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Species-specific calcification response of Caribbean corals after 2-year transplantation to a low aragonite saturation submarine spring

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Coral calcification is expected to decline as atmospheric carbon dioxide concentration increases. We assessed the potential of *Porites astreoides*, *Siderastrea siderea* and *Porites porites* to survive and calcify under acidified conditions in a 2-year field transplant experiment around low pH, low aragonite saturation (Ω_{arag}) submarine springs. Slow-growing *S. siderea* had the highest post-transplantation survival and showed increases in concentrations of Symbiodiniaceae, chlorophyll *a* and protein at the low Ω_{arag} site. Nubbins of *P. astreoides* had 20% lower survival and higher chlorophyll *a* concentration at the low Ω_{arag} site. Only 33% of *P. porites* nubbins survived at low Ω_{arag} and their linear extension and calcification rates were reduced. The density of skeletons deposited after transplantation at the low Ω_{arag} spring was 15–30% lower for all species. These results suggest that corals with slow calcification rates and high Symbiodiniaceae, chlorophyll *a* and protein concentrations may be less susceptible to ocean acidification, albeit with reduced skeletal density. We postulate that corals in the springs are responding to greater energy demands for overcoming larger differences in carbonate chemistry between the calcifying medium and the external environment. The differential mortality, growth rates and physiological changes may impact future coral species assemblages and the reef framework robustness.

1. Introduction

Anthropogenic activities are increasing the amount of carbon dioxide (CO_2) entering the atmosphere. A large fraction of this CO_2 dissolves in the ocean and lowers ocean pH and carbonate ion concentrations [1]. Low carbonate ion concentrations decrease the aragonite saturation state (Ω_{arag}) of seawater and the availability of carbonate ions needed for deposition of calcium carbonate skeletons [2]. A meta-analysis of experimental studies found a decrease of approximately 15% in coral calcification rates per unit decrease in Ω_{arag} [3]. Data from laboratory experiments and modelling suggest that coral reef carbonate accretion diminishes at Ω_{arag} lower than 3.3 [4], a level likely to be reached in much of the surface ocean by 2100 [1,5]. Many laboratory experiments have reported reduced coral growth under low Ω_{arag} [6], but other experiments show little impact on calcification [7,8]. These conflicting results reflect the limitations of short-term single-organism manipulative experiments since, while laboratory experiments allow precise control of physico-chemical parameters, they do not fully reflect the complexity of the natural environment

[9]. Moreover, such experiments are often too short for organisms to fully acclimate [10].

Scleractinian corals have been observed living in low pH waters near CO_2 vents (pH < 7.7 [11]), low Ω_{arag} submarine springs ($\Omega_{arag} < 1$ [12]), upwelling areas (Ω_{arag}: 2.27–4.18 [13]) and mangrove lagoons (Ω_{arag}: 1.47–2.02 [14]), although the taxonomic richness and calcification at some of these sites is lower than at nearby sites with ambient carbonate chemistry. At high CO_2 vents in the Mediterranean, temperate corals were absent at Ω_{arag} less than 2.5 [15], although two species that were transplanted to these low pH vents survived and grew at rates typical to the species used [16]. We investigated the survival, physiological response and calcification rate of three species of Caribbean corals in a two-year long, *in situ* transplantation experiment leveraging naturally occurring low pH, low Ω_{arag} submarine springs.

Off the Yucatán peninsula, Mexico, submarine groundwater springs discharge low pH, low Ω_{arag} water ($\Omega_{arag} \sim 0.5$) into the back-reef lagoon of the Mesoamerican Barrier Reef. These springs, locally referred to as 'ojos', can be used for testing the potential of corals to acclimatize or adapt to low Ω_{arag} conditions, although the 'ojos' are not perfect analogues for future ocean acidification. In this region, the groundwater mixes with seawater before discharging [17], and sensor and discrete sampling demonstrate that the discharged water has relatively high dissolved inorganic carbon (DIC), high alkalinity and slightly lower than ambient salinity, in addition to the low pH, resulting in low Ω_{arag} [12,18–22]. Only three scleractinian coral species (*Siderastrea siderea*, *Porites astreoides* and *Porites divaricata*) grow within the areas continuously exposed to low Ω_{arag} conditions, while taxonomic richness is higher outside the influence of discharge [12]. It has been shown that coral colonies living in the low Ω_{arag} seawater are smaller than those exposed to ambient seawater [12] and have lower skeleton densities and calcification rates [19].

We assessed the potential of three coral species to survive and calcify under low Ω_{arag} conditions by conducting an *in situ* transplant experiment with genetically identical coral fragments (nubbins). We monitored the corals for 2 years and collected surviving nubbins for analyses of skeletal characteristics (density, linear extension, calcification rate), and physiological characteristics (coral tissue protein content, and cell densities and chlorophyll *a* concentration of photosymbiotic algae Symbiodiniaceae). We then explored responses that may allow coral populations to survive and calcify at very low Ω_{arag} conditions by comparing coral responses between transplantation sites (reflecting putative environmental control) and among sites of origin (reflecting putative genotypic influence).

2. Material and methods

(a) Study area

The Yucatán peninsula, Mexico, is a karstic region where rainfall flows through a complex aquifer system that discharges low pH, low Ω_{arag} water at submarine springs (ojos) in the lagoon approximately 500 m offshore [23]. The conditions creating low pH seawater at the ojos differ from those of the ocean acidification scenario, as the high CO_2 in the discharging water is derived from brackish water that has interacted with soil and limestone. The spring water is characterized by lower pH, higher DIC and higher total alkalinity compared to the ambient conditions. Nutrient concentrations vary between ojos but, at the transplant site, the spring waters only have higher silicate (not N or P). The corals at these ojos are constantly exposed to the discharging water, as discussed in detail in [12,19,20] and they represent settings with persistent low Ω_{arag}. We note that Ω_{arag} is the main parameter that affects calcification, and when calculating Ω_{arag} in our study, all relevant environmental parameters were considered (e.g.: higher alkalinity and DIC in addition to the low pH). Ojos occur along the entire Yucatan karst platform and have been discharging water since the rise in sea level associated with the retreating ice sheets after the last glacial maximum [24]. The specific transplantation site at ojo Laja, Puerto Morelos (20.8°N, 86.8°W) was selected to minimize as much as possible the differences between the spring water and the surrounding seawater so that the main difference is in carbonate chemistry parameters.

