

NOTE

Resilience of Black Sea Bass Embryos to Increased Levels of Carbon Dioxide

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Abstract

After a decade of research on how embryonic fish will respond to the increased dissolved carbon dioxide (pCO_2) levels predicted for the next century, no uniform response to near future acidification has been observed among marine species. We exposed Black Sea Bass *Centropristes striata* (BSB) embryos to varied levels of pCO_2 (microatmospheres [μatm]) for 48 h during seasonal experiments conducted in 2013–2015 to compare embryonic response among multiple broodstocks. The relationship between pCO_2 concentration and hatching success was inconsistent among years, with a nonlinear, inverse relationship noted in 2014 only, explaining 13% of observed variance. Conversely, pCO_2 was a good predictor of unhatched BSB embryos after 48 h for all years combined (39%) and for 2013 (38%). The pCO_2 concentration was a good predictor of the frequency of vertebral column anomalies for individual years (2013: 40%; 2014: 12%; 2015: 38%) but not when data were pooled for all years. In 2013 and 2015, vertebral column anomalies were relatively consistent below 1,000 μatm and were elevated above that threshold. Preliminary results suggest that BSB embryos may demonstrate resilience to future pCO_2 levels, but the results also highlight the challenges associated with drawing broad conclusions given observed variability in results obtained from different broodstocks and study years.

Ocean acidification (OA), a decrease in ocean pH, is a result of increased anthropogenic carbon dioxide (CO_2) in the atmosphere (Feely et al. 2010; Hönnisch et al. 2012; Duarte et al. 2013), which causes dissolved CO_2 (pCO_2) and bicarbonate ions (HCO_3^{2-}) to increase, while carbonate ions (CO_3^{2-}), pH, and calcium carbonate saturation

levels (Ω) decrease (Feely et al. 2004, 2010; Hönnisch et al. 2012; Duarte et al. 2013). Coastal zones, in addition to increasing atmospheric CO_2 fluxes, can experience acidification attributable to other mechanisms, including river water inputs (Salisbury et al. 2008), upwelling (Feely et al. 2008), and nutrient loading (Wallace et al. 2014). Accordingly, inshore areas often are exposed to conditions that are not expected to occur in the open ocean for hundreds of years (Cai et al. 2011; Melzner et al. 2013; Carstensen et al. 2018). For example, the Chesapeake Bay estuary has longitudinal and vertical pH and Ω signals that vary seasonally, with both highest and lowest pH (7.31–8.18) and Ω (0.31–3.93) values reported in summer (Cai et al. 2017; Brodeur et al. 2019). Declines in pH and Ω are known to affect molecular, physiological, developmental, and behavioral processes in marine organisms (Seibel and Fabry 2003; Orr et al. 2005; Fabry et al. 2008; Siedlecki et al. 2021). Studying the effects of OA upon marine organisms is complex because the response to different pCO_2 concentrations may vary with life stage and across species.

Juvenile and adult fish are reported to be more resilient to acidification than calcifying marine invertebrates (Pörtner et al. 2004; Melzner et al. 2009; Hurst et al. 2012; Perry et al. 2015; Davis et al. 2016); however, fish embryos, which are still developing physiological controls, may be vulnerable to OA (Murray et al. 2014; Gobler et al. 2018). Previous studies have suggested species-dependent responses to OA for fish. The early life stages of some fish species are highly sensitive to OA. In a study of two

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different stocks of Atlantic Cod *Gadus morhua* exposure to high pCO_2 levels (~1,100 microatmospheres [μatm]) resulted in a doubling of daily mortality rates. This was true for larvae at all feeding levels, even among fish that were fed ad libitum prey densities, where energy resources should have been sufficient for regulation of acid–base processes (Stiasny et al. 2016). Experiments with Inland Silverside *Menidia beryllina* (Baumann et al. 2012; Gobler et al. 2018), Summer Flounder *Paralichthys dentatus* (Chambers et al. 2013), and Atlantic Cod (Stiasny et al. 2016) showed direct reductions in growth and survival when early life stages were exposed to elevated pCO_2 concentrations (Chambers et al. 2013; Stiasny et al. 2016). In contrast, Walleye Pollock *Gadus chalcogrammus* (Hurst et al. 2012, 2013, 2021) and European Bass *Dicentrarchus labrax* (Crespel et al. 2017) showed little to no sensitivity and no effect of elevated pCO_2 on growth and survival. Conversely, survival of larval Atlantic Herring *Clupea harengus* exposed to combinations of high pCO_2 (400 and 900 μatm) and temperature (10°C and 12°C) showed no consistent response (Sswat et al. 2018). A negative effect of pCO_2 on survival was detected at 12°C, whereas the effect was observed to be positive at 10°C, suggesting that synergistic effects of multiple environmental factors can elicit varying responses to OA in young fish (Sswat et al. 2018). These previous studies highlight the complex response of marine fish to OA but were limited by the scope of pCO_2 exposure levels, generally focusing on two or three treatments within a narrow range (400, 800, and 1,200 μatm ; Baumann et al. 2012; Chambers et al. 2013; Hurst et al. 2013; Espinel-Velasco et al. 2018). The best practice guide for OA experiments recommends that multiple pCO_2 levels ranging from preindustrial (<300 μatm) to predicted future levels (>1,200 μatm) are optimal for examining the physiological response of organisms to OA (Riebesell et al. 2010).

An assessment of fish and invertebrate species found on the Northeast U.S. Continental Shelf has identified Black Sea Bass *Centropristes striata* (BSB) as potentially susceptible to climate change (Hare et al. 2016). The BSB is an economically valued species in the mid-Atlantic United States, with a 2018 recreational fisheries harvest of 5.44 million kg (12 million lb) and commercial fisheries landings of 1.45 million kg (3.2 million lb; NOAA Fisheries 2019). Historically, BSB have ranged from Massachusetts to the Gulf of Mexico, with the stock centered in the Middle Atlantic Bight (Roy et al. 2012; Slesinger et al. 2019). More recently, the northern stock of BSB has shifted northward into the Gulf of Maine, with the shift attributed to warming water temperatures (Bell et al. 2015; Hare et al. 2016; Kleisner et al. 2017; McMahan et al. 2020). Adults migrate seasonally from January through July for spawning, moving into coastal estuaries (e.g., Cape Hatteras, Chesapeake Bay, and the Delaware and

Hudson River estuaries; Drohan et al. 2007), where OA levels may be more variable than in the open ocean.

Currently, little is known regarding the effects of elevated pCO_2 upon BSB, especially during early life stages, when fish may be more sensitive to OA effects (Ishimatsu et al. 2008; Murray et al. 2014). In this preliminary study, we exposed embryos to a range of pCO_2 levels (182.7–2,252.6 μatm) and measured the proportions of hatched larvae and unhatched viable embryos after 48 h of exposure. Our hypothesis was that pCO_2 concentrations would be an accurate predictor of hatching success. Larvae were also assessed for vertebral column anomalies (scoliosis and/or lordosis) and were classified as normal or abnormal.

METHODS

Adult male and female BSB broodstock were held at the National Oceanic and Atmospheric Administration (NOAA) Milford Laboratory (Milford, Connecticut) under ambient seawater conditions for Long Island Sound (41°12'43.6788"N, 73°3'7.47"W) during 2012–2013, 2013–2014, and 2014–2015. The BSB broodstock used in 2013 (7 females, 4 males) were held for approximately 375 d, those used in 2014 (10 females, 6 males) were held for 265 d, and those used in 2015 (11 females, 6 males) were held for 630 d. The mean surface pH values \pm SE recorded at 16 stations distributed within Connecticut waters from the eastern to western end of Long Island Sound ($n = 32$ per year) were 7.65 ± 0.04 in 2013, 7.70 ± 0.04 in 2014, and 7.81 ± 0.04 in 2015 (Connecticut Department of Energy and Environmental Protection; Water Quality Portal, <https://www.waterqualitydata.us/portal/#mimeType=csv>). These values varied seasonally, ranging from 7.59 to 7.71 in 2013, from 7.64 to 7.76 in 2014, and from 7.74 to 7.87 in 2015. Adults were fed squid during conditioning and spawning. Gamete development in fish occurred naturally, and spawning took place in holding tanks during July of each year. Outflow water from the broodstock tank passed through a 500- $\mu\text{m} \times 30.5\text{-cm}$ round screen (placed in a basin) to collect the eggs. To reduce variation in embryonic stage between treatments and across years, all eggs were examined under an inverted microscope to select only those eggs that had reached blastula development (~6 h after fertilization). These embryos were then exposed to pCO_2 levels for 48 h. Eight experimental trials were conducted during 2013 ($n = 3$), 2014 ($n = 4$), and 2015 ($n = 1$). One trial conducted in 2014 was removed from analysis because hatching was less than 1%, which was highly atypical compared to the results for the other trials. A trial run consisted of exposing embryos to one of three pCO_2 levels (low, medium, or high), with three experimental beakers for each pCO_2 level, resulting in nine independent pCO_2 concentrations. For one trial in

2013, there were only enough blastula-stage embryos for two pCO₂ levels.

