scénarios futurs. En outre, Kleypas et al. (1999), Gattuso et Buddemeier (2000), et Langdon (2000) ont utilisé le même ensemble de données sur les changements prévus de l'Ω_{ara} de l'eau de mer pour prédire une diminution de 17 à 35 % de la calcification des récifs au cours du siècle prochain. Ces auteurs ont conclu que "*plusieurs études ont montré que les taux de calcification des coraux et des algues corallines qui construisent les récifs sont réduits par l'augmentation des niveaux de CO_2...*", et que "*la diminution de l'état de saturation pourrait entraîner une réduction des taux de calcification*".

Sur la base de ces preuves expérimentales, les scientifiques ont envoyé un message alarmant sur l'avenir des récifs coralliens face à l'AO (Hoegh-Guldberg et al. 2007 ; Erez et al. 2011). Les taux de calcification des coraux scléractiniaires diminueront considérablement d'ici la fin de ce siècle en raison de la réduction de la concentration en carbonate de l'eau de mer due à un doublement des niveaux préindustriels de CO_2 atmosphérique (Kleypas et al. 2006). En outre, un déclin de 40 à 83 % de la calcification des récifs (Langdon et al. 2005) et un changement global des récifs coralliens d'une accrétion nette à une dissolution nette ont été prédits pour l'année 2065 (Silverman et al. 2009). Par exemple, une étude menée par Jury et Thomas (2001) a examiné les effets d'une réduction du pH sur les taux de calcification de *Porites astreoides*, une espèce corallienne commune des Caraïbes. Les chercheurs ont constaté que la diminution du pH de l'eau de mer entraînait une baisse significative du taux de calcification des coraux. Cohen et ses collègues (2009) ont constaté qu'une baisse du pH réduisait le taux de calcification du corail *Porites astreoides*, qui construit les récifs. De même, une étude d'Albright et al. (2018) a démontré que des niveaux de pH plus faibles réduisaient les taux de calcification et modifiaient la structure du squelette du corail *Porites cylindrica*. Les impacts du pH sur la calcification corallienne sont encore aggravés par d'autres facteurs de stress, tels que l'augmentation de la température de l'eau de mer et la pollution par les nutriments, qui peuvent interagir en synergie avec l'acidification des océans. Cependant, les preuves d'une relation systématique entre Ω_{ara} et la calcification corallienne n'ont pas toujours été bien étayées et sont en désaccord avec les conclusions de Lough et Barnes (2000) qui n'ont pas détectés de diminution à long terme (1903-1979) de la calcification dans les carottes de *Porites*, un corail à longue durée de vie, prélevées sur la Grande Barrière de Corail. En outre, Atkinson et al. (1999) ont signalé des taux de croissance élevés à long terme pour 57 espèces de coraux

poussant dans de l'eau de mer relativement riche en nutriments et à très faible pH (environ 7,6), ce qui indique que les coraux peuvent continuer à calcifier à des concentrations de CO_3^{2-} extrêmement faibles.
Après plus de 20 ans d'intenses recherches expérimentales dans le domaine de l'AO, il semble que le consensus sur l'effet de l'AO sur la calcification des récifs coralliens ait persisté jusqu'à très récemment, même si plusieurs études ont mis en évidence les idées fausses et les biais méthodologiques du passé, et qu'il a été démontré que plusieurs espèces résistent aux effets néfastes de l'AO. Par exemple, les coraux d'eau froide se développent bien et sans diminuer leur taux de calcification près de l'horizon de saturation en aragonite (c'est-à-dire $\Omega_{ara} = 1$) (références dans Rodolfo-Metalpa et al. 2015) ; certaines espèces tempérées semblent calcifier à des taux normaux ou même plus élevés dans de l'eau de mer acidifiée (e. Rodolfo-Metalpa et al. 2011 ; Ries et al. 2009) ; plusieurs études ont montré des réponses très variables des espèces tropicales, y compris l'absence d'effet (par exemple Houlbrèque et al. 2012 ; Reynaud et al. 2003).
La calcification corallienne est-elle altérée par l'AO? Les récifs coralliens vont-ils se dissoudre comme on le prétend ? Les connaissances croissantes acquises au cours des dernières années, en particulier sur les mécanismes de calcification, ont démontré que les prédictions communément rapportées sur les effets néfastes de l'AO - réduction de la calcification et augmentation de la dissolution - pourraient devoir être révisées (Roleda et al. 2012).

Ici, je révise le consensus sur l'effet de l'AO sur les taux de calcification et de dissolution des coraux à la lumière de quelques études marquantes (par exemple, Venn et al. 2012, 2011 ; McCulloch et al. 2012a, 2012b ; Comeau et al. 2015a, 2015b, 2014a, 2014b, 2013 ; Jokiel 2011a, 2011b ; Jokiel et al. 2014 ; Murillo et al. 2014 ; etc) qui ont grandement amélioré nos connaissances sur les réponses des récifs coralliens à l'AO, et des métanalyses très récentes (e.g. Leung et al. 2022 ; Connell and Leung 2023). Tout au long de cette étude, l'effet délétère du réchauffement ne sera que partiellement mentionné, mais il est clair que les augmentations de température prévues au cours de ce siècle ont le potentiel d'affecter grandement les récifs coralliens, en combinaison ou non avec l'acidification. Bien que les effets exacts de l'AO sur les principaux mécanismes physiologiques fassent encore l'objet de débats, il ne fait aucun doute que les récifs coralliens sont particulièrement sensibles aux augmentations de température et que le réchauffement sera mortel pour la plupart des espèces (p. ex. Rodolfo-Metalpa et al. 2011).

3.2. Effet sur les taux de calcification à l'échelle de l'organisme. La calcification corallienne est-elle altérée par l'AO ?

L'une des principales activités physiologiques affectées pendant une période de stress qui définit l'état de santé d'un corail est sa croissance somatique. La calcification corallienne est un processus vital qui joue un rôle crucial dans la croissance et la survie des récifs coralliens. Il s'agit de la capacité des coraux à construire leur squelette de carbonate de calcium, qui constitue la base structurelle du récif. Le maintien d'un taux élevé de calcification corallienne est crucial pour la croissance et la résilience des récifs coralliens, car il leur confère une intégrité structurelle et une protection contre les perturbations physiques. La calcification est étroitement liée à la photosynthèse (Gattuso et al. 1999) et à l'efficacité de certaines Symbiodiniaceae à transférer des composés photosynthétiques à leurs hôtes (Cooper et al. 2011). Il est essentiel de comprendre le rôle des différentes formes de carbonate (CO_2, HCO_3^-, CO_3^{2-}) sur les mécanismes de calcification et le métabolisme en général pour établir des prévisions éclairées sur les futurs impacts de l'AO sur les récifs coralliens. Une mauvaise compréhension des mécanismes de calcification impliqués a entravé les progrès dans ce débat. Même la question fondamentale de savoir quelle source de carbone inorganique est privilégiée par les coraux pour leur

minéralisation reste partiellement sans réponse (Allemand et al. 2011). Le processus de calcification corallienne implique l'absorption de carbone inorganique dissous (CID) de l'eau environnante, la conversion du CID en ions bicarbonate et la précipitation de cristaux de carbonate de calcium dans les tissus du corail (Cohen et McConnaughey 2003). Cette régulation est essentielle au bon fonctionnement des processus cellulaires impliqués dans la calcification et est fortement liée à la capacité du corail à maintenir une gamme de pH interne. L'évaluation des rôles du CO_2, du HCO_3^-, et du CO_3^{2-}dans l'apport de carbone inorganique dissous (DIC) pour la calcification des coraux représente un défi important pour l'avancement de ce domaine de recherche, notamment en termes de description des mécanismes de calcification sur lesquels les études ultérieures de l'AO peuvent être basées, et de détermination de la réponse des coraux à l'AO.
À ce jour, plusieurs hypothèses ont été proposées concernant l'origine du CID nécessaire à la calcification, mais elles restent controversées. Il semble que la plupart des études aient pris pour acquis que la formation de $CaCO_3$ résulterait de la combinaison de Ca^{2+} et de CO_3^{2-} dissous dans l'eau de mer. Étant donné que les taux de calcification et de dissolution des espèces marines sont liés à la chimie des carbonates de l'eau de mer, des relations empiriques entre Ω_{ara} ou [CO_3^{2-}] et le dépôt de $CaCO_3$ ont été utilisées pour prédire les futurs taux de calcification et de dissolution en réponse à l'AO (par exemple, Erez et al. 2011 ; Kleypas et al. 2006 ; Kleypas et al. 1999). Étant donné que la concentration de Ca^{2+} est presque conservatrice dans l'eau de mer, Ω_{ara} est largement déterminé par [CO_3^{2-}] dans l'eau de mer, qui à son tour est l'un des éléments constitutifs du carbonate de calcium (Gattuso et Buddemeier 2000).