(b) Coral species and transplant experiment design

We collected two genetically identical fragments (i.e. two nubbins collected from the same colony) from 10 *P. astreoides* colonies and 10 *S. siderea* colonies from areas impacted by the ojo discharge, and from 10 *P. astreoides* colonies and 9 *S. siderea* colonies from adjacent control sites away from the influence of discharge (a total of 39 colonies) (see electronic supplementary material, table S1 for collection details). Both species are found throughout the lagoon, including within low Ω_{arag} ojo discharge plumes [12]. We also collected two nubbins each from 10 *Porites porites* colonies, a species not found in the lagoon, and from 10 additional *S. siderea* colonies from the main barrier reef structure. From each colony, one nubbin was transplanted to a low Ω_{arag} site (ojo) and the second was transplanted to an ambient Ω_{arag} site (control) in the lagoon, approximately 5 m away from the discharge site. A total of 59 colonies and 118 nubbins were transplanted (see electronic supplementary material, table S2 for transplantation details and figure 1 for transplant experiment design). One nubbin of *P. porites* transplanted to low Ω_{arag} ojo was lost in the process and was not included in further analyses.

Nubbins of approximately 2–3 cm in length of massive *P. astreoides* and *S. siderea* were extracted by coring the upper horizontal surface with a submersible pneumatic drill. *P. porites* nubbins (small upright branches approx. 3–5 cm long) were broken off each colony with pliers. Each nubbin was mounted on a 5 × 5 cm square of stainless-steel mesh with epoxy putty that also covered all exposed skeletal surfaces to prevent bioerosion. Each nubbin was individually labelled with a tag tied to the mesh. The nubbins were placed in shallow trays with seawater and alizarin red S dye for 6–8 h to stain the surface and mark the beginning of new skeleton growth. After staining, each nubbin was photographed, and then randomly assigned to one of the transplantation sites (low Ω_{arag} or ambient Ω_{arag}). Within transplant sites, nubbins were randomly assigned to positions on stainless-steel grids and attached to the grids with cable ties (electronic supplementary material, figure S1). The grids were secured at the location of maximum discharge and approximately 5 m away from the influence of the discharging water.

Divers monitored the nubbins weekly for the first two months and then 14 times over the following 22 months. At the end of the transplant experiment, all surviving nubbins were collected and preserved in liquid nitrogen.

(c) Water chemistry

Water samples were collected weekly for the first two months of the experiment and then seven times during the following 22 months. Water samples were collected by divers, during the day time (9:00–13:00) into HDPE bottles, filtered (0.2 µm) into glass bottles and preserved with saturated mercuric chloride solution following Dickson, Sabine [25]. Temperature was measured with a handheld probe (YSI model 63). DIC was measured with a CM5011 Carbon Coulometer (UIC, Inc.).

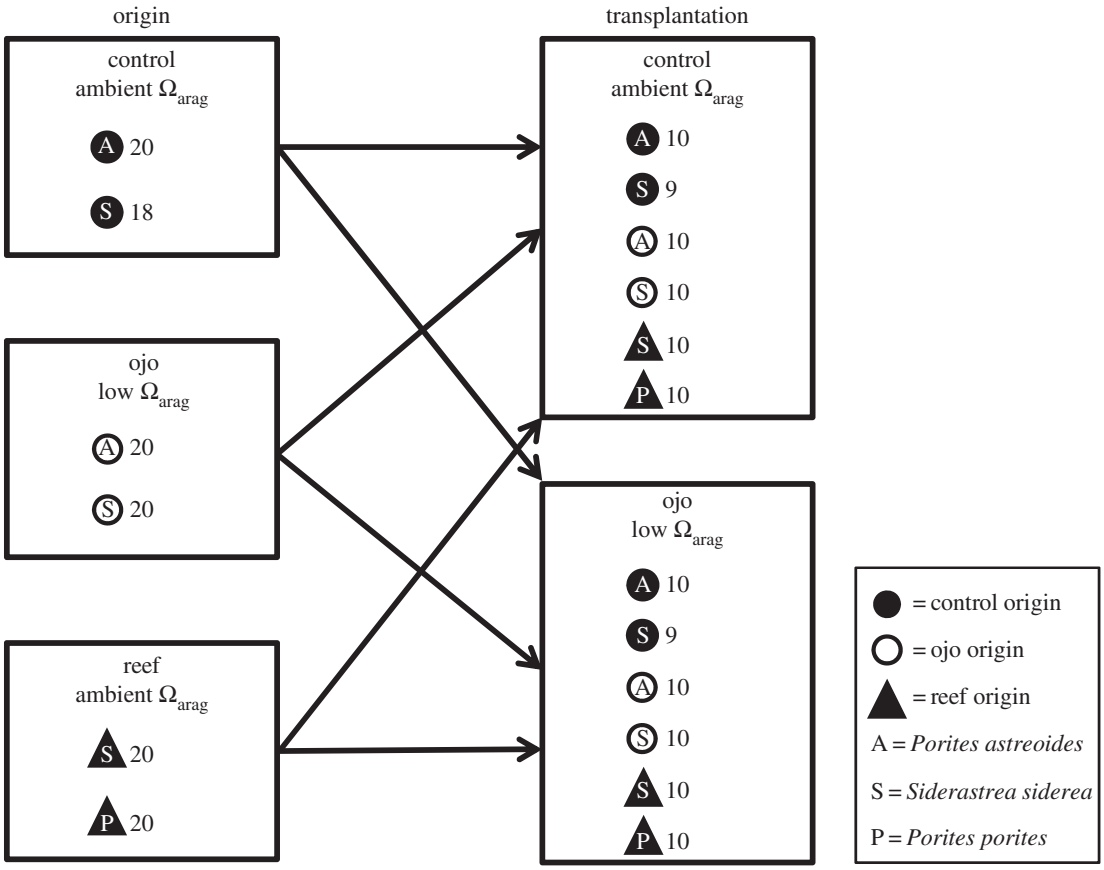

Figure 1. Transplant experiment design. Numbers are numbers of nubbins from each origin.

