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Vulnerability of early life stage Northwest Atlantic forage fish to ocean acidification and low oxygen

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ABSTRACT: Global oceans are undergoing acidification and deoxygenation, yet the concurrent effects of low oxygen and acidification on marine fish are unknown. This study quantified the separate and combined effects of low pH and low oxygen on 4 vital early life-history traits (time-to-hatch, hatching success, post-hatch survival, and growth) of 3 ecologically important estuarine fish species (*Menidia beryllina*, *Menidia menidia*, and *Cyprinodon variegatus*). Offspring were exposed from the egg through the early larval stages to ideal (pH_T [pH total scale] = 7.9, DO [dissolved oxygen] = 9.0 mg l^{-1}), hypoxic (DO = 1.6–2.5 mg l^{-1}), acidified (pH_T = 7.4), and hypoxic + acidified (pH_T = 7.4, DO = 1.6–2.5 mg l^{-1}) conditions. Hypoxia alone significantly delayed hatching of embryos by 1 to 3 d and reduced hatching success of all 3 species by 24 to 80%. Acidification alone significantly depressed the survival of *M. beryllina*. Acidification and hypoxia had an additive negative effect on survival of *M. beryllina*, a seasonal, synergistic negative effect on survival of *M. menidia*, and no effect on survival of *C. variegatus*. Acidification and hypoxia had an additive negative effect on length of larval *M. beryllina*, while hypoxia alone significantly reduced length of *M. menidia* and *C. variegatus* from 15 to 45%. Our findings suggest a greater sensitivity of early life estuarine fish to low oxygen compared to low pH conditions, while also demonstrating that the co-occurrence of both stressors can yield both additive and synergistic negative effects on survival and other fitness-related traits. The reduced fitness of forage fish when experiencing acidification and hypoxia may limit the productivity of higher trophic organisms that depend on them as prey.

KEY WORDS: Ocean acidification hypoxia · Fish · Early life history · Eutrophication

INTRODUCTION

The burning of fossil fuels since the industrial revolution has increased atmospheric and oceanic CO_2 concentrations, thus reducing average open-ocean pH over the past 200 yr by ~0.1 units (Caldeira & Wickett 2003). However, within coastal and continental shelf waters, cycles in biological productivity, upwelling, and river discharge are important and more dynamic drivers of pH (Feely et al. 2010, Duarte et al. 2013, Waldbusser & Salisbury 2014). Furthermore, eutrophication in coastal waters is known to promote algal growth, which is followed by an increase in microbial respiration and, in some cases, hypoxia (Rabalais et al. 2002, Diaz & Rosenberg 2008, Wallace et al. 2014). This microbial activity also produces CO_2 that can depress pH levels. The co-occurrence of hypoxia and acidification is known to take place seasonally (Cai et al. 2011, Wallace et al. 2014, Baumann et al. 2015); yet the combined effects of these stressors on coastal marine organisms are poorly understood (Gobler et al. 2014).

Ocean acidification and its associated impacts on ocean chemistry, such as a decrease in the concentra-

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tion of carbonate ions, can pose a significant physiological challenge, particularly for calcifying organisms. Hence, the largest body of experimental studies currently exists for calcifying invertebrates, such as corals, mollusks, and echinoderms (Ries et al. 2009, Talmage & Gobler 2009, 2010, Gazeau et al. 2013). Fewer studies have evaluated the effects of ocean acidification on marine teleosts, with outcomes ranging from no discernible effects to directly reduced survival at elevated CO_2 levels. *Theragra chalcogramma* (walleye pollock) proved resistant to elevated CO_2, with little to no effect on hatch size or larval and juvenile growth (Hurst et al. 2012, 2013). In multiple tropical reef fish, exposure to elevated pCO_2 levels impairs olfaction and thus leads to detrimental settlement and predator avoidance behavior (Munday et al. 2009, 2010, Dixson et al. 2010). *Gadus morhua* (Atlantic cod) from the Norwegian coast experienced temporary tissue damage when exposed from the egg stage to acidified water, while the same species from the Baltic Sea showed no effects (Frommel et al. 2012, 2013). Direct survival reductions due to elevated CO_2 exposure have thus far been observed during the early life stages of inland silversides (*Menidia beryllina*; Baumann et al. 2012), cinnamon anemonefish (*Amphiprion melanopus*; Miller et al. 2012), summer flounder (*Paralichthys dentatus*; Chambers et al. 2014), and Atlantic silversides (*M. menidia*; Murray et al. 2014). In *M. beryllina*, acidification had the greatest negative effect on larval growth and survival when exposure was initiated in the egg stage rather than the larval stage (Baumann et al. 2012). Hence, the current empirical evidence regarding CO_2 effects on fish allows few generalizations, while pointing at highly species- and/or population-specific sensitivities to ocean acidification, which may need to be assessed on a case-by-case basis.

The effects of low oxygen on marine organisms have been studied extensively. Much focus has been on climate-change-induced expansion of oxygen minimum zones resulting from subsurface microbial activity in the open ocean (Keeling et al. 2010), as well as hypoxic 'dead zones' that result from excessive coastal nutrient loading and are particularly detrimental to benthic organisms (Rabalais et al. 2002, Diaz & Rosenberg 2008, Vaquer-Sunyer & Duarte 2008). Ecosystem composition is greatly impacted by dead zones, with many organisms being replaced or migrating out of their preferred habitat (Rabalais et al. 2002). Reduced oxygen can be lethal to fish at concentrations even above the generally accepted hypoxia threshold of 2 mg l^{-1} and can elicit a range of sublethal responses, including decreased activity, swimming speed, and growth rate (Breitburg, 2002, Vaquer-Sunyer & Duarte 2008, Ekau et al. 2010).