Experiments were conducted in 3-L beakers. Each beaker was placed in a temperature-controlled (mean \pm SD = 20.3 \pm 0.6°C) water table. Salinity in each beaker was measured using a Yellow Springs Instruments Model 85 handheld meter at time 0 and at 48 h, with no detectable change in salinity. Artificial seawater was combined with natural seawater (salinity of 27) from Milford Harbor (41°12'43.6788"N, 73°3'7.47"W) to raise the salinity to 32.32 \pm 0.20. Target pCO₂ levels represented a range of conditions from preindustrial to projected future levels (182.7–2,252.6 μ atm). Mass-flow controllers (Aalborg Instruments and Controls, Orangeburg, New York) delivered a mixture of research-grade CO₂ and compressed air that passed through a CO₂ absorber (Puregas, Broomfield, Colorado). This CO₂/air mixture was delivered by an air stone to each beaker to maintain pCO₂ levels that had been measured and set prior to the addition of embryos. Each 3-L beaker was stocked with 50 blastula-stage embryos in 2013 and 100 blastula-stage embryos in 2014 and 2015.

Black Sea Bass are known to hatch within 48 h at 20°C (Perry et al. 2007). To quantify hatching success, embryos and larvae were removed from each beaker after 48 h of pCO₂ exposure and were preserved in 10% buffered formalin for analysis. Fish larvae and embryos that are preserved in 10% formalin are known to remain stable for up to 29 weeks with well-developed features (Klinger and van Den Avyle 1993). Viable embryos were those observed to be alive at sampling, while nonviable embryos sank to the bottom (i.e., were dead) and had no development beyond the blastula stage. Black Sea Bass were examined under a microscope to count hatched larvae and unhatched viable embryos and to assess larvae for vertebral column abnormalities. Each preserved larva was assessed for anomalies by two different individuals for quality control to ensure accuracy and consistency. Hatching success (%) was calculated as the number of BSB larvae divided by the total stocking density \times 100. To assess whether increased pCO₂ levels reduced hatching in BSB, the percentage of unhatched viable embryos was calculated as the number of viable embryos divided by the total stocking density \times 100. Newly hatched larvae with poorly differentiated skeletal tissue were scored as abnormal if they showed sublethal vertebral column anomalies, including lordosis (V-shaped dorsal–ventral curvature) or scoliosis (lateral curvature; Roo et al. 2009; Boglione et al. 2013). Vertebral column anomalies (%) were calculated as the number of abnormal larvae divided by the total number of larvae \times 100.

Seawater samples collected from each beaker prior to the addition of the BSB embryos and when the experiment

ended (time = 48 h) were analyzed for pH (U.V. Spectrometer; Ocean Optics, Winter Park, Florida) and dissolved inorganic carbon (DIC; Apollo SciTech, Newark, Delaware) immediately upon collection, without the addition of preservatives. The DIC and pH values showed low variation (<0.2–1.5%) over the 48 h; therefore, the mean value was reported and was used in CO2SYS (Pierrot et al. 2006). Certified reference materials, analyzed along with samples, measured within 1 SD for total DIC (\pm 2.7 μ mol/kg) replicates and pH (\pm 0.0014; seawater scale). This protocol was part of a previous international, interlaboratory DIC comparison exercise that measured within 0.5% of assigned values (Bockmon and Dickson 2015). The DIC and pH values were entered into CO2SYS (Pierrot et al. 2006) to calculate pCO₂ (μ atm), Ω_{calcite} , and $\Omega_{\text{aragonite}}$ using the following constants: K_1 and K_2 from Lueker et al. (2000), K of hydrogen sulfate from Dickson (1990), and total boron from Lee et al. (2010).

During 2013, experimental conditions for pH and DIC at in situ temperature (20°C) ranged from 7.242 to 8.277 and from 1,642.66 to 2,188.69 μ mol/kg, respectively (Table 1). The calculated levels of pCO₂ ranged from 177 to 2,698 μ atm, while Ω_{calcite} measured from 5.60 to 0.72. In 2014, pH at in situ temperature ranged from 7.197 to 7.934, while DIC measured from 1,875.79 to 2,208.76 μ mol/kg. This corresponds to a calculated pCO₂ level between 480 and 2,942 μ atm and Ω_{calcite} between 3.15 and 0.64. Treatments were similar in 2015, with a pH that ranged from 7.445 to 7.979 and DIC values of 1,842.83 to 2,018.86 μ mol/kg; calculated pCO₂ was between 423 and 1,641 μ atm, and Ω_{calcite} ranged from 3.39 to 1.12.

All statistical analyses were performed with R version 4.1.1 (R Foundation for Statistical Computing, Vienna; www.r-project.org) using robust methods described by Wilcox (2017) and employing R source library version 39 (downloaded from <https://dornsife.usc.edu/labs/rwilcox/software/>). A running interval smoother was used to assess the explanatory power of pCO₂ concentration in predicting BSB metrics (Wilcox 2017). Smoothers are advantageous over the ordinary least-squares regression or Pearson's product-moment correlation approaches because they are robust to conditions of nonnormality or heteroscedasticity as well as to the presence of outliers in the data. Smoothers also do not assume a linear relationship and have a better fit to nonlinear data while still maintaining a good fit for relationships that are linear or zero. Smoothers are particularly effective over classical regression techniques in threshold responses where abrupt changes in the relationship between the predictor and response variables may occur (Wilcox 2017). Previous studies (Barry et al. 2010; Ries et al. 2010) have shown that the biological metric relationship with pCO₂ can be zero or linear up to a certain threshold pCO₂ concentration before changing abruptly in a nonlinear fashion; thus,

TABLE 1. Chemical parameters for carbonate chemistry in blastula-stage Black Sea Bass embryos that were exposed to ρCO_2 levels for 48 h. Dissolved inorganic carbon (DIC; $\mu\text{mol/kg}$) and pH values were entered into CO2SYS (Pierrot et al. 2006) to calculate ρCO_2 (microatmospheres [μatm]) and calcium carbonate saturation (Ω_{calcite}) using the following constants: K_1 and K_2 from Lueker et al. (2000), K of hydrogen sulfate from Dickson (1990), and total boron from Lee et al. (2010). The reported pH value is at 20°C.

Date	ρCO_2 level	Salinity	DIC	pH	ρCO_2	Ω_{calcite}
Jul 13, 2013	Low	32	1,642.66	8.28	177.03	5.60
	Low	32	1,748.84	8.23	213.21	5.43
	Low	32	1,755.51	8.21	222.54	5.28
Jul 13, 2013	Medium	32	1,897.49	7.71	823.56	1.95
	Medium	32	1,989.64	7.73	831.82	2.12
	Medium	32	2,005.07	7.73	832.33	2.15
Jul 13, 2013	High	32	2,097.42	7.39	1,912.30	1.04
	High	32	2,020.73	7.37	1,943.06	0.95
	High	32	2,101.50	7.36	2,074.90	0.96
Jul 20, 2013	Low	32.5	1,819.20	7.85	566.30	2.55
	Low	32.5	1,813.66	7.85	568.56	2.52
	Low	32.5	1,835.64	7.80	646.93	2.29
	Low	32.5	1,830.81	7.81	632.69	2.33
Jul 20, 2013	High	32.5	1,936.69	7.48	1,455.82	1.17
	High	32.5	1,956.76	7.40	1,753.52	0.99
Jul 23, 2013	Low	32.3	1,933.70	7.99	432.93	3.62
	Low	32.3	1,899.99	8.00	415.32	3.63
	Low	32.3	1,866.66	8.01	393.56	3.68
Jul 23, 2013	Medium	32.3	1,993.36	7.78	745.22	2.35
	Medium	32.3	1,946.55	7.76	766.32	2.19
	Medium	32.3	1,960.48	7.76	765.07	2.22
Jul 23, 2013	High	32.3	2,188.69	7.45	1,753.36	1.23
	High	32.3	2,094.08	7.24	2,697.91	0.72
	High	32.3	2,083.33	7.42	1,797.63	1.09
Jul 3, 2014	Low	32	1,878.20	7.84	610.12	2.52
	Low	32	1,888.66	7.82	638.47	2.44
	Low	32	1,875.79	7.84	600.61	2.55
Jul 3, 2014	Medium	32	2,008.27	7.48	1,508.67	1.20
	Medium	32	2,022.98	7.39	1,886.44	0.97
	Medium	32	2,008.38	7.44	1,652.44	1.10
Jul 3, 2014	High	32	2,072.09	7.21	2,881.65	0.66
	High	32	2,065.03	7.25	2,598.90	0.73
	High	32	2,064.29	7.20	2,941.96	0.64
Jul 5, 2014	Low	32.5	1,876.28	7.93	486.17	3.10
	Low	32.5	1,876.62	7.93	480.12	3.13
	Low	32.5	1,893.18	7.93	486.37	3.15
Jul 5, 2014	Medium	32.5	2,088.18	7.44	1,700.85	1.16
	Medium	32.5	2,094.54	7.43	1,762.47	1.13
	Medium	32.5	2,086.13	7.45	1,689.08	1.17
Jul 5, 2014	High	32.5	2,208.76	7.39	2,039.22	1.08
	High	32.5	2,177.54	7.29	2,540.44	0.84
	High	32.5	2,184.87	7.23	2,911.53	0.73
Jul 12, 2014	Low	32.5	1,907.94	7.90	529.58	2.96
	Low	32.5	1,911.73	7.89	544.97	2.90
	Low	32.5	1,901.66	7.88	549.24	2.85
Jul 12, 2014	Medium	32.5	2,019.44	7.48	1,530.29	1.21
	Medium	32.5	2,028.83	7.49	1,482.71	1.26
	Medium	32.5	2,024.52	7.56	1,254.54	1.48