Alkalinity was measured with an automated open-cell, potentiometric titrator (Orion model 950). Salinity was measured with a salinometer (Portasal Model 8410, Guild Line). Certified CO_2 seawater standards (UC San Diego # 108 and 135) were used as reference material. Ω_{arag} was calculated using the CO_2SYS program [26] with constants from Mehrbach *et al.* [27] refit by Dickson & Millero [28]. In addition, SeapHOx sensors were deployed for the duration of the experiment at the transplant sites (electronic supplementary material, figure S1) and collected temperature, conductivity, pressure, oxygen and pH every hour.

(d) Tissue extraction and skeletal analysis

Coral tissues were extracted with an airbrush using artificial seawater. Aliquots of the homogenized tissue were used for protein, symbiont cells and chlorophyll *a* measurements following the procedures of Krief *et al.* [29]. Coral skeletons were then submerged in 10% sodium hypochlorite overnight to remove residual tissue. Each nubbin was sliced vertically through the primary growth axis. Linear extension above the alizarin red mark was measured under a microscope. A piece (surface area: approx. 2 cm^2) of the coral skeleton was cut from the top of the alizarin red mark to the top of the coral, and its volume was calculated following the procedure of Smith *et al.* [30] to determine the bulk density using the buoyant weight method [31]. The annual calcification rate (g cm^{-2} $year^{-1}$) was calculated as the product of linear extension (cm $year^{-1}$) and density (g cm^{-3}).

(e) Tissue analysis

Symbiodiniaceae concentrations were determined in triplicate with a hemocytometer and symbiont counts were normalized to skeleton surface area (Symbiodiniaceae cells cm^{-2}). Chlorophyll *a* was extracted in 90% acetone and concentration was measured with a spectrophotometer (Genesys 10s UV–VIS, ThermoScientific), calculated with equations in Jeffrey & Humphrey [32], and the chlorophyll *a* concentrations were then divided by Symbiodiniaceae cell concentrations (µg $cell^{-1}$). Protein concentration was determined with a Nanodrop 2000 spectrophotometer and then divided by skeleton surface area (mg cm^{-2}).

(f) Statistical analysis

Statistical analyses were performed with R v. 3.4.3 [33] and plots were made with the 'ggplot2' package in R [34]. The 'survival' package [35] was used to plot Kaplan–Meier survival curves and Mantel–Cox log-rank tests were used for statistical comparisons among survival curves. Mann–Whitney *U* non-parametric tests were used when comparing two groups (i.e. transplantation sites) and Kruskal–Wallis non-parametric tests for comparing three groups (i.e. coral origins) followed by pairwise Mann–Whitney *U post hoc* tests. Non-parametric tests were used after visual assessment of normality with histograms and *Q*–*Q* plots.

3. Results

(a) Water chemistry

Total alkalinity and DIC were higher at the ojo than at the control site (total alkalinity: Mann–Whitney *U*, $W = 244$, $p < 0.001$; DIC: $W = 252$, $p < 0.001$) (table 1). Average total alkalinity was 2759 µmol kg^{-1} at the ojo site and 2365 µmol kg^{-1} at the control site and average DIC was 2621 µmol kg^{-1} at the ojo and 2019 µmol kg^{-1} at the control site. Salinity and calculated Ω_{arag} and pH were lower at the ojo than at the control site (salinity: $W = 6$, $p < 0.001$; Ω_{arag}: $W = 32$, $p < 0.01$; pH: $W = 50$, $p < 0.01$). The average salinity, Ω_{arag} and pH were 33.3, 2.10 and 7.70, respectively, at the ojo site and 35.6, 3.53 and 8.17 at the control site. Temperature fluctuated throughout the experiment but did not differ among transplantation sites

