



Exposure to elevated $p\text{CO}_2$ does not exacerbate reproductive suppression of *Aurelia aurita* jellyfish polyps in low oxygen environments

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ABSTRACT: Eutrophication-induced hypoxia is one of the primary anthropogenic threats to coastal ecosystems. Under hypoxic conditions, a deficit of O_2 and a surplus of CO_2 will concurrently decrease pH, yet studies of hypoxia have seldom considered the potential interactions with elevated $p\text{CO}_2$ (reduced pH). Previous studies on gelatinous organisms concluded that they are fairly robust to low oxygen and reduced pH conditions individually, yet the combination of stressors has only been examined for ephyrae. The goals of this study were to determine the individual and interactive effects of hypoxia and elevated $p\text{CO}_2$ on the asexual reproduction and aerobic respiration rates of polyps of the scyphozoan *Aurelia aurita* during a manipulative experiment that ran for 36 d. $p\text{CO}_2$ and $p\text{O}_2$ were varied on a diel basis to closely mimic the diel conditions observed in the field. Exposure to low dissolved oxygen (DO) reduced asexual budding of polyps by ~50% relative to control conditions. Under hypoxic conditions, rates of respiration were elevated during an initial acclimation period (until Day 8), but respiration rates did not differ between DO levels under prolonged exposure. There was no significant effect of increased $p\text{CO}_2$ on either asexual reproduction or aerobic respiration, suggesting that elevated $p\text{CO}_2$ (reduced pH) did not exacerbate the negative reproductive effects of hypoxia on *A. aurita* polyps.

KEY WORDS: Hypoxia · Ocean acidification · Scyphozoan · Aerobic respiration

INTRODUCTION

Hypoxia appears to be expanding along many of the world's coastal regions (Diaz & Rosenberg 2008), and global oceanic oxygen content has decreased over 2% in the past 50 yr (Schmidtke et al. 2017). This has major implications for sustaining life in the coastal ocean because most marine animals and plants require dissolved oxygen (DO) to aerobically respire and survive. Eutrophication facilitates the development of hypoxia (typically defined as $<2.0\text{--}5.0\text{ mg O}_2\text{ l}^{-1}$; Vaquer-Sunyer & Duarte 2008) or

anoxia ($<0.5\text{ mg O}_2\text{ l}^{-1}$; Zogorski et al. 2006) in many coastal waters because the input of excess nutrients increases organic matter production, which in turn is decomposed by microbes. This increased microbial aerobic metabolism depletes oxygen from the water column and causes hypoxia (Wallace et al. 2014). Hypoxia can also greatly influence the flow of energy through food webs by shifting energy from mobile predators to microbe communities, because microbes can efficiently support their metabolism via anaerobic pathways (Diaz & Rosenberg 2008).

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In microbial-driven hypoxic waters, the pH is lower because enhanced respiration elevates $p\text{CO}_2$ (Brewer & Peltzer 2009, Cai et al. 2011, Wallace et al. 2014, Gobler & Baumann 2016). Reduced pH conditions (i.e. elevated $p\text{CO}_2$ drives lower pH values relative to ambient conditions) can affect acid–base regulation (Pörtner & Reipschläger 1996, Cecchini et al. 2001, Fabry et al. 2008), metabolic rates (Hand 1991, Pörtner & Reipschläger 1996, Guppy & Withers 1999, Fabry et al. 2008), and protein synthesis and growth (Hand 1991, Fabry et al. 2008) and therefore can potentially have detrimental effects on rates of survival and reproduction of organisms. Levels of DO and pH naturally fluctuate on diel cycles, increasing during the day when rates of photosynthesis exceed respiration, and decreasing at night when photosynthesis ceases. In eutrophic regions, diel variability in DO and $p\text{CO}_2$ can be extreme (e.g. 0.22–6.5 mg O_2 l^{-1} DO, and ~330–4000 μatm $p\text{CO}_2$; Baumann et al. 2015), but it is unclear whether these variations will cause additional environmental stress for coastal organisms (Keppel et al. 2016) or potentially provide sporadic relief (Frieder et al. 2014).

Despite observations that hypoxia and reduced pH are tightly linked in hypoxic ecosystems, studies of hypoxia have rarely considered the potential interactive effects of low DO and reduced pH (but see Frieder et al. 2014, Gobler et al. 2014, DePasquale et al. 2015, Algueró-Muñiz et al. 2016). In laboratory settings, hypoxia is usually created by sparging seawater with N_2 gas to displace oxygen. An artifact of this method is that pH is elevated (up to 8.6) due to simultaneous displacement of CO_2 , creating different conditions to those that occur in the field (Gobler et al. 2014, Gobler & Baumann 2016). Therefore, studies of hypoxia that have sparged seawater with N_2 (Condon et al. 2001, Decker et al. 2004, Grove & Breitburg 2005, Thuesen et al. 2005, Miller & Graham 2012) may have produced results that are inconsistent with the response of biota in the natural environment.

