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Combined effects of ocean acidification and low temperature on two range expanding hermatypic corals: *Acropora solitaryensis* and *Porites heronensis*

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Declaração de autoria de trabalho

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Declaro ser oa autora deste trabalho, que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam da listagem de referências incluída,

Klervi Lugué

(Klervi Lugué)

Abstract

A recent poleward shift of hermatypic corals' distribution has been reported and was attributed to the increase in sea temperature since the pre-industrial revolution. Ocean acidification and predicted increasing variability of sea surface temperatures, may together limit this shift in the future. The objective of this study was to investigate whether a tipping point exists in the physiological and metabolic responses of *Acropora solitaryensis* and *Porites heronensis*, to CO_2, under average winter temperature and under cold event temperature (cold stress; -4°C decrease).We studied the effects of increased partial pressures of CO_2 (pCO_2) from 294 ppm to 5018 ppm, on a set of metabolic parameters. The light and dark calcification, skeletal growth rate, chlorophyll and protein concentrations decreased linearly as a function of increasing partial pCO_2 in *A. solitaryensis*. In comparison only the dark calcification and skeletal growth rate decreased linearly as a function of increasing partial pCO_2 in *P. heronensis*. For both species, the cold stress acted as an additional stress to the pCO_2 exposure, except for the respiration in *P. heronensis*. No physiological tipping point has been identified, beyond which these coral species were no longer capable of carrying out the functions necessary to their survival. The lack of a clear tipping point, as well as the emergence of potential 'ecological winners', here *P. heronensis*, in the face of decreasing pH and cold temperature stress, indicate that in the coming decades the species composition of coral reefs is likely to slowly change, to a new composition in which surviving in marginal high latitudes are those that show the required potential for adaptation. Our study highlights the substantial advantages of the regression method to predict the impacts of ocean acidification. Resolving high resolution relationships between metabolism and pCO_2 could greatly improve the accuracy of models describing the effects of future ocean acidification on calcifying organisms and marine ecosystems.

Keywords: Hermatypic corals, Climate Change, Ocean Acidification, Cold stress, Japan

Resumo

Nos últimos anos, o aquecimento global e a acidificação dos oceanos (OA) devido às emissões antropogénicas de CO_2 tornaram-se algumas das questões ambientais mais prementes do mundo, representando uma ameaça fundamental para os recifes de coral tropicais. Impulsionados pelas condições ameaçadoras nos trópicos devido ao aquecimento global em curso, os limites biogeográficos das espécies de corais de águas quentes têm vindo a mudar. O aumento da temperatura da superfície do mar (SST) a grande altitude permite que os habitats de coral se expandam para os pólos, encontrando refúgio em novas regiões temperadas quentes adequadas, ao mesmo tempo que limita o seu habitat nos trópicos devido a um branqueamento excessivo. O Japão cobre uma grande amplitude latitudinal, estendendo-se de áreas subtropicais a temperadas, e é definido como uma "zona de transição subtropical-temperada" para a distribuição geográfica de uma grande diversidade de espécies de coral. Esta zona, na vanguarda para as espécies subtropicais e na vanguarda para as espécies temperadas, proporciona uma oportunidade única para o estudo das mudanças e/ou expansões da gama de espécies devido ao aquecimento climático, numa grande escala espacial. No entanto, a acidificação oceânica projectada e a crescente variabilidade das temperaturas da superfície do mar (por exemplo, El Niño Southern Oscillation), podem, em conjunto, limitar as mudanças da gama de habitats de corais tropicais-subtropicais, nos mares que rodeiam o Japão.

Estudámos aqui, em condições controladas, o efeito da acidificação oceânica sob a temperatura média de Inverno e sob a temperatura do evento frio (stress frio; -4°C de diminuição), em duas espécies de coral: o coral temperado quente e incrustado *Porites heronensis*, e a espécie *Acropora solitaryensis* de mudança de vara Acropora. Investigámos os efeitos de uma vasta gama de pressões parciais de CO_2 (p CO_2) (i.e. "abordagem de regressão"), de 294 ppm a 5018 ppm, sobre um conjunto completo de parâmetros metabólicos (níveis de pCO_2 abrangendo as condições actuais, projecções do IPCC até ao ano 2100 e seguintes). O objectivo do estudo era: i) testar se a existência de um ponto de viragem fisiológico para além do qual as espécies de coral já não são capazes de desempenhar as funções necessárias à sua sobrevivência; ii) determinar se uma exposição a pH baixo pode afectar fortemente a sobrevivência dos corais durante um evento de frio extremo.

Como resultado da experiência de 7 semanas, não foram identificados eventos de branqueamento, mas sim efeitos lineares significativos do aumento do pCO_2 (isto é, acidificação oceânica) sobre a fisiologia e metabolismo, diferindo entre as espécies. A calcificação clara e escura, a taxa de crescimento do esqueleto, as concentrações de clorofila e

proteínas diminuíram linearmente em função do aumento parcial do $p\mathrm{CO}_2$ em *A.solitaryensis*. Enquanto que apenas a calcificação escura e a taxa de crescimento do esqueleto diminuíram linearmente em função do aumento parcial de $p\mathrm{CO}_2$ em *P.heronensis*. Os efeitos dos níveis de $p\mathrm{CO}_2$ no metabolismo do coral não diferiram em função da temperatura, mas os dois combinados resultaram numa importante diminuição. Em *A. solitaryensis*, o stress do frio actuou como um stress adicional à exposição ao $p\mathrm{CO}_2$, tanto no metabolismo de zooxanthellae como no do hospedeiro. Em *P. heronensis*, o zooxanthellae só mostrou respostas significativamente mais baixas após o choque frio, enquanto que taxas respiratórias mais elevadas foram registadas. Não foi identificado nenhum ponto de viragem fisiológica, para além do qual estas espécies de coral já não eram capazes de desempenhar as funções necessárias à sua sobrevivência.

Este estudo destacou a heterogeneidade na resposta dos calcificadores de recife ao $p\mathrm{CO}_2$ elevado e ao stress adicional do frio, levando ao aparecimento de potenciais "vencedores ecológicos", tais como aqui a *P. heronensis*. De facto, os nossos resultados são consistentes com a afirmação de estudos anteriores, identificando *A. solitaryensis* como parte do grupo de "alta sensibilidade" ao $p\mathrm{CO}_2$, previsto ser severamente limitado geograficamente pela acidificação oceânica; e *P. heronensis* como parte do grupo de "baixa sensibilidade" ao $p\mathrm{CO}_2$, mostrando uma alta resiliência a níveis elevados de CO_2. Mesmo que não tenha sido revelada qualquer interacção entre os níveis de $p\mathrm{CO}_2$ e os tratamentos de temperatura para estas duas espécies de coral, os nossos resultados sugerem que o impacto do stress frio extremo a curto prazo (~ 12 dias abaixo de 15°C) na fisiologia e no equilíbrio energético dos organismos do recife. É provável que o metabolismo dos corais, expostos ao stress da temperatura fria, seja ainda mais enfraquecido se estiverem sujeitos a ameaças adicionais como a qualidade da água (por exemplo, metais pesados, eutrofização) e pressões antropogénicas. Os resultados obtidos sublinham a necessidade de considerar a combinação da acidificação oceânica combinada com o evento de frio extremo, num contexto de expansão dos corais para os pólos. Os nossos resultados sugerem que o deslocamento para a poleward *A. solitaryensis* seria severamente limitado pela acidificação oceânica e apenas o coral temperado quente e incrustado *P. heronensis*, já estabelecido nestas altas latitudes, poderia manter taxas de crescimento adequadas sob futura acidificação oceânica e stress frio. A falta de um ponto de viragem claro, bem como a emergência de potenciais vencedores enfrente à diminuição do pH, indicam que nas próximas décadas a composição das espécies de recifes de coral irá provavelmente mudar lentamente, sem transições abruptas, para uma nova composição em que as espécies têm o

potencial necessário para se adaptarem nas altas latitudes marginais. A redistribuição dos recifes de coral e o declínio da estrutura estruturalmente complexa que forma os corais (por exemplo *Acropora solitaryensis*) nas altas latitudes marginais poderia causar modificações graves e fundamentais dos ecossistemas (por exemplo, redução da disponibilidade de habitat para peixes e muitos invertebrados) e, portanto, afectar fortemente: o funcionamento dos ecossistemas, o bem-estar humano (desenvolvimento económico, meios de subsistência, doenças emergentes, segurança alimentar), e a própria dinâmica das alterações climáticas (feedbacks, sequestro de carbono). Nestas circunstâncias actuais, a necessidade de avaliar os controlos ambientais sobre os recifes de coral e prever a distribuição global em cenários futuros de alterações climáticas é fundamental.

O nosso estudo considerou apenas o aspecto fisiológico para a determinação da gama de distribuição futura, que pode não ser suficiente para uma previsão correcta. Contudo, a abordagem de regressão, utilizada neste estudo, demonstrou ser um método perspicaz para melhor prever os impactos da OA em comparação com a "abordagem de cenário", resolvendo a tendência geral e a forma da relação entre metabolismo e $p\mathrm{CO}_2$. A descrição destas relações para uma vasta gama de espécies que compõem as comunidades de recifes de coral, poderia expandir grandemente a nossa compreensão dos mecanismos empregados pelos corais, e melhorar grandemente a precisão dos modelos que descrevem os efeitos da OA prevista sobre os organismos calcificadores e os ecossistemas, sob as alterações climáticas.

Palavras-chave : Corais Hermatypic, Alterações Climáticas, Acidificação Oceânica, Stress frio, Japão

Table of contents

Index of Figures

CHAPTER 2 : Combined effects of ocean acidification and low temperature on two range expanding hermatypic corals : *Acropora solitaryensis* and *Porites heronensis*

Index of Tables

CHAPTER 2 : Combined effects of ocean acidification and low temperature on two range expanding hermatypic corals : *Acropora solitaryensis* and *Porites heronensis*

List of Abbreviations

DO – Dissolved Oxygen

GHG – Greenhouse gases

IPCC – Intergovernmental Panel on Climate Change

OA – Ocean acidification

pCO_2 – Atmospheric carbon dioxide concentration

RCP – Representative Concentration Pathways

SST – Sea Surface Temperature

TA – Total alkalinity

$\Omega_{\text{Aragonite}}$ – Saturation state of aragonite

CHAPTER 1 : Introduction

1. Climate-driven biogeographic shifts

Since the beginning of life on Earth, living organism's geographical distribution has always been closely related to the environmental changes on different spatial and temporal scales (Davis and Shaw, 2001). To stay within their preferred environmental conditions, species shift their geographical range limits, which can be identified as dynamic and fluctuating over time according to the climate changes. Moreover, the sustainability of a specie over time, is highly related to its capacity to shift their distributions in response to tectonic, oceanographic, or climatic events (Rosen,1984).

Nowadays, the increasing anthropogenic carbon dioxide (CO_2) emission has driven rapid and major climatic changes, such as global warming and ocean acidification (IPCC, 2019). These anthropogenic-driven environmental changes have already generated a rapid changes in ocean properties, profoundly affecting species' biogeography and phenology, as well as ecosystem dynamics and biogeochemical cycling (Hoegh-Guldberg et al., 2014; Pörtner et al., 2014).The rapid modification in fundamental physico-chemical (e.g. warming and ocean acidification) and biological properties of the oceans, affect strongly the marine organism's biogeography (i.e. what lives where).

Following the RCP8.5 scenario of GHG emission (i.e. high greenhouse gas emission scenario in the absence of policies to combat climate change), atmospheric and ocean surface temperature in 2100 is predicted to rise by 4°C by the year 2100 (IPCC, 2019). One of the way for marine organisms to respond to this important environmental change, is to shift in location at the cooler extremes of their distributions. Therefore, species are moving poleward, while their range limits are contracting at their warmer range edge, where environmental conditions are no longer tolerable. (Pecl et al., 2017). Major biogeographic shifts are already underway and reported in a large range of marine organisms, from copepods to sea birds (Figueira & Booth, 2010; Last et al., 2011; Ling et al., 2009; Wernberg et al., 2011; Pitt et al., 2010).

1.1. Poleward range expansion of corals

Hermatypic corals (i.e. reef builders) are one of the world's most important species, being not only primary producers, but also by being ecosystem engineers. These organisms are providing food and habitat to a large range of marine organisms, as well as providing many other ecological goods and services (Lilley and Schiel, 2006). Corals host in their endoderm endosymbiotic microalgae of the *Symbiodinaceae* family, commonly known as zooxanthellae, and depend on their photosynthesis as corals get 90% of their energy requirements from the translocation of photosynthetic products (Edmunds and Davies, 1986; Leletkin, 2000). These symbiotic reef building corals have been identified as being extremely vulnerable to ocean warming (Hoegh-Guldberg, 1999).

When corals are stressed by too high sea surface temperature (SST), symbiotic zooxanthellae inside them are weakened and the zooxanthellae will be released or degraded internally, and corals bleached (i.e. disintegration of the coral–dinoflagellate symbiosis; Hoegh-Guldberg and Smith, 1989). Such predicted a rise in sea surface temperatures, due to climate change, threaten corals in the tropics, leading to increasing frequency and severity of coral bleaching events with negative consequences for coral survival, growth, and reproduction. Mass mortality of most known tropical coral reefs would lead to the declines in coral abundance and biodiversity (Pandolfi et al., 2003; Heron et al., 2016; Hughes et al., 2017). With high temperature stress due to climate change threatening corals in the tropics, cooler high latitudes may become a potential refuge. To face these threatening conditions in the tropics, warm-water coral species are moving their biogeographic limits northward (Kayanne et al., 1999). Increases sea surface temperature (SST) at high-latitude allow coral habitats to expand poleward, finding refuge in new suitable warm temperate regions areas (Precht and Aronson, 2004; Yamano et al., 2011), while at the same time, limit their habitat in the tropics due to excessive bleaching (Guinotte et al., 2003; Meissner et al., 2012). Therefore, this climate-driven redistribution is characterized by a poleward range shifts and/or expansions of corals in temperate areas, from regional to global scale (Yamano et al., 2011; Baird et al. 2012; Kiessling et al., 2012; Muir et al., 2015; Grupstra et al., 2017).