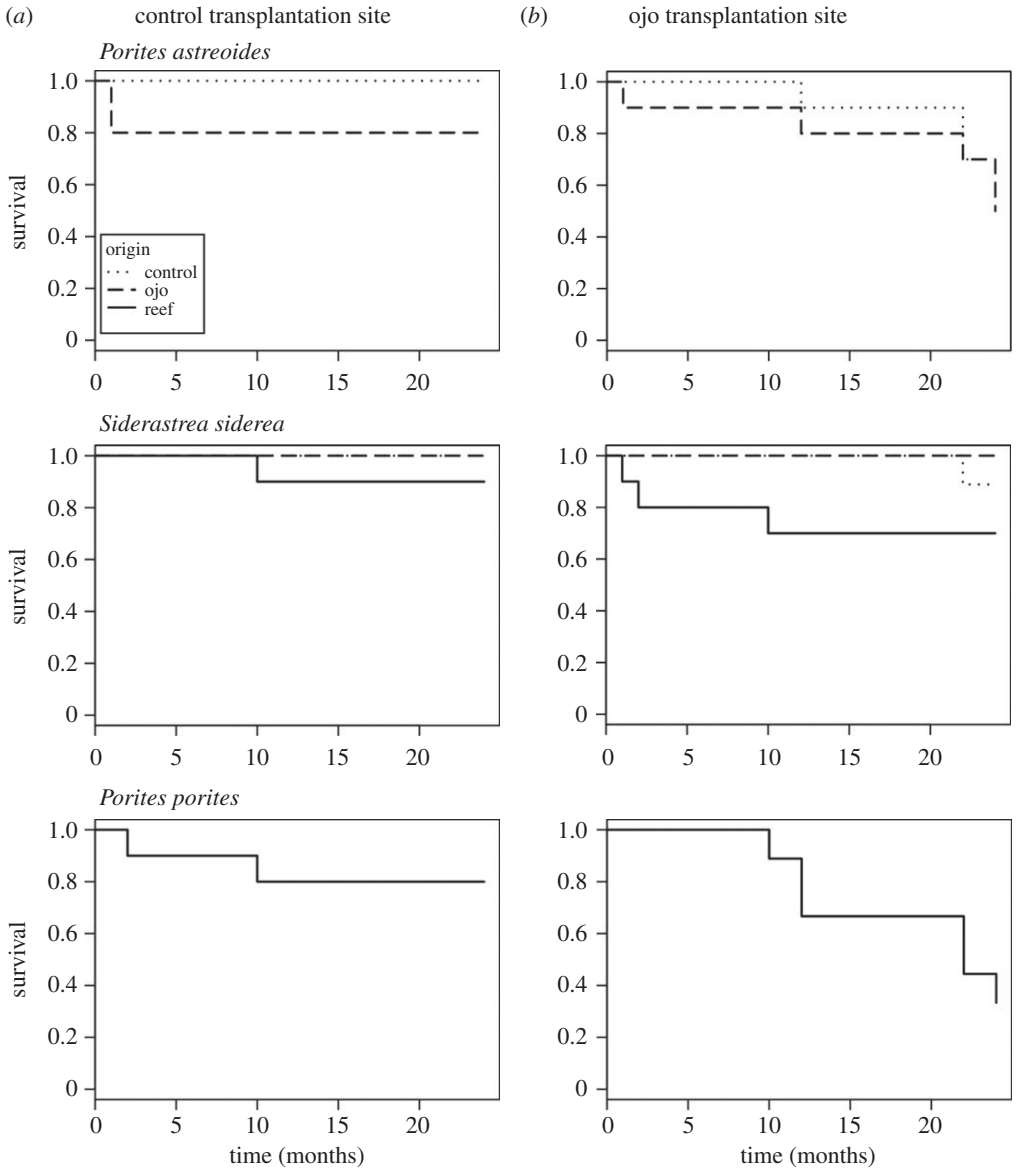

Figure 2. Survival curves of nubbins over 24 months after transplanting into (*a*) ambient Ω_{arag} control site and (*b*) low Ω_{arag} ojo: *Porites astreoides* ($n = 40$), *Siderastrea siderea* ($n = 58$) and *Porites porites* ($n = 19$). Solid and dashed lines indicate collection origins of the corals.

Table 1. Chemistry parameters of water samples collected throughout the 2-year experiment at the ambient Ω_{arag} lagoon transplantation site (control) and the low Ω_{arag} submarine spring transplantation site (ojo) where coral nubbins were transplanted. Data are mean ± s.e.

transplant site	*N*	alkalinity (µmol kg^{-1})	DIC (µmol kg^{-1})	pH[a]	Ω^{a}_{arag}	Temperature (°C)	Salinity
control	15	2365 ± 32	2019 ± 12	8.17 ± 0.07	3.53 ± 0.43	26.9 ± 0.3	35.6 ± 0.1
ojo	17	2759 ± 104	2621 ± 93	7.70 ± 0.14	2.10 ± 0.35	27.1 ± 0.2	33.2 ± 0.5

[a]Calculated using CO_2Sys.

(approx. 27°C ± 0.3 s.e.; $W = 122$, $p = 0.676$). The temperature, pH and conductivity data recorded by the SeapHOx sensors were within the range of values measured in our discreet samples. These data were used to confirm that our spot analyses are representative, but were not used to calculate Ω_{arag} since total alkalinity and DIC data were not available.

(b) Survival

Two years after transplantation, the survival of *P. astreoides* nubbins transplanted to the ojo was lower (60%) than those transplanted to the control site (90%) (log-rank test: $\chi^2 = 4.1$, d.f. = 1, $p = 0.042$; figure 2; electronic supplementary material, table S3). The survival of *P. astreoides* nubbins originating from ojos was qualitatively lower (65%) than those originated from control sites (85%), although the survival curves did not differ significantly ($\chi^2 = 2.2$, d.f. = 1, $p = 0.141$). *S. siderea* survival was similar among transplantation sites (control: 96%; ojo: 86.2%; log-rank test: $\chi^2 = 2$, d.f. = 1, $p = 0.16$), but survival of nubbins originating from the reef was marginally lower (80%) than those originating from ojos (100%) and controls (94%) ($\chi^2 = 5.6$, d.f. = 2, $p = 0.062$). All *P. porites* nubbins originated from the reef, and