TABLE 1. Continued.

Date	pCO ₂ level	Salinity	DIC	pH	pCO ₂	Ω _{calcite}
Jul 12, 2014	High	32.5	2,105.50	7.35	2,135.91	0.94
	High	32.5	2,076.86	7.40	1,869.73	1.04
	High	32.5	2,094.55	7.30	2,358.74	0.84
Jul 10, 2015	Low	32	1,885.25	7.97	440.67	3.39
	Low	32	1,846.61	7.97	431.42	3.32
	Low	32	1,842.83	7.98	423.35	3.37
Jul 10, 2015	Medium	32	1,979.09	7.68	938.42	1.86
	Medium	32	1,957.34	7.64	1,025.27	1.67
	Medium	32	1,972.94	7.63	1,047.24	1.66
Jul 10, 2015	High	32	2,016.87	7.45	1,631.18	1.12
	High	32	2,018.86	7.45	1,639.06	1.12
	High	32	2,016.43	7.45	1,641.00	1.12

we have employed smoothers as the approach most likely to detect a broad range of potential relationships between the predictor and response variables. The running interval smoother applied here enabled the use of a robust analog of the sample mean—the 20% trimmed mean—as a measure of location to calculate a series of *y*-values (fish metric) moving across the observed predictor *x*-values (pCO₂ concentration). The 20% trimmed mean was chosen as the measure of location in the response data because this metric has been shown to be much more robust to the presence of outliers than the standard sample mean (Wilcox 2017). The running interval smoother uses a robust analog of the SD—the median absolute deviation—to determine which *x*-values are considered close to a given point for the purpose of calculating the 20% trimmed means, and the span was set to *f* = 1. Each beaker was considered a replicate, with beaker-specific measurements of pCO₂ concentration paired to all biological metrics. Running interval smooths were generated for each biological metric (hatching success, unhatched viable embryos, vertebral column anomalies) for data collected within each individual year (2013: *n* = 24; 2014: *n* = 27; 2015: *n* = 9). Data were also pooled across all 3 years to allow for an examination of broader trends in the data (*n* = 60). Although the running interval smoother does not provide a test of significance or generate a predictive equation, it does provide a measure of explanatory power, and its flexibility makes it useful as an exploratory data analysis tool. Smooths with an explanatory power greater than zero were selected for further analysis and examined visually for evidence of response thresholds. If the smooth suggested a consistent trend across the range of observed pCO₂ concentrations, the robust Theil–Sen regression estimator was used to generate a predictive equation, and the significance of the estimator was tested using a percentile bootstrap method, which does not assume normality or

equality of variance in the data (Wilcox 2017). If the smooth suggested a threshold response in the data, we separated the data into two groups based on the threshold pCO₂ concentration, and those groups were compared using 20% trimmed means as the measure of location and a percentile bootstrap method for hypothesis testing (Wilcox 2017).

RESULTS

Overall, hatching success showed no relationship with pCO₂ concentration when all years were combined or for the 2013 and 2015 individual year data sets (Table 2). During 2014, pCO₂ concentration explained 13% of the variation in hatching success. Regression analysis suggested a negative relationship between hatching success and pCO₂ concentration (slope = -4.9×10^{-5} ; intercept = 0.53), but the slope was not statistically significant (*P* = 0.073). The percentage of hatched larvae ranged from 6% to 63% in 2013, from 16% to 70% in 2014, and from 36% to 95% in 2015 (Figure 1).

When all years were pooled, pCO₂ explained 39% of the variation in the percentage of unhatched viable embryos within 48 h (Table 2) and the smooth was curvilinear in shape (Figure 2). Regression analysis suggested a positive relationship between pCO₂ concentration and the percentage of unhatched viable embryos (slope = 4.9×10^{-2} ; intercept = 6.2×10^{-5}), and the slope was statistically significant (*P* = 0.03). The explanatory power of the Theil–Sen regression estimator (16%) was less than half that of the smooth (39%), likely due to the curvature in the data. In 2013, the pCO₂ concentration explained 38% of the variation in the percentage of unhatched embryos, and 0–20% of the embryos did not hatch (Figure 2). Regression analysis suggested a positive relationship between pCO₂ concentration and the percentage of unhatched viable

TABLE 2. Explanatory power of pCO_2 concentration in predicting Black Sea Bass hatching success, percentage of unhatched viable embryos, and frequency of vertebral column anomalies based on a running interval smoother.

Metric	Data set	n	Explanatory power
Hatching success	2013	24	0.00
	2014	27	0.13
	2015	9	0.00
	All years	60	0.00
Unhatched viable embryos	2013	24	0.38
	2014	27	0.00
	2015	9	0.00
	All years	60	0.39
Frequency of anomalies	2013	24	0.00
	2014	27	0.40
	2015	9	0.12
	All years	60	0.38

embryos ($\text{slope} = 1.5 \times 10^{-5}$; $\text{intercept} = 3.6 \times 10^{-2}$), but the slope was not statistically significant ($P = 0.33$). In 2014 and 2015, there was no relationship between pCO_2 concentration and the percentage of unhatched viable embryos. Approximately 11–55% of the embryos did not hatch in 2014, while 0–8% did not hatch in 2015.

The frequency of vertebral column anomalies showed no trend when data were pooled across years (explanatory power = 0; Table 2). Results across individual years were mixed. The pCO_2 concentration explained 40% of the variation in vertebral column abnormalities in 2013, and a visualization of the smooth suggested a declining trend at low pCO_2 but an increasing trend above 1,000 μatm (Figure 3). Regression analysis over the full range of pCO_2 concentrations yielded a positive overall slope, although it was not significant ($\text{slope} = 8.0 \times 10^{-5}$; $\text{intercept} = 0.41$; $P = 0.32$). Data were also separated into two groups based on a 1,000- μatm threshold, but statistical comparison of these groups was not significant ($P = 0.10$). The pCO_2 concentration explained 13% of the variability in vertebral column abnormalities in 2014, but the smooth suggested a declining trend. Regression analysis yielded a negative slope ($\text{slope} = -1.34 \times 10^{-5}$; $\text{intercept} = 0.46$), but the slope was not statistically significant ($P = 0.62$). The concentration of pCO_2 explained 38% of the variability in vertebral column abnormalities in 2015, and the smooth suggested a consistent increase across pCO_2 concentrations. Regression analysis yielded a positive but nonsignificant slope ($\text{slope} = 1.6 \times 10^{-4}$; $\text{intercept} = 0.55$; $P = 0.15$). The frequency of vertebral column anomalies ranged from 22% to 90% in

2013, from 8% to 80% in 2014, and from 46% to 86% in 2015 (Figure 3).