Néanmoins, au moins trois réactions principales peuvent expliquer la formation de $CaCO_3$:

$Ca^{2+} + CO_3^{2-} = CaCO_3$ (1)
$Ca^{2+} + HCO_3^- = CaCO_3 + H^+$ (2)
$Ca^{2+} + CO_2 + H_2O = CaCO_3 + 2H^+$ (3)
Il est rare que les ions bicarbonate (éq. 2) ou le dioxyde de carbone (éq. 3), qui peuvent se déplacer librement à travers les membranes cellulaires et sont disponibles grâce au processus de photosynthèse, aient été proposés comme substrat pour la calcification dans les études sur l'AO. Un réexamen de la littérature antérieure montre que l'éq. 1 ne contribue pratiquement pas à la formation de $CaCO_3$ (Allemand et al. 2011) car le rapport [CO_3^{2-}]/[HCO_3^-] à un pH physiologique (entre 7,5 et 9,0 unités de pH) est extrêmement faible (Venn et al. 2009).
Il est connu que les coraux accumulent leurs structures de $CaCO_3$ dans un milieu rempli de fluide appelé milieu subcalicoblastique (Allemand et al. 2011) sous-jacent à l'épithélium calicoblastique qui est le tissu de calcification. Dans l'espace de calcification de l'organisme, une élévation de plus d'une unité de pH par rapport au pH ambiant rend la calcification possible (Allemand et al. 2011 ; Trotter et al. 2011 ; Venn et al. 2011). Le pH interne est élevé par l'activité de l'antiport Ca^{2+}/H^+ ATPase, qui transfère le Ca^{2+} vers le site de calcification en échange de protons. L'échange de H^+ entraîne l'équilibre vers la formation de $CaCO_3$, convertissant HCO_3^- en CO_3^{2-} et augmentant ainsi l'état de saturation (Ω) dans le fluide calcifiant (Cohen et McConnaughey 2003). En utilisant différentes approches, Trotter et al. (2011), McCulloch et al. (2012a), et Venn et al. (2011) ont montré que certains coraux tropicaux, tempérés et profonds maintiennent de forts gradients de pH entre l'eau de mer environnante et l'espace corallien interne de calcification pour une gamme de valeurs de pH de l'eau de mer, y compris les valeurs projetées à la fin du siècle. Par conséquent, la précipitation du carbonate dans les structures calcifiées ne provient pas directement du carbonate de l'eau, mais est générée ou modulée par plusieurs réactions à partir de HCO_3^- et/ou de CO_2 dans le compartiment alcalin aux taux de calcification. La manière dont les deux autres formes de CID (CO_2 et HCO_3^-) peuvent être acheminées vers les sites de calcification et utilisées pour la

calcification a été largement discutée (Allemand et al. 2011 ; Jokiel 2011a, 2011b), et leurs effets directs sur les taux de calcification ont été prouvés pour certaines espèces (par exemple Comeau et al. 2013 ; Jury et al. 2010 ; Marubini et al. 2008 ; Herfort et al. 2008 ; Schneider et Erez 2006).
Dans une première tentative de séparer provisoirement la contribution des différents composants du système de carbonate de l'eau de mer, les coraux ont été incubés dans de l'eau de mer où les paramètres de carbonate ont été manipulés en ajoutant de l'acide, des bases ou les deux. Certaines études (par exemple Schneider et Erez 2006) ont montré une augmentation de la croissance des coraux après une augmentation du CID, suggérant que la concentration ambiante en CID de l'eau de mer peut limiter les taux de calcification des coraux hermatypiques. Pour cela, Marubini et al. (2008) ont manipulé séparément la concentration de HCO_3^- et le pH pour étudier les mécanismes physiologiques qui permettent la calcification des coraux. Ils ont constaté que *Stylophora pistillata* se développait plus rapidement dans une eau de mer enrichie en bicarbonate, indépendamment des conditions de pH (pH 7,6 à 8,2), ce qui suggère que la calcification est limitée par le carbone. Bien que le CO_3^{2-} soit l'espèce ionique déposée dans le site de calcification, il semble clair que ce dépôt dans le site de calcification dépend du transport de HCO_3^- à partir du milieu extérieur. Les coraux peuvent obtenir le carbone nécessaire à la calcification à partir du HCO_3^- présent dans l'eau de mer ou du CO_2 produit par leur métabolisme (Furla et al. 2000). L'anhydrase carbonique située dans le cytoplasme du corail convertit le HCO_3^-, qui constitue la majorité du CID intracellulaire de l'hôte (Venn et al. 2009), en CO_2 et HCO_3^-, qui sont ensuite utilisés pour la photosynthèse et la calcification, respectivement. En manipulant la concentration en HCO_3^- à pH constant (8,2) sur les coraux Acropora sp. et *Porites porites*, Herfort et al. (2008) ont confirmé le rôle du HCO_3^- dans la stimulation de la calcification à la fois sur les coraux exposés à la lumière et à l'obscurité, et pas seulement en stimulant la photosynthèse. Cependant, dans cette expérience, HCO_3^- et CO_3^{2-} covarient et leur effet ne pouvait pas être séparé. C'est ce qu'ont fait Jury et al. (2009), qui ont constaté que la calcification du corail *Mandracis mirabilis* non seulement n'était pas affectée par l'AO, mais était renforcée par l'ajout de HCO_3^-. Comeau et al. (2013) ont conçu une expérience de manipulation pour tester les rôles de HCO_3^- et CO_3^{2-} sur la calcification du corail *Porites rus* et de l'algue corallienne crustose *Hydrolithon onkodes* à la lumière et à l'obscurité. Les auteurs ont démontré que les deux formes sont impliquées dans la calcification, et de nouveaux modèles conceptuels de la calcification ont été proposés. En mesurant la géochimie du bore du squelette, Allison et al. (2014) ont confirmé que pour les *Porites* spp. massives, HCO_3^- contribue principalement au pool CID utilisé pendant la calcification.
Par conséquent, la calcification ne semble pas toujours dépendre de la concentration en ions carbonate de l'eau de mer, comme on le supposait auparavant (voir Erez et al. 2011). Roleda et al. (2012) ont souligné que, bien que la compréhension globale des mécanismes de calcification corallienne ait été difficile au début de l'ère de la recherche sur l'AO, les connaissances étaient suffisantes pour affirmer que la calcification des coraux est biologiquement modulée et ne dépend pas simplement de la combinaison du CO_3^{2-} et du Ca^{2+} dans un milieu d'eau de mer. Il semble que les études pionnières sur l'AO aient négligé les connaissances les plus récentes pour expliquer, de manière plus simpliste, comment la diminution du CO_3^{2-} et du Ω_{ara} a entraîné la diminution observée des taux de calcification des coraux.

Alors pourquoi plusieurs études ont-elles montré qu'à l'échelle des organismes individuels, les taux de calcification corallienne étaient affectés par des diminutions élevées ou même modérées du pH, du CO_3^{2-} ou de l'Ω_{ara} ? La calcification corallienne est-elle altérée par l'AO?
Il est important de noter que les études ont utilisé une gamme de conditions physiques et chimiques qui auraient pu donner des résultats différents, et que la sensibilité des coraux à l'AO est plus spécifique à l'espèce que ce que l'on pensait jusqu'à présent. Pandolfi et al. (2011) et

Edmunds et al. (2012) ont examiné les raisons possibles de cette grande variabilité entre les études et les espèces. En général, ils ont constaté que la variance de la réponse à une pCO_2 élevée peut être attribuée non seulement à des mécanismes physiologiques spécifiques et à des différences entre les espèces de coraux (coraux à croissance rapide ou lente, p. ex. Rodolfo-Metalpa et al. 2010), mais aussi aux limites des méthodologies expérimentales (p. ex. manipulation de la chimie de l'eau de mer), à la durée de l'expérience, au dispositif expérimental (flux d'eau, irradiance lumineuse, e.g. Comeau et al. 2014b), et à l'origine des échantillons (e.g. échantillons collectés sur des plaines récifales où les variations de pH varient plus que dans d'autres habitats, Comeau et al. 2014c). Une méta-analyse sur la sensibilité de la calcification corallienne aux changements d'Ω_{ara} (Chan et Connoly 2013) a montré que les réponses à l'AO pourraient être moins sévères que ce qui avait été suggéré précédemment. Leur analyse suggère que la grande variabilité entre les études n'a pas été expliquée par les différentes manipulations de la chimie de l'eau de mer, ni par les taux de calcification corallienne ou les niveaux d'irradiance de culture. Il est intéressant de noter que les auteurs ont constaté que les études mesurant la calcification par la méthode de l'alcalinité (voir ci-dessous) ont révélé des baisses de calcification significativement plus importantes que les études utilisant la pesée dans l'eau, probablement parce que cette dernière intègre à la fois la calcification à la lumière et à l'obscurité, et qu'elle mesure l'ensemble de la croissance du corail.