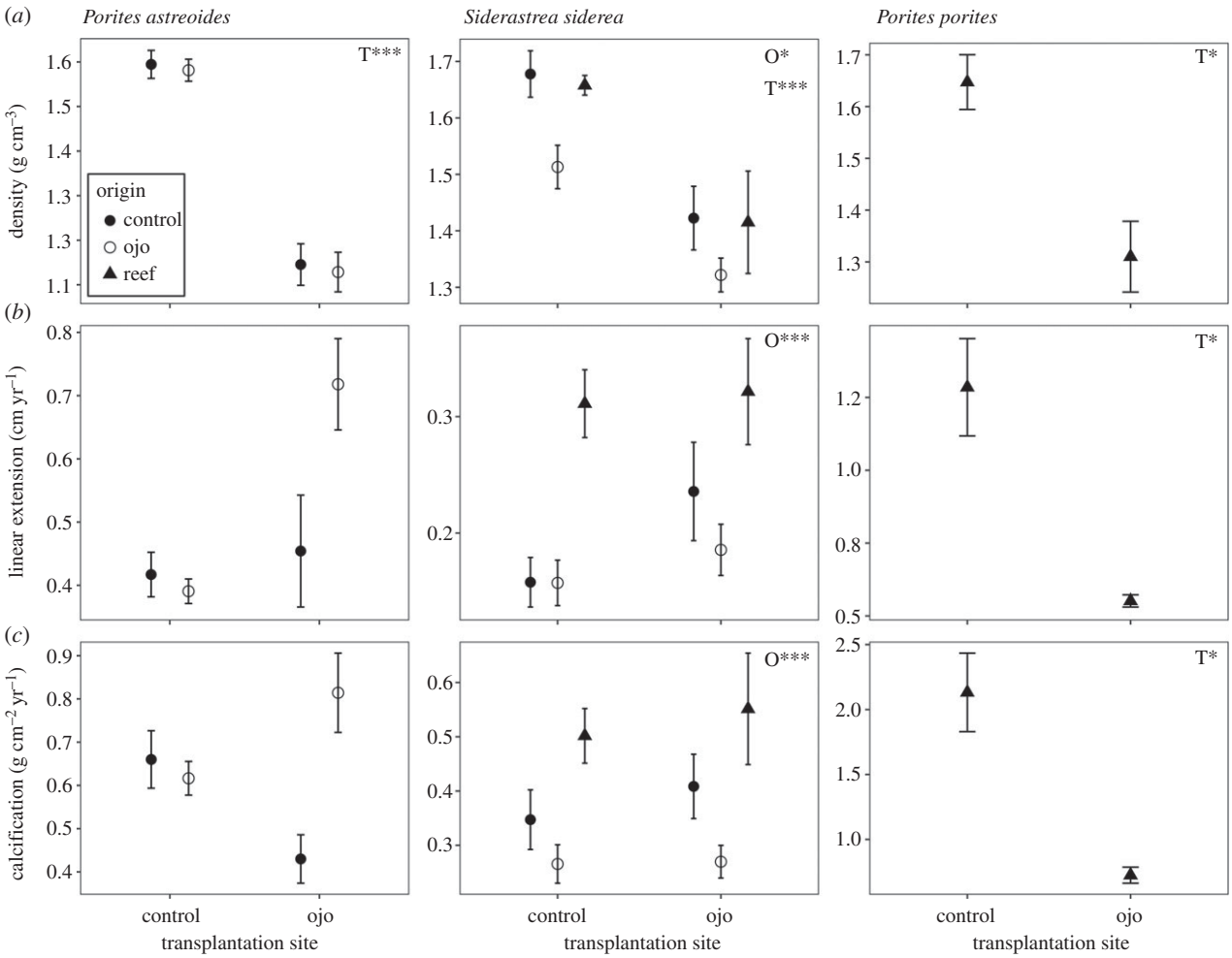

Figure 3. (*a*) Bulk density, (*b*) linear extension and (*c*) calcification rates of skeletons deposited during the two years after transplantation. Symbols indicate collection origins. Note the different ranges on *y*-axes. Data are mean ± s.e. Letters indicate overall comparisons of origin (O) and transplantation (T). Asterisks indicate statistical significance at *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$.

their survival at the ojo transplantation site was significantly lower (33%) than those at the control site (80%), but their survival curves were not different ($\chi^2 = 2.9$, d.f. = 1, $p = 0.087$).

(c) Density

The skeletal density of corals transplanted to the ojo was lower than that of corals transplanted to the control site for all three species (figure 3*a*; electronic supplementary material, table S3). The median density of coral skeletons transplanted to ojo and control sites, respectively, was 1.10 g cm^{-3} ± 0.03 s.e. and 1.58 g cm^{-3} ± 0.02 s.e. for *P. astreoides*, (Mann–Whitney *U*: $W = 150$, $p < 0.001$); 1.38 g cm^{-3} ± 0.03 s.e. and 1.63 g cm^{-3} ± 0.02 s.e. for *S. siderea* ($W = 250$, $p < 0.001$); and 1.29 g cm^{-3} ± 0.07 s.e. and 1.72 g cm^{-3} ± 0.05 s.e. for *P. porites* ($W = 20$, $p = 0.033$). Skeletal density of *P. astreoides* originating from ojos and control sites was similar ($W = 85$, $p = 0.728$). Skeletal density of *S. siderea* differed based on nubbin origin (Kruskal–Wallis: $H = 7.783$, d.f. = 2, $p = 0.020$). Density of *S. siderea* nubbins originating from ojos was lower than that of nubbins originating from the reef ($p = 0.017$) but was not different than nubbins originating from control sites ($p = 0.163$). Density of *S. siderea* nubbins originating from control sites and from the reef did not differ ($p = 0.508$).

(d) Linear extension

Linear extension of *P. astreoides* did not vary among transplantation sites (Mann–Whitney *U*: $W = 59.5$, $p = 0.079$) or origins ($W = 77$, $p = 0.245$; figure 3*b*; electronic supplementary material, table S3). *S. siderea* linear extension did not vary among transplantation sites ($W = 257$, $p = 0.302$) but varied based on nubbin origin (Kruskal–Wallis: $H = 16.679$, d.f. = 2, $p < 0.001$). Linear extension of *S. siderea* nubbins originating from the reef was higher (0.31 cm year^{-1} ± 0.02 s.e.) than nubbins originating either from control (0.19 cm year^{-1} ± 0.02 s.e., $p = 0.004$) or ojo sites (0.16 cm year^{-1} ± 0.01 s.e., $p < 0.001$). Linear extension of *P. porites* nubbins transplanted to the ojo was half that of nubbins transplanted to the control site ($W = 21$, $p = 0.017$).