The egg stage of several fishes has proven to be highly susceptible to hypoxia, with numerous deleterious effects after hatching, including reduced length and impaired swimming abilities (Ekau et al. 2010). For example, exposure to low dissolved oxygen (DO) during somitogenesis of *Pagrus major* (red sea bream) and *Seriola dumerili* (amberjack) resulted in centrum defects that, in turn, led to morphological defects post-hatch (Hattori et al. 2004, Sawada et al. 2006). Larval *M. beryllina* experience up to 90% mortality when exposed to low DO during the egg stage (Miller et al. 2002). While the effects of hypoxia on early life stage fish have been well studied, the effects of hypoxia coupled with other estuarine stressors have not been extensively studied.

Estuaries are susceptible to hypoxia and acidification due to eutrophication (Feely et al. 2010, Cai et al. 2011, Wallace et al. 2014). Considering the absence of research on the combined effects of acidification and hypoxia on marine life (Gobler et al. 2014), it is important to study the combined effects of these co-occurring stressors on the estuarine organisms most likely to encounter them. Interactions between environmental stressors are increasingly studied, because biological outcomes are often not predictable from the effects of the individual stressors (Pörtner et al. 2005, Melzner et al. 2011, Pansch et al. 2014). While 2 parameters can simply have additive effects (i.e. no interaction; Fig. 1B), wherein organismal responses are proportional to the outcomes of each individual parameter, synergistic (Fig. 1C) and antagonistic interactions among parameters can produce more or less intense outcomes, respectively, than would be predicted by the effects of each individual stressor. For example, tolerance of hypoxia decreases for organisms outside their optimal temperature range while acidification can result in a narrowing of thermal tolerance (Pörtner 2008, 2010).

Here we quantified the effects of individual and co-occurring acidification and hypoxia during the early life stages of 3 forage fish species: inland silverside *Menidia beryllina*, Atlantic silverside *Menidia menidia*, and sheepshead minnow *Cyprinodon variegatus*. As abundant members of nearshore habitats along most of the US Atlantic seaboard (Able & Fahay 1998), these species are of particular ecological importance due to their intermediary trophic position between zooplankton and larger piscivores (Present & Conover 1992, Conover et al. 2005, Pikitch

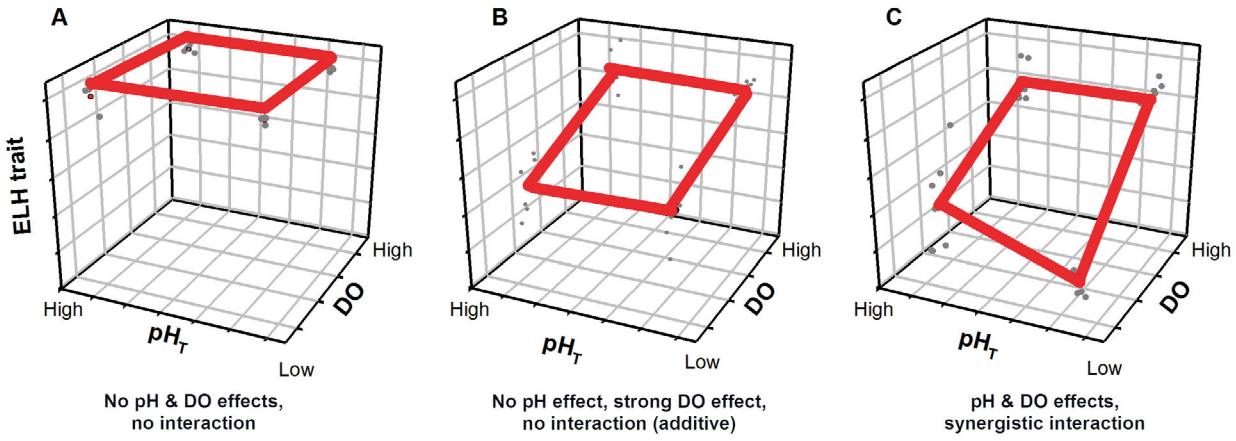

Fig. 1. Schematic response shapes for 3 potential scenarios of total pH (pH_T), dissolved oxygen (DO), and pH & DO effects on an early life-history trait (ELH traits; e.g. survival). (A) No effects and no interaction, (B) no pH effect, strong DO effect but no interaction, and (C) synergistic pH and DO effects. Grey dots are example data for replicate means; red lines connect overall treatment means

et al. 2014). Newly fertilized embryos were reared in quadruplicates under control, hypoxic, acidified, and hypoxic + acidified conditions until the end of the most vulnerable early larval stage. Our goal was to assess how time-to-hatch, hatching success, post-hatch survival, and growth in response to acidification and hypoxia would vary among species.

METHODS

Seawater chemistry

Hypoxia and acidification were examined as 2 treatments in a 2 × 2 experimental design. We established 4 sets of replicate (n = 4), 8 l, opaque polyethylene experimental vessels with lids: control, hypoxic, acidified, and hypoxic + acidified. Target pH_T (pH, total scale) / pCO_2 levels were 7.9 / ~450 µatm (control) and 7.4 / ~2000 µatm (acidified), and target DO levels were ~8.5 mg l^{-1} (control) and ~2.5 mg l^{-1} (hypoxic; Fig. 2). These acidified and hypoxic conditions mimic the range of DO and pH observed in eutrophic estuaries during the spring and summer spawning seasons (Wallace et al. 2014, Baumann et al. 2015). The 16 experimental vessels were filled with 0.45-µm-filtered seawater from Old Fort Pond in Southampton, NY, USA, which generally maintains a salinity of 30, a recommended salinity for all 3 species (Middaugh et al. 2009). Vessels were placed in water-filled baths that kept temperatures between ~22 and 24°C (Middaugh et al. 2009). Gas-proportioning systems (Cole-Parmer®) were used to control the rate of gas flow into each vessel, depending on the treatment. The control vessels were bubbled with