Pour expliquer le paradigme entre la réduction observée de la calcification dans des conditions d'augmentation de l'AO et le rôle incertain des différents composants du CID , Jokiel (2011a, 2013) n'a pas étudié le processus de calcification corallienne comme l'ont fait les chercheurs précédents, essayant ainsi de comprendre quels composants sont nécessaires aux coraux pour la calcification, mais à partir de la fin du processus : quels déchets les coraux doivent éliminer pour permettre la calcification. L'auteur a formulé une histoire élégante, « l'hypothèse du flux de protons », selon laquelle la calcification des coraux est régulée par le taux d'efflux net de H^+ produits au cours du processus de calcification (équation 2), du tissu corallien dans l'eau environnante. Les coraux sont séparés de l'eau de mer par une couche limite, appelée couche limite de diffusion ("DBL"), dont la force influence l'élimination du H^+, ainsi que d'autres déchets métaboliques des coraux. Étant donné que l'augmentation de l'AO entraîne une augmentation de la concentration de H^+ dans l'eau de mer, le gradient devient moins favorable, ce qui réduit le flux de protons hors du corail et diminue son taux de calcification. Le modèle révèle que le HCO_3^- est le substrat de la calcification, obtenu soit directement à partir de l'eau de mer, soit dérivé du CO_2 mitochondrial qui est converti par l'anhydrase carbonique en HCO_3. L'hypothèse du flux de protons a été intégrée dans le "modèle de flux de protons à deux compartiments" (Jokiel 2011b), dans lequel l'auteur a unifié les théories et paradoxes existants de la biologie des coraux dans une contribution majeure. Étant donné que les processus de photosynthèse et de calcification sont en concurrence pour le carbone inorganique disponible dans les coraux (Gattuso et al. 1999), ils ont développé une morphologie qui sépare la zone de calcification rapide (ZC) de la zone de photosynthèse rapide (ZP), la ZC étant située entre la ZP, les couches limites de diffusion (DBL) et l'eau de mer externe. Dans le DBL, le transport des ions et des gaz vers et depuis la surface de l'organisme est limité par la diffusion, qui dépend à son tour du gradient de concentration à travers le DBL. Parmi les avantages pour le métabolisme du corail, cette disposition spatiale permet une translocation rapide de l'apport énergétique en carbone fixe de la ZP à la ZC et l'efflux de protons hors du corail, régulant ainsi la calcification et la photosynthèse. En outre, Jokiel (2013) a utilisé les données de Comeau et al. (2013) pour tester l'hypothèse du flux de protons, affirmant que le CO_3^{2-} et l'Ω_{ara} n'ont pas de signification physiologique fondamentale pour la calcification. Au contraire, la calcification est bien corrélée avec le rapport entre la concentration de CID et de protons ([DIC] : [H^+]).

La contribution de Hohn et Merico (2015), qui a amélioré les modèles mathématiques précédents pour la calcification corallienne, constitue une avancée. Elle suggère que la

combinaison du transport ionique transcellulaire et de la voie paracellulaire est le mécanisme le plus probable pour la calcification corallienne à un coût énergétique réduit. Leur modèle indique que la voie transcellulaire favoriserait le transport du carbone à travers les cellules coralliennes via la diffusion libre du CO_2 à travers les membranes cellulaires, tandis que la diffusion paracellulaire assurerait l'échange d'eau de mer au niveau du fluide calcifiant directement. Les calculs de leur modèle conceptuel révèlent que la majorité du carbone (environ 98 %) pénètre dans le liquide de calcification par diffusion du CO_2 métabolique, tandis que seulement 0,8 % est transporté activement sous forme de bicarbonate. Le dioxyde de carbone qui peut ensuite réagir avec H_2O pour former HCO_3^- et H^+ (catalysé par l'enzyme carbonic anhydrase) pourrait donc être une source alternative de carbone inorganique pour la calcification dans des taxons particuliers.
Hohn et Merico (2015) ont également évoqué une question clé concernant le coût de la compensation des différences de pH dans les conditions d'AO, donc dans le maintien ou non des taux de calcification (Erez et al. 2011 ; Pandolfi et al. 2011). Ce processus est très énergivore (Allemand et al. 2011), l'augmentation du coût métabolique étant estimée à environ 10 % pour chaque diminution de 0,1 unité de pH de l'eau de mer (McCulloch et al. 2012b). Si la calcification devient énergétiquement plus coûteuse sous pCO_2 élevé en raison d'une diminution de Ω_{ara}, alors l'énergie supplémentaire nécessaire au maintien de la calcification pourrait être puisée en augmentant les taux d'alimentation et/ou en puisant dans les réserves d'énergie. Cette hypothèse est particulièrement importante pour les coraux aposymbiotiques tels que les coraux d'eau froide et d'eau profonde qui sont uniquement hétérotrophes. Cependant, en général, les quelques études réalisées jusqu'à présent n'ont pas trouvé de diminution des taux de calcification des coraux d'eau froide, ni de leurs réserves de lipides, ni d'augmentation de leurs taux de respiration (revue dans Rodolfo-Metalpa et al. 2015). McCulloch et al. (2012a) ont calculé qu'une diminution du pH de l'eau de mer de 8,1 à 7,7 unités entraîne un coût supplémentaire pour la calcification des coraux symbiotiques constructeurs de récifs, qui est toutefois insignifiant (<1 %) par rapport à l'énergie totale produite par leur photosynthèse. En conséquence, Schoepf et al. (2013) ont constaté que les réserves énergétiques de quatre espèces tropicales ne diminuaient pas avec l'augmentation de l'AO au cours de manipulations expérimentales en aquarium, ce qui suggère que les réserves énergétiques ne jouent pas de rôle dans le maintien de la calcification dans des conditions d'AO, ou que les coûts énergétiques accrus du maintien de la calcification dans des conditions d'AO sont relativement insignifiants.

3.3. Effect on the reef net community calcification. Will coral reefs dissolve by future AO levels?

Parmi les quelques études utilisées dans une première tentative pour définir la menace émergente de l'AO sur la calcification des récifs coralliens, certaines ont été menées à l'échelle de la communauté, considérant donc l'ensemble de l'habitat formé par les espèces et les substrats (références dans Langdon et al. 2010, et Gattuso et al. 1999). Ces études ont utilisé des mésocosmes expérimentaux où une partie du récif corallien a été reproduite dans de grands aquariums et où les conditions attendues dans le futur ont été simulées. Il est important de noter que ce type d'approche a été fondamental pour évaluer l'effet du changement climatique sur les récifs coralliens, car il peut fournir des informations essentielles concernant les effets interactifs potentiels des principaux paramètres biologiques et environnementaux dans une configuration plus réaliste et plus pertinente sur le plan écologique. Les expériences en mésocosme intègrent un panel d'organismes, des paramètres environnementaux naturels et leur variabilité, ainsi que la disponibilité de la nourriture et les types de substrats pour simuler l'environnement naturel. Malgré son importance, un nombre limité d'études ont été menées jusqu'à présent à l'échelle de l'écosystème en utilisant des mésocosmes expérimentaux (p. ex. Comeau et al. 2015a, 2015b ; Andersson et al. 2009), probablement en raison de la complexité méthodologique de la

reproduction de la structure et de la diversité des communautés benthiques et de l'entretien de tels systèmes. Alors que les résultats des expériences menées sur des coraux individuels ont montré des réponses variables allant de l'absence d'effet (par exemple Reynaud et al. 2003) à des effets négatifs (par exemple Erez et al. 2011), voire positifs (par exemple Rodolfo-Metalpa et al. 2011), en général, les études sur l'effet de l'AO sur l'ensemble de la communauté récifale ont constamment démontré des effets négatifs sur les taux de calcification nets de la communauté corallienne (NCC). Par exemple, Leclerq et al. (2000) ont constaté que le taux de calcification du mésocosme de Monaco était une fonction linéaire de Ω_{ara} et qu'il diminuait de 21% en réponse à l'acidification. Dans une étude à long terme, bien que les coraux individuels soient restés sains et activement calcifiés, Andersson et al (2009) ont constaté une diminution du NCC et de la dissolution nette en réponse aux niveaux d'AO attendus à la fin de ce siècle.
Les expériences en mésocosme ont considérablement renforcé le consensus dans la littérature sur l'AO selon lequel la calcification est inhibée par une réduction du $[CO_3^{2-}]$ de l'eau de mer, et donc de l'Ω_{ara} (Kleypas et al. 2006). De même, les mesures de la variabilité naturelle quotidienne de la chimie des carbonates sur les récifs coralliens ont renforcé l'utilisation de l'Ω_{ara} comme l'un des principaux facteurs contrôlant le NCC des récifs, et les résultats ont été utilisés pour obtenir des prévisions fiables à l'échelle mondiale. Les processus métaboliques et physiques qui se déroulent dans un récif, du moins pour ceux qui sont situés dans des lagons semi-fermés et des habitats récifaux peu profonds, modifient la chimie des carbonates de l'eau de mer environnante, atteignant parfois les niveaux prévus pour l'avenir. Par conséquent, la relation entre la variabilité naturelle de la chimie du carbonate de l'eau de mer et la NCC du récif peut être utilisée pour prédire les réponses futures à l'AO (Shaw et al. 2012).
En général, les résultats des environnements naturels, des mésocosmes expérimentaux et des prévisions des modèles ont montré qu'il existe un fort couplage positif entre l'Ω_{ara} de l'eau de mer et le NCC des récifs (Silverman et al. 2009). Avec les niveaux d'acidification prévus, la NCC quotidienne sera considérablement réduite par rapport au taux et au dépôt de $CaCO_3$ dans les conditions ambiantes, et une dissolution nette à très faible Ω_{ara} sera très probable, ce qui signifie que la NCC de nombreux systèmes récifaux pourrait devenir négative parce que la production de $CaCO_3$ sera dépassée par sa dissolution (Langdon 2000 ; Andersson et al. 2009). Sur la base de la relation observée entre Ω_{ara} et la calcification récifale, il a été prédit qu'en moyenne, la calcification de la communauté diminuerait de 60 % par unité de diminution de Ω_{ara}, avec des valeurs allant d'environ 15 % à 130 % (références dans Andersson et Gledhill 2013).