1.2. Poleward range expansion recorded in Japan

The poleward range expansion of several tropical corals with increasing SST has already been reported in Japan (Yamano et al., 2011; Yara et al., 2011), and in several places over the world (e.g. in the Caribbean (Precht & Aronson, 2004), in Australia (Hughes et al., 2012)).

Japan covers a large latitudinal range, extending from subtropical to temperate areas, and is defined as a “subtropical-temperate transition zone”. The distribution of corals along the Pacific coast of Japan ranges from the southernmost islands of the Ryukyu Archipelago (24 °N) to Amatsukominato in Chiba Prefecture (34 °N). The presence of hermatypic corals at these high latitudes is mainly to the strong Kuroshio current, which brings warm water as far as Tateyama (34°N) (Yamano et al., 2011). Latitudinal geographical distribution limits of a highly diverse coral species are overlapping over this area (Veron, 1992b; Yamano et al., 2001). This zone, at the leading-edge for subtropical species and the trailing-edge for temperate species, provides a unique opportunity for examining species range shifts and/or expansions due to climatic warming, over a large spatial scale (Yamano et al., 2011).

Yamano et al. (2011) demonstrated, on the basis of long-term coral observations, that such northward expansion has already occurred along the coastlines of Japan, at a high speed (14 km/year). Yara et al. (2009, 2011) investigated the potential future development of this northward expansion along the coasts of Japan, suggesting that this trend, at relatively smaller speeds (1 to 4 km/year), might continue for several decades. Moreover, Kumagai et al. (2018) demonstrated the implication of the Kuroshio (warm-water) current in the rapid biogeographic coral shifts in Japan, by providing a high connectivity across latitudes (by enhancing the spread of larvae). These observations suggest that coral reefs might shift to higher latitudes of Japan, becoming therefore a potential refuge for scleractinian corals, and possibly counterbalancing the loss of coral reefs at lower latitudes (tropics) (Nakabayashi et al., 2019). Thus, these studies provide the evidence about the potential sustainability of coral reefs in the future, leading to reduced concern. However, Yamano et al. (2011) and Yara et al. (2009, 2011) took only into account the change in temperature (SST), but not changes in the aragonite saturation state and changing intensities as well as frequencies of extreme cold events at high latitudes.

2. Climat change mitigation

Over the 21st century, the ocean is projected to become profoundly affected by the anthropogenically-induced greenhouse gas emission, resulting in significant increases of the global sea surface temperature mean (SST + 4.3°C by the year 2100, under RCP8.5), increases in the frequency and intensity of marine heatwaves (MHWs), further acidification of the open ocean surface pH (- 0.3 pH unit by the year 2100, under RCP8.5), and numerous other impacts (e.g oxygen decline, greater upper ocean stratification, sea level rises, and increases in the frequency of extreme El Niño and La Niña events) (IPCC 2019).

2.1. Ocean acidification

Carbonate System in Seawater

Due to their dependence on the formation of an aragonite skeleton, corals are also sensitive to the carbonate ion concentration, $[CO_3^{2-}]$, and to the aragonite saturation state, $\Omega_{\text{Aragonite}}$. The process of calcification is strongly linked to seawater carbonate chemistry and calcium carbonate. Atmospheric carbon dioxide reacts with water. The equations describing this carbonate system are:

$$CO_2\ (g) \leftrightarrow CO_2\ (aq) \qquad (1)$$

$$CO_2\ (aq) + H_2O\ (l) \leftrightarrow H^+(aq) + HCO_3^-\ (aq) \qquad (2)$$

$$HCO_3^-\ (aq) \leftrightarrow H^+(aq) + CO_3^{2-}\ (aq) \qquad (3)$$

the notations (g), (l) and (aq) show the state, i.e., a gas, a liquid, and in aqueous solution respectively (Riebesell et al., 2011; Kleypas and Langdon, 2006). Calcifying organisms, including corals, make skeletons using carbonate ions and calcium ions in seawater with the following equilibrium:

$$CO_3^{2-}\ (aq) + Ca^{2+}\ (aq) \leftrightarrow CaCO_3\ (s) \qquad (4)$$

where (s) indicates the solid phase (Riebesell et al., 2011). A direct consequence of the increasing partial pressure of CO_2 in the atmosphere is the increase of total dissolved inorganic carbon in seawater, decreasing therefore the seawater pH (i.e. net increase in protons H^+; Equation 2). However, in order to maintain charge balance, some carbonate ions will be converted to bicarbonate (HCO_3^-), lowering the carbonate ions concentration (CO_3^{2-} ; Equation (3)). One of the important seawater parameters for coral is $\Omega_{Aragonite}$. $\Omega_{Aragonite}$ shows the saturation state of aragonite, which is one of the common crystal forms of calcium carbonate in seawater, with the following equation :

$$\Omega_{Aragonite} = [Ca^{2+}][CO_3^{2-}] / K_{sp}(aragonite) \qquad (5)$$

When $\Omega_{Aragonite}$ is lower than 1, aragonite dissolution is thermodynamically enhanced. On the other hand, aragonite precipitation is favoured when $\Omega_{Aragonite}$ is higher than 1 (Riebesell et al., 2011). The geographic distribution of corals reefs shows that corals need sufficient calcium carbonate saturation to form reefs ($\Omega_{Aragonite} > 3$; Kleypas et al., 1999).

Seawater carbonate chemistry changes implications

With almost one third of released CO_2 directly absorbed by the oceans, the world average seawater pH is predicted to decrease by 0.3 by the year 2100, under RCP8.5 (IPCC, 2019). Following the chemical equilibrium stated above, as increased atmospheric carbon dioxide dissolves in the ocean, the concentration of bicarbonate ions and hydrogen ions in seawater increases while the concentration carbonate ions decreases. $\Omega_{Aragonite}$ also decreases with the concentration of carbonate ions. This change in ocean chemistry is commonly refers to as ocean acidification (OA).

Because coral reef ecosystems are confined to the upper mixed layer of the ocean, they will be readily exposed to these shifts in the ocean carbonate equilibrium. Under OA, the current saturation states may cross the threshold supporting biogenic calcification ($\Omega_{\text{Aragonite}} > 3$; Kleypas et al., 1999), driving to a decline in calcification rates of corals, and, in the worst case scenario, to the net dissolution of carbonate structures (coral reefs) ($\Omega_{\text{Aragonite}} < 1$) (Albright et al., 2016). These changes in ocean chemistry presents a major challenge for marine calcifying organisms. Numerous scientific studies already highlighted the severe threat of low pH and $\Omega_{\text{Aragonite}}$ for reef-building organisms, affecting especially their calcification rates and their overall productivity (Kleypas et al., 1999; Anthony et al., 2008), but also reproduction and development (Kurihara, 2008), acid-base regulation (Pörtner, 2008), photosynthesis (Anthony et al., 2008), respiration (Kaniewska et al., 2012), and tolerances of other stressors (Hoegh-Guldberg et al., 2007). Hoegh-Guldberg et al. (2007) have suggested that a threshold of atmospheric carbon dioxide concentration (i.e. $p\text{CO}_2$; 500ppm) exists, above which coral reef calcification will be dramatically reduced.

Poleward shifts implications

At high-latitude regions, seawater temperatures are colder, resulting into an increase of the CO_2 solubility, and thus, lower carbonate ion concentrations and $\Omega_{\text{Aragonite}}$. High-latitude regions will therefore reach first critically low levels of seawater saturation states (Kleypas et al.,1999b; Orr et al.,2005; Fabry et al., 2014). By reducing the growth potential and survivorship of corals, decreasing $\Omega_{\text{Aragonite}}$ is predicted to cause the shift of corals distribution northern limit southward (Guinotte et al., 2003; Hoegh-Guldberg et al., 2007; Yara et al., 2012), countering the advantages of ocean warming (rising SST) in a context of range shifts and/or expansions of corals northward (Yara et al., 2012).

Yara et al. (2012) found that coral habitats will be shifting constantly poleward by the end of 2100, due to the northward expansion of the average SST isolines, making seas surrounding Japan suitable to tropical-subtropical coral communities. Moreover, they projected that ocean acidification will give rise to the southward expansion of isolines of $\Omega_{\text{Aragonite}}$, pushing current marine ecosystems, around Japan, below the $\Omega_{\text{Aragonite}} = 3$ threshold (i.e. needed to support sufficiently high calcification rates; Kleypas et al.; 1999). As a result they projected that coral habitats will become “sandwiched” between high temperature regions (Tropical regions; where the frequency of coral bleaching will increase), and low aragonite saturation states regions (temperate to subpolar latitudes; where the calcification rates will be reduced), leading to a significant reduction of suitable coral habitat around Japan.

By investigating marine communities at volcanic seeps (off the Pacific Japanese coast), Agostini et al. (2018) found that an increased mean levels of *p*CO2 results in profound community-level changes, shifting sub-tropical ecosystems from carbonate to fleshy algal dominated systems. They observed a significant biodiversity loss, due to a decline in key habitat-forming species (e.g. scleractinian corals) and an increase in low-profile fleshy algae. Overall, this study highlights the expected implication of ocean acidification on the simplification coastal marine communities at the seas surrounding Japan, but also throughout East Asia. Regarding More recently, Agostini et al. (in preparation) combined field surveys and transplantation experiments at natural analogues of present day and future conditions. Their results suggest that an extensive loss in foundation kelp species will occur, and will not be replaced by scleractinian corals, under the projected scenario of combined warming and acidification. They show the direct evidence that the new suitable regions (at higher latitudes) of some coral species, will be severely limited by ocean acidification (e.g. Acroporids), leading to fundamental changes within biotic communities and rapid changes in ecosystem functioning and services. Therefore, this study highlights the severe threat of an unprecedent loss of ecosystem services, caused by a simplification of the warm temperate region ecosystems.

2.2. Extreme cold stress

Bleaching is commonly defined as the loss of the symbiotic algae and/or its pigments. Severe and prolonged bleaching events can cause mass mortality of corals (Hughes et al., 2018; Kayanne et al., 2017; Sampayo et al., 2008). Over the last decade, records of coral bleaching events have been increasing in frequency and spatial scales (Gates et al. 1992, Brown et al. 1994), due to elevated sea surface temperatures (Hoegh-Guldberg 1999). However, bleaching events have also been correlated with cold sea surface temperatures (Coles & Jokiel 1977, Gates et al. 1992; Higuchi et al., 2015; Higuchi et al., 2020), leading sometimes to mass mortality (Leriorato and Nakamura, 2019). Bleaching susceptibility has been demonstrated to varies greatly among coral taxa (Marshall and Baird, 2000), leading to strong selective mortality and marked directional shifts in the structure of coral assemblages (e.g., Pratchett et al., 2011).

ENSO events in the Pacific are predicted to increase in intensities as well as frequencies (Urban et al. 2000) suggesting that La Niña events may increase in frequency. La Niña events are defined by colder years, in which sea temperatures can be cooler than long-term averages, and different wind patterns. Notably the passage of cold air masses from the Arctic has been shown to have rapid cooling effects on shallow water carbonate environments, due to increased chilling and mixing of water bodies by strong winds (Roberts et al. 1982). In addition, extreme weather events (Wernberg et al., 2013, IPCC 2019); including extreme heat events (marine heat waves, MHWs) and extreme cold events (marine cold spells, MCSs); are expected to increase over time, leading to severe impact on the structure of ecosystems (Jentsch et al. 2007).

Cold bleaching and mortalities events recorded in Japan

Japan host high latitudes coral communities, with the world northernmost coral reefs reported at Iki Island, Japan Sea (33°48’N; Yamano et al., 2001). Cold bleaching, and mortality events have already been recorded in marginal high-latitude coral communities in Japan (Veron and Minchin, 1992; Yamano and Namizaki, 2009), leading at times to high mortality rates of corals during winter. Recently, Leriorato and Nakamura (2019), recorded an extreme cold event, during the winter 2018 (SST < 15 °C, from 10 days to 2 months), at the coastal waters of Tosa Bay (Japan). This extreme cold event, enhanced by inflows region polar continental air masses (influenced by La Niña), caused an unprecedent massive coral bleaching event and subsequent mortality of more than 90% of corals in the bay. This study highlights the potential instability of temperate waters, acting as a refuge for range-shifting reef-building corals, due to the predicted increasing extreme climatic events, implicated in the severe declines and the potential disappearance of various coral species.

Poleward shifts implications

In the last hundred years, some coral species have expanded their range in Japan at tremendous speed, up to 14 km per year (Yamano et al., 2011). While the future increase in temperature under global warming may allow more species of corals to colonise higher latitudes, cold winter events will certainly occur and may cause extended mortalities of coral species less resilient to cold stress. This could severely limit the number of species that will be able to find refuges in higher latitudes, causing fundamental changes in corals associated ecosystem functioning and services. However other species do not show such expansion which suggests some inherent physiological characteristics to allow the survival in higher latitudes. Higuchi et al. (2020) showed the resistance of two hermatypic corals; commonly found in the temperate regions of Japan; to cold stress, inferring specific adaptations allowing their survival in the marginal high latitudes (e.g. reduction in basal metabolism and shift to alternative source of energy). This resistance and resilience to cold stress, and the ability to colonise reef habitats in the aftermath of severe bleaching events, could be a common trait of corals found in high latitudes marginal coral communities (Chen et al., 2016; Howe and Marshall, 2001; Ross et al., 2018). Through their endemicity and potential adaptations, marginal coral communities, already established at high latitudes, represent a reservoir of biodiversity (Veron, 1992). These communities could therefore play a determinant role as a refuge for coral diversity under climate change (Makino et al., 2014).