DISCUSSION

Absence of an organ system and limited homeostatic regulation in embryos suggest that developing eggs may be more vulnerable to OA than other life stages. Hatching often presents a critical bottleneck in the life history of marine fish (Köster et al. 2003; Houde 2008; Dahlke et al. 2020a). Reduced hatching in response to high pCO_2 has been observed in some marine fish, including Senegalese Sole *Solea senegalensis* (Pigmental et al. 2014; Faria et al. 2017), White Seabream *Diplodus sargus*, and Meagre *Argyrosomus regius*, as well as crustaceans, including Florida stone crab *Menippe mercenaria* (Gravinese 2018), Antarctic krill *Euphausia superba* (Kawaguchi et al. 2013), and the copepod *Calanus finmarchicus* (Mayor et al. 2007).

We observed no relationship between pCO_2 and hatching during our 2013 and 2015 trials, and while the running interval smoother suggested a slight declining trend in hatching success with increasing pCO_2 in 2014 (explanatory power = 13%), subsequent regression analysis was not statistically significant ($P = 0.07$). These results are similar to those of other studies (Atlantic Herring *Clupea harengus*: Franke and Clemmesen 2011; Atlantic Cod: Frommel et al. 2013; Yellowtail Jack *Seriola lalandi*: Munday et al. 2016), which found that embryos and larvae can tolerate a variety of pCO_2 levels that are well above those predicted for the next century. For example, Yellowtail Jack from 12 h postfertilization embryos to 3 d posthatch larvae exhibited tolerance to pCO_2 levels as high as 1,700 μatm (Munday et al. 2016). Duration of the embryonic and larval periods may play a role in the CO_2 sensitivity of early stage marine fish (Baumann 2019). Species with quickly developing embryos and larvae, like the BSB, may be less susceptible to effects of elevated pCO_2 than fish with longer-duration early life stages (Murray et al. 2019), which might explain why an overall relationship of pCO_2 with hatching success was not consistently observed for all 3 years combined. Furthermore, BSB are migratory fish that move offshore and south in the fall, returning north and inshore to estuaries and bays in the spring. These movements expose them to a variety of pCO_2 levels, and for this reason, BSB may tolerate a broader range of environmental conditions than do open-ocean fish species (Baumann 2019).

Exposure to pCO_2 may affect time to hatch for BSB embryos. The pCO_2 concentration explained 39% of the variability in the percentage of viable unhatched embryos that we observed at 48 h postfertilization when data were combined across all 3 years; there was a significant increasing trend with increased pCO_2 and the response was clearly nonlinear. Failure to hatch within this time

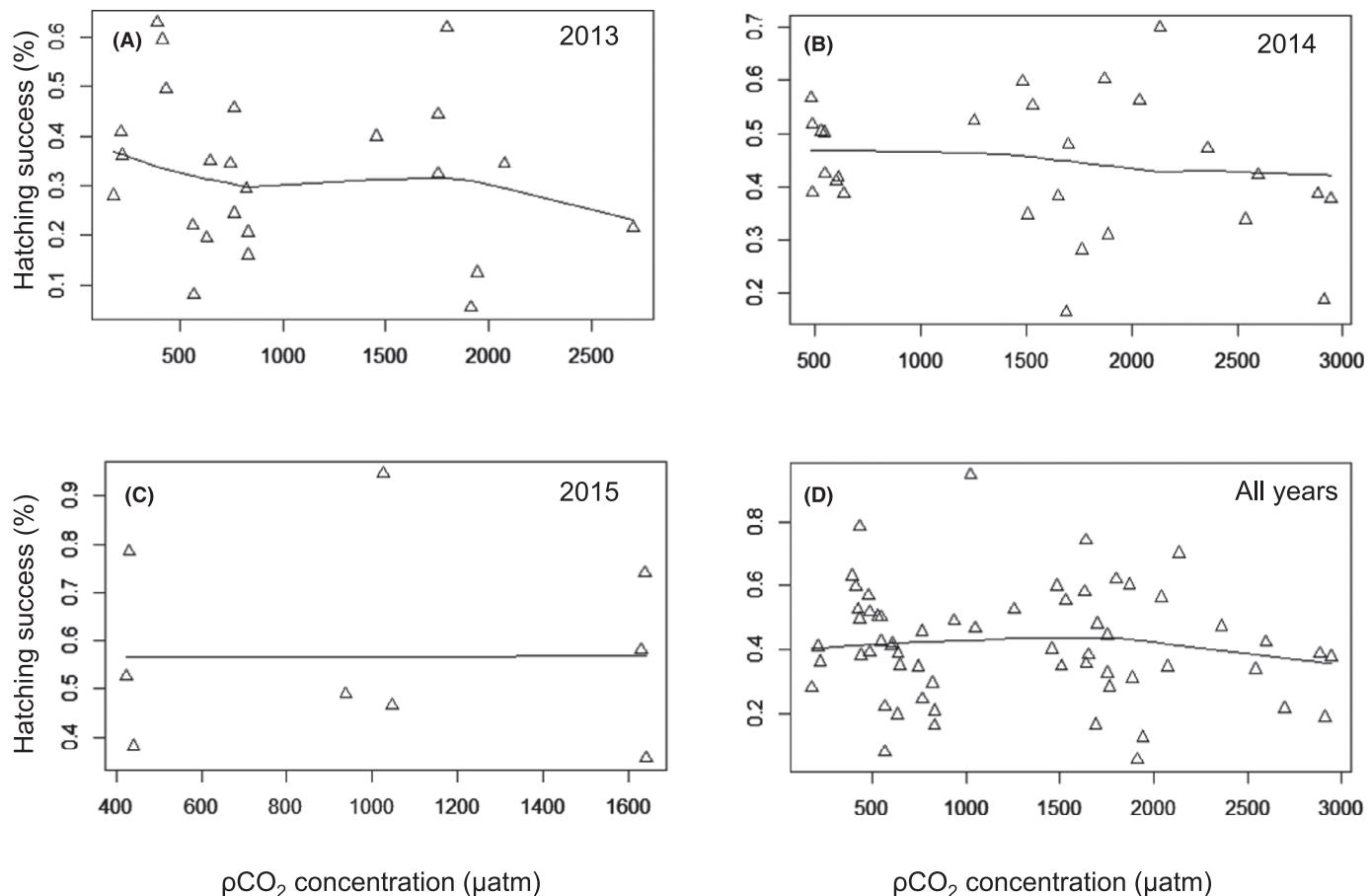


FIGURE 1. Running interval smooths for predicting Black Sea Bass hatching success (%) from the pCO_2 concentration (microatmospheres [μatm]). Smooths were generated for data from each individual year and all years pooled. Hatching success was calculated as the total number of larvae divided by the total stocking density of fertilized eggs $\times 100$.

frame may indicate a disruption in normal hatching processes, since BSB eggs typically hatch within 48 h at temperatures of 19–20°C (Perry et al. 2007; Watanabe et al. 2021). Although embryos of many marine fish demonstrate tolerance to elevations in pCO_2 (Munday et al. 2016; Rodrigues-Dominguez et al. 2018), this ability may relate to how quickly their ion channels can respond to varying pCO_2 levels (Melzner et al. 2009). Embryos do not have gills to maintain acid–base balance; therefore, protons and ions play a critical role in responding to increased pCO_2 conditions (Melzner et al. 2009). Under elevated pCO_2 , the ability of embryos to exchange gas ions via transport by channels and pumps may be impaired, which could affect time to hatch (Melzner et al. 2009; Carter et al. 2013; Pan et al. 2015), and this mechanism may account for the increase in unhatched viable embryos with elevated pCO_2 . A possible disruption to hatching enzyme levels might have also contributed to the delay in time to hatch for BSB embryos. Levels of hatching enzymes are known to be dependent on the pH of the

water (Swain et al. 2020) and may have been affected by elevations in pCO_2 . Although it was beyond the scope of this study to explore all of the possible mechanisms that could relate to variation in hatching, our trials suggest that time to hatch in BSB may be prolonged at high pCO_2 concentrations. Further studies are needed to better understand intrinsic factors that may influence the response to pCO_2 , including fluctuations in egg quality, ion channel and pump response times, sensitivity of hatching enzymes to pH, and the influence of parental lineage.

