Prolonged exposure to elevated CO$_2$ promotes growth of the algal symbiont Symbiodinium muscatinei in the intertidal sea anemone Anthopleura elegantissima

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Summary

Some photosynthetic organisms benefit from elevated levels of carbon dioxide, but studies on the effects of elevated PCO$_2$ on the algal symbionts of animals are very few. This study investigated the impact of hypercapnia on a photosynthetic symbiosis between the anemone Anthopleura elegantissima and its zooxanthella Symbiodinium muscatinei. Anemones were maintained in the laboratory for 1 week at 37 Pa PCO$_2$ and pH 8.1. Clonal pairs were then divided into two groups and maintained for 6 weeks under conditions naturally experienced in their intertidal environment, 45 Pa PCO$_2$, pH 8.1 and 231 Pa PCO$_2$, pH 7.3. Respiration and photosynthesis were measured after the 1-week acclimation period and after 6 weeks in experimental conditions. Density of zooxanthellal cells, zooxanthellal cell size, mitotic index and chlorophyll content were compared between non-clonemate anemones after the 1-week acclimation period and clonal anemones at the end of the experiment. Anemones thrived in hypercapnia. After 6 weeks, A. elegantissima exhibited higher rates of photosynthesis at 45 Pa (4.2 μmol O$_2$ g$^{-1}$ h$^{-1}$) and 231 Pa (3.30 μmol O$_2$ g$^{-1}$ h$^{-1}$) than at the initial 37 Pa (1.53 μmol O$_2$ g$^{-1}$ h$^{-1}$). Likewise, anemones at 231 Pa received more of their respiratory carbon from zooxanthellae (CZAR = 78.2%) than those at 37 Pa (CZAR = 66.6%) but less than anemones at 45 Pa (CZAR = 137.3%). The mitotic index of zooxanthellae was significantly greater in the hypercapnic anemones than in anemones at lower PCO$_2$. Excess zooxanthellae were expelled by their hosts, and cell densities, cell diameters and chlorophyll contents were not significantly different between the groups. The response of A. elegantissima to hypercapnic acidification reveals the potential adaptation of an intertidal, photosynthetic symbiosis for high PCO$_2$.

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Introduction

Anthropogenic emissions of carbon dioxide are not only warming the climate but also decreasing the pH of the ocean (Vitousek et al., 1997; Orr et al., 2005). At 39.4 Pa, the present partial pressure of CO$_2$ (PCO$_2$) in the atmosphere is nearly 40% higher than in the prior 800,000 years (Lüthi et al., 2008).

Elevated levels of PCO$_2$ can increase photosynthesis and growth rates of many different kinds of plants (e.g. Kets et al., 2010; Mateos-Naranjo et al., 2010; Moulinho-Pereira et al., 2009; Norikane et al., 2010). In the marine environment, elevated PCO$_2$ stimulates photosynthesis and/or growth rates in macroalgae (Gao et al., 1993; Kübler et al., 1999; Xu et al., 2010) and seagrass (Palacios and Zimmerman, 2007; Jiang et al., 2010). Photosynthesis in some free-living microalgae is positively affected by elevated PCO$_2$ (Beardall et al., 2009; Wu et al., 2010; Van de Waal et al., 2011), but only a few studies have looked at the effects of elevated PCO$_2$ on photosynthesis in the microalgae of alga-cnidarian symbioses. To date, those studies have all been carried out on calcifying symbioses (Schneider and Erez, 2006; Anthony et al., 2008; Crawley et al., 2010).

