RRH: OCEAN ACIDIFICATION AND HYPOXIA IMPACTS

LRH: WIT AND OTHERS

A SHORT-TERM SURVIVAL EXPERIMENT ASSESSING IMPACTS OF OCEAN ACIDIFICATION AND HYPOXIA ON THE BENTHIC FORAMINIFER *GLOBOBULIMINA TURGIDA*

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ABSTRACT

The oceans are absorbing increasing amounts of carbon dioxide (CO_2) as a result of rising anthropogenic atmospheric CO_2 emissions. This increase in oceanic CO_2 leads to the lowering of seawater pH, which is known as ocean acidification (OA). Simultaneously, rising global temperatures, also linked to higher atmospheric CO_2 concentrations, result in a more stratified surface ocean, reducing exchange between surface and deeper waters, leading to expansion of oxygen-limited zones (hypoxia). Numerous studies have investigated the impact of one or the other of these environmental changes (OA, hypoxia) on a wide variety of marine organisms, but few experimental studies focus on the simultaneous effects of these two stressors. Foraminifera are unicellular eukaryotes (protists) that live in virtually every marine environment and form an important link in the benthic food web. Here we present results of a short-term (3.5 week) study in which both CO_2 (OA) and O_2 (hypoxia) were manipulated to evaluate the influence of these parameters on the survival of the benthic foraminifer *Globobulimina turgida*. Elevated CO_2 concentrations did not impact short-term survivorship of this species, and furthermore, *G. turgida* had higher survival percentages under hypoxic conditions (0.7 ml/l) than in well-aerated water, regardless of CO_2 concentration.

INTRODUCTION

The anthropogenic increase in atmospheric CO_2 is driving increases in global temperatures and in the net CO_2 uptake by the oceans. As a result, present day open-ocean pH values are already ~0.1 unit lower than pre-Industrial values (Prentice et al., 2001; Feely et al., 2004; Hauri et al., 2009) and are projected to decrease an additional 0.3–0.4 units by the end of this century (Prentice et al., 2001; Orr et al., 2005; Feely et al., 2009). These lower pH values lead to a reduced calcite saturation state [$\Omega_{calcite} = ([Ca^{2+}][CO_3^{2-}] / K^*_{sp})$, in which K^*_{sp} is the solubility product at a given temperature, salinity and pressure]. The phenomenon of decreasing pH in our seas is termed "ocean acidification" (OA).

At the same time, oxygen is less soluble in warmer waters compared to colder waters, and global warming enhances the stratification of the upper ocean. These two effects reduce the supply of oxygen to the deeper parts of the ocean, expanding zones in which oxygen concentrations are low (hypoxia ($[O_2]$<1.4 ml/l) (Pörtner et al., 2005; Feely et al., 2010; Keeling et al., 2010; Stramma et al., 2010). Furthermore, eutrophication can exacerbate hypoxia in coastal settings (e.g., Levin et al., 2009).

Numerous studies have investigated the biological impact of both of these environmental changes separately. For example, OA often negatively impacts marine calcifying organisms in terms of lowering their calcification and growth rates (reviewed in Doney et al., 2009). Corals in particular, have received much study; typically higher pCO_2 concentrations yield lower calcification rates (e.g., Hoegh-Guldberg et al., 2007; Pandolfi et al., 2011). In coccolithophores, calcification is known to decrease due to OA, both in lab cultures (Riebesell et al., 2000) and in

field assemblages compared to pre-Industrial communities (Meier et al., 2014). Pteropods have been similarly shown to decrease calcification in response to OA (Comeau et al., 2009), as have planktonic foraminifera (Moy et al., 2009). There are exceptions, however, where certain coccolithophore populations can be heavily calcified in low pH waters (Beaufort et al., 2011). In multi-generation experiments, coccolithophores have been shown to adapt to OA by increasing growth rates (Lohbeck et al., 2012). Larval molluscs had lower survival and growth rates when exposed to enriched pCO_2 (Andersen et al., 2013; Gobler et al., 2014). In sum, while most calcareous organisms have lower net calcification rates in response to lower pH, some taxa can be have higher net calcification rates while growing in more acidic waters (Ries et al., 2009).

The impact of hypoxia on marine aerobes is also well studied, in part because low oxygen concentrations can occur naturally in the oceans, especially in Oxygen Minimum Zones, silled basins and estuaries (e.g., Keeling et al., 2010). Physiological responses to hypoxia vary widely, depending on organism type (plant, vertebrate, invertebrate, protist, bacteria) (e.g., Pörtner et al., 2005; Seibel et al., 2014). For species that are not normally subject to oxygen depletion, hypoxia can impact behaviors and distributions (biogeography) in addition to growth and reproduction (e.g., Zhang et al., 2010).

Fewer studies published to date have focused on responses to simultaneous decreases in pH and $[O_2]$ (e.g., Gobler et al., 2014; Hernroth et al., 2015; Jakubowska & Normant, 2015). It is important to determine the impacts of multiple stressors because environmental parameters do not change in isolation and the effects of multiple stressors can be additive, greater than additive (synergistic) or less than additive (antagonistic) (Breitburg et al., 2015). For example, an antagonistic response would be where a given species increases its reproductive yield due to

warming but OA negatively impacts its reproduction, so simultaneous OA and warming appear to invoke no significant response for that taxon in terms of reproduction.