We found that pCO_2 concentration was a poor predictor of the frequency of vertebral column anomalies when data were pooled across years, and we obtained mixed results when data were examined by individual year. The smooth generated for the 2013 data set had good explanatory power (40%) and suggested the presence of a possible threshold around 1,000 μatm , with different responses on either side of that point, but statistical comparison of the data above and below 1,000 μatm produced nonsignificant results. A linear regression of the data yielded a positive

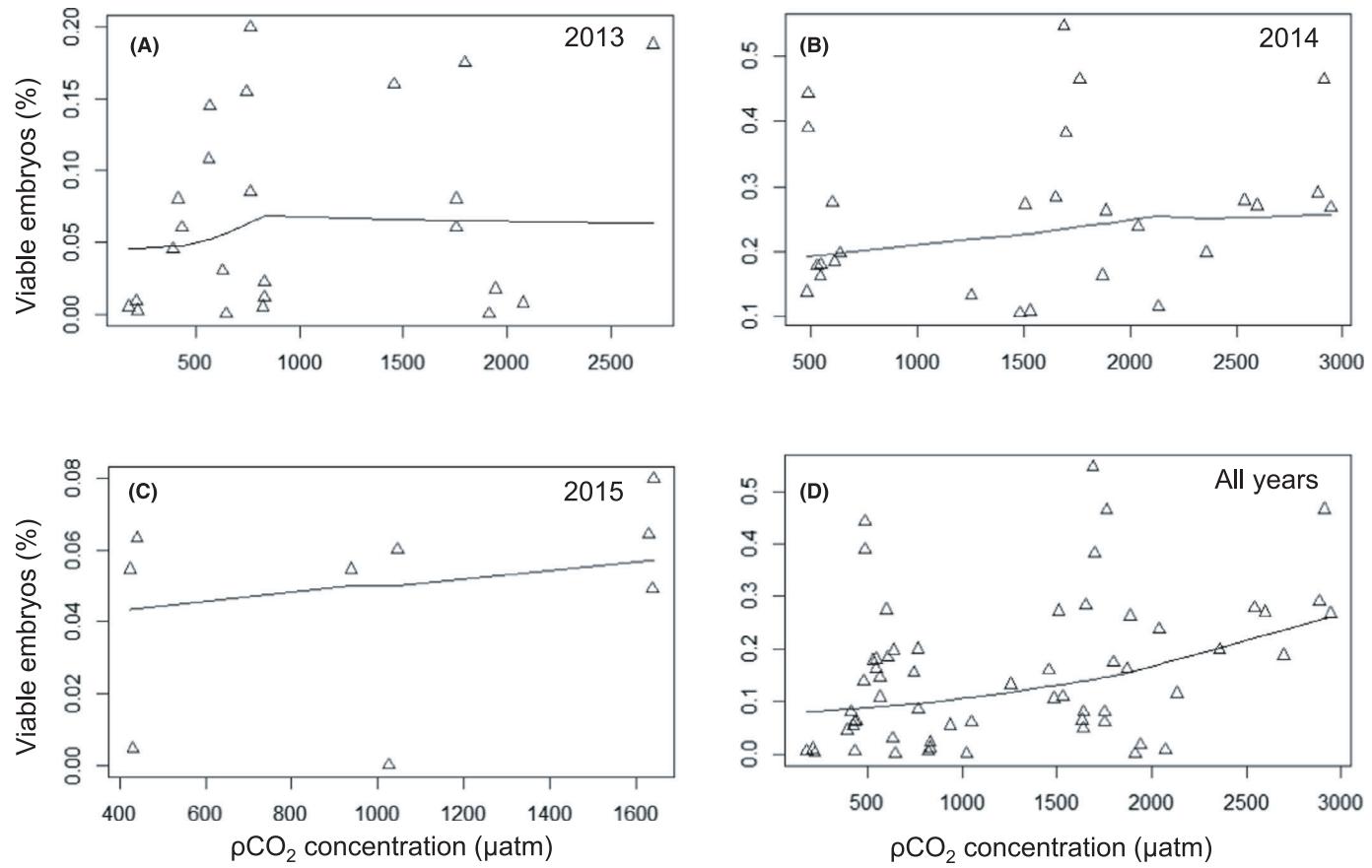


FIGURE 2. Running interval smooths for predicting the percentage of unhatched viable Black Sea Bass embryos at 48 h from the pCO_2 concentration (microatmospheres [μatm]). Smooths were generated for data from each individual year and all years pooled. The percentage of unhatched viable embryos was calculated as the total number of live embryos that had not hatched after 48 h divided by the total stocking density $\times 100$.

slope, but the slope was not significant and had poor explanatory power (8%) relative to the smooth. Results from 2014 were not significant, and the smooth suggested a slight decreasing trend in vertebral column anomalies with increasing pCO_2 . In 2015, the smooth generated had good explanatory power (38%) and the overall trend was increasing vertebral column anomalies with increased pCO_2 , but the data set was small ($n=9$) and the slope of the subsequent regression line was not significant.

Under culture conditions, the presence of vertebral column anomalies can be high in marine fish larvae under ambient pCO_2 . For example, in Red Porgy *Pagrus pagrus*, up to 50% of larvae within a cohort can be affected (Roo et al. 2009), while Atlantic Salmon *Salmo salar* have been shown to experience wide variation (7–94%) in the prevalence of anomalies (Grini et al. 2011). Evidence of vertebral column anomalies under increased pCO_2 has also been observed in adult fish from shallow CO₂ vents (Mirssole et al. 2017, 2021) and at the early life stages (egg to larvae) in Senegalese Sole (Pimentel et al. 2014) and

Gilthead Bream *Sparus auratus* (Pimentel et al. 2016). Many factors can contribute to abnormalities, as larvae are sensitive to environmental and handling conditions during early osteological development. Alterations in vertebral column development can result from suboptimal environmental conditions (Incardona et al. 2004; Boglione et al. 2013) and nutritional deficiencies (Hamre et al. 2013; Rønnestad et al. 2013) and can be influenced by hereditary factors (Castro et al. 2008; Boglione et al. 2013). The ecophysiology of larval fish may be compromised by vertebral column anomalies (Pimentel et al. 2014), which can (1) affect their ability to position themselves in the water column, (2) impede swimming and foraging, and (3) interfere with predator–prey interactions (Powell et al. 2009). Functional transitions, such as hatching and metamorphosis, are critical periods during development in which physical defects may result in mortality or reduce the competency of larval or juvenile fish (Dahlke et al. 2016, 2020b). Future studies should focus on long-term effects of vertebral anomalies.

