pH variability at volcanic CO 2 seeps regulates coral calcifying fluid chemistry

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running title: Coral calcifying fluid chemistry at CO_2 seeps

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Abstract

Coral reefs are iconic ecosystems having immense ecological, economic and cultural value, but globally their carbonate-based skeletal construction is threatened by ocean acidification. Identifying coral species that have specialised mechanisms to maintain high rates of calcification in the face of declining seawater pH is of paramount importance to predicting future species composition, and growth of coral reefs. Here, we studied multiple coral species from two distinct volcanic CO_2 seeps in Papua New Guinea to assess their capacity to control their calcifying fluid chemistry. Several coral species living under conditions of low mean seawater pH but with either low or high variability in seawater pH were examined and compared with those living under 'normal' (non-seep) ambient seawater pH. We show that when mean seawater pH is low but highly variable, corals have a greater ability to maintain constant pH_{cf} in their calcifying fluid, but this characteristic was not linked with changes in abundance. Under less variable low seawater pH, corals with limited reductions in pH_{cf} at the seep sites compared to controls tended to be more abundant at the seeps site than at the control site. However, this finding was strongly influenced by a single species (*Montipora foliosa*), able to maintain complete pH_{cf} homeostasis. Overall, while our findings indicate that there might be an association between ecological success and greater pH_{cf} homeostasis, further research with more species and at more sites with differing seawater pH regimes is required to solidify inferences regarding coral ecological success under future ocean acidification.

Keywords

Calcifying fluid, ocean acidification, abundance, coral, Dissolved inorganic carbon, Coral reefs, Papua New Guinea, Calcification

Introduction

Ocean acidification (OA) is caused by a shift in ocean carbonate chemistry resulting from increased atmospheric CO_2 concentrations, and is one of the major threats to the future of coral reefs (Hoegh-Guldberg et al., 2017). Declining seawater pH and altered relative concentrations of the different forms of dissolved inorganic carbon are expected to reduce the capacity of corals, the main reef-building taxon, to precipitate calcium carbonate (Kleypas & Yates, 2009). Indeed, laboratory experiments demonstrate that decreases in pH expected by the end of the century will cause an average ~ 15-20% decrease in coral calcification (Chan & Connolly, 2013; Cornwall et al., 2021). However, coral responses are highly species-specific, with some species being more resistant to OA, while others are highly sensitive (Comeau, Cornwall, DeCarlo, et al., 2019; Comeau et al., 2014a; Kornder et al., 2018). Lack of understanding of why certain species are resistant to OA, while others are not, limits reliable projections of how the species composition and ecological functioning of reefs is likely to change in the future.

The physiological mechanisms that control species' capacity to tolerate OA are still unclear. Coral calcification is a key physiological and ecological process that enable corals to form large three-dimensional structures. New coral skeleton made of aragonite is formed via biomineralization of the calcifying fluid (CF) that lies between aboral coral tissues layers and the existing calcium carbonate skeleton (Tambutté et al., 2011). To form their skeleton, corals have the ability to modify the chemical conditions of the calcifying fluid to facilitate the mineralization process. In the CF, pH is maintained at values well above that in seawater (pH_{cf} ~ 8.2–8.9, McCulloch et al., 2012; Venn et al., 2019), and the dissolved inorganic carbon (DIC) is increased to values about 1.5–2 times higher than in ambient seawater (Sevilgen et al., 2019). As a result, the saturation state of calcium carbonate in the CF is elevated to values that thermodynamically favor its precipitation (i.e., Ω_{cf} ~ 12, DeCarlo et al., 2017), which is being catalyzed by a set of organic molecules (e.g., CARPs, Drake et al., 2018; Mass et al., 2013). Decreasing seawater pH under OA generally decreases pH_{cf} (Comeau et al., 2017; Holcomb et al., 2014; Venn et al., 2013). Similarly, increasing seawater DIC under OA elevates DIC_{cf} (Comeau et al., 2018). While this increase in DIC_{cf} could partially alleviate the negative effects of decreasing pH_{cf} (Cornwall et al., 2018; Schoepf et al., 2017) large uncertainties exist in the magnitude and

physiological controls of these effects. Physiological compensating mechanisms under OA, which are being used to maintain optimal conditions within the CF (i.e., pH homeostasis vs DIC upregulation vs calcium upregulation), are species-specific and can be modulated by environmental conditions (Comeau, Cornwall, Pupier, et al., 2019). In this study we quantify the capacity of different coral species to control their pH_{cf}, and assess whether and how this capacity changes among locations subject to either stable or variable seawater pH.

Both the average pH of seawater, and the magnitude of pH variability, have been suggested to modulate the response of marine organisms to OA on coral reefs (Rivest et al., 2017). However, a large range of coral responses to treatments with different levels of pH variability has been reported in laboratory experiments, ranging from no measurable impacts (Camp et al., 2016) to positive offsets against OA (Comeau et al., 2014b). This range of impacts could arise due to species-specific responses to pH variability, but also because of differences among studies in the frequency and magnitude of pH fluctuations used in the experiments. To date, only one laboratory study has specifically addressed the effect of a regular diel pH variability on the CF of corals and coralline algae, where mean seawater pH was the main driver of the CF chemistry (Cornwall et al., 2018). However, resolving these apparently conflicting results requires an understanding of the physiological mechanisms involved in regulating the composition of the CF, and the factors that constrain those mechanisms.

Field observations at naturally acidified sites such as volcanic CO_2 seeps, semi-enclosed lagoons, and upwelling regions provide unique opportunities to investigate the effects of ocean acidification and pH variability on time scales that cannot be matched by laboratory experiments (i.e., years to decades). At most of these sites, the pH level, alongside other abiotic parameters, fluctuate around mean pH values similar to those predicted for the global ocean by the end of this century (pH ~ 7.7-7.8, Fabricius et al., 2011; Teixidó et al., 2020). So far, contradictory results describing the effects of OA on benthic marine calcifying taxa have emerged from these naturally acidified sites. For instance, in a semi-enclosed lagoon in New Caledonia with persistent low pH (mean pH ~ 7.6), coral communities are diverse and some species can maintain calcification rates as high as the ones from control sites (Camp et al., 2017). In contrast, deleterious effects of decreasing pH on the physiology, abundance, and diversity of calcareous organisms were reported at seep

sites in Papua New Guinea (Fabricius et al., 2011, 2017). Specifically, at the CO_2 seeps in Normanby, naturally acidified reef areas have reduced species diversity and evenness compared to control sites, and were reported to be mostly dominated by resistant species such as massive *Porites* (Fabricius et al., 2011). This dominance of *Porites* spp. could be due to its capacity to maintain elevated pH_{cf} under a large range of seawater pH, as demonstrated both *in situ* (Wall et al., 2016; Wall et al., 2019a) and *ex situ* (Comeau, Cornwall, DeCarlo, et al., 2019). Furthermore, the ability to elevate DIC_{cf} under low pH could be an additional mechanism that favours the presence of certain coral species in naturally acidified sites (Wall et al., 2019a; Wall et al., 2019b). Both mechanisms lead to constant Ω_{cf} in corals from low pH conditions.

Some locations with naturally-occurring low pH conditions host abundant and diverse hard-coral assemblages, such as in Palau (Barkley et al., 2017; Golbuu et al., 2016; Shamberger et al., 2018), Papua New Guinea (Pichler et al., 2019), West Australia (Dandan et al., 2015; Schoepf et al., 2015), New Caledonia (Camp et al., 2017), and the Virgin Islands (Yates et al., 2014). At one of the CO_2 seep sites in Papua New Guinea (Upa-Upasina, Normanby), more than 100 coral species were observed near the seep during our expeditions (Hoogenboom M. and Rodolfo-Metalpa R., Pers. Obs.) coexisting with the dominant mound-shaped massive *Porites* colonies. This high species richness under OA conditions was confirmed also at the CO_2 seeps of Ambitle Island, where around 100 species were found in a large, acidified bay in addition to massive *Porites* spp. (Shlesinger T. and Rodolfo-Metalpa R., Pers. Obs.). Previous studies have shown that corals cannot acclimatise within one year to low pH conditions, and that the ability to resist changes in pH_{cf} under low seawater pH (i.e., pH homeostasis) is a species-specific inherent trait (Comeau, Cornwall, DeCarlo, et al., 2019). Therefore, environments with regular high pH could promote species that calcify at enhanced rates during periods of elevated pH. Accordingly, we hypothesize that corals living in consistently low pH their entire lifetime will show stronger control over their calcifying fluid chemistry than corals living under variable or different pH regimes.

While laboratory research has enabled us to understand more of the physiological mechanisms responsible for resistance to low pH, they are unable to provide information regarding how or if this translates to ecological success under OA. Here, we aimed firstly to understand whether changes in mean seawater pH and in the magnitude of variability in seawater pH affects the control of coral calcifying

fluid in the field for multiple species. Secondly, we aimed to explore whether the capacity of species to better regulate CF chemistry is correlated to species relative abundances at sites with different pH and DIC conditions. To that end, we utilised two natural CO_2 seeps locations with distinct pH characteristics. Using physiological measurements and field observations of coral species abundances, we tested three complementary hypotheses: 1) corals growing in acidified sites have the capacity to maintain chemical conditions optimal for calcification in their calcifying fluid (i.e., pH_{cf} homeostasis), 2) the most abundant corals in acidified sites are the ones with the best control on their calcifying fluid, and 3) seawater pH variability will alter both ecological outcomes and corals' CF chemistry.

Materials and Methods

Study sites, surveys, and sample collections

Two reefs surrounding CO_2 seeps and adjacent control reefs (i.e., sites with ambient conditions) in Papua New Guinea were repeatedly visited between September 2016 and October 2019: Upa-Upasina Reef (Normanby Island, Milne Bay Province) and Tutum Bay (Ambitle Island, New Ireland Province) (Fig. 1).