In most instances, exposure to both reduced pH and low DO produces additive (sum of individual effects) or synergistic (response greater than sum of effects) negative effects across a wide variety of taxa (Kim et al. 2013, Gobler et al. 2014, DePasquale et al. 2015, Jansson et al. 2015, Steckbauer et al. 2015). For example, the combination of low DO and reduced pH elicited additive negative effects on growth and survival in 3 larval fish species (inland silverside *Menidia beryllina*, Atlantic silverside *M. menidia*, and sheepshead minnow *Cyprinodon variegatus*; DePasquale et al. 2015) and 2 early life stage bivalves (juvenile hard clams *Mercenaria mercenaria* and larval

bay scallops *Argopecten irradians*; Gobler et al. 2014). Synergistic negative effects on growth were observed for juvenile red abalone (*Haliotis rufescens*) (Kim et al. 2013). This pattern also manifests as metabolic responses (Steckbauer et al. 2015). According to the conceptual model presented by Gobler & Baumann (2016) (originally proposed by Pörtner et al. 2005), aerobic performance (within a thermal range) will be reduced in hypoxic environments, and further reduced in environments with both low oxygen and elevated $p\text{CO}_2$. Indeed, combining reduced pH with hypoxia caused both additive and synergistic reduction in respiration compared to hypoxia alone across a suite of invertebrate taxa (Steckbauer et al. 2015).

Jellyfish tolerance to hypoxia is considered to be a competitive advantage in systems that experience seasonal hypoxia. Some studies have determined the independent effects of hypoxia (Condon et al. 2001, Decker et al. 2004, Grove & Breitburg 2005, Thuesen et al. 2005, Miller & Graham 2012), and reduced pH (Klein et al. 2014, Lesniewski et al. 2015, Tills et al. 2016) on gelatinous zooplankton, with the majority concluding that various gelatinous taxa are relatively tolerant to constant exposure to both stressors in isolation (but see Tills et al. 2016). For example, consumption and digestion rates of the ctenophore *Mnemiopsis leidyi* on copepods were unaffected by hypoxia (Decker et al. 2004), which benefits gelatinous organisms over other plankton when oxygen is low. Scyphozoan jellyfish, however, have complex life histories that comprise alternating sexual and asexual generations. Medusae sexually produce microscopic planula larvae that attach on the seafloor and transform into benthic polyps. Polyps can asexually reproduce ('bud' new polyps), as well as strobilate, or produce stacks of ephyrae that are released into the water column to become medusae that grow into adult jellyfish. Consequently, benthic life history stages (i.e. benthic polyps) may experience DO and pH conditions that differ from those experienced by pelagic life history stages (i.e. ephyrae and medusae). Low DO had minimal effect on the growth of the scyphomedusa *Chrysaora quinquecirrha* (Grove & Breitburg 2005) because scyphozoan medusae may stockpile oxygen within the mesoglea (Thuesen et al. 2005). Scyphomedusae and ctenophores are mobile and may also be able to escape bottom hypoxia, but benthic polyps are sessile and remain exposed to bottom conditions. Polyps may be similarly tolerant to hypoxia, however, since both *Chrysaora* sp. and *Aurelia* sp. polyps can survive and asexually reproduce, albeit at reduced rates, during prolonged exposure to hypoxia (Condon et al. 2001, Miller & Graham

2012). Polyps may equally adapt to reduced pH conditions, since polyps of the cubozoan *Alatina nr mordens* survived and asexually reproduced, although more slowly, in pH 7.61 (Klein et al. 2014), and growth and carbon content of scyphozoan polyps *Cyanea capillata* and *Chrysaora hysoscella* were unaffected by increased CO_2 (Lesniewski et al. 2015). Taken together, these studies suggest that gelatinous zooplankton are generally tolerant to hypoxia and acidification in isolation.

Despite the inextricable link between hypoxia and acidification in coastal marine ecosystems, only 2 studies have investigated the potential interactive effects of hypoxia and acidification on jellyfish, and these studies demonstrated inconsistent responses to the dual stressors. Klein et al. (2017) compared the response of symbiotic and aposymbiotic polyps of the upside-down jellyfish *Cassiopea* sp. under reduced DO, elevated $p\text{CO}_2$, and the stressors in combination, and reported that hypoxia alone reduced asexual reproduction of polyps by $\sim 22\%$ regardless of $p\text{CO}_2$ concentration or the presence of symbionts. Ephyrae of *A. aurita* exposed to hypoxia and acidification simultaneously, however, exhibited slightly lower carbon content than those exposed to the stressors individually, suggesting a negative, albeit small, synergistic response of the dual stressors (Algueró-Muñiz et al. 2016). Although ephyrae are supposedly the most vulnerable life stage of most scyphozoans (Algueró-Muñiz et al. 2016), bottom hypoxia and subsequent acidification in stratified coastal waters may arguably affect benthic polyps more severely.

The moon jellyfish *A. aurita* is a cosmopolitan jellyfish species whose populations occur worldwide. This species can form intense blooms in bays and coastal areas (Lucas 2001). The goals of this study were to determine the individual and interactive effects of hypoxia and elevated $p\text{CO}_2$ on survival, asexual reproduction, and aerobic respiration rates of polyps of the cosmopolitan scyphozoan *A. aurita*. Specifically, we hypothesized that polyps exposed to hypoxia or elevated $p\text{CO}_2$ individually would have reduced rates of asexual reproduction and survival

and increased rates of respiration, and that the combination of the 2 stressors would generate additive or synergistic responses.

MATERIALS AND METHODS

Experimental set-up

Aurelia aurita polyps were obtained from the commercial aquarium 'Underwater World,' Sunshine Coast, Australia, and were acclimated to temperature- and light-controlled laboratory conditions ($26.32 \pm 0.008^\circ\text{C}$ and 12:12 h light:dark cycle) for 1 wk in $10 \mu\text{m}$ filtered seawater (36.21 ± 0.06 ppt) sourced from the Gold Coast Seaway, Queensland, Australia (27.56°S , 153.25°E). Five polyps were gently transferred into each of 16 individual plastic petri dishes. One additional polyp was transferred into each of 16 glass scintillation vials (40 ml volume), each fitted with a PreSens Fibox 4 sensor spot. Each petri dish and vial were randomly placed in 1 l glass aquaria ($n = 16$).