(e) Calcification

P. astreoides calcification did not differ among sites (Mann–Whitney *U*: $W = 72$, $p = 0.815$) or origins ($W = 46$, $p = 0.143$; figure 3*c*; electronic supplementary material, table S3). Calcification rates of *S. siderea* also did not vary with transplantation site ($W = 144$, $p = 0.762$), but they did differ with nubbin origin (Kruskal–Wallis: $H = 15.113$, d.f. = 2, $p < 0.001$). Calcification of *S. siderea* nubbins originated from the reef was almost double those from ojo sites ($p < 0.001$), and approximately 35% more than nubbins originated from control sites ($p = 0.058$). The calcification rate of *P. porites* was approximately 66% lower at the ojo than at the control site ($W = 21$, $p = 0.017$).

(f) Symbiodiniaceae concentration

Symbiodiniaceae concentration per surface area of *S. siderea* was higher at the ojo (median: 1.26 × 10^7 cell cm^{-2} ± 3.6 × 10^6 s.e.) than at the control site (2.50 × 10^6 cell cm^{-2} ± 1.8 ×

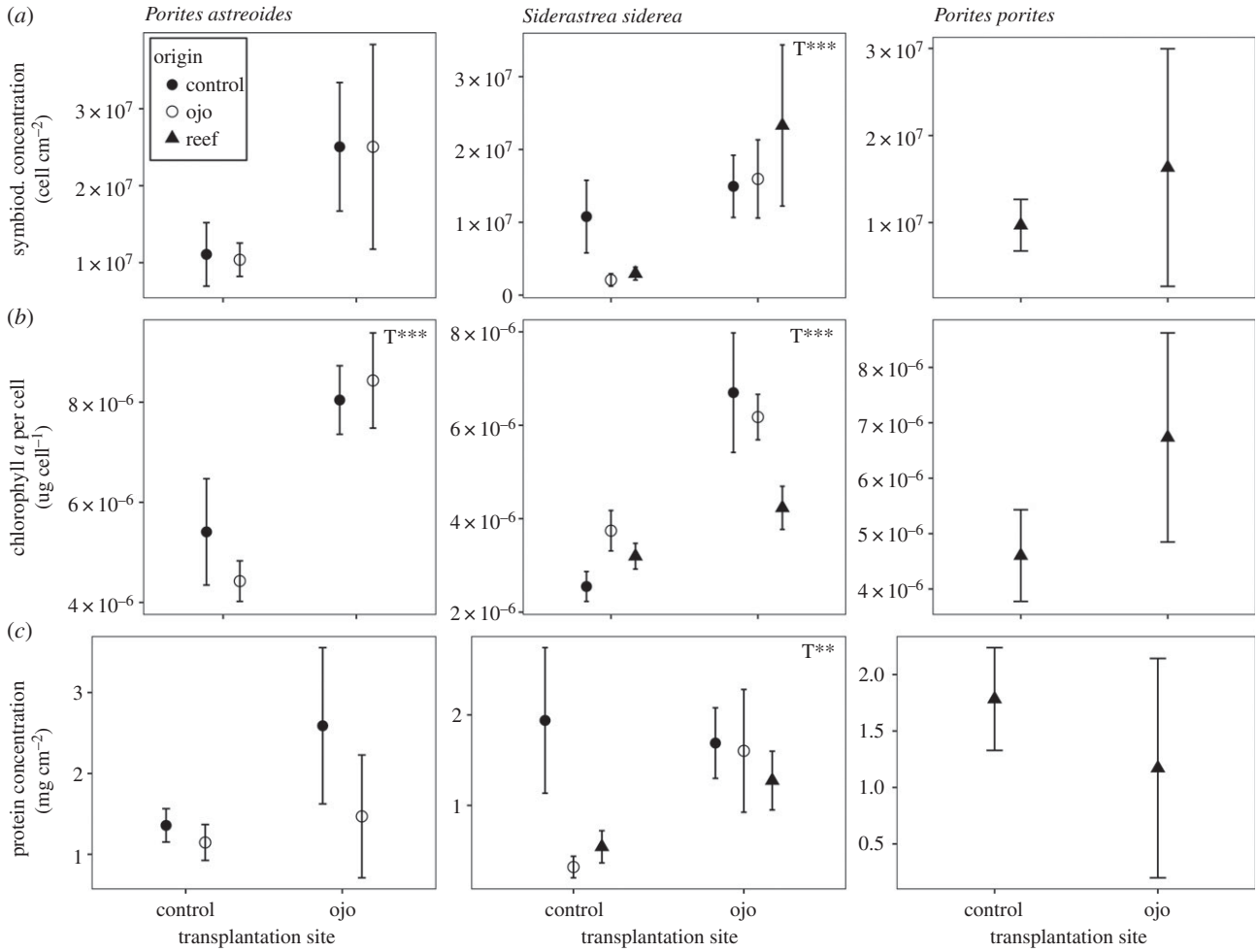

Figure 4. (*a*) Symbiodiniaceae, (*b*) chlorophyll *a* and (*c*) protein concentrations of surviving coral nubbins two years after transplantation at ambient Ω_{arag} control and low Ω_{arag} ojo sites. Data are mean ± s.e. Letters and significance values are denoted as in figure 3.

10^6 s.e., Mann–Whitney *U*: $W = 75$, $p < 0.001$; figure 4*a*; electronic supplementary material, table S4). Concentrations at the ojo were higher for *P. astreoides* and *P. porites* but the difference between transplantation sites for either *P. astreoides* ($W = 52$, $p = 0.183$) or *P. porites* ($W = 4$, $p = 0.857$) was not statistically significant. No coral species differed in Symbiodiniaceae concentration based on collection origin (*P. astreoides*: $W = 73$, $p = 0.81$; *S. siderea*: Kruskal–Wallis, $H = 2.469$, d.f. = 2, $p = 0.291$).

