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

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Abstract

Ocean acidification (OA) is a severe threat to coral reefs mainly by reducing their calcification rate. Identifying the resilience factors of corals to decreasing seawater pH is of paramount importance to predict the survivability of coral reefs in the future. This study compared corals adapted to variable pH (i.e., 7.23-8.06 pH_T units) from the semi-enclosed lagoon of Bouraké, New Caledonia, to corals adapted to more stable seawater pH (i.e., 7.90-8.18 pH_T units). In a 100-day aquarium experiment, we examined the physiological response and genetic diversity of Symbiodiniaceae from three coral species (*Acropora tenuis*, *Montipora digitata* and *Porites* sp.) from both sites under three stable pH conditions (i.e., 8.11, 7.76, 7.54 pH_T units) and fluctuating pH conditions (i.e., between 7.56 and 8.07 pH_T units). Bouraké corals consistently exhibited higher growth rates than corals from the stable pH environment, with specific ITS2 intragenomic variant profiles. While OA generally decreased coral calcification by ca. 16%, Bouraké coralsshowed higher growth rates (21 to 93% increase, depending on species with all pH conditions pooled) than those from the stable pH environment. This superior performance coincided with divergent ITS2-like profiles with better consistency for both variable and low pH conditions. This response was not gained by corals from the more stable environment exposed to variable pH during the four-month experiment, suggesting that such a kind of plasticity is time dependent. Future long-term experiments should address the exposure duration required to confer fitness benefits for sustained calcification, hopefully fast enough to cope with the ongoing rapid OA.

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Abstract

Ocean acidification (OA) is a severe threat to coral reefs mainly by reducing their calcification rate. Identifying the resilience factors of corals to decreasing seawater pH is of paramount importance to predict the survivability of coral reefs in the future. This study compared corals adapted to variable pH (i.e., 7.23-8.06

pH_T units) from the semi-enclosed lagoon of Bouraké, New Caledonia, to corals adapted to more stable seawater pH (i.e., 7.90-8.18 pH_T units). In a 100-day aquarium experiment, we examined the physiological response and genetic diversity of Symbiodiniaceae from three coral species *(Acropora tenuis* , *Montipora digitata* and *Porites*sp.) from both sites under three stable pH conditions (i.e., 8.11, 7.76, 7.54 pH_T units) and fluctuating pH conditions (i.e., between 7.56 and 8.07 pH_T units). Bouraké corals consistently exhibited higher growth rates than corals from the stable pH environment, with specific ITS2 intragenomic variant profiles. While OA generally decreased coral calcification by ca. 16%, Bouraké coralsshowed higher growth rates (21 to 93% increase, depending on species with all pH conditions pooled) than those from the stable pH environment. This superior performance coincided with divergent ITS2-like profiles with better consistency for both variable and low pH conditions. This response was not gained by corals from the more stable environment exposed to variable pH during the four-month experiment, suggesting that such a kind of plasticity is time dependent. Future long-term experiments should address the exposure duration required to confer fitness benefits for sustained calcification, hopefully fast enough to cope with the ongoing rapid OA.

Introduction

Coral reefs have been suggested to be at risk of disappearance in the coming decades due to their sensitivity to the deadly trio of stressors including ocean warming (OW), acidification (OA), and deoxygenation (OD) (e.g., Hoegh-Guldberg et al., 2017; D. J. Hughes et al., 2020; T. P. Hughes et al., 2018). These stressors are expected to exceed optimal levels for the good functioning of coral reefs as early as 2050 (Hoegh-Guldberg et al., 2017; IPCC Report, 2021), when most coral reefs are expected to dramatically decline. However, species-specific responses in the reefs' environment (Altieri et al., 2021; Comeau et al., 2022; Jury & Toonen, 2019; Rodolfo-Metalpa et al., 2011), and in vitro experiments (Comeau et al., 2019; Cornwall et al., 2018) have shown that some corals seem to be resilient to one stressor or to the combination of more stressors.

Among the most likely hypotheses to explain coral resilience to OA, is the phenomenon that corals chronically exposed to major environmental changes are more resilient to future changes (Comeau et al., 2022; Enochs et al., 2020; Schoepf et al., 2020). Possible mechanisms that could support resilience include physiological plasticity, epigenetics, and/or host microbiome shifts (Camp et al., 2017, 2020; Putnam et al., 2016; Ros et al., 2021; Voolstra & Ziegler, 2020), although coral response remains equivocal (Camp et al., 2016; Cornwall et al., 2018; Enochs et al., 2020; Rathbone et al., 2021; Rivest et al., 2017).

Corals thriving under variable seawater pH and temperature conditions in mangrove lagoons have showed a shift in dominant host-associated Symbiodiniaceae taxa (Ros et al., 2021), likely contributing to the persistence of corals in such extreme environments (Camp et al., 2018, 2020; Haydon et al., 2021; Howells et al., 2012; Voolstra & Ziegler, 2020). Interestingly, some corals from marginal environments have associated with *Durusdinium* (clade D) (Schoepf et al., 2015) that is considered a 'stress-tolerant' taxa, although this is not always the case (Camp et al., 2019, 2020). Whilst corals with a stable host-Symbiodiniaceae association have been suggested to be physiologically more resilient under stressful conditions (Grottoli et al., 2018; Howells et al., 2020), it remains unclear how stability (i.e., fidelity) and flexibility in the Symbiodiniaceae diversity benefit host fitness under stress conditions (McIlroy et al., 2020; Smith et al., 2017; Ziegler et al., 2015).

More generally, coral resilience to extreme conditions includes trade-offs in the main physiological traits, such as reduced calcification rate (Bay & Palumbi, 2016; Camp et al., 2020; Cunning et al., 2015) and photosynthesis (Camp et al., 2020), increased photodamage (Silverstein et al., 2017), and change in the autotrophic nutrition capacities (Wall et al., 2020). For instance, corals with thermotolerant symbionts have been reported to resist bleaching but grow more slowly (Jones & Berkelmans, 2010). Most of these trade-offs remain poorly tested on corals already adapted to conditions close to or even worse than the ones predicted for the end of this century. Yet, extreme environments such as mangrove lagoons have been identified to be faithful natural laboratories for studying species living under the combination of future extreme conditions in OA, OD, and OW (Camp et al., 2018). The semi-enclosed lagoon of Bouraké in New Caledonia is one of these extreme environments, exhibiting fluctuating acidified, warm and deoxygenated conditions, where a well-diversified coral reef thrives (Camp et al., 2017; Maggioni et al., 2021). The environmental variability in the dissolved oxygen, temperature and pH in Bouraké is directly related to the tidal cycle with changes

on a single day by up to 4.91 mg O_2 L^{-1}, 6.50°C and 0.69 pH_T units (Maggioni et al., 2021), respectively. Such environmental conditions likely enhanced the coral respiration rates and total chlorophyll contents, while reducing photosynthesis and calcification rates (Camp et al., 2017; Jacquemont et al., 2022). Although species-specific, the differences in Symbiodiniaceae types found in three coral species between Bouraké and an adjacent reef (Camp et al., 2020) highlight their potential role on coral resilience to environmental stress, and raises the question of the fidelity of resistant Symbiodiniaceae described in locally adapted species (Quigley et al., 2017, 2019). Corals in Bouraké have likely either adapted or established key trade-offs to achieve resilience in an environment somewhat worse than future climate change projections.