The experiment consisted of 2 orthogonal factors, each with 2 levels: oxygen concentration (ambient: $6.5\text{--}7.0 \text{ mg O}_2 \text{ l}^{-1}$, and hypoxic: $2.0\text{--}2.5 \text{ mg O}_2 \text{ l}^{-1}$), and pH (ambient: $7.95\text{--}8.05$, and reduced pH: $7.62\text{--}7.72$). $p\text{CO}_2$ and $p\text{O}_2$ were varied on a diel basis to reflect the natural variability in water chemistry that results from the cessation of photosynthesis at night (Tables 1 & 2). Ambient DO and pH values and magnitude of diel cycles were based on empirical measurements of diel variability locally at Moreton Bay, Australia (27.13°S , 153.07°E), in November 2014, during which time no *in situ* hypoxia was observed. Levels of pH and DO, and the magnitude of diel fluctuations, can also vary substantially among hypoxic ecosystems worldwide (Cai et al. 2011, Gobler et al. 2014, Gobler & Baumann 2016). We therefore chose hypoxic DO levels based on the mean value of hypoxia derived from the literature (see Vaquer-Sunyer & Duarte 2008), and pH conditions based on pH values that accompanied naturally hypoxic waters in the northern

Table 1. Target and diel experimental pH conditions ($\pm\text{SD}$). Calculated $p\text{CO}_2$ was established from total alkalinity (TA), pH, and temperature measurements. Experimental water temperature was kept stable throughout the experiment ($26.32 \pm 0.008^\circ\text{C}$)

Treatment	Local time (h)	pH target	pH measured	TA ($\mu\text{mol l}^{-1}$)	Calculated $p\text{CO}_2$ (μatm)
Control pH	10:00–14:00	8.05	8.02 ± 0.007	2444.36 ± 170.53	665.66 ± 91.15
	18:00–06:00	7.95	7.97 ± 0.021		
Reduced pH	10:00–14:00	7.72	7.69 ± 0.006	2458.45 ± 148.68	1624.34 ± 227.09
	18:00–06:00	7.62	7.63 ± 0.009		

Table 2. Target and diel experimental dissolved oxygen (DO) conditions (\pm SD). Experimental water temperature was kept stable throughout the experiment ($26.32 \pm 0.008^\circ\text{C}$)

Treatment	Local time (h)	Target DO ($\text{mg O}_2 \text{ l}^{-1}$)	Measured DO ($\text{mg O}_2 \text{ l}^{-1}$)
Control DO	10:00–14:00	7.0	7.14 ± 0.34
	18:00–06:00	6.5	6.55 ± 0.40
Low DO	10:00–14:00	2.5	2.24 ± 0.13
	18:00–06:00	2.0	2.21 ± 0.11

Gulf of Mexico (Cai et al. 2011), a region that supports seasonal proliferation of gelatinous zooplankton (Robinson & Graham 2013).

A series of gas mass controllers were used to deliver CO_2 , N_2 , and O_2 gas to seawater to achieve the desired water chemistry of the 4 treatments (see Bockmon et al. 2013). Desired gas compositions (CO_2 , N_2 , O_2) were mixed from individual gas cylinders, using 4 sets of 3 Omega[®] mass flow controllers (FMA-5400s, $0\text{--}20 \text{ ml min}^{-1} [\text{CO}_2]$, $0\text{--}5 \text{ l min}^{-1} [\text{N}_2]$, $0\text{--}2 \text{ l min}^{-1} [\text{O}_2]$). A desktop PC running NI LabVIEW[™] software (32-bit version) was used to operate and monitor functions of the mass flow controllers. The PC and mass flow controllers communicated via an Omega Expandable Modular Data Acquisition System[®] (iNET-400) that was connected with 3 Omega[®] wiring boxes with screw terminals (iNET-510). After the appropriate flow for each gas was achieved, the desired proportions of CO_2 , N_2 , and O_2 gas were subsequently mixed in a stainless steel manifold, and the gas line was then split to provide identical gas mixtures to the replicate aquaria. Flow rates of the desired gas compositions to replicate aquaria were manually adjusted using secondary stainless steel manifolds with control valves. For each treatment, 2 gas compositions (day and night) were used to closely mimic diel fluctuations in water chemistry in the field. NI LabVIEW[™] was used to linearly transition between day and night gas compositions for each of the 4 treatments. Specifically, gas compositions were held constant overnight (18:00–06:00 h local time) and during the day between 10:00 and 14:00 h local time; Tables 1 & 2).

The desired gas compositions for each treatment were continuously delivered to each aquarium using plastic air stones. Lids were placed loosely over each replicate aquarium with a header space of $\sim 10 \text{ mm}$ to minimize evaporation and subsequent changes in water chemistry. All aquaria were exposed to 12 h of light d^{-1} to accurately mimic diel patterns during summer.