Mais s'il est vrai que la calcification corallienne n'est pas toujours affectée par l'AO comme on le pensait auparavant, pourquoi plusieurs études ont-elles montré qu'à l'échelle de la communauté récifale, les taux de calcification corallienne étaient affectés par une diminution du CO_3^{2-} ou de l'Ω_{ara} ? Les récifs coralliens vont-ils se dissoudre comme on le prétend ?
Il est important de noter que les taux de NCC ont été estimés sur la base de mesures de la chimie du carbone dans l'eau courante au-dessus de communautés de récifs naturels, de mésocosmes ou de flumes. Cette méthode part du principe que, bien qu'une communauté ne puisse pas être décrite comme présentant un métabolisme en soi, il est possible de décrire la performance intégrée de l'écosystème en termes de vitesse à laquelle les espèces chimiques métaboliquement pertinentes (par exemple CID, AT) sont modifiées dans la colonne d'eau sus-jacente. Les estimations récentes de la NCC basées sur le métabolisme des communautés de différents environnements récifaux donnent une large gamme de taux, de 22 mmol $CaCO_3$ m^{-2} $jour^{-1}$ à 331 mmol $CaCO_3$ m^{-2} $jour^{-1}$. Ces écarts importants reflètent les différences entre les types de récifs, les heures de la journée, les saisons, les structures des récifs et leur composition. Le premier problème potentiel de cette méthode est que les taux de calcification des récifs sont calculés à partir des changements dans l'état des bases acides de l'eau par la technique de

l'alcalinité (Chisholm et Gattuso 1991) qui suppose que pour deux moles d'alcalinité totale (AT) consommées, une mole de $CaCO_3$ est produite. La réaction inverse se produit lors de la dissolution. Bien que cette technique ait été validée pour des incubations de courte durée (Chisholm et Gattuso 1991) et pour des organismes de petite taille (Langdon et al. 2010), les processus métaboliques et de régulation acido-basique qui interfèrent jettent un doute sur les taux absolus déterminés. Ceci est particulièrement vrai lorsque cette technique est utilisée pour mesurer la calcification d'organismes ayant de grandes biomasses qui excrètent des déchets métaboliques importants, affectant probablement les valeurs d'AT et donc le NCC du récif. D'autres processus liés aux récifs coralliens, tels que la production de matière organique par photosynthèse, la diagenèse anaérobie dans les sédiments et l'activité microbienne, ainsi que la transformation des nutriments (références dans Murillo et al. 2014, qui ont récemment révisé la méthode), peuvent affecter l'AT. Plus important encore, lorsqu'elle est appliquée pour évaluer la calcification de l'ensemble du récif, cette approche intègre la réponse à la calcification de toutes les espèces composant le récif et inclut l'effet de la dissolution de tous les substrats biogéniques et de leur bioérosion vers la CCN du récif. En effet, à l'échelle de la communauté, il est presque impossible de séparer la contribution de la dissolution de la calcification nette, car il est impossible de mesurer la calcification brute du récif. En revanche, à l'échelle de l'organisme, l'effet de la dissolution sur la calcification corallienne nette peut être mesuré par la différence entre les taux de calcification brute et nette. Les expériences de marquage avec le radio-isotope ^{45}Ca en aquarium sont la seule technique susceptible de fournir des mesures de la calcification brute, et elle a été appliquée pour la première fois pour tester l'effet de l'AO sur la calcification de deux coraux tempérés : *Cladocora caespitosa* et *Balanophyllia europaea* (Rodolfo-Metalpa et al. 2011). Alors que les taux de calcification nets de *C. caespitosa*, dont des parties du squelette sont exposées à l'eau de mer environnante, diminuent de manière significative à $pH_T < 7,8$ et que le corail souffre d'une grave dissolution du squelette, *B. europaea*, dont le squelette est entièrement recouvert de tissus, n'a pas été affecté. En mesurant à la fois la calcification nette et la calcification brute, il a été possible de montrer que les deux coraux étaient remarquablement capables de maintenir des taux de calcification brute à des niveaux de pH attendus jusqu'à la fin de ce siècle et au-delà. Ces résultats suggèrent que le tissu corallien protège le squelette sous-jacent de la dissolution dans des conditions d'AO, comme cela a été confirmé pour le corail constructeur de récifs *Stylophora pistillata* (Tambutté et al. 2015). En mesurant la calcification brute de *S. pistillata* maintenu dans des aquariums à pH_T 7,8, Houlbréque et al. (2012) n'ont trouvé aucun effet de l'AO. Ceci est en accord avec Cohen et Fine (comm. pers.) qui n'ont pas trouvé de différences dans les taux de calcification nets et bruts des nubbins de *S. pistillata* maintenus pendant 16 mois à pH_T 7,6. Dans une autre étude, Rodolfo-Metalpa et al. (2015) n'ont pas constaté de différences dans les taux nets, bruts de calcification et de dissolution du corail d'eau froide *Desmophyllum dianthus* maintenu à des valeurs pH_T comprises entre 8,1 et 7,7 unités. Il ne s'agit là que de quelques exemples montrant que certains coraux peuvent se calcifier dans des conditions d'AO et que la dissolution des structures squelettiques exposées pourrait être plus importante que l'effet sur la calcification, en fonction du rôle protecteur joué par les tissus dans la prévention de la dissolution. Bien qu'il soit spéculatif d'extrapoler les réponses basées sur les organismes aux réponses de l'ensemble de l'écosystème, il est évident que la dissolution du squelette et la bioérosion contribuent de manière importante à la diminution observée dans le NCC récifal soumis à des conditions d'AO. C'est ce qu'ont montré Shamberger et al. (2014), qui ont constaté une couverture et une diversité coralliennes relativement élevées sur le récif de la baie à faible pH de Palau. En outre, Barkley et al. (2015) ont montré que si les taux de calcification corallienne sur ce site n'étaient pas affectés par l'AO, la bioérosion corallienne était multipliée par 11 à mesure que le pH diminuait. Les récifs étant constitués d'habitats divers avec des compositions minérales et des communautés benthiques variées, le calcul de la NCC à partir des variations de l'AT ne permet

pas de distinguer les habitats récifaux qui contribuent le plus à la dissolution nette finale (par exemple, les sédiments, les coraux, les macroalgues). Cette méthode ne permet pas, par exemple, de déchiffrer l'importance des taux de calcification par rapport aux taux de dissolution des différentes espèces et substrats formant un récif. Il est probable que le point de basculement calculé à partir duquel la production de carbonate récifal passe de la calcification nette à la dissolution (par exemple, Andersson et al. 2009 ; Silverman et al. 2009) dépende principalement de la contribution des différents habitats récifaux. Les récifs avec des fractions prédominantes de sédiments, de pavement corallien (c'est-à-dire un substrat de $CaCO_3$ cimenté recouvert d'un assemblage de turf et d'algues corallines) ou de macroalgues sont des composants importants des systèmes récifaux (par ex. lagon, backreef, front de mer). Dans des conditions naturelles, et en particulier la nuit, lorsque la respiration métabolique entraîne une diminution de l'Ω_{ara} de l'eau de mer, la dissolution des récifs est en effet principalement attribuable à des processus se produisant dans les sédiments carbonatés. Dans des conditions de faible Ω_{ara}, Andersson et al. (2009) ont constaté que la majeure partie de la dissolution se produisait très probablement dans la fine couche de sédiments présente au fond de leur mésocosme. Murillo et al. (2014), en mesurant le NCC de différents composants récifaux dans des expériences en flume, ont suggéré que la technique de l'alcalinité totale n'était valable que pour les environnements dominés par les coraux. Lorsque les sédiments et les algues sont inclus, le rapport entre AT et Ca^{2+} s'écarte de la valeur théorique de 2,0, ce qui signifie que ces autres substrats contribuent largement en tant que sources d'AT. Ils ont constaté que pour une communauté mixte (c'est-à-dire algues, sédiments et coraux), la méthode AT estimera une calcification nette plus faible que pour une communauté uniquement corallienne en raison du recyclage de l'AT. L'importance de la fraction sédimentaire dans la diminution observée du taux de NCC des récifs a été confirmée (Eyre et al. 2014). Dans d'autres expériences en flume, la NCC a été réduite de 59 % et 49 % (Comeau et al. 2015a et Comeau et al. 2015b, respectivement) dans des conditions d'AO. Dans ces études, la dissolution des sédiments et du revêtement récifal expliquait respectivement environ 50 % et 78 % des diminutions observées. En utilisant les données du NCC disponibles dans 233 endroits, dont 183 récifs autour du globe, Cornwall et al. (2023) ont étudié dans une méta-analyse les taux nets de production de carbonate et les réponses des taux de calcification et de bioérosion des taxons de récifs coralliens aux changements prévus de la couverture corallienne induits par le changement climatique d'ici à 2050 et à 2100. Les auteurs ont constaté que les scénarios futurs entraîneront une diminution nette de la calcification et une augmentation de l'érosion des récifs. Ces diminutions résultent en grande partie de la réduction de la couverture corallienne due aux épisodes de blanchissement plutôt que des effets directs du réchauffement ou de l'acidification des océans sur la calcification ou la bioérosion.