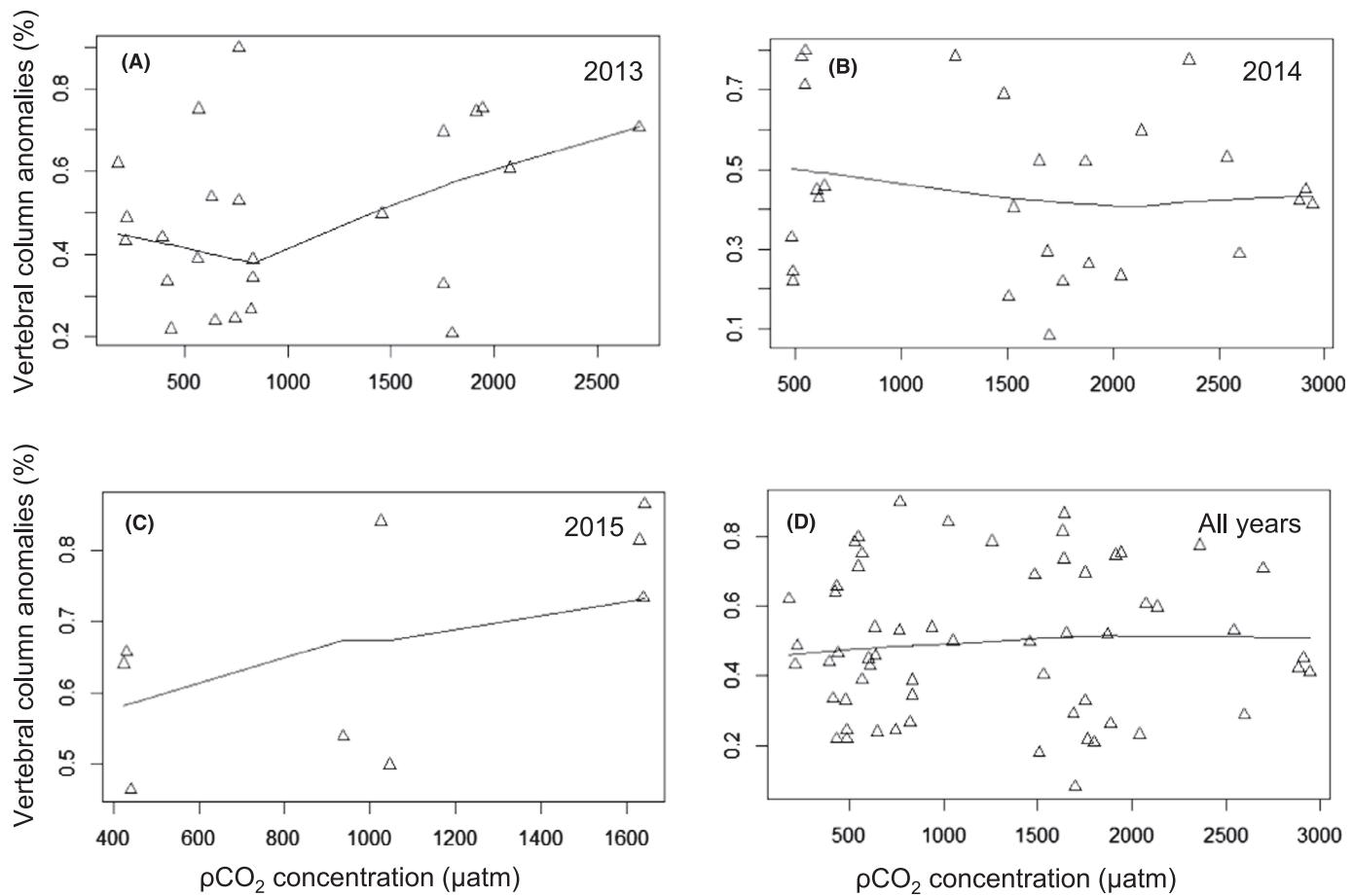


FIGURE 3. Running interval smooths for predicting the percentage of Black Sea Bass larvae with vertebral column anomalies (%) from the pCO₂ concentration (microatmospheres [μatm]). Smooths were generated for data from each individual year and all years pooled. The frequency of vertebral column anomalies was calculated as the number of larvae that had lordosis and/or scoliosis divided by the total number of larvae × 100.

Although our preliminary results found variability in the response of BSB embryos to pCO₂, the early life stages of BSB appear generally resilient to the pCO₂ levels that are predicted to occur over the next 100 years (800–1,000 μatm). We found no apparent effects of pCO₂ upon hatching, but we did observe a modest increase in time to hatch and mixed results in vertebral column anomalies with rising levels of pCO₂ exposure during some trials. Observed effects generally were nonlinear, with curvature observed in the response of time to hatch and one potential threshold observed in 2013 for vertebral column anomalies. Interannual variability in the response of BSB embryos to pCO₂ exposure, however, highlights the challenges of documenting robust predictive relationships between pCO₂ concentration and biological metrics. Variability in egg quality between broodstocks has been reported in the literature and may have contributed to differences in the embryo responses we observed across study years. Further research should focus on critical life history stages that may be key in determining developmental

resilience to a range of environmental conditions and should be conducted concurrently with exposure to other climate change variables (i.e., temperature).

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REFERENCES

Barry, J. P., J. M. Hall-Spencer, and T. Tyrrell. 2010. In situ perturbation experiments: natural venting sites, spatial/temporal gradients in

ocean pH, manipulative in situ pCO₂ perturbations. Pages 123–136 in U. Riebesell, V. J. Fabry, L. Hansson, and J. P. Gattuso, editors. Guide to best practices for ocean acidification research and data reporting. Publications Office of the European Union, Luxembourg City, Luxembourg.

Baumann, H., S. Talmage, and C. Gobler. 2012. Reduced early life growth and survival in a fish in direct response to increased carbon dioxide. *Nature Climate Change* 2:38–41.

Baumann, H. 2019. Experimental assessments of marine species sensitivities to ocean acidification and co-stressors: how far have we come? *Canadian Journal of Zoology* 97:399–408.

Bell, R. J., D. E. Richardson, J. A. Hare, P. D. Lynch, and P. S. Fratantoni. 2015. Disentangling the effects of climate, abundance, and size on the distribution of marine fish: an example based on four stocks from the northeast US shelf. *ICES (International Council for the Exploration of the Sea) Journal of Marine Science* 72:1311–1322.

Bockmon, E. E., and A. G. Dickson. 2015. An inter-laboratory comparison assessing the quality of seawater carbon dioxide measurements. *Marine Chemistry* 171:36–43.

Boglione, C., E. Gisbert, P. Gavaia, P. E. Witten, M. Moren, S. Fontagne, and G. Koumoundouros. 2013. Skeletal anomalies in reared European fish larvae and juveniles. Part 2: main typologies, occurrences and causative factors. *Reviews in Aquaculture* 5:S121–S167.

Brodeur, J. R., B. Chen, J. Su, Y.-Y. Xu, N. Hussain, K. M. Scaboo, Y. Zhang, J. M. Testa, and W.-J. Cai. 2019. Chesapeake Bay inorganic carbon: spatial distribution and seasonal variability. *Frontiers in Marine Science* [online serial] 6:99.

Cai, W.-J., X. Hu, W.-J. Huang, M. C. Murrell, J. C. Lehrter, S. E. Lohrenz, W.-C. Chou, W. Zhai, J. T. Hollibaugh, and Y. Wang. 2011. Acidification of subsurface coastal waters enhanced by eutrophication. *Nature Geoscience* 4:766–770.

Cai, W. J., W. J. Huang, G. W. Luther, D. Pierrot, M. Li, J. Testa, M. Xue, A. Joesoef, R. Mann, J. Brodeur, and Y. Y. Xu. 2017. Redox reactions and weak buffering capacity lead to acidification in the Chesapeake Bay. *Nature Communications* [online serial] 8:article 369.

Carstensen, J., M. Chierici, B. G. Gustafsson, and E. Gustafsson. 2018. Long-term and seasonal trends in estuarine and coastal carbonate systems. *Global Biogeochemical Cycles* 32:497–513.

Carter, H. A., L. Ceballos-Osuna, N. A. Miller, and J. H. Stillman. 2013. Impact of ocean acidification on metabolism and energetics during early life stages of the intertidal porcelain crab *Petrolisthes cinctipes*. *Journal of Experimental Biology* 216:1412–1422.

Castro, J., A. Pino-Querido, and M. Hermida. 2008. Heritability of skeleton abnormalities (lordosis, lack of operculum) in Gilthead Seabream (*Sparus aurata*) supported by microsatellite family data. *Aquaculture* 279:18–22.

Chambers, R. C., A. C. Candelmo, E. A. Habeck, M. E. Poach, D. Wieczorek, K. R. Cooper, C. E. Greenfield, and B. A. Phelan. 2013. Ocean acidification effects in the early life-stages of Summer Flounder, *Paralichthys dentatus*. *Biogeosciences Discussion* 10:13897–13929.

Crespel, A., J.-L. Zambonino-Infante, D. Mazurais, G. Koumoundouros, S. Frakouli, P. Quazuguel, C. Huelvan, L. Madec, A. Servili, and G. Claireaux. 2017. The development of contemporary European Sea Bass larvae (*Dicentrarchus labrax*) is not affected by projected ocean acidification scenarios. *Marine Biology* [online serial] 164(7):article 155.

Dahlke, F. T., S. N. Politis, I. A. Butts, E. A. Trippel, and M. A. Peck. 2016. Fathers modify thermal reaction norms for hatching success in Atlantic Cod, *Gadus morhua*. *Journal of Experimental Marine Biology and Ecology* 47:148–155.

Dahlke, F., M. Lucassen, U. Bickmeyer, S. Wohlrab, V. Puvanendran, A. Mortensen, M. Chierici, H. O. Pörtner, and D. Storch. 2020a. Fish embryo vulnerability to combined acidification and warming coincides with a low capacity for homeostatic regulation. *Journal of Experimental Biology* 223:jeb212589.

Dahlke, F. T., S. Wohlrab, M. Butzin, and H. O. Pörtner. 2020b. Thermal bottlenecks in the life cycle define climate vulnerability of fish. *Science* 369:65–70.

Davis, B. E., N. A. Miller, E. E. Flynn, and A. E. Todgham. 2016. Juvenile Antarctic Rockcod (*Trematomus bernacchii*) are physiologically robust to CO₂-acidified seawater. *Journal of Experimental Biology* 219:1203–1213.

Dickson, A. G. 1990. Thermodynamics of the dissociation of boric acid in synthetic seawater from 273.15 to 318.15 K. *Deep Sea Research Part A: Oceanographic Research Papers* 37:755–766.

Drohan, A. F., J. P. Manderson, and D. B. Packer. 2007. Essential fish habitat source document: Black Sea Bass, *Centropristes striata*, life history and habitat characteristics, 2nd edition. NOAA Technical Memorandum NMFS-NE-200.