Benthic foraminifera comprise an important component of the food web (Lee & Anderson, 1991; Legendre & le Févre, 1995; Sen Gupta, 1999; Murray; 2006; van Oevelen et al., 2006). The response of foraminifera to either OA (Dissard et al., 2010; Fujita et al., 2011; Haynert et al., 2011; Uthicke & Fabricius, 2012; Keul et al., 2013; McIntyre-Wressnig et al., 2013, 2014; Uthicke et al., 2013; Haynert et al., 2014) or hypoxia (e.g., Bernhard & Reimers, 1991; Bernhard, 1992; Jorissen et al., 1995; Bernhard & Alve, 1996; Bernhard et al., 1997; Geslin et al., 2004) is species specific, in part because benthic foraminifera live in many environments. In general, most foraminiferal species are negatively impacted by OA (e.g., decreased calcification, species shifts to non-calcareous forms; reviewed in Keul et al., 2013), but a few foraminiferal species do not appear to be negatively impacted by OA (e.g., McIntyre-Wressnig et al., 2013; 2014). Where the effects of multiple stressors on benthic foraminifera have been tested, warming and OA were investigated with respect to calcification in the shallow-water foraminifer *Ammonia tepida* (Cushman); both stressors decreased shell weights (Dissard et al., 2010).

Globobulimina turgida (Bailey) is a benthic foraminiferal species that produces a calcium carbonate test, the congeners of which are commonly reported in the literature to have a deep-infaunal sediment habitat (Corliss, 1985, 1991; Risgaard-Petersen et al., 2006; Piña-Ochoa et al., 2010; Koho et al., 2011). These habitats are often characterized by low pH and low [O_2], as a result of the microbial decomposition of sedimentary organic matter (e.g., Emerson and Bender, 1981; Hales et al., 1994). *Globobulimina* Cushman species thus live in oxygen-depleted, low pH environments, and can survive periods of no oxygen (anoxia) due to their ability to perform

complete denitrification (Risgaard-Petersen et al., 2006; Piña-Ochoa et al., 2010; Koho et al., 2011). Thus, we hypothesize that *G. turgida* may be insensitive to certain aspects of climate change (i.e., hypoxia, acidification). In assessing impacts of such global change, it is critical to identify both sensitive and insensitive taxa to gain a more complete vision of future ecosystem functioning. Here, we present results from a study in which both CO_2 (OA) and O_2 (hypoxia) were controlled, to evaluate the influence of both parameters (individually or concurrently) on the survival of the benthic foraminifer *Globobulimina turgida*. As such, this contribution is the first description of the survival response of a benthic foraminiferal species to these two stressors.

MATERIAL AND METHODS

Sediment samples were recovered during a 4-day cruise in May 2013 aboard the R/V *Endeavor* to the "Mud Patch" region of the New England continental shelf (approximately 41°30'N, 70°30'W, ~80-m water depth, Bothner et al., 1981). A Soutar boxcorer and multicorer (Ocean Instruments MC800) were used to collect undisturbed sediments containing live foraminifera. On board, the top ~2 cm of boxcored sediment was siphoned off and washed through a stack of sieves (53 μm, 90 μm, 125 μm and 500 μm). The different size fractions, including the <53-μm fraction, were stored immediately in an onboard 5°C environmental room and transported to Woods Hole Oceanographic Institution (WHOI) after the cruise. These foraminifera-bearing sediments were the source of specimens for our experiment (see below). During the cruise, CTD casts were performed to collect bottom-water temperature, salinity and oxygen data, as well as samples for bottom-water alkalinity and Dissolved Inorganic Carbon (DIC) analyses (Table 1).

Working in a refrigerated shipboard van, the top 3 cm of two multicores with undisturbed sediments were sampled at 1-cm resolution. Samples (0–1, 1–2, 2–3 cm) from one multicore were incubated in the environmental room in the viability indicator CellTracker® Green CMFDA (5-chloromethylfluorescein diacetate; hereafter referred to as "CTG", Life Technologies) with a final concentration of 1 μM in seawater, following the approach of Bernhard et al. (2006). After 12 h, samples were preserved in a ~3.8% paraformaldehyde solution buffered with sodium borate (Borax®) to avoid calcite dissolution. In the shore-based laboratory, these sediment samples were sieved over a 125-μm screen and the coarser fraction analyzed for fluorescent (living) specimens of *G. turgida*. Organisms, including *G. turgida*, that were alive during the CTG incubation had taken up the solution in their cytoplasm, where it is ultimately cleaved into a number of compounds, of which one is the fluorescent compound fluorescein. In living specimens, the cytoplasm had a green fluorescence when viewed under a Leica MZ FLIII epifluorescence stereomicroscope equipped with optics for fluorescein detection (~480 nm excitation; 520 nm emission). Some specimens fluoresced orange/yellow due to combined fluorescence of CTG (green) and chlorophyll autofluorescence (red) from algal ingestion (feeding).

In the ship's wet lab, samples of 1-cm sections of a separate multicore were centrifuged, filtered with a 0.45-μm syringe filter, and poisoned with a saturated solution of mercuric chloride (10 μl) to obtain pore-water samples for later alkalinity and DIC analyses. On shore, alkalinity values were determined by automated Gran titrations of 1 ml samples, standardized using certified reference materials obtained from Dr. A. Dickson (Scripps Institution of Oceanography). The relative standard deviation of alkalinity analyses of replicate samples from the culture experiments (below) averaged ~0.9% (n = 39). Dissolved inorganic carbon

concentrations were determined manometrically on ~ 5 ml samples, using an automated vacuum extraction system. The relative standard deviation of DIC analyses of replicate samples from the experiments averaged ~1.1% (n = 47). Calculations of carbonate system parameters were performed using a spreadsheet version of the CO2SYS program (Lewis & Wallace, 1998) using the total pH scale and the dissociation constants of Mehrbach et al. (1973), as refit by Dickson & Millero (1987), and the calcite solubility of Mucci (1983).

