Tolerance of allogromiid Foraminifera to severely elevated carbon dioxide concentrations: Implications to future ecosystem functioning and paleoceanographic interpretations

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Abstract

Increases in the partial pressure of carbon dioxide (pCO_2) in the atmosphere will significantly affect a wide variety of terrestrial fauna and flora. Because of tight atmospheric-oceanic coupling, shallow-water marine species are also expected to be affected by increases in atmospheric carbon dioxide concentrations. One proposed way to slow increases in atmospheric pCO_2 is to sequester CO_2 in the deep sea. Thus, over the next few centuries marine species will be exposed to changing seawater chemistry caused by ocean-atmospheric exchange and/or deep-ocean sequestration. This initial case study on one allogromiid foraminiferal species (*Allogromia laticollaris*) was conducted to begin to ascertain the effect of elevated pCO_2 on benthic Foraminifera, which are a major meiofaunal constituent of shallow- and deep-water marine communities. Cultures of this thecate foraminiferan protist were used for 10-14-day experiments. Experimental treatments were executed in an incubator that controlled CO_2 (15 000; 30 000; 60 000; 90 000; 200 000 ppm), temperature and humidity; atmospheric controls (i.e., ~375 ppm CO_2) were executed simultaneously. Although the experimental elevated pCO_2 values are far above foreseeable surface water pCO_2, they were selected to represent the spectrum of conditions expected for the benthos if deep-sea CO_2 sequestration becomes a reality. Survival was assessed in two independent ways: pseudopodial presence/absence and measurement of adenosine triphosphate (ATP), which is an indicator of cellular energy. Substantial proportions of *A. laticollaris* populations survived 200 000 ppm CO_2 although the mean of the median [ATP] of survivors was statistically lower for this treatment than for that of atmospheric control specimens. After individuals that had been incubated in 200 000 ppm CO_2 for 12 days were transferred to atmospheric conditions for ~24 hours, the [ATP] of live specimens (survivors) approximated those of the comparable atmospheric control treatment.

Incubation in 200 000 ppm CO_2 also resulted in reproduction by some individuals. Results suggest that certain Foraminifera are able to tolerate deep-sea CO_2 sequestration and perhaps thrive as a result of elevated pCO_2 that is predicted for the next few centuries, in a high-pCO_2 world. Thus, allogromiid foraminiferal "blooms" may result from climate change. Furthermore, because allogromiids consume a variety of prey, it is likely that they will be major players in ecosystem dynamics of future coastal sedimentary environments.

Keywords: *Allogromia laticollaris*, CO_2 injection, deep-sea, climate change, hypercapnia

1. Introduction

The partial pressure of carbon dioxide (pCO_2) in the atmosphere has oscillated between 200 and 280 ppm over the past ~420,000 years (Prentice et al., 2001; EPICA Community Members, 2004), until the Industrial Revolution in the mid-1800s. Since then, atmospheric pCO_2 has increased to today's concentration of ~375 ppm. The atmospheric pCO_2 has increased dramatically in the past several hundred years due to both anthropogenic causes and natural processes (e.g., Sabine et al., 2004). Because CO_2 is a greenhouse gas that contributes to global warming, society would benefit from the identification of effective means to minimize the increase of atmospheric pCO_2. Several methods of CO_2 sequestration have been proposed, including geological sequestration in aquifers or subsurface deposits, terrestrial biological sequestration via copious planting of trees, and oceanic sequestration via iron seeding or deep-ocean injection (reviewed in Yamasaki, 2003). Deep-ocean injection of CO_2 extracted from gases released by industrial activities could potentially slow and minimize global warming by removing CO_2 from emission sources and pumping it as a gel-like liquid into mid-ocean depths or sea-floor depressions where it could dissipate over millennial time scales (e.g., Haugan and Drange, 1992; Brewer et al., 1998; 2005).

Because a large percentage of Earth's carbon dioxide is naturally sequestered in the oceans, deep-ocean CO_2 sequestration continues to receive dedicated study as a feasible method to minimize rates of atmospheric pCO_2 rise (e.g., Hove and Haugan, 2005; White et al., 2006; Wannamaker and Adams, 2006; Brewer et al., 2006). Although carbon dioxide is also naturally stored in the oceans, the effect of artificially elevated levels of pCO_2 on the ocean's inhabitants and global ecosystem balance is unclear. An increase in pCO_2 results in decreases in both pH and carbonate ion concentration, each of which has important biological implications.

Biochemical factors to contemplate when considering exposure to high pCO_2, or hypercapnia, are acid-base imbalances, the potential toxicity of CO_2 on respiration via disruption of oxygen-transport mechanisms, and metabolic suppression that retards growth and reproduction (e.g., Seibel and Walsh, 2001; Pörtner et al., 2004). Acid-base imbalances are especially important for taxa with calcareous exoskeletons because the more acidic conditions in the ocean's water column will lead to shell dissolution. Sub-lethal effects of elevated pCO_2 in animals include reduced activity and loss of consciousness; if impeded oxygen transport persists, death may result (Seibel and Walsh, 2001). Thus, while the deep sea may provide an appealing destination for CO_2 disposal to mitigate global warming, the negative effects of the elevated pCO_2 may outweigh the advantages (e.g., Tyler, 2003; Pörtner et al., 2005). By understanding the effects of deep-sea CO_2 sequestration on all major taxa, we can better predict future ecosystem functioning to enable better-informed policy decisions.

The effect of carbon dioxide disposal on deep-sea organisms has received much study over the last few years but is still poorly understood. It has been established that fish and other megafauna, even though mobile, do not necessarily escape enhanced pCO_2 (Tamburri et al., 2000); severe physiological effects can be long lasting or lethal (e.g., Seibel and Walsh, 2003). There is limited knowledge of the effects of deep-ocean CO_2 sequestration on microbial communities, although it has been shown that CO_2 sequestration inhibits marine nitrification (Huesemann et al., 2002). Information on the metazoan meiofaunal community response to deep-sea CO_2 disposal suggests that copepod biodiversity is negatively affected (e.g., Kurihara et al., 2004; Thistle et al., 2005), harpacticoid copepod attempt to escape CO_2-rich seawater (Thistle et al., 2007), and nematode abundance and specimen size decrease (Fleeger et al., 2006). Other studies assessing the effects of CO_2 sequestration on deep-sea meiofauna agree that

metazoan abundances decrease (Barry et al., 2004; Ishida et al., 2005) but, interestingly, bacterial respiration rates increase (Ishida et al., 2005). Thus, the effect of CO_2 sequestration on the deep-sea benthos is a complex relationship (Ishida et al., 2005).