In the present study, we conducted a 100-day OA experiment in aquaria using three coral species (i.e., *Acropora tenuis* , *Montipora digitata* and *Porites* sp.) from Bouraké and an adjacent Reference reef to assess i) whether corals adapted to either ambient or fluctuating pH conditions alter their rates of photosynthesis, metabolism and calcification under different levels of OA, and ii) whether Symbiodiniaceae communities are distinct between habitats and treatments at the end of the experiment. In addition, we considered both static (average values) and variable (taking into accounting the natural diel variability measured at Bouraké) pH conditions to assess the role that fluctuating pH may play in the success of Bouraké corals, and whether corals adapted to stable pH can quickly acclimate when exposed to variable pH. We hypothesize that corals from Bouraké exhibit enhanced physiological rates and a reorganization in their associated Symbiodiniaceae community, compared with corals from the adjacent reef, and that the natural diel fluctuations in seawater pH promote their resilience to OA.

2. Materials and Methods

2.1 Study sites, coral collection and acclimation

All corals were collected at the end of January 2020 in the semi-enclosed lagoon of Bouraké, New Caledonia (Fig. S1), and at one reference reef adjacent to Bouraké (St R1, 4 km away). In Bouraké, dissolved oxygen (DO) and pH regularly fluctuated according to the tide (ca. 1 m tidal range), from 1.87 to 7.24 mg O_2L^{-1} and from 7.23 to 8.06 pH_T (in total scale), respectively. Seawater temperature varied from 17.50°C in winter to 33.80°C during summer, when temperature might change by up to 6.50°C in a single day. In comparison, DO, pH and temperature were more stable at the reference site and yearly averaged 6.45 ± 0.95 mg O_2 L^{-1}, 8.05 ± 0.04 pH_T, and 25.25 ± 1.89°C (Camp et al., 2017; Maggioni et al., 2021). Notwithstanding such extreme and chronic conditions, 66 coral species form healthy reefs all along the Bouraké lagoon (Maggioni et al., 2021). These reefs spend 44% of the time at $pH_T$of 7.7-7.8 all year long, and 71% of the time in summer at temperatures predicted for the end of the century under scenario RCP4.5 (i.e., 31-32°C; (Camp et al., 2017)).

Fifteen mature colonies (30 - 60 cm diameter) of the branching*Acropora tenuis* (Dana, 1846) and *Montipora digitata* (Dana, 1846), and the massive *Porites* sp. were sampled at each site at ca. 2 meter depth. Only colonies at a distance of at least 5 m from each other were collected to limit the risks of clonality in our sampling. From each branching colony, four terminal portions of branches (ca. 3 - 5 cm long) were collected using a plier. For *Porites* sp., four samples (mean of ca. 8 cm^2) were collected from each colony using a 3-cm-diameter steel tube and a hammer. Coral fragments were transported in a cooler to the "Aquarium des Lagons" (Nouméa), which is 2 h distant from Bouraké, in individual hermetic zip bags (one for each colony of each species) containing seawater from the collection site. At the laboratory, fragments of *A. tenuis* and *M. digitata* were attached on nylon wires and suspended in two 200 L recovering tanks, one for each site of collection (see below). Fragments of *Porites* sp. were mounted on labeled 2x2 cm PVC plates using epoxy resin (Holdfast, Aquarium Systems) and placed at the bottom of the tanks. Exposed skeleton was covered with the resin to avoid turf algae proliferation and potential skeletal dissolution. All 360 fragments (2 sites, 3 species, 15 colonies per species, 4 samples per colonies) were allowed to recover for three weeks in the two aquaria settled at the same temperature (26.0 ± 0.5°C), which was close to values measured*in situ* at both sites at the time of collection. Seawater pH and carbonate chemistry replicated mean values of Bouraké and reference sites (ca. 7.7 and 8.1 pH_{NBS} units, respectively). Temperature and pH were kept constant using heaters and bubbling pure CO_2 in each tank. All tanks were connected to an IKS logger

system (IKS, Karlsbad, accuracy ± 0.05 pH unit and 0.1 °C). During the recovery period, coral fragments received the same lighting intensity using four Aquablue Plus neon bulbs (15.000K, Giesemann, Germany), and they were fed once a week with *Artemia salina* nauplii (Houlbrèque et al., 2015). At the end of the recovery period, the naked skeleton of branching corals were already covered by the tissue, while new tissue was visible on the resin embedding*Porites* sp.

2.2 Aquarium setup and physiological measurements

2.2.1 Aquarium conditions and experimental set-up

At the end of the recovery period, corals from each site of collection (called origin hereafter) were assigned to one of the 12 experimental tanks as follows. Three replicate 10 L tanks were set up for each of the four conditions of pH (Table 1): i) reference reef pH (Control; pH_{NBS} 8.11 ± 0.05; p CO_2 474 µatm); ii) future reef pH based on the RCP7.0 IPCC scenario (IPCC Report, 2021) (Future; pH_{NBS} 7.76 ± 0.07;p CO_2 1192 µatm); iii) extreme stable pH (Extreme; pH_{NBS} 7.54 ± 0.08; p CO_2 2115 µatm); and iv) variable pH reproducing the daily variation of pH in Bouraké (Variable; mean pH_{NBS} ranging from 7.56 ± 0.07 to 8.07 ± 0.07; p CO_2 ranging from 1968 to 533 µatm). While Extreme stable pH were arbitrarily chosen, Control and Variable pH were settled based on the mean and variance data collected respectively at reference and Bouraké reef between 2016 and 2020 (see Maggioni et al., 2021). Five fragments per coral species (n = 3) and per origin (n = 2) were randomly positioned in each tank. *Porites*samples were positioned on the bottom of the tanks while the branching corals were suspended.