(g) Chlorophyll *a* concentration

Chlorophyll *a* concentrations per Symbiodiniaceae (µg per Symbiodiniaceae cell) of both *P. astreoides* and *S. siderea* were higher at the ojo than at the control site (*P. astreoides*: Mann–Whitney *U*, $W = 15$, $p < 0.001$; *S. siderea*: $W = 58$, $p < 0.001$; figure 4*b*; electronic supplementary material, table S4). Chlorophyll *a* concentration per Symbiodiniaceae of *P. astreoides* and *S. siderea* did not differ based on origins (*P. astreoides*: $W = 98$, $p = 0.719$; *S. siderea*: Kruskal–Wallis, $H = 4.135$, d.f. = 2, $p = 0.126$). Chlorophyll *a* concentration per Symbiodiniaceae of *P. porites* was higher at the ojo but the difference was not statistically significant among transplantation sites ($W = 4$, $p = 0.262$).

(h) Protein concentration

The protein concentration (mg cm^{-2}) of *S. siderea* nubbins transplanted to the ojo was twice that of nubbins transplanted to the control (Mann–Whitney *U*: $W = 119$, $p = 0.006$; figure 4*c*; electronic supplementary material, table S4), but differences were not statistically significant among transplantation sites for either *P. astreoides* ($W = 75$, $p = 0.936$) or *P. porites* ($W = 7$, $p = 0.571$). Protein concentration did not differ among nubbin origins for *P. astreoides* ($W = 98$, $p = 0.267$) or *S. siderea* (Kruskal–Wallis, $H = 4.807$, d.f. = 2, $p = 0.090$).

4. Discussion

This study demonstrated that after 2 years of *in situ* transplantation, three Caribbean coral species (*Porites astreoides*, *Siderastrea siderea* and *Porites porites*) were able to survive and calcify at very low Ω_{arag}; however, their skeletal density decreased while net linear extension and calcification rates were maintained. *S. siderea* nubbins had the highest survival and responded to low Ω_{arag} by increasing the concentration of Symbiodiniaceae, chlorophyll *a* and protein.

(a) Survival of slow-growing corals was higher than fast-growing corals transplanted to low Ω_{arag}

The survival of slow-growing massive *P. astreoides* and *S. siderea* was higher at low Ω_{arag} than faster-growing branching *P. porites* (figure 2), indicating a species-specific tolerance to low Ω_{arag} water discharging at the ojo. This is consistent with the coral assemblages naturally found at our study site:

massive colonies of *P. astreoides* and *S siderea* are abundant at low Ω_{arag} ojos, the only branching species found at the ojos is *P. divaricata* and it is present in very low numbers, and branching *P. porites* is absent [12]. Similarly, massive *Porites* corals were twice as abundant, while the abundance of structurally complex (e.g. branching) corals was a third lower at high pCO_2 volcanic seeps than at ambient Ω_{arag} sites in Papua New Guinea [11], and massive *Pavonid* corals were least sensitive to acidification in Panama [36]. While these results suggest massive corals have an advantage over branching corals at low Ω_{arag} sites, this may be attributed to differential growth rates rather than morphologies, since morphology was found to be unrelated to calcification of corals experimentally incubated at high pCO_2 [37,38]. Fast-growing corals may be more affected by low Ω_{arag} because their rapid calcification makes them more sensitive to low carbonate ion availability [8] and requires them to remove protons from the calcification site fast enough to maintain an adequate inner pH [37], with a concomitant increased energy demand.

(b) Skeletal density was lower at the low Ω_{arag} transplantation site

While calcification rates were not consistently lower at the ojo transplantation site, the skeletal density for all species was 15–30% lower. This suggests these corals maintained their linear extension at the expense of density (figure 3). Linear extension is inversely correlated with density because a faster extension rate results in less time for skeleton thickening [39]. Phenotypically plastic skeletons have been described in many corals under a variety of environmental factors, with high-density skeletons associated with high Ω_{arag} [40], high temperature [41], low cloud coverage and rainfall [42], low turbidity and sediment load [43] and high hydraulic energy [30]. In field studies, several environmental variables often covary and isolating the effect of a single environmental factor is challenging. In our experiment, most parameters except carbonate chemistry were similar at both transplantation sites, which emphasizes the role of low Ω_{arag} in reducing density. Similar density and porosity differences occur naturally in *P. astreoides* sampled at ojos and control sites as measured with computerized tomography [19], suggesting that the differences in our study were not artefacts of the transplantation process, and that corals experiencing low Ω_{arag} conditions for their entire lives do not achieve the skeletal densities of their counterparts in ambient Ω_{arag} water. Field and laboratory studies collectively indicate that coral growth at low Ω_{arag} waters usually results in precipitation of lower density skeletons [19,40,44,45]. Our data show that when moved to a more favourable environment, the corals can acclimatize and increase their skeletal density.

Decoupling extension and density, and the processes that control each, will increase our understanding of coral calcification and potential acclimatization in low Ω_{arag} waters. Neither calcification rate nor skeletal growth alone fully reflects the effects of low Ω_{arag} on the coral skeleton; it is necessary to consider density, linear extension and calcification rate together [44,45] to avoid conflicting results on the effects of ocean acidification on calcification.

Although all the *S. siderea* nubbins originating from the ojos survived the 2-year transplantation experiment, their skeletal density, linear extension and calcification rates were lower than that of nubbins originating from the reef site. It is possible that the early life history of corals is affected by low pH (e.g. post settlement growth) [46], impacting the ability of *S. siderea* originating from the ojos to respond to changing conditions later in life, potentially reflecting poor overall health state for corals that were obtained from sites that have less than optimal growth conditions, impairing their ability to overcome further stress. It is also possible that the populations have been physically isolated for an extended time allowing some genetic divergence. Indeed, a recent study [47] reported intraspecific variability of calcification rates of *Acropora digitifera* originating from two distinct locations after exposure to high CO_2 in a laboratory experiment, suggesting different populations of the same coral species have different susceptibility to ocean acidification.