This study investigates the effects of elevated CO_2 on a ubiquitous protistan constituent of marine microbial systems: allogromiid benthic Foraminifera. The response of marine protists to pCO_2 sequestration has only been recently documented via field studies (e.g., Barry et al., 2005; Ricketts et al., in revision). In this laboratory study, Foraminifera were targeted for a number of reasons. First, they are an important link in the marine food web (e.g., Legendre and Le Févre, 1995; van Oevelen et al., 2006; Rowe et al., in press). Second, they can be the dominant meiofaunal taxon in deep-sea sediments (e.g., Coull et al., 1977; Snider et al., 1984; Gooday et al., 2000). Third, because of their relatively slow migration rates (e.g., Gross 2000), elevated pCO_2 may be more deleterious to them compared to mobile metazoans. Fourth, certain Foraminifera are easily cultured in the laboratory, and thus, provide plentiful populations for experimentation (e.g., Lee and Pierce, 1963; Hintz et al., 2004). Of the three types of Foraminifera (i.e., calcareous, agglutinated, thecate), this initial case study used a thecate shallow-water species to allow experimentation at room temperature and atmospheric pressure.

2. Materials and methods

Allogromia laticollaris cultures, which were obtained originally in 2004 from J. Travis, were grown in a mixture of 32 ‰ seawater and Alga-Gro® seawater medium (Carolina Biological Supply) in 20 ml glass tubes with loose caps (modified from Travis and Allen, 1981). Each week, each culture tube received ~2 mL of concentrated algae comprised of an equal

proportion of both *Dunaliella tertiolecta* and *Isocrysis galbana* to serve as foraminiferal food. Cultures were maintained at ~23°C and in a 12 hour light-dark cycle.

For each experiment, the full size spectrum of ~100 *A. laticollaris* were removed from a culture tube via pipette; ~25-30 individuals and minimal residual algal material were placed in each of three 3.5-cm diameter Petri dishes containing 32‰ artificial seawater. Two of these dishes were placed in a Nuaire US Autoflow CO_2 Water-Jacketed incubator (NU4950) that was attached to a Thermo RTE740 refrigerated bath to maintain temperature at 22-23°C for the duration of the experiment (i.e., 10-14 days). The darkened incubator was maintained at 75-85% humidity during each experiment. One of these Petri dishes was denoted the experimental (CO_2) treatment and the other as the Rebound (see below). The third of these dishes was placed in a humid chamber, which was housed in a darkened cabinet for the duration of the experiment; for these dishes, the temperature ranged from 21-23°C and humidity ranged from 90-95% throughout each experiment. This treatment was denoted the atmospheric (ATM) control. After the enriched CO_2 incubation, the Rebound Petri dish was also placed in the humid chamber. Thus, it was allowed to "rebound" to atmospheric conditions for ~24 hours prior to subsequent analyses.

Experiments were run at five enhanced CO_2 concentrations: 15 000; 30 000; 60 000; 90 000; and 200 000 ppm. These concentrations were selected because an original aim was to determine survival thresholds in response to massive CO_2 releases on the seafloor, where benthic fauna would be directly exposed to CO_2 hydrate or in the immediate vicinity of such release; the maximum setting of the incubator is 200 000 ppm. Experiments were duplicated at all but the 30 000 ppm concentration; only one 60 000 experiment had a Rebound treatment. Based on the diffusion rate of CO_2, surface area of seawater media in the experimental dishes, temperature,

incubator pCO_2, and some assumptions about carbonate chemistry, it is estimated that the pCO_2 of the seawater media equilibrated with the incubator atmosphere within a few days (D. McCorkle, pers. comm. 2007; but see also Results). Thus, the experimental durations of 10-14 days do not necessarily equate to the length of time specimens were exposed to the experimental pCO_2 value, but because transferring specimens into a pre-equilibrated solution would likely shock the individuals and perhaps kill them, we opted for the slower equilibration approach.

At the end of each experiment, both Petri dishes housed in the CO_2 incubator were removed and one was placed within the humid chamber to serve as the Rebound treatment. Due to the small available volume of incubation media (seawater), we used pH indicator paper (3-8, 0.4 pH unit increments) to obtain a rough approximation of pH within 1 minute of dish removal from the incubator; the wet pH paper was digitally imaged along with color scale to allow later value determination. Individuals in the second Petri dish served as CO_2 specimens and within 10-20 minutes after removing the dish from the incubator, individuals were haphazardly selected using a Nikon SMZ-2B stereo dissecting microscope, cleaned of adherent debris, measured for their diameter (rounded to the nearest 20 μm), and extracted for Adenosine Triphosphate (ATP) in 1.0 mL boiling phosphate-citrate buffer (DeLaca, 1986) for 5 minutes, after which extracts were frozen for later analysis. ATP is an indicator of cellular energy that has been used to quantify benthic foraminiferal populations (e.g., DeLaca, 1986; Bernhard and Reimers, 1991; Bernhard, 1992; Bernhard et al., in press). In general, 25 specimens were extracted individually per treatment, although occasionally an individual sample was lost due to human error. Some experiments had only 20 specimens per treatment (depending on culture abundance at the time). Not all specimens were used per treatment because (1) extra individuals were initially included to allow selection of those not firmly encased in bacterial biofilms, which necessitates potentially

detrimental cleaning of the foraminifer and (2) sometimes specimens reproduced during the experiments, resulting in increased population size.

Individuals in the ATM treatments were examined extensively with the stereomicroscope to survey for presence / absence of pseudopods. Presence of pseudopods indicates specimen survival, although lack of pseudopods does not necessarily indicate specimen death. Thus, pseudopodial presence / absence was used as a qualitative indicator of foraminiferal survival and activity. The next day, specimens in the Rebound Petri dish, which had been allowed to return toward atmospheric CO_2 for ~24 hours, were similarly measured, examined for pseudopods, photographed, and extracted for ATP.