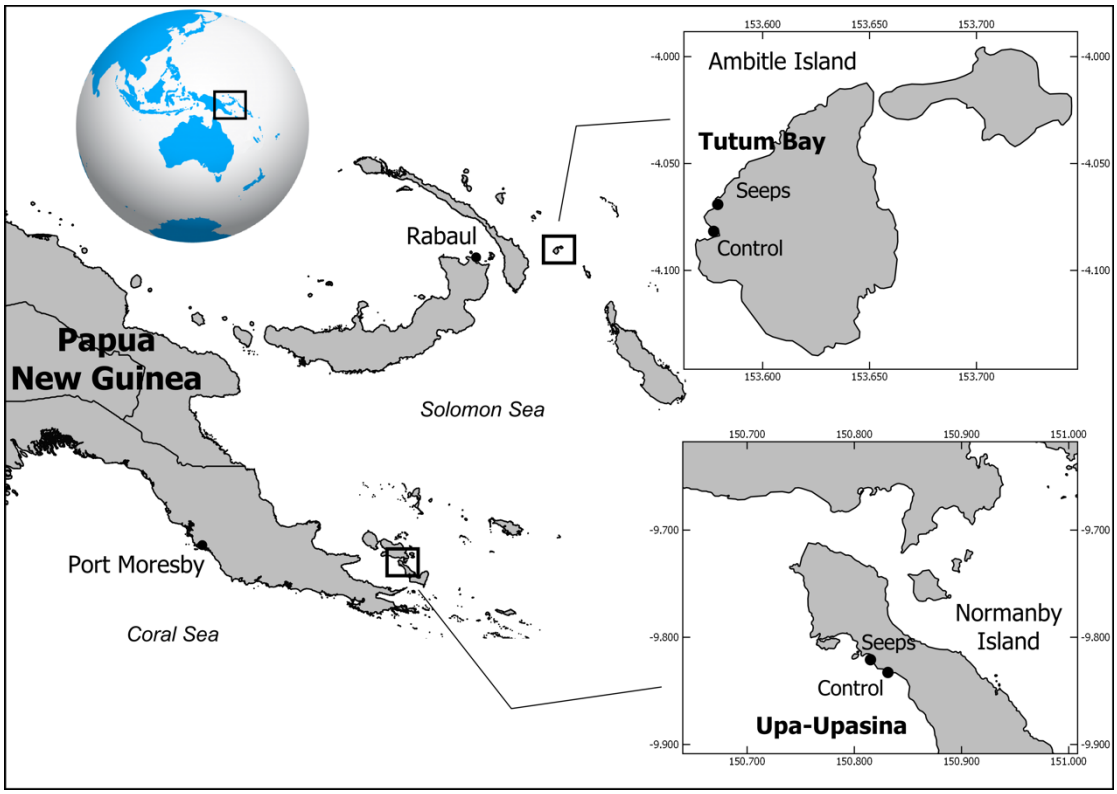


Figure 1. Maps showing the sampling and surveyed sites. Corals were collected from Ambitle Island and Normanby Island at both control sites and seeps sites in Tutum

Bay in Ambitle and Upa-Upasina Reef in Normanby (map redrawn from Biscéré et al. 2019).

The seawater carbonate chemistry of the seep areas, and of the adjacent control reefs, at both locations was characterized continuously during each of the seven 10-day trips performed between 2016 and 2019 (i.e., 4 trips to Ambitle and 3 trips to Normanby). Most of the seawater carbonate chemistry data from Normanby has been already reported (Fabricius et al., 2011; Fabricius et al., 2014), and several subsequent studies (e.g., Fabricius et al., 2017). Most of these studies were conducted on the same reef areas as visited during this study, and where hundreds of discrete water samplings verified that the median pH was close to 7.8 pH_T units as projected for 2100. However, only a limited number of studies to date have logged pH at high frequency to characterise pH variability at the Normanby study site (Fabricius et al., 2014; Smith et al., 2017; Uthicke et al., 2016). The seeps site in Ambitle Island was only recently studied and an exhaustive dataset of seawater physical and chemical conditions have been published (Biscéré et al., 2019; Pichler et al., 2019).

During our seven field works at the Normanby and Ambitle seeps, we measured the extent to which pH fluctuated at the study sites because of its potential importance in affecting the coral ability to control their calcifying fluid chemistry. At both Normanby and Ambitle seeps, and at the reference (control) sites at each location, we used three pH loggers (SeaFET V2, Sea-Bird Scientific, Bellevue, WA 98005, USA) recording every 10 minutes (i.e., Tutum Bay in Ambitle; Fig. 1 in Pichler et al., 2019; see all Supplementary Data, ttps://ars.els-cdn.com/content/image/1-s2.0-S0025326X1830780X-mmc1.pdf). At each seeps site, we collected continuous pH data for a duration of 10 days at each of 4 fixed stations located in the area where the coral samples were collected, and an additional 20 24-h measurements all around the seeps area. Seawater samples were filtered through 0.45-µm Whatman filters using a Nalgene vacuum system and stored at 4 °C in the dark for further testing at the Institut de Recherche pour le Développement (IRD) in New Caledonia. Total alkalinity (A_T) was determined using an auto titrator (TIM865 Titralab, Radiometer). Three replicate 20 mL sub-samples were analysed at 25 °C using an open cell potentiometric method. Total alkalinity was calculated from the Gran function applied to pH from 4.2 to 3.0, as mEq L^{-1} from the slope of the pH *vs* HCl curve. Results were corrected against A_T standards provided by A.G. Dickson

(batch #155). Parameters of the carbonate system were calculated from pH_T (median, 5th and 95th percentile); median A_T, temperature and salinity (34) using the R package seacarb. A full description of the carbonate chemistry at the two locations and sites is presented in the Table S1.

During the expedition of 2016, we collected fragments of 14 and 8 coral species at Normanby Island and Ambitle Island, respectively, from both the seeps and control sites (CITES collection permits n. 016132 and 017027). The 14 species sampled in Normanby Island were: *Acropora cytherea, Acropora millepora, Acropora samoensis, Acropora tenuis, Favites halicora, Favites pentagona, Galaxea fascicularis, Merulina ampliata, Pachyseris speciosa, Pocillopora verrucosa, Porites rus, Seriatopora caliendrum, Tubastraea* sp.*,* and *Turbinaria reniformis*. The 8 species sampled in Ambitle Island were: *Acropora nana, Acropora tenuis, Echinopora lamellosa, Galaxea fascicularis, Montipora foliosa, Pocillopora damicornis, Porites lutea, Psammocora contigua*. For each species, one branch tip or a piece of skeleton (3–7 cm long) was collected from three spatially separated colonies (5–20 m distant) using a bone cutter or a hammer and chisel for branching or massive and foliose species, respectively. Coral tissues were removed using high pressure water and they were dried in air over 48 h before being carefully preserved in individual bags.

To quantify the abundance of the focal species mentioned above, field surveys were performed in May 2017 in Normanby Island and in June 2018 in Ambitle Island. We used 10 x 1 m belt transects haphazardly positioned at a depth range of ~2–10 m at both seeps and control sites in each location. Every colony identified as one of the studied species (except for massive or encrusting *Porites* that were grouped as *Porites* spp.) was counted along 15 belt transects at each site in Normanby Island, and along 18 belt transects at each site in Ambitle Island, and the species abundances are presented as the number of colonies per transect.

Analyses of calcifying fluid pH and DIC

Calcifying fluid pH (pH_{cf}) and dissolved inorganic carbon (DIC_{cf}) were estimated respectively using the $\delta^{11}B$ proxy method (Trotter et al., 2011) and the $\delta^{11}B$ and B/Ca method (Holcomb et al., 2016; M. T. McCulloch et al., 2017). Fragments of coral colonies were collected from 3 to 4 colonies per species, and the tissues were removed by water pressure before being sun dried. The skeletons were then shipped to the

University of Western Australia, where they were cleaned with mQ water, bleached for 24 hours in 6.25 % NaClO to remove any tissue left at the surface of the samples, and dried in a drying oven for 48 h at 50 °C.

For branching corals (e.g., *Acropora*, *Seriatopora*, *Pocillopora*), geochemical analyses were performed on a ~ 1 cm long piece of skeleton from the tip of each of three branches per colony. The three tips were then crushed together to smooth potential differences in calcifying fluid chemistry within colonies and were considered as one sample (for a total of three samples per species and condition). For massive corals (e.g., *Favites*, *Galaxea*), a fragment of 0.5 cm^3 located ~ 0.5 cm below the surface was selected using a dental drill to avoid the area of the skeleton where tissues were present (first mm of the skeleton). For the foliose corals (e.g., *Echinopora*, *Pachyseris*, *Turbinaria*), a section of the skeleton close to the growing edge of laminae of the colonies was sampled using cutting pliers. The selected portions of skeletons were bleached for an additional 24 hours in 6.25 % NaClO and then rinsed three times with mQ water to remove any residual traces of organic matter and bleach. Bleached samples were dried for 48h at 50°C. Skeleton samples from all colonies were then crushed in a mortar with a pestle to powder prior to analysis.

In the clean laboratory of the Advanced Geochemical Facility for Indian Ocean Research (AGFIOR, University of Western Australia), 10 mg of powdered skeleton from each coral sample was prepared for dissolution and dilution to 10-ppm Ca solutions. The 10 mg samples were dissolved in 0.51 N HNO_3, and the boron was quantitatively separated on ion exchange columns. $\delta^{11}B$ was measured on a multicollector inductively coupled plasma mass spectrometer (NU II). Measurements of the international carbonate standard JCP-1 yielded a mean value of 24.43 ± 0.08 ‰ (mean ± SE, n = 10), which was similar to the 24.33 ± 0.11 ‰ (SE) reported previously. pH_{cf} was estimated from $\delta^{11}B$ using the calculations described by Trotter et al. (2011), as:

$$pH_{cf} = pK_B - \log\left[\frac{(\delta^{11}B_{SW} - \delta^{11}B_{carb})}{\left(\alpha_{(B3-B4)}\delta^{11}B_{carb} - \delta^{11}B_{SW} + 1000\,(\alpha_{(B3-B4)} - 1)\right)}\right] \quad (1),$$

where pK_B is the dissociation constant dependent on temperature and salinity as measured at the site of coral collection, $\delta^{11}B_{sw}$ = 39.61(Foster et al., 2010), and $\alpha_{(B3\text{-}B4)}$ is the boron isotopic fractionation factor for the pH dependent equilibrium of the borate ($B(OH)_4^-$) relative to the boric acid ($B(OH)_3$) species in the calcifying fluid, with a value of 1.0272 (Klochko et al., 2006).

B/Ca ratios were determined on the same aliquot of the solution used for $\delta^{11}B$. Both B/Ca and $\delta^{11}B$ were utilized to determine $[CO_3^{2-}]$ and then [DIC] at the site of calcification $[DIC]_{cf}$ following (McCulloch et al., 2017). Estimates of carbonate ion concentrations in the calcifying fluid were calculated using the following equation:

$$[CO_3^{2-}]_{cf} = K_D[B(OH)_4^-]_{cf}/\left(^{B}/_{Ca}\right)_{CaCO_3} \quad (2),$$

where $K_D = K_{D.0}\exp(-k_{KD}[H^+]_T)$ with $K_{D.0}= 2.97 \pm 0.17\times10^{-3}$ (± 95% CI) and $k_{KD} = 0.0202 \pm 0.042$. The concentration of DIC_{cf} was then calculated from estimates of pH_{cf} and $[CO_3^{2-}]_{cf}$.

T-tests were used to assess differences in the estimates of pH_{cf} and DIC_{cf} between the seeps and the control samples at both locations, with locations analysed separately. T-tests were used separately for each species because we were interested in whether there is a CO_2 effect for each species rather than an effect between species. All of the analyses and visualizations were done in R v4.0.2. All data are presented as mean ± SE. The data that support the findings of this study are archived in the Pangaea database (https://doi.pangaea.de/10.1594/PANGAEA.939651).

Relationship between coral abundance and carbonate chemistry

We used two complementary approaches to determine if the most abundant coral species at the seeps sites were the species with the best control on their calcifying fluid. First, we investigated the relationships between coral abundance at the seeps sites and corals pH_{cf} or DIC_{cf}. Second, to consider the differences in species abundance between the seeps and control sites, we calculated the proportional change in their abundance at the seeps relative to the control at each location. We then examined the relationship between the differences in the corals' abundance among sites and their calcifying fluid carbonate chemistry using a linear regression. Differences for each species in mean abundance between the sites were calculated as the relative change in mean abundance as follows:

Relative change = (mean abundance in the seeps – mean abundance in the control) / mean abundance in the control

Principal Component Analyses were used to evaluate how relative change in mean abundance, vent pH_{cf} and DIC_{cf}, delta pH_{cf} (pH_{cf} vent – pH_{cf} control) and delta DIC_{cf} (pH_{cf} vent – pH_{cf} control) were correlated.

