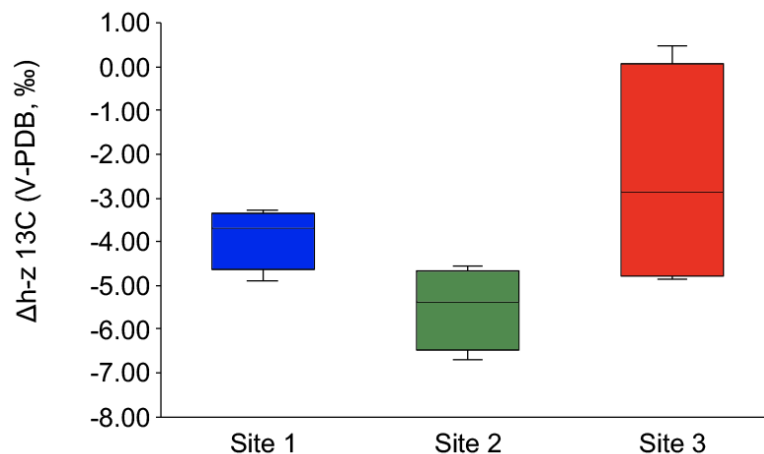


Acclimatization of a coral-algal mutualism at a CO₂ vent

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SUPPLEMENTARY MATERIALS

Supplementary Figures



Supplementary Figure 1. Average $\Delta^{h-z} {}^{13}\text{C}$ (V-PDB, ‰) values in *B. europaea* from Sites 1 (control), 2 (intermediate pCO₂) and 3 (high pCO₂). The boxes indicate the 25th and 75th percentiles and the line within the boxes mark the medians. Whisker length is equal to 1.5 × interquartile range (IQR).

SUPPLEMENTARY TABLES

Supplementary Table 1. Seawater carbonate chemistry data for each study site. Data are presented as mean values with 95% CIs in brackets. N = number of observations; T = temperature (number of observations); TA = total alkalinity; pCO₂ = carbon dioxide partial pressure; HCO₃⁻ = bicarbonate; CO₃²⁻ = carbonate; DIC = dissolved inorganic carbon; Ω_{arag} = aragonite saturation; NS = not significant; *p<0.05, ***p<0.001, Kruskal-Wallis equality-of- populations rank test. pH_{Ts}, T, TA, and salinity were used to calculate the carbonate system parameters, denoted by (a). Pairwise comparisons between Sites were performed with Mann-Whitney U test. **p< 0.01, ***p< 0.001, NS = not significant

| | pH _{Ts} | Salinity (‰) | T (°C) | TA (μmol/kg) | pCO ₂ (μatm) ^a | HCO ₃ ⁻ (μmol/kg) ^a | CO ₃ ²⁻ (μmol/kg) ^a | CO ₂ (μmol/kg) ^a | DIC (μmol/kg) ^a | Ω _{calc} ^a | Ω _{arag} ^a |
|-----------------------------|---------------------|---------------------|---------------------|---------------------|---|---|---|---|-------------------------------|--------------------------------|--------------------------------|
| Site 1 | 7.97 (7.95-7.99) | 35.6 (35.5-35.8) | 21.1 (20.5-21.7) | 2368 (2277-2459) | 437 (415-459) | 1888 (1867-1909) | 207 (199-216) | 13.7 (13.0-14.4) | 2109 (2094-2124) | 4.9 (4.7-5.1) | 3.2 (3.1-3.4) |
| | N = 198 | N = 195 | N = 198 | N = 26 | N = 195 | N = 195 | N = 195 | N = 195 | N = 195 | N = 195 | N = 195 |
| Site 2 | 7.86 (7.83-7.90) | 35.5 (35.3-35.7) | 21.1 (20.5-21.8) | 2288 (2217-2359) | 654 (589-720) | 1927 (1902-1952) | 157 (150-165) | 20.4 (18.5-22.3) | 2105 (2082-2127) | 3.7 (3.3-3.9) | 2.5 (2.3-2.6) |
| | N = 185 | N = 185 | N = 185 | N = 25 | N = 185 | N = 185 | N = 185 | N = 185 | N = 185 | N = 185 | N = 185 |
| Site 3 | 7.64 (7.55-7.77) | 35.6 (35.4-35.8) | 21.1 (20.5-21.7) | 2348 (2296-2399) | 1161 (832-1490) | 1995 (1973-2018) | 146 (138-154) | 35.7 (26.1-45.2) | 2177 (2155-2199) | 3.5 (3.3-3.7) | 2.3 (2.2-2.4) |
| | N = 189 | N = 184 | N = 189 | N = 22 | N = 184 | N = 184 | N = 184 | N = 184 | N = 184 | N = 184 | N = 184 |
| Pairwise comparisons | | | | | | | | | | | |
| 1 vs 2 | *** | NS | NS | NS | *** | *** | *** | *** | NS | *** | *** |
| 1 vs 3 | *** | NS | NS | NS | *** | *** | *** | *** | ** | *** | *** |
| 2 vs 3 | *** | NS | NS | NS | *** | *** | *** | *** | ** | NS | NS |