Frozen ATP extracts were thawed and subsequently analyzed using a Berthold Lumat LB 9507 luminometer and luciferin-luciferase reaction per standard protocol (e.g., Bernhard, 1992). Cell volume, which was calculated using the recorded cell diameter data and volume of a sphere ($v=4/3\pi r^3$), was used to normalize ATP data. Specimens were considered to be living if their [ATP] exceeded 145 ng/mm^3, which corresponds to the live-dead threshold determined for deep-sea benthic Foraminifera by Bernhard (1992).

A partial hierarchical ANOVA model (Brownlee, 1965) was used to test whether the mean proportion of survivors differed among treatments (ATM, CO_2, Rebound) and among the 15 000, 90 000 and 200 000 ppm experiments. In a second analysis, median [ATP] was determined for each treatment in each experimental trial, and the same ANOVA model was used to test whether mean (of the median) ATP concentrations (ng/mm^3) of survivors differed between treatments and experiments. The 30 000 and 60 000 ppm experiments were not included in either statistical analysis because only one experiment was executed at 30 000 ppm and only one 60 000 experiment included all three treatments. A Tukey-Kramer multiple comparisons test

was used to determine which means differed. Statistical significance was declared at an alpha level of 0.05.

3. Results

Both ATP data and microscopic examination indicate that considerable populations of *Allogromia laticollaris* survived exposure to all experimental treatments of elevated pCO_2, even both replicates of the 200 000 ppm CO_2 experiments (Figures 1-2). In all experiments where Rebound specimens were examined microscopically, at least a few individuals had extended pseudopodial networks (Figure 1A, C) that were similar in appearance to those deployed by specimens exposed to atmospheric pCO_2 (not shown). Furthermore, at least three specimens reproduced during exposure to either 90 000 ppm or 200 000 ppm CO_2 (Figure 1B, C); such reproduction was observed only once in an atmospheric treatment. Specimens extracted for ATP ranged in diameter from ~80-400 μm; survivors included specimens from ~80-350 μm (data not shown).

Survivorship data show that *Allogromia laticollaris* from atmospheric treatments had an average survivorship of 83% (with a range of 60-100%; Figure 2). Statistically, the mean of *A. laticollaris* survival did not differ significantly between treatments (i.e., ATM, pCO_2, Rebound) within the 15 000 ppm, 90 000 ppm and 200 000 experiments (Figure 3, Table 1, across rows in Table 2). The overall mean proportion of Foraminifera that survived the 200 000 ppm experiments (48%), however, was significantly lower than the mean proportion that survived the 15 000 ppm (86%) and 90 000 ppm (90%) experiments (last column in Table 2). Mean survival in the latter two experiments did not differ significantly. Mean survivorship of the specimens exposed to 200 000 ppm CO_2 (33%) was significantly less than the mean survivorship of

specimens exposed to 15 000 ppm CO_2 (90%; Table 2). The mean survivorship of 200 000 ppm CO_2 Rebound specimens was significantly less than that of Rebound specimens exposed to 90 000 ppm CO_2 but was not significantly less than the Rebound specimens exposed to 15 000 ppm.

Comparing the average concentrations of ATP in populations from the different treatments can also be used to assess impacts of elevated pCO_2 on foraminiferal energy levels, which can be used to infer "health" in the context of population stability, sustainability, and resilience. In each experiment's ATM treatment, the median value for all live specimens (i.e., those exceeding the ATP live-dead threshold) generally was between 350-500 ng ATP/mm^3 (Figure 4). There is one case, however, where the median [ATP] value for the atmospheric population was much higher (i.e., 1164 ng ATP/mm^3; 60 000 ppm experiment; Figure 4E).

In general, the median [ATP] of Rebound populations approached that of each respective experiment's ATM treatment. In other words, for experiments where the median [ATP] in the CO_2 treatment was higher than ATM populations (Figure 4 A-C), the Rebound [ATP] was lower than CO_2 treatments but higher than or nearly equal to that of atmospheric controls. For experiments where the median [ATP] in the CO_2 treatment was lower than in the ATM populations (Figure 4 E-I), the Rebound [ATP] was approximately equal to or higher than CO_2 treatments but lower than ATM treatments. The exception to this generality is the Rebound of the 30 000 ppm experiment, where the median [ATP] of the Rebound population far exceeded that of both the ATM and CO_2 treatment populations (Figure 4C).

The mean ATP concentration of foraminiferal survivors in the 15 000 ppm experiments did not differ between treatments (Fig. 5, Tables 1 and 3), but in the 90 000 ppm experiments, mean [ATP] of survivors was significantly lower in the CO_2 treatment than in the ATM and the Rebound treatments (Table 3). The mean [ATP] of survivors in the 200 000 ppm experiments

was significantly lower in CO_2 treatments than in the ATM treatments. Survivors of the Rebound treatment, however, did not significantly differ in mean [ATP] from those of the 200 000 ppm or the ATM treatments.

Within the three replicated CO_2 treatments (columns in Table 3), the mean [ATP] of survivors of the 15 000 ppm treatment (501.4 ng/mm^3) was significantly higher than the mean [ATP] of survivors of the 90 000 ppm (256.6 ng/mm^3) and 200 000 ppm (248.0 ng/mm^3) treatments. The means of these later two treatments did not differ significantly. There was no statistically significant difference between the means of the three ATM treatments or between the three Rebound treatments.

The insignificant differences between ATP concentrations of Rebound and ATM populations (Table 3) indicate that the ~24 hour exposure to atmospheric conditions subsequent to incubation in elevated pCO_2 permitted equilibration with atmospheric conditions. We infer the reverse also holds, namely that approximately one day is required for the pCO_2 of seawater media in Petri dishes to equilibrate with the incubator's atmosphere. Thus, our estimate of a few days for equilibration (see Section 2) represents a maximum and we conclude that the duration of elevated pCO_2 incubations was 9-13 days (i.e., one day less than experimental duration to allow for equilibration).

The pH in CO_2 treatments was lower than those of the ATM and Rebound treatments, but values are not reported because we are not confident that the pH indicator paper was accurate for our purposes. We know the pH drop in other experiments of the same duration, done on calcareous and agglutinated foraminiferans but run at 6^{o}C (Eisenkolb and Bernhard, unpubl), showed an asymptotic decline with an increase in pCO_2. At the maximum pCO_2 value investigated for those experiments (i.e., 20 000 ppm), the pH was 6.36. From a different

perspective, our observations on pH further support the conclusion that Petri dishes equilibrated to the incubator's atmosphere within a day because the pH of Rebound treatments approximated that of ATM treatments.