3. High latitudes coral communities

Because different species respond at different rates and to different degrees, key interactions between species are disrupted within the ecological communities, and allow new interactions to develop (Pecl et al., 2017). These shifts are expected to continue with projected ocean warming (Jones and Cheung, 2015; Wisz et al., 2015) , causing potentially permanent changes within marine ecosystems, including: local extinctions (leading to "winners" and "losers" species) (Jones and Cheung, 2015), novel biotic communities and rapid changes in ecosystem functioning and services (Pecl et al., 2017).

Tropical corals are under severe stresses at temperature under 18°C (Colella et al., 2012; Kleypas et al., 1999), defining therefore the geographic limits for the formation of coral reefs (Kleypas et al., 1999). Nevertheless, a few warm-water hermatypic coral species can extend their northern limit to regions presenting minimum temperatures reaching 10°C (Veron and Minchin, 1992). Marginal coral communities have been observed at high latitudes where surface seawater temperature regularly drops down to 10 °C (Denis et al., 2013; Dimond et al., 2013; Yamano et al., 2001, 2011; Yamano and Namizaki, 2009). Such marginal communities could potentially represent a "refugia" , allowing corals to survive under a warming climate (Beger et al., 2014; Makino et al., 2014).

3.1. *Acropora solitaryensis*

Acroporid corals are abundant in the tropics and exhibit the highest diversity among corals, with approximately 180 species in the genus Acropora (5). In Japan, acroporid species can be observed even in the northernmost reefs, although their diversity decreases significantly at the highest latitudes (i.e. near Tateyama (34 °N)). For instance, *Acropora solitaryensis* have expanded its distribution northward in Japan (Yamano et al., 2011; Nakabayashi et al., 2019), but still has a more southerly range than other coral species belonging to the genus Acropora.

Bleaching of *A. solitaryensis* and other tabular acroporids was reported from high-latitude coral communities near Nagasaki (32.5 °N) after 12 days of low temperatures (< 13 °C) in 2013 (Suzuki et al.,2013). Higuchi et al., (2015) investigated the resistance and resilience of the genus *Acropora* to cold bleaching. They highlighted the importance of cold resistance and recovery from cold bleaching, in determining the northern distribution limits of Acropora coral species. Low resistance to cold stress in *A. solitaryensis* was observed, due to a lack of protection against cold stress-induced damage, explaining why is less common at higher latitudes. Recently, Agostini et al. (in preparation) conducted field surveys, at the seas surrounding Japan, to assess the existing coral communities at present day and future conditions (warming and combined warming/acidification conditions). The Acroporid *A. solitaryensis*, was only found at the warming condition. Therefore, this study showed the direct evidence of the high sensitivity to $p\mathrm{CO}_2$, and suggesting that the latitudinal shift of this fast growing Acropora species will be limited in the future by ocean acidification.

3.2. *Porites heronensis*

One of the dominant corals in temperate regions of Japan is the slow growing *P.heronensis* (Veron, 1985; Nishihira and Veron, 1995). This coral species can be found in more tropical areas, but it is more commonly found in higher latitudes. *P. heronensis* distribution range from Okinawa mainland (24°N) to Tateyama in Chiba prefecture (34 °N).

Physiologic thermal limits of this species (Huey & Stevenson, 1979) were found to be 12 °C for the lower critical temperature as most of the corals bleached and died at this temperature. Higuchi et al. (2020) investigated the effect of cold temperature on *P.heronensis* through field transplantation and *in situ* incubations (slow and natural decrease in temperature). After 243 days at temperature under 18°C, (i.e. limit for the development of reefs) and 110 days under 15°C , bleaching was observed but no specimens died. This study highlighted the high resistance of this temperate coral species to cold stress, through perhaps specific adaptations allowing it survival in the marginal high latitudes. Previous experiments showed also that corals of the genus *Porites* have a high resistance to high CO_2 levels (Edmunds, 2011; Fabricius et al., 2011). Recently, Agostini et al. (in preparation), investigated the effect of ocean acidification and warming on high latitudes coral communities, under natural conditions. They observed that the warm temperate encrusting coral *P. heronensis* was consistently found in all locations. Therefore, this study showed the direct evidence that the slow growing coral species, such as *P. heronensis*, already established at these latitudes, could maintain suitable growth rates under the near-future projected ocean acidification.

4. Research questions

Under these current circumstances, the study of the impact of ocean acidification and cold stress on the changing coral species' biogeographic distributions is fundamental, in order to permit a better conservation and protection against the anthropogenic threats and pressures, and thus, ensure the fundamental coral's ecological goods and services.

Coral populations exhibit variability in tolerance to external stress, within and between coral species (Hoegh-Guldberg and Salvat 1995, Loya et al. 2001, Coles and Brown 2003). Moreover, these differences in tolerance can occur within a single site (Oliver and Palumbi 2009) or across geographic regions (Coles et al. 1976, Hoegh-Guldberg 1999) showing the direct evidence that the evolution of stress tolerance, has already occurred in the past. Therefore, it is important to emphasize that the definition of "coral habitats" must considers potential acclimation and adaptation processes, allowing them to expand outside their current ranges and/or reduce their sensitivity to ocean warming and ocean acidification (Pandolfi et al., 2011). Recent studies demonstrated that corals are able to alleviate the potential impact of warming and ocean acidification (Pandolfi et al., 2011). For example, Cooper et al. (2012) pointed out that ocean warming would permit coral communities to migrate poleward, but also, may have a strong positive effect on coral calcification, potentially allowing them to overcome the negative effect of ocean acidification.

Given that the warm temperate encrusting coral *Porites heronensis,* and the fast growing Acropora species *Acropora solitaryensis*; show different biological responses to ocean acidification, we posit the following questions :

What is the impact of ocean acidification on the biological processes of the two coral species?
Is there a tipping point beyond which these coral species are no longer able to perform the functions necessary for their survival?
Does cold temperature shock have a significant effect on coral survival? And if so, are corals exposed to acidified seawater more sensitive?
Is there an acclimation process (e.g. shift in energy allocation, thermal compensation) that would allow them to reduce their sensitivity to the ocean acidification and/or cold stress, and thus expand outside their current ranges?

5. Objectives

For this experimentation, we use two coral species: the coral *Acropora solitaryensis,* identified as being a specie severely sensitive to ocean acidification and cold stress; and the coral *Porites heronensis,* identified as being a species remaining in acidified areas (Agostini et al., 2018; Agostini et al., in preparation).

The aim of the study is to : i) to investigate under controlled conditions, the effects of a wide range of seawater pH by using the “regression approach” (Comeau et al., 2013; Dorey et al.,2013; Ventura et al.,2016), (6 targeted pH conditions - covering present conditions and IPCC’s projections by the year 2100) on a full set of metabolic/physiological parameters of two coral species; and ii) study the effect of a cold temperature shock on the coral survival at the end of the pH exposure period.

By using the “regression approach”, we expect to identify a high-resolution relationship between the pH and the measured parameter. Moreover, we expect to identify the physiological tipping point beyond which these coral species are no longer capable of carrying out those functions necessary to their survival. By identifying potential tipping points, and by comparing regression models of different metabolic responses, we could have a better understanding of the coral’s energy allocation strategy (i.e. acclimation processes, e.g. thermal compensation) under perturbated climate states (OA).

By creating a thermal (cold) shock at the end of the pH exposure period, we expect to determine if a lower pH exposure can affect strongly the coral’s survival during thermal extreme event (acting synergistically?), in a context of poleward expansion of corals. Indeed, we hypothesize that a chronic acidified condition exposure will affect the coral’s metabolism and will have negative consequences on their temperature tolerance.

And finally, by comparing the response of the two species, to the combined ocean acidification and extreme cold event, we would improve the knowledge about the future biogeographic distribution shift and assemblage structure of hermatypic corals at high latitudes, in the seas around Japan.

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CHAPTER 2 : Combined effects of ocean acidification and low temperature on two range expanding hermatypic corals : *Acropora solitaryensis* and *Porites heronensis*

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Abstract

A recent poleward shift of hermatypic corals' distribution has been reported and was attributed to the increase in sea temperature since the pre-industrial revolution. Ocean acidification and predicted increasing variability of sea surface temperatures, may together limit this shift in the future. The objective of this study was to investigate whether a tipping point exists in the physiological and metabolic responses of *Acropora solitaryensis* and *Porites heronensis*, to CO_2, under average winter temperature and under cold event temperature (cold stress; -4°C decrease).We studied the effects of increased partial pressures of CO_2 (pCO_2) from 294 ppm to 5018 ppm, on a set of metabolic parameters. The light and dark calcification, skeletal growth rate, chlorophyll and protein concentrations decreased linearly as a function of increasing partial pCO_2 in *A. solitaryensis*. In comparison only the dark calcification and skeletal growth rate decreased linearly as a function of increasing partial pCO_2 in *P. heronensis*. For both species, the cold stress acted as an additional stress to the pCO_2 exposure, except for the respiration in *P. heronensis*. No physiological tipping point has been identified, beyond which these coral species were no longer capable of carrying out the functions necessary to their

survival. The lack of a clear tipping point, as well as the emergence of potential ‘ecological winners’, here *P. heronensis*, in the face of decreasing pH and cold temperature stress, indicate that in the coming decades the species composition of coral reefs is likely to slowly change, to a new composition in which surviving in marginal high latitudes are those that show the required potential for adaptation. Our study highlights the substantial advantages of the regression method to predict the impacts of ocean acidification. Resolving high resolution relationships between metabolism and $p\mathrm{CO}_2$ could greatly improve the accuracy of models describing the effects of future ocean acidification on calcifying organisms and marine ecosystems.

Introduction

Anthropogenic carbon dioxide (CO_2) emission has driven rapid and major climatic changes, such as global warming and ocean acidification (IPCC, 2019). These anthropogenic-driven environmental changes (e.g. ocean warming and ocean acidification) are profoundly affecting the biogeography (i.e. what lives where) and phenology of marine species, as well as ecosystem dynamics and biogeochemical cycling (Hoegh-Guldberg et al., 2014; Pörtner et al., 2014). These high environmental changes velocities have already led to rapid regional and global-scale range shifts of marine organisms (Poloczanska et al., 2013; Garcia et al., 2015; Poloczanska et al., 2016). Marine species are moving poleward (at the cooler extremes of their distributions), while their range limits are contracting at their warmer range edge, where environmental conditions are no longer tolerable. (Pecl et al., 2017).

Pushed by the threatening conditions in the tropics due to the on-going global warming, the biogeographic limits of warm-water coral species have been shifting (Baird et al. 2012; Muir et al., 2015; Grupstra et al., 2017). Increases sea surface temperature (SST) at high-latitude allow coral habitats to expand poleward, finding refuge in new suitable warm temperate regions areas (Precht and Aronson, 2004; Yamano et al., 2011), while at the same time, limit their habitat in the tropics due to excessive bleaching (Guinotte et al., 2003; Meissner et al., 2012). The poleward range expansion of several tropical corals with increasing SST has already been reported in several places over the world (e.g. Caribbean (Precht & Aronson, 2004), Australia (Hughes et al., 2012)), including in Japan (Yamano et al., 2011; Yara et al., 2011). Japan covers a large latitudinal range, extending from subtropical to temperate areas, and is defined as a “subtropical-temperate transition zone” for the geographical distribution of a highly diverse coral species. The presence of hermatypic corals at these high latitudes is principally due to the

Kuroshio current, which brings warm water as far as Tateyama (Yamano et al., 2011) and high connectivity across latitudes (Kumagai et al., 2018).This zone, at the leading-edge for subtropical species and the trailing-edge for temperate species, provides a unique opportunity for the study of species range shifts and/or expansions due to climatic warming, over a large spatial scale (Yamano et al., 2011).

Scientific studies are predicting that the tropical-subtropical coral habitat range shifts, in the seas surrounding Japan, could be severely limited due climate change related events (Yara et al.; 2012, Leriorato and Nakamura (2019), Agostini et al. (in preparation)). Projected ocean acidification will lead to the southwardshift of isolines of Ωarag, pushing current marine ecosystems, around Japan, below the Ωarag=3, the minimum level for the maintenance of sufficient growth rates of corals and the formation of reefs(Kleypas et al.; 1999; Yara et al.; 2012). As a result, coral habitats are projected to be "sandwiched" between high temperature regions (Tropical regions; where the frequency of coral bleaching will increase), and low aragonite saturation states regions (temperate to subpolar latitudes; where the calcification rates will be reduced). In addition, extreme cold events; which are predicted to increase in frequency and intensity (Urban et al. 2000); have been previously recorded in Japanese coastal waters, resulting in unprecedented massive coral bleaching and mortality events (Leriorato and Nakamura, 2019). Previous studies have shown that long term exposure to cold temperatures lead to higher mortality rates in tropical corals than high temperatures (Jockiel and Coles, 1977). However, the combined effects of ocean acidification and cold stress, predicted to severely limit the poleward biogeographic limits of coral species, remain poorly understood.

The "scenario" approach is almost systematically used in the literature to describe the effects of ocean acidification on the physiology of marine organisms. This method consists of measuring the physiological response of organisms under a small number of pH conditions (generally 2-3), in replicates (i.e. representing ambient conditions and those expected for the end of the century). However, this method has limitations that have been pointed out recently, including the poor interpretive capability with the comparison of only a few environmental conditions (Vargas et al., 2017). Inferences of bias due to the arbitrary choice of pH conditions; generally unrealistic compared to the natural variability remaining largely unknown today; have been emitted (Vargas et al., 2017). Finally, the protocols are often not standardized and do not allow comparisons between different studies (Gazeau et al., 2013). To face these limitations, the "regression" method was proposed as an alternative (Dorey et al., 2013; Comeau et al., 2013; Ventura et al., 2016). The organisms are exposed to a vast gradient of different conditions, without any replication, in order to model each physiological response by regression as a function of $p\mathrm{CO}_2$. One can thus determine the existence or not of tipping-point, threshold from which the capacities of acclimatization are no longer sufficient and where a physiological parameter is impacted by the acidification of the oceans. This promising and largely under-used approach has been chosen in our study.