Une autre mise en garde fondamentale dans la prévision des réponses d'écosystèmes récifaux entiers à un changement de la chimie de l'eau de mer est que la relation linéaire supposée entre le NCC et Ω_{ara} est en fait modifiée par les taux de photosynthèse et de respiration des récifs, qui varient considérablement entre les récifs et tout au long du cycle journalier. De fortes relations entre la production nette de la communauté récifale (NCP) et la NCC ont été trouvées (par exemple Jokiel 2014 ; Andersson et Gledhill 2013 ; Cyronak et al. 2013 ; McMahon et al. 2013) conduisant à un modèle d'hystérésis diurne dans la NCC par rapport à l'Ω_{ara}. Jokiel (2014) a mesuré pendant des cycles journaliers la chimie des carbonates de l'eau de mer, le NCC et le NCP dans des mésocosmes contenant des composants mixtes du récif (corail, algues et les deux composants). Les résultats ont montré que les changements dans les Ω_{ara} de l'eau de mer sont une conséquence des changements dans le NCC et le NCP du récif plutôt qu'un moteur du NCC. Ces résultats ont des implications importantes car s'ils sont confirmés, ils invalideront la plupart des modèles prédictifs qui supposent que le NCC du récif est contrôlé par l'Ω_{ara} de l'eau de mer.

3.4. Ma contribution au débat sur l'AO

Ce qui a été surprenant au cours des 15 premières années de recherche sur le débat sur l'AO, c'est que les études montrant des réponses "différentes" à l'AO, s'écartant ainsi du consensus, ont timidement augmenté en nombre, mais n'ont pas contribué à modifier le message de manière substantielle. Cela ne veut pas dire que l'AO n'affectera pas la calcification, l'intégrité de la structure et la diversité des récifs coralliens, mais l'effet sera probablement moins dramatique que ce qui a été prédit jusqu'à présent. Curieusement, au cours de cette phase intense de recherche, qui était uniquement axée sur l'effet d'un seul facteur, l'AO, nous avons tous oublié que l'avenir sera la combinaison d'au moins deux facteurs, l'AO et le réchauffement, ce dernier étant certainement le plus dangereux.

Ma recherche a d'abord contribué au consensus mais a rapidement dévié vers un effet plus nuancé ou pas d'effet du tout. En effet, deux premières études (Hall-Spencer et al. 2008 ; Martin et al. 2008) ont évalué les habitats intertidaux et les herbiers marins exposés pendant des siècles à l'eau de mer acidifiée émise par des resurgences de CO_2 en eaux peu profondes dans la mer Méditerranée. Nos données ont révélé un changement dans les communautés, avec la réduction d'organismes importants tels que les oursins et les patelles et l'absence de coraux scléractiniaires dans des conditions de pH réduit. Les algues corallines, qui jouent un rôle crucial dans les écosystèmes d'herbiers marins, ont disparu dans des conditions acidifiées, entraînant une réduction significative du carbonate de calcium épiphyte. Ces résultats soulignent la sensibilité des organismes calcaires à des niveaux élevés de pCO_2 et, en accord avec la plupart des études publiées à partir d'expériences en mésocosme et en laboratoire, mettent en évidence les conséquences potentielles de l'AO à l'échelle de l'écosystème. Je pourrais honnêtement reconnaître, grâce à mes recherches sur d'autres resurgences de CO_2 en Papouasie-Nouvelle-Guinée, que l'une des critiques les plus courantes concernant l'utilisation d'un tel système naturel pour étudier l'effet de l'AO est que l'effet est exacerbé par la variabilité du CO_2. En effet, nous avons constaté que le "point de basculement" du pH provoquant le passage des algues calcaires aux algues non calcaires se situait autour du pH 7,83, mais il est possible que ce changement ait été causé par la grande variabilité du pH sur le site d'étude, avec des valeurs inférieures à 7,4. Cette sous-saturation de l'eau de mer est probablement à l'origine de la dissolution observée des coquilles et autres structures carbonatées. Cela dit, d'une part, cette étude a été honnête dans la présentation des données et de ses limites, d'autre part, elle a été la première preuve du danger que l'AO pourrait représenter pour tout un écosystème. C'est pourquoi d'autres resurgences de CO_2, surtout dans la zone tropicale, ont été utilisées de la même manière, renforçant le consensus sur l'effet de l'AO sur les récifs coralliens (voir Fabricius et al. 2011, pour exemple).

Cependant, dans Rodolfo-Metalpa et al. (2010), ma première étude sur l'effet du niveau d'AO prévu jusqu'en 2100, seul ou en combinaison avec une température de +3°C, n'a pas donné les résultats escomptés, puisque nous avons constaté que l'incubation à court et à long terme n'affecte pas fondamentalement le taux de calcification (et les taux métaboliques) du corail méditerranéen *Cladocora caespitosa*. Ce résultat est surprenant. Il a introduit une nouvelle dimension au sujet en démontrant que tous les organismes ne réagissent pas de la même manière à l'augmentation des niveaux de pCO_2. Certaines espèces, en particulier celles qui ont un taux de croissance lent, peuvent ne pas présenter de taux de calcification réduits. L'article souligne que des facteurs tels que les changements de température saisonniers jouent un rôle important dans la détermination de la réponse des organismes à l'AO, et que l'impact n'est peut-être pas aussi généralisé qu'on le pensait jusqu'à présent. Pendant que nous finalisions cet article, l'étude la plus célèbre de Ries et al (2009) a été publiée, montrant des résultats similaires sur le corail *Oculina arbuscula*, et d'autres organismes calcifiants. L'un des biais de mon étude est que le dispositif expérimental a été mis en place pour étudier les algues calcaires CCA