Containers with foraminifera-bearing sediment fractions were connected to a recirculating seawater system at 7^{o}C at WHOI. Sediments from the 125–500-μm size fraction were picked for *G. turgida*. Individual specimens of *G. turgida* that had green/brown coloration, indicating algal uptake (Piña-Ochoa et al., 2010; Koho et al., 2011), were deemed alive and introduced to the experimental setup. In total ~650 individuals were selected, and divided among the 6 treatments in this experiment (Table 2; ~100–110 specimens per treatment). Foraminifera were kept in 140-ml plastic tubs, containing a ~1-cm layer of sediment (grain size <53 μm) and 120 ml of bottom water collected by Niskin bottles on a CTD rosette at the sample site. Bottom-water salinity of collected seawater increased from 34.2 to 35.5, potentially as a result of vacuum filtration over a 0.2-μm filter. Salinity was subsequently adjusted from 35.5 to 35 by adding deionized water (pH ~6.0) and was uniform across all treatments. Any decline in pH due to the added small volume of deionized water would quickly be equilibrated to the atmosphere in each chamber, as described below.

The experiment was conducted in a darkened climate-controlled room maintained at 7^{o}C, colder than the Mud Patch site at the time of sample collection, but typical of winter temperatures at this site. The room was typically lit <1 h per day. Each treatment was housed in a Biospherix C-Chamber to control the atmosphere of the different treatments. The treatments

included combinations of four O_2 [0.7 (A, B), 4.9 (C), 5.9 (D) and 7.1 (E,F) ml/l] and five CO_2 [122 (A), 275 (C), 902 (E), 2000 (B, D) or 3500 (F) ppmv] concentrations (Table 2). The low CO_2 concentration in Treatment A (122 ppmv) was inadvertently caused by simultaneous loss of CO_2 while lowering [O_2], without a CO_2 sensor. Treatment E was intended to serve as a control, but high CO_2 concentrations (~900 ppmv) in the 7°C climate room resulted in elevated CO_2 concentrations in Treatment E. Dissolved oxygen concentrations were assumed to be in equilibrium with the treatment gas and calculated using the known % O_2 and temperature (7°C). Each chamber was connected to a gas source (either 1% CO_2/99% N_2, 100% N_2 or 100% CO_2) and, in 5 of the 6 cases, via a ProCO2 and/or ProOx controller (Biospherix) to monitor (using CO_2 and/or O_2 sensors) and manipulate its atmospheric composition. In the sixth case (F, 3500 ppmv CO_2), the C-Chamber was not controlled with any Biospherix sensors because it was housed in a Nuaire incubator (Autoflow CO_2 Water-Jacketed incubator (NU4950) attached to a Thermo RTE740 refrigerated bath to maintain temperature at 7ºC), which controlled CO_2 via an internal sensor. The C-Chamber atmosphere was linked to the controlled Nuaire atmosphere. Tests from a previous study that used the Biospherix CO2 system showed that the atmosphere and seawater are in equilibrium within 40 h after introduction to the controlled atmosphere within the chamber (McIntyre-Wressnig et al., 2014). All treatments were fed weekly with 1 ml of a concentrated mixture of live *Dunaliella tertiotecta* Butcher and *Isochrysis galbana* Parke. The calcium carbonate content of the <53-µm experiment sediment was determined on an aliquot of excess sediment by weighing air-dried samples before and after a 12 h submersion in ~2.7 M HCl.

Experimental seawater was sampled for DIC and alkalinity at the end of weeks 1 and 2 and at the end of the experiment (3.5 weeks) to monitor the carbonate system. Water samples

were taken directly from each treatment container, which held seawater, fine-grained sediment and *Globobulimina turgida*. For each time point, we removed 18 ml (8 ml for DIC; 10 ml for alkalinity) and replenished the volume with pre-equilibrated seawater from the same chamber. Carbonate system parameters (pH, $\Omega_{calcite}$, $[CO_3^{2-}]$, pCO_2) for the experiment were calculated from alkalinity and DIC values using CO2SYS (Lewis & Wallace, 1998), as described above for the field samples.

The experiment was terminated after 3.5 weeks to evaluate short-term survivorship of *G. turgida*. Before termination, each treatment was incubated for 24 h in the viability indicator CTG, using the same methods employed for the multicore samples, as described above. Before microscope analyses, samples were sieved over a 63-μm mesh with seawater. The >63-μm fraction of the CTG-incubated samples was evaluated for living (fluorescent) foraminifera (Bernhard et al. 2006). Both green and orange/yellow fluorescing individuals of *G. turgida* were regarded as alive at the end of the experiment.

RESULTS

Bottom-water temperature and salinity at the sample site were ~10.4^{o}C and ~34.2, respectively, with little variability between the different CTD deployments, which were taken over 3 days (Table 1). Measured bottom-water DIC and alkalinity values were consistent and varied between 2091–2092 μmol/kg for DIC and 2273–2285 μmol/kg for alkalinity (Table 1). Calculated *in situ* pH (Total scale) and calcite saturation state ($\Omega_{calcite}$) averaged ~7.97 and 3.2, respectively. Bottom waters were aerated, with oxygen concentrations between 5.9 and 6.0 ml/l (Table 1).