Duarte, C. M., I. E. Hendriks, T. S. Moore, Y. S. Olsen, A. Steckbauer, L. Ramajo, J. Carstensen, J. A. Trotter, and M. McCulloch. 2013. Is ocean acidification an open-ocean syndrome? Understanding anthropogenic impacts on seawater pH. *Estuaries and Coasts* 36:221–236.

Espinel-Velasco, N., L. Hoffmann, A. Agüera, M. Byrne, S. Dupont, S. Uthicke, N. Webster, and M. Lamare. 2018. Effects of ocean acidification on the settlement and metamorphosis of marine invertebrate and fish larvae: a review. *Marine Ecology Progress Series* 606:237–257.

Fabry, V. J., B. A. Seibel, R. A. Feely, and J. C. Orr. 2008. Impacts of ocean acidification on marine fauna and ecosystem processes. *ICES (International Council for the Exploration of the Sea) Journal of Marine Science* 65:414–432.

Faria, A. M., F. Soraia, A. F. Lopes, A. P. Oliveira, E. J. Goncalves, and L. Ribeiro. 2017. Effects of high pCO₂ on early life development of pelagic spawning marine fish. *Marine and Freshwater Research* 68:2106–2114.

Feely, R. A., S. R. Alin, J. Newton, C. L. Sabine, M. Warner, A. Devol, C. Krembs, and C. Maloy. 2010. The combined effects of ocean acidification, mixing, and respiration on pH and carbonate saturation in an urbanized estuary. *Estuarine, Coastal and Shelf Science* 88:442–449.

Feely, R. A., C. L. Sabine, J. M. Hernandez-Ayon, D. Ianson, and B. Hales. 2008. Evidence for upwelling of corrosive “acidified” water onto the continental shelf. *Science* 320:1490–1492.

Feely, R. A., C. L. Sabine, K. Lee, W. Berelson, J. Kleypas, V. J. Fabry, and F. J. Millero. 2004. Impact of anthropogenic CO₂ on the CaCO₃ system in the oceans. *Science* 305:362–366.

Franke, A., and C. Clemmensen. 2011. Effect of ocean acidification on early life stages of Atlantic Herring (*Clupea harengus* L.). *Biogeosciences* 8:3697–3707.

Frommel, A. Y., A. Schubert, U. Piatkowski, and C. Clemmensen. 2013. Egg and early larval stages of Baltic Cod, *Gadus morhua*, are robust to high levels of ocean acidification. *Marine Biology* 160:1825–1834.

Gobler, C. J., L. R. Merlo, B. K. Morrell, and A. W. Griffith. 2018. Temperature, acidification, and food supply interact to negatively affect the growth and survival of the forage fish, *Menidia beryllina* (Inland Silverside), and *Cyprinodon variegatus* (Sheepshead Minnow). *Frontiers in Marine Science* 5:86.

Gravinese, P. M. 2018. Ocean acidification impacts the embryonic development and hatching success of the Florida stone crab, *Menippe mercenaria*. *Journal of Experimental Marine Biology and Ecology* 500:140–146.

Grini, A., T. Hansen, A. Berg, A. Wargelius, and P. G. Fjelldal. 2011. The effect of water temperature on vertebral deformities and vaccine-induced abdominal lesions in Atlantic Salmon, *Salmo salar* L. *Journal of Fish Diseases* 34:531–546.

Hamre, K., M. Yúfera, I. Rønnestad, C. Boglione, L. E. Conceição, and M. Izquierdo. 2013. Fish larval nutrition and feed formulation: knowledge gaps and bottlenecks for advances in larval rearing. *Reviews in Aquaculture* 5:S26–S58.

Hare, J. A., W. E. Morrison, M. W. Nelson, M. M. Stachura, E. J. Teevers, R. B. Griffis, M. A. Alexander, J. D. Scott, L. Alade, and R. J. Bell. 2016. A vulnerability assessment of fish and invertebrates to climate change on the northeast US continental shelf. PLOS (Public Library of Science) ONE [online serial] 11:e0146756.

Hönisch, B., A. Ridgwell, D. N. Schmidt, E. Thomas, S. J. Gibbs, A. Sluijs, R. Zeebe, L. Kump, R. C. Martindale, and S. E. Greene. 2012. The geological record of ocean acidification. *Science* 335:1058–1063.

Houde, E. D. 2008. Emerging from Hjort's shadow. *Journal of Northwest Atlantic Fishery Science* 41:53–70.

Hurst, T. P., E. R. Fernandez, and J. T. Mathis. 2013. Effects of ocean acidification on hatch size and larval growth of Walleye Pollock (*Theragra chalcogramma*). ICES (International Council for the Exploration of the Sea) *Journal of Marine Science* 70:812–822.

Hurst, T., E. Fernandez, J. Mathis, J. Miller, C. Stinson, and E. Ahgeak. 2012. Resiliency of juvenile Walleye Pollock to projected levels of ocean acidification. *Aquatic Biology* 17:247–259.

Hurst, T. P., L. A. Copeman, J. F. Andrade, M. A. Stowell, C. E. Al-Samarrie, J. L. Sanders, and M. L. Kent. 2021. Expanding evaluation of ocean acidification responses in a marine gadid: elevated CO₂ impacts development, but not size of larval Walleye Pollock. *Marine Biology* 168:119.

Incarnona, J. P., T. K. Collier, and N. L. Scholz. 2004. Defects in cardiac function precede morphological abnormalities in fish embryos exposed to polycyclic aromatic hydrocarbons. *Toxicology and Applied Pharmacology* 196:191–205.

Ishimatsu, A., M. Hayashi, and T. Kikkawa. 2008. Fishes in high-CO₂, acidified oceans. *Marine Ecology Progress Series* 373:295–302.

Kawaguchi, S., A. Ishida, R. King, B. Raymond, N. Waller, A. Constable, S. Nicol, M. Wakita, and A. Ishimatsu. 2013. Risk maps for Antarctic Krill under projected Southern Ocean acidification. *Nature Climate Change* 3:843–847.

Kleisner, K., M. Fogarty, S. McGee, J. Hare, S. Moret, C. Perretti, and V. Saba. 2017. Marine species distribution shifts on the U.S. Northeast Continental Shelf under continued ocean warming. *Progress in Oceanography* 153:24–36.

Klinger, R. C., and M. J. van Den Avyle. 1993. Preservation of Striped Bass eggs: effects of formalin concentration, buffering, stain, and initial stage of development. *Copeia* 1993:1114–1119.

Köster, F. W., C. Möllmann, S. Neuenfeldt, M. Vinther, M. A. St. John, J. Tomkiewicz, R. Voss, H. H. Hinrichsen, G. Kraus, and D. Schnack. 2003. Fish stock development in the Central Baltic Sea (1976–2000) in relation to variability in the environment. ICES (International Council for the Exploration of the Sea) *Marine Science Symposia* 219:294–306.

Lee, K., T. W. Kim, R. H. Byrne, F. J. Millero, R. A. Feely, and Y. M. Liu. 2010. The universal ratio of boron to chlorinity for the North Pacific and North Atlantic oceans. *Geochimica et Cosmochimica Acta* 74:1801–1811.

Lueker, T. J., A. G. Dickson, and C. D. Keeling. 2000. Ocean pCO₂ calculated from dissolved inorganic carbon, alkalinity, and equations for K_1 and K_2 : validation based on laboratory measurements of CO₂ in gas and seawater at equilibrium. *Marine Chemistry* 70:105–119.

Mayor, D. J., C. Matthews, K. Cook, A. F. Zuur, and S. Hay. 2007. CO₂-induced acidification affects hatching success in *Calanus finmarchicus*. *Marine Ecology Progress Series* 350:91–97.

McMahan, M. D., G. D. Sherwood, and J. H. Grabowski. 2020. Geographic variation in life-history traits of Black Sea Bass (*Centropristes striata*) during a rapid range expansion. *Frontiers in Marine Science* [online serial] 7:567758.

Melzner, F., S. Gobel, M. Langenbuch, M. A. Gutowska, H. O. Portner, and M. Lucassen. 2009. Swimming performance in Atlantic Cod (*Gadus morhua*) following long-term (4–12 months) acclimation to elevated seawater PCO₂. *Aquatic Toxicology* 92:30–37.

Melzner, F., J. Thomsen, W. Koeve, A. Oschlies, M. A. Gutowska, H. W. Bange, H. P. Hansen, and A. Körtzinger. 2013. Future ocean acidification will be amplified by hypoxia in coastal habitats. *Marine Biology* 160:1875–1888.