We studied, under controlled conditions, the effect of ocean acidification and the effect of combined acidification and cold stress, on two coral species: the warm temperate encrusting coral *Porites heronensis*, and the poleward shifting Acropora species *Acropora solitaryensis*. We investigated a wide range of $p\mathrm{CO}_2$ (six targeted $p\mathrm{CO}_2$ - covering present conditions, IPCC's projections by the year 2100 and further; regression approach) on a full set of metabolic parameters. This approach was used in order to test for the existence of critical thresholds and to have a better understanding of potential acclimation processes in corals species, under disrupted climate states. The results obtained highlight the need to consider the combination of combined ocean acidification and extreme cold event, in a context of poleward expansion of corals.

Materials and Methods

Coral collection and laboratory conditions. Colonies of *Porites heronensis* (n=3) were collected in Shidagaura, Shimoda city, Shizuoka prefecture, Japan (34°39'58.1"N, 138°56'33,6"E) (ca 3 m depth). Colonies of *Acropora solitaryensis* (n=3) were collected offshore Shikine Island, Tokyo, Japan (34°19′17″ N, 139°12′17″ E) (ca 5 m depth). Coral colonies were then transported to the Shimoda Marine Research Center, University of Tsukuba, Shimoda city, Shizuoka prefecture, Japan (34°40'3,92"N, 138°56'7,704"E). Coral colonies were maintained, for several months, in an outdoor tank (1.8m x 0.5m x 0.9m, volume = 810 L) supplied continuously with seawater (flow rate = 42 $\mathrm{L.min^{-1}}$) pumped from 5 m depth, in front of the research center.

Thereafter, each coral colony was sectioned into 8 comparatively sized nubbins (ca 3 to 4 cm long) and glued with epoxy to cover the exposed skeleton, incorporating a plastic cable tie, so the corals maintained an upright position. Coral specimens (one nubbin per species) were randomly assigned to one of the 24 independent experimental units, and allowed to recover for 30 days before the different treatments were applied. Each experimental unit consisted of one 12L experimental tank, supplied continuously by raw seawater in open flow (flow rate = 0.13

L.min^{-1}; turnover = 64%.h^{-1}), pumped from 5 m depth, in front of the research center. The laboratory conditions were recreating the ambient seawater conditions, with a salinity of 34, temperature of 16.6 ± 0.36 °C , pH$_{NBS}$ 8.22 ± 0.06. LED Aquarium Lights (ZP4000-1200M, Zetlight®) provided an irradiance close to a natural cycle experienced by the corals in field in winter with a peak at ca 100 µmol photons m^{-2}s^{-1} reached at 12:00, over a 14 h period of light. An air bubbler was used to maintain a dissolved oxygen saturation state in excess (7.79 ± 0.3 mg O_2 l^{-1}) in each tank. Food supply was not regulated by external input. Experimental tanks were cleaned once a week to remove the growing algae. Coral nubbins were visually inspected each day of the recovery period and no evidence of bleaching or disease was observed.

Experimental setup and Seawater carbonate chemistry. Once the recovery period was achieved, pH conditions were randomly assigned to the experimental tanks. Six targeted pH conditions were tested, ranging from 8.2 (control condition) to 7.2 pH unit, with an increment of 0.2 pH unit between each condition. Three tanks were assigned to each condition except for 8.2 and 7.8 pH, representing the present-day conditions and the RCP8.5 IPCC projection by the year 2100, for which six tanks were assigned. This configuration allowed an intermediate coral sampling (n=3 per species and per pH condition) before the cold shock, for these two relevant pH conditions.

Coral nubbins were progressively acclimated for 7 days, during which the seawater acidity was gradually decreased in the experimental tanks (0 to - 0.28 pH unit.day^{-1}) to reach their specific targeted pH condition. Once the acclimatation phase completed, coral nubbins were held at their specific pH condition for 28 days (four weeks). After 20 days, a thermal cold shock was applied on the remaining coral nubbins. The cold shock consisted of 8 days of temperature decrease (average of 0.4 °C.day^{-1}), and 12 days under 15°C (Figure 2.2).

The pH conditions were obtained by bubbling pure CO_2 within each tank via an air stone cylinder with the input of CO_2 constrained using a needle valve on each aquarium. In each tank, the bubbling was regulated by a pH-stat system (Apex, Neptune Systems, USA) that controlled a solenoid valve. The pH probes (Neptune Systems, USA), connected to the Apex interface (via a PM1 module and an Apex Base Unit; Neptune Systems, USA) continuously measured the pH in each tank (measure every 10 minutes) (Figure 2.1). To avoid over switching the solenoid valves a range of pH of ± 0.1 unit was allowed, meaning that the opening of the solenoid valve was triggered when the pH was 0.1 higher than the target and the valve was closed when the pH read -0.1 from the target. Minimum variations in pH were obtained by reducing the flow of CO_2 in each aquarium using the needle valve. Each pH probe was calibrated once per week with NBS buffered solutions (National Bureau of Standards) at pH 7. 0 and 10.1 (Thermo Fisher, USA). In addition, to verify the pH measurement of the pH probes within the tanks, pH measurements were done weekly using a ROSS pH electrode (8102BNUWP, Thermo Fisher, USA) calibrated on the pH NBS scale.

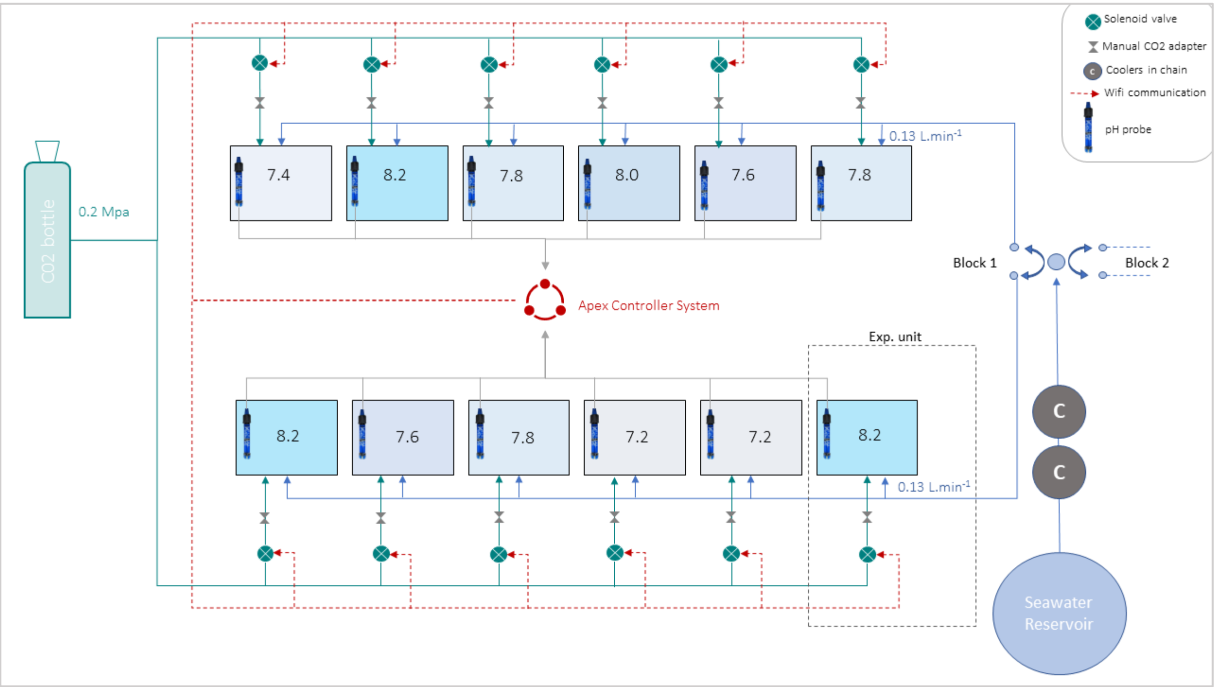

Figure 2.1. Schematic representation of the experimental system for Block 3

For the cold shock, the temperature condition was obtained by diverting the general input seawater into a cooler chain connection (Aquarium cooler ZC-13000α, Zensui, Japan; Figure X). Temperature probes (Neptune Systems®), connected to the Apex interface (Neptune Systems, USA), were placed in each tank, and continuously measured the temperature (measure every 10 minutes) (Figure 1). The temperature setup of the coolers were adjusted to reach the targeted temperature in the aquarium.

Calcification, photosynthetic and respiration rates. Metabolism was measured at 0, 28 (i.e. before the cold shock), and 48 (i.e. after the cold shock) days after the start of experimentation. For metabolic rates measurements, the main water reservoir supply was stopped, and coral specimens were incubated in 300ml and 250ml beaker (container; for *Acropora solitaryensis* and *Porites heronensis* respectively) fully filled with seawater, well mixed with a magnetic stirrer, and sealed with a watch glass, to remove the air-seawater exchange surface. Incubations were conducted for 2h under light condition followed by 3h under dark condition. In between the two incubations, the containers were refilled with continuous supply of seawater for over one hour under dark condition. The pH $_{NBS}$, temperature, dissolved oxygen (DO) and seawater samples for the measurement of total alkalinity (ca. 50ml) were measured and collected from each container, at the beginning and at the end of each incubation. Measurements were done with a multimeter (Orion 4 stars, Thermo Fisher, USA) calibrated on the pH $_{NBS}$ scale and using a RDO® Optical Dissolved Oxygen Sensor (RDO, Thermo Fisher, USA) and a ROSS pH electrode (8102BNUWP, Thermo Fisher, USA). Seawater samples were collected with 50 ml syringes, filtered at 0.45 µm membrane (GF/F Whatman), and stored in plastic reagent bottles.

Gross photosynthesis and respiration rates were calculated from the difference between the final and the initial DO values of the light and the dark incubation, respectively. The light and the dark calcification rates were estimated using the alkalinity anomaly technique (Chisholm and Gattuso, 1991) as the difference between the final and the initial Total alkalinity (TA) values of each incubation. TA (µmol kg^{-1}) was measured by titration of the sampled seawater, with 0.1 M HCl using a Metrohm titration system (916 Ti-Touch, Metrohm®), and calculated using the titration points from pH 3.9 to 2.9 by non linear regression (function “at” of the seacarb R package; function. The measurements were validated by comparison to certified reference materials for TA provided by A.G. Dickson (batch 121).

The pH, temperature, TA and salinity measured during the metabolism experiments were used as the input variables for calculation of the other parameters using CO2SYS (Pierrot, Lewis, and Wallace, 2006) with the constant from Roy et al. (1993), KSO_4 using Dickson (1990), and total borate concentration from Uppstorm (1974).

Photosynthetic efficiency. At 0, 28 and 48 days after the start of experimentation, the photosynthetic efficiency (F_v/F_m) of the Photosystem II (PSII) of zooxanthellae *in hospite* were measured, following the incubation measurements. Measurements (in the dark) were done using a JUNIOR-PAM chlorophyll fluorometer (Walz, Germany) (n = 5 for each species and each tank), following 30 min of dark adaptation.

Skeletal growth rates. Coral specimens were weighted at 0, 28 and 48 days, using the buoyant weight technique (Davies, 1989). Samples were weighed using an electronic balance (1mg precision, *ASONE*® AS PRO ASP123F), in seawater of known density. The net buoyant weight of the corals was converted into dry weight using the density of the pure aragonite

(2.94 g cm^{-3}). The skeletal growth rates were calculated as the daily change in dry weight between the initial and the final weight and expressed in $mg.d^{-1}$.

Zooxanthellae, Chlorophyll *a* and Protein content. At 28 and 48 days, coral nubbins were collected and frozen (-80°C) for measurements of zooxanthellae density, chlorophyll a, protein and surface areas. Tissues were removed from coral skeletons, using an airbrush compressor filled with 34 g l^{-1} solution of NaCl. The slurry was homogenized with a Glass Teflon Potter Homogenizer, and the total obtained volume was measured. About 1ml of homogenized tissues samples were centrifuged (at 1000 g for 10 min at 5 °C), and the zooxanthellae fraction was fixed with 5% formalin and used to count zooxanthellae cells on a Neubauer hemocytometer. To extract the chlorophyll a, 1ml of the homogenized slurry was filtered on a 25 mm GF/F filter, which was then extracted in 90% acetone overnight. Absorbances were read at 664 and 647 nm, using a spectrophotometer (UV-1280, Shimadzu, Japan). Chlorophyll *a* concentrations were computed according to the spectrometric equations from Ritchie (2006). Total soluble protein concentration of the host fraction was then determined using the Bradford protein assay (Bradford, 1976). Surface area of each coral was determined using the Wax Coating technique which consists in the dipping of corals in paraffin wax and measuring the increase in weight (Stimson and Kinzie, 1991).

Statistical analysis . For statistical analysis, all parameters were normalized by the surface area of the coral skeleton, except for Fv/Fm ratio. All data were tested for the assumptions of normality and homoscedasticity using Shapiro Wilk's test and Bartlett's test respectively. After verification, two-way ANOVAs were used to test the effects of pCO_2 and temperature on the zooxanthellae density, chlorophyll-a concentration, total soluble protein concentration, within and between the two targeted pH conditions 8.2 and 7.8. When the ANOVA determined a significant difference, a Tukey's honest significant difference test (HSD) was used to attribute differences between specific factors.

An Akaike Information Criterion (AIC) approach was used to determine which model best described the relationship between metabolic parameters and pCO_2, before and after the cold shock, for each species. Two different models were tested: linear and polynomial. This statistical analysis, following the "regression approach", used the mean over the whole experiment duration of the measured ppm values of each independent tank (Table 2.6, i.e. not the mean response of each targeted pCO_2 condition), to obtain a high resolution regressions. To compare the response to pCO_2 prior and after the cold shock, analyses of covariance

(ANCOVA) were performed on the regression models within each species and each metabolic response. All data are expressed as the mean ± SE. The R statistical environment was used for these analyses (Team, 2011).