Seawater in the tanks was renewed at a rate of 16.5 L h^{-1} (renewal rate of ca. 165% h^{-1}) and mixed using a submersible pump (micro-jet MC 320, Aquarium system, USA). Each tank was supplied with seawater pumped from one to three 60 L sump tanks settled at different pH (7.4, 7.7, and 8.1). In addition, three more sump tanks were set up respectively at pH 7.6, 7.8 and 7.9 and were used to better simulate the pH variation in the variable treatment (see below). Each sump tank was continuously supplied with 50 µm-filtered seawater pumped at 5 m depth in front of the Aquarium des Lagons (Baie des Citrons, Nouméa). Each sump contained a submersible pump (23 W Eheim), a heater and an external refrigerating system, both connected to an IKS logger system (IKS, AquaStar, Germany), and temperature and pH probes. Sumps were all maintained at 26.3 ± 0.7°C, and at one of the 6 pH conditions, automated by IKS (AquaStar, Germany). Seawater pH was set to the desired value by bubbling pure CO_2 gas. Each experimental tank received seawater at the right temperature and pH from the respective sump tank. In contrast, for the variable pH treatment, the time function of the IKS system was used to simulate the pH changes measured at Bouraké. Briefly, tanks received seawater either from one or simultaneously from different sumps according to a predetermined timetable (Supplementary Table S1; Fig. S2). Irradiance in all tanks increased from 0 to ca. 250-300 µmol photons m^{-2} s^{-1} on a 12:12 h light:dark cycle (6:00-18:00 h lighting vs 18:00-6:00 h darkness) using LED lights (Mitras LX6100, GHL Germany; see Supplementary Table S2 for led brightness wave simulation) according to Biscéré et al., (2019), Houlbrèque et al., (2015) and Jacquemont et al., (2022). Irradiance was monitored during 48-h cycles using a NKE PAR with LI-193 spherical quantum sensor and showed maximum values ranging from ca. 270 to 320 µmol photons m^{-2} s^{-1} (Supplementary Fig. S3). Tanks' positions were repeatedly changed during the duration of the experiment to minimize any potential spatial variability in the light intensity received by corals. A Seabird SeaFET pH logger and YSI 600 OMS-M probes were periodically used to measure over a 48-h cycle seawater temperature, pH_T, salinity and dissolved oxygen (DO) in the experimental tanks. Colonies were fed with *Artemia salina nauplii* once a week and were maintained under these experimental conditions for 100 days.

Seawater pH and temperature values were continuously monitored by the IKS pH probes and verified daily using a portable pH-meter (827 pH lab Metrohm). Total alkalinity (A_T) and nutrients were measured twice a month on each of the 12 tanks (see Supplementary Informations).

2.2.2 Growth rate

After the recovery period and at the end of the experiment, each coral fragment was weighted using the buoyant weight technique (Spencer Davies, 1989). Samples were weighted using a Sartorius ENTRIS 224i-

1S electronic balance (readability 0.1 mg) in seawater of known density (calculated from temperature and salinity). Dry skeleton weight was calculated using the density of pure aragonite (2.94 g cm^{-3}); growth rates (in term of calcification rates) were calculated as the change in dry weight between the initial and the final weight and expressed either in mg $g^{-1}d^{-1}$ or mg cm^{-2} d^{-1}depending on the species.

2.2.3 Photosynthetic efficiency

During the last week of the 100 day experiment, photosynthesis efficiency (F_v/F_m) and the relative electron transport rate (rETR) of the photosystem II (PSII) of symbionts *in hospite* were measured on all corals using a pulse amplitude modulation (PAM) fluorometer (DIVING-PAM, Walz, Germany, Schreiber et al., 1986). Before measurements, coral fragments were dark adapted during 15 minutes (Hoegh-Guldberg & Jones, 1999). Measurements were performed at dark (no light source); the 8 mm optical fiber was maintained perpendicular to the fragment's surface using a black-jacket at a fixed distance of 5 mm. The initial (F_0) and maximal (F_m) fluorescence of symbionts was measured by applying respectively a weak pulse of red light (LED 650 nm, width 3 µs) and a saturating pulse of actinic light (max. intensity reaching 8000 µmol photon m^{-2} s^{-1}, width 800 µs) on coral fragments. Variable fluorescence (F_v) was calculated as F_m-F_0 and maximal quantum yield as F_v/F_m. F'_v/F'_m represent the effective quantum yield at the irradiance (PAR) experienced by the corals. Relative electron transport rate (rETR=F_v/ F_mx0.5xPAR) versus PAR curves were established according to Ralph et al. (1999).Comparisons between treatments and origin were done on the maximum quantum yieldF_v/F_m and the maximum $rETR_{max}$.

2.2.4 Photosynthesis and respiration rates

Seven corals for each pH condition (n= 4), each origin (n= 2) and each species (n= 3) were randomly selected (n= 168 total) and their oxygen production and consumption rates were measured daily in the light and dark during four consecutive days. Corals were individually placed in 100 mL Pyrex glass beakers which were filled with seawater from their respective tank treatment and hermetically sealed underwater with transparent cellophane and a rubber band (Jacquemont et al., 2022). Measurements of corals incubated at variable pH were performed two times, first at the lowest pH and then, after one day of recovery, at the highest pH value (i.e., 7.4 and 8.1 pH_{NBS} values). Two control beakers without coral fragments were used to measure any metabolic activity of microbes in the water. We were able to process 28 samples simultaneously (plus two empty beakers) using two 15-place magnetic stirring plates (Telesystem 15, Thermo Scientific). The 30 beakers were semi-immersed in a water bath positioned above the stirring plates and settled at a temperature of 26.0°C ± 0.5°C using one heater and two submersible water pumps to homogenize water temperature. Each beaker had one O_2 sensor spot (PreSens) fixed on the glass, and contained a stirring bar and a bridge made of plastic mesh to separate the coral fragment from the bar. Corals were first incubated in the light for 50 min under ca. 250 µmol photons m^2s^{-1} and then in the dark for the same time (Biscéré et al., 2019; Jacquemont et al., 2022). Incubation time was preliminary defined to avoid both hyperoxic and hypoxic conditions in the beakers. Temperature and dissolved oxygen (DO) in mg L^{-1} were measured at the beginning and at the end of each incubation in each beaker using an optical fiber (PreSens Fibox 4 trace). Before measurement, corals were left for 10 min under either light or dark conditions and then DO was measured. At the end of the light incubation, each beaker was opened and seawater volume was measured using a graduated cylinder. Fresh seawater from the corresponding experimental tank was then added, before the beakers were resealed and placed on the stirring plates under dark conditions. At the end of each incubation pair (dark and light) for all pH conditions, coral fragments were frozen at -20°C for further analysis of the chlorophyll, symbiont, clades and protein contents, and to determine their skeletal surface area.

