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Effect of ocean acidification on growth and otolith condition of juvenile scup, *Stenotomus chrysops*

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Abstract

Increasing amounts of atmospheric carbon dioxide (CO_2) from human industrial activities are causing changes in global ocean carbonate chemistry, resulting in a reduction in pH, a process termed "ocean acidification." It is important to determine which species are sensitive to elevated levels of CO_2 because of potential impacts to ecosystems, marine resources, biodiversity, food webs, populations, and effects on economies. Previous studies with marine fish have documented that exposure to elevated levels of CO_2 caused increased growth and larger otoliths in some species. This study was conducted to determine whether the elevated partial pressure of CO_2 (pCO_2) would have an effect on growth, otolith (ear bone) condition, survival, or the skeleton of juvenile scup, *Stenotomus chrysops*, a species that supports both important commercial and recreational fisheries. Elevated levels of pCO_2 (1200–2600 µatm) had no statistically significant effect on growth, survival, or otolith condition after 8 weeks of rearing. Field data show that in Long Island Sound, where scup spawn, in situ levels of pCO_2 are already at levels ranging from 689 to 1828 µatm due to primary productivity, microbial activity, and anthropogenic inputs. These results demonstrate that ocean acidification is not likely to cause adverse effects on the growth and survivability of every species of marine fish. X-ray analysis of the fish revealed a slightly higher incidence of hyperossification in the vertebrae of a few scup from the highest treatments compared to fish from the control treatments. Our results show that juvenile scup are tolerant to increases in seawater pCO_2, possibly due to conditions this species encounters in their naturally variable environment and their well-developed pH control mechanisms.

Introduction

Human industrial and agricultural activities during the past 250 years have increased atmospheric concentrations of carbon dioxide (CO_2) by about 100 parts per million (Feely et al. 2008). The oceans have absorbed about one-third of the anthropogenic CO_2, significantly altering their carbonate chemistry and decreasing pH, leading to the expression "ocean acidification" (OA). Models project that by the year 2100, seawater partial pressure of CO_2 (pCO_2) will double from current levels to 750 ppm, resulting in a decrease in oceanic surface water pH of ~0.4 by the end of the century, and a 50% decrease in carbonate ion concentration (Feely et al. 2004, 2008). Some regions of the ocean, such as estuaries and basins, already experience levels of CO_2 that are higher than

those that are predicted to occur by the end of the century (Frommel et al. 2013; McElhany and Busch 2013; Murray et al. 2014). Understanding the impacts of elevated levels of CO_2 on marine resources and ecosystems is important for predicting an organism's ability to adapt to these increases and for developing a coordinated plan to address the challenges to ecosystems and the management of commercially important species.

Many studies have indicated a variability in the response of marine organisms to elevated levels of CO_2, including research on corals, crustaceans, algae, bivalves, worms (Ries et al. 2009; Kroeker et al. 2010), sea urchins (Dupont et al. 2013), echinoderms, coccolithophores, pteropods, foraminifera (Fabry et al. 2008), marine fish (Ishimatsu et al. 2008; Munday et al. 2011a,b), and mollusks (Talmage and Gobler 2009). Fish may be among the more resilient marine species to OA, due to their high capacity for acid–base regulation (Melzner et al. 2009). Also, some marine fish may be more tolerant of OA due to exposure to natural fluctuations in ambient CO_2 in the habitats occupied by different life stages (Munday et al. 2011b; Hurst et al. 2012). However, studies have reported the effects of OA can be highly variable among marine fish, depending on the species, the sensitivity of the life stage, its life history habits, and parental exposure to high-CO_2 conditions (Miller et al. 2012; Murray et al. 2014; Bignami et al. 2013a,b).

Studies have demonstrated positive, negative, or no effects of elevated levels of CO_2 on the growth and survival of marine teleost fish. Juvenile Atlantic cod showed reduced weight gain, growth rate, and condition factor with increasing CO_2, but survival was not affected (Moran and Stottrup 2011). In a study by Frommel et al. (2011), Atlantic cod larvae exposed to increased levels of CO_2 had severe tissue damage in many internal organs, but larvae from the high treatment attained more weight than the control fish. Candelmo et al. (2013) found fertilization success and survival to hatch significantly increased with increasing CO_2 for winter flounder (*Pseudopleuronectes americanus*); however, their larvae were susceptible to sublethal effects. In another study exposing eggs and larvae of Baltic cod, *Gadus morhua*, to high levels of CO_2, there were no effects on hatching, survival, development, and otolith size at any stage of development (Frommel et al. 2013). Eggs and larvae of the orange clown fish raised in seawater simulating CO_2 acidification scenarios predicted to occur in the next 50–100 years had no detectable effect on embryonic duration, egg survival, and size at hatching; however, OA did have a tendency to increase the growth rate of the larvae (Munday et al. 2009). Examinations on the early life stages of Atlantic herring (*Clupea harengus*) exposed to elevated pCO_2 revealed no effects on embryogenesis, hatch rate, total length, dry weight, and yolk sac area of newly hatched larvae (Franke and Clemmesen 2011).

Hurst et al. (2013) investigated the effects of OA on hatch size and larval growth of walleye pollock (*Theragra chalcogramma*) and found only minor effects of CO_2 level on size and growth rate; but fish in the elevated CO_2 treatments tended to be larger and there were no effects on survival.