Results

Coral fragments recovered and grew during the acclimation period in the experimental tanks, covering the exposed skeletons with new tissue. No mortality events were observed throughout the experiment. The corals did not show any signs of bleaching prior to the cold and only minor paling after the cold shock. pH_{NBS} conditions within the tanks were precisely regulated across time (standard errors ranging from 0.04 to 0.09 pH unit; Figure 2.2). The relation between the measured physiological parameters and levels of CO_2 were best explained using a linear regression than a polynomial regression as shown by the lower AIC values obtained for linear regressions compared to polynomial regression (Table 2.1).

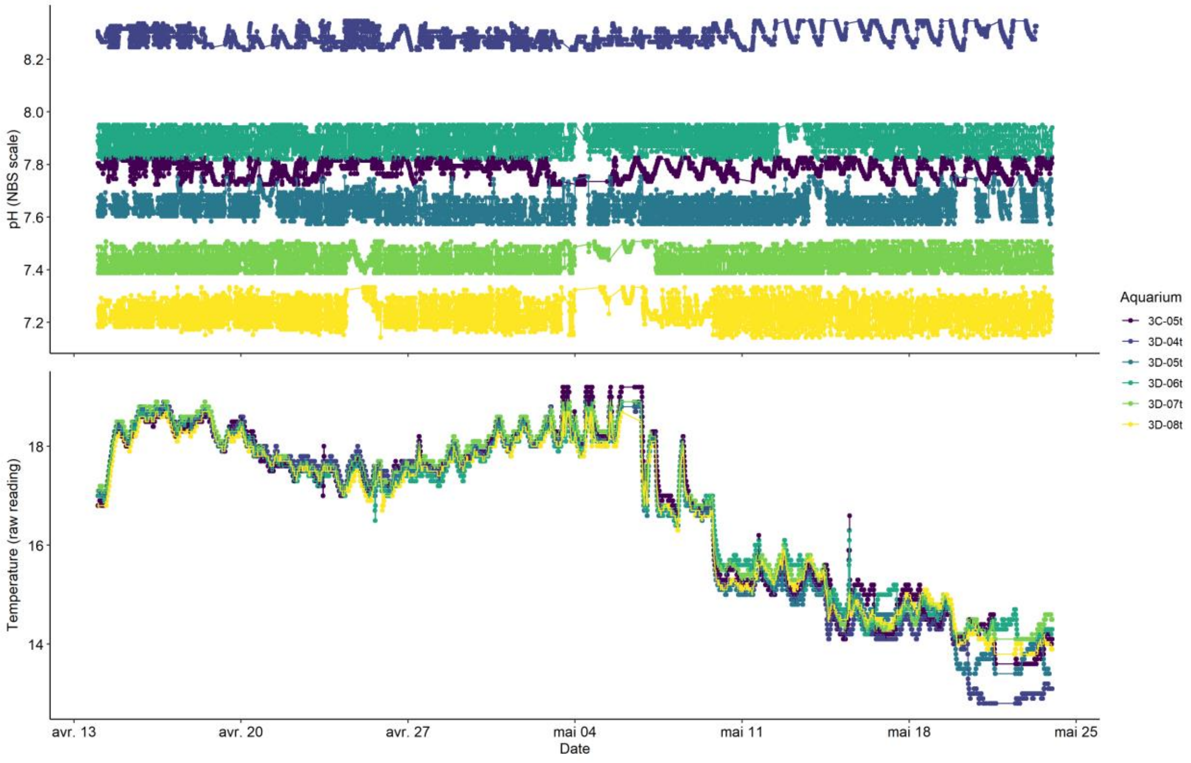

Figure 2.2. Continuous data of pH_{NBS} and temperature within 7 weeks of the experiment, for 6 aquaria from the Block 3

Table 2.1. Summary of the Akaike Information Criterion (AIC) used to determine the best model type (linear vs. polynomial) describing the relationship between measured metabolic parameters and pCO_2. The regression model revealing the minimum AIC value was considered the best fitting.

Metabolic responses	Regression model	*Acropora solitaryensis*		*Porites heronensis*	
	(Cold shock)	Linear	Polynomial	Linear	Polynomial
Photosynthesis efficiency	Prior	- 95	- 92.8	- 110	- 102.8
	After	- 56	- 55.2	- 76	- 73.9
Gross Photosynthesis	Prior	- 35	- 32.2	- 21	-17.6
	After	- 3	- 1.12	30	32.7
Respiration	Prior	- 98	- 95.1	- 59	134.7
	After	- 43	- 39.9	- 33	83.6
Light Calcification	Prior	- 43	132.1	- 8.1	- 5.4
	After	- 67	67.3	- 25	- 24
Dark Calcification	Prior	- 65	- 63	- 15	- 13.9
	After	- 42	- 41.8	3.6	-33.5
Skeletal growth rate	Prior	- 33	- 32.8	3.6	4.1
	After	- 40	- 39.4	8.4	9.6
Zooxanthellae density	After	16	17	46	42.3
Clorophyll	After	81	81.8	120	122.8
Protein	After	200	202.7	270	271.6

Zooxanthellae metabolism. For *A. solitaryensis* and *P. heronensis* there were no significant linear declines in photosynthetic efficiency as a function of increasing pCO_2 (Figure 2.3 (a), Table 2.2). The ANCOVA revealed significant different intercepts between the two regression models (i.e. before and after the cold shock; Table 2.3, $p < 0.001$) in the two corals species, which indicate a significant negative effect of the cold temperature stress on the photosynthetic efficiency. The ANCOVA did not reveal differences in the slopes (Table 2.3).

For both species, no significant effect of the increasing pCO_2 on the gross photosynthesis has been identified (Figure 2.3 (b), Table 2.2). The outcomes of the ANCOVA showed significant higher intercepts of the gross photosynthesis -pCO_2 regression models after the cold shock than before cold shock (Table 2.3, $p < 0.001$). Before the cold shock, the highest gross photosynthesis, for *A. solitaryensis* and *P. heronensis*, were recorded at 0.60 and 0.59 µmol O_2 h^{-1} cm^{-2} (at 744 ppm and 437 ppm respectively). Whereas, after the cold shock, the highest gross photosynthesis was recorded at 0.90 and 1.72 µmol O_2 h^{-1} cm^{-2} (at 737 ppm and 388 ppm respectively). No significant different slopes were identified between the response to pCO_2 prior and after cold shock (Table 2.3).

After the cold thermal treatment, *A. solitaryensis* exhibited no significant response of zooxanthellae density, neither as a linear decrease as a function of increasing $p\mathrm{CO_2}$ (Figure 2.6 (a1), Table 2.2), nor between and within the mean zooxanthellae densities of the targeted pH condition 7.8 and 8.2 (Figure 2.5 (a1), Table 2.5). *P. heronensis* showed no significant linear model as a function of $p\mathrm{CO_2}$ (Figure 2.6 (a2), Table 2.2), but a significant difference of zooxanthellae density between the two temperature treatments, for the targeted pH condition 7.8 and 8.2 (Figure 2.5 (a2), Table 2.5, $p < 0.01$).

For the chlorophyll *a* concentration measured after the cold shock, the two species displayed contrasting responses to $p\mathrm{CO_2}$. *A. solitaryensis* exhibited a linear decrease of chlorophyll *a* concentration as a function of increasing $p\mathrm{CO_2}$ (slope = -5.3 µg chlorophyll *a* $\mathrm{cm^{-2}}$ per 10^5 ppm of $p\mathrm{CO_2}$; Figure 2.6 (b1), Table 2.2, $p = 0.014$) with a minimum concentration of 4,27 µg chlorophyll *a* $\mathrm{cm^{-2}}$ recorded at 3532 ppm. *P. heronensis* showed unaffected chlorophyll *a* concentration as a function of increasing $p\mathrm{CO_2}$ (Figure 2.6 (b2), Table 2.2). According to the scenario results comparing the targeted pH condition 7.8 and 8.2 (i.e. control condition) before and after the cold shock; no significant differences of chlorophyll *a* concentration have been identified for *A. solitaryensis* (Figure 2.5 (b1), Table 2.5). *P. heronensis* showed a significant difference in chlorophyll *a* concentration before and after the cold shock; for the targeted condition 7.8 and 8.2 (Figure 2.5 (b2), Table 2.5, $p < 0.01$), although no significant linear regression model was identified.

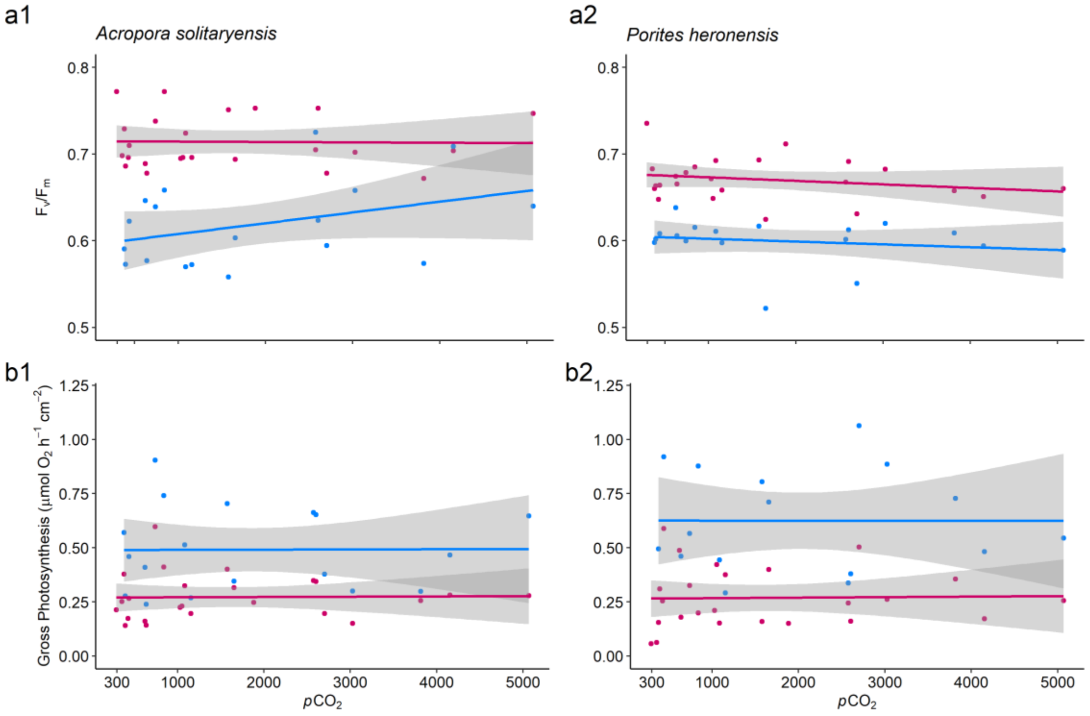

Figure 2.3. Zooxanthellae metabolic responses as a function of $p\mathrm{CO_2}$ (ppm) in the two studied species (1: *A. solitaryensis*; 2: *P. heronensis*): (a) Photosynthetic efficiency (Fv/Fm) ; (b) Gross photosynthesis (µmol 0_2 $\mathrm{h^{-1}}$ $\mathrm{cm^{-2}}$). Purple straight lines: coral response prior to the cold shock; Blue straight lines: coral response after the cold shock. Grey bands represent the 95% CIs of the linear regressions.

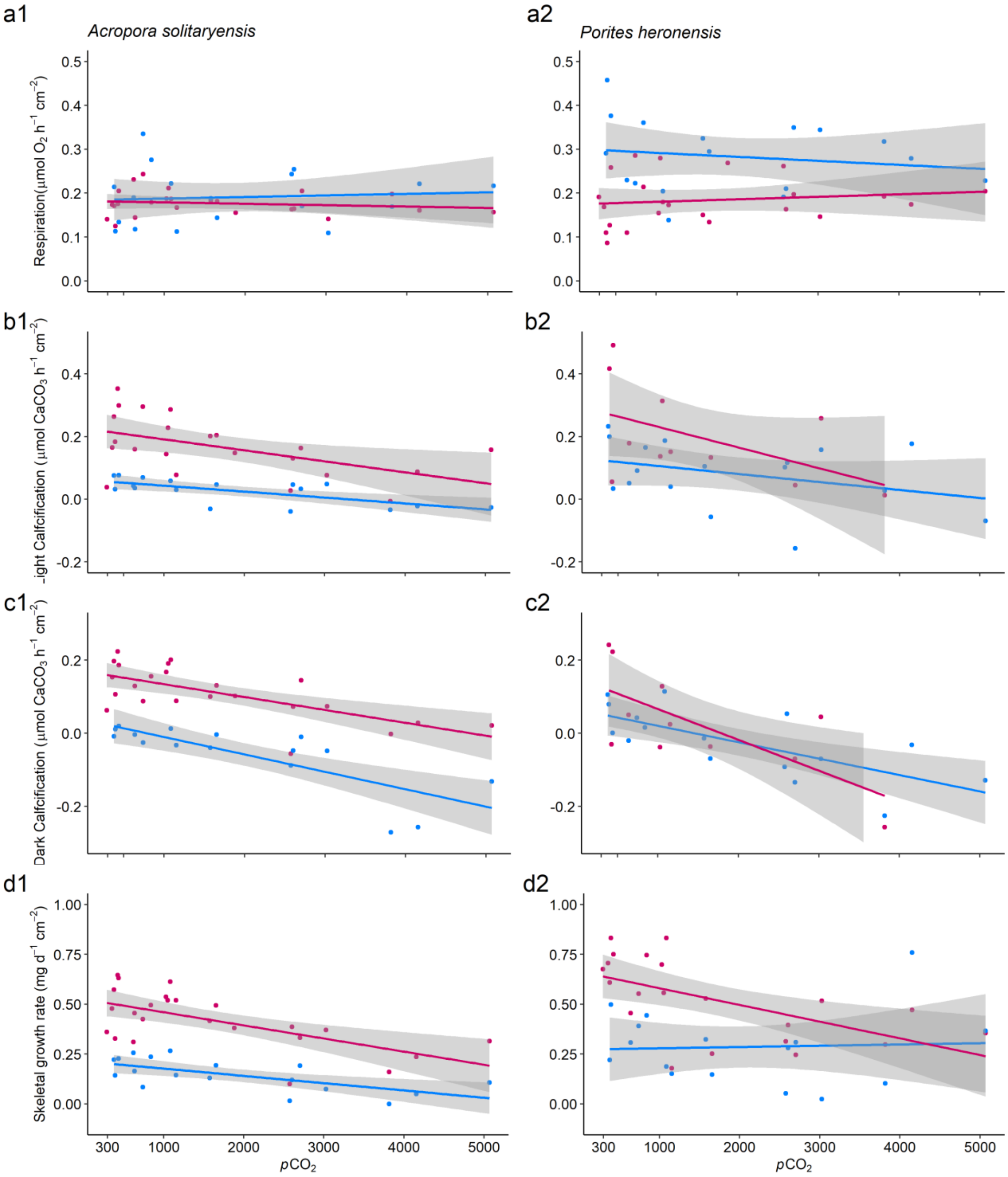

Figure 2.4. Host metabolic responses as a function of $p\mathrm{CO}_2$ (ppm) in the two studied species (1: *A. solitaryensis*; 2*: P. heronensis*): (a) Respiration (µmol 0_2 $\mathrm{h}^{-1}\mathrm{cm}^{-2}$); (b) Light calcification (µmol CaCO_3 h^{-1} cm^{-2}); (c) Dark calcification (µmol CaCO_3 h^{-1} cm^{-2}); (d) Skeletal growth rate (mg d^{-1} cm^{-2}). Purple straight lines: coral responses prior to the cold shock; Blue straight lines: coral response after the cold shock. Grey bands represent the 95% CIs of the linear regressions.