Rates of net photosynthesis (P_n) and respiration in the dark (R_{dark}) were calculated using the change in DO concentrations in each beaker corrected by the mean of the microbial activity measured in the two empty beakers, and normalized by the incubation duration (hours), the volume of seawater in each beaker (L), and the coral's surface (cm^2). Rates of gross photosynthesis (P_g) were calculated as:

$P_g = P_{net} + |R_{dark}|$ (1)

Data were normalized per surface area of the fragment as described below. P_n, P_g and $R_{dark}$are expressed in mg O_2 $cm^{-2}h^{-1}$ then converted in µmol O_2cm^{-2} h^{-1}.Photosynthesis to respiration ratio (P_g:R) was calculated using the value of daylight hours equal to 12 as follows:

$$P_g : R = \frac{P_g \times hours\ of\ daylight}{|R_{\mathrm{dark}}| \times 24} \quad (2)$$

2.2.5 Tissue and surface measurements

All fragments used to assess the coral photosynthesis and respiration rates were prepared and analyzed for their Symbiodiniaceae and chlorophyll contents. Protein measurements were performed on the same individual used during photosynthesis and respiration incubations for*Acropora tenuis* and *Montipora digitata* and from a new individual for *Porites* sp. Then, their skeleton's surface areas were measured. Coral tissue was extracted from the skeleton using an air pick in 20 mL filtered seawater and homogenized with a Potter tissue grinder. For symbiont density measurement, 2 mL of the slurry was sampled to count the number of Symbiodiniaceae (n count = 8) using a Neubauer's cell under a stereomicroscope. Ten mL subsamples were centrifuged at 5,000 g for 10 min, the supernatant was discarded, and the pellet containing the symbiont was re-suspended in 10 mL of pure acetone to extract during 24 h at 4°C in darkness the chlorophylls$a$ and c_2 . The solution was then centrifuged at 10,000 g for 15 min and the supernatant was sampled to measure its absorbance at 630, 663 and 750 nm using a spectrophotometer (Evolution 201, Thermo Scientific). Chlorophyll a andc_2 concentrations were calculated using the spectrophotometric equations of Jeffrey & Humphrey, (1975). Chlorophyll$a$ and c_2 are given as total chlorophyll.

Protein content was estimated using a BCA assay kit (Uptima, Interchim). Total protein was extracted according to (Hoogenboom et al., 2010) by incubating each fragment in a sodium hydroxide solution (1 N) maintained in a water bath for 30 min at 90°C. Samples were then diluted by a factor of 15 before being transferred into 96-well microplates and incubated with a dye reagent (Uptima Reagents, Interchim) for 30 min at 60°C. Bovine serum albumin (BSA, Interchim) was used as a protein standard with concentrations of 0, 50, 100, 200, 350, 500, 750 and 1000 µg ml^{-1}. Samples and standards were homogenized for 30 s on a microplate shaker within the spectrophotometer (Biotek ELx808); absorbances were measured at 563 nm, and the protein contents were calculated according to the standard equation.

The skeletal surface areas of samples were estimated using the paraffin wax-dipping method (Naumann et al., 2009; Stimson & Kinzie, 1991) for branching species (i.e., *Acropora tenuis* and *Montipora digitata*) and the aluminum foil technique (Marsh, 1967) for the massive species *Porites* sp.

2.3 Metagenomic analysis

2.3.1 DNA extraction, PCR amplification and sequencing

Fragments of corals (n= 2-6, depending on pH treatment) were collected and stored at -20°C. Total coral holobiont DNA (i.e., Symbiodiniaceae, polyp and associated microorganisms DNAs) was extracted using a 2% CTAB-based protocol adapted from (Mieog et al., 2009). The quantity and quality of extracted DNA were checked using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, MA). Extracted DNA was then diluted to a range of 30-70 ng μL^{-1} for PCR amplification. The Symbiodiniaceae nuclear DNA ribosomal internal transcribed spacer (ITS2) region was amplified with the forward primer ITS2-DINO [5'-*TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG* GTGAATTGCAGAACTCCGTG-3'] (Pochon et al., 2001) and reverse primer ITS2Rev2 [5'-*GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG* CCTCCGCTTACTTATATGCTT-3'] (Stat et al., 2009). The underlined segments represent Illumina adapter overhangs (Illumina, San Diego, CA, USA). The PCRs were conducted in 25 µL reactions using 12.5 µL of AmpliTaq 360 Master Mix, 1 µL of each 10 µM primer mix, 1 µL of 360 GC Enhancer, 2 µL of DNA template and DNAse-free water to adjust the reaction volume. The amplification cycle was set and adjusted from (Arif et al., 2014) as follows: 94°C for 15 min; 35 cycles each at 95°C for 30 s, 49°C for 1 min, and 72 °C for 30 s; and a final extension at 72°C for 10 min. To check amplification success, 3 µL of each PCR product was run on a 1% agarose gel. The resulting amplicons were sequenced using the Illumina MiSeq platform (2 x 300 bp) (Australian Genome Research Facility, Victoria, Australia). Returned

demultiplexed FASTQ files were analyzed via the SymPortal analytical framework (Hume et al., 2019). The SymPortal framework predicts ITS2 type profiles from specific sets of defining intragenomic ITS2 sequence variants (DIVs) based on genetically differentiated Symbiodiniaceae taxa. Quality control was assessed using MOTHUR 1.39.5 (Schloss et al., 2009), BLAST+ suite of executables (Camacho et al., 2009) and minimum entropy decomposition (MED; Eren et al., 2015) to predict Symbiodiniaceae taxa from the ITS2 marker.