Supplementary Table 2. Average concentration of sulphate (N = 5), phosphate (N = 4) and inorganic nitrogen (N = 4) in the seawater at the four sites. Data are presented as mean \pm Standard Deviation (SD). The analytical error for sulphate is < 5%. Pairwise comparisons between Sites were performed with Mann-Whitney U test. *p < 0.05, **p < 0.01, NS = not significant

| | SO₄²⁻ ($\mu\text{mol L}^{-1}$) | PO₄³⁻ ($\mu\text{mol L}^{-1}$) | NO_x(NO₂+NO₃) ($\mu\text{mol L}^{-1}$) |
|-----------------------------|--|--|--|
| Site 1 | 36852 (± 1243) | 0.802 (± 1.355) | 0.515 (± 0.450) |
| Site 2 | 35686 (± 2188) | 0.158 (± 0.162) | 0.113 (± 0.184) |
| Site 3 | 31855 (± 1001) | 0.393 (± 0.419) | 0.758 (± 0.997) |
| Pairwise comparisons | | | |
| 1 vs 2 | NS | NS | NS |
| 1 vs 3 | ** | NS | NS |
| 2 vs 3 | * | NS | NS |

Supplementary Table 3. Number of coral specimens measured by PAM fluorometry in the three Sites across the sampling period.

| Date | Time interval | Number of corals | | | |
|-----------|---------------|------------------|------------|------------|------------|
| | | Site 1 | Site 2 | Site 3 | Total |
| 22/7/2019 | 18:00-20:00 | 10 | 10 | 10 | 30 |
| 23/7/2019 | 9:00-13:00 | 17 | 13 | 15 | 45 |
| 23/7/2019 | 20:00-22:00 | 11 | 8 | 11 | 30 |
| 24/7/2019 | 9:00-13:00 | 21 | 22 | 25 | 68 |
| 24/7/2019 | 20:00-22:00 | 14 | 9 | 15 | 38 |
| 25/7/2019 | 18:00-20:00 | 22 | 19 | 20 | 61 |
| 26/7/2019 | 18:00-20:00 | 19 | 19 | 19 | 57 |
| 14/2/2020 | 9:00-13:00 | 8 | 9 | 7 | 24 |
| | TOTAL | 122 | 109 | 122 | 353 |
| | 9:00-13:00 | 46 | 44 | 47 | 137 |
| | 18:00-20:00 | 51 | 48 | 49 | 148 |
| | 20:00-22:00 | 25 | 17 | 26 | 68 |

Supplementary Table 4. Average values for the three fluorescence parameters $\Delta F/F_m'$, F, and F' at the three sites in the three time intervals.