Table 2.2. Parameters of the linear regressions used to estimate the relationship between metabolic responses and $p\mathrm{CO_2}$. *P*-values of the slopes and intercepts, as well as R^2, for the linear regressions are given; intercept *p*-values were considered significant when $p < 0.05$. The stars represent significant differences at $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***).

Metabolic responses	Regression model	*Acropora solitaryensis*			*Porites heronensis*		
	(Cold shock)	Values	*p*	Adj-*R*²	Values	*p*	Adj-*R*²
Photosynthesis efficienc	Prior	$- 4.3\ 10^{-7} x + 0.71$	0.930	-0.04	$- 8.2\ 10^{-6} x + 0.68$	0.240	0.02
	After	$1.2\ 10^{-5} x + 0.60$	0.130	0.08	$- 4.8\ 10^{-6} x + 0.60$	0.550	-0.04
Gross Photosynthesis	Prior	$- 1.5\ 10^{-6} x + 0.27$	0.940	-0.05	$8.4\ 10^{-6} x + 0.26$	0.830	-0.04
	After	$9.5\ 10^{-7} x + 0.49$	0.980	-0.06	$- 1.9\ 10^{-4} x + 0.96$	0.230	0.03
Respiration	Prior	$- 3.2\ 10^{-6} x + 0.18$	0.480	-0.02	$2.3\ 10^{-6} x + 0.19$	0.900	-0.04
	After	$3.6\ 10^{-6} x + 0.18$	0.750	-0.05	$- 1.8\ 10^{-5} x + 0.30$	0.490	0.03
Light Calcification	Prior	$- 3.5\ 10^{-5} x + 0.23$	0.015 *	0.23	$- 6.6\ 10^{-5} x + 0.30$	0.110	0.17
	After	$- 1.9\ 10^{-5} x + 0.06$	<0.001 ***	0.44	$- 2.6\ 10^{-5} x + 0.13$	0.170	0.06
Dark Calcification	Prior	$- 3.5\ 10^{-5} x + 0.17$	<0.001 ***	0.43	$- 8.4\ 10^{-5} x + 0.15$	0.015 *	0.45
	After	$- 4.8\ 10^{-5} x + 0.04$	<0.001 ***	0.59	$- 4.5\ 10^{-5} x + 0.06$	<0.001 ***	0.47
Skeletal growth rate	Prior	$- 0.003 x + 11.0$	0.031 *	0.37	$- 1.0\ 10^{-4} x + 0.68$	0.015 *	0.22
	After	$- 1.2\ 10^{-4} x + 0.52$	<0.001 ***	0.20	$- 3.1\ 10^{-5} x + 2.30$	0.520	-0.05
Zooxanthellae density	After	$- 2.0\ 10^{-4} x + 2.10$	0.068	0.14	$2.4\ 10^{-4} x + 2.10$	0.310	-0.01
Clorophyll	After	$- 5.3\ 10^{-5} x + 0.19$	0.014 *	0.31	$- 8.5\ 10^{-3} x + 2.30$	0.610	-0.04
Protein	After	$- 0.032 x + 500$	0.034 *	0.22	$0.025 x + 1200$	0.730	-0.05

Table 2.3. Results of the ANCOVA used to test for differences in slopes and intercepts in the relationships between metabolic responses and $p\mathrm{CO_2}$ in the two coral species, tested by thermal treatment pairs (i.e. prior - after cold shock). The stars represent significant differences at $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***).

Species	Metabolic responses	Residuals df	Slope		Intercept	
			F	*p*	*F*	*p*
Acropora solitaryensis	Photosynthesis efficiency	38	2.741	0.106	65.594	<0.001 ***
	Gross Photosynthesis	38	0.018	0.893	20.801	<0.001 ***
	Respiration	38	0.454	0.504	0.834	0.367
	Light Calcification	35	0.857	0.361	42.920	<0.001 ***
	Dark Calcification	35	1.613	0.212	79.386	<0.001 ***
	Skeletal growth rate	39	2.192	0.147	87.105	<0.001 ***
Porites heronensis	Photosynthesis efficiency	38	0.026	0.872	81.285	<0.001 ***
	Gross Photosynthesis	37	1.156	0.289	28.800	<0.001 ***
	Respiration	37	1.132	0.294	20.348	<0.001 ***
	Light Calcification	26	1.158	0.293	5.463	0.210
	Dark Calcification	23	1.690	0.207	0.927	0.346
	Skeletal growth rate	36	1.523	0.225	4.308	0.067

Host metabolism. In the two symbiotic corals, no significant effect of the increasing *p*CO_2 on the respiration has been identified for the two temperature treatments (Table 2.2). The ANCOVA revealed a significantly higher intercept for the linear regression after the cold shock (Figure 2.4 (a2), Table 2.3, $p < 0.001$), for *P. heronensis*. Before the cold shock, the highest respiration rate, for *P. heronensis*, was recorded at 0.22 µmol O_2 h^{-1} cm^{-2} at 748 ppm. Whereas, after the cold shock, the highest respiration rate was recorded at 0.46 µmol O_2 h^{-1} cm^{-2} at 388 ppm. No significant different slopes were identified (Table 2.3).

The light calcification declined significantly and linearly as a function of increasing *p*CO_2 for *A. solitaryensis* , with the strongest *p*CO_2 dependent decline measured after the cold shock (with respective slopes of - 3.5 and - 1.9 µmol $CaCO_3$ h^{-1} cm^{-2} per 10^5 ppm of *p*CO_2 , pre and post -cold shock) (Figure 2.4 (b1), Table 2.2). In addition, the ANCOVA results showed a significant higher intercept of the pre-cold shock regression model, but no differences in slopes (Table 2.3, $p < 0.001$ and $p = 0.361$ respectively). Before the cold shock, the highest light calcification rate was recorded at 0.35 µmol $CaCO_3$ h^{-1} cm^{-2} at 463 ppm. After the cold shock the highest light calcification rate was recorded at 0.07 µmol $CaCO_3$ h^{-1} cm^{-2} at 441 ppm. No significant decline in light calcification rate as a function of *p*CO_2 has been identified for *P. heronensis* (Figure 2.4 (b2), Table 2.2), and no differences of intercepts and slopes between the regression models (Table 2.3).

The response of dark calcification as a function of increasing *p*CO_2 was significantly negated in *A. solitaryensis* and *P. heronensis*. The strongest *p*CO_2 dependent decline was measured for *P.heronensis* before the cold shock (respective slopes of - 3.5 and -8.4 µmol $CaCO_3$ $h^{-1}cm^{-2}$ per 10^5 ppm of *p*CO_2; Table 2.2), and *A. solitaryensis* after the cold shock (respective slopes of - 4.8 and - 4.5 µmol $CaCO_3$ $h^{-1}cm^{-2}$ per 10^5 ppm of *p*CO_2 ; Figure 2.4 (c), Table 2.2). The ANCOVA confirmed the significantly higher intercept of the relationship dark calcification - *p*CO_2 before the cold shock, for *A. solitaryensis* and no significant differences in slopes (Table 2.3).

Before the cold shock, the skeletal growth rate decreased significantly as a function of increasing *p*CO_2 , in *A. solitaryensis* and *P. heronensis*, with the strongest *p*CO_2 dependent decline measured for *A. solitaryensis* (respective slopes of - 30 and - 1.7 µmol h^{-1} cm^{-2} per 10^4 ppm of *p*CO_2; Figure 2.4 (d), Table 2.2). After the cold shock, the skeletal growth rate was negated by *p*CO_2 only in *A. solitaryensis* (slope = -1.2 µmol h^{-1} cm^{-2} per 10^4 ppm of *p*CO_2; Table 2.3). Significant different intercepts, between the two temperature treatment regressions, have been revealed for *A. solitaryensis*, but not for slopes (Table 2.3). When submitted to the cold shock, the skeletal growth rates decreased by an average of 65% in *A. solitaryensis*, relative to the rates prior to the cold shock.

Regarding the total soluble protein concentrations after the cold shock, the two studied species demonstrated dissimilar responses to $p\mathrm{CO}_2$. *A. solitaryensis* exhibited a significant decrease as a function of increasing $p\mathrm{CO}_2$ (slope = - 3.2 µg cm^{-2} per 10^2 ppm; Figure 2.6 (c1), Table 2.2, $p = 0.034$), and a significant difference of concentration between the two temperature treatments, for the targeted pH condition 7.8 and 8.2 (Figure 2.5 (c1), Table 2.5, p = 0.045). *P. heronensis* ,demonstrated no significant response of protein concentration, neither as a linear decrease as a function of increasing $p\mathrm{CO}_2$ (Figure 2.6 (c2), Table 2.5), nor between and within the targeted pH condition 7.8 and 8.2 (Figure 2.5 (c2), Table 2.5).

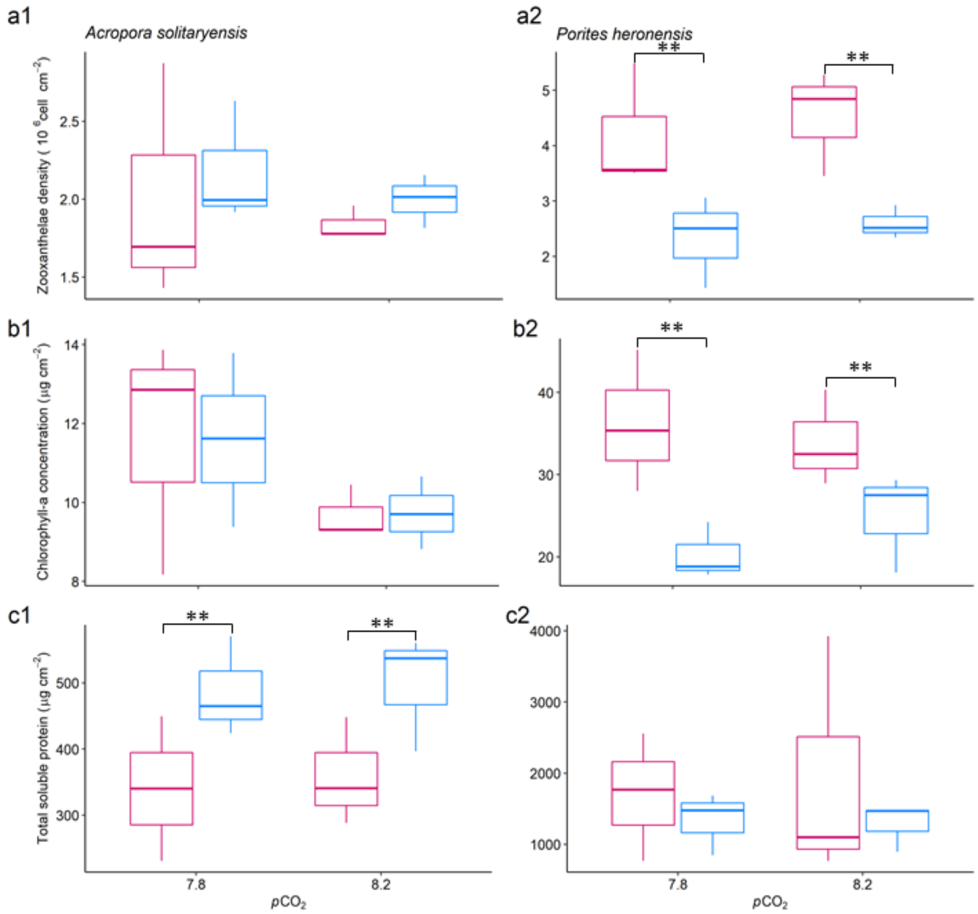

Figure 2.5. Scenario approach. Mean metabolic responses as a function of targeted pH conditions ($\mathrm{pH}_{\mathrm{NBS}}$) in the two studied species (1: *A. solitaryensis*; 2: *P. heronensis*): (a) Zooxanthellae density (10^6 cells cm^{-2}); (b) Chlorophyll *a* concentration (µg cm^{-2}) ; (c) Total soluble protein concentration (µg cm^{-2}). Purple boxplots: coral response prior to the cold shock; blue boxplots: coral response after the cold shock. The stars represent significant differences at $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***), computed by Tukey HSD test at 95% interval of confidence.