2.4 Statistical analyses and data presentation

Statistical analyses were conducted, and figures were produced using RStudio (R Development Core Team, version 4.1.0, 2021), including the packages: "ggplot2", "ggpubr", "ARTool", "car", "vegan", and "pairwiseAdonis". Homogeneity and the normality of variance distributions were tested using respectively the Levene test and the Shapiro-Wilkinson test, and graphically verified with Q-Q plots. Statistical analyses were performed separately for each species as the three species we used are morphologically different. Firstly, differences in physiological measurements between replicate tanks were tested for each pH condition and origin. Either t-test, Wilcoxon, ANOVA or Kruskal-Wallis tests were used depending on the number of replicates and verification of the homogeneity and normality of data for each parameter. No significant differences were found between the three replicate pH tanks (e.g. no tank effect) for a given measurement, thus data was pooled between tanks. Two-way ANOVAs were used on physiological parameters to test the effect of pH (4 levels: Control, Future, Extreme and Variable), coral's origin (two levels: Bouraké and Reference) and their interactions. A two-way ANOVA type II was performed when no interaction was found between origin and pH. When an interaction was found, a parametric two-way ANOVA type I was run with origin as the first interaction term. Post hoc Tukey HSD was run to assess significant interactions (p-levels were not adjusted). In contrast,F_v/F_m data did not meet the assumption of normality, and they were compared using the non-parametric two-way Aligned Rank Transformed (ART) ANOVA (Type III) followed by a Bonferroni p-levels adjusted post hoc. Data were described as box plots using median values ± 25^{th} and 75^{th}percentiles (box), minimum and maximum values (whiskers) and dots as outliers, otherwise specified. Differences in Symbiodiniaceae ITS2 profiles were analyzed on square-root transformed data using three-factorial permutational multivariate analysis of variance (PERMANOVA) with 999 permutations of residuals and based on Bray-Curtis distances to test for differences between sites, pH conditions and species (three levels).

3. Results

3.1 Seawater parameters

Seawater pHNBS, temperature and carbonate chemistry were maintained at the target experimental values during the 100-day experiment (Table 1; Table S3 for data in each replicate tank; Table S4 for data from the sump tanks). Averaged temperatures were similar between conditions (ranging from 26.18°C to 26.41°C). Minimum and maximum values were recorded in the extreme (23.90°C) and variable treatment tanks (28.30°C), respectively, due to a temporary malfunction of the temperature control system. On a daily scale, seawater pH values varied by ca. 0.60-0.65 pH_T units in the variable condition, and by only 0.05-0.1 pH_T units in the other conditions (Fig. 1). Dissolved oxygen (DO) showed synchronized diel fluctuations in all conditions with an increase of up to 1 mg O_2L^{-1} at daylight.

Nutrients concentrations were rather similar between conditions (Table S5). Averaged values (± SD) of NO_x varied from 0.59 (± 0.21) to 0.67 (± 0.25) µmol L^{-1} for control and future conditions, respectively. PO_4^{3-}concentrations were equal to 0.30-0.31 µmol L^{-1} for all treatments, while $Si(OH)_4$ varied from 2.79 (± 1.06) to 2.89 (± 1.18) µmol L^{-1} for variable and future conditions, respectively.

3.2 Growth rate

Growth rates of the three coral species were significantly different according to corals' origin and pH conditions, with the exception of*M. digitata* showing a p -value = 0.086 for pH (2-way ANOVA, Table 2 and Table S6 for all data analyses). In general, corals from Bouraké showed higher growth rates than individuals from the Reference site, irrespective of the pH condition (Fig. 2). Pooling all pH conditions, this increase was higher of 21, 93 and 55% (*A. tenuis* , *M. digitata* and *Porites* sp., respectively) relative to pooled pH

conditions of the Reference site. Two coral species showed significantly higher growth rates in the control condition, compared with the variable (*A. tenuis*) and extreme (*Porites* sp.) conditions (Table S6).

3.3 Photosynthetic efficiency and electron transport rate

The photosynthetic efficiency (F_v/F_m) and the maximum relative electron transport rates ($rETR_{max}$) were significantly different for *A. tenuis* only (Table 2; Table S6). TheF_v/F_m of the latter differed according to both corals' origin and pH conditions, whereas only corals' origin had an influence on $rETR_{max}$. Both $rETR_{max}$ (Fig. 3) andF_v/F_m (Fig. S4) were higher for*A. tenuis* individuals from Bouraké, as compared to individuals from the Reference site, whatever the pH treatment. TheF_v/F_m for this species also showed higher values for future and extreme pH conditions than for control and variable pH conditions.

3.4 Photosynthesis and respiration rates

Metabolic rates (i.e., P_g, R_{dark}, P_g:R) of the three coral species did not significantly vary according to corals' origin. In contrast, ANOVAs showed significant differences between pH conditions in the P_g, R_{dark} and their ratio P_g:R for *A. tenuis* , in R_{dark} for *M. digitata* , and in P_g for *Porites* sp. (Table 2; Table S6). Overall, P_g and R_{dark} values were higher for future and extreme pH conditions (Table S6; Fig. 4). The P_g:R ratios were significantly different between pH conditions for *A. tenuis* (Fig. S5), contrary to the other species. This result was due to the higher P_g:R ratio values calculated for *A. tenuis* corals maintained at the variable pH condition, when P_g and R were measured at a pH of 8.1 (Table S6).

3.5 Symbiodiniaceae, chlorophyll and protein content

Symbiodiniaceae density and total chl contents did not significantly differ between pH conditions (Tables 2 and S6) for the three coral species, although differences were almost significant for *A. tenuis* . Significant differences in the Symbiodiniaceae density (*A. tenuis* and *Porites* sp.) and total chl content (*A. tenuis*) were found according to individual origin (Table 2; Fig. 5 and S6). Most of these differences were driven by higher data variability in the extreme condition, especially for corals originating from the Reference site (Fig. 5 and S6; Table S6). In contrast, both*A. tenuis* and *Porites* sp. protein contents significantly differed between pH conditions (Tables 2 and S6), and although values were very similar (Fig. 5), post hoc comparisons showed higher values for corals kept at the control pH condition compared to the variable (*A. tenuis*) and future (*Porites* sp.) pH conditions.