méditerranéennes vivant à 20-30 m de profondeur, et les températures que nous avons testées étaient relatives à cette profondeur et non à la surface où les eaux plus chaudes menacent les écosystèmes. Alors que *C. caespitosa* vit à partir de 5 m de profondeur jusqu'aux 40 premiers mètres, la plupart d'entre eux sont communs dans les 10-15 premiers mètres et, par conséquent, soumis à des températures plus élevées (et qui augmentent avec le réchauffement en cours), en particulier pendant l'été. C'est pourquoi la température plus élevée testée dans mon étude, 25,4°C, n'était réaliste que pour la population profonde, et incapable de tester l'effet combiné du réchauffement et de l'AO. Malgré ce biais, cette étude a été ma première recherche montrant que les réponses à l'AO sont plus complexes qu'on ne le pense généralement.
Dans une autre étude (Rodolfo-Metalpa et al. 2010, Marine Ecology), j'ai souligné l'importance de prendre en compte les effets simultanés de l'acidification et de la température, en utilisant cette fois une vague de chaleur inattendue lors d'une expérience de transplantation *in situ*. Des colonies vivantes et mortes du bryozoaire ramifié *Myriapora truncata* ont été transplantées dans des conditions normales (pH 8,1), élevées (pH moyen 7,66, valeur minimale 7,33) et extrêmement élevées en CO_2 (pH moyen 7,43, valeur minimale 6,83) près des resurgences de CO_2 de l'île d'Ischia (mer Tyrrhénienne, Italie). Les taux de calcification nette des colonies vivantes et les taux de dissolution des colonies mortes ont été estimés par pesée après 45 jours (mai-juin 2008) et après 128 jours (juillet-octobre). Les données ont permis d'évaluer si les niveaux élevés de CO_2 affectent différemment la croissance et la survie des bryozoaires dans des conditions de pH modérée et d'eau chaude. Il semble que *M. truncata* puisse maintenir son taux de calcification lorsqu'il est exposé à un pH de l'eau de mer assez bas mais à une température saisonnière normale, mais lorsqu'elle est combinée à une température élevée, cette combinaison peut entraîner la mortalité d'organismes qui semblaient initialement résistants à l'AO. Cela suggère que les facteurs du changement climatique, y compris l'augmentation des températures, peuvent exacerber les défis posés par l'AO et doivent être pris en compte dans la prévision des réponses futures. Cet étude sur le bryozoaire *M. truncata* a soulevé la question de la capacité de certaines espèces à calcifier même à un pH très bas, tout en ayant potentiellement leur squelette dissous en raison de la valeur inférieure de l'état de saturation en aragonite (ou calcite) de l'eau de mer. Dans l'étude précédente, j'ai calculé la calcification brute en ajoutant simplement la quantité de squelette dissous à la calcification nette mesurée, ce qui est très imprécis. En revanche, dans Rodolfo-Metalpa et al. (2015, *Nature Climate Change*), j'ai mesuré à la fois la calcification nette et la calcification brute sur des coraux, des moules et des patelles transplantés. Cette étude a exploré les mécanismes de protection employés par certains organismes calcifiants pour limiter la dissolution du squelette lorsqu'ils sont exposés à des conditions acides. Elle a montré que certaines espèces, lorsqu'elles sont exposées à des niveaux élevés de CO_2, peuvent calcifier et croître encore plus rapidement que dans des conditions normales, et suggère qu'il s'agit d'un mécanisme qu'elles utilisent pour réparer leur squelette. Cependant, à mesure que les niveaux de pH diminuent, la dissolution des coquilles et des squelettes devient un risque, ce qui souligne l'importance des tissus et des couches organiques dans la protection de ces structures contre l'eau de mer corrosive. Nos expériences de transplantation montrent que chacune des espèces examinées était capable de calcifier et que certaines calcifiaient plus rapidement à des valeurs de pH bien inférieures à celles prévues pour les eaux de surface mondiales d'ici 2100. Leur tolérance à l'acidification dépendait de leur capacité à maintenir cette protection à des niveaux élevés de CO_2. Nous avons montré que, bien que certains organismes puissent augmenter la calcification à des niveaux de saturation en carbonate inférieurs, ils s'appuient sur des couches organiques protectrices pour éviter la dissolution. Par conséquent, les niveaux attendus d'AO sont susceptibles d'augmenter l'érosion des structures carbonatées biogéniques non protégées. Aussi dans cette étude, il est inquiétant de constater que les organismes calcifiants côtiers que nous avons transplantés le long des gradients naturels de CO_2 étaient plus vulnérables aux effets de l'AO lorsque l'eau était la plus

chaude, ce qui indique que l'AO exacerbera probablement les événements de mortalité benthique massive qui ont été enregistrés de plus en plus fréquemment dans la mer Méditerranée qui se réchauffe. Ces coraux n'ont pas montré de taux de calcification impactés sous les niveaux de pCO_2 prévus pour 2100. Les résultats soulignent l'importance de prendre en compte de multiples facteurs, y compris la température et les mécanismes de protection, lors de l'évaluation de la vulnérabilité des organismes calcifiants et des écosystèmes aux changements futurs de la chimie des océans.

Dans une autre étude (Rodolfo-Metalpa et al. 2015, *Global Change Biology*), nous avons donné un aperçu de la résilience des coraux d'eau froide à l'AO. Certains craignaient que les coraux d'eau froide et profonde, soient encore plus vulnérables car ils vivent dans des zones où la saturation en aragonite (Ω_{ara}) est plus faible que sous les tropiques et diminue rapidement en raison des émissions de CO_2. Cependant, la plupart de nos connaissances sur le métabolisme des coraux d'eau froide (CWC) et des coraux d'eau profonde (DSC) ont été mesurées dans des conditions artificielles en laboratoire, car les données *in situ* sur la réponse physiologique des CWC aux variations environnementales sont difficiles à obtenir d'un point de vue logistique. Cela a certainement entravé notre capacité à prévoir leur sort face à l'augmentation des niveaux de CO_2. En conséquence, il est possible que la plupart des données publiées sur l'effet de l'AO sur ces coraux soient simplement biaisées par le régime nutritionnel donné pendant l'expérience en aquarium puisque rien n'est connu sur leur régime alimentaire, et qu'il est prouvé que la nutrition affecte la performance des organismes ("Fed corals are happy corals" : source F. Houlbrèque). Ainsi, dans cette expérience, trois espèces de CWC ont été maintenues et nourries artificiellement dans des aquariums, et leur calcification nette et brute a été mesurée. Pour tester l'effet de ces conditions artificielles, le seul moyen était de transplanter les coraux dans leur environnement naturel, à la fois à un pH normal et bas de l'eau de mer. Le seul site qui se prêtait à ce type d'expérience était le site volcanique d'Ischia, où j'ai transplanté une espèce corallienne (*Desmophyllum dianthus*). Ischia étant un site peu naturel pour ces coraux, car trop peu profond, j'ai transplanté les mêmes espèces (plus deux autres) à 300 m de profondeur, où le pH était normal et, surtout, où les coraux pouvaient bénéficier d'une nutrition naturelle. J'ai constaté que les trois espèces de coraux ne présentaient aucun changement dans leur calcification brute dans les conditions de l'AO. L'espèce maintenue à 300 m avait le même taux de calcification que son homologue maintenu à Ischia dans des conditions de pH très bas. Cette étude confirme qu'il faut considérer avec prudence les résultats obtenus en aquarium, en particulier lorsque la nutrition de l'organisme étudié est inconnue. En outre, les résultats obtenus en aquarium et *in situ* confirment que l'acidification n'a pas d'effet dramatique sur la croissance des coraux.

D'autres observations faites sur le terrain au niveau des resurgences de CO_2 à Vulcano renforcent l'hypothèse selon laquelle certaines espèces savent déjà comment contrer les niveaux futurs d'AO. Près des resurgences de CO_2, dans une eau au pH très bas, et enfouie dans les sédiments où le pH est encore plus bas que dans l'eau de mer environnante, j'ai trouvé des coquilles de deux espèces de gastéropodes, dissoutes par l'eau de mer sous-saturée, mais toujours vivantes. Dans cette étude (Rodolfo-Metalpa et al. 2015, *Nature Climate Change*), nous avons montré que i) deux espèces de gastéropodes adaptées à l'eau de mer acidifiée dans les resurgences de CO_2 en eaux peu profondes étaient plus petites que celles trouvées dans des conditions de pH normales ; ii) elles grandissaient systématiquement moins qu'au pH de contrôle, et iii) elles avaient une consommation d'énergie spécifique à la masse plus élevée mais une demande d'énergie métabolique de l'animal entier significativement plus faible. Ces changements physiologiques ont permis aux animaux de survivre, bien qu'à une taille réduite, et de maintenir le mécanisme de calcification nécessaire pour réparer partiellement la dissolution de la coquille. Ces observations des effets chroniques à long terme de l'augmentation des niveaux de CO_2 soutiennent l'hypothèse selon laquelle l'AO a contribué aux événements d'extinction passés et suggèrent que certaines espèces pourraient s'adapter par la

nanification (c'est-à-dire l'"effet Lilliput"), ce qui leur conférerait des avantages physiologiques à mesure que le taux d'émissions de CO_2 continue d'augmenter.
Bien que la plupart de mes études aient montré un effet limité de l'AO sur certaines espèces, i) elles n'étaient valables que pour les espèces méditerranéennes ; ii) elles mélangeaient des observations faites sur des sites uniques avec une forte variabilité de pCO_2 et dans des conditions d'expérience non naturelles en laboratoire. Par conséquent, ma recherche, bien qu'innovante, ne pouvait pas ébranler le consensus formé autour des dizaines d'études étonnantes produites dans les resurgences de CO_2 de l'île Normanby, le laboratoire naturel que Katharina Fabricius a découvert, en collaboration avec les meilleurs spécialistes de l'effet de l'AO sur les coraux et d'autres organismes récifaux. Le consensus était que l'AO contribuira à affecter les récifs coralliens et que seules quelques espèces gagnantes survivraient. Mes résultats ont donc été considérés comme des écarts curieux et intéressants par rapport à la vérité.