4. Discussion

4.1. Allogromia laticollaris response to elevated pCO_2

Foraminiferal pseudopods are used for locomotion (reviewed in Travis and Bowser, 1991), feeding (e.g., Lipps 1983), and are surmised to be sites of respiration (Doyle, 1935). Thus, the fact that at least some *Allogromia laticollaris* specimens extended pseudopods during their incubation in very high pCO_2 (i.e., 200 000 ppm) indicates their ability to normally feed, move, and respire in those conditions. In addition, imminent reproduction in other specimens exposed to the same conditions indicates their reproductive capacity was not lost during such exposure. Furthermore, because the diameter of the survivors of CO_2 treatments ranged over hundreds of microns, apparently both young (small) and adult (larger) individuals tolerated enhanced pCO_2 for the experimental durations.

Although *A. laticollaris* survivorship is negatively impacted by high pCO_2, this foraminiferal species survives extremely high pCO_2 for 9-13 days (see above), which is a substantial portion of their 16-26-day life cycle (McEnery and Lee 1976). Importantly, the reversibility of high pCO_2 exposure, which is illustrated in the insignificant differences observed in ATP concentrations of Rebound and ATM populations (Table 3), indicates that *A. laticollaris* is resilient to increased pCO_2. Furthermore, because ATP concentrations were slightly higher in populations exposed to our moderately elevated pCO_2 (i.e., 15 000 and 30 000 ppm) compared to populations maintained at atmospheric concentrations, it could be asserted that *A. laticollaris'*

population sustainability may not be adversely impacted in areas affected by deep-sea CO_2 sequestration and in physicochemical conditions of a high-pCO_2 world (see below).

4.2. Implications to deep-sea CO_2 sequestration

Allogromiid Foraminifera are very diverse and abundant in the deep sea (e.g., Gooday, 2002; Sabbatini et al., 2002; Gooday et al., 2004) and can comprise a dominant group of deep-sea meiofauna (e.g., Gooday et al., 2000; Cornelius and Gooday, 2004). Thus, results of these initial experiments of benthic foraminiferal response to elevated pCO_2 can be interpreted to indicate that allogromiid foraminiferal populations will tolerate or even thrive in response to deep-sea CO_2 disposal depending on the proximity of the population to the source or deposit, at least for relatively short-term exposures (days to weeks). It is imperative to note, however, that the species we utilized in our experiments, *Allogromia laticollaris*, is a shallow-water species (Arnold, 1955) and its response to elevated pCO_2 does not necessarily reflect the response of allogromiid species that are endemic to bathyal and abyssal water depths. For example, it is well established that bonafide deep-sea species have lower metabolic rates, in general, than their shallow-water counterparts, and thus are expected to have lower capacity for pH buffering and low concentrations of ion-transport proteins, leading to large intracellular changes in pH and pCO_2 (Seibel and Walsh, 2003). Indeed, a recent comparison of the physiologic response of shallow- and deep-water crab species indicate that the deep-sea species is more affected by elevated pCO_2 than its shallow-water counterpart (Pane and Barry, 2007). While it would have been desirable to execute similar experiments on deep-sea allogromiids, to our knowledge, no deep-sea allogromiid species is presently being successfully cultured or maintained. Preliminary results of our field studies indicate, however, that abyssal allogromiid Foraminifera may indeed

survive month-long exposure to CO_2 pumped onto the deep-sea floor (Bernhard et al., in prep.), suggesting that our experiments on shallow-water allogromiid Foraminifera may be relevant to and useful for preliminary interpretations regarding allogromiid response to increased pCO_2 in the deep-sea.

4.3. Implications regarding the High pCO_2 World

Although *A. laticollaris* is perhaps not the best analog to study the effects of deep-sea CO_2 sequestration on deep-sea endemic Foraminifera, *A. laticollaris* is an appropriate species to predict the response of shallow-water thecate Foraminifera to predicted increases in atmospheric pCO_2, given its isolation from a shallow-water semi-tropical setting (Arnold, 1955; McEnery and Lee, 1976). Thus, results indicate that at least some foraminiferal species will tolerate pCO_2 values that are one to two orders of magnitude higher than those predicted for the next few centuries (i.e., 560-840 ppm; Prentice et al., 2001) and even the extreme case for which the burning of all fossil fuels results in atmospheric pCO_2 approaching 2000 ppm by the year 2300 (Caldiera and Wickett, 2003).

Our results further imply that allogromiid Foraminifera will compete well in foreseeable environmental conditions and ecosystems. Allogromiid fitness will be enhanced relative to calcareous organisms because of their expected negative responses to ocean acidification resulting from a high pCO_2 world, such as those observed experimentally for shallow-water gastropods and sea urchins (e.g., Shirayama and Thornton, 2005). Furthermore, the combined effects of increased pCO_2, increased temperature, and hypoxia (i.e., oxygen depletion) are hypothesized to enhance sensitivity of marine animals to environmental extremes (Pörtner et al., 2005). Because it is established that allogromiid foraminifers can inhabit anoxic, sulfidic

sediments (Bernhard et al., 2006), our argument that allogromiid Foraminifera will compete well in future ecosystems is further bolstered. Finally, because allogromiid Foraminifera consume a variety of prey, including bacteria (e.g., Muller and Lee, 1969; Langezaal et al., 2005) and their biofilms (Bernhard and Bowser, 1992), unicellular algae (e.g., Lee et al., 1966; Gooday et al., 1995), and small metazoans (Bowser et al., 1992), predictably they will be major players in ecosystem dynamics of future coastal sedimentary environments.