Table 2.4. Means of parameters of the carbonate chemistry, for the targeted pH_{NBS} condition 8.2 and 7.8, and for each temperature treatment (i.e. before and after cold shock). pH_{NBS} (continuous measurement), temperature (continuous measurement) and total alkalinity (n = 13) are measured values; and pCO_2, HCO_3, CO_3^{2-}, and $\Omega_{Aragonite}$ are calculated values using CO_2sys. Values are presented as mean ± S.E..

Temperature treatment	Targeted pH NBS condition	Measured pH_{NBS}	Temperature (°C)	Total Alkalinity (µmol kg^{-1})	pCO_2 (µatm)	HCO_3 (µmol kg^{-1})	CO_3^{2-} (µmol kg^{-1})	Ω_{Ar} in
Prior to cold shock	8.2	8.12 ± 0.07	17.9 ± 0.02	2269.8 ± 1.2	507 ± 119	1859.4 ± 45.8	168.4 ± 18.7	2.61 ± 0.29
	7.8	7.76 ± 0.06	17.9 ± 0.03	2268.9 ± 3.1	1247 ± 232	2068.3 ± 24.7	82.9 ± 10.1	1.29 ± 0.16
After cold shock	8.2	8.09 ± 0.07	15.13 ± 0.05	2261.4 ± 1.1	574 ± 119	1890.7 ± 42.9	151.5 ± 17.5	2.33 ± 0.27
	7.8	7.83 ± 0.04	15.34 ± 0.02	2259.9 ± 1.2	997 ± 106	2051.0 ± 16.7	86.2 ± 6.8	1.33 ± 0.10

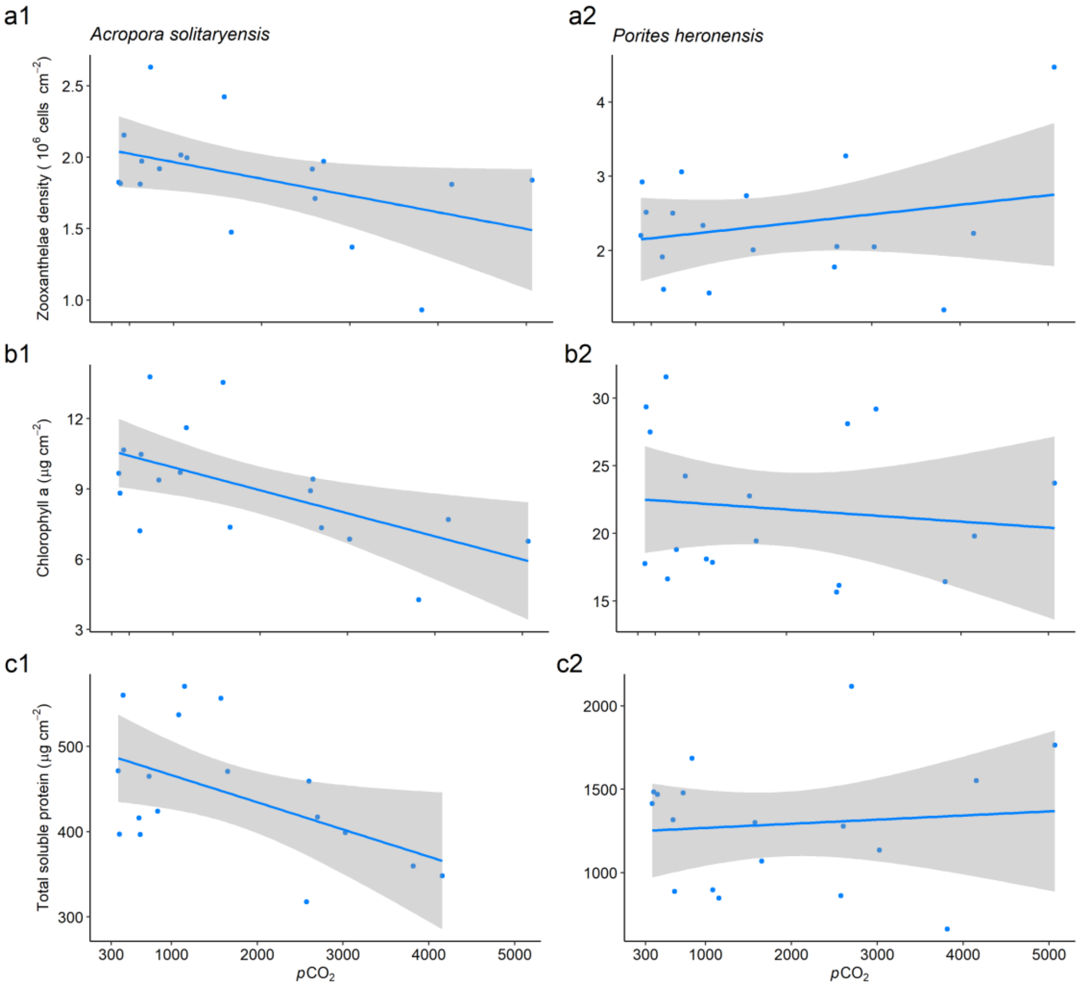

Figure 2.6. Coral metabolic responses as a function of pCO_2 (ppm), after the cold shock, in the two studied species (1: *A. solitaryensis*; 2: *P. heronensis*): (a) Zooxanthellae density (10^6 cells cm^{-2}); (b) Chlorophyll *a* concentration (µg cm^{-2}) ; (c) Total soluble protein concentration (µg cm^{-2}). Grey bands represent the 95% CIs of the linear regressions.

Table 2.5. Results of the ANOVA for the effects of temperature and pCO_2 on the two coral metabolic responses. *P*-values were considered significant when $p < 0.05$. The stars represent significant differences at $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***).

Metabolic responses		df	*Acropora solitaryensis*		*Porites heronensis*	
			F	*p*	*F*	*p*
Zooxanthellae density	temperature	1	0.443	0.524	14.59	0.005 **
	pH	1	0.462	0.516	0.363	0.563
	temp* pH	1	0.002	0.963	0.005	0.944
Chlorophyll	temperature	1	3.481	0.095	11.84	0.008 **
	pH	1	2.839	0.130	0.112	0.746
	temp* pH	1	0.001	0.973	0.928	0.363
Protein	temperature	1	5.900	0.045 *	0.049	0.829
	pH	1	0.065	0.806	0.023	0.882
	temp* pH	1	0.004	0.950	0.059	0.813

Discussion

Effects of OA. In the present study, based on a 7-week experiment, only light paling was observed. In addition to cold stress, significant linear effects of increasing $pCO_2$was observed on some of the physiological and metabolic parameters investigated , but the response differed among the species.

The strongest effect of increasing pCO_2 was measured for *A. solitaryensis*, on the total soluble protein concentration (-32 µg cm^{-2} per 10^3 ppm of pCO_2), whereas no significant effect of the increasing pCO_2 was identified for *P. heronensis*. Impairment of protein metabolism was shown for several coral species (Edmunds and Wall., 2014). In addition protein reserves are important indicators of the energy balance and the overall health of corals (Anthony et al., 2008). A significant decrease in protein contents suggests that the coral host is compensating a loss of energetic supply (i.e. photosynthates translocation from the photosynthesis) by using its own reserve (Rodrigues & Grottoli, 2007). OA was also shown to affect protein metabolism in some corals.

Increasing pCO_2 led to significant reduction in dark calcification and skeletal growth rates for the coral species *P. heronensis*, but not for the light calcification. Further reductions in skeletal growth, light and dark calcification rates were revealed for the coral *A. solitaryensis*. Calcification rates, in both species, reached from near zero to negative rates under high pCO_2 conditions (ΩAragonite < 1, circa $pCO2$ of 1500 ppm), indicating severe decreases in calcification and even the net dissolution of the skeleton. These results, in respect to calcification, suggest that the two corals are sensitive to pCO_2 under average winter temperature

(~18°C), with a slightly higher resistance for *P. heronensis*. The effect of elevated *p*CO_2 on calcification in corals have been well documented (Gattuso et al., 1999; Langdon et al., 2003; Marubini et al., 2003). Some studies highlighted the ability of the massive *Porites spp*. to resist the effects of high *p*CO_2 exposure under warm temperatures (> 22°C) (Edmunds et al., 2011; Fabricius et al., 2011). Moderately elevated temperatures can stimulate enzyme activity (Marshall and Clode 2004) and metabolic rate (Edmunds 2005), including calcification in corals (Marshall and Clode 2004). The reduction of calcification under elevated *p*CO_2 in *P. heronensis*, is likely to have been due to the combination of the low winter temperature and the effect of elevated *p*CO_2 on calcification. Similar strongly reduced calcification rates or net dissolution are commonly observed during the lowest temperature period (i.e. winter) in coral species found at high latitudes (Howe and Marshall, 2001; Higuchi et al., 2015; Higuchi et al., 2020).

Significant loss pigmentation (i.e. chlorophyll *a* contents) was observed in *A. Solitaryensis* under elevated *p*CO_2, and neither gross photosynthesis nor photosynthetic efficiency (Fv/Fm) significantly increased for both species. These results suggest that zooxanthellae are resistant to short-term high *p*CO_2 exposure. This hypothesis is consistent with the study by Langdon *et al.* (2003) which did not find change in productivity response to elevated *p*CO_2, suggesting that symbiotic algae are able to rely on bicarbonate for photosynthesis (Burris et al., 1983; Goiran et al., 1996), and the additional inorganic carbon provided by the increased *p*CO_2 is likely too small to lead in a significant increase of photosynthesis.

Overall, our results are consistent with previous studies, identifying *P. heronensis* as part of the "low-sensitivity" group to *p*CO_2, showing a high resistance to high CO_2 levels (Edmunds, 2011; Fabricius et al., 2011); and *A. solitaryensis* as part of "high-sensitivity" group to *p*CO_2 , predicted to be severely limited geographically by ocean acidification, in a context of poleward shift (Agostini et al. 2018; Agostini et al., in preparation). Such resistance to increasing *p*CO_2 potentially may be due to heterotrophy and changes in biomass (Edmunds, 2011), and/or to the coral ability to up-regulate pH at the site of calcification (Herfort et al., 2008; McCulloch et al. 2012), which might be a critical factor in determining the dominant species in the predicted high *p*CO_2 oceans.

Our study highlights the different possible interpretations of the effect of *p*CO_2 on coral metabolisms, according to the used approach : "regression" versus "scenario". The "scenario" method; consisting of measuring the physiological response of organisms under a small number of pH conditions (generally 2-3) in replicates; has been pointed out recently for its limitations (Vargas et al., 2017; Gazeau et al., 2013). To face these limitations, the "regression" method; consisting of investigate responses under a vast gradient of different conditions, without any replication; was proposed as an alternative (Dorey et al., 2013; Comeau et al., 2013; Ventura et al., 2016). In the present study, the regression approach revealed significant linear models demonstrating the effect of $p\mathrm{CO_2}$ on the chlorophyll-a and protein contents, for *A.solitaryensis,* after the cold shock (p = 0.014 and 0.034 respectively); whereas the scenario approach did not identity significant differences of chlorophyll-a and proteins between the two targeted pH conditions 8.2 and 7.8 (covering the present condition and IPCC's projections by the year 2100, respectively) (Figure 2.5). It is likely that the regression approach is an insightful method to better predict the impacts of OA in comparison with the "scenario" approach, by resolving the general trend and the shape of the relationship between metabolism and $p\mathrm{CO_2}$. The description of these relationships could greatly expand our understanding of potential acclimation mechanisms in corals (i.e. by comparing the regression models within the metabolic parameters; e.g. thermal compensation, energy allocation shift), and greatly improve the accuracy of models describing the effects of the predicted OA on calcifying organisms and ecosystems.

No physiological tipping point were identified, beyond which these coral species were no longer capable of carrying out the functions necessary to their survival. These observations, regarding the calcification, do not match with previous studies suggesting a $p\mathrm{CO_2}$ threshold at 500 ppm (Hoegh-Guldberg et al. 2007), above which calcification of coral reefs is projected to be catastrophically reduced. However our results are consistent with the previous study of Comeau et al. (2013), who identified no tipping-points within the responses of eight coral reef calcifiers to increasing $p\mathrm{CO_2}$, and stressed the necessity to consider a multidimensional environment (multiple drivers) to conclude about experimental studies. Indeed, it is likely that both biological and methodological effects can explain the reason why the effects of seawater acidification on the metabolism of *A. solitaryensis* and *P. heronensis*, has been limited in the present study, regarding bleaching, productivity and calcification responses, compared to previous studies (Anthony et al., 2008). One explanation is that in this study, low-light intensities have been used (maximum of 100 µmol photons $m^{-2}s^{-1}$), which did not trigger the synergic exacerbation of photoinhibition (Foyer et al. 1994, Long et al.1994). These light levels are lowered than those use in most coral studies but here temperate coral species were considered, compared to tropical species, and light levels of 100 µmol photons $m^{-2}s^{-1}$ are frequent in winter at the depth where coral are found in high latitudes. Indeed, $p\mathrm{CO_2}$ concentration and high light irradiance (irradiance of >1000µmol photons $m^{-2}s^{-1}$) interaction have been demonstrated to trigger bleaching (Dunne and Brown, 2001; Jones & Hoegh-Guldberg 2001) and different light regime have been shown to change the response of corals to elevated $p\mathrm{CO_2}$ (Comeau et al., 2016, 2013; Dufault et al., 2013; Suggett et al., 2013; Wall et al., 2017). Moreover, our experiment was carried out by supplying non filtered seawater to the experimental tanks. This could have resulted in the supply of energy-rich compounds (POC/nutrients) to corals that would have contributed to a mitigation of the deleterious effects of low pH (Edmunds, 2011). Therefore, if food supply is abundant and light irradiance moderated, which are typical conditions in temperate latitudes, corals may still be capable of calcifying at sufficient rates to maintain growth. This also suggests that coral calcification, at least to a certain seawater acidity and duration of exposure thresholds, is mainly an energy-limited process and is still possible even at considerably low pH.