Many marine ecosystems experience naturally high levels of PCO$_2$, including midwater oceanic oxygen minimum layers (Childress and Seibel, 1998; Paulmier et al., 2011), estuaries (Kempe, 1982; Frankignoule et al., 1998; Hinga, 2002) and tide pools (Ganning, 1971; Truchot and Duhamel-Jouve, 1980; Morris and Taylor, 1983). Diel cycles of photosynthesis and respiration strongly affect PCO$_2$ in tide pools, both seasonally (Truchot and Duhamel-Jouve, 1980) and on a daily basis (Morris and Taylor, 1983). In isolated tide pools with dense populations of organisms, daytime PCO$_2$ can fall below 1 Pa due to the use of CO$_2$ by photosynthesis; at night, organismal respiration can drive PCO$_2$ values above 355 Pa as CO$_2$ accumulates (Morris and Taylor, 1983). An 8-year study of coastal waters of the northeastern Pacific Ocean found that pH typically varied by 0.24 units over 24 hours and by more than one pH unit annually due to fluctuations in PCO$_2$ (Wootton et al., 2008). The region is also subject to coastal upwelling, which can bring in seawater with PCO$_2$ at 100 Pa (Feely et al., 2008). The combined effects of upwelling with daily and seasonal fluctuations periodically subject coastal organisms to levels of pH and PCO$_2$ that approximate the conditions expected by the year 2300.
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M˙O2 were also observed in individuals after six weeks of incubation in elevated CO2 conditions (paired t-test, p<0.001 for both groups) (Table 1; Fig. 1A). Furthermore, P˙g was greater at 45 Pa when compared to their clonemates at 231 Pa (paired t-test, p=0.03) (Fig. 1A). Significant increases in M˙O2 were also observed in individuals after six weeks of incubation in 45 and 231 Pa CO2 (paired t-tests, p<0.01 and 0.05, respectively) (Table 1; Fig. 1B). However, there were no significant differences in M˙O2 of 45 Pa CO2 when compared to their clonemates at 231 Pa CO2 (paired t-test, p>0.05) (Fig. 1B). As in the case of P˙g, there were significant increases in daily net photosynthesis after six weeks in 231 Pa CO2 with even greater increases among anemones in 45 Pa (paired t-test, p<0.001 for both groups) (Table 1; Fig. 1C).

Significant increases in P˙g:M˙O2 ratios were also observed in individuals after six weeks of incubation in 45 and 231 Pa CO2 (paired t-tests, p<0.001 and 0.01, respectively) (Table 1; Fig. 2A). Mean P˙g:M˙O2 ratios were greater in 45 Pa than at 231 Pa CO2, however the difference was not significant (paired t-test, p=0.1). The contribution of organic carbon by zooxanthellae to animal respiration (CZAR) (Fig. 2B) was greatest in 45 Pa CO2 (CZAR = 143.6%). This was significantly higher than after one week at 37 Pa CO2 (CZAR = 68.2%, paired t-test, p<0.001) and also higher than that of clonemates incubated at 231 Pa CO2 (CZAR = 81.8%, paired t-test, p<0.01).

Results

All anemones thrived during the experimental period, and three individuals among both the 45 Pa specimens and 231 Pa specimens reproduced through bilateral fission. These new clones were combined and treated as single individuals for final measurements. There were no significant differences in mass of the anemones (paired t-test, p>0.4) after 6 weeks of incubations in elevated CO2, and there were no significant effects of body mass on either P˙g or M˙O2 over the size range of specimens used in this study (3.33–12.09 g; linear regressions not shown).

Photosynthesis, respiration and CZAR

Significant increases in P˙g were observed in individuals after six weeks of incubation in 45 and 231 Pa PCO2 (paired t-test, p<0.001 for both groups) (Table 1; Fig. 1A). Furthermore, P˙g was greater at 45 Pa when compared to their clonemates at 231 Pa (paired t-test, p=0.03) (Fig. 1A). Significant increases in M˙O2 were also observed in individuals among both the 45 Pa specimens and 231 Pa specimens reproduced through bilateral fission (Ayre and Grosberg, 1995), and experiments examined the effects of elevated PCO2 through paired comparisons of genetically identical but separated clonal couplets.