4.4. Paleoceanographic Implications

Past atmospheric pCO_2 are debated and pCO_2 model estimates throughout the Proterozoic and Phanerozoic range considerably. In general, however, there were Phanerozoic episodes when atmospheric pCO_2 is thought to have been considerably higher than today. For example, estimates of atmospheric pCO_2 indicate very high values in the Cambrian (~8200 ppm; Berner and Kothavala, 2001) and Cretaceous (~4500-5100 ppm; Bice and Norris, 2002, Retallack, 2001; although more recent estimates suggest values <3500 ppm; Bice et al., 2006). Concentrations decreased during the Eocene into the Miocene from ~1000-2000 ppm CO_2 to approximately pre-industrial values (~280 ppm; Pagani et al., 2005). Our results suggest that, throughout the Phanerozoic, oceanic pCO_2 would not have impeded the ecological success of allogromiid Foraminifera, which are considered to be basal forms whose ancestors evolved ~0.69-1.15 bya (Pawlowski et al., 2003). Indeed, maximum pCO_2 estimates for the Phanerozoic (i.e., 8200 ppm; Berner and Kothavala, 2001) may have enhanced allogromiid competitiveness, given the enhanced [ATP] of *A. laticollaris* during exposure to pCO_2 of 15 000-30 000 ppm. Furthermore, evidence suggests that atmospheric pCO_2 may have been as high as ~75 000 ppm at 1.4 bya in the Proterozoic (Kaufman and Xiao, 2003), suggesting that the earliest evolving Foraminifera

may have been originally adapted to a high pCO_2 world or even to conditions approaching those of Snowball Earth (i.e., 120 000 ppm CO_2; Hoffman et al., 1998).

4.5. Future Directions

Future experiments utilizing calcareous benthic Foraminifera will augment our understanding of the effects of CO_2 sequestration on biomineralizing microbes. While it is established that the calcification rate of planktonic foraminiferal calcite decreases during exposure to elevated pCO_2 (e.g., Bijma et al., 1999), other experimental work indicates that at least some coccolithophore species are not similarly impacted by increased pCO_2 (Langer et al., 2006). Preliminary experiments using our approach were executed with deep-sea calcareous and agglutinated foraminifers. Those results indicate survival of bathyal agglutinated and calcareous species up to 2000 ppm CO_2, even after calcareous tests (shells) decalcified (Eisenkolb and Bernhard, unpubl). It is unclear, however, if these specimens could feed, migrate, or reproduce. In addition, before confident conclusions can be made regarding the response of any foraminiferal species to elevated pCO_2, it will be important to determine survivorship and normal life function (e.g., feeding, reproduction, migration) over time periods that equal or exceed that species' life expectancy and, for deep-sea species, during exposure to appropriate pressure. Such experiments will require either sophisticated laboratory systems or logistically complicated field approaches.

5. Conclusions

Results indicate that at least one species of allogromiid benthic Foraminifera is able to survive 10-14-day exposure to elevated pCO_2 as high as 200 000 ppm. Although experiments

were conducted solely on one shallow-water foraminiferal species, data suggest that seafloor carbon dioxide sequestration would not impose a significant negative impact on population dynamics of these thecate Foraminifera. Furthermore, allogromiid foraminiferal "blooms" may ensue from predicted increases pCO_2 in oceanic surface waters. Because positive responses to predicted surface-water pCO_2 increases are rarely noted in eukaryotes, as demonstrated in this study, our contribution emphasizes the need for additional dedicated study of protistan response to hypercapnia and ocean acidification.

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8. Figure Captions

Figure 1 (view in color). Transmitted light micrographs of *Allogromia laticollaris*. A. Rebound specimen after exposure to 200 000 ppm CO_2 for 12 days, with extended pseudopodial network. B. Specimens, exposed to 90 000 ppm CO_2 for 14 days, encasing dozens of offspring. C. Individual (lower center) that reproduced during exposure to 90 000 ppm CO_2 for 14 days, four days after return to atmospheric conditions. Note that many offspring have dispersed, one of which is visible with extended pseudopods; approximately 6 remain in the parental test. Scale bars: A = 100 μm; B, C = 50 μm.

Figure 2. *A. laticollaris* survivorship for each treatment, presented for each experimental replicate. Each row is a replicated pCO_2 experiment (thus, there is only one replicate for 30 000 ppm CO_2). Reb = Rebound treatment.

Figure 3. A plot of the mean proportion that survived ($\pm$ 1 standard deviation) each treatment for the three replicated CO_2 experiments. N = 2 trials.

Figure 4. Median [ATP] of *A. laticollaris* survivors, presented for each treatment, in each experimental replicate. Each row is a replicated pCO_2 experiment (thus, there is only one replicate for 30 000 ppm CO_2). Note the change in scale for E; Reb = Rebound treatment.

Figure 5. A plot of the mean of the median [ATP] ($\pm$ 1 standard deviation) for each treatment for the three replicated CO_2 experiments. N = 2 trials.

Table 1. Results of partial hierarchical ANOVAs examining the effects of CO_2 (15 000, 90 000 or 200 000; a fixed factor) and Treatment (Atmospheric, %CO_2 and Rebound; a fixed factor) on the proportion of specimens that survived and on their median ng ATP/mm^3. Two trials (random factor) were run in each CO_2 experiment. Bold typeface represents significant values.

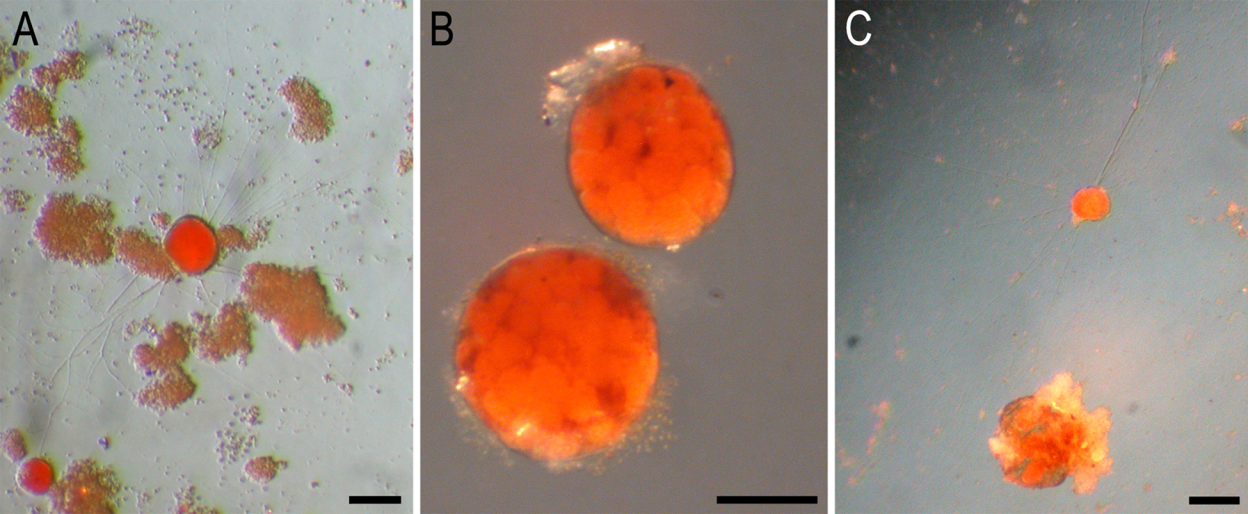
		Proportion survived			Median ng ATP/mm^3		
Source	df	Mean-square	F-ratio	P	Mean-square	F-ratio	P
[CO_2] Experiment	2	0.325	14.239	**0.029**	10 786.9	0.624	0.593
Trial ([CO_2] Experiment)	3	0.023	1.597	0.286	17 280.6	11.535	**0.007**
Treatment	2	0.050	3.465	0.100	31 564.5	21.070	**0.002**
[CO_2]Experiment * Treatment	4	0.028	1.983	0.216	20 863.9	13.927	**0.003**
Error	6	0.014			1 498.1		