Effects of temperature. Coral bleaching events (i.e. loss of pigmentation), correlated with decreases in sea surface temperatures, have been widely reported in coral reefs (Coles and Jokiel 1977, Gates et al. 1992; Higuchi et al.,2015; Higuchi et al., 2020). In marginal high latitude coral communities, cold bleaching events have been recorded in winter after unusually cold weather, leading to high mortality rates of corals (Yamano and Namizaki, 2009; Leriorato and Nakamura, 2019). In the present study no bleaching and mortality events have been identified for the two coral species as the result of cold shock. These results highlight a resistance of these two hermatypic corals to a short-term cold stress. However, the survival strategies to the cold temperature seem to differ among the species.

In *P.heronensis,* decreased *Fv/Fm* (i.e. indicating increased photoprotection; Jones and Hoegh-Guldberg 2001), zooxanthellae density and chlorophyll content; along with a significant increase in photosynthesis wa observed. Calcification rates of *P.heronensis* under low seawater temperature did not decrease significantly; even under ΩAragonite < 1 conditions (i.e. enhancing the aragonitic skeleton dissolution); and a significant increased of respiration rates was observed. These results are consistent with the wide temperature tolerance range of the warm temperate coral *P.heronensis* (Higuchi et al., 2020), and suggest a faster acclimation to cold temperatures, compared to its symbionts. In addition, the significant increase of respiration rates after the cold stress suggests that a thermal compensation in metabolism rates may have occurred. Increased respiration rates and production of enzymes to defend against stress, have been observed in corals (Jacques et al., 1983; Howe and Marshall, 2001), and in other organisms

(Sassaman and Mangum, 1970; Mangum et al., 1972). However, our results do not match with previous studies on *P.heronensis,* showing great resistance to cold stress by means of a reduction in basal metabolism (decrease of photosynthesis, calcification, respiration, ETSA; Higuchi et al., 2020). Nevertheless, such resistance to cold stress potentially may be due to endemicity and potential adaptations, which might be a critical factor in determining the dominant species in the high latitude marginal coral communities of the future. Regarding *A.solitaryensis,* decreases of photosynthetic efficiency and calcification (i.e. skeletal growth rate, light and dark calcification rates) were observed, although a significant increase of photosynthesis was observed. However, these results do not accord with previous studies, which showed a higher sensitivity to cold stress in *A.solitaryensis* (severe bleaching and mortality when exposed at 13 °C; Higuchi et al., 2015).

The limited paling and absence of bleaching in our study may be linked to the low-light intensities that have been used (maximum of 100 µmol photons $m^{-2}s^{-1}$).These could have contributed to the limited effect of the cold shock treatment. Also, the experimental period of cold stress (below 15°C) lasted 12 days, which was shorter than the one recorded by Higuchi *et al.* (2020) (i.e. 110 days), or by Leriorato and Nakamura (2019) (i.e. 58 days); thereby playing a major role in the ultimate survival of corals. Nevertheless, our results suggest that *P.heronensis* has a higher tolerance to cold temperatures than *A.solitaryensis,* in line with previous studies identifying the fast growing *A.solitaryensis* as low resilient coral to cold stress, and *P.heronensis* highly resilient to cold stress and already established at high latitudes (Higuchi et al., 2015; Higuchi et al., 2020). This study highlights the diversities of response among corals to cold temperature events; and the need to better understand the response of these high latitude marginal coral communities, for better conservation of this future reservoir of biodiversity, in a context of global change.

Combined effects of OA and temperature. Even if no interaction between $p\text{CO}_2$ levels and temperature treatments has been revealed for these two coral species (i.e. no different slopes between regression models), this study highlights the additional effect of cold stress, on the weakened metabolism of coral exposed to acidified seawater. Skeletal growth rates in *A. solitaryensis,* decreased by an average of 65% when exposed to the cold shock (23 days). It is likely that coral colonies exposed to acidified seawater will be even more weakened if they are subjected to additional threats (i.e. water quality, anthropogenic pressures). When corals are exposed to a poor seawater quality, it has been widely demonstrated that they stop their growth and expel their zooxanthellae (e.g. Hoegh-Guldberg, 1999; Ferrier-Pages et al., 2000), so a cold thermal stress would exacerbate these phenomena (Saxby et al., 2003).

Conclusion

The variety of responses to high $p\mathrm{CO}_2$ and additional cold stress reported in this 7 week-incubation experiment and elsewhere demonstrate heterogeneity in the response of reef calcifiers (Pandolfi et al. 2011; Higuchi et al., 2015; Higuchi et al., 2020; Agostini et al., in prepa). These different responses could lead to the emergence of potential “ecological winners”, such as *P. heronensis* here. In a context of poleward shift, our results suggest that poleward shifting *A. solitaryensis* would be severely limited by ocean acidification and only the warm temperate encrusting coral *P. heronensis*, already established at these high latitudes, could maintain suitable growth rates under future ocean acidification and cold stress. The results of this study suggest that the impact of short-term extreme cold stress (12 days under 15°C) on the physiology and energy balance of reef organisms, is acting as an additional stress to acidification threats. The lack of a clear tipping point, as well as the emergence of potential winners in the face of declining pH, indicate that in the coming decades the species composition of coral reefs is likely to slowly change, without abrupt transitions, to a new composition in which species do have the required potential for adaptation in the marginal high latitudes. The redistribution of coral reefs and the decline in structurally complex framework forming corals (e.g. *Acropora solitaryensis*) at the marginal high latitudes could cause severe and fundamental ecosystem modifications (e.g. reduction of habitat availability for fish and many invertebrates; Sunday et al., 2017; Cattano et al., 2020) and thus, strongly affects: ecosystem functioning, human well-being (economic development, livelihoods, emerging diseases, food security; Hall-Spencer and Harvey, 2019), and the dynamics of climate change itself (feedbacks, carbon sequestration; Pecl et al., 2017). Under the current changing environment, the need to evaluate the environmental controls on coral reefs and predict global distribution under future climate change scenarios is fundamental.

Our study only considers the physiological aspect for the determination of the future distribution range which may not be sufficient for a correct prediction (Pearson & Dawson, 2003). However, the regression approach, used within this study, has been demonstrated to be an insightful method to better predict the impacts of OA in comparison with the “scenario approach”, by resolving the general trend and the shape of the relationship between metabolism and $p\mathrm{CO}_2$. The description of these relationships for a wide range of species composing coral reef communities at low and high latitudes, could improve the accuracy of models describing the effects of the predicted OA on calcifying organisms and ecosystems, under climate change.

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Supplementary materials

Table 2.6. Parameters of the carbonate chemistry, for each tank, prior and after the cold shock (n = 24 and n = 18 respectively). pH_{NBS} (continuous measurement), temperature (continuous measurement) and total alkalinity (n = 79 and n = 50 respectively) are measured values; and pCO_2, HCO_3, CO_3^{2-}, and $\Omega_{Aragonite}$ are calculated values using CO_2sys. Values are presented as mean ± S.E..

Temperature treatment	Block	Tank	Targeted pH_{NBS} condition	Measured pH_{NBS}	Temperature (°C)	Total Alkalinity (µmol kg^{-1})	pCO_2 (µatm)	HCO_3^- (µmol kg^{-1})	CO_3^{2-} (µmol kg^{-1})	CO_2 (µmol kg^{-1})	Ω_{Ar} in
Prior to the cold shock	3	3C-04	7.4	7.1490 ± 0.0004	18.153 ± 0.003	2269.6 ± 1.0	5126	2219.2	20.9	175.3	0.32
	3	3C-05	8.2	7.7894 ± 0.0002	18.153 ± 0.003	2269.6 ± 1.0	1091	2063.3	84.9	37.3	1.31
	3	3C-06	7.8	7.9397 ± 0.0003	18.153 ± 0.003	2269.6 ± 1.0	743	1988.3	115.6	25.4	1.79
	3	3C-07	8	8.1854 ± 0.0001	18.153 ± 0.003	2269.6 ± 1.0	385	1816.3	186.0	13.2	2.88
	3	3C-08	7.6	7.4224 ± 0.0004	18.153 ± 0.003	2269.6 ± 1.0	2679	2176.4	38.4	91.6	0.59
	3	3C-09	7.8	7.4778 ± 0.0005	18.153 ± 0.003	2269.6 ± 1.0	2345	2164.2	43.4	80.2	0.67
	3	3D-04	8.2	8.2815 ± 0.0001	18.153 ± 0.003	2269.6 ± 1.0	294	1730.3	221.1	10.0	3.43
	3	3D-05	7.6	7.6341 ± 0.0002	18.153 ± 0.003	2269.6 ± 1.0	1604	2121.4	61.0	54.8	0.94
	3	3D-06	7.8	7.8851 ± 0.0002	18.153 ± 0.003	2269.6 ± 1.0	856	2017.9	103.5	29.2	1.60
	3	3D-07	7.2	7.4304 ±0.0002	18.153 ± 0.003	2269.6 ± 1.0	2628	2174.7	39.1	89.9	0.60
	3	3D-08	7.2	7.2275 ± 0.0002	18.153 ± 0.003	2269.6 ± 1.0	4260	2209.3	24.9	145.7	0.38
	3	3D-09	8.2	8.2106 ± 0.0001	18.153 ± 0.003	2269.6 ± 1.0	359	1794.8	194.8	12.3	3.02
	4	4C-04	7.8	7.737 ± 0.018	18.153 ± 0.003	2269.6 ± 1.0	1242	2084.7	76.1	42.4	1.18
	4	4C-05	7.8	7.764 ± 0.032	18.153 ± 0.003	2269.6 ± 1.0	1162	2074.0	80.5	39.7	1.25
	4	4C-06	7.8	7.768 ± 0.038	18.153 ± 0.003	2269.6 ± 1.0	1151	2072.3	81.1	39.3	1.26
	4	4C-07	7.2	7.242 ± 0.040	18.153 ± 0.003	2269.6 ± 1.0	4108	2207.2	25.8	140.5	0.40
	4	4C-08	8.2	8.139 ±0.029	18.153 ± 0.003	2269.6 ± 1.0	437	1853.2	170.9	14.9	2.65
	4	4C-09	7.4	7.349 ± 0.030	18.153 ± 0.003	2269.6 ± 1.0	3190	2190.3	32.7	109.1	0.50
	4	4D-04	8	7.978 ± 0.009	18.153 ± 0.003	2269.6 ± 1.0	672	1965.3	125.0	22.9	1.94
	4	4D-05	8.2	8.118 ± 0.026	18.153 ± 0.003	2269.6 ± 1.0	464	1870.2	164.0	15.8	2.54
	4	4D-06	7.6	7.608 ±0.006	18.153 ± 0.003	2269.6 ± 1.0	1710	2129.5	57.7	58.4	0.89
	4	4D-07	8.2	8.169 ±0.013	18.153 ± 0.003	2269.6 ± 1.0	402	1829.3	180.7	13.7	2.80
	4	4D-08	7.4	7.355 ±0.046	18.153 ± 0.003	2269.6 ± 1.0	3145	2189.3	33.1	107.5	0.51
	4	4D-09	8	8.025 ± 0.047	18.153 ± 0.003	2269.6 ± 1.0	594	1935.7	137.1	20.3	2.13
After cold shock	3	3C-04	7.4	7.1435 ± 0.0005	15.337 ± 0.006	2261.6 ± 0.6	5016	2216.4	18.6	186.3	0.28
	3	3C-05	8.2	7.7822 ± 0.0002	15.337 ± 0.006	2261.6 ± 0.6	1079	2076.1	75.9	40.1	1.16
	3	3C-06	7.8	7.9336 ± 0.0003	15.337 ± 0.006	2261.6 ± 0.6	736	2007.2	104.0	27.3	1.60
	3	3C-07	8	8.1884 ± 0.0001	15.337 ± 0.006	2261.6 ± 0.6	375	1841.5	171.5	13.9	2.64
	3	3C-08	7.6	7.4318 ± 0.0005	15.337 ± 0.006	2261.6 ± 0.6	2534	2175.0	35.5	94.1	0.54
	3	3D-05	7.6	7.6342 ± 0.0002	15.337 ± 0.006	2261.6 ± 0.6	1555	2126.5	55.3	57.7	0.85
	3	3D-06	7.8	7.8877 ± 0.0002	15.337 ± 0.006	2261.6 ± 0.6	828	2030.2	94.6	30.7	1.45
	3	3D-07	7.2	7.4335 ± 0.0002	15.337 ± 0.006	2261.6 ± 0.6	2524	2174.7	35.6	93.7	0.54
	3	3D-08	7.2	7.2332 ± 0.0003	15.337 ± 0.006	2261.6 ± 0.6	4062	2206.2	22.7	150.9	0.35
	4	4C-06	7.8	7.753 ± 0064	15.337 ± 0.006	2261.6 ± 0.6	1159	2087.0	71.4	43.0	1.10
	4	4C-07	7.2	7.292 ± 0.025	15.337 ± 0.006	2261.6 ± 0.6	3535	2198.3	25.9	131.3	0.40
	4	4C-08	8.2	8.129 ± 0.034	15.337 ± 0.006	2261.6 ± 0.6	441	1886.3	153.3	16.3	2.36
	4	4C-09	7.4	7.379 ± 0.028	15.337 ± 0.006	2261.6 ± 0.6	2870	2184.5	31.6	106.6	0.48
	4	4D-04	8	8.009 ± 0.036	15.337 ± 0.006	2261.6 ± 0.6	605	1965.1	121.2	22.5	1.86
	4	4D-06	7.6	7.621 ± 0.029	15.337 ± 0.006	2261.6 ± 0.6	1604	2130.2	53.8	59.6	0.82
	4	4D-07	8.2	8.176 ±0.011	15.337 ± 0.006	2261.6 ± 0.6	388	1851.2	167.6	14.4	2.58
	4	4D-08	7.4	7.478 ±0.022	15.337 ± 0.006	2261.6 ± 0.6	2268	2165.7	39.3	84.2	0.60
	4	4D-09	8	7.981 ± 0.015	15.337 ± 0.006	2261.6 ± 0.6	652	1981.5	114.5	24.2	1.76