In the eastern North Pacific Ocean, the anemone Anthopleura elegantissima Brandt (Cnidaria: Anthozoa) harbors the dinoflagellate Symbiodinium muscatinei LaJeunesse and Trench (Dinomastigota: Dinophyceae), which is congeneric with symbiotic dinoflagellates found in hermatypic corals (LaJeunesse and Trench, 2000). In its intertidal habitat, Anthopleura elegantissima is regularly exposed to fluctuations of PCO2 and pH due to fresh water input, tidal exchanges, emersion and localized consumption and production of CO2. To better understand how hypercapnia affects metabolic processes in an intertidal, photosynthetic symbiosis, which does not produce a calcium carbonate skeleton, this study examined the metabolic effects of increased PCO2 on A. elegantissima. An experiment was designed to measure a suite of characteristics at naturally-occurring elevated PCO2 levels (45 and 231 Pa) with the lower PCO2 condition serving as a control. This anemone is arguably the most well studied alga-invertebrate symbiosis and much is known about its ecology, genetics, physiology, etc. (Muscatine et al., 1981; Fitt et al., 1982; Ayre and Grosberg, 1995; LaJeunesse and Trench, 2000; Secord and Augustine, 2000; Lewis and Muller-Parker, 2004; Verde and McCloskey, 2007). Anthopleura elegantissima forms aggregations of genetically identical clones through bilateral fission (Ayre and Grosberg, 1995), and experiments examined the effects of elevated PCO2 through paired comparisons of genetically identical but separated clonal couplets.

Significant increases in P˙g:M˙O2 ratios were also observed in individuals after six weeks of incubation in 45 and 231 Pa CO2 (paired t-tests, p<0.001 and 0.01, respectively) (Table 1; Fig. 1B). However, there were no significant differences in M˙O2 at 45 Pa CO2 (paired t-test, p=0.05) (Fig. 1B). As in the case of P˙g, there were significant increases in respiration (CZAR) (Fig. 2B) was greatest in 45 Pa CO2 (CZAR = 143.6%). This was significantly higher than after one week at 37 Pa CO2 (CZAR = 68.2%, paired t-test, p<0.001) and also higher than that of clonemates incubated at 231 Pa CO2 (CZAR = 81.8%, paired t-test, p<0.01).

Fig. 1. Mean mass-specific rates of gross photosynthesis (A), respiration (B) and net photosynthesis (C) in Anthopleura elegantissima following incubations of one week at PCO2 of 37 Pa and six weeks at PCO2 of 45 and 231 Pa. Net photosynthesis was calculated on a 14:24 light:dark basis. Bars of the same color represent the same individuals, while different colors represent genetically identical clones. Error bars represent one standard error; n=11 for all experiments. Letters designate statistically significant differences between individuals at two different PCO2 (a=p<0.001, b=p<0.01, c=p<0.05) using paired t-tests, while asterisks represents significant differences (*=p<0.05; **=p<0.01) between clonemates at two different PCO2.

Fig. 2. Significant increases in P˙g were observed in individuals after six weeks of incubation in 45 and 231 Pa CO2 (paired t-test, p<0.001 for both groups) (Table 1; Fig. 1A). Furthermore, P˙g was greater at 45 Pa when compared to their clonemates at 231 Pa (paired t-test, p=0.03) (Fig. 1A). Significant increases in M˙O2 were also observed in individuals after six weeks of incubation in 45 and 231 Pa CO2 (paired t-tests, p<0.01 and 0.05, respectively) (Table 1; Fig. 1B). However, there were no significant differences in M˙O2 of 45 Pa CO2 when compared to their clonemates at 231 Pa CO2 (paired t-test, p>0.05) (Fig. 1B). As in the case of P˙g, there were significant increases in daily net photosynthesis after six weeks in 231 Pa CO2 with even greater increases among anemones in 45 Pa (paired t-test, p<0.001 for both groups) (Table 1; Fig. 1C).