| | | N | Mean (95% CI) |
|-------------|-----------------------------------|----------|----------------------|
| 9:00-13:00 | $\Delta F/F_m'$ | | |
| | Site 1 | 46 | 0.52 (0.49-0.55) |
| | Site 2 | 44 | 0.53 (0.50-0.57) |
| | Site 3 | 47 | 0.55 (0.53-0.58) |
| | F | | |
| | Site 1 | 46 | 258 (234-282) |
| | Site 2 | 44 | 303 (272-333) |
| | Site 3 | 47 | 314 (289-340) |
| | Fm' | | |
| | Site 1 | 46 | 563 (503-623) |
| | Site 2 | 44 | 718 (604-832) |
| | Site 3 | 47 | 745 (658-832) |
| 18:00-20:00 | $\Delta F/F_m'$ | | |
| | Site 1 | 51 | 0.60 (0.59-0.62) |
| | Site 2 | 48 | 0.61 (0.60-0.63) |
| | Site 3 | 49 | 0.59 (0.58-0.60) |
| | F | | |
| | Site 1 | 51 | 351 (321-381) |
| | Site 2 | 48 | 443 (404-462) |
| | Site 3 | 49 | 421 (400-443) |
| | Fm' | | |
| | Site 1 | 51 | 849 (783-916) |
| | Site 2 | 48 | 1113 (1049-1212) |
| | Site 3 | 49 | 1037 (984-1091) |
| 20:00-22:00 | $\Delta F/F_m'$ | | |
| | Site 1 | 25 | 0.58 (0.56-0.61) |
| | Site 2 | 17 | 0.59 (0.54-0.64) |
| | Site 3 | 26 | 0.59 (0.57-0.62) |
| | F | | |
| | Site 1 | 25 | 190 (172-208) |
| | Site 2 | 17 | 279 (248-310) |
| | Site 3 | 26 | 306 (288-324) |
| | Fm' | | |
| | Site 1 | 25 | 482 (421-543) |
| | Site 2 | 17 | 740 (636-843) |
| | Site 3 | 26 | 769 (711-828) |

Supplementary Table 5. Results of the PERMANOVA analysis for the three fluorescence parameters. Pairwise tests are shown for significant factors. df: degrees of freedom; SS: sum of squares; MS: mean squares. Significant differences are indicated in bold.

| <i>$\Delta F/F_m'$</i> | | | | | | <i>F</i> | | | | | | <i>F_m'</i> | | | | | |
|---|-----|-------|-------|--------------|--------------|---|-----|---------|-------|--------------|--------------|---|-----|--------|-------|--------------|--------------|
| Source | df | SS | MS | Pseudo-F | P(perm) | Source | df | SS | MS | Pseudo-F | P(perm) | Source | df | SS | MS | Pseudo-F | P(perm) |
| Site | 2 | 0.006 | 0.003 | 0.548 | 0.553 | Site | 2 | 0.424 | 0.212 | 28.588 | 0.001 | Site | 2 | 3.597 | 1.798 | 28.417 | 0.001 |
| Hour | 2 | 0.336 | 0.168 | 29.254 | 0.001 | Hour | 2 | 1.298 | 0.649 | 87.590 | 0.001 | Hour | 2 | 9.560 | 4.780 | 75.537 | 0.001 |
| Site x Hour | 4 | 0.026 | 0.006 | 1.115 | 0.357 | Site x Hour | 4 | 0.044 | 0.011 | 1.484 | 0.198 | Site x Hour | 4 | 0.343 | 0.086 | 1.356 | 0.232 |
| Res | 344 | 1.978 | 0.006 | | | Res | 344 | 2.550 | 0.007 | | | Res | 344 | 21.769 | 0.063 | | |
| Total | 352 | 2.345 | | | | Total | 352 | 4.3202 | | | | Total | 352 | 35.516 | | | |
| Pairwise tests for the factor "Hour" | | | | | | Pairwise tests for the factor "Hour" | | | | | | Pairwise tests for the factor "Hour" | | | | | |
| Groups | | t | | P(perm) | | Groups | | t | | P(perm) | | Groups | | t | | P(perm) | |
| 9:00-13:00 vs 18:00-20:00 | | 7.323 | | 0.001 | | 9:00-13:00 vs 18:00-20:00 | | 10.008 | | 0.001 | | 9:00-13:00 vs 18:00-20:00 | | 10.392 | | 0.001 | |
| 9:00-13:00 vs 20:00-22:00 | | 3.980 | | 0.001 | | 9:00-13:00 vs 20:00-22:00 | | 2.843 | | 0.008 | | 9:00-13:00 vs 20:00-22:00 | | 0.295 | | 0.783 | |
| 18:00-20:00 vs 20:00-22:00 | | 1.416 | | 0.155 | | 18:00-20:00 vs 20:00-22:00 | | 11.537 | | 0.001 | | 18:00-20:00 vs 20:00-22:00 | | 10.653 | | 0.001 | |
| Pairwise tests for the factor "Site" | | | | | | Pairwise tests for the factor "Site" | | | | | | | | | | | |
| Groups | | t | | P(perm) | | Groups | | t | | P(perm) | | | | | | | |
| Site 1 vs Site 2 | | 5.497 | | 0.001 | | Site 1 vs Site 2 | | 6.16 | | 0.001 | | | | | | | |
| Site 1 vs Site 3 | | 7.388 | | 0.001 | | Site 1 vs Site 3 | | 7.4738 | | 0.001 | | | | | | | |
| Site 2 vs Site 3 | | 0.743 | | 0.460 | | Site 2 vs Site 3 | | 0.31678 | | 0.769 | | | | | | | |