Data collection

Polyps were counted and aerobic respiration was measured on Days 0, 2, 4, and then every fourth day. Measurements were made during the 'daytime' conditions in the diel cycle. Petri dishes containing polyps were removed from the aquaria, and the total number of polyps in each dish was counted using a dissecting microscope. Aerobic respiration rates were measured using a PreSens Fibox 4 Optical Oxygen Meter (sensitivity range $15\text{--}45 \text{ ppm O}_2$) and sensor spots adhered to the inside walls of the glass scintillation vials (Warkentin et al. 2007, Kragh et al. 2008). Prior to measurements, any newly produced polyps were removed so that only the original parent polyp remained. Water within each vial was replaced with $0.2 \mu\text{m}$ filtered seawater of the appropriate water chemistry. Each vial was filled to overflowing and sealed. DO measurements were taken before and after vials were incubated in the dark for 4 h, to determine aerobic respiration rate ($\text{mg O}_2 \text{ l}^{-1} \text{ h}^{-1}$). Aerobic respiration rates were then converted to carbon-specific respiration rates ($\mu\text{g C polyp}^{-1} \text{ h}^{-1}$), assuming a respiratory quotient of 1.

Maintenance and monitoring of experiment

Polyps were fed 20–30 newly hatched brine shrimp (*Artemia* sp.) nauplii after completion of measurements, and were allowed to feed for approximately 1 h. During feeding, lids were loosely placed on dishes and vials to minimize gas exchange and maintain treatment conditions. Feeding occurred during the diurnal part of the cycle when conditions were least extreme and feeding likely to be maximal to ensure that polyps were able to feed to satiation. Excess brine shrimp and other feeding debris were then gently removed using a pipette. Prior to returning polyps to their aquaria, 25% of water was replaced in each aquarium with water of the appropriate chemistry. To ensure that the desired water chemistry was maintained, twice during the experiment 100% of the water in each aquarium was replaced with ambient seawater and allowed to equilibrate to experimental conditions for 2 h before replacing polyp dishes and vials. DO, pH (NBS scale), and temperature were recorded daily using a Mettler Toledo SevenGo pro[™] SG9 optical DO meter and an SG8 pH/ion meter. Every 2–4 d, DO, pH, and temperature were monitored hourly from 06:00–20:00 h local time, to ensure the full cycle of diel changes in DO and pH (including the increase

from 'night' to 'day' conditions, and the decrease from 'day' to 'night' conditions) was achieving target conditions (see Fig. A1 in the Appendix). Every 3–5 d, a 50 ml water sample was collected for analysis of total alkalinity (TA) from each replicate aquarium. Samples of seawater were collected in clean glass amber bottles using a drawing tube and overfilled for ~20 s to minimize gas exchange between the sample water and the atmosphere. All samples were sealed tightly and stored at experimental temperature (~26°C) until analyzed, within 8 h of collection. TA samples were analyzed using an automatic 848 Titrino Plus Total Alkalinity Titrator (Metrohm®). The concentration of HCl used in titrations was verified using the APHA 2320A standard titration method (Eaton et al. 2005), and TA values were subsequently corrected using the confirmed HCl concentration. Measurements of temperature, salinity, pH, and TA were used to calculate the $p\text{CO}_2$ in the treatments using the on-line software CO2SYS (Pierrot et al. 2011), employing GEOSECS constants (NBS scale) and constants from Mehrbach et al. (1973) (Table 1). Values of $p\text{CO}_2$ and $p\text{O}_2$ were then used to calculate the respiratory index (RI = $\log_{10}[p\text{O}_2/p\text{CO}_2]$; Brewer & Peltzer 2009) for each treatment (Table 3).

Data analysis

Repeated measures linear mixed-effects models were used to analyze the effects of hypoxia and pH on asexual budding (average number of polyps) and aerobic respiration rate ($\mu\text{gC polyp}^{-1} \text{h}^{-1}$). Aerobic respiration rates were square-root transformed before running the model. DO level (control: 6.5–7.0 mg $\text{O}_2 \text{ l}^{-1}$, and low DO: 2.0–2.5 mg $\text{O}_2 \text{ l}^{-1}$), and pH level (control: 7.95–8.05, and reduced pH: 7.62–7.72; Tables 1 & 2) were modeled as fixed effects. Time was included as the repeated measure. An individual replicate identifier was included as a random effect

Table 3. Respiratory index (RI, $\pm\text{SD}$) for each treatment combination, where $\text{RI} = \log_{10}(p\text{O}_2/p\text{CO}_2)$ (Brewer & Peltzer 2009). Treatment details are given in Tables 1 & 2. DO: dissolved oxygen

Treatment	RI
Control pH + Control DO	2.43 \pm 0.07
Reduced pH + Control DO	2.02 \pm 0.10
Control pH + Low DO	1.89 \pm 0.11
Reduced pH + Low DO	1.51 \pm 0.13

to account for differences between experimental dishes or vials and the repeated measures on these experimental units. Various goodness-of-fit statistics (e.g. Akaike information criterion) were compared to determine the best-fit model for each analysis. Normality of residuals was confirmed using quantile-quantile plots (Wilk & Gnanadesikan 1968), and homoscedasticity was confirmed with standardized residual plots. When significant differences were detected, post hoc estimated marginal means were used to determine which means differed. Mixed-effects models were fit using the 'nlme' package (Pinheiro et al. 2017) in R (R Core Team 2015), and post hoc analyses were performed in SPSS (IBM).