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CO₂ effects on Symbiodinium

Fig. 3. Characteristics of the zooxanthella, Symbiodinium muscatinei, in Anthopleura elegantissima following incubations of one week at PCO₂ of 37 Pa and six weeks at PCO₂ of 45 and 231 Pa. (A) Cell diameter. (B) Cell density. (C) Mitotic index. (D) Chlorophyll a concentration. Bars of the same color represent zooxanthellae from the same individuals, while different color bars represent those from genetically identical clones. Grey bars represent those from different individuals collected at the same time from the same population of anemones. Error bars represent ± one standard error; n=11 for all measurements. Letters designate statistically significant differences using ANOVA of arcsine-transformed data (not shown) followed by Fisher’s LSD post-hoc analyses between individuals (a=p<0.001, b and c=p<0.05).

Zooxanthellae

There were no significant differences in zooxanthellar cell diameters, cell densities and Chl a concentrations among any of the PCO₂ conditions (ANOVA, p>0.05 for all comparisons) (Table 2; Fig. 3). In contrast, the number of dividing zooxanthellar cells (mitotic index) was progressively greater among the three PCO₂ (ANOVA, p<0.001) (Fig. 3C). Visual observations of anemones in the highest PCO₂ tank revealed that anemones released large amounts of mucus-bound zooxanthellae. When these zooxanthellae were examined under a microscope, a very high ratio of doublet cells was observed. In contrast, specimens in the lowest PCO₂ tank released much less mucus that contained zooxanthellae.

Discussion

Much work has been done investigating tidepool organisms’ strategies to withstand intermittent hypoxia (e.g. Richards, 2011), but investigations distinguishing the effects of hypercapnia are lacking. This study demonstrated that the non-calcifying cnidarian Anthopleura elegantissima and its photosynthetic dinoflagellate Symbiodinium muscatinei can thrive in hypercapnic seawater for 6 weeks. The initial rates of Pₕ and MO₂ in the current study are similar to those reported by others when adjusted for dry weight/wet weight differences (Shick and Dykens, 1984), however, both Pₕ and MO₂ increased after 6 weeks under both PCO₂ conditions. The consistent feeding schedule in our laboratory study likely contributed to the rise in metabolic activity after six weeks. Such increases are in agreement with previous studies that have shown that laboratory maintenance under conditions of consistent temperature and lighting, low UV exposure, absence of desiccation, and reduced wave stress all contribute to increased physiological performance of anemones (Shick, 1991; Verde and McCloskey, 1996b). In a study by Fitt et al., MO₂ of well-fed A. elegantissima was twice the rate of starved animals (Fitt et al., 1982); CZAR averaged 13% for fed anemones compared to 45% for starved or newly collected anemones. This suggests that the CZAR in the current study may under-estimate the potential increase in CZAR of wild, underfed anemones living in hypercapnia. These experiments have demonstrated that A. elegantissima can tolerate not only periodic hypercapnia, but thrive for extended periods of time at PCO₂ exceeding 5 times normocapnia.

While MO₂ of anemones was not significantly different between 45 and 231 PCO₂ after six weeks, Pₕ of specimens held in 231 PCO₂ was lower than that of specimens held in 45 PCO₂. The increases in Pₕ at both elevated PCO₂ levels offset the much smaller, corresponding increases in MO₂, thus, the range of P:R ratios (1.52–1.83) and CZAR (78–137%) of specimens held at 45 and 231 Pa PCO₂ are greater than the P:R ratios (0.54–0.86) and CZAR (13–126%) reported previously for A. elegantissima in normocapnic conditions (cf. Muller-Parker and Davy, 2001).