Results

Seawater pH variability

All seawater carbonate chemistry and seawater pH variability measured at fixed stations are in Supplementary Table S1. Ambient mean pH values were 8.01 and 7.96 in the control sites, and 7.64 and 7.51 in the seeps sites in Ambitle and Normanby, respectively (Fig. 2). pH variability was considerably larger at the Normanby seep site where the pH dropped down as low as 6.64 pH units. During the entire time frame of pH logging, corals were exposed to low pH conditions (i.e., pH ranging between 7.6–7.8) for 60% of the time at Ambitle, and for 31% of the time at Normanby. Very low pH values (i.e., pH < 7.6) were less frequent in Ambitle than in Normanby (24% and 43% of the time, respectively). Similarly, high pH values (> 7.8) were less frequent in Ambitle than in Normanby (16% and 26% of the time, respectively).

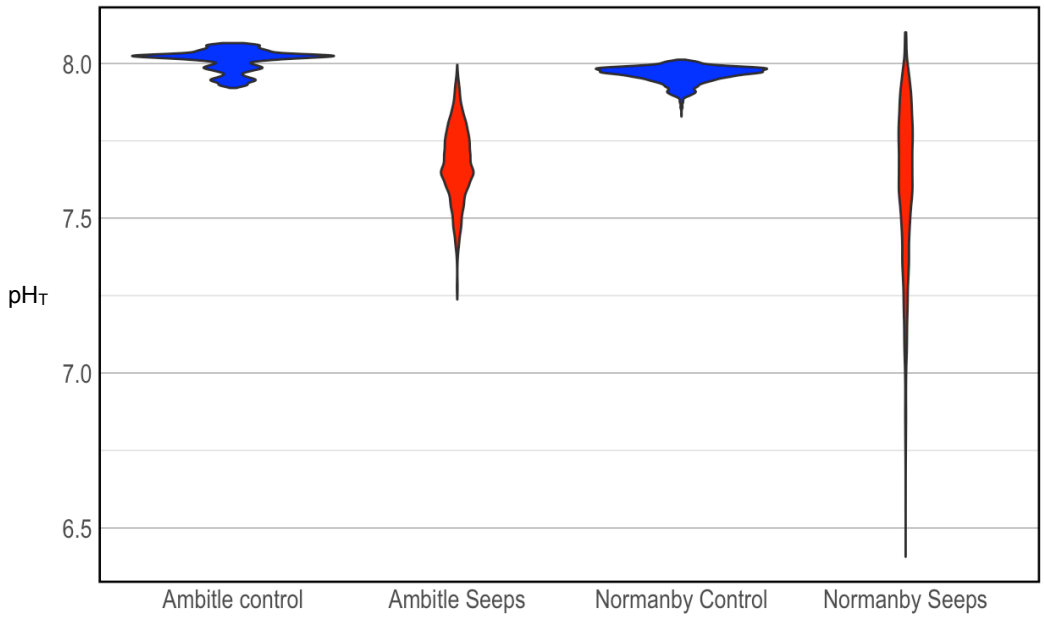


Figure 2. Violin plot showing the *in situ* pH (n > 15,000 for each site) measured using autonomous pH sensors SeaFET at both Ambitle and Normanby seeps and respective control sites during fieldwork in September 2016 and May 2017.

pH of coral calcifying fluid

Across the 14 coral species studied in Normanby average pH_{cf} was higher in the control site (8.43 ± 0.02, mean ± SE, n = 42) than in the seeps site (8.39 ± 0.02, mean ± SE, n = 42, t-test, $p < 0.05$). The highest pH_{cf} was observed in *Galaxea fascicularis* at the control site (8.57 ± 0.01, n = 3), while the lowest was measured in *Favites halicora* at the seeps site (8.27 ± 0.01). pH_{cf} was significantly lower at the seeps site compared with the control site in five species (Fig. 3A; t-test, $p < 0.05$ for all five), with delta pH_{cf} the lowest in *Acropora samoensis* (-0.15, Fig. 3B). There was no difference in pH_{cf} between sites for eight species, while pH_{cf} was higher at the seeps site in *Seriatopora caliendrum* (Fig. 3A; t-test, $p < 0.001$), with a positive delta pH_{cf} of 0.07 (Fig. 3B).

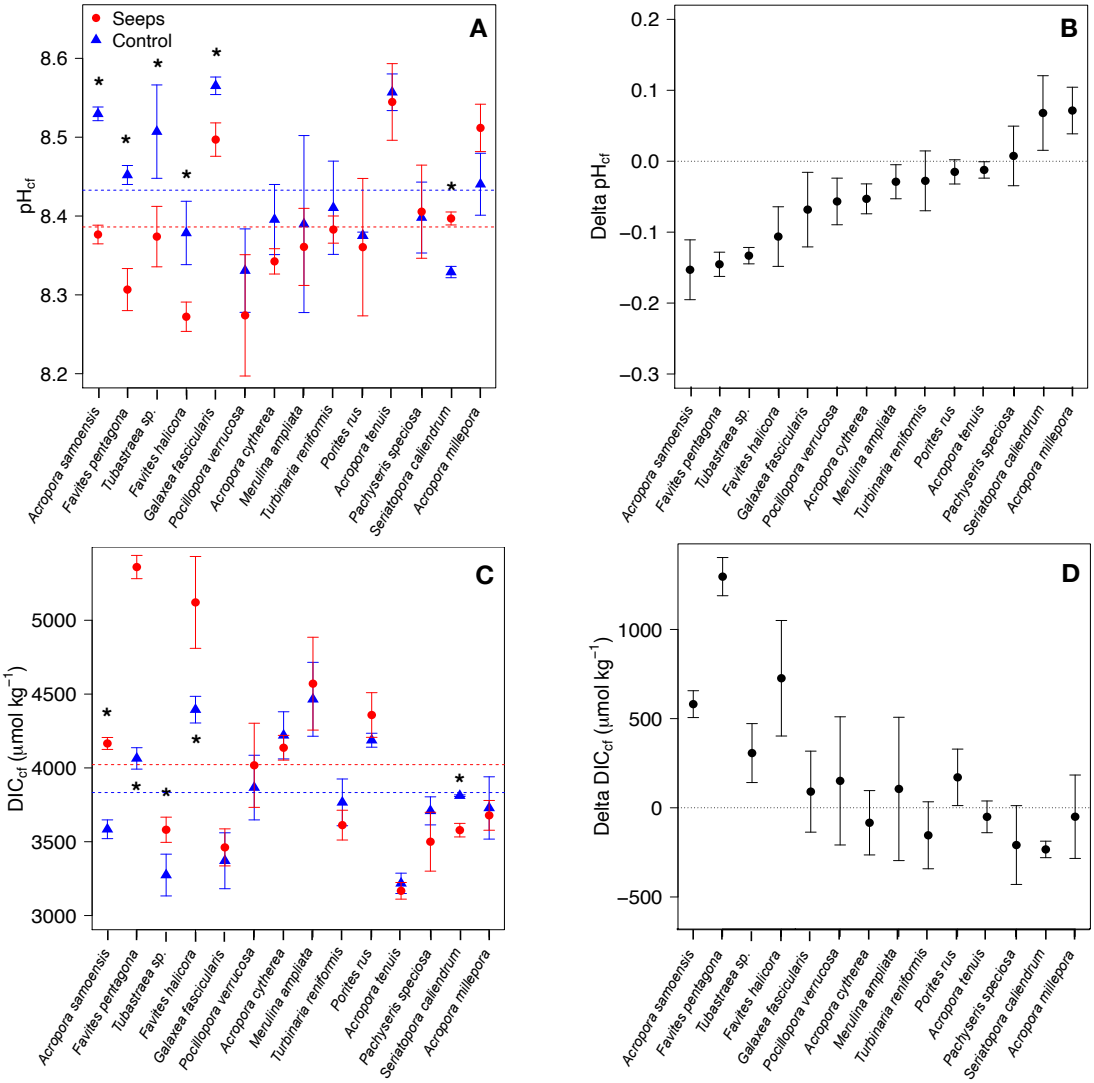


Figure 3. Calcifying fluid carbonate chemistry estimates of 14 coral species from the control and seeps sites in Normanby Island. A) pH of the calcifying fluid (pH_{cf}); B) Difference in mean pH_{cf} between control and seeps sites; C) Dissolved inorganic

carbon at the site of calcification (DIC_{cf}); and D) Difference in mean DIC_{cf}. Blue and red colours indicating the control and seeps data, respectively. Dashed lines in (A) and (C) represent the pooled pH_{cf} and DIC_{cf} mean across all species in each site. Asterisks indicating species in which significant differences were found. All data presented as mean ± SE, with n = 3.

In Ambitle, for the 8 species pooled together, pH_{cf} was on average significantly higher (t-test, $p < 0.05$) in the control site (8.46 ± 0.02, n = 24) than in the seeps site (8.35 ± 0.02, n = 24). The highest pH_{cf} was measured in *Acropora tenuis* at the control site (8.54 ± 0.04), while the lowest was found in *Echinopora lamellosa* at the seeps site (8.23 ± 0.03). pH_{cf} was higher in the control site compared with the seeps site in all species (t-test, $p < 0.05$ for all) but one, *Montipora foliosa*, which showed no differences (Fig. 4A). As a result, delta pH_{cf} which is equal to $pH_{cf\ seeps} - pH_{cf\ ambient}$ varied between -0.19 in *Echinopora lamellosa* and -0.02 in *Montipora foliosa* (Fig. 4B).