In fish, the otoliths comprise an important organ consisting of aragonite–protein bilayers that sense sound, orientation, and acceleration and can be used for documenting age and growth. Aragonite is a form of calcium carbonate ($CaCO_3$), which may be susceptible to the effects of OA. Changes in calcification may affect the shape, size, and mass of the otolith, thus affecting behavior, including feeding (NOAA 2010). Studies involving the effects of OA on fish otoliths have been mostly conducted on larval developmental stages. Recent studies with larval orange clown fish and larval Atlantic cod have shown elevated levels of CO_2 caused an increase in otolith area and maximum length, but no effects on symmetry between the left and right otoliths (Munday et al. 2011a; Maneja et al. 2013). Other studies have shown elevated levels of CO_2 have enhanced otolith growth, resulting in larger otoliths. Elevated levels of CO_2 caused an increase in otolith size and density in larval cobia (*Rachycentron canadum*) (Bignami et al. 2013a,b) and significantly larger otoliths in larval white sea bass (*Atractoscion nobilis*) (Checkley et al. 2009); however, there were no effects on otolith shape in either species.

Conversely, studies examining the effects of OA on larval Baltic cod, larval Atlantic herring, and larval mummichogs (*Fundulus heteroclitus*) have determined there were no effects on otolith size (Franke and Clemmesen 2011; Frommel et al. 2013; Stoneman 2013). In a study by Munday et al. (2011b), juvenile spiny damselfish exposed to elevated levels of CO_2 also showed no effect on otolith size, shape, or symmetry. In species where otolith growth is maintained despite increased levels of pCO_2, there may be physiological trade-offs in terms of reduced growth.

Scup or porgy, *Stenotomus chrysops,* are an important commercial and recreational fish in the northeast United States (National Marine Fisheries Service (NMFS) 2002, Terceiro 2009, 2012). They are found locally in Long Island Sound and in coastal waters of the USA from Cape Cod, Massachusetts to Cape Hatteras, North Carolina (Bigelow and Schroeder 1953). Scup are managed by the Atlantic States Marine Fisheries Commission (ASMFC) and the Mid-Atlantic Fishery Management Council (MAFMC) under Amendment 8 to the Summer Flounder, Scup, and Black Sea Bass Fishery Management Plan (FMP). Recent studies conducted at the National Oceanic and Atmospheric Administration (NOAA) Fisheries, Milford Laboratory, examined growth rates of juvenile scup fed commercial diets and found them to acclimate quickly to tank conditions in the laboratory,

and to exhibit rapid growth rates (Perry et al. 2009, 2013). As scup are regulated under a fishery management plan, acclimate well to laboratory conditions, and reside in Long Island Sound where they are exposed to natural fluctuations in ambient CO_2, they are an appealing marine species for studying the effects of OA on somatic growth and otolith condition. It is important to determine whether scup are susceptible to the effects of increased levels of CO_2 in the marine environment because of their economic importance as a fisheries species and as a potential candidate species for commercial aquaculture (Perry et al. 2013). Furthermore, the younger and, perhaps, more vulnerable life stages of scup may be especially sensitive to the effects of OA, leading to potential impacts on stocks.

A study involving juvenile scup was conducted at the NMFS laboratory in Milford, Connecticut, during the summer of 2011 to evaluate the effects of OA on young scup. This study conducted measurements of somatic growth (length and weight); performed digital X-ray analysis of skeletons; and examined otolith shape, symmetry (between left and right side), size, and mass from young-of-the-year (YOY) scup that were exposed to three levels of CO_2.

Materials and Methods

Fish collection and experimental design

During early August 2011, approximately 400 YOY scup were collected off Milford, Connecticut, from the R/V Loosanoff by otter trawling with a fine-mesh (1.61 cm square) liner in the net. Fish were transported to the Milford Laboratory in seawater with aeration where they were weighed, measured, and sorted by size.

Thirty-five fish (0.5–1.5 g each) were randomly stocked into nine separate 76-L aquaria. The aquaria were randomly placed into one large black tank (5.9 × 1.2 × 0.5 m). Light intensity (1000 lux) and photoperiod (9 L: 15 D) were held constant for the duration of the experiment. Carbon dioxide and air were delivered to equilibrium chambers from a tank of compressed research grade CO_2 and an air compressor, respectively, using mass flow controllers (Aalborg Instruments and Controls, Orangeburg, NY) (Fig. 1). The equilibrium chambers contained flow-through temperature-controlled (21.4 ± 0.07°C) sand-filtered seawater that provided each aquarium with a supply of seawater. The same mixture of air (control) and air–CO_2 was also constantly bubbled into each corresponding aquarium.

There were two treatments and one control group in the experiment, with three replicate aquaria per treatment and the control. Seawater flow rates were maintained at 1 L/min/replicate during the study.

The mean values of pCO_2 used were 1205 µatm control (pH = 7.56), a mid-level value of 1726 µatm (pH = 7.42), and a high value of 2614 µatm (pH = 7.24) (Table 1). The control and mid-level values of pCO_2 were selected to mimic values that are already occurring in Long Island Sound where the fish were collected. The high value of pCO_2 was selected to expose the fish to a level that they may encounter under projected future OA conditions.