3.6 Metagenomic results

The three coral species were consistently associated with Symbiodiniaceae of the genus*Cladocopium* but were dominated by distinct ITS2 type profiles. The latter were significantly different between species, coral origin, and their interaction (Table 3; Fig. 6). The major ITS2 type profiles of corals from the Reference site comprised 58.1-91.1%, 42.6-87.5% and 76.4-96.1% of the total sequences in each sample for *Acropora tenuis, Montipora digitata and Porites* sp.*,* respectively. Under control condition, *A. tenuis* had 4 major ITS2 DIV type profiles, with C3k-C3bo-C50a-C3ba-C50q most common (3 of 5 samples), while*Porites* sp. had 3 major type profiles, with C15-C15ce-C15cc-C15n-C15cf-C15l-C15qh most common (3 of 5 samples). Two major type profiles were found in *Montipora digitata*(C15/C15vi-C15vj-C15f-C15he and C73-C73a-C21). For corals from Bouraké, the major ITS2 type profiles comprised 72.6-92.5%, 64.5-96.2% and 82.2-93.3% of the total sequences in each sample for *A. tenuis, M. digitata and Porites* sp.*,* respectively. For *A. tenuis* , the major type profile was C1-C1b-C42.2-C1c-C1bh-C1br-C1cb (one sample was also C3/C15), while *Porites* sp. was predominantly C15-C15bq-C15iq (one replicate was C15-C15ce-C15cc-C15n-C15cf-C15l-C15qh). *M. digitata* had 9 major type profiles, with C15/C15vi-C15vj-C15f-C15he the most common (found in 50% of the samples).

The pH treatment did not result in a significant change in the Symbiodiniaceae major type profiles for any coral species (Table 3). However, an interesting observation was that under low (i.e., Future and Extreme conditions) and variable pH conditions, corals from Bouraké had more consistent major ITS2 type profiles between replicate colonies, than corals from the Reference site (Table 3; Fig. 6). Individuals of the three

coral species originating from the Reference site had 3 times more type profiles when maintained at future or variable pH conditions than individuals under the control pH.

4. Discussion

Corals from the Bouraké lagoon have evolved over generational time scales in environmental conditions (pH, temperature, dissolved oxygen) chronically exceeding those predicted by the IPCC scenarios for the end of this century (IPCC Report, 2021). While the hydrodynamic and topographic features that make environmental parameters at this site so particular have not yet been investigated, with no doubt the organisms living in this lagoon have been exposed to extreme environmental conditions throughout their lifetime. In fact, the Bouraké geomorphology, coupled with an intense oxidative activity in the mangrove sediments, have been shown to cause seawater acidification, deoxygenation, and warming in the lagoon (Maggioni et al., 2021). Despite the extreme levels of acidification measured, which should not allow corals to calcify (Kleypas et al., 1999), an abundant, well-diversified and healthy coral reef thrives in the lagoon. By assessing the holobiont physiological responses and the Symbiodiniaceae profiles of three coral species from both Bouraké and a Reference reef to a large range in pH, we found that corals from Bouraké constantly exhibited higher growth rates and had a specific and more consistent ITS2 majority sequence than corals from the Reference reef under low and variable pH conditions. It seems likely that such patterns were linked to the strong life-long environmental fluctuations, which might have promoted coral resilience as previously suggested (e.g., Brown et al., 2022; Comeau et al., 2022; Enochs et al., 2020; Rivest et al., 2017; Schoepf et al., 2020). We are aware that this study explored only a few of the compensatory mechanisms that might be at the origin of the resilience observed for corals from Bouraké. We also admit that these coral populations may have developed such mechanisms in a much longer time scale than corals that will have to cope with rapid climate change. However, results from this study would suggest that adaptation to future OA conditions could be possible in the wild.

Coral potential resilience to ocean acidification: what do we learn from lifetime adapted corals?

In agreement with the consensus on the effect of OA on coral calcification (Gattuso et al., 1999), recently revised by Leung et al., (2022), we found that calcification rates of both Reference and Bouraké corals were affected incrementally when exposed to future and extreme pH levels. For instance, calcification rates of *Acropora tenuis* ,*Montipora digitata* , and *Porites* sp. originating from the Reference site decreased by 9.3%, 19.0% and 17.6%, respectively, in the Future pH (pH 7.76) condition and by 15.4%, 53.5%, and 20.7% in the Extreme pH (pH 7.54), compared to the Control pH (pH 8.11). In contrast, *Acropora tenuis* and *Montipora digitata*originating from Bouraké showed quite stable calcification rates (4.0% and 2.2% increases, respectively) at Future condition. However, lower rates were observed at Extreme pH (15.2% and 9.6% decreases, respectively), compared to the corals maintained at Control pH. Only*Porites* sp. from Bouraké always exhibited decreased calcification rates as for the Reference corals (21.5% and 41.0% decreases at Future and Extreme pH, respectively). These results are consistent with coral species-specific responses to OA with respect to calcification. Relatively small effects of OA on some coral species were previously observed in aquaria experiments (Bell et al., 2022; Comeau et al., 2013; Ries et al., 2009) and recently reviewed (Bove et al., 2020; Cornwall et al., 2021; Leung et al., 2022).

A reaction norm better shows to what extent coral calcification changes according to coral origin, when individuals are exposed to different pH levels. We assumed that corals from Bouraké might originate from seawater at either pH 7.7 (Future pH) or 7.4 (Extreme pH). Then, it appears that all three coral species from Bouraké calcified from 19.0% (*A. tenuis*) to 67.3% (*Porites* sp.) more than their counterpart from the Reference site, when maintained at Control pH (Fig. 7a,b). This unexpected finding suggests that Bouraké corals, adapted to OA because they have been exposed to extreme conditions throughout their lives, regain even higher rates of calcification than the same coral counterparts adapted to normal conditions, once at normal pH.

Our experiment has at least two limitations since the corals are isolated from the reef and kept in aquaria,

which is an artificial environment, and exposed to the experimental conditions for a medium-term period, although long enough to overcome the unrealistic shock effect as measured during past experiments (Comeau et al., 2018). With this in mind, the decline in calcification that we measured on corals from the Reference site gives us only a limited estimate of the effect of OA, which however may becomes highly significant when the effect is extrapolated to the long term. Nevertheless, the innovative aspect of our study is that we compared corals adapted to ambient seawater pH with corals likely adapted, or at least fully acclimated, to an extreme and fluctuating environment overlaying future climate scenarios. This study demonstrated that Bouraké corals have become more resilient to OA, likely using plastic and/or assimilated mechanisms (Camp et al., 2020). Brown et al. (2022) incubated coral*Pocillopora damicornis* from both a flat and a sloping reef, where seawater pH variability differed, in aquaria with stable and variable pH. As in our study, they found higher rates of calcification for corals from a more variable environment, probably because they maintained less intracellular pH acidosis, as previously found for other corals (Comeau et al., 2022; Cornwall et al., 2018; Gibbin & Davy, 2014). Clearly, this compensatory mechanism might have a cost. Among the potential hypothese explaining how corals can cope with the additional energy required to maintain calcification such as proton pumping (Guillermic et al., 2021; McCulloch et al., 2012), it is known that corals: (i) might boost their endosymbiotic algal production (Barott et al., 2015; Castillo et al., 2014; Schoepf et al., 2013), (ii) use their energy reserves, such as proteins and lipids (Edmunds & Wall, 2014; Towle et al., 2015), and (iii) increase heterotrophy (Edmunds, 2011; Houlbrèque et al., 2015).