Concentrations of living *G. turgida* at the sample site were 242, 138 and 128 specimens/50 cm^3 for the 0–1, 1–2, 2–3 cm depth intervals, respectively.

Pore-water alkalinity and DIC values varied between 2240–2450 and 2393–2478 μmol/kg, respectively (Table 3). Calculated pore water pCO_2, pH (Total scale) and $\Omega_{calcite}$ were between 1986–4143 ppm, 7.01–7.36 and 0.39–0.92, respectively (Table 3).

In the experimental cultures, carbonate system parameters varied both between treatments and within treatments over time; DIC ranged from 1610–2393 μmol/kg, and alkalinity ranged from 1927–2392 μmol/kg (Fig. 1, Table 2). Possible causes for the variability in alkalinity are discussed below. The pH varied across treatments, between 7.30–8.42. The calcite saturation state was as low as 0.58 and as high as 4.8. The calcium carbonate content of experimental sediment was 4.1% dry weight.

Globobulimina turgida survivorship at the end of the 3.5-week experiment varied between ~60–90% (Fig. 2). Survival correlated negatively with oxygen content of each treatment ($R^2 = 0.88$, $p<0.01$, t-test), but did not show a significant correlation with carbonate system chemistry ($p>0.10$, t-test) (Fig. 3).

DISCUSSION

FIELD ABUNDANCES

Globobulimina turgida is among the most common species at our sampling site (Lang, 2013). Specifically, *G. turgida* comprised 21% of rose-Bengal stained specimens (> 63 μm) in May 2010, when the community was sampled for a different study. Other common species at that time were *Elphidium excavatum* (Terquem) (29%), *Stainforthia fusiformis* (Williamson) (13%), *Trochammina inflata*

(Montagu) (10%), *Bulimina marginata* d'Orbigny (7%) and *Aubignyna hamblensis* Murray, Whittaker and Alve (7%).

Globobulimina turgida was quite abundant at the sampling site when we sampled in May 2013, with over 240 specimens/50 cm^3 in the surface cm. These abundances far exceed other published abundances for this species. For example, in the Pacific, the abundances of congener *G. pacifica* Cushman were <5 specimens/50 cm^3 (Shepherd et al., 2007). Another Pacific site reported abundances of ~180 rose-Bengal stained *G. pacifica*/50 cm^3 and ~70 live conspecifics/50 cm^3 as determined by ATP assay (Bernhard, 1992). In the NE Atlantic, abundances of *Globobulimina* spp. were all were below ~125 specimens/50 cm^3 (Koho et al., 2008) and *G. affinis* (d'Orbigny) were below 40 specimens/50 cm^3 (Fontanier et al., 2002). In the Gullmar Fjord, *G. pseudospinescens* (Emiliani) abundances were <60 specimens/50 cm^3 (Risgaard-Petersen et al., 2006). The high abundances of *G. turgida* at our sampling site indicate that this taxon is particularly well adapted to the Mud Patch area.

Because *Globobulimina* is known to denitrify (Risgaard-Petersen et al., 2006), it might be expected that this taxon will have a subsurface peak in abundance, as noted in some publications (e.g., Corliss, 1985; Fontanier et al., 2002; Koho et al., 2008). In our case, the maximum abundance was in the top 1 cm. Surface maxima for *Globobulimina* have also been documented in the literature (e.g., Risgaard-Petersen et al., 2006; Koho et al., 2008; Shepherd et al., 2007). While subsurface maxima also occur, in cases where both types of distributions were noted, surface abundances exceeded subsurface distributions (Koho et al., 2008; Shepherd et al., 2007). Surface maxima of *Globobulimina* most likely are linked to steep redox gradients, as suggested by Koho et al. (2011).

EXPERIMENTAL CONDITIONS

Alkalinity and DIC varied among individual treatments. The DIC values of the treatments were expected to differ due to the range of experimental pCO_2, whereas alkalinity is not affected

directly by changes in pCO_2 (Table 2, Fig. 1). We suspect that the observed differences in alkalinity between treatments were caused by dissolution of calcium carbonate within the sediment. The maximum change in alkalinity between treatments was ~400 μmol/kg, which corresponds to the dissolution or precipitation of 200 μmol/kg calcium carbonate per treatment (Table 2). Each sample contained ~10 grams of sediment corresponding to about 4100 μmol $CaCO_3$ at a calcium carbonate content of 4.1% (as determined for our experiment sediments). The observed increases in alkalinity in the low-saturation-state treatments (B, F) are only a fraction of the total $CaCO_3$ present.

We do not think that photosynthesis in the culture containers can explain the observed alkalinity increases; the experiments were conducted in a nearly-dark, climate-controlled room, and although photosynthesis would increase alkalinity slightly, it would cause a much larger decrease in DIC, which did not occur. Although decomposition of organic matter via sulfate reduction or iron oxide reduction can lead to increases in both alkalinity and DIC (Berner et al., 1970), anaerobic processes are unlikely to have been significant even within the thin sediment layer (<1-cm thick) of the containers exposed to low-oxygen treatments.