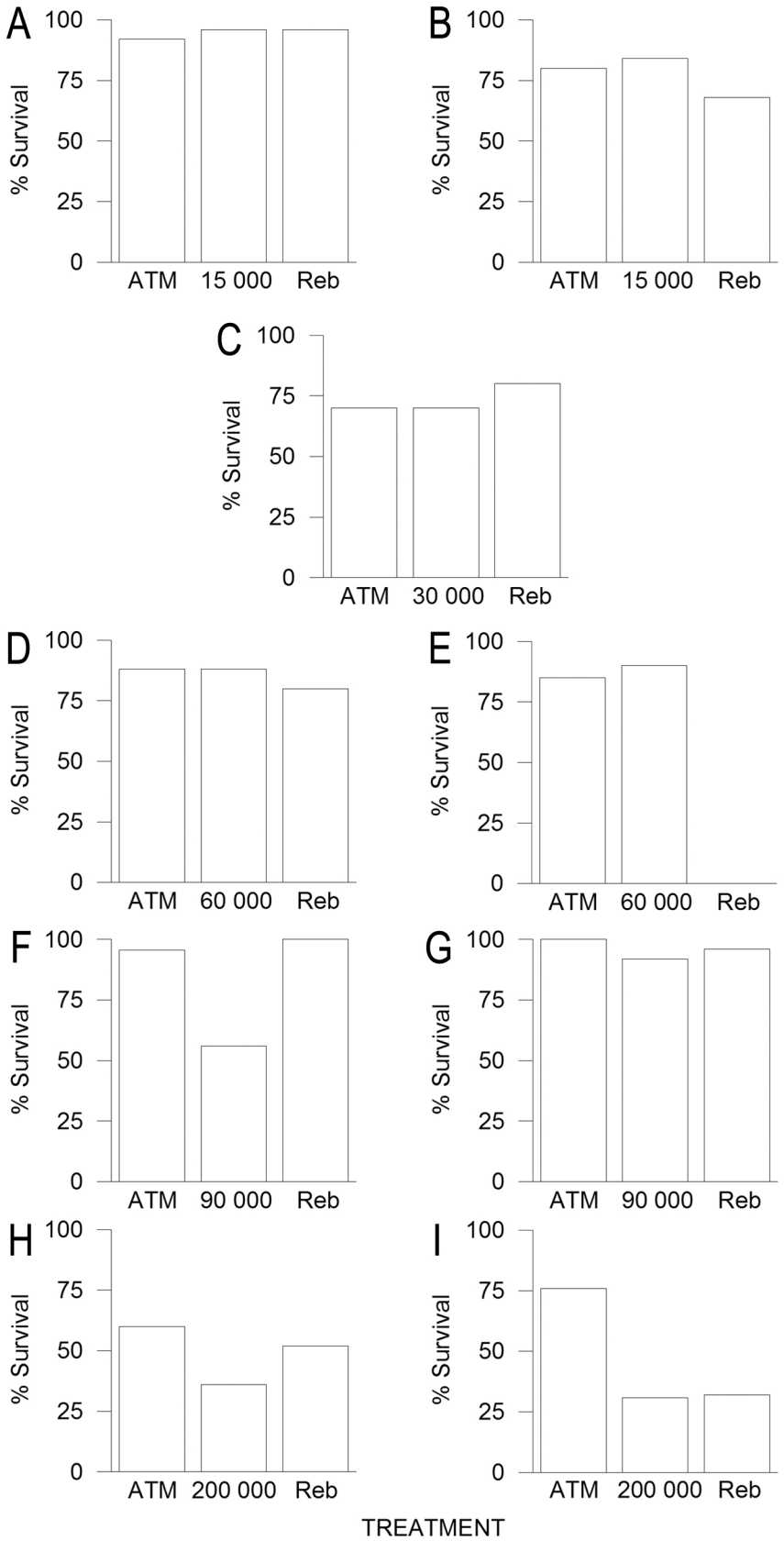
Table 2. Mean proportion of Foraminifera that survived in each treatment in each experiment (n = 2). Underlining indicates values (means) that are not significantly different from one another within an experiment (within a row). Similar letters denote experimental means within a treatment (within a column) that are not significantly different from one another, as established by Tukey-Kramer analysis.