To satisfy the needs of zooxanthellae for photosynthesis, animal hosts must actively accumulate dissolved inorganic
carbon (DIC) in their tissues. Although some free-living dinoflagellates can use CO$_2$, HCO$_3^-$, or both, as their inorganic source of carbon (Hansen et al., 2007), the mechanism whereby DIC is made available to zooxanthellae is still being elaborated (Yellowlees et al., 2008; Venn et al., 2009). It is generally thought that zooxanthellae receive ~15% of their CO$_2$ from host respiration, and the remaining carbon needs are met by active transport and facilitated diffusion of bicarbonate through host tissues (Allemend et al., 1998; Furla et al., 2005). Even so, zooxanthellae in anemones remain carbon limited under normocapnia (Weis, 1993; Verde and McCloskey, 2007). For _A. elegantissima_, this study has suggested that CO$_2$ can diffuse through host tissues to reach symbiont cells at elevated PCO$_2$. Although cell size, cell-specific amounts of chlorophyll _a_ and cell densities of _S. muscatinei_ did not change in specimens maintained in the hypercapnic conditions for six weeks, zooxanthellae were affected by increasing PCO$_2$, as evidenced by the pattern of higher mitotic indices. Population densities of zooxanthellae in anthozoans are maintained under host control through the active expulsion of symbionts and chemically-signalized arrest of algal reproduction (Trench, 1987; Baghdasarian and Muscatine, 2000). The high amounts of dividing algal cells observed in excreted mucus in this study indicate the anemone cannot regulate algal reproduction in hypercapnia and an increase in the rate of expulsion to maintain normal densities of rapidly reproducing algal cells is needed in order to avoid toxicity from excess oxidative products (Furla et al., 2005). This suggests that the extra carbon acquired by hypercapnic anemones due to CO$_2$-enhanced photosynthesis was lost to the symbiosis due to expulsion. The small but significant differences in _P_$_S$ and MI between specimens held in 45 and 231 Pa PCO$_2$ indicate that the upper limits of performance in extended hypercapnia were approached at 231 Pa PCO$_2$. It is generally accepted that nutrient limitation is one mechanism that hosts may use to regulate algal symbiont populations (Yellowlees et al., 2008). Under high PCO$_2$ conditions in the present study, _A. elegantissima_ was apparently not able to maintain nutrient-limited conditions or other photosynthesis- and algal growth-inhibiting mechanisms.

These anemones have a high tolerance for internal hypercapnic conditions, and this trait is shared with animals that have high metabolic rates and need to tolerate high internal PCO$_2$ owing to exercise-induced fluctuations of CO$_2$ (Seibel and Walsh, 2003; Melzner et al., 2009a). This has been demonstrated in active organisms with a high capacity for ion regulation such as teleost fish (Melzner et al., 2009a), brachyuran crabs (Pane and Barry, 2007; Spicer et al., 2007) and cuttlefish (Gutowska et al., 2008). Animals that currently cope with internal hypercapnia may be pre-adapted to survive future increases in environmental PCO$_2$ as well. An environmental study of the species composition at a high PCO$_2$ volcanic vent community off Ischia, Italy, also suggests that non-calculifying alga-invertebrate symbioses may be pre-adapted to hypercapnia (Hall-Spencer et al., 2008). Along a natural pH gradient from 8.2 to 7.4 at this site, gastropods, sea urchins and epiphytic coralline algae were diminished or completely absent in areas below pH 7.7 (Hall-Spencer et al., 2008; Martin et al., 2008). Although several species of symbiont-containing scleractinian corals are common to the region, photosynthetic anemones were the only cnidarians found in zones with elevated PCO$_2$ (Hall-Spencer et al., 2008).