De 2016 à 2019, dans le cadre du projet CARIOCA, j'ai visité 3 fois le célèbre site volcanique avec les resurgences de CO_2 étudié par K. Fabricius, et 4 fois un nouveau site en PNG, l'île d'Ambitle. J'ai tardé à publier les données que nous avons recueillies et qui montrent i) la production primaire plus élevée des coraux de ces sites (Bisceré et al. 2019 ; Meunier et al. 2021) ; ii) l'association gagnante des coraux vivants sur les resurgences de CO_2 avec des souches particulières de diazotrophes (Geissler et al. 2021) conférant une plus grande résistance thermique à l'hôte (Meunier et al. 2022). En outre, les données recueillies en collaboration avec M. Hoogenboom et T. Shlesinger (encore à publier) ont démontré que l'OA ne diminuait que partiellement la diversité et l'abondance des coraux et suggèrent que la faible diversité observée à Normanby pourrait être spécifique au site, et non une réponse générale des récifs coralliens à l'OA. Bien que je n'aie pas mesuré les taux de croissance des coraux transplantés sur ces sites, en utilisant des techniques géochimiques telles que la signature du bore dans le squelette de plusieurs espèces de coraux, nous avons montré (Comeau et al. 2022, Global Change Biology) que les coraux provenant des suintements de CO_2 étaient toujours capables de maintenir un pH plus élevé au site de calcification, donc de se calcifier, que les coraux du récif de contrôle. Cette capacité semble conférer un avantage écologique au corail puisque les espèces qui ont montré une plus grande capacité à maintenir un pH interne élevé étaient également les plus abondantes près des resurgences de CO_2. En outre, la mesure du pH de l'eau de mer à haute fréquence, sur chaque site et au cours de chaque mission, suggère que les coraux de Normanby sont soumis à des changements soudains de pH par rapport à Ambitle et qu'ils sont exposés à une très grande variabilité du pH de l'eau de mer. Bien que le pH soit en moyenne similaire, Normanby était plus variable qu'Ambitle, et a probablement causé certains des effets précédemment rapportés.
En Nouvelle-Calédonie, grâce à la découverte de Bouraké, où la géomorphologie du site fait varier le pH, l'oxygène, la température et d'autres paramètres environnementaux et chimiques de l'eau de mer, j'ai mené plusieurs expériences pour tester l'effet de l'AO en combinaison avec d'autres facteurs sur la calcification corallienne, et pas seulement. La preuve la plus surprenante sur un tel site est que les coraux sains qui y vivent semblent bien se développer à un pH qui varie régulièrement de la normale à 7,3 (à l'échelle totale) ! La question s'est donc posée de savoir comment ils pouvaient continuer à calcifier avec un pH et un état de saturation de l'aragonite aussi bas, sans oublier les autres stresseurs. Dans la première étude, réalisée avec des collègues de l'UTS (Australie ; E. Camp et D. Suggett), nous avons d'abord caractérisé les conditions environnementales pendant 2 semaines et les premières mesures de la réponse physiologique de trois espèces de coraux. En utilisant la technique de l'alcalinité (incubations courtes), nous avons constaté que les coraux de Bouraké calcifiaient, bien que moins que les coraux de référence, et respiraient davantage, probablement en raison de leur capacité hétérotrophique plus élevée qui compense le coût plus élevé de la calcification. Après cela, j'ai effectué une transplantation réciproque de quatre espèces entre 2 stations à Bouraké et 2 à

l'extérieur, comme référence (non publié) et j'ai trouvé, en utilisant la technique de pesée dans l'eau (c'est-à-dire la différence de poids du corail à long terme) que i) les coraux de Bouraké ont toujours grandi plus que leurs homologues du site de référence ; ii) les coraux des sites de référence ont mieux grandi lorsqu'ils se trouvaient à l'intérieur de Bouraké ; iii) les coraux de Bouraké ont souffert sur les sites de référence. Ces données préliminaires (expérience d'un an) suggèrent que la population de Bouraké s'est adaptée aux conditions environnementales et que les conditions environnementales elles-mêmes peuvent favoriser leur croissance. Pour tester l'hypothèse selon laquelle la condition hétérotrophe plus élevée du lagon de Bouraké est à l'origine du taux de croissance plus élevé observé, et donc mieux étudier le potentiel d'adaptation de ces coraux, et également pour tester l'effet de la variabilité du pH de l'eau de mer, précédemment suggéré pour augmenter la résilience des coraux, avec mon doctorant Clément Tanvet, nous avons réalisé une expérience de 100 jours en aquarium en testant uniquement l'effet du pH (constant et variable) sur 3 espèces de coraux du Bouraké et du site de référence. Tous les coraux ont été nourris de la même manière. Cette étude a confirmé mes données précédentes sur les coraux transplantés et a ajouté une nouvelle pièce cruciale au puzzle. En effet, i) elle exclut que l'hétérotrophie soit le principal facteur à l'origine de la forte croissance à Bouraké puisque les deux populations ont reçu le même niveau d'alimentation en aquarium ; ii) elle met en lumière la capacité des coraux de Bouraké à calcifier sur un site de référence (c.-à-d. à un pH normal).). En effet, alors que lors de ma première transplantation, j'ai mesuré une diminution de la calcification des coraux transplantés de Bouraké vers le site de référence, les mêmes coraux maintenus en aquarium à un pH normal (conditions de référence) ont grandi encore plus vite ; iii) cela confirme que les coraux de Bouraké se sont en quelque sorte adaptés aux conditions extrêmes puisqu'ils ont toujours maintenu une croissance plus élevée, quel que soit le pH de l'eau de mer ; iv) cela confirme (après les résultats préliminaires de Camp et al. 2020) que l'un des mécanismes d'adaptation était la diversité des Symbiodiniaceae hébergées dans leurs tissus. Ces résultats ont été confirmés par la thèse de Cinzia Alessi qui a réalisé une transplantation de 2 espèces de coraux provenant de 2 sites de référence à Bouraké.

Si, lors d'observations *in situ* et d'expériences en laboratoire, j'ai constaté des effets variables et parfois limités de l'AO sur les coraux adultes, l'un des problèmes pour l'état des récifs est leur capacité à se reproduire, à s'installer et à former continuellement des récifs persistants. Les récifs coralliens sains et fonctionnels dépendent directement de leur capacité à libérer des larves et de leur succès de recrutement qui, à son tour, dépend de l'abondance de leur substrat de fixation préféré, à savoir les algues coralliennes encroutantes (CCA). On prévoit que l'acidification des océans affectera grandement les différentes étapes de ce mécanisme naturel et complexe. Ma première étude dans ce domaine de recherche portait sur la capacité du corail *Pocillopora damicornis* de Bouraké à se reproduire, à s'installer et à croître en laboratoire dans des conditions acidifiées (Rodolfo-Metalpa et al, non publié). Cet article a été rejeté trois fois par les mêmes évaluateurs, dans trois revues différentes. Je l'ai laissé dans un coin, mais il semble que nous n'ayons pas eu de chance. En effet, le principal problème est que nous avons comparé les performances des recrutes de colonies provenant de deux sites différents, Bouraké et un récif témoin, pour lesquels nous ne connaissons pas le moment du pic de planulation et, par conséquent, les phénotypes larvaires optimaux par rapport aux larves sous-optimales. L'évaluateur a conclu par cette phrase encourageante : "*En outre, les conclusions de ce manuscrit proviennent de la comparaison de pommes et d'oranges sur les deux sites différents, étant donné l'impact bien documenté des temps de libération des larves sur le phénotype larvaire*". Eh bien, *Pocillopora damicornis* libère des larves d'octobre à mai, et manifestement à des rythmes différents d'un site à l'autre, d'une colonie à l'autre et d'une année à l'autre. Il me faudra des années pour disposer d'un ensemble de données valables sur le moment du pic de planulation et leur phénotype larvaire pour les deux sites étudiés. Nous avons cherché à évaluer

si le préconditionnement tout au long de la vie à un environnement fluctuant contrasté peut modifier la tolérance de la progéniture à l'OA et au réchauffement pendant 28 jours d'exposition à une pCO_2 manipulée (500 et 1500 ppm) et à une température (26 et 29°C). À la fin de l'incubation, nous avons mesuré la survie des recrues après leur fixation et pendant 1 mois, la croissance latérale et le bourgeonnement de nouveaux polypes. Il est probable que cette étude dise quelque chose que l'évaluateur n'a pas aimé, à savoir l'absence de différences entre les deux populations, l'une adaptée à des conditions extrêmes et l'autre provenant d'un récif frangeant normal. Nos mesures ont confirmé l'effet négatif d'une pCO_2 élevée sur la croissance latérale et les taux de bourgeonnement des recrues, et nous n'avons trouvé aucune preuve que les individus provenant d'un environnement plus variable gagnent en performances physiques et en tolérance à des conditions similaires à celles du futur. La microstructure du squelette des recrues des deux environnements n'a pratiquement pas été affectée, bien que la pCO_2 élevée ait été testée. Nous avons insisté sur l'observation que l'investissement reproductif était plus important chez les coraux de Bouraké parce que les colonies produisaient beaucoup plus de planulas que les colonies du site de référence. Mais nous avions raison comme nous l'avons démontré dans l'étude réalisée par mon doctorant Clément Tanvet sur l'effet des conditions extrêmes de Bouraké sur la couverture de CCA installée sur des carreaux de céramique artificiels, et sur les taux de recrutement corallien (Tanvet et al. 2021, *Frontiers*). Dans cette étude, nous avons évalué les effets des conditions environnementales extrêmes et fluctuantes à Bouraké sur l'abondance du CCA, du turf algal et des recrues coralliennes. Pour atteindre cet objectif, nous avons obtenu des tuiles couvertes de CCA après exposition pendant 7 mois dans un récif et les avons déplacées à Bouraké et dans deux sites de récifs côtiers frangeants présentant des conditions environnementales distinctes. Sur la base de l'effet rapporté de l'AO sur la couverture CCA et les taux de recrutement corallien, nous avons émis l'hypothèse qu'à Bouraké, les deux seraient fortement affectés par les conditions extrêmes. Bien que nous ayons confirmé les résultats précédents sur la sensibilité des CCA à l'AO, puisque leur couverture a diminué à Bouraké, nous avons constaté avec surprise que les taux de recrutement corallien à Bouraké étaient plus élevés que dans les deux récifs frangeants. Nos conclusions contredisent les résultats de plusieurs expériences d'OA en laboratoire et renforcent l'idée que le recrutement corallien est possible même dans des conditions extrêmes et que, en particulier pour le site de Bouraké, les coraux peuvent s'être adaptés à des conditions environnementales aussi variables et extrêmes.