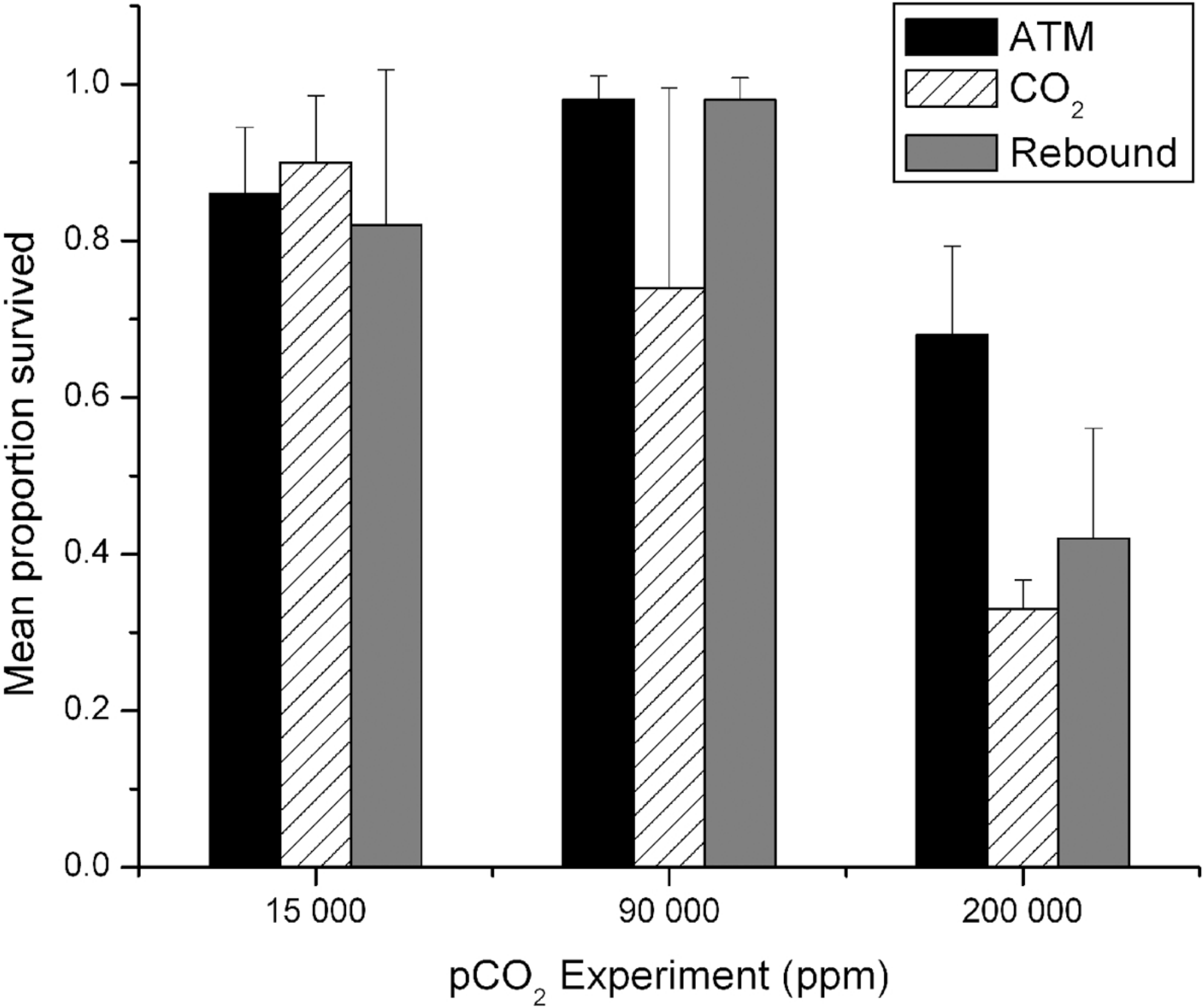
CO_2 experiment	Treatment Means [Proportion survived]			
(ppm)	ATM	Rebound	CO_2	Overall Expt mean
15 000	$\underline{0.86^{A}}$	$\underline{0.82^{AB}}$	$\underline{0.90^{A}}$	0.86^{A}
90 000	$\underline{0.98^{A}}$	$\underline{0.98^{A}}$	$\underline{0.74^{AB}}$	0.90^{A}
200 000	$\underline{0.68^{A}}$	$\underline{0.42^{B}}$	$\underline{0.33^{B}}$	0.48^{B}

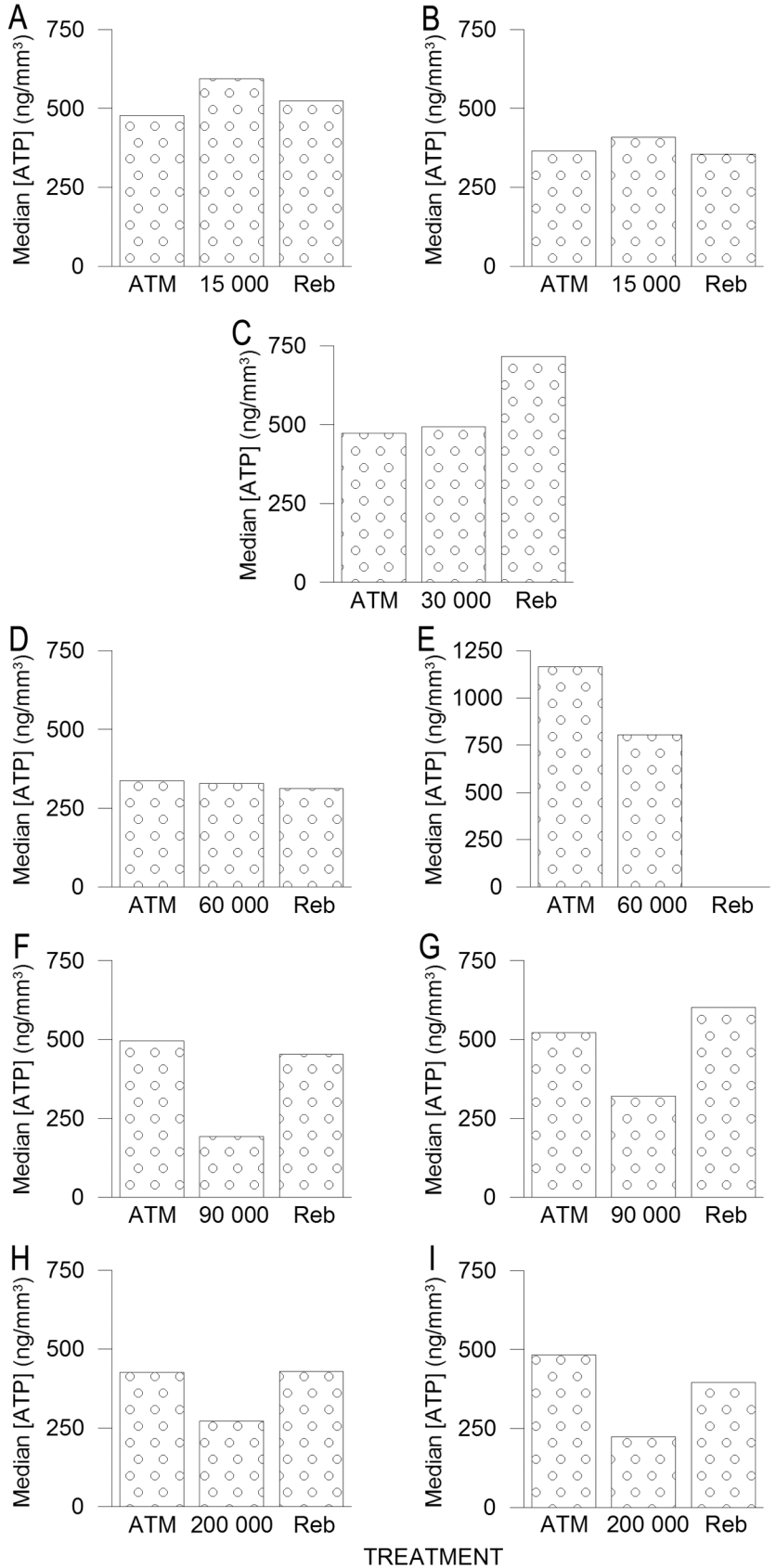
Table 3. Mean [ATP] for each treatment in each experiment (n = 2). Lines underline means that are not significantly different from one another within an experiment (compares means within a row). Similar letters denote experimental means within a treatment (within a column) that are not significantly different from one another, as established by Tukey-Kramer analysis.