The influence of hypercapnia on photosynthesis in calcifying anthozoans with algal symbionts is variable. The branching coral _Acropora intermedia_ increased rates of photosynthetic productivity (at temperatures increased by 3°C) at moderately increased levels of CO$_2$ (53–71 Pa) (Anthony et al., 2008) similar to the increases observed for _A. elegantissima_ in the current study; however, productivity in the massive coral _Porites lobata_ was diminished (Anthony et al., 2008). Very high PCO$_2$ (101–132 Pa) decreased productivity to near zero in both corals. The authors speculate that the increase in productivity in _P. lobata_ may have resulted directly from an increase in CO$_2$ supply but that at higher concentrations, the effects of hypercapnia were offset by physiological disruption from acidification. As in _A. intermedia, A. elegantissima_ had the highest rate of photosynthesis at moderate levels of PCO$_2$ (45 Pa); however, unlike _A. intermedia, A. elegantissima_ also displayed a higher photosynthetic rate at very high PCO$_2$ (231 Pa) than at 39 Pa. Since _A. elegantissima_ routinely encounters PCO$_2$ of this magnitude in its environment, it appears to be better poised to make the physiological adjustments necessary to support photosynthesis at higher PCO$_2$.

In studies on _Acropora formosa_, chlorophyll content of zooxanthellae increased but cell density did not following 4 days incubation at 57 Pa PCO$_2$ (Crawley et al., 2010). The Mediterranean coral _Cladocora caespitosa_ displayed no differences in rates of photosynthesis under hypercapnia (~70 Pa) for one month, but chlorophyll content and density of zooxanthellae increased due to hypercapnia during winter experiments (Rodolfo-Metalpa et al., 2010). None of these studies examined the effects of elevated PCO$_2$ on mitotic indices of zooxanthellae or expulsion rates.

Anthropogenic hypercapnia in marine environments will have various effects on many different kinds of organisms, not only calcifying species (Fabry et al., 2008). The results of the present study suggest that _A. elegantissima_ can tolerate, and possibly benefit from, environmental hypercapnic acidification and highlights the adaptation of _A. elegantissima_ to the broad ranges of PCO$_2$ and pH that are characteristic of both its intertidal habitat and photosynthetic lifestyle. This study used a PCO$_2$ slightly above the current world oceanic mean as a control. Future studies using fluctuating PCO$_2$ levels that more closely represent the natural conditions of this intertidal organism will cast further light on intertidal adaptations to hypercapnia. Much more remains to be understood about mechanisms used by organisms that are “pre-adapted” to high PCO$_2$ due to the demands of life in periodically hypercapnic environments.

**Materials and Methods**

**Specimen collection**

Specimens of zooxanthellate _Anthopleura elegantissima_ were collected during April, 2008 from Point Grenville, Washington, USA (47°18.2’N, 124°16.2’W). This anemone harbors two different types of photosynthetic symbiont, the dinoflagellate _Symbiodinium muscatinei_ and a unicellular trebouxiothycean green alga (Lewis and Muller-Parker, 2004). To exclude green algal symbionts, anemones were collected from colonies at ~1.5–2.0 m above mean low low water (Secord and Augustine, 2000). Clonemates from contiguous colonies and additional individual specimens were collected and transported to the laboratory at The Evergreen State College, Olympia, Washington in separate, plastic bags filled with seawater. No clonemate displayed acrorhagial aggression toward its respective clonemate: an indication that they are genetically identical (Ayre and Grosberg, 2005).

**Specimen maintenance**

120-l aquaria were prepared with natural seawater (~27 psu) adjusted up to 30 psu with a combination of Instant Ocean® synthetic seawater and a carbonate-free synthetic seawater (Bidwell and Spotte, 1985) to maintain targeted pH, PCO$_2$ and CO$_2$ effects on _Symbiodinium_ 618
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photosynthesis:respiration 6
Respiratory rate 0.88
Net photosynthetic rate 0.67
Gross photosynthetic rate 1.54
Chlorophyll a (pg cell
cell diameter (µm) 8.08
Cell diameter (10^6 cells
Cell density (10^6 cells
Mitotic Index (% cells
Chlorophyll a (pg cell
Table 2. Characteristics of the zooxanthella Symbiodinium muscateni in Anthopleura elegantissima following one week in P(\text{CO}_2) of 37 Pa and after 6 weeks at 45 and 231 Pa. Values are mean ± S.E.; n=11 for all analyses.