Our data do not consistently suggest that Bouraké corals have acquired a particular mechanism that accounts for better physiological plasticity to cope with low pH conditions, thereby maintaining higher calcification rates. Indeed, we did not find a clear effect of coral origin, through an increase in photosynthetic rates, higher contents of Symbiodiniaceae, chlorophylls, and protein in Bouraké corals. However, this study confirms previous findings on the positive effect of high$p$ CO_2 on photosynthetic rates. It is well known that under acidified conditions, the photosynthetic activity of algal symbionts in coral increases up to exceeds the maximum level of CO_2 consumed by the algae (e.g., Barott et al., 2015; Gattuso et al., 1999; Gibbin & Davy, 2014). Although CO_2-induced photosynthetic fertilization of symbionts may be less effective for *M. digitata* , *A. tenuis* and*Porites* sp. showed higher photosynthesis under OA scenarios compared to the Control condition. This was accompanied by higher ETR_{max}, F_v/F_mvalues, and a better exploitation of lower light intensities for*A. tenuis* . This species also reveals the highest growth rates, which highlights the symbionts involvement in coral growth. Higher productivity was supported by an increased concentration in the Symbiodiniaceae density and/or chlorophylls, but not consistently among species. Moreover, the respiration rates were higher under acidified conditions for *A. tenuis* and *M. digitata* . The high photosynthetic rates are consistent with the beneficial effect of elevated p CO_2 on the productivity of corals living in CO_2 vents in Papua New Guinea (Biscéré et al., 2019). These trends of increasing photosynthetic and respiration rates under OA do not match with the observations made by Jacquemont et al. (2022), for whom OA had no effect on photosynthetic and respiration rates of Bouraké corals, although, unlike our study, they found significantly different rates between Bouraké and the Reference site. Differences between the two studies could be due to the different setup of the two experiments as Jacquemont et al. (2022) measured coral metabolic rates under conditions mimicking the Bouraké environment, at high and low tide, and using seawater collected directly during both tidal periods. We measured the effect of medium-term incubation at different seawater pH on corals from Bouraké and the Reference site, while Jacquemont et al. (2022) tested the effect of a combination of factors, including pH, oxygen and organic matter.

This study also found significant depletion of protein levels in all pH treatments, although this loss remained equivalent between the two corals origins, suggesting an alternative energy source used by Bouraké corals to maintain higher growth rates. Interestingly, *A. tenuis*and *Porites* sp. had lower protein levels under variable pH, suggesting that corals must bear an additional cost to cope with the large change in pH measured at Bouraké (see below). The negative effect of OA on coral protein metabolism could be the result of accelerated proteins catabolism at elevated p CO_2 (Edmunds & Wall, 2014), which can be exacerbated when pH fluctuates over time. The loss of proteins under acidified conditions could also be explained by the species-specific ability of corals to preferentially allocate energy either toward inorganic growth (calcification)

or somatic growth (tissues) when facing elevated p CO_2 (Agostini et al., 2021). We recognize that protein content can only partially describe the change in the coral energy reserves, since lipids and carbohydrates were not measured. However, exposure to higher$p$ CO_2 does not deplete coral tissue of lipids (Grottoli et al., 2018); because the corals were fed once a week with*Artemia salina* nauplii, they were able to enhance their energy storage through heterotrophic inputs to maintain their skeletal growth under high p CO_2 levels (Drenkard et al., 2013; Edmunds, 2011; Houlbrèque et al., 2015). The artificial diet we used, although limited compared to previous studies, may have helped corals maintain appropriate energy expenditure and calcify under acidified conditions, as previously observed (Cohen & Holcomb, 2009; Houlbrèque et al., 2015; Houlbrèque & Ferrier-Pagès, 2009). This is one of the potential limitations of most existing experiments in aquaria, and it limits their ecological value. Indeed, it is difficult, even utopian to think of being able to imitate the natural contribution of zooplankton to the diet of corals in an aquarium. However, all individuals were fed in the same manner and the nutrient levels (i.e., NO_x, PO_4^{3-} and $Si(OH)_4$) measured in the aquaria were quite similar between tanks and throughout the experiment. Thus, the diet, although unnatural, could perhaps explain some of the apparent resistance of corals to OA, but not the different responses between Bouraké and the Reference site.

To what extent do pH fluctuations improve the physiological performances of corals?

One of our hypotheses to explain the success of Bouraké corals was the potential positive effect that diurnal pH fluctuations could have on coral metabolism, as previously found for corals in GBR mangrove lagoons (Camp et al., 2019), St Vincent and the Grenadines CO_2vents (Enochs et al., 2020), and for others incubated in mesocosms (Brown et al., 2022). Our results show that short-term exposure (i.e. acclimation of Reference site corals during our experiment) to fluctuating pH has a negative effect on coral calcification as all Reference site coral species decreased their growth when incubated at variable pH (Fig. 7c). In contrast, Bouraké corals (i.e. acclimatized and/or long adapted to local conditions) exposed to variable pH calcified 7.6% (*A. tenuis*) to 116.2% (*M. digitata*) more than their counterpart from the Reference site. Furthermore Bouraké corals incubated at future and extreme pH maintained these higher growth rates and even increased them when grown under Control conditions, confirming their resilience to OA. Although the duration of our experiment was longer than most OA experiments (i.e., 77% lasted 1-11 weeks; Brown et al., 2022; Ziegler et al., 2021), we acknowledge that the duration of variable pH exposure experienced by corals from the Reference site during the course of the experiment was too short to compare with that experienced by corals at Bouraké. Corals exposed to a variable environment throughout their life are physiologically more plastic than corals adapted to stable environments (Kenkel & Matz, 2017); such plasticity is probably time-dependent. Future experiments should take into account the length of exposure to variable pH (as well as other environmental parameters) that the corals experienced before the experiment.

Does their Symbiodiniaceae community raise the physiology of Bouraké corals?