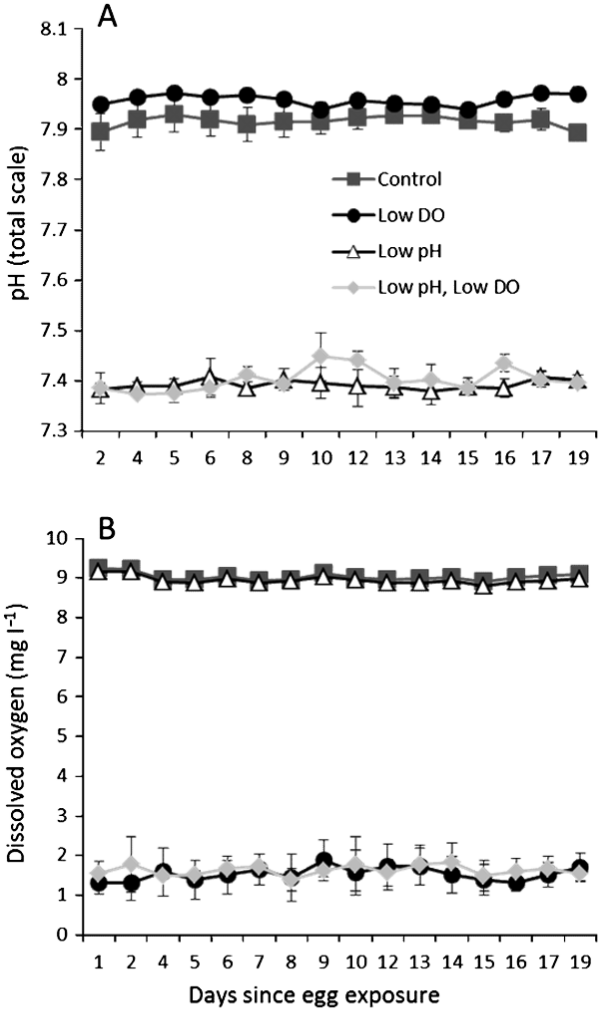

Fig. 2. Time series of daily mean (A) pH and (B) DO values from the second *Menidia beryllina* experiment. Points represent means ± standard deviation (n = 4)

compressed air; the hypoxic vessels were bubbled with air and tanked N_2 gas pre-mixed with 400 µatm pCO_2; the acidified vessels were bubbled with air blended with tanked CO_2; and the acidified + hypoxic vessels were bubbled with air, tanked N_2 (without pre-mixed 400 µatm pCO_2), and tanked 5% CO_2.

Daily measurements of pH (e.g. Fig. 2A) were made with a Honeywell© Durafet Ion Sensitive Field Effect Transistor pH sensor calibrated with Tris buffer in synthetic seawater on the total pH scale (Dickson et al. 2007). Consistent with our prior studies (Wallace et al. 2014), pH measurements made with this device were not significantly different from those made spectrophotometrically using m-cresol purple (Dickson et al. 2007). Daily DO measurements (e.g. Fig. 2B) were made with a Hach© optical dissolved oxygen probe calibrated in water-saturated air. DO measurements made with this sensor have been found to be indistinguishable from those made via Winkler titrations (Grasshoff et al. 1983, Gobler et al. 2014).

To assess carbonate chemistry in the experimental vessels, seawater was bubbled with gases as described above for at least 24 h before being analyzed at the beginning and end of each experiment using a Liqui-Cel® Membrane (Membrana) to separate the gas phase from seawater and an EGM-4 Environmental Gas Analyzer® (PP Systems) to quantify total dissolved inorganic carbon (DIC). Measured levels of DIC, pH_T (i.e. pH measured on the total scale), temperature, salinity, and first and second dissociation constants of carbonic acid in seawater according to Roy et al. (1993) were used in the program CO2SYS (http://cdiac.ornl.gov/ftp/co2sys/) to calculate pCO_2, total alkalinity, $\Omega_{calcite}$, $\Omega_{aragonite}$, and concentration of CO_3^{2-}. As a quality assurance measure, certified reference material for oceanic pCO_2 measurements from the Marine Physical Laboratory at Scripps Institution of Oceanography (Batches 117, 123, and 132) was analyzed at the start and end of each analytical DIC run (mean percent recovery: 102 ± 4%). Details of carbonate chemistry during experiments can be found in Tables S1–S5 in the Supplement at www.int-res.com/articles/suppl/m523p145_supp.pdf.

Rearing of fish embryos and larvae

Menidia beryllina embryos (<24 h old) were obtained from a large commercial broodstock (Aquatic Research Organisms, Hampton, NH). Eggs attached to clusters of yarn were quantified so that strands containing a total of 80 eggs were suspended in each replicate vessel. After hatch, *M. beryllina* larvae were fed *Brachionus plicatilis* (rotifer) ad libitum for the first 5 d and then newly hatched *Artemia* nauplii ad libitum for the remainder of the experiment (Middaugh et al. 1987). *M. menidia* offspring were obtained by strip-spawning wild ripe adults (20+ individuals per sex) caught by beach seine on the north shore of Long Island, NY (Poquot, 40° 58.12′ N, 73° 5.28′ W). Since larval *M. menidia* have been shown to be susceptible to acidification early in their spawning season, but resistant later in the season (Murray et al. 2014), experiments were performed in the months bracketing these scenarios — May and June. Females were strip-spawned into plastic dishes containing clean seawater, sperm, and cut-out sections of window screen (1 mm mesh size). Within 15 min, fertilized eggs attached to the window screen via chorionic filaments, while unfertilized eggs sank to the bottom of the dish. Successfully fertilized eggs were then readily quantified, and pieces of window screen containing a total of 80 eggs were suspended in each replicate experimental vessel within 4 h of fertilization. Hatched *M. menidia* larvae were immediately provided with ad libitum rations of newly hatched *Artemia* nauplii; in addition, larval powder food (Otohime Marine Weaning Diet, Size A, Reed Mariculture®) was given during the first 3 d. *C. variegatus* embryos (<24 h old) were obtained from Aquatic BioSystems in Fort Collins, Colorado; and 80 loose eggs were randomly distributed into each replicate vessel. Post-hatch, *C. variegatus* were fed ad libitum rations of newly hatched *Artemia* nauplii (Middaugh et al. 2009).