| Cell Parameter | Incubation P(\text{CO}_2) (Pa) |
|----------------|--------------------------------|
|                | 37  | 45  | 231 |
| Cell diameter (µm) | 11.33±0.15 | 10.94±0.11 | 11.21±0.11 |
| Cell density (10^6 cells mg protein ^{-1}) | 0.22±0.03 | 0.30±0.05 | 0.27±0.03 |
| Mitotic Index (% cells dividing) | 0.24±0.05 | 0.39±0.04 | 0.62±0.08 |
| Chlorophyll a (pg cell ^{-1}) | 3.59±0.53 | 4.32±0.39 | 3.74±0.28 |

Table 3. Seawater parameters in three incubation conditions used to investigate effects of hypercapnic acidification on the intertidal sea anemone Anthopleura elegantissima. NBS: National Bureau of Standards, TA: Total Alkalinity. Values are mean ± S.D.

| pHNBS | P(\text{CO}_2) (Pa) | TA (µmol/kg SW) |
|-------|-------------------|-----------------|
| 8.10  | (n=2)             | 37.2            | 2403 |
| 8.08±0.04 (n=14) | 45.0±4.7 | 2566±120 |
| 7.35±0.02 (n=14) | 231±10.1 | 2119±43 |

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with the duration of cytokinesis $t_d$ equal to 28 (McCloskey et al., 1996) and $f$ equal to the fraction of cells in the division phase as determined from mitotic index. The zooxanthellal carbon-specific growth rates ($C_m$) were determined with the formula supplied by Verde and McCloskey (Verde and McCloskey, 1996a):

$$C_m = \left(\frac{SS}{C_{cell}}\right)^{-1}\left[\frac{m}{d}\right].$$

Standing stock (SS) was estimated from zooxanthellal cell densities, assuming that 90% of zooxanthellae are harbored in the oral disk and tentacles (Shick, 1991). Carbon per zooxanthellal cell was calculated as reported by Menden-Deuer and Fricke (1999): pg C-cell$^{-1}$ = 0.760 (cell volume$^{0.819}$).

Zooxanthellal measurements

Characteristics of Symbiodinium muscaeae were measured to gauge the effects of hypercapnic acidification on the photosynthetic symbionts. Previously frozen oral disks from laboratory-acclimated, non-clonemate anemones and post-experimental hypercapnic acidification on the photosynthetic symbionts. Previously frozen oral disks from laboratory-acclimated, non-clonemate anemones and post-experimental hypercapnic acidification on the photosynthetic symbionts. Previously frozen oral disks from laboratory-acclimated, non-clonemate anemones and post-experimental hypercapnic acidification on the photosynthetic symbionts. Previously frozen oral disks from laboratory-acclimated, non-clonemate anemones and post-experimental hypercapnic acidification on the photosynthetic symbionts. Previously frozen oral disks from laboratory-acclimated, non-clonemate anemones and post-experimental hypercapnic acidification on the photosynthetic symbionts.

Statistical analyses

Data were analyzed with JMP Statistical Discovery Software, version 7.0. Paired t-tests were performed on data collected on the same individuals before and after 6-week incubations and between clonemates at each PCO2 to determine if 6 weeks of hypercapnia affected metabolism and photosynthesis. Analyses were carried out on mass-specific rates that were not adjusted for mass. Because symbionts extracted from non-clonemate animals, ANOVA analyses were performed to examine differences related to zooxanthellae. ANOVA tests were followed with Fisher’s LSD post hoc comparisons. CZAR percentage data were not transformed, because data were normally distributed around the mean and not bounded by 100. Mitotic index (%) data were arcine transformed for ANOVA.

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Competing Interests

The authors declare that there are no competing interests.

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