It is thought that species-specific metabolic responses to environmental stress could be due to different symbiont communities hosted by corals (Barott et al., 2015; Ziegler et al., 2015). Our data suggest that seawater pH level, whether stable or variable, at future or extreme levels, does not affect the Symbiodiniaceae major ITS2 type profiles of corals (at least over a 100-day period), since at the end of the experiment we found no significant effect. Interestingly however, Bouraké corals exhibited more consistent major ITS2 type profiles between replicate colonies at low and variable pH than corals from the Reference site. Microbiome stability (both for symbionts and/or bacteria) was linked to greater physiological resilience to OA (Ge et al., 2021; Grottoli et al., 2018; Quigley et al., 2017, 2019; Ros et al., 2021), suggesting that consistent major ITS2 type profiles for Bouraké corals under pH treatments could facilitate their success; however, further work will be needed to verify this hypothesis.

We observed distinct major ITS2 type profiles between coral species and native habitat (e.g. Bouraké versus Reference site). All coral species in this study were associated with the genus *Cladocopium* (LaJeunesse et al., 2018). We could have expected to find the Symbiodiniaceae genus*Durusdunium* as it is often found in stress-tolerant corals (Haydon et al., 2021; Hoadley et al., 2019; Lajeunesse et al., 2014). However, some species of *Cladocopium* seem to be competitively dominant against *Durusdunium* in a multi stressors environment

such as Bouraké (Barshis et al., 2010; Hume et al., 2016). For example, *Cladocopium* was found to be the dominant genus on corals species such as *Porites lutea* from the mangrove lagoon of Woody Isles (Australia, Camp et al., 2019), and both *Acropora pulchra* and *A. muricata* in Bouraké (Camp et al., 2020). In Bouraké, distinct symbiont type profiles for each coral host species, across environments, support previous hypotheses of species-specific strategies of environmental adaptation (Camp et al., 2020):

(i) *A. tenuis* in Bouraké had a major type profile of C1-C1b-C42.2-C1c-C1bh-C1br-C1cb. Corals associated with C1 as a major ITS2 sequence have been found across many environments (LaJeunesse et al., 2003), and exhibit apparent capabilities to adapt to locally stressful and/or fluctuating environments (Howells et al., 2012; Ng & Ang, 2016; Schoepf et al., 2015). Corals from Bouraké exhibited lower Symbiodiniaceae density than the Reference corals, but had higher photosynthetic efficiency. Corals associated with *Cladocopium goreaui* (C1) have previously shown elevated photosynthetic efficiency (Cantin et al., 2009; Morgans et al., 2020). It is therefore possible that elevated photosynthesis is a common trait within some taxa from the C1 radiation and may explain the higher photosynthetic efficiency mechanism of C1 to face OA as observed by Ge et al. (2021) in*Acropora valida* . The elevated photosynthetic capacity of *A. tenuis* in Bouraké, coupled with the dominance of major ITS2 sequence C1, could explain their significantly higher growth rates, as previously observed in *A. tenuis* juveniles (Cantin et al., 2009; Little et al., 2004). The C1 ITS2 sequence was still dominant for some colonies of*A. tenuis* from the Reference site (albeit different ITS2 type profiles that suggest different species), but in most instances, ITS2 sequences from the C3 radiation (i.e., C3k and C3bo) were most abundant; these are not among the most efficient in photosynthetic capabilities (Hoadley et al., 2016). We note the uncommon prevalence of C15 in*A. tenuis* (Fig. S7). We are confident from our negative control gels that this is real (Fig. S8), and could perhaps result from expelled symbionts from the other coral taxa during the stress experiment. Further work would need to validate this hypothesis.

(ii) *Porites* sp. from both Bouraké and the Reference site maintained an association with*Cladocopium* of the C15 radiation, but there were more numerous C15 major ITS2 type profiles at the Reference site. Interestingly, discrete C15 genotypes (distinct ITS2 type profiles) were observed across habitats (i.e., Extreme vs Reference) in *Porites lutea*from Bouraké (Camp et al., 2020) and from a mangrove lagoon on the Great Barrier Reef (Camp et al., 2019). This is in agreement with the recent finding of Hoadley et al. (2021) demonstrating that within C15, diverged lineages exist for *Porites* sp. living in different reef habitats and that it brings physiological differences.

(iii) M. digitata from Bouraké had nine major ITS2 type profiles, with C15/C15vi-C15vj-C15f-C15he the most common. *Cladocopium* of the C15 radiation are considered physiologically resistant (Fisher et al., 2012; Fitt et al., 2009; Nitschke et al., 2018). The same C15 major type profile was also found for the Reference corals, alongside C73-C73a-C21. Coral association with C73 is rarely found and appears to be present according to relative light levels (Stat et al., 2008). With this in mind and according to the equivalent light irradiance measured between tanks in our experiments, we could assume that light regimes of the native environment of corals modified their Symbiodiniaceae community associations. For instance, turbid environments such as Bouraké have been observed to limit the diversity of the associated symbiont community and could lead to predominant local adaptive genotypes (Smith et al., 2020). While it is beyond the intent of this study to determine which environmental drivers shape symbiotic communities, our data supports the growing body of evidence (Camp et al., 2020; Howells et al., 2016; Ziegler et al., 2017) that corals from distinct environments often have unique symbiotic partners that could be crucial to support their survival.

While we cannot define the role of each Symbiodiniaceae ITS2-profile in the coral stress response, the difference in symbiotic partners' genotypes between sites highlights how important local environmental conditions are and how symbiotic stability (fidelity) versus flexibility ultimately enhances holobiont resilience (Epstein et al., 2019; Grottoli et al., 2018; Putnam et al., 2012).

In conclusion, we show under that OA scenarios tested here, corals living their entire lives in an extreme and variable environment somehow have systematically higher calcification rates than conventional reef corals. Although physiological measurements did not reveal significant differences between coral's origin, Bouraké corals have displayed more consistent Symbiodiniaceae communities. Ultimately, upcoming studies should

explore the effects of the exposure duration (i.e., short vs long-term) to variable pH on coral biomineralization mechanisms, which already appear to differ between more stable and fluctuating environmental conditions of pH (Comeau et al., 2022). One might believe that the different dynamics of symbiotic partners greatly influence calcification processes. The use of isotopic tools in the coral skeleton would help that way, via tracing and defining the inputs of specific symbiotic partners into biomineralization mechanisms.

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Author Contributions

CT and RRM conceived and designed the project. Sampling, material preparation, aquaria experiment and data collection were performed by CT and RRM. CT conducted the data analysis. EFC contributed to metagenomic data analysis, interpretation and funding. CT led the writing of the manuscript in collaboration with all co-authors.

Data Availability Statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author/s.

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