Mirasole, A., B. M. Gillanders, P. Reis-Santos, F. Grassi, G. Capasso, G. Scopelliti, A. Mazzola, and S. Vizzini. 2017. The influence of high pCO₂ on otolith shape, chemical and carbon isotope composition of six coastal fish species in a Mediterranean shallow CO₂ vent. *Marine Biology* [online serial] 164:article 191.

Mirasole, A., G. Scopelliti, C. Tramati, G. Signa, A. Mazzola, and S. Vizzini. 2021. Evidences on alterations in skeleton composition and mineralization in a site-attached fish under naturally acidified conditions in a shallow CO₂ vent. *Science of the Total Environment* [online serial] 761:143309.

Munday, P. L., S. A. Watson, D. M. Parsons, A. King, N. G. Barr, I. M. Mcleod, B. J. Allan, and S. M. Pether. 2016. Effects of elevated CO₂ on early life history development of the Yellowtail Kingfish, *Seriola lalandi*, a large pelagic fish. ICES (International Council for the Exploration of the Sea) *Journal of Marine Science* 73:641–649.

Murray, C., A. J. Malvezzi, C. Gobler, and H. Baumann. 2014. Offspring sensitivity to ocean acidification changes seasonally in a coastal marine fish. *Marine Ecology Progress Series* 504:1–11.

Murray, C. S., D. Wiley, and H. Baumann. 2019. High sensitivity of a keystone forage fish to elevated CO₂ and temperature. *Conservation Physiology* 7(1):coz084.

NOAA (National Oceanic and Atmospheric Administration) Fisheries. 2019. Landings [online database]. NOAA Fisheries, Silver Spring, Maryland. Available: <https://foss.nmfs.noaa.gov/apexfoss/f?p=215:200:9482349827593:NO>. (April 2020).

Orr, J. C., V. J. Fabry, O. Aumont, L. Bopp, S. C. Doney, R. A. Feely, A. Gnanadesikan, N. Gruber, A. Ishida, and F. Joos. 2005. Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature* 437:681–686.

Pan, T.-C. F., S. L. Applebaum, and D. T. Manahan. 2015. Experimental ocean acidification alters the allocation of metabolic energy. *Proceedings of the National Academy of Sciences of the USA* 112:4696–4701.

Perry, D. M., D. A. Nelson, D. H. Redman, S. Metzler, and R. S. Katersky. 2007. Growth of Black Sea Bass (*Centropristes striata*) in recirculating aquaculture systems. NOAA Technical Memorandum NMFS-NE-206.

Perry, D. M., D. H. Redman, J. C. Widman Jr., S. Meseck, A. King, and J. J. Pereira. 2015. Effect of ocean acidification on growth and otolith condition of juvenile Scup, *Stenotomus Chrysops*. *Ecology and Evolution* 5:4187–4196.

Pierrot, D., E. Lewis, and D. W. R. Wallace. 2006. MS Excel program developed for CO₂ system calculations. U.S. Department of Energy, Oak Ridge National Laboratory, Carbon Dioxide Information Analysis Center, ORNL/CDIAC-105a, Oak Ridge, Tennessee.

Pimentel, M. S., F. Faleiro, G. Dionisio, T. Repolho, P. Pousão-Ferreira, J. Machado, and R. Rosa. 2014. Defective skeletogenesis and oversized otoliths in fish early stages in a changing ocean. *Journal of Experimental Biology* 217:2062–2070.

Pimentel, M. S., F. Faleiro, T. Marques, R. Bispo, G. Dionisio, A. M. Faria, J. Machado, M. A. Peck, H. Portner, P. Pousão-Ferreira, E. J. Goncalves, and R. Rosa. 2016. Foraging behaviour, swimming performance and malformations of early stages of commercially important fishes under ocean acidification and warming. *Climate Change* 137:495–509.

Pörtner, H. O., M. Langenbuch, and A. Reipschläger. 2004. Biological impact of elevated ocean CO₂ concentrations: lessons from animal physiology and earth history. *Journal of Oceanography* 60:705–718.

Powell, M. D., M. A. Jones, and M. Lijalad. 2009. Effects of skeletal deformities on swimming performance and recovery from exhaustive exercise in triploid Atlantic Salmon. *Diseases of Aquatic Organisms* 85:59–66.

Riebesell, U., V. J. Fabry, L. Hansson, and J.-P. Gattuso. 2010. Guide to best practices for ocean acidification research and data reporting. European Commission, Brussels, Belgium.

Ries, J. B., A. L. Cohen, and D. C. McCorkle. 2010. A nonlinear calcification response to CO₂-induced ocean acidification by the coral *Oculina arbuscula*. *Coral Reefs* 29:661–674.

Rodriguez-Dominguez, A., S. Connell, C. Baziret, and I. Nagelkerken. 2018. Irreversible behavioural impairment of fish starts early: embryonic exposure to ocean acidification. *Marine Pollution Bulletin* 133:562–567.

Rønnestad, I., M. Yúfera, B. Ueberschär, L. Ribeiro, Ø. Sæle, and C. Boglione. 2013. Feeding behaviour and digestive physiology in larval fish: current knowledge, and gaps and bottlenecks in research. *Reviews in Aquaculture* 5:S59–S98.

Roo, F. J., C. M. Hernández-Cruz, J. A. Socorro, H. Fernández-Palacios, D. Montero, and M. S. Izquierdo. 2009. Effect of DHA content in rotifers on the occurrence of skeletal deformities in Red Porgy *Pagrus pagrus* (Linnaeus, 1758). *Aquaculture* 287:84–93.

Roy, E., J. Quattro, and T. Greig. 2012. Genetic management of Black Sea Bass: influence of biogeographic barriers on population structure. *Marine and Coastal Fisheries: Dynamics, Management, and Ecosystem Science* [online serial] 4:391–402.

Salisbury, J., M. Green, C. Hunt, and J. Campbell. 2008. Coastal acidification by rivers: a threat to shellfish? *Eos, Science News by AGU* 89:513–528.

Seibel, B. A., and V. J. Fabry. 2003. Marine biotic response to elevated carbon dioxide. *Advances in Applied Biodiversity Science* 4:59–67.

Siedlecki, S. A., J. Salisbury, D. K. Gledhill, C. Bastidas, S. Meseck, K. McGarry, C. W. Hunt, M. Alexander, D. Lavoie, Z. A. Wang, J. Scott, D. C. Brady, I. Mlsna, K. Azetsu-Scott, C. M. Liberti, D. C. Melrose, M. M. White, A. Pershing, D. Vandemark, D. W. Townsend, C. Chen, W. Mook, and R. Morrison. 2021. Projecting ocean acidification impacts for the Gulf of Maine to 2050: new tools and expectations. *Elementa: Science of the Anthropocene* [online serial] 9 (1):00062.

Slesinger, E., A. Andres, R. Young, B. Seibel, V. Saba, B. Phelan, J. Rosendale, D. Wieczorek, and G. Saba. 2019. The effect of ocean warming on Black Sea Bass (*Centropristes striata*) aerobic scope and hypoxia tolerance. *PLOS (Public Library of Science) ONE* [online serial] 14(6):e0218390.

Sswat, M., M. H. Stiasny, F. Jutfelt, U. Riebesell, and C. Clemmesen. 2018. Growth performance and survival of larval Atlantic Herring, under the combined effects of elevated temperatures and CO₂. *PLOS (Public Library of Science) ONE* [online serial] 13(1):e0191947.

Stiasny, M. H., F. H. Mittermayer, M. Sswat, R. Voss, F. Jutfelt, M. Chierici, V. Puvanendran, A. Mortensen, T. B. H. Reusch, and C. Clemmesen. 2016. Ocean acidification effects on Atlantic Cod larval survival and recruitment to the fished population. *PLOS (Public Library of Science) ONE* [online serial] 11(8):e0155448.

Swain, S., P. B. Sawant, N. K. Chadha, E. M. Chhandaprajnadasini, and M. B. Katare. 2020. Significance of water pH and hardness on fish biological processes: a review. *International Journal of Chemical Studies* 8:330–337.

Wallace, R. B., H. Baumann, J. S. Grear, R. C. Aller, and C. J. Gobler. 2014. Coastal ocean acidification: the other eutrophication problem. *Estuarine, Coastal and Shelf Science* 148:1–13.

Watanabe, W. O., P. M. Carroll, M. S. Alam, C. F. Dumas, J. E. Gabel, T. M. Davis, and C. D. Bentley. 2021. The status of Black Sea Bass, *Centropristes striata*, as a commercially ready species for U.S. marine aquaculture. *Journal of the World Aquaculture Society* 52:541–565.

Wilcox, R. R. 2017. Understanding and applying basic statistical methods using R. Wiley, Hoboken, New Jersey.