RESULTS

Asexual reproduction

Polyps in all treatments reproduced asexually during the experiment, and no polyps encysted. Asexual reproduction rates varied through time, but were slower in hypoxic conditions (Table 4, Fig. 1). Under ambient oxygen levels, numbers of polyps increased consistently, and at the end of the experiment, twice as many polyps occurred in the ambient than hypoxic treatments (Fig. 1). Polyps exposed to hypoxia initially increased in number and, until Day 16, numbers of polyps did not differ between ambient and hypoxic treatments. After Day 16, some polyps in the hypoxia treatment died and average numbers declined, but significantly more polyps remained at the end of the experiment compared to the start (paired t -test, $p = 0.002$). Variation in pH had no effect on reproduction, either independently or in combination with hypoxia (Table 4).

Table 4. Linear mixed model results comparing asexual reproduction of *Aurelia aurita* (average number of polyps) between treatments. Treatment details are given in Tables 1 & 2. Values in **bold** are significant at $p < 0.05$; DO: dissolved oxygen

	Numerator df	Denominator df	F	p
pH	1	19.441	0.454	0.508
DO	1	19.441	17.074	0.001
Time	10	105.191	10.111	<0.001
pH \times DO	1	19.441	2.434	0.135
pH \times Time	10	105.191	1.196	0.302
DO \times Time	10	105.191	3.45	0.001
pH \times DO \times Time	10	105.191	1.263	0.261

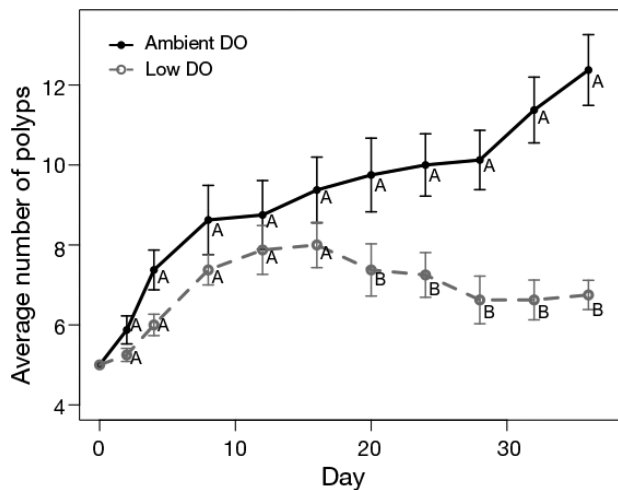


Fig. 1. Average number of *Aurelia aurita* polyps (\pm SE) in the control dissolved oxygen (DO) treatments (solid black) and low DO treatments (dashed gray). Different letters (A,B) on a given day indicate significant differences ($p < 0.05$) between groups; treatment details are given in Table 2

Aerobic respiration

Rates of aerobic respiration varied between oxygen treatments, but patterns were not consistent through time (Table 5, Fig. 2). During the first 4 d of the experiment, rates of respiration in the hypoxic treatment were more than twice those in the ambient DO treatment. Rates of respiration also initially increased in the ambient DO treatment but at a slower rate than in the hypoxic treatment. By Day 7, however, rates of respiration were similar in both treatments. After Day 12, rates of respiration decreased in both treatments, and at the end of the experiment, rates of respiration were slightly higher in the hypoxic than ambient treatments. There was no influence of pH on rates of respiration.

Table 5. Linear mixed model results comparing *Aurelia aurita* polyp respiration rate ($\mu\text{gC polyp}^{-1} \text{h}^{-1}$) between treatments. Data were square-root(x) transformed. Treatment details are given in Tables 1 & 2. Values in **bold** are significant at $p < 0.05$; DO: dissolved oxygen

	Numerator df	Denominator df	F	p
pH	1	23.286	2.418	0.133
DO	1	23.286	21.352	<0.001
Time	9	75.204	35.321	<0.001
pH \times DO	1	23.286	0.918	0.348
pH \times Time	9	75.204	0.747	0.665
DO \times Time	9	75.204	5.434	<0.001
pH \times DO \times Time	9	75.204	0.935	0.501

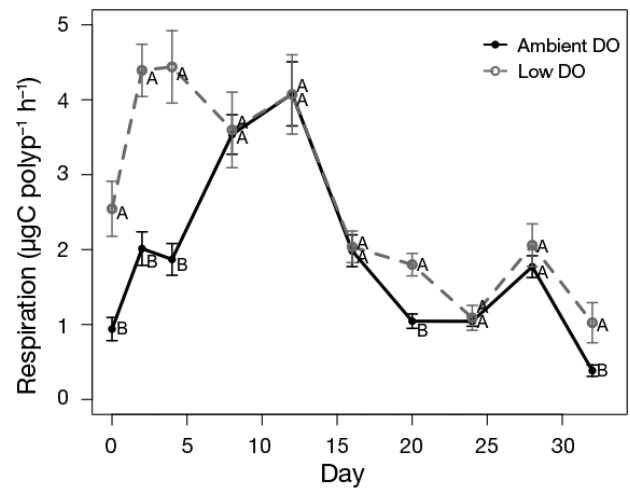


Fig. 2. Carbon-specific aerobic respiration rates ($\mu\text{gC polyp}^{-1} \text{h}^{-1} \pm$ SE) of *Aurelia aurita* polyps in the control dissolved oxygen (DO) treatments (solid black) and low DO treatments (dashed gray). Different letters (A, B) on a given day indicate significant differences ($p < 0.05$) between groups; treatment details are given in Table 2

DISCUSSION

Effects of hypoxia and elevated $p\text{CO}_2$ on polyp development and metabolism