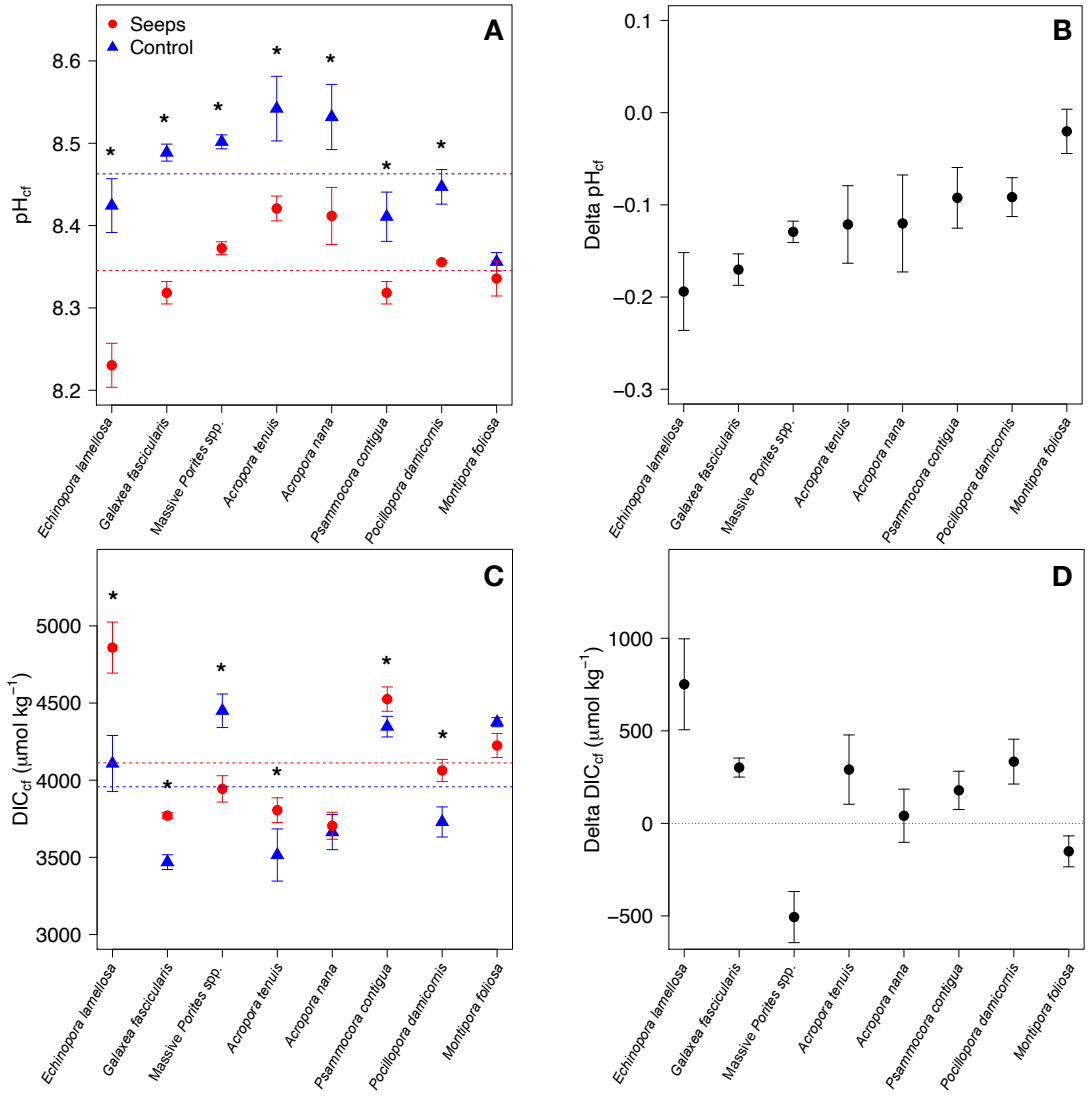


Figure 4. Calcifying fluid carbonate chemistry estimates of 8 coral species from the control and seeps sites in Ambitle Island. A) pH of the calcifying fluid (pH_{cf}); B) Difference in mean pH_{cf} between control and seeps sites; C) Dissolved inorganic carbon at the site of calcification (DIC_{cf}); and, D) Difference in mean DIC_{cf}. Blue and red colours indicating the control and seeps data, respectively. Dashed lines in (A) and (C) represent the pooled pH_{cf} and DIC_{cf} mean across all species in each site. Asterisks indicating species in which significant differences were found. All data presented as mean ± SE, with n = 3.

Dissolved inorganic carbon in coral calcifying fluid

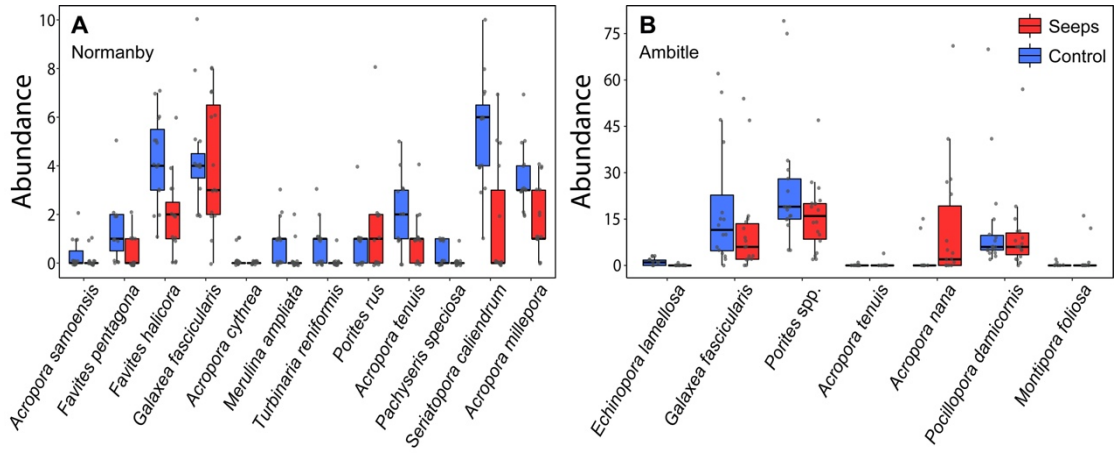
In Normanby, for the 14 species pooled together, mean DIC_{cf} was 3833 ± 105 µmol kg^{-1} and 4022 ± 173 µmol kg^{-1} at the control and seeps site (n = 42 for both), respectively, with no statistical differences (t-test, p = 0.268). DIC_{cf} was more

elevated at the seeps site compared with the control site in four species (*A. samoensis*, *F. pentagona*, *Tubastraea* sp., and *F. halicora*; t-test, $p < 0.05$ for all), while it was more elevated in the control site for *S. caliendrum* (t-test, $p < 0.05$). There was no difference in DIC_{cf} between sites in the nine other species (Fig. 3C). There was a large range of species-specific delta $DIC_{cf} = DIC_{cf\ seeps} - DIC_{cf\ ambient}$ with the maximal increase in delta DIC_{cf} at the seeps found in *Favites pentagona* (1200 μmol kg^{-1}, Fig. 3D). In contrast, delta DIC_{cf} 233 μmol kg^{-1} at the seeps site in *Seriatopora caliendrum* (Fig. 3D), which was also one of the only species with higher pH_{cf} at the seeps.

Across the 8 coral species studied in Ambitle, mean DIC_{cf} was 3957 ± 144 μmol kg^{-1} and 4112 ± 143 μmol kg^{-1} at the control and seeps site (n = 24 for both), respectively, with no statistical differences (t-test, $p = 0.206$). DIC_{cf} was more elevated at the seeps site compared with the control sites in five species (*E. lamellosa, G. fascicularis, A. tenuis*, *Psammocora* sp., and *P. damicornis*; t-test, $p < 0.05$ for all), while it was lower at the seeps sites for only massive *Porites* spp. (t-test, $p < 0.05$) despite lower pH_{cf} also found at the seeps. There was no difference between sites in DIC_{cf} for *Acropora nana* and *Montipora foliosa* (Fig. 4C). The delta DIC_{cf} ranged from -506 μmol kg^{-1} in massive *Porites* spp. to 751 μmol kg^{-1} in *Echinopora lamellosa* (Fig. 4D).

Coral abundance and calcifying fluid chemistry

In Normanby, most of the studied species were either similarly abundant (absolute abundance) in both sites or more abundant at the control site than at the seeps site (Fig. 5A). By contrast, this pattern was not observed in Ambitle (Fig 5B), where three species were more abundant at the control site than at the seeps site (*G. fascicularis*, *P. damicornis*, and massive *Porites* spp.) but two other species were more abundant at the seeps site than at the control site (*A. nana* and *M. foliosa*). Although being relatively rare in Ambitle, two more species had opposing abundances: *A. tenuis* was more abundant at the seeps site than at the control site (0.22 ± 0.9 and 0.06 ± 0.2 mean abundance per transect, respectively) while *E. lamellosa* was more abundant at the control site than at the seeps site (1 ± 1.1 and 0.05 ± 0.2 mean abundance per transect, respectively).

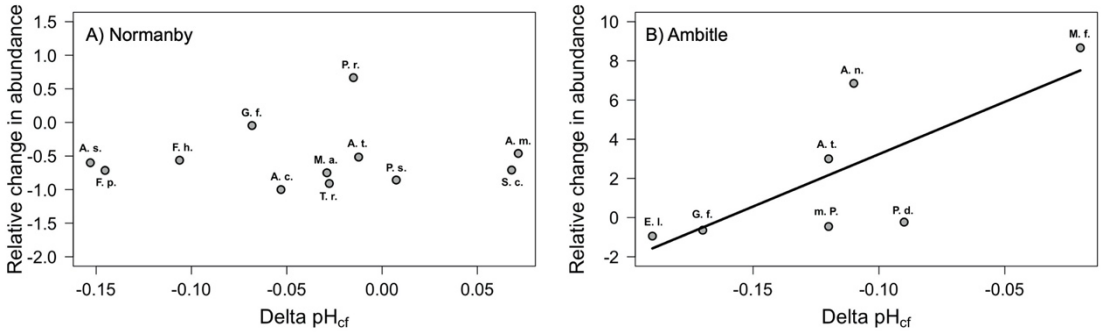


405

406 **Figure 5.** Abundance of the studied species in A) Normanby Island; and, B) Ambitle
407 Island. The abundance is presented as the number of colonies per belt transect with
408 points indicating individual belt transect data (n = 15 at each site in Normanby, and n
409 = 18 at each site in Ambitle). To aid the visualization of panel B, three outlying data
410 points were excluded. Two of these points were values > 100 for *G. fascicularis* in the
411 control and the third was a value > 80 for *Porites* spp. in the control. Blue and red
412 colours indicating the control and seeps data, respectively. Center lines of the box
413 plots indicate the medians, boxes indicate the lower and upper quartiles, and whiskers
414 indicate 1.5x interquartile range.

415

416 There was no relationship between delta pH_{cf} or delta DIC_{cf} and the abundance
417 of corals at the seeps sites of both Normanby and Ambitle (Fig. S1) (Table S2).
418 Similarly, there was also no significant relationship between delta pH_{cf} and the
419 relative change in species abundance between the seeps site and the control site in
420 Normanby (Fig. 6A). By contrast, a relationship between delta pH_{cf} and the relative
421 change in abundance was found in Ambitle (Fig. 6B; linear regression, p-value of the
422 slope = 0.05, p-value of the intercept = 0.02, $R^2 = 0.55$), although it was appears to be
423 largely driven by one species, *M. Foliosa*. The delta DIC_{cf} had no significant
424 relationship with the relative change in abundance at both Normanby and Ambitle
425 (Fig. S2).



426

427 **Figure 6.** Relationship between the difference in mean pH_{cf} between the control and
428 seeps sites (i.e., Delta pH_{cf}) and the mean relative change in coral abundance between
429 the control and the seeps sites. Positive values indicate an increase in relative
430 abundance between the seeps and the control. There was no relationship in Normanby
431 (A), while there was a linear relationship in Ambitle (B) (p-value of the slope = 0.05,
432 p-value of the intercept = 0.02, $R^2 = 0.56$). Species names are indicated as initials on
433 the figure.

434

435 These results were confirmed by the PCA, which demonstrated no association
436 between proportional abundance and the calcifying chemistry parameters in
437 Normanby (Fig. S3A). In contrast, the PCA for Ambitle showed a clear negative
438 association between delta pH_{cf} at the seep site and relative change in abundance there
439 (Fig. S3B).