There were 2 experiments each with *M. beryllina* and *M. menidia*, and 1 experiment with *C. variegatus*. DO and pH_T were near the target values (see above) in each experiment except for one with *M. beryllina*, which had a more intense hypoxic treatment ~1.6 mg l^{-1}, a level commonly found in more eutrophic estuaries during summer (Wallace et al. 2014, Baumann et al. 2015). For each experiment, hatched live larvae were counted daily in triplicates by placing a plastic divider into the experimental vessels to separate the fish into smaller groups. Half of the water in each experimental vessel was replaced with new filtered seawater twice weekly, and detritus was siphoned off the bottom of each vessel daily. Experiments generally lasted for 10 d after the majority of fish had hatched in each treatment. At the end of the experiment, remaining fish were preserved in 10% buffered formalin (final concentration: 3%), and standard lengths were later measured with calibrated digital images and image analysis software (ImageJ 1.45s). Mean time-to-hatch (days) was

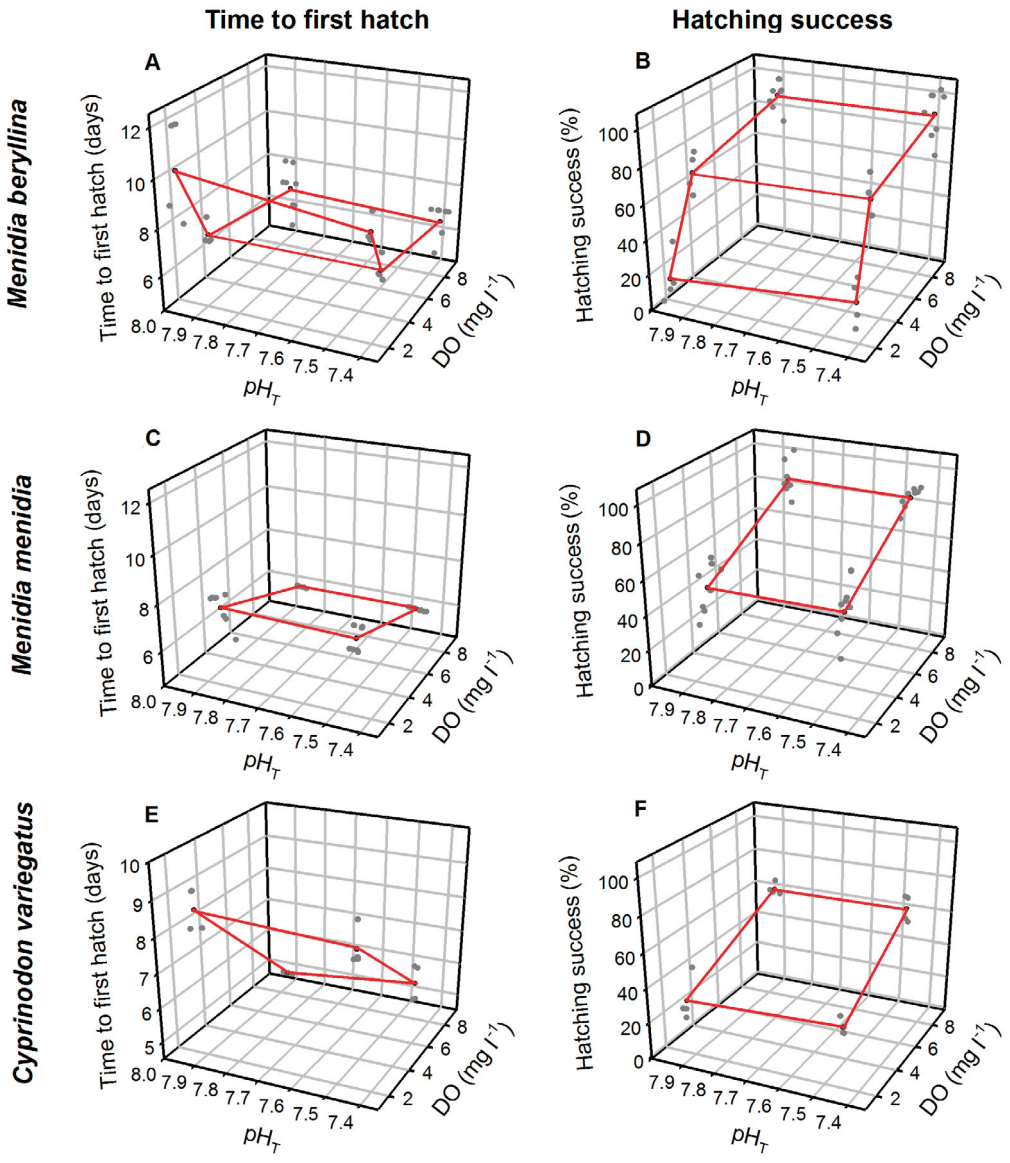

Fig. 3. Combined effects of pH and DO on time to first hatch and hatching success of (A,B) *Menidia beryllina*, (C,D) *M. menidia*, and (E,F) *Cyprinodon variegatus*. Grey dots depict individual replicates; red lines connect treatment means. Panels A to D contain data from 2 experiments each. In A & B, normoxic treatments were pooled, but given the different responses to low and very low oxygen conditions, the 2 hypoxic treatments were left separate

calculated from the start of the experiment until the first eggs hatched in each experimental vessel and then averaged across treatments. This time generally varied by no more than 2 d within a treatment. Percent hatching was calculated by dividing the maximum number of larvae in each vessel by the initial total number of eggs (80), averaged across treatments. Percent post-hatch survival was calculated by dividing the number of survivors at the end of an experiment by the maximum number of larvae observed in each vessel, averaged across the replicates in a treatment. For statistical analyses (SigmaPlot 11.0©), percent survival and hatching success were arcsine square root transformed. These transformed percentages were analyzed, along with time-to-hatch and standard length, by 2-way ANOVAs to assess the effects of pH, DO, and their interaction on each parameter. For *M. menidia*, control and treatment conditions did not differ significantly between experiments (Tables S3 & S4), and hence all 8 replicates between experiments were pooled to describe the overall response shapes of the 4 early life-history traits for this species (Figs. 3 & 4). Similarly, replicates were pooled across the 2 *M. beryllina* experiments for the control and low pH treatments, but not for the 2 different low DO levels (1.6 and 2.7 mg l^{-1}).