Our results do not support the need to consider elevated $p\text{CO}_2$ (reduced pH) in conjunction with hypoxia for *Aurelia aurita* polyps. We predicted that simultaneous exposure to both hypoxia and elevated $p\text{CO}_2$ would result in decreased asexual reproduction and increased aerobic respiration rates as compared to hypoxic conditions alone. Contrary to these hypotheses, we report that the addition of elevated $p\text{CO}_2$ did not exacerbate the negative reproductive effects of hypoxia alone, and that aerobic respiration was minimally affected by the various treatments tested. Many experimental studies that investigate the impacts of hypoxia on jellyfish and other marine species manipulate O_2 concentrations with N_2 gas (but see Gobler & Baumann 2016), creating conditions that are hypoxic but less (not more) acidic (Gobler et al. 2014). Although our results suggest that elevated $p\text{CO}_2$ did not affect the response of the 2 variables we measured for *A. aurita* polyps to hypoxia, we cannot directly determine whether our observations are consistent with studies that manipulate O_2 levels using N_2 gas because we did not account for low DO and high pH in combination. We therefore advocate that subsequent studies use environmentally (and ecologically) relevant treatment

conditions that better reflect conditions likely to be experienced by organisms as conditions change

Although asexual reproductive rates were low and some mortality was observed, the average number of polyps in the hypoxic treatments was slightly greater at the end of the 36 d experiment than at the start. This indicates that populations of *A. aurita* polyps are able to survive prolonged (>1 mo) exposure to low oxygen conditions, which has been previously shown for scyphozoan polyps (Condon et al. 2001, Miller & Graham 2012). However, reproduction rates in the hypoxic treatments were about half those in the control treatments. Scyphozoan jellyfish have complex life histories of alternating sexual and asexual generations, and the growth and survival of the asexual stage (i.e. polyps) may directly determine the size of adult populations. To form blooms, scyphozoans such as *A. aurita* rely on the production of large numbers of polyps and ephyrae, as well as successful recruitment of ephyrae into the adult stage (Mills 2001). Reduced asexual reproduction in polyps experiencing hypoxia may reduce the ability of medusae to form blooms as compared to those in normoxic waters. However, reproduction under hypoxic conditions still occurred, and blooms of *A. aurita* have been observed in areas prone to hypoxia (Robinson & Graham 2013).

Asexual reproductive success is often used as the primary measure for determining the response of polyps to experimental treatments (Condon et al. 2001, Winans & Purcell 2010, Miller & Graham 2012, Klein et al. 2014, 2016, Schiariti et al. 2015), but direct observations of state variables do not capture information on short-term metabolic processes, which are required to determine mechanisms of responses to environmental conditions. We found that the metabolic response of polyps in terms of aerobic respiration did not mirror the trends observed in asexual reproduction.

According to the conceptual models by Gobler & Baumann (2016) and Carlson et al. (2007), aerobic performance will be reduced under hypoxic environments and further reduced in environments with both low oxygen and elevated $p\text{CO}_2$ (see Gobler & Baumann 2016), and cell-specific respiration will increase with increasing 'hostility' of the environment (see Carlson et al. 2007, their Fig. 3). Additionally, elevated CO_2 could inhibit aerobic respiration when the concentration of CO_2 is high proportional to the oxygen concentration, even if the $p\text{O}_2$ is considered in the normoxic range (Brewer & Peltzer 2009). While *A. aurita* polyp respiration rates varied over the first 4 d of the experiment, beginning on Day

8, all treatments exhibited similar aerobic respiration rates for the remainder of the experiment. This suggests that polyps require just a few days to acclimate to changes in oxygen conditions. If polyps are responding to environmental stress through decreased aerobic performance and increased cell-specific respiration as proposed (Carlson et al. 2007, Gobler & Baumann 2016), this response is short-lived. Additionally, the RIs defined by Brewer & Peltzer (2009) ($\text{RI} = \log_{10}[p\text{O}_2/p\text{CO}_2]$) were outside the zone of aerobic stress (0.7–1.0 RI, see Brewer & Peltzer 2009, their Fig. 1), even in the combined hypoxic + reduced pH treatment (Table 3). At the $p\text{CO}_2$ concentrations experienced in the reduced pH treatment in this study, $p\text{O}_2$ concentrations would need to be $<0.5 \text{ mg l}^{-1}$ (i.e. anoxic) or $p\text{CO}_2$ would have to greatly increase ($>5500 \mu\text{atm}$) to drive the RI below 1. At $p\text{CO}_2$ and $p\text{O}_2$ concentrations relevant to relatively shallow (i.e. not deep sea) coastal waters, respiration may not be significantly affected.

Combined hypoxia and elevated $p\text{CO}_2$ and multiple stressor studies

In this study, we observed that the net production of polyps was less in hypoxic conditions, and the combination with reduced pH did not significantly alter the overall reproductive success of *A. aurita* polyps in hypoxic conditions alone. These results are contrary to the additive and/or synergistic effects observed for other invertebrates exposed to combined hypoxia and reduced pH conditions. For example, growth rates and survival of the juvenile red abalone *Haliotis rufescens* were reduced in hypoxic conditions, but exposure to the dual stressors of hypoxia and reduced pH produced synergistic negative effects on growth (Kim et al. 2013). For a suite of benthic invertebrates in coastal Chile (2 anthozoans, 9 mollusks, 4 crustaceans, 2 echinoderms) respiration was decreased (a sign of metabolic depression) in low oxygen conditions, and the addition of reduced pH caused either additive or synergistic reduction in respiration, dependent on species (Steckbauer et al. 2015). In early life stage bivalves, hypoxia alone negatively affected the survival of juvenile hard clams *Mercenaria mercenaria* and the growth of larval bay scallops *Argopecten irradians*, yet the combination of hypoxia and reduced pH had additive negative effects on growth and survival of both species (Gobler et al. 2014).