440

441 **Discussion**

442 We observed major site-specific differences in the responses of corals'
443 calcifying fluid chemistry. At the consistently low pH seeps site (i.e., at Ambitle
444 Island) most coral species had lower pH_{cf} at the CO_2 seeps compared to the control
445 site. This mirrors observations made on laboratory grown corals under well-controlled
446 and consistently low pH (McCulloch et al., 2012). Conversely, this pattern was less
447 clear at the inconsistently low pH seeps site (i.e., at Normanby Island) when
448 compared to the control site. Additionally, at Ambitle, coral species with limited
449 reductions in pH_{cf} at the seep sites compared to controls (i.e. greater pH homeostasis)
450 also tended to be more abundant at the seeps site than at the control site. This
451 association was influenced heavily by the species with complete pH_{cf} homeostasis,
452 which also increased in abundance more than any other species between the control
453 and seep site at Ambitle. However, three out of four of the species that increased in

abundance also had the highest pH homeostasis. Three of the four species with the least pH_{cf} homeostasis also had the greatest declines in abundance between the control and seeps. Such trends were not observed at Normanby. We suggest that the disparity between responses of pH_{cf} in the different locations is most likely due to the larger range and variability in pH at Normanby. Nonetheless, we cannot rule out other factors such as differences in light, temperature, and flow that can affect the calcifying fluid chemistry (Comeau, Cornwall, Pupier, et al., 2019). As correlation does not imply causation, further assessments at additional sites with consistently low pH are warranted to assess the logical association between coral pH_{cf} homeostasis and directionality of abundance change under consistently low pH that our study suggests.

Controlling pH in the calcifying fluid well above external seawater pH is one of the key mechanisms that corals have developed to favour the precipitation of calcium carbonate. pH homeostasis, where pH in the calcifying fluid remains constant independent of changes in external seawater pH, has therefore been suggested as a mechanism for corals to cope with ocean acidification (Georgiou et al., 2015; McCulloch et al., 2017). To date, pH homeostasis in corals has been demonstrated only in a handful of species: On fragments of *Porites cylindrica* during an *in situ* experiment (Georgiou et al., 2015), on massive *Porites* during a flume experiment (Comeau, Cornwall, DeCarlo, et al., 2019), and on massive *Porites*, *Porites astreoides*, and *Balanophyllia europaea* at naturally low pH sites (Wall et al., 2016, 2019a, 2019b). Using a large range of taxa and morphologies, our study confirms the difficulties associated with determining the resistance to ocean acidification based on coral phylogeny and/or morphology (Comeau et al., 2014a; Okazaki et al., 2017).

While it has been suggested that certain coral genera, such as *Porites*, are particularly resistant to OA, we found that some species of other coral genera that are largely regarded as highly susceptible to OA (Kavousi et al., 2016; Kornder et al., 2018), such as *Acropora* and *Montipora*, can also exert strong control over their calcifying fluid chemistry. However, a large range of species-specific responses within a genus was found. For example, *Acropora millepora* was one of the species with the best apparent control over its pH_{cf} at Normanby while *Acropora samoensis* was the species exhibiting the largest decline in pH_{cf} at this location. Similarly, coral morphology was not correlated with pH homeostasis in the calcifying fluid. Although

massive species have been suggested to be more resistant to OA, we found that many of the species that are able to maintain homeostasis were branching (*e.g., Acropora cytherea*, *Acropora millepora*, *Pocillopora verrucosa*) and foliose (e.g., *Merulina ampliata*, *Montipora foliosa*, *Pachyseris speciosa*, *Turbinaria reniformis*) while some of the species with massive growth morphologies had poor control over their calcifying fluid chemistry (e.g., *Favites pentagona*, *Galaxea fascicularis*). However, these trends might be confounded by the differences in seawater pH variability at the two locations.

The trends in pH control may only be physiologically meaningful for sites where seawater pH was consistently low. Apparent pH homeostasis was much more common in Normanby, where eight out of 14 taxa experienced pH_{cf} homeostasis (and one coral had higher pH_{cf} at the seeps than in the control site). In Ambitle, homeostasis was found only for *Montipora foliosa*, while pH_{cf} was lower at the CO_2 seep site in the other seven species investigated. *Montipora foliosa* was also the species with the highest proportional increase in abundance between the control and the seeps site. This suggests that the capacity of this species to control its pH_{cf} could represent an ecological advantage. However, it is important to note that this species was relatively rare in Ambitle with a total of three colonies recorded in the surveys at the control site and 29 colonies at the seeps site. Therefore, even small increases in abundance equate to large relative increases. Additionally, three of the four species with the least control over their pH_{cf} also had the greatest declines in relative abundance. Together, these results explain the clear association between pH_{cf} homeostasis and relative change in abundance found with the PCA. They also show that rather than the absolute value of pH_{cf} at the vents, it is a greater control over pH_{cf} that could give some ecological advantage in a future high CO_2 ocean. However, further assessment at additional sites where seawater pH is low and not highly variable is now needed to confirm these results.

In contrast, a relationship between pH_{cf} control and proportional change in abundance was not found in Normanby. While different species were studied at both sites, this difference between locations was unlikely to be solely because of taxonomic composition as the two species studied at both locations (*Acropora tenuis* and *Galaxea fascicularis*) also exhibited lower pH_{cf} in Ambitle's seeps site compared to

Normanby's seep site. Furthermore, these results are based on a large range of coral genera and morphologies representative of the local diversity. Thus, variability in seawater pH and time spent by corals in different pH levels can likely explain the discrepancies between locations. Despite similar mean pH at both CO_2 seeps, corals in Normanby experienced much larger variations in seawater pH. This includes frequent records of pH as low as 6.6, but also values close to ambient conditions (pH 8.0) very frequently. pH in Normanby occasionally varied by as much as one pH unit in less than one hour. This large variability in pH is likely driven by the shallow topography of the Upa-Upasina reef, where depth varies between ~1–4 m, which makes seawater pH extremely dependent on water mixing caused by local wind conditions (Fabricius et al., 2011). In contrast, Pichler et al. (2019) showed that in Tutum Bay (Ambitle Island) the main seep and other associated sparse seeps change the seawater carbonate chemistry of the whole bay (1-8 m deep). In Tutum Bay, pH variability is mostly driven by tides, with lower pH associated with low tides. Apparent pH_{cf} homeostasis in Normanby in most coral species studied could therefore result from the high variability in pH at this location. Moreover, it is likely that this apparent pH homeostasis in Normanby might represent calcification occurring predominantly during these high pH events associated with high tide. Other environmental parameters such as temperature, light, and flow are known to affect the composition of the calcifying fluid chemistry (Comeau, Cornwall, Pupier, et al., 2019; Guo, 2019). However, they did not impact the present results, as all environmental parameters other than seawater pH were similar between control and seeps sites (Table S1). Therefore, the different responses observed in Normanby and Ambitle can likely be attributed to differing seawater pH (and DIC) variability.

Greater pH variability could elicit at least three distinct responses. 1) Periods of elevated seawater pH could allow calcification to occur unabated as pH_{cf} is elevated during these time periods, but pH_{cf} is then decreased when seawater pH is reduced. Since calcification rates are higher when pH_{cf} is higher, a greater proportion of boron would be incorporated during the periods of time when pH_{cf} is elevated, which would be reflected by elevated pH_{cf} at CO_2 seeps, as in our data. This might further explain the absence of a relationship between relative change in abundance of species and their ability to maintain constant pH_{cf} at the CO_2 seeps in Normanby, where the reef might be dominated by species that are able to rapidly calcify in brief

periods when seawater pH is high. This hypothesis is also supported by the two co-occurring species at Ambitle/Normanby possessing lower pH_{cf} at Ambitle, indicative of calcification occurring more rapidly in higher seawater pH. However, this cannot be tested with the present data, as boron isotopes only provide indication on the mean pH_{cf} during the precipitation of calcium carbonate over several weeks and other methods, such as dyes and microelectrodes, cannot be used *in situ* presently. 2) Periods of extremely low pH could cause physiological stress and even dissolution of resident organisms, especially for any individuals where skeletal $CaCO_3$ material becomes exposed. For example, the low pH values found in Upa-Upasina reef (Normanby Island) could also explain the deleterious effects of seawater pH on coral skeletal characteristics reported at this site (Prada et al. preprint). In this scenario, dissolution-resistant species would be favoured at Normanby. 3) Greater pH variability itself could cause deleterious effects, such as reduction of calcification as observed in the coralline alga *Arthrocardia corymbosa* (Cornwall et al. 2013). The only study on the effect of pH variability on coral CF chemistry has shown that pH_{cf} of the coral *Goniopora* sp. was driven by the mean seawater pH and was not affected by diurnal variability in seawater pH (Cornwall et al., 2018). However, this supports a null hypothesis 4) that responses at Normanby are equivalent to those that will occur under future ocean acidification. While this contradicts what is found here, it is important to note that this study was restricted to only one species exposed to a regular diurnal variability in pH (low pH at night and high pH during the day), which could elicit much different responses than those occurring at our sites. Collectively, the effects of differences in pH variability on calcification physiology is also highly species-specific (Rivest et al., 2017), and thus extremely high pH, low pH and differences in pH variability itself could have altered both the pH_{cf} and the ecological outcomes at Normanby (and even Ambitle to a lesser extent) in ways that are difficult to predict from our available data.

DIC_{cf} is another important parameter of the calcifying physiology of corals (McCulloch et al., 2017; Ross et al., 2018; Wall et al., 2019) and is generally inversely correlated with pH_{cf}, whereby DIC_{cf} is elevated when pH_{cf} decreases. This trend also persists when corals are grown under low pH in the laboratory (Cornwall et al., 2018; Schoepf et al., 2017; Sevilgen et al., 2019) and occurs on seasonal cycles *in situ* (McCulloch et al., 2017; Ross et al., 2017). Here, the average DIC_{cf} across all

590 coral species in each site was similar between the control and seeps sites at both
591 locations, and only 4 species out of 14 in Normanby and 5 out 8 in Ambitle had
592 higher DIC_{cf} at the seeps compared to the control. As for pH_{cf}, the lack of differences
593 in DIC_{cf} at the seeps compared to the control at Normanby in most corals could have
594 resulted from the high variability in seawater DIC, because seawater DIC and pH are
595 highly correlated in most conditions, i.e. where total alkalinity is similar. The present
596 results also showed that the relative change in abundance was not associated with
597 either DIC_{cf} or delta DIC_{cf}. This is not surprising, as previous studies have shown that
598 while increasing DIC_{cf} can help to partially mitigate the negative effects of ocean
599 acidification on corals calcification, seawater pH and its impact on pH_{cf} is the main
600 driver of the calcifying fluid chemistry (Comeau et al. 2018). Nevertheless, there was
601 a significant relationship between pH_{cf} and DIC_{cf} at both locations when all the
602 samples were assessed (Fig. S4). Elevation of DIC_{cf} by some species has been
603 invoked as one potential mechanism whereby Ω_{cf} could be increased under OA,
604 thereby reducing the negative effects of OA on calcification (Cornwall et al., 2018;
605 Schoepf et al., 2017; Wall et al., 2019). However, we did not find a significant
606 relationship between DIC_{cf} elevation and coral abundance or relative change in
607 abundance at either locations. This result therefore suggests that while the control of
608 DIC_{cf} could help corals to sustain calcification in low pH under specific
609 circumstances, this mechanism likely plays a minor role compared to the control of
610 pH_{cf}.
611 Overall, our results support the idea that species-specific coral physiology
612 controls responses to OA *in situ* (as observed with seaweed inorganic carbon use
613 previously; Cornwall et al., 2017) with no or minor relations to coral phylogeny and
614 morphological traits. Moreover, our findings suggest that coral control of carbonate
615 chemistry in the calcifying fluid might influence their ecological success under OA.
616 This manifested in Ambitle, where pH variability is low and where corals with the
617 highest control on their calcifying fluid pH generally had a higher change in relative
618 abundance between the CO_2 seeps and control sites. However, these traits only
619 provide partial information and further research at a more extensive set of sites is now
620 required. In contrast, our study also shows that large pH variability, such as the one
621 found in Normanby, could mask the link between species physiological traits and
622 ecological success, highlighting the importance of characterizing environmental
623 conditions *in situ* at high temporal resolution. By combining geochemical, ecological,

and chemical approaches, our study demonstrates that even under seawater pH lower than that predicted by the end of the century because of climate change, a variety of corals that exert strong control on their calcifying fluid might still be able to calcify, grow, and persist.