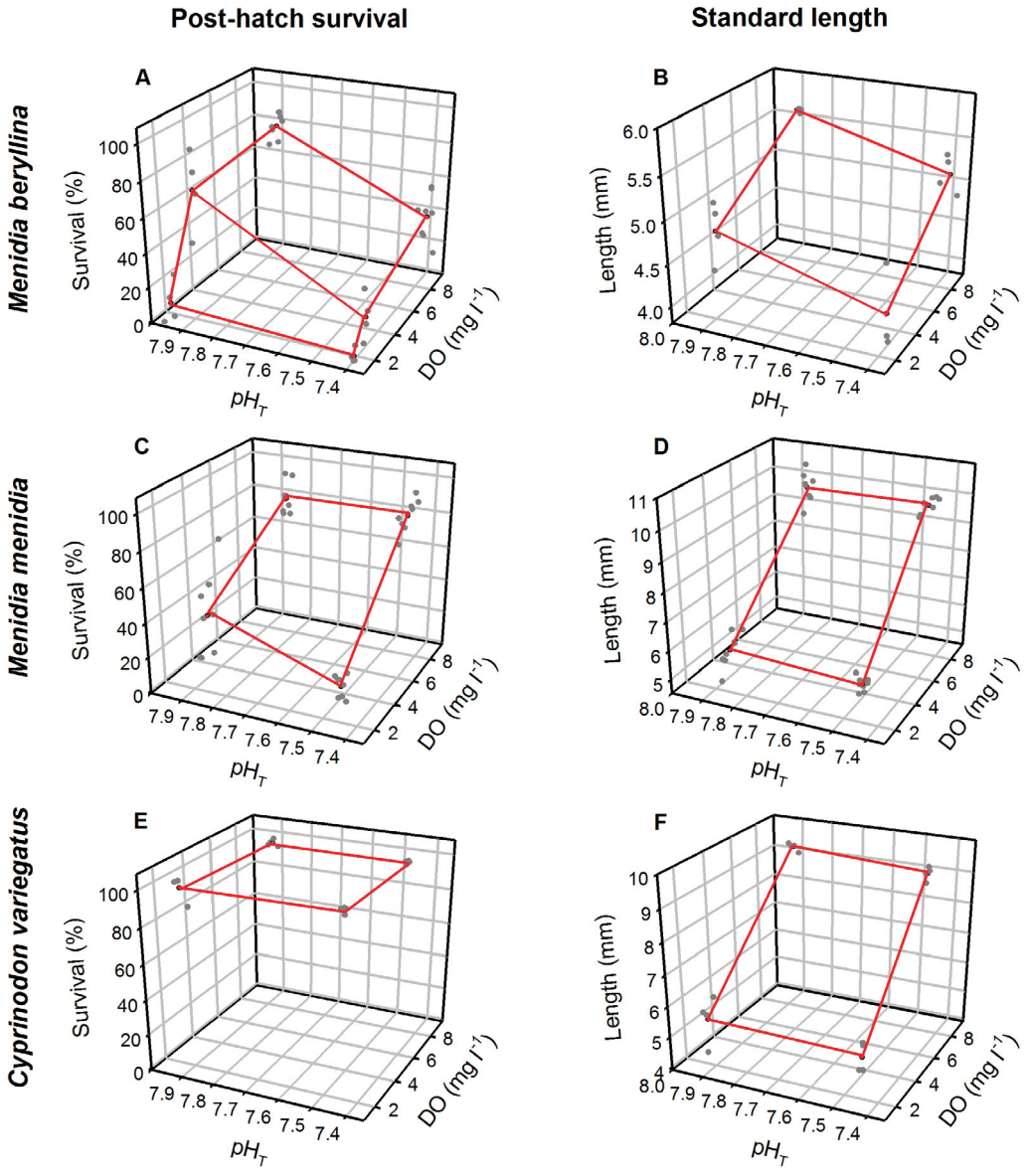

Fig. 4. Combined effects of pH and DO on post-hatch survival (hatch to ~10 days post-hatch [dph]) and standard length (~10 dph) of (A,B) *Menidia beryllina*, (C,D) *M. menidia*, and (E,F) *Cyprinodon variegatus*. Grey dots depict individual replicates; red lines connect treatment means. Panels A to D contain data from 2 experiments each. In A, normoxic treatments were pooled, but given the different responses to low and very low oxygen conditions, the 2 hypoxic treatments were left separate

RESULTS

Menidia beryllina

Hypoxia significantly delayed hatching in *M. beryllina*, as eggs exposed to low DO (1.6 mg l^{-1}) hatched after ~10 d, whereas eggs exposed to normal oxygen levels hatched in 7 d ($p < 0.001$; Table 1, Fig. 3A). In contrast, exposure to slightly higher DO levels (~2.7 mg l^{-1}) did not delay hatching (Fig. 3A). Low DO levels also affected hatching success, with embryos exposed to 1.6 and 2.7 mg DO l^{-1} having significantly lower hatching success compared to controls ($p < 0.001$; Table 1, Fig. 3B). However, while hatching success at ~2.7 mg DO l^{-1} was 71 ± 11% (mean ± SD) compared to 94 ± 4% in the control treatment, DO levels of ~1.6 mg l^{-1} resulted in only 16% hatching success compared to 82% in the control treatment (Fig. 3B). Exposure to acidification alone (~2000 µatm CO_2, $pH_T = 7.4$) did not affect the timing or the success of hatching, and there was no interaction between the stressors (Table 1, Fig. 3A,B). In contrast, post-hatch survival was significantly reduced by low DO and low pH levels, both separately and combined (Table 1,