A growing body of evidence suggests that interactions between stressors can suppress the experimen-

tal response as compared to each individual stressor (Frieder et al. 2014, Ramajo et al. 2016). For example, low oxygen in combination with semidiurnal pH fluctuation reduced the effects of acidification alone for 2 mytilid mussel species (Frieder et al. 2014). Moderate hypoxia ($\sim 3.0 \text{ mg O}_2 \text{ l}^{-1}$) had positive effects on the growth and survival of juvenile Baltic clams *Macoma balthica*, and in combination with reduced pH had increased survival and synergistic positive effects on growth (Jansson et al. 2015). By stimulating these types of positive feedback, multiple stressors can potentially invoke different responses than single stressors, and thus, global change studies that investigate the effects of singular stressors may not accurately reflect the response of biota in the natural environment (Byrne & Przeslawski 2013).

Role of the gelatinous body plan in mitigating effects of hypoxia and elevated $p\text{CO}_2$

Jellyfish possess a gelatinous body plan that is characterized by high water and low organic carbon content (Acuña 2001, Condon et al. 2011, Lucas et al. 2011, Pitt et al. 2013). In this study, we saw no effect of increased $p\text{CO}_2$, either independently or in combination with low DO. The anions present in seawater buffer against changes in pH, and a similar ionic composition is present within the mesoglea of scyphozoans (Wright & Purcell 1997). Osmotic and ionic adjustments in the mesoglea can lead to ionic regulation in these organisms (Wright & Purcell 1997), and if the mesoglea stores oxygen (Thuesen et al. 2005), hydroxide ions would be plentiful. In laboratory conditions, the internal pH of non-symbiotic polyps reflects the pH of the surrounding seawater under both reduced pH and control pH conditions (Klein et al. 2017). Due to the ion concentration and buffering capacity of seawater, we offer an alternative hypothesis in which the high inorganic salt content of polyps (Pitt et al. 2013) and the chemical speciation present in the colloidal mesoglea would provide similar buffering capabilities and lead polyps to be relatively robust to external increases in $p\text{CO}_2$.

Food quality and quantity may mitigate effects of reduced pH for some organisms (Lesniewski et al. 2015, Ramajo et al. 2016). Increased food supply also enhanced resistance to ocean acidification in calcifying organisms (Ramajo et al. 2016), and for 2 species of scyphozoan polyps (Lesniewski et al. 2015). Effects of food quality can be observed through multiple trophic levels (Boersma et al. 2008, Lesniewski et al. 2015), so shifts in nutrient composition of primary

producers may be seen in higher-order consumers. Ocean acidification may indirectly favor some organisms due to stoichiometric changes in nutritional quality of food from increased carbon (Lesniewski et al. 2015). In this experiment, the polyps were not food-restricted, potentially facilitating their relative fitness in reduced pH conditions.

The gelatinous body plan is accompanied by unique physiological characteristics, including the high turnover of organic matter and fast growth rates (Acuña 2001, Condon et al. 2011, Lucas et al. 2011, Pitt et al. 2013). Physiologically, this favors low rates of aerobic respiration, and leads to high growth efficiencies. As an oxyregulator (Rutherford & Thuesen 2005), *Aurelia* sp. is able to maintain a constant respiratory rate despite changes in available oxygen. Therefore, it is not surprising that aerobic respiration rates were relatively low and unaffected by exposure to low DO and/or pH after the initial period of acclimation. Potential storage of O_2 in the mesoglea, albeit reduced in content in polyps relative to medusae, would supplement aerobic and maintenance metabolic processes when required by the organism (Thuesen et al. 2005). While hypoxia (manipulated with N_2 bubbling) significantly affects a suite of physiological and molecular responses in *Aurelia* sp.1 medusae, the response in polyps is only a slight up-regulation of fructose-bisphosphate aldolase, suggesting an increase in glycolysis (Wang et al. 2017). It is also possible that *A. aurita* polyps can utilize anaerobic pathways in low oxygen conditions, as previously proposed (Childress & Seibel 1998, Condon et al. 2001). Anaerobic pathways are less energetically favorable, so the differences observed in asexual reproduction and aerobic respiration rates may be due to utilization of these pathways. Additionally, polyps did not encyst, indicating that cells were still viable under these conditions. Indeed, these results suggest that polyps may have a greater tolerance than medusae to hypoxia at the same levels of low oxygen (Wang et al. 2017).

While the unique characteristics of the gelatinous body plan are shared among all scyphozoans, *A. aurita* may not necessarily represent other scyphozoan species. *A. aurita* is a widely distributed jellyfish species that often occurs abundantly in bays and coastal areas (Lucas 2001). The distribution of *Aurelia* spp. probably reflects its tolerance for wide ranges of environmental factors such as temperature, salinity, light, and nutrient supply (Schiariti et al. 2015), and therefore, *Aurelia* might be more robust than other scyphozoans to various stressors such as hypoxia and reduced pH. Whilst the most likely future ocean acid-

ification models project 400–500 ppm of CO_2 by the year 2100 (Hughes et al. 2017), many coastal ecosystems already exhibit CO_2 conditions far beyond these levels on a diel basis (Hofmann et al. 2011, Wallace et al. 2014, Baumann et al. 2015). Consequently, biota may be regularly exposed to reduced DO and pH, and these conditions act may act to select for organisms that are able to rapidly acclimate or adapt to the dual stressors.