The observed bottom-water pCO_2 values at the coring site (380–394 ppmv) were close to present day atmospheric pCO_2, while the pore-water alkalinity and DIC values were substantially higher than bottom-water values (Tables 1 and 3). These elevated pore-water concentrations are consistent with diagenetic processes (organic matter decomposition and carbonate dissolution) in the organic carbon-rich sediments of the Mud Patch (~2% organic carbon; Bothner et al., 1981). The decomposition of organic matter at the sediment-water interface and within the top few centimeters of the sediment causes an increase in pore-water DIC (Emerson & Bender, 1981; Reimers et al., 1986; Archer et al., 1989). The DIC increase causes higher pore-water pCO_2 and

lower pore-water pH values, and can cause undersaturation of pore-waters with respect to carbonate minerals ($\Omega_{carbonate}$ <1) (Table 3). This undersaturation will tend to dissolve sedimentary calcium carbonate, increasing pore-water alkalinity and ultimately reducing the degree of carbonate undersaturation (Berner, 1980).

In organic-rich sediments, the amount of organic matter that can be decomposed depends in part on the oxygen concentration of the overlying bottom water. Given the measured bottom-water oxygen concentrations (Table 1), and assuming that the pore-waters in the top 1–3 cm were hypoxic but not anoxic (i.e., assuming a pore-water oxygen concentration of 0.7–1.1 ml/l), then approximately 4.9–5.3 ml/l O_2 was consumed by aerobic decomposition of organic matter within the top few cm. This decomposition would increase pore-water DIC by ~350 μmol/kg, assuming a Redfield C:O_2 ratio (106:138) and an O_2:DIC diffusivity ratio of 2. This DIC increase would lead to pCO_2, pH (total scale) and $\Omega_{calcite}$ of ~5000 ppmv, 7.0 and 0.28, respectively, assuming no "compensation" by calcium carbonate dissolution. If dissolution of sedimentary calcium carbonate is assumed to proceed until $\Omega_{calcite}$ increases to a value of 1.0, the pore-water pCO_2 would be ~2000 ppmv pCO_2, and the pH (total scale) would be ~7.5. The observed pore-water carbonate system values (Table 3) in the shallowest sample are comparable to the "uncompensated" (decomposition only) predictions, and the deeper pore waters are consistent with *in situ* carbonate dissolution acting to compensate for the addition of respiration-derived DIC. The pCO_2 levels of the experimental treatments were chosen to yield $\Omega_{calcite}$ values of 0.6 to 4.8 (Table 2), spanning the range of observed bottom water and pore-water $\Omega_{calcite}$. Even higher pCO_2 levels were used in a recent long-term OA culture experiment with benthic foraminifera, to establish treatment levels that encompassed the range of carbonate chemistry experienced by benthic foraminifera in the field (Haynert et al., 2014).

Studies of foraminiferal responses to hypoxia typically do not control [O_2] as stringently as in our experiment. For example, whole cores were used to assess impact of "strong" hypoxia on survival of a foraminiferal community from a 35-m deep Adriatic site (Pucci et al., 2009). In that study, profiles of oxygen indicate steep gradients, with [O_2] as low as ~0.1 ml/l in some cores for part of the experiment. Likewise, an 8-month experiment had [O_2] <0.2 ml/l for a portion of that experiment, but oxygen levels drifted and were not stringently controlled (Alve & Bernhard, 1995).

SURVIVORSHIP

Benthic foraminiferal microhabitats are predominantly controlled by the availability of oxygen and food (Corliss, 1985, 1991; Bernhard & Reimers, 1991; Bernhard, 1992; Jorissen et al., 1995; Bernhard & Alve, 1996; Sen Gupta, 1999; Murray, 2006), which in turn influence bottom- and pore-water carbonate chemistry. For this experiment with *G. turgida*, we compared survivorship to oxygen concentration and carbonate-system chemistry. No dietary comparison was made because all treatments received identical amounts of food from the same source.

Survival rates of foraminifera in prior OA experiments are not always determined, depending on the scope of the study. A direct comparison of foraminiferal survival rates can be made with the studies of McIntyre-Wressnig et al. (2013, 2014), although survival was determined in those studies using a different method (ATP analysis). After six weeks, survival rates for *Bulimina marginata,* also collected from the Mud Patch, were ~73–89% among control populations and 68–82% in populations exposed to enhanced pCO_2 (1000 and 2000 ppmv; McIntyre-Wressnig et al., 2014). These survival rates for *B. marginata* are similar to those observed in this study for *G. turgida* (60–90%). Survival rates for the temperate bathyal *Bolivina*

argentea Cushman and the reef dwelling *Amphistegina gibbosa* d'Orbigny after six weeks were remarkably similar to each other (~35–78% among both species and all treatments; McIntyre-Wressnig et al., 2014; 2013, respectively) and a bit lower than observed for *G. turgida*.

Survival percentages for *G. turgida* at the end of the experiment were strongly correlated with the oxygen concentration of the treatment chamber's atmosphere, with higher survivorship at lower [O_2] (Fig. 3). Aerobic organisms often show little response to decreasing [O_2] above a threshold concentration of about 1.4 ml/l (Levin et al., 2009; Melzner et al., 2013). Small changes in near-saturated oxygen conditions are not expected to impact survivorship of aerobic foraminifera. Previous studies indicated that *Globobulimina* can live in a low-oxygen environment and is able to survive short periods of anoxia (Corliss, 1985, 1991; Risgaard-Petersen et al., 2006; Piña-Ochoa et al., 2010; Koho et al., 2011). The ability of *G. turgida* to survive such conditions depends, at least partly, on denitrification, using nitrate stored in an internal pool within its cytoplasm (Piña-Ochoa et al., 2010; Koho et al., 2011). This nitrate pool needs replenishment after periods of anoxia; a maximum period for this species of up to 56 days has been reported, although the length of this period hypothetically depends on other environmental parameters such as temperature and pH (Piña-Ochoa et al., 2010; Koho et al., 2011). Interestingly, the survival rates of *G. turgida* were similar in oxic and anoxic treatments (without acidification), as long as the treatment included nitrate (Piña-Ochoa et al., 2010). It is also known that nitrate acquisition in *G. turgida* is slower in treatments with oxygen as opposed to treatments lacking oxygen (Koho et al., 2011). In sum, it is not clear why our *G. turgida* had lower survival rates in aerated treatments compared to hypoxic treatments, but it may be related to denitrification physiology. Other laboratory experiments indeed showed that *G. turgida*

actively migrates from well-oxygenated sediments towards low-oxygen conditions (Koho et al., 2011).