Table 1. *Menidia beryllina*, *M. menidia*, *Cyprinodon variegatus*. Average change (%) relative to controls of 4 early life-history traits and 3 estuarine forage fish species when reared at hypoxic (H; 1.6–2.7 mg l^{-1}), acidified (A; pH_T = 7.4), and combined hypoxic/acidified conditions (H & A). Significant interactions ($p < 0.05$) between hypoxia and acidification were only detected for post-hatch survival in *M. menidia* (2-way ANOVA, in **bold** print)

	Menidia beryllina	*Menidia menidia*	*Cyprinodon variegatus*
Time-to-hatch			
H	+30	+25	+70
A	−4	0	+1
H & A	+22	+25	+65
Hatching success			
H	−50	−41	−57
A	+2	−1	+3
H & A	−43	−37	−51
Post-hatch survival			
H	−50	−51	−1
A	−52	+1	0
H & A	−88	**−79**	+2
Length			
H	−15	−41	−44
A	−9	0	−2
H & A	−25	−42	−45

Fig. 4A). Exposure to ~1.6 mg l^{-1} of DO significantly reduced survival to 9 ± 12% compared to 75 ± 6% in the control treatment ($p < 0.001$; Fig. 4A). At ~2.7 mg l^{-1} of DO, post-hatch survival was not significantly different from that in the control ($p = 0.16$; Fig. 4A). Exposure to low pH alone resulted in 27 to 49% compared 75 to 81% post-hatch survival in the control ($p < 0.001$; Table 1, Fig. 4A), and there was no statistically significant interaction between both stressors ($p = 0.08$; Table 1, Fig. 4A). Larval length at 9 d post-hatch was significantly reduced by low DO and low pH, with no significant interaction (Table 1, Fig. 4B), as offspring exposed to low pH, low DO (2.7 mg l^{-1}), and both stressors measured 5.1 ± 0.2, 4.7 ± 0.4, and 4.2 ± 0.5 mm, respectively, whereas control fish were 5.6 ± 0.03 mm. DO levels of 1.6 mg l^{-1} resulted in too few survivors for conclusive length analyses.

Menidia menidia

M. menidia embryos were sensitive to hypoxia, experiencing a significant delay in hatching and a reduction in hatching success when exposed to ~2.5 mg l^{-1} of DO versus ~8.5 mg l^{-1} in the control treatment (Table 1, Fig. 3C,D). In both experiments, low DO delayed hatching from 6 d (control) to ~7.5 d, while low pH (7.46 versus control pH = 7.85) had no effect on the time of hatch, and there was no interaction between the stressors (Table 1, Fig. 3C). In addition, low DO significantly reduced hatching success from 86 ± 10% in the control to 51 ± 13%. Low pH had no effect on hatching success, and there was no interaction between DO and pH (Table 1, Fig. 3D). In contrast, pH and DO significantly affected post-hatch survival in a synergistic negative way (Table 1, Fig. 4C). While low DO conditions alone reduced survival by 51% (control: 79.6%; low DO: 39.2%) and low pH conditions alone did not (80.3%), the 2 stressors together lowered survival by almost 80% (low DO/low pH: 16.4%). This finding, in particular, emphasizes the importance of multi-stressor experiments for identifying potential synergistic effects. Larval length, on the other hand, was negatively affected only by low DO levels, but was robust to low pH conditions, and there was no interaction between both stressors (Table 1, Fig. 4D).

Offspring strip-spawned from wild adults collected in May and June exhibited some differences in sensitivities to low DO, low pH, and the 2 stressors combined (Fig. 5). While time-to-hatch and larval length did not show month-to-month differences (Fig. 5A,D), hatching success was considerably higher in June compared to May across all treatments (Fig. 5B). Post-hatch survival differed between May and June, particularly in the low DO treatment (Fig. 5C). Low DO in May reduced survival from 74.7% (control) to 56.7%, whereas in June the reduction in survival due to low DO alone was much greater (control: 84.5%; low DO: 21.7%). Since survival with the combined stressor treatment was equally low in May (17.5%) and June (15.4%), the significant negative interaction between both stressors (described above; Table 1, Fig. 4C) was mainly driven by the survival pattern during the May experiment.

Cyprinodon variegatus

Low DO (~2.4 mg l^{-1}) significantly delayed hatching of *C. variegatus* from ~5 to ~8.5 d, while acidification had no effect, and there was no interaction between both stressors (Table 1, Fig. 3E). Low DO also reduced hatching success from 62 ± 4% in the control to 27 ± 13% (Table 1, Fig. 3F). Hatching success at low pH (64 ± 8%) was similar to that of the control, while low pH and low DO combined resulted in 31 ± 5% hatching; hence, there was no interaction between DO and pH (Table 1, Fig. 3F). Interestingly,