Acknowledgements

This study was partially funded by the French National Research Agency (ANR; project CARIOCA grant agreement no. ANR15CE02-0006-01, 2015), by the French grant scheme Fonds Pacifique (project AMBITLE grant agreement no. 1598, 2016), by the Australian ARC Centre of Excellence in Coral Reef Studies (CE140100020) and by the Flotte Océanographique Française for use of the research vessel Alis. We are grateful to the population of Tutum Bay in Ambitle and Upa-Upasina in Normanby for allowing us to conduct the study on their reefs, and to the National Research Institute, the Milne Bay Provincial Research Committee, the New Ireland Provincial Administration, and the Conservation and Environment Protection Authority of Papua New Guinea for permits. Thanks to the crew of the R/V Alis, and M/B Chertan. Thanks to K. Fabricius and S. Noonan (AIMS) for their invaluable contributions. A-M Comeau-Nisumaa and J-P D'Olivo provided vital laboratory support. C. Tanvet helped for graphical preparation. MTM was supported by an ARC Laureate Fellowship (LF120100049), S. C. was supported by an ARC DECRA (DE160100668).

References

Barkley, H. C., Cohen, A. L., McCorkle, D. C., & Golbuu, Y. (2017). Mechanisms and thresholds for pH tolerance in Palau corals. *Journal of Experimental Marine Biology and Ecology*, *489*, 7–14. https://doi.org/10.1016/j.jembe.2017.01.003

Biscéré, T., Zampighi, M., Lorrain, A., Jurriaans, S., Foggo, A., Houlbrèque, F., & Rodolfo-Metalpa, R. (2019). High pCO_2 promotes coral primary production. *Biology Letters*, *15*(7), 20180777. https://doi.org/10.1098/rsbl.2018.0777

Camp, E. F., Nitschke, M. R., Rodolfo-Metalpa, R., Houlbreque, F., Gardner, S. G., Smith, D. J., Zampighi, M., & Suggett, D. J. (2017). Reef-building corals thrive within hot-acidified and deoxygenated waters. *Scientific Reports*, *7*(1), 2434. https://doi.org/10.1038/s41598-017-02383-y

Camp, E. F., Suggett, D. J., Gendron, G., Jompa, J., Manfrino, C., & Smith, D. J. (2016). Mangrove and seagrass beds provide different biogeochemical services for corals threatened by climate change. *Coral Reef Research*, 52. https://doi.org/10.3389/fmars.2016.00052

Chan, N. C. S., & Connolly, S. R. (2013). Sensitivity of coral calcification to ocean acidification: A meta-analysis. *Global Change Biology*, *19*(1), 282–290. https://doi.org/10.1111/gcb.12011

Comeau, S., Cornwall, C. E., DeCarlo, T. M., Doo, S. S., Carpenter, R. C., & McCulloch, M. T. (2019). Resistance to ocean acidification in coral reef taxa is not gained by acclimatization. *Nature Climate Change*, *9*(6), 477–483. https://doi.org/10.1038/s41558-019-0486-9

669 Comeau, S., Cornwall, C. E., DeCarlo, T. M., Krieger, E., & McCulloch, M. T. (2018).
670 Similar controls on calcification under ocean acidification across
671 unrelated coral reef taxa. *Global Change Biology*, *24*(0), 4857–4868.
672 https://doi.org/10.1111/gcb.14379
673 Comeau, S., Cornwall, C. E., & McCulloch, M. T. (2017). Decoupling between the
674 response of coral calcifying fluid pH and calcification to ocean
675 acidification. *Scientific Reports*, *7*(1), 7573.
676 https://doi.org/10.1038/s41598-017-08003-z
677 Comeau, S., Cornwall, C. E., Pupier, C. A., DeCarlo, T. M., Alessi, C., Trehern, R., &
678 McCulloch, M. T. (2019). Flow-driven micro-scale pH variability affects the
679 physiology of corals and coralline algae under ocean acidification.
680 *Scientific Reports*, *9*(1), 1–12. https://doi.org/10.1038/s41598-019-
681 49044-w
682 Comeau, S., Edmunds, P. J., Spindel, N. B., & Carpenter, R. C. (2014a). Diel pCO_2
683 oscillations modulate the response of the coral *Acropora hyacinthus* to
684 ocean acidification. *Marine Ecology Progress Series*, *501*, 99–111.
685 https://doi.org/10.3354/meps10690
686 Comeau, S., Edmunds, P. J., Spindel, N. B., & Carpenter, R. C. (2014b). Fast coral
687 reef calcifiers are more sensitive to ocean acidification in short-term
688 laboratory incubations. *Limnology and Oceanography*, *59*(3), 1081–1091.
689 https://doi.org/10.4319/lo.2014.59.3.1081
690 Cornwall, C. E., Comeau, S., DeCarlo, T. M., Moore, B., D'Alexis, Q., & McCulloch, M.
691 T. (2018). Resistance of corals and coralline algae to ocean acidification:
692 Physiological control of calcification under natural pH variability. *Proc. R.*
693 *Soc. B*, *285*(1884), 20181168. https://doi.org/10.1098/rspb.2018.1168

694 Cornwall, C. E., Comeau, S., Kornder, N. A., Perry, C. T., Hooidonk, R. van, DeCarlo,
695 T. M., Pratchett, M. S., Anderson, K. D., Browne, N., Carpenter, R., Diaz-
696 Pulido, G., D'Olivo, J. P., Doo, S. S., Figueiredo, J., Fortunato, S. A. V.,
697 Kennedy, E., Lantz, C. A., McCulloch, M. T., González-Rivero, M., … Lowe, R.
698 J. (2021). Global declines in coral reef calcium carbonate production
699 under ocean acidification and warming. *Proceedings of the National*
700 *Academy of Sciences*, *118*(21). https://doi.org/10.1073/pnas.2015265118
701 Cornwall, C. E., Revill, A. T., Hall-Spencer, J. M., Milazzo, M., Raven, J. A., & Hurd, C.
702 L. (2017). Inorganic carbon physiology underpins macroalgal responses
703 to elevated CO_2. *Scientific Reports*, *7*(1), 46297.
704 https://doi.org/10.1038/srep46297
705 Dandan, S. S., Falter, J. L., Lowe, R. J., & McCulloch, M. T. (2015). Resilience of coral
706 calcification to extreme temperature variations in the Kimberley region,
707 northwest Australia. *Coral Reefs*, *34*(4), 1151–1163.
708 https://doi.org/10.1007/s00338-015-1335-6
709 DeCarlo, T. M., D'Olivo, J. P., Foster, T., Holcomb, M., Becker, T., & McCulloch, M. T.
710 (2017). Coral calcifying fluid aragonite saturation states derived from
711 Raman spectroscopy. *Biogeosciences*, *14*(22), 5253–5269.
712 https://doi.org/10.5194/bg-14-5253-2017
713 Drake, J. L., Schaller, M. F., Mass, T., Godfrey, L., Fu, A., Sherrell, R. M., Rosenthal,
714 Y., & Falkowski, P. G. (2018). Molecular and geochemical perspectives on
715 the influence of CO_2 on calcification in coral cell cultures. *Limnology and*
716 *Oceanography*, *63*(1), 107–121. https://doi.org/10.1002/lno.10617
717 Fabricius, K. E., De'ath, G., Noonan, S., & Uthicke, S. (2014). Ecological effects of
718 ocean acidification and habitat complexity on reef-associated

719 macroinvertebrate communities. *Proceedings of the Royal Society B:*
720 *Biological Sciences*, *281*(1775), 20132479.
721 https://doi.org/10.1098/rspb.2013.2479
722 Fabricius, K. E., Langdon, C., Uthicke, S., Humphrey, C., Noonan, S., De'ath, G.,
723 Okazaki, R., Muehllehner, N., Glas, M. S., & Lough, J. M. (2011). Losers and
724 winners in coral reefs acclimatized to elevated carbon dioxide
725 concentrations. *Nature Climate Change*, *1*(3), 165–169.
726 https://doi.org/10.1038/nclimate1122
727 Fabricius, K. E., Noonan, S. H. C., Abrego, D., Harrington, L., & De'ath, G. (2017).
728 Low recruitment due to altered settlement substrata as primary
729 constraint for coral communities under ocean acidification. *Proceedings of*
730 *the Royal Society B: Biological Sciences*, *284*(1862), 20171536.
731 https://doi.org/10.1098/rspb.2017.1536
732 Foster, G. L., Pogge von Strandmann, P. a. E., & Rae, J. W. B. (2010). Boron and
733 magnesium isotopic composition of seawater. *Geochemistry, Geophysics,*
734 *Geosystems*, *11*(8), Q08015. https://doi.org/10.1029/2010GC003201
735 Georgiou, L., Falter, J., Trotter, J., Kline, D. I., Holcomb, M., Dove, S. G., Hoegh-
736 Guldberg, O., & McCulloch, M. (2015). pH homeostasis during coral
737 calcification in a free ocean CO_2 enrichment (FOCE) experiment, Heron
738 Island reef flat, Great Barrier Reef. *Proceedings of the National Academy of*
739 *Sciences*, *112*(43), 13219–13224.
740 https://doi.org/10.1073/pnas.1505586112
741 Golbuu, Y., Gouezo, M., Kurihara, H., Rehm, L., & Wolanski, E. (2016). Long-term
742 isolation and local adaptation in Palau's Nikko Bay help corals thrive in