Survivorship of *G. turgida* showed no significant correlation with carbonate system chemistry (Fig. 3). The high pCO_2, low pH, and low calcite saturation state of the pore-water habitat of *G. turgida* (shallow to deep infaunal) may explain the lack of significant effect of increased pCO_2 on short-term survival percentages, as this species presumably is adapted to high pCO_2 / low pH environments. The same may be true for other infaunal benthic foraminifera, and short-term experiments have showed no significant effect of OA on survival for *Ammonia* spp. (Dissard et al., 2010; Haynert et al., 2011; Keul et al., 2013), *Bulimina marginata* and *Bolivina argentea* (McIntyre-Wressnig et al., 2014). It seems likely that the energetic cost of building and maintaining a calcium carbonate test will become too high when the environmental carbonate saturation state or pH values fall below some critical level. Although survivorship provides one measure of the *Globobulimina turgida* response to multiple stressors and suggests this species is insensitive to two major stressors of climate change at the conditions we tested, studies that determine the response of a range of factors, such as growth and calcification rates, test morphology, motility, and reproductive capacity, to these same concurrent stressors are needed to assess this species' full response to ocean acidification and hypoxia.

CONCLUSIONS

Neither reduced $[O_2]$ (hypoxia) nor elevated CO_2 (OA) lowered *G. turgida* survivorship; in fact, survival percentages for low-oxygen treatments were significantly higher than those for high-oxygen treatments. This hypoxia tolerance may reflect the ability of *G. turgida* to perform

complete denitrification, and its adaptation to the low and variable pH of its natural pore-water habitat. To anticipate changes in ocean ecosystems in the decades ahead, it is imperative to understand the impacts of multiple concurrent stressors on marine fauna, and to identify species that may benefit from climate change; *Globobulimina turgida* may be one of those species. It remains to be determined whether prolonged periods of severe oxygen limitation combined with OA might negatively impact survivorship and calcification of *G. turgida* , and thus whether *G. turgida* will emerge as an ecological winner or loser as a result of intensifying ocean acidification and the expansion of hypoxic zones.

ACKNOWLEDGMENTS

We thank captain and crew of the R/V *Endeavor* and the science party for their assistance with collecting samples during cruise EN524. We are thankful to Marti Jeglinski, Dave Beaudoin and Inge van Dijk for their help with selecting live specimens for the experiment. Katherine Hoering and Elizabeth Bonk are thanked for performing the DIC and alkalinity analyses, as are two anonymous reviewers for comments on an earlier manuscript version. This research was supported by US NSF grant OCE-1219948 to JMB.

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Received 8 October 2014

Accepted 20 April 2015

TABLE CAPTIONS

Table 1. Bottom-water values for three different CTD casts (events) at the sample site. Values for temperature, salinity, O_2, alkalinity and DIC were measured, while pCO_2, $pH_{(total\ scale)}$, CO_3^{2-} and $\Omega_{calcite}$ were calculated using CO2SYS (Lewis & Wallace, 1998).

Table 2. The water chemistry of each experimental treatment. Treatment designations are listed from low to high O_2 and low to high CO_2 in case of equal O_2-levels. Values for pCO_2, O_2, alkalinity and DIC were measured, while $pH_{(total\ scale)}$, CO_3^{2-} and $\Omega_{calcite}$ were calculated using CO2SYS (Lewis & Wallace, 1998). Treatment F has no error bars for carbonate system parameters because only one sample was successfully measured for alkalinity and DIC.

Table 3. Pore-water carbonate-system parameters as calculated from measured DIC and alkalinity using CO2SYS (Lewis & Wallace, 1998). Values for pH are reported on the Total scale.

FIGURE CAPTIONS

Figure 1. Carbonate-chemistry parameters (alkalinity (A), CO_3^{2-} (B), DIC (C) and $\Omega_{calcite}$ (D)) for the different treatments over the course of the experiment. Alkalinity (A) and DIC (C) were measured, while CO_3^{2-} (B) and $\Omega_{calcite}$ (D) were calculated using CO2SYS (Lewis & Wallace, 1998). Treatment F has only a single point over the course of the experiment because only one sample was successfully measured for DIC and alkalinity. Carbonate-system parameters varied between treatments and over the course of the experiment, which might be related to the precipitation/dissolution of calcium carbonate within each treatment.