(c) Concentrations of symbiodiniaceae, chlorophyll *a* and proteins were higher at the low Ω_{arag} ojo transplantation site

The higher concentrations of Symbiodiniaceae, chlorophyll *a* and tissue protein of *S. siderea* transplanted to the ojo (figure 4) may be responsible for its ability to survive and calcify at low Ω_{arag}. Calcification requires transport of carbonate and calcium ions and proton removal from the extracellular calcifying medium to maintain a high Ω_{arag} at the calcification site [48]. If the external seawater has a lower Ω_{arag}, the coral may have to expend more energy for raising the internal Ω_{arag} to promote calcification [49]. Tropical corals obtain the energy required to move these ions through heterotrophy and symbiotic algal photosynthesis. Although feeding rates were not measured in this study, corals can modify their heterotrophic feeding to increase their energy budget under low Ω_{arag} conditions [49]; nevertheless, changes in heterotrophy are species-specific [50]. It is possible that nubbins transplanted to the low Ω_{arag} ojo enhanced symbiont concentrations that provided more energy resources needed to cope with the greater difference between the external and internal carbonate chemistry. Indeed, the higher symbiont concentrations may have allowed *S. siderea* nubbins transplanted to the ojo to maintain calcification rates similar to those of nubbins transplanted to the control site, as observed in laboratory experiments [7,29,45]. It is also possible that photosynthates derived from endolithic algae may promote calcification under low Ω_{arag} as they could also provide similar photosynthates to corals at low Ω_{arag}. Collectively, our data and data from previous research suggest that the energy provided by the symbionts can be used to overcome the larger gradient of proton and carbonate ion between the environment and the calcification solution in order to maintain net growth at least in some coral species.

The chlorophyll *a* concentration per Symbiodiniaceae cell of *P. astreoides* and *S. siderea* was higher after 2 years of transplantation at low Ω_{arag} (figure 4*b*), which suggests an increased capacity for the photosynthetic activity that could provide additional energy to the corals. The increase in primary production may mean that more photosynthates are produced and assimilated by the host in such a manner that the coral can keep calcification rates under low Ω_{arag}. Nevertheless, further research is needed to elucidate the effects of low pH on chlorophyll *a* concentrations and assess the effect of chlorophyll *a* concentrations on calcification, since several authors have reported mixed results in laboratory experiments with *Stylophora pistillata* [7,29,45]. It is possible that the increase in

chlorophyll *a* concentration is due to an increase in cyanobacteria rather than Symbiodiniaceae; the cyanobacteria may promote photosynthesis through nitrogen fixation [51] and ultimately, provide the needed energetic resources to facilitate calcification under low Ω_{arag}.

The protein content per surface area was higher in *S. siderea* nubbins transplanted to the ojo than those at the control site (figure 4*c*), suggesting that these corals may be thickening their organic matrix protein content to promote calcification under low Ω_{arag} seawater [45]. Several experimental studies have also reported an increase in host tissue and organic matrix protein concentrations under reduced pH in several species [29,45]. While we did not measure tissue thickness, thicker tissue has been observed in low Ω_{arag} corals at this field site [19] and has been proposed to confer an advantage against coral bleaching [52] and low pH [16], since it serves as a protective barrier against hot or corrosive seawater.

(d) Shifting species assemblages in an acidified ocean

The differential sensitivity across populations and species to low Ω_{arag} suggest that, as ocean acidification progresses, the relative abundances of different coral species and populations in reef ecosystems may change towards the dominance of slow-growing species, and hence changes in coral communities and reef structure are also likely, with potential cascading effects. This finding is particularly critical for reefs currently dominated by fast-growing, branching species, such as *Acropora* and *Stylophora pistillata* in Indo-Pacific shallow reefs [53]. As sea level is expected to rise by 26 to 55 cm by 2100 (scenario RCP2.6 of IPCC), it is possible that coral reefs dominated by slow-growing species may not be able to grow fast enough to avoid drowning [54]. In addition, reduced skeletal density has been linked to increased bioerosion and boring [4,19,55] and low Ω_{arag} waters also weaken cementation of reef structures, which further promotes coral erosion [56] and leads to less cohesive reef frameworks that are more susceptible to storms. Differences in fecundity and recruitment among species can also shape the reef's species assemblage. All three species used in this study brood larvae able to settle soon after release [57] which increases the likelihood that larvae may settle in parental habitats and which would be advantageous if selection among adults has favoured adaptations for low pH. In particular, *P. astreoides* has additional advantages: it is hermaphroditic with potentially high rates of self-fertilization [58] and also appears capable of producing parthenogenic or asexual larvae [59], all of which are mechanisms for propagating maternal genotypes. Furthermore, *P. astreoides* appears capable of adaptation to thermal stress [60] further enhancing its potential for greater abundance in a warmer and more acidified future.

In summary, while corals can calcify and grow under ocean acidification conditions, the possible synergistic effects of sea-level rise on slow-growing corals; high temperature on Symbiodiniaceae concentrations; high nutrient concentrations on skeletal bioerosion; or increased storm intensity on less dense coral skeletons predict a challenging future for coral survival and growth. On the other hand, the differences in survival and calcification among coral species may be useful for studies of human-assisted evolution projects [61] and implementation of restoration programmes as a strategy to augment the capacity of reef organisms to tolerate stress and to facilitate recovery after disturbances.

Data accessibility. Data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.3pm80bp [62].

Authors' contributions. A.P. and D.C.P. jointly conceived and led this project and designed the experimental set-up. A.M., L.H., M.R.-V., A.P. and D.C.P. participated in the fieldwork and A.M. conducted the laboratory and data analyses. All authors contributed to the interpretation of the data. A.M. wrote the paper with contribution from all authors.

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