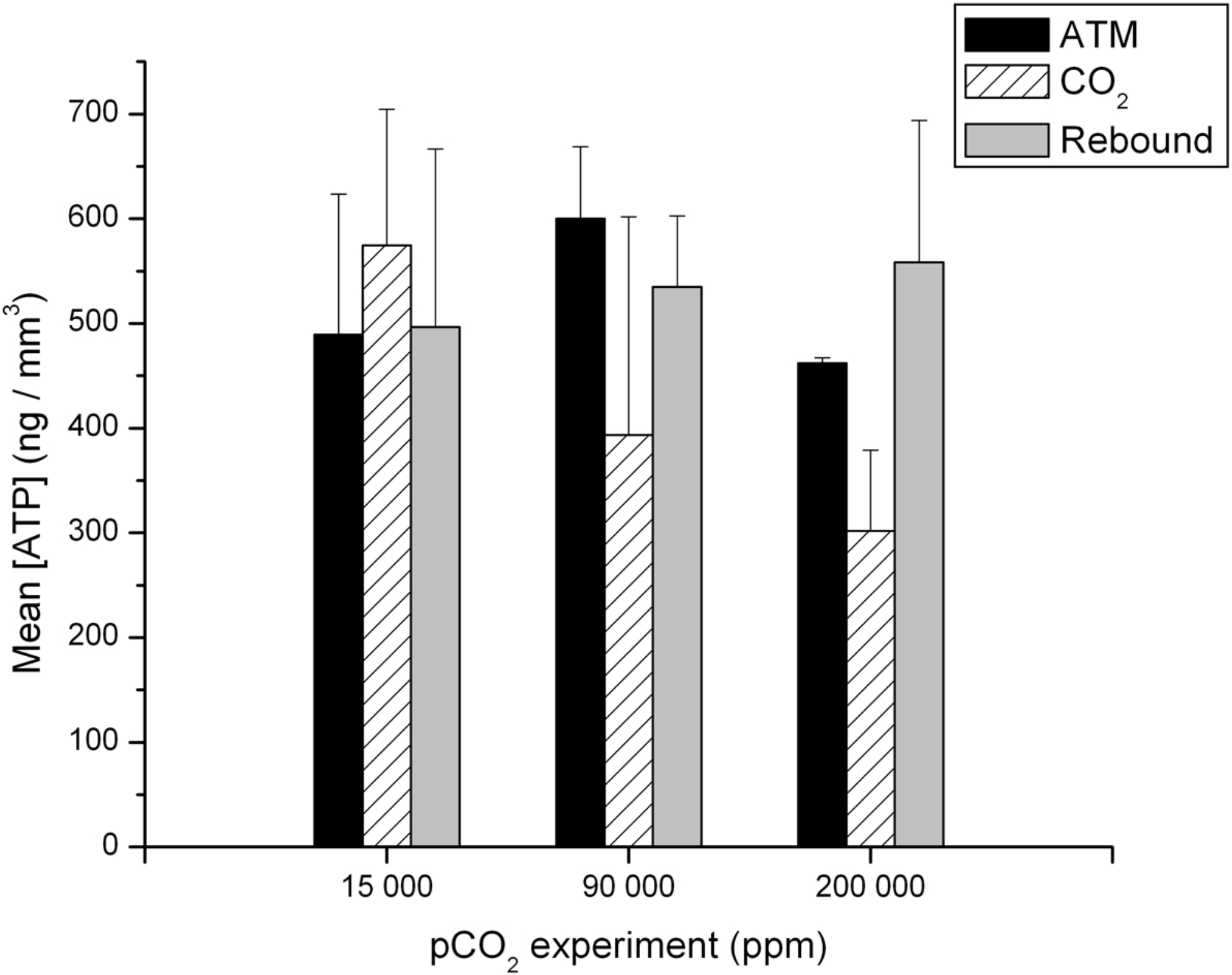
CO_2 experiment	Treatment Means [ATP]		
(ppm)	ATM	Rebound	CO_2
15 000	421.6^{A}	439.6^{A}	501.4
90 000	509.1^{A}	527.6^{A}	256.6^{A}
200 000	455.0^{A}	412.9^{A}	248.0^{A}


A
B
C


A
B
C
D
E
F
G
H
I
% Survival
100
75
50
25
0
ATM
15 000
30 000
60 000
90 000
200 000
Reb
TREATMENT


ATM
CO2
Rebound
Mean proportion survived
1.0
0.8
0.6
0.4
0.2
0.0
15 000
90 000
200 000
pCO2 Experiment (ppm)


A
B
C
D
E
F
G
H
I
Median [ATP] (ng/mm³)
750
500
250
0
1250
1000
ATM
15 000
30 000
60 000
90 000
200 000
Reb
TREATMENT


ATM
CO2
Rebound
700
600
500
400
300
200
100
0
Mean [ATP] (ng / mm3)
15 000
90 000
200 000
pCO2 experiment (ppm)