CONCLUSIONS

Eutrophication is reducing the oxygen concentration of many coastal waters through microbial respiration. Less well recognized, however, is that net respiration produces CO_2 to simultaneously reduce pH in coastal systems. Our research demonstrates that *Aurelia aurita* polyps can survive and asexually reproduce during prolonged exposure to hypoxia, although at half the rate as in normoxic conditions. Furthermore, respiration rates were fairly consistent between DO levels during prolonged exposure (>4 d), and there was no significant effect of increased $p\text{CO}_2$ on either asexual reproduction or aerobic respiration. While other studies (Kim et al. 2013, Gobler et al. 2014, DePasquale et al. 2015, Jansson et al. 2015, Steckbauer et al. 2015) highlight the need to consider elevated $p\text{CO}_2$ in conjunction with hypoxia, our results show that for *A. aurita* polyps, combining pH with hypoxic conditions has no significant effect on net asexual reproduction and metabolism. Multiple stressors have the capacity to invoke different responses than single stressors, but results may differ for some gelatinous organisms that appear more robust to environmental changes than other metazoans (Kim et al. 2013, Gobler et al. 2014, DePasquale et al. 2015, Jansson et al. 2015, Steckbauer et al. 2015).

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Appendix

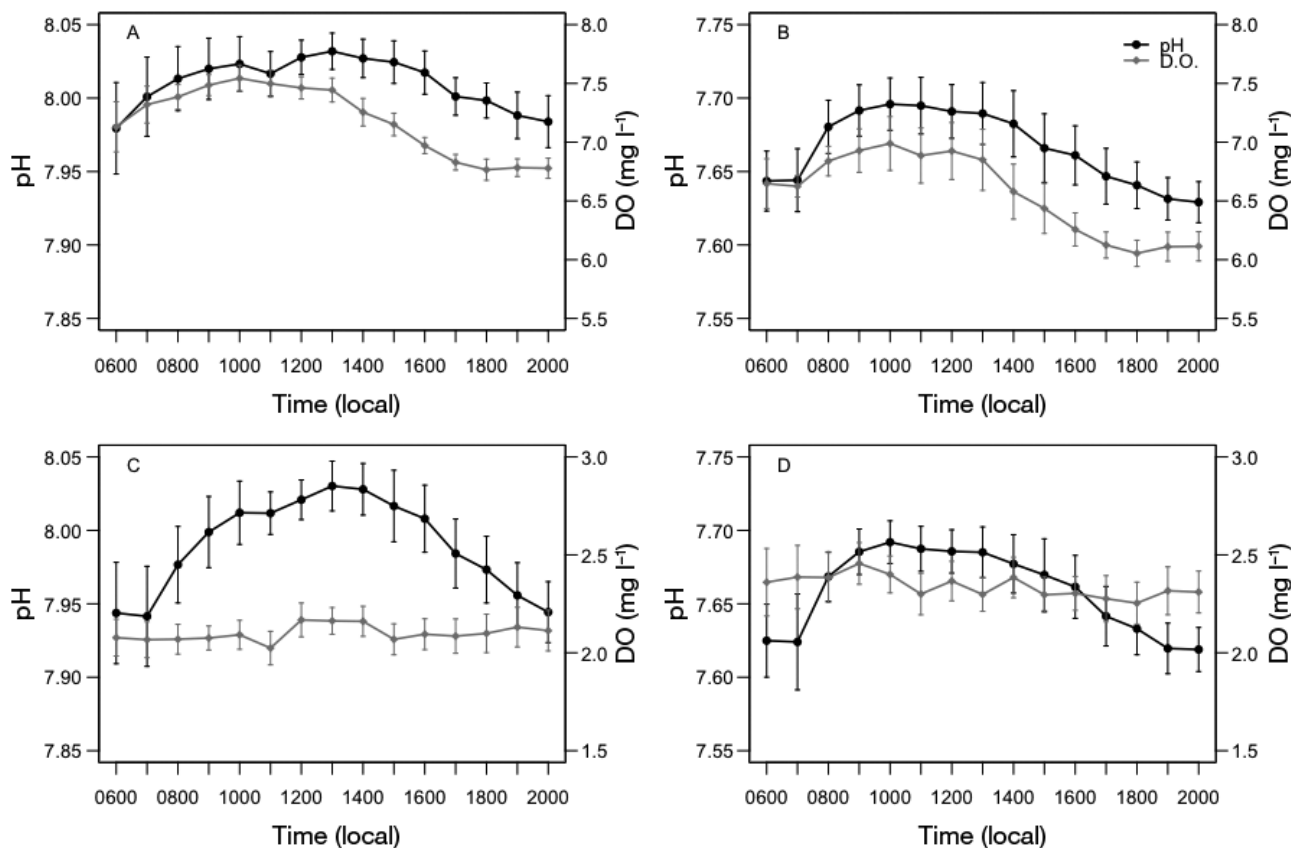


Fig. A1. Mean (\pm SE) pH and DO measurements taken at hourly intervals throughout the day from 06:00 to 20:00 h local time in (A) control pH and dissolved oxygen (DO), (B) reduced pH and control DO, (C) control pH and low DO, and (D) reduced pH and low DO treatments