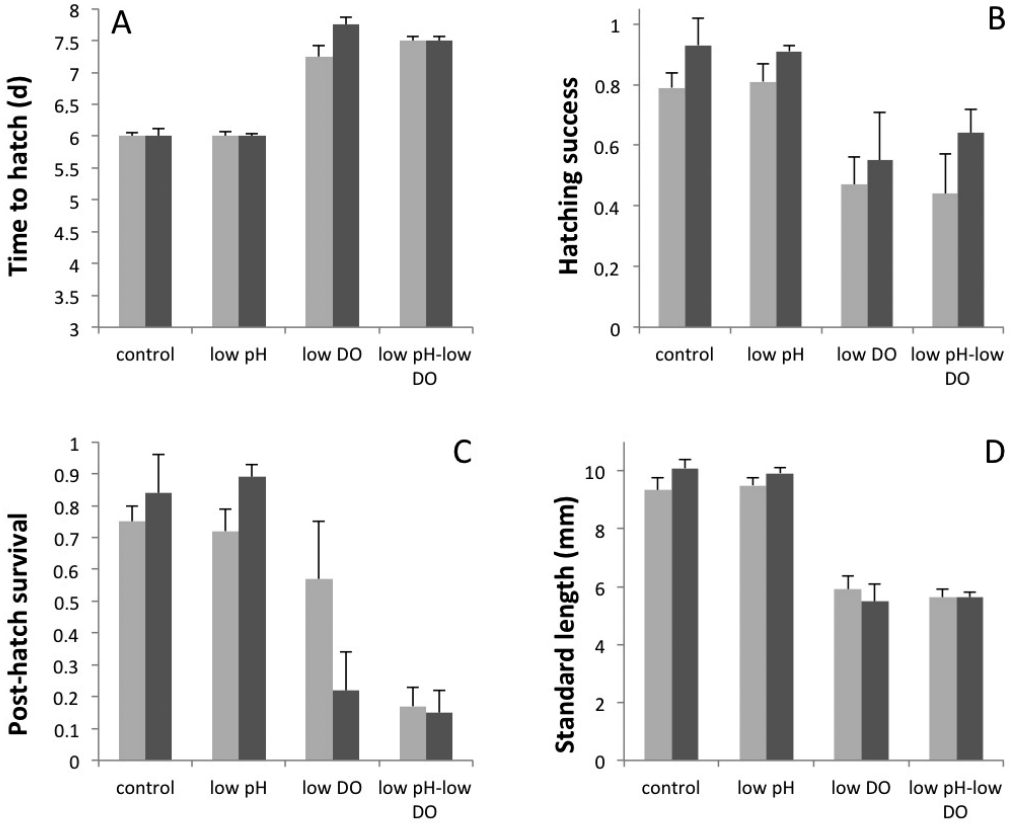

Fig. 5. Effects of low pH, low DO, and both stressors combined on (A) time-to-hatch, (B) hatching success, (C) post-hatch survival, and (D) standard length of *Menidia menidia* offspring from wild adults caught in May (light grey bars) and June 2013 (dark grey bars). Bars represent means ± 1 SD

post-hatch survival (10 d post-hatch) was robust to low DO, low pH, and the 2 stressors combined, as all treatments had survival exceeding 95% (Table 1, Fig. 4E). Standard length, however, was significantly reduced in the low DO, but not in the low pH treatment, and there was no interaction between the stressors (Table 1, Fig. 4F). Fish from the control and low pH treatments averaged ~9 mm, while individuals from the low DO and low DO + low pH treatments averaged ~5 mm.

DISCUSSION

We used a multi-stressor, experimental approach to assess early life sensitivity to co-occurring acidification and hypoxia in 3 species of estuarine fish. We found that hypoxia consistently delayed hatching and reduced hatching success and larval length across species, whereas patterns of post-hatch survival differed among species. Tolerance to acidification was generally higher in all species, but increased considerably from *Menidia beryllina* (sensitive) to *M. menidia* (less sensitive) and *Cyprinodon variegatus* (tolerant). For co-occurring stressors, both additive and synergistic negative effects were observed, thus illustrating the need for such multi-stressor approaches.

To examine effects of hypoxia on marine organisms, experiments have traditionally evacuated the oxygen by bubbling N_2 gas into rearing vessels (e.g. Breitburg et al. 1997, Jordan & Steffensen 2007, Clark et al. 2013). Since this approach also removes CO_2 and thus increases pH, it creates a laboratory condition (low DO, high pH; Gobler et al. 2014) that does not exist in nature where hypoxic zones are acidified (Wallace et al. 2014). To avoid such unrealistic basification in rearing vessels, this study used N_2 gas mixed with CO_2 and air to maintain pH at ambient or low DO levels, allowing for isolation of the effects of low oxygen from the combined effects of low oxygen and low pH (Gobler et al. 2014). The additively negative effect of hypoxia and acidification on survival of *M. beryllina* and the synergistically negative effect of the 2 stressors on survival of *M. menidia* demonstrate the importance of assessing

hypoxia effects under the pH conditions that are likely to occur in nature. Hypoxia, for example, could lead to a reduction in aerobic performance and thereby impair an organism's ability to cope with the additional stress of acidification (Pörtner et al. 2005, Pörtner & Knust 2007, Pörtner 2008). As such, the true effects of hypoxia on fish might be more severe than those reported in prior studies using N_2 gas and not controlling pH levels.

Hypoxic conditions have become more prevalent in marine ecosystems in recent decades (Diaz & Rosenberg 2008), and the effects of hypoxia on marine fish populations can be profound (Breitburg 2002, Vaquer-Sunyer & Duarte 2008, Ekau et al. 2010). Consistent with these findings, hypoxia depressed the post-hatch survival of both *Menidia* spp. during this study. Hypoxia also delayed hatching and reduced hatching success up to 80% in all 3 species, relative to control treatments (*M. beryllina*). Short-term exposure to hypoxia near the end of the egg stage can induce hatching in fish, which is likely due to the activation of hatching enzymes in near-mature embryos (Oppen-Berntsen et al. 1990, Czerkies et al. 2001, Ciuhandu et al. 2005). In contrast, prolonged exposure to hypoxia during the egg stage typically reduces viability and delays development, resulting in a smaller, less developed individual at a common age (Ciuhandu et al. 2005, Ekau et al. 2010), with the latter being generally linked to a larger predation risk for surviving individuals (Sogard 1997). The levels of oxygen used during our experiments were within the range typically observed in a temperate coastal ecosystem during warmer months (Rabalais et al. 2002, Diaz & Rosenberg 2008, Wallace et al. 2014), suggesting the effects observed during this study may commonly occur within an ecosystem setting.

Hypoxia reduced larval size in all 3 study species, an outcome that could have a negative impact on recruitment success, with a higher risk of predation mortality (Houde 1997). Hypoxia is known to cause prolonged states of elevated metabolism after feeding, and this energetic cost may have contributed to the smaller size of fish in those treatments (Jordan & Steffensen 2007). Additionally, hypoxia can result in elevated ventilation rates, cardiovascular stress, and reduced mobility, hence, placing greater metabolic demands on fish while decreasing the time spent seeking food (Hughes & Saunders 1970, Randall 1982, Jordan & Steffensen 2007, Pörtner 2010). Given that growth and mortality rates are coupled in fish early life stages (Houde 1997), any reductions in growth resulting from hypoxia may negatively impact recruitment success. While adult fish may tolerate or evade hypoxic zones, their eggs and larvae are more stationary and susceptible to the hypoxic conditions that tend to occur in coastal habitats during spring and summer months, when many fish species spawn (Feely et al. 2010, Wallace et al. 2014, Baumann et al. 2015). The physiological and behavioral impacts of hypoxia, especially when combined with acidification, reduce the competitiveness of affected organisms in the wild, where survival under climate change will depend on physiological performance and adaptability (Pörtner & Farrell 2008).