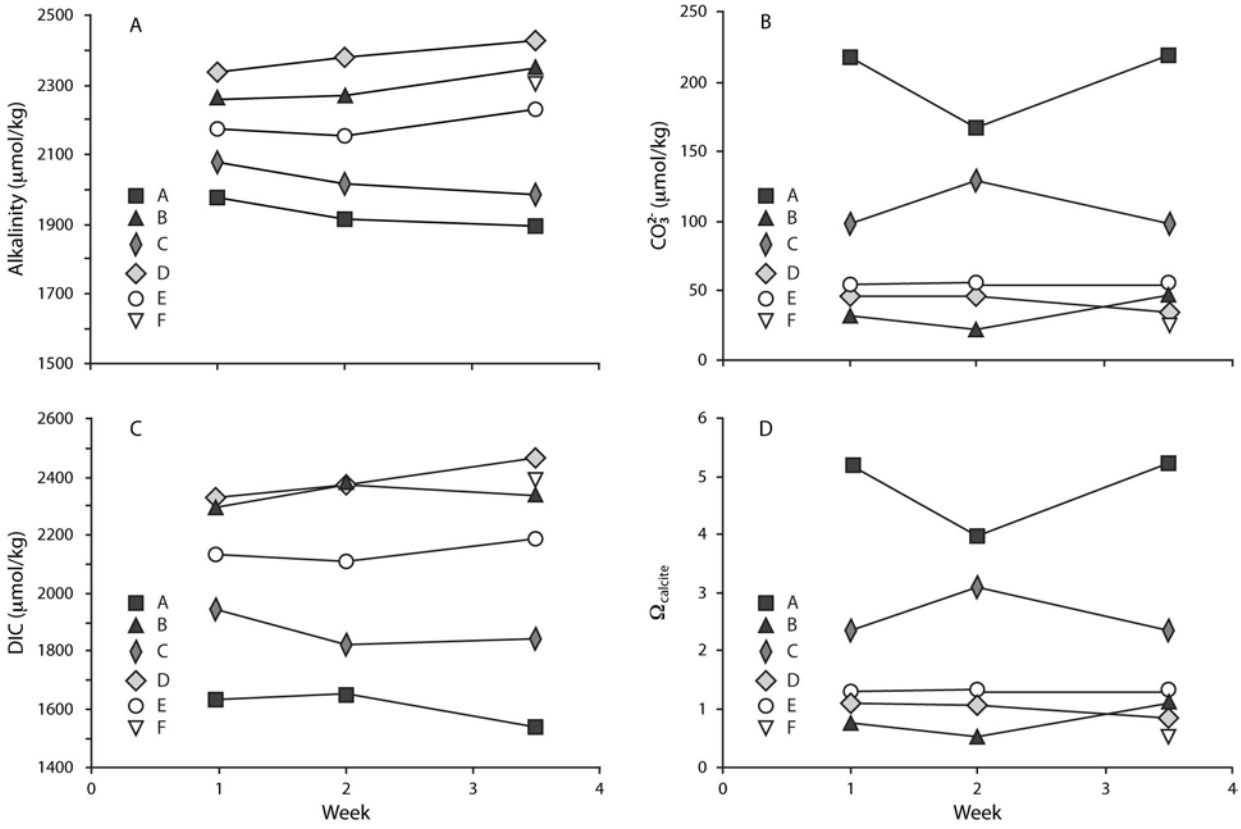
Figure 2. Survivorship (%) for *G. turgida* from the different experiment treatments presented by $[O_2]$ and pCO_2. Shading of the bars relates to $[O_2]$, with darker shades being more depleted in oxygen.

Figure 3. *Globobulimina turgida* survivorship (%) versus $[O_2]$ (A), alkalinity (B), $\Omega_{calcite}$ (C) and pCO_2 (D). Survivorship was significantly correlated with oxygen (A), while no significant relation existed with the carbonate-system parameters (B, C, D).

Event	Temperature	Salinity	O_2	Alkalinity	DIC	pCO_2	pH	CO_3^{2-}	$\Omega_{calcite}$
	°C		ml/l	µmol/kg	µmol/kg	ppmv		µmol/kg	
EN524-1	10.64 ± 0.02	34.25	5.87 ± 0.007	2283 ± 3	2091 ± 3	380 ± 13	7.97 ± 0.01	139 ± 4	3.28 ± 0.09
EN524-5	10.74 ± 0.02	34.28	5.87 ± 0.10	2285 ± 4	2092 ±1	380 ± 6	7.098 ± 0.01	140 ± 2	3.30 ± 0.05
EN524-12	10.15 ± 0.11	34.05	5.96 ± 0.060	2273 ± 4	2091 ± 1	394 ± 8	7.95 ± 0.01	132 ± 2	3.12 ± 0.05

Treatment	O_2	pCO_2	Alkalinity	DIC	pH	CO_3^{2-}	$\Omega_{calcite}$
	ml/l	ppmv	µmol/kg	µmol/kg		µmol/kg	
A	0.7 ± 0.02	122 ± 30	1927 ± 45	1610 ± 59	8.42 ± 0.09	201 ± 29	4.8 ± 0.70
B	0.7 ± 0.02	2000 ± 40	2291 ± 49	2334 ± 36	7.42 ± 0.17	33 ± 13	0.79 ± 0.30
C	4.9 ± 0.12	275 ± 6	2024 ± 50	1869 ± 66	8.06 ± 0.08	109 ± 18	2.59 ± 0.43
D	5.9 ± 0.14	2000 ± 40	2392 ± 47	2393 ± 70	7.56 ± 0.02	45 ± 6	1.08 ± 0.15
E	7.1 ± 0.17	902 ± 40	2184 ± 39	2142 ± 41	7.70 ± 0.01	56 ± 0.8	1.33 ± 0.02
F	7.1 ± 0.17	3500 ± 70	2308	2392	7.30	24	0.58