Enfin, ma doctorante Cinzia Alessi a mesuré pendant trois ans la période de ponte et la fécondité d'*Acropora tenuis*, *Montipora digitata*, et *M. stellata* de Bouraké et de trois sites de référence situés à des distances variables de Bouraké. Il est intéressant de noter qu'aucune réduction du nombre d'œufs par polype fécondé ou de la proportion de polypes fécondés n'a été observée à Bouraké par rapport aux sites de référence, quelle que soit l'espèce. Cependant, *A. tenuis* et *M. digitata* avaient des œufs plus petits à Bouraké par rapport aux sites de référence, avec des répercussions possibles sur le développement des premiers stades de l'histoire de la vie et sur l'état de santé des coraux. Cinzia a également mesuré moins de recrues coralliennes sur les carreaux de céramique installés à Bouraké au cours des années 2020-2022, ce qui contraste avec nos conclusions précédentes dans Tanvet et al. (2021) au cours des années 2017-2019. Il est probable que cette différence soit due à l'effet des trois années consécutives de La Niña en Nouvelle-Calédonie et à la mortalité massive des coraux à Bouraké qui en a résultée, mais ceci est une autre histoire….

3.5. Conclusions et perspectives

L'AO pourrait affecter la calcification corallienne, surtout sur les espèces qui ne peuvent pas réguler leur pH interne et faciliter le dépôt de $CaCO_3$ comme le font d'autres espèces. L'AO pourrait avoir un effet sur les sédiments et les squelettes des récifs, entraînant leur dissolution, ce qui sera d'autant plus important si les coraux meurent et si les récifs se dégradent à l'avenir. L'AO pourrait affecter d'autres taxons que les coraux, certains profiteront des niveaux élevés de CO_2, comme les macroalgues, peut-être les éponges ; d'autres seront fortement affectés, comme les espèces de plancton. L'AO pourrait avoir un effet indirect sur les paramètres abiotiques qui, à leur tour, régulent les échanges complexes entre le métabolisme des coraux et le milieu environnant. L'AO peut interagir avec d'autres paramètres environnementaux et se combiner pour produire un effet négatif synergique. L'exemple le plus frappant est sa combinaison avec la température élevée, qui semble elle-même être le facteur causal de la prochaine extinction massive des coraux.

Cela dit, à la lumière de mes recherches et de certaines meta-analyses récentes de la production scientifique sur ce sujet, je suis persuadé que l'effet de l'AO sur la calcification sera marginal sur la survie des coraux face au réchauffement climatique en cours.
Un lecteur attentif aura certainement remarqué que ce mémorandum, qui vise à répondre à la question "Pourquoi les coraux devraient-ils se préoccuper de l'acidification des océans ? est déjà obsolète. En fait, j'ai commencé à écrire une histoire pour faire la lumière sur l'effet de l'AO en 2016. Année après année, j'ai ajouté des articles sans jamais trouver le courage de publier ce qui devrait être un document de réflexion, peut-être par peur, peut-être par opportunisme, puisque je travaille sur ce sujet depuis près de 20 ans.

Mes recherches se sont concentrées sur l'effet de l'AO sur les coraux et d'autres organismes, en particulier les coraux, surtout en ce qui concerne leur capacité à calcifier. Bien sûr, j'ai étudié d'autres organismes, bien sûr pas seulement la calcification, bien sûr en utilisant à la fois des aquariums et des laboratoires naturels, mais en fin de compte ma recherche principale a été la transplantation de coraux et la mesure de leur calcification. J'ai commencé cette approche lorsque le thème était très chaud et que les attentes étaient dramatiques, car on s'attendait à ce que le problème de l'AO dévaste les recifs. Heureusement, il a été démontré que l'effet était moins important que ce qui avait été annoncé et mes études ont partiellement contribué à améliorer ce domaine de recherche. Il convient de mentionner que mes recherches démontrent le rôle protecteur joué par les tissus coralliens (et du périostracum sur les bivalves) dans la prévention de la dissolution du squelette. Un autre résultat que je considère comme très important a été la mesure de la calcification brute des coraux (excluant donc toute contribution de la dissolution du squelette). Pour ce faire j'ai utilisé la technique du ^{45}Ca à l'AIEA. Pour la première fois, il a été démontré que les coraux peuvent calcifier à la même vitesse dans des conditions d'AO. Mais ce qui a fondamentalement fait évoluer ma compréhension de l'effet de l'AO sur les coraux, c'est de voir qu'en fait, les coraux peuvent prospérer dans des environnements extrêmes tels que les resurgences de CO_2 et d'autres analogues naturels tels que Bouraké et la baie de Nikko à Palau. La chance que j'aie eu d'utiliser ces sites spéciaux a certainement amélioré mes connaissances sur la résilience de divers organismes aux conditions futures et nous a suggéré les mécanismes potentiels utilisés par certaines espèces pour faire face à ces environnements extrêmes. Les analogues naturels sont des systèmes imparfaits pour tester l'effet de conditions environnementales futures sur des organismes résidents, probablement adaptés. Malgré les limitations potentielles, telles que la variabilité des paramètres abiotiques, les effets confondants d'autres paramètres inattendus, la difficulté de reproduire les experimentations sur d'autres sites, etc., les laboratoires naturels sont le meilleur moyen d'améliorer notre capacité à mieux tester la résilience et l'adaptation des coraux au changement

climatique. En utilisant ces laboratoires naturels, j'ai fourni des éléments de preuve indiquant que l'adaptation est probablement possible. Ce que je n'ai pas pu démontrer est, est-ce que les coraux qui ont pu survivre se sont simplement acclimatés ou adaptés aux conditions difficiles. Dans les deux cas, il est crucial de mieux comprendre si ces mécanismes sont rapides ou s'ils prennent plusieurs générations pour garantir la survie du corail dans des conditions futures. Cela devient essentiel pour donner un peu d'espoir à la survie des espèces de coraux et des écosystèmes récifaux du monde entier face au changement rapide en cours. Mon projet ERC, qui n'a pas été financé, aborde cette question. Les installations de l'aquarium que je suis en train de construire à Nouville seront un formidable outil pour stimuler ce projet, malgré l'absence du financement necessaire. Je ferai de mon mieux pour cela.

Le projet se concentrera sur les espèces présentes à la fois à l'intérieur (environnement sous-optimal) et à l'extérieur (environnement de référence) de l'analogue naturel de Bouraké. Mon ambition est de cibler au moins cinq espèces de coraux qui se sont développées et ont grandi dans ces conditions contrastées tout au long de leur cycle de vie, éventuellement sur plusieurs générations (ce qui est la grande ambition de ce projet). Une approche expérimentale multidisciplinaire sera utilisée pour quantifier les variations de la physiologie, du patrimoine génétique et des traits fonctionnels (i) entre les espèces, (ii) au sein des espèces en utilisant des colonies collectées à l'intérieur et à l'extérieur du système de Bouraké pour évaluer l'acclimatation et/ou l'adaptation à long terme, et (iii) entre les clones et les génotypes en utilisant des individus transplantés réciproquement d'un environnement à l'autre. Des expériences de transplantation réciproque seront menées en utilisant des fragments clonaux de génotypes multiples pour chaque espèce afin d'évaluer leur niveau d'acclimatation par rapport à l'adaptation génétique. Ce projet sera le premier à expérimenter avec des coraux qui ont été exposés de manière chronique, tout au long de leur vie, à des conditions sous-optimales (température, acidification et désoxygénation) dans un environnement naturel. La transplantation à long terme de colonies adultes et de leurs recrues sera réalisée *in situ* au cours d'expériences transgénérationnelles, ce qui permettra d'évaluer de manière robuste la capacité de tampon phénotypique et la propension à l'adaptation des coraux.