Since hypoxia delayed hatching for all 3 species, the resulting shorter larval period may have contributed to the large difference in size between low and ambient oxygen treatments. However, while *M. beryllina* from the hypoxic treatment ~10 d post-hatch were significantly larger than day-of-hatch larvae from supplementary control vessels (4.7 ± 0.4 mm compared to 3.4 ± 0.2 mm), and *C. variegatus* from the hypoxic treatment were significantly larger than day-of-hatch lengths from the literature (5.2 ± 0.8 mm compared to 4 mm; Kuntz 1916), *M. menidia* from the hypoxic treatment were not significantly larger than day-of-hatch lengths from the literature (5.9 ± 0.5 and 5.5 ± 0.6 mm compared to 5.4 ± 0.4 mm; Murray et al. 2014). It is possible, therefore, that *M. menidia* in the hypoxic treatment did not grow when exposed to low oxygen conditions.

Similar levels of hypoxia (2.4–2.7 mg l^{-1}) from the egg stage through ~10 d post-hatch had different effects on each fish. Only *M. menidia* was negatively impacted by this level of DO in all 4 traits quantified here (time-to-hatch, percent hatch, percent survival of larvae, size of larvae), a finding consistent with prior research showing that of these 3 species, *M. menidia* is the least tolerant of hypoxia (Able & Fahay 1998, Smith & Able 2003). While ~2.7 mg DO l^{-1} only reduced hatching success in *M. beryllina*, a lower DO of ~1.6 mg l^{-1} reduced hatching success in addition to delaying hatching and reducing survival of this species. Such a distinction in results based on a shift in DO of 1 mg l^{-1} hints at a threshold response consistent with the observation of severe hypoxia effects in coastal ecosystems after DO levels decrease below 2 mg l^{-1} (Rabalais et al. 2002, Diaz & Rosenberg 2008, Vaquer-Sunyer & Duarte 2008).

The existing empirical evidence on the sensitivity of fish offspring to ocean acidification suggests highly species-specific or even population-specific responses that may, in part, stem from environmental conditions already experienced in the wild (Munday et al. 2009, 2010, Baumann et al. 2012, Chambers et

al. 2014, Murray et al. 2014). The present study confirmed the highly diverse nature of responses to acidification between allopatric species, as *C. variegatus* was resistant to acidification, while *M. beryllina* and *M. menidia* were not. Like both *Menidia* species, *C. variegatus* spawns in estuaries. However, this fish is generally known to be tolerant of a wide range of seawater conditions (Able & Fahay 1998). Furthermore, *C. variegatus* is known to lay demersal eggs in shallow pools or marsh areas (Able & Fahay 1998), which generally have lower levels of pH and DO compared to open waters (Wallace et al. 2014, Baumann et al. 2015) where both *Menidia* species lay their eggs (Conover & Ross 1982, Able & Fahay 1998). These differences in life-history traits may contribute to a higher tolerance for acidification and hypoxia in the early life of *C. variegatus* (Ekau et al. 2010). Further research is needed to resolve the mechanisms potentially facilitating the susceptibility and resistance to acidification in fish.

M. menidia offspring collected in May versus June responded differently to acidification (hatching success and post-hatch survival). These results may reflect rapidly declining pH values in spring, which have been shown to promote offspring resistant to acidification later in the spawning season (Murray et al. 2014). Other recent studies have similarly highlighted the potential for parental environmental histories to influence offspring performance via transgenerational plasticity (Miller et al. 2012, Salinas et al. 2013). Given that *M. beryllina* and *C. variegatus* were obtained from a hatchery broodstock, further experiments should be conducted with eggs from wild-caught adults to assess potential effects of domestication (Bobe & Labbé 2010).

Hypoxia has long received attention as an environmental threat to marine animals, including fish populations (Breitburg 2002, Vaquer-Sunyer & Duarte 2008, Ekau et al. 2010). Given that these hypoxic zones are also acidified (Feely et al. 2010, Cai et al. 2011, Wallace et al. 2014) and that most prior experimental studies of hypoxia applied basification of pH rather than acidification (Gobler et al. 2014), this represents the first assessment of how environmentally realistic hypoxia (e.g. combined with acidification) affects fish. The response of fish to these stressors contrasts with those of bivalve larvae that are more sensitive to acidification than hypoxia (Gobler et al. 2014). However, in a manner similar to that of the Atlantic silversides (*M. menidia*) studied here, the growth of juvenile clams (*Mercenaria mercenaria*) was synergistically, negatively affected by hypoxia and acidification (Gobler et al. 2014). Collectively, these studies suggest that ecosystem-wide effects of low oxygen and acidification on coastal marine food webs are likely to be both significant and complex.

Given the significant declines in the growth and survival of forage fish exposed to low oxygen and acidification, these conditions may translate into significant biomass losses in eutrophic ecosystems, which commonly experience these conditions (Wallace et al. 2014). Such changes likely have important implications for coastal fisheries, which depend on the successful recruitment of early life stage fish to adulthood (Houde 1997) and are particularly dependent on forage fish as key prey (Present & Conover 1992, Conover et al. 2005, Pikitch et al. 2014). Given that coastal ecosystems are often low in oxygen and more acidified during months when fish spawn, it will be important to expand and refine our understanding of the effects of these and other climate-change stressors on fish to best predict how fisheries will respond as climate change exacerbates these conditions in the near future (Pörtner 2008, 2010). Further studies should also assess the long-term effects of climate-change stressors on fish to determine if physiology and behaviors are affected in adulthood or first-generation offspring.

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