Event	Depth	Alkalinity	DIC	CO_2	pH	CO_3^{2-}	$\Omega_{calcite}$
	cm	µmol/kg	µmol/kg	ppmv		µmol/kg	
EN524-10	0-1	2241	2393	4143	7.01	16	0.39
EN524-10	1-2	2450	2478	1986	7.36	39	0.92


A
Alkalinity (µmol/kg)
2500
2300
2100
1900
1700
1500
B
CO_3^{2-} (µmol/kg)
250
200
150
100
50
0
C
DIC (µmol/kg)
2600
2400
2200
2000
1800
1600
1400
D
$\Omega_{calcite}$
6
5
4
3
2
1
0
0
1
2
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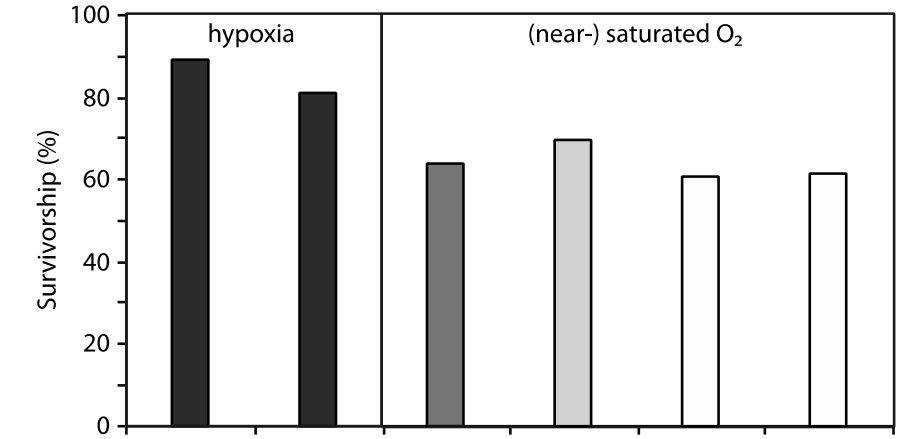
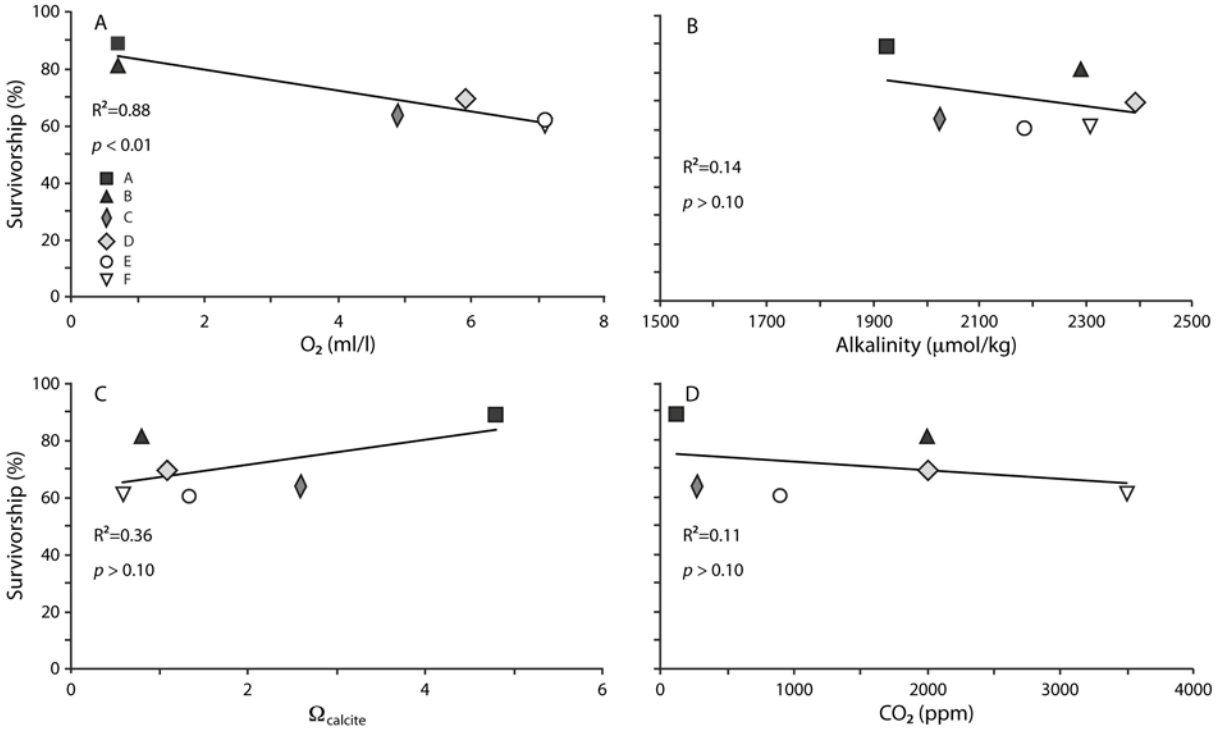
Week
A
B
C
D
E
F



Treatment	A	B	C	D	E	F
O_2 (ml/l)	0.7	0.7	4.9	5.9	7.1	7.1
pCO_2 (ppmv)	122	2000	275	2000	902	3500


A
Survivorship (%)
R²=0.88
p < 0.01
A
B
C
D
E
F
O₂ (ml/l)
B
R²=0.14
p > 0.10
Alkalinity (µmol/kg)
C
R²=0.36
p > 0.10
Ωcalcite
D
R²=0.11
p > 0.10
CO₂ (ppm)