Supplementary Table 6. $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and C/N ratios for host tissue and algal symbionts in Sites 1 (control), 2 (intermediate pCO_2) and 3 (high pCO_2). Pairwise comparisons between Sites were performed with a post-hoc Fisher least significant differences test (LSD). N = 4 specimens for each Site. NS: Not Significant, ** $p < 0.01$, *** $p < 0.001$.

| | | Pairwise comparison | | | | |
|---------------------------|--------|---------------------|----------------|--------|--------|----------------|
| | | Tissue | Algal symbiont | Sites | Tissue | Algal symbiont |
| $\delta^{13}\text{C}$ (‰) | Site 1 | -19.55 | -16.27 | 1 vs 2 | NS | NS |
| | | -19.99 | -15.09 | | | |
| | | -19.20 | -15.64 | | | |
| | | -18.08 | -14.26 | | | |
| | Site 2 | -18.89 | -13.95 | 1 vs 3 | NS | NS |
| | | -19.13 | -14.57 | | | |
| | | -21.41 | -15.59 | | | |
| | | -19.68 | -12.98 | | | |
| | Site 3 | -17.95 | -13.43 | 2 vs 3 | NS | NS |
| | | -17.93 | -18.40 | | | |
| | | -19.41 | -18.21 | | | |
| | | -19.55 | -14.67 | | | |
| $\delta^{15}\text{N}$ (‰) | Site 1 | 3.57 | 3.10 | 1 vs 2 | *** | NS |
| | | 3.05 | 2.31 | | | |
| | | 3.92 | 1.69 | | | |
| | | 3.86 | 1.53 | | | |
| | Site 2 | 0.80 | 1.50 | 1 vs 3 | *** | NS |
| | | -0.64 | 1.82 | | | |
| | | -0.71 | 1.92 | | | |
| | | 0.03 | 0.65 | | | |
| | Site 3 | 0.93 | 0.56 | 2 vs 3 | NS | NS |
| | | 0.83 | 0.49 | | | |
| | | -0.04 | 0.74 | | | |
| | | 0.03 | 3.26 | | | |
| C/N ratio | Site 1 | 5.60 | 6.56 | 1 vs 2 | *** | ** |
| | | 4.95 | 6.36 | | | |
| | | 5.76 | 6.40 | | | |
| | | 6.16 | 7.45 | | | |
| | Site 2 | 1.14 | 8.67 | 1 vs 3 | *** | *** |
| | | 0.32 | 8.10 | | | |
| | | 0.09 | 7.56 | | | |
| | | 0.19 | 9.78 | | | |
| | Site 3 | 1.26 | 11.25 | 2 vs 3 | NS | ** |
| | | 1.94 | 11.00 | | | |
| | | 0.24 | 10.72 | | | |
| | | 0.46 | 9.86 | | | |