743 acidic waters. *Coral Reefs*, *35*(3), 909–918.
744 https://doi.org/10.1007/s00338-016-1457-5
745 Guo, W. (2019). Seawater temperature and buffering capacity modulate coral
746 calcifying pH. *Scientific Reports*, *9*(1), 1189.
747 https://doi.org/10.1038/s41598-018-36817-y
748 Hoegh-Guldberg, O., Poloczanska, E. S., Skirving, W., & Dove, S. (2017). Coral reef
749 ecosystems under climate change and ocean acidification. *Frontiers in*
750 *Marine Science*, *4*. https://doi.org/10.3389/fmars.2017.00158
751 Holcomb, M., DeCarlo, T. M., Gaetani, G. A., & McCulloch, M. (2016). Factors
752 affecting B/Ca ratios in synthetic aragonite. *Chemical Geology*, *437*, 67–76.
753 https://doi.org/10.1016/j.chemgeo.2016.05.007
754 Holcomb, M., Venn, A. A., Tambutté, E., Tambutté, S., Allemand, D., Trotter, J., &
755 McCulloch, M. (2014). Coral calcifying fluid pH dictates response to ocean
756 acidification. *Scientific Reports*, *4*, 5207.
757 https://doi.org/10.1038/srep05207
758 Kavousi, J., Tanaka, Y., Nishida, K., Suzuki, A., Nojiri, Y., & Nakamura, T. (2016).
759 Colony-specific calcification and mortality under ocean acidification in the
760 branching coral *Montipora digitata*. *Marine Environmental Research*, *119*,
761 161–165. https://doi.org/10.1016/j.marenvres.2016.05.025
762 Kleypas, J., J., & Yates, K. (2009). Coral reefs and ocean acidification.
763 *Oceanography*, *22*(4), 108–117.
764 Klochko, K., Kaufman, A. J., Yao, W., Byrne, R. H., & Tossell, J. A. (2006).
765 Experimental measurement of boron isotope fractionation in seawater.
766 *Earth and Planetary Science Letters*, *248*(1–2), 276–285.
767 https://doi.org/10.1016/j.epsl.2006.05.034

768 Kornder, N. A., Riegl, B. M., & Figueiredo, J. (2018). Thresholds and drivers of
769 coral calcification responses to climate change. *Global Change Biology*.
770 https://doi.org/10.1111/gcb.14431
771 Mass, T., Drake, J. L., Haramaty, L., Kim, J. D., Zelzion, E., Bhattacharya, D., &
772 Falkowski, P. G. (2013). Cloning and characterization of four novel coral
773 acid-rich proteins that precipitate carbonates in vitro. *Current Biology: CB*,
774 *23*(12), 1126–1131. https://doi.org/10.1016/j.cub.2013.05.007
775 McCulloch, M., Falter, J., Trotter, J., & Montagna, P. (2012). Coral resilience to
776 ocean acidification and global warming through pH up-regulation. *Nature*
777 *Climate Change*, *2*(8), 623–627. https://doi.org/10.1038/nclimate1473
778 McCulloch, M. T., D'Olivo, J. P., Falter, J., Holcomb, M., & Trotter, J. A. (2017). Coral
779 calcification in a changing World and the interactive dynamics of pH and
780 DIC upregulation. *Nature Communications*, *8*, 15686.
781 https://doi.org/10.1038/ncomms15686
782 Okazaki, R. R., Towle, E. K., Hooidonk, R. van, Mor, C., Winter, R. N., Piggot, A. M.,
783 Cunning, R., Baker, A. C., Klaus, J. S., Swart, P. K., & Langdon, C. (2017).
784 Species-specific responses to climate change and community composition
785 determine future calcification rates of Florida Keys reefs. *Global Change*
786 *Biology*, *23*(3), 1023–1035. https://doi.org/10.1111/gcb.13481
787 Pichler, T., Biscéré, T., Kinch, J., Zampighi, M., Houlbrèque, F., & Rodolfo-Metalpa,
788 R. (2019). Suitability of the shallow water hydrothermal system at
789 Ambitle Island (Papua New Guinea) to study the effect of high pCO_2 on
790 coral reefs. *Marine Pollution Bulletin*, *138*, 148–158.
791 https://doi.org/10.1016/j.marpolbul.2018.11.003

792 Rivest, E. B., Comeau, S., & Cornwall, C. E. (2017). The role of natural variability in
793 shaping the response of coral reef organisms to climate change. *Current*
794 *Climate Change Reports*, 1–11. https://doi.org/10.1007/s40641-017-
795 0082-x
796 Ross, C. L., Falter, J. L., & McCulloch, M. T. (2017). Active modulation of the
797 calcifying fluid carbonate chemistry (δ 11 B, B/Ca) and seasonally
798 invariant coral calcification at sub-tropical limits. *Scientific Reports*, *7*(1),
799 13830. https://doi.org/10.1038/s41598-017-14066-9
800 Ross, C. L., Schoepf, V., DeCarlo, T. M., & McCulloch, M. T. (2018). Mechanisms and
801 seasonal drivers of calcification in the temperate coral *Turbinaria*
802 *reniformis* at its latitudinal limits. *Proceedings of the Royal Society B:*
803 *Biological Sciences*, *285*(1879), 20180215.
804 https://doi.org/10.1098/rspb.2018.0215
805 Schoepf, V., Jury, C. P., Toonen, R. J., & McCulloch, M. T. (2017). Coral calcification
806 mechanisms facilitate adaptive responses to ocean acidification. *Proc. R.*
807 *Soc. B*, *284*(1868), 20172117. https://doi.org/10.1098/rspb.2017.2117
808 Schoepf, V., Stat, M., Falter, J. L., & McCulloch, M. T. (2015). Limits to the thermal
809 tolerance of corals adapted to a highly fluctuating, naturally extreme
810 temperature environment. *Scientific Reports*, *5*, 17639.
811 https://doi.org/10.1038/srep17639
812 Sevilgen, D. S., Venn, A. A., Hu, M. Y., Tambutté, E., Beer, D. de, Planas-Bielsa, V., &
813 Tambutté, S. (2019). Full in vivo characterization of carbonate chemistry
814 at the site of calcification in corals. *Science Advances*, *5*(1), eaau7447.
815 https://doi.org/10.1126/sciadv.aau7447

816 Shamberger, K. E. F., Lentz, S. J., & Cohen, A. L. (2018). Low and variable
817 ecosystem calcification in a coral reef lagoon under natural acidification.
818 *Limnology and Oceanography*, *63*(2), 714–730.
819 https://doi.org/10.1002/lno.10662
820 Smith, J. N., Richter, C., Fabricius, K. E., & Cornils, A. (2017). Pontellid copepods,
821 *Labidocera* spp., affected by ocean acidification: A field study at natural
822 CO2 seeps. *PLOS ONE*, *12*(5), e0175663.
823 https://doi.org/10.1371/journal.pone.0175663
824 Tambutté, S., Holcomb, M., Ferrier-Pagès, C., Reynaud, S., Tambutté, É., Zoccola,
825 D., & Allemand, D. (2011). Coral biomineralization: From the gene to the
826 environment. *Journal of Experimental Marine Biology and Ecology*, *408*(1),
827 58–78. https://doi.org/10.1016/j.jembe.2011.07.026
828 Teixidó, N., Caroselli, E., Alliouane, S., Ceccarelli, C., Comeau, S., Gattuso, J.-P., Fici,
829 P., Micheli, F., Mirasole, A., Monismith, S. G., Munari, M., Palumbi, S. R.,
830 Sheets, E., Urbini, L., Vittor, C. D., Goffredo, S., & Gambi, M. C. (2020). Ocean
831 acidification causes variable trait-shifts in a coral species. *Global Change*
832 *Biology*, *26*(12), 6813–6830. https://doi.org/10.1111/gcb.15372
833 Trotter, J., Montagna, P., McCulloch, M., Silenzi, S., Reynaud, S., Mortimer, G.,
834 Martin, S., Ferrier-Pagès, C., Gattuso, J.-P., & Rodolfo-Metalpa, R. (2011).
835 Quantifying the pH 'vital effect' in the temperate zooxanthellate coral
836 *Cladocora caespitosa*: Validation of the boron seawater pH proxy. *Earth*
837 *and Planetary Science Letters*, *303*(3–4), 163–173.
838 https://doi.org/10.1016/j.epsl.2011.01.030
839 Uthicke, S., Ebert, T., Liddy, M., Johansson, C., Fabricius, K. E., & Lamare, M.
840 (2016). Echinometra sea urchins acclimatized to elevated pCO_2 at volcanic

vents outperform those under present-day pCO_2 conditions. *Global Change Biology*, *22*(7), 2451–2461. https://doi.org/10.1111/gcb.13223

Venn, A. A., Tambutté, E., Caminiti-Segonds, N., Techer, N., Allemand, D., & Tambutté, S. (2019). Effects of light and darkness on pH regulation in three coral species exposed to seawater acidification. *Scientific Reports*, *9*(1), 2201. https://doi.org/10.1038/s41598-018-38168-0

Venn, A. A., Tambutté, E., Holcomb, M., Laurent, J., Allemand, D., & Tambutté, S. (2013). Impact of seawater acidification on pH at the tissue–skeleton interface and calcification in reef corals. *Proceedings of the National Academy of Sciences*, *110*(5), 1634–1639. https://doi.org/10.1073/pnas.1216153110

Wall, M., Fietzke, J., Crook, E. D., & Paytan, A. (2019). Using B isotopes and B/Ca in corals from low saturation springs to constrain calcification mechanisms. *Nature Communications*, *10*(1), 3580. https://doi.org/10.1038/s41467-019-11519-9

Wall, M., Fietzke, J., Schmidt, G. M., Fink, A., Hofmann, L. C., Beer, D. de, & Fabricius, K. E. (2016). Internal pH regulation facilitates *in situ* long-term acclimation of massive corals to end-of-century carbon dioxide conditions. *Scientific Reports*, *6*, 30688. https://doi.org/10.1038/srep30688

Wall, M., Prada, F., Fietzke, J., Caroselli, E., Dubinsky, Z., Brizi, L., Fantazzini, P., Franzellitti, S., Mass, T., Montagna, P., Falini, G., & Goffredo, S. (2019). Linking internal carbonate chemistry regulation and calcification in corals growing at a Mediterranean CO_2 vent. *Frontiers in Marine Science*, *6*. https://doi.org/10.3389/fmars.2019.00699

Yates, K. K., Rogers, C. S., Herlan, J. J., Brooks, G. R., Smiley, N. A., & Larson, R. A. (2014). Diverse coral communities in mangrove habitats suggest a novel refuge from climate change. *Biogeosciences*, *11*(16), 4321–4337. https://doi.org/10.5194/bg-11-4321-2014

Supplementary materials

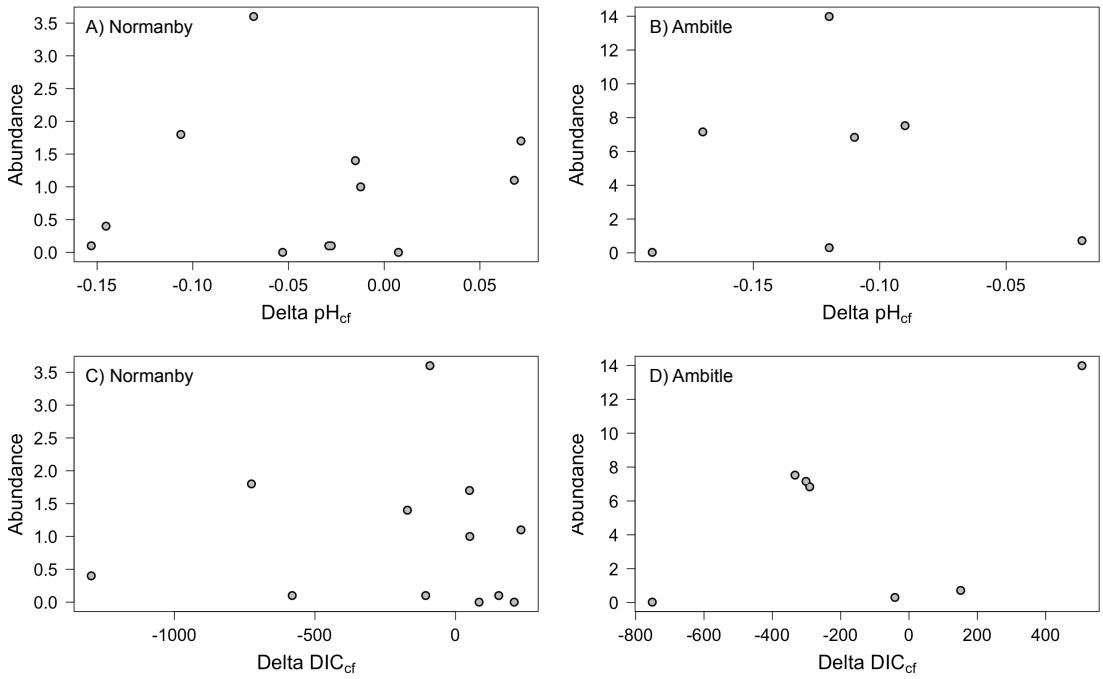


Figure S1. Relationship between the difference in pH_{cf} and DIC_{cf} between the control and seeps sites (i.e., Delta pH_{cf} and Delta DIC_{cf}) and the abundance of corals at the seeps sites. There was no relationship with pH_{cf} in Normanby (A) and Ambitle (B). Similarly, there was no relationship with DIC_{cf} in Normanby (C) and Ambitle (C).

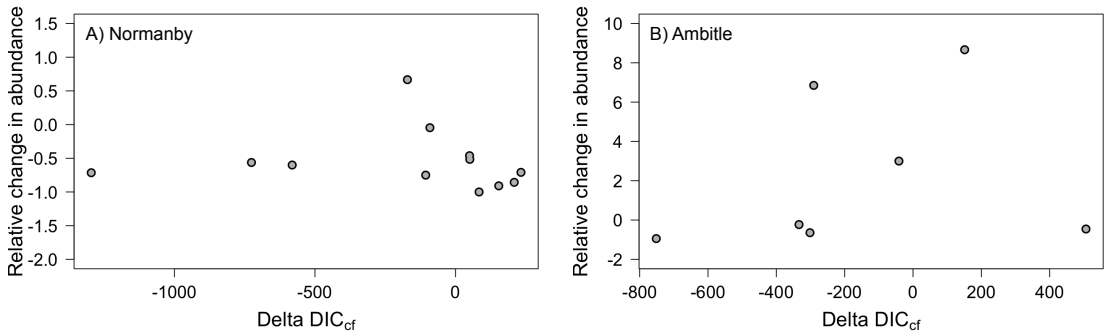


Figure S2. Relationship between the difference in DIC_{cf} between the control and seeps sites (i.e., Delta DIC_{cf}) and the relative change in coral abundance between the control and the seeps sites. There was no relationship in Normanby (A) and Ambitle (B).

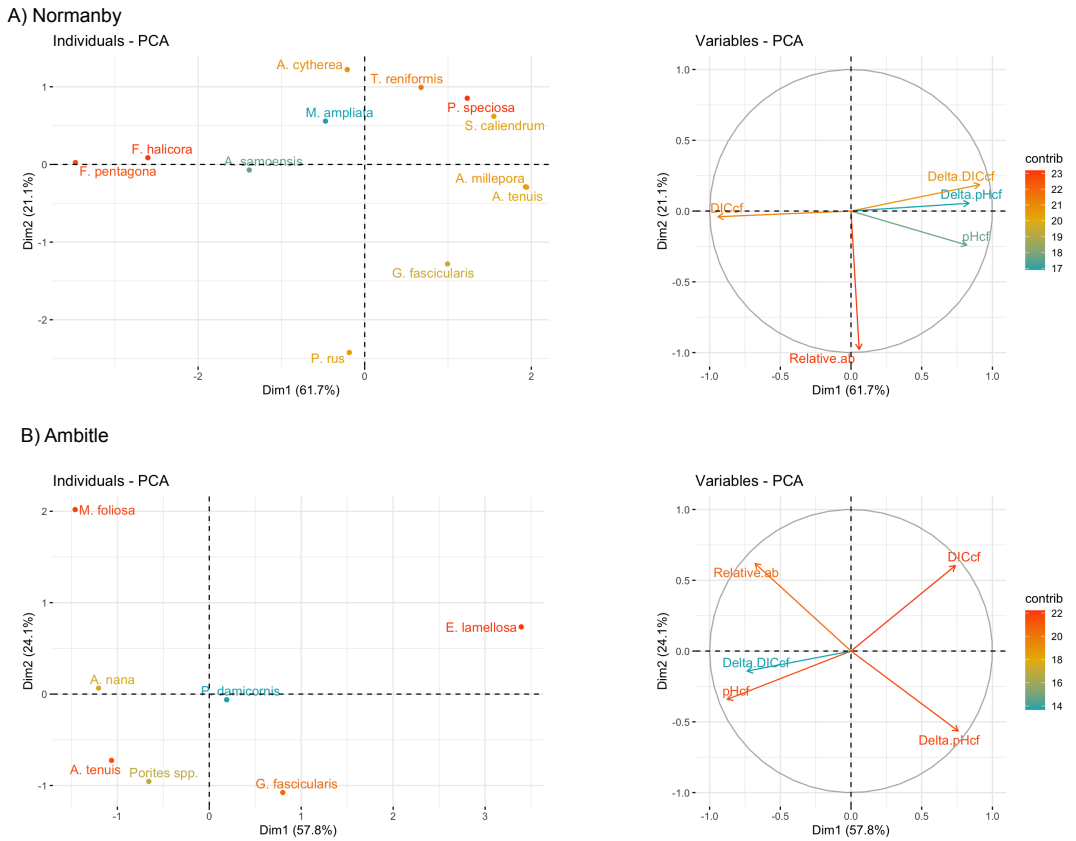


Figure S3. Principal Component Analyses showing the correlation between change in relative mean abundance (Prop_ab), vent pH_{cf} and DIC_{cf}, delta pHcf (pH_{cf} vent – pH_{cf} control; delta pH on the figure) and delta DIC_{cf} (pH_{cf} vent – pH_{cf} control; delta DIC on the figure) were correlated.

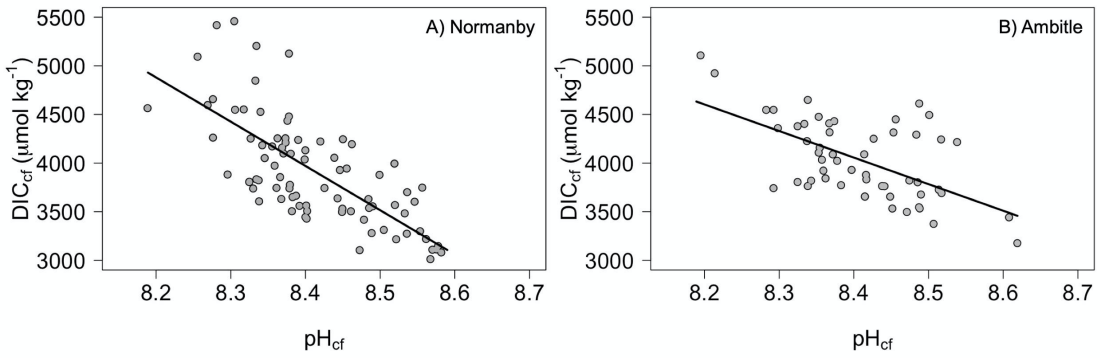


Figure S4. Relationship between pH_{cf} and DIC_{cf} in corals from Normanby and Ambitle. Corals from the control and the seeps sites were pooled together. A) relationship in Normanby Island (Linear regression: p-value of the slope < 0.001, R^2 = 0.50). B) relationship in Ambitle Island (Linear regression: p-value of the slope < 0.001, R^2 = 0.36).

Table S1. Seawater carbonate chemistry in Ambitle and Normanby at the control and the seeps sites. Medians, 5th and 95th percentile (between parentheses) of the measured pH_T, total alkalinity (A_T) and temperature (T) are shown. pCO_2, C_T, and Ω_{arag} were calculated using the median values of A_T and T and the median, 5th and 95th percentile of pH_T.

Site	pH_T	A_T (µmol kg^{-1})	T	pCO_2 (µatm)	C_T (µmol kg^{-1})	Ω_{arag}
Ambitle Control	8.02 (7.94 – 8.06)	2315 (2149-2349)	30.3 (30.3-30.6)	422 (376-530)	1980 (1955 2029)	3.88 (3.37-4.15)
Ambitle Seeps	7.65 (7.47-7.80)	2349 (2183-2382)	30.5 (30.2–30.7)	1178 (793 -1863)	2204 (2135-2276)	2.95 (2.03-3.95)
Normanby Control	7.97 (7.91-8.00)	2163 (2107-2192)	30.5 (30.1-30.8)	454 (416-537)	1872 (1855-1905)	3.33 (2.99-3.51)
Normanby Seeps	7.66 (7.19-7.92)	2221 (2184-2276)	30.2 (26.9-30.9)	1084 (538-3495)	2080 (1956-2246)	1.89 (0.70-3.10)

Table S2. Summary of the mean changes in delta pH_{cf} (pH_{cf} seeps – pH_{cf} ambient), delta DIC_{cf} (DIC_{cf} seeps – DIC_{cf} ambient) and relative change in abundance at the two locations.

Location	Species	Delta pH_{cf}	Delta DIC_{cf}	Change in relative abundance
Normanby	*Acropora samoensis*	-0.15	581	-0.6
	Favites pentagona	-0.14	1296	-0.71
	Tubastrea sp.	-0.13	306	NA
	Favites halicora	-0.10	726	-0.56
	Galaxea fascicularis	-0.07	90	-0.04
	Pocillopora verrucosa	-0.06	150	NA
	Acropora cytherea	-0.05	-83	-1
	Merulina ampliata	-0.03	106	-0.75
	Turbinaria reniformis	-0.03	-154	-0.91
	Porites rus	-0.02	170	0.66
	Acropora tenuis	-0.01	-50	-0.52
	Pachyseris speciosa	0.01	-208	-0.86
	Seriatopora caliendrum	0.07	-233	-0.70
	Acropora millepora	0.07	-50	-0.46
Ambitle	Echinopora lamellosa	-0.19	751	-0.94
	Galaxea fascicularis	-0.17	301	-0.64
	Massive Porites sp.	-0.13	-506	-0.45
	Acropora tenuis	-0.12	290	3
	Acropora nana	-0.10	28	6.85
	Psammocora contigua	-0.09	178	NA
	Pocillopora damicornis	-0.09	333	-0.23
	Montipora foliosa	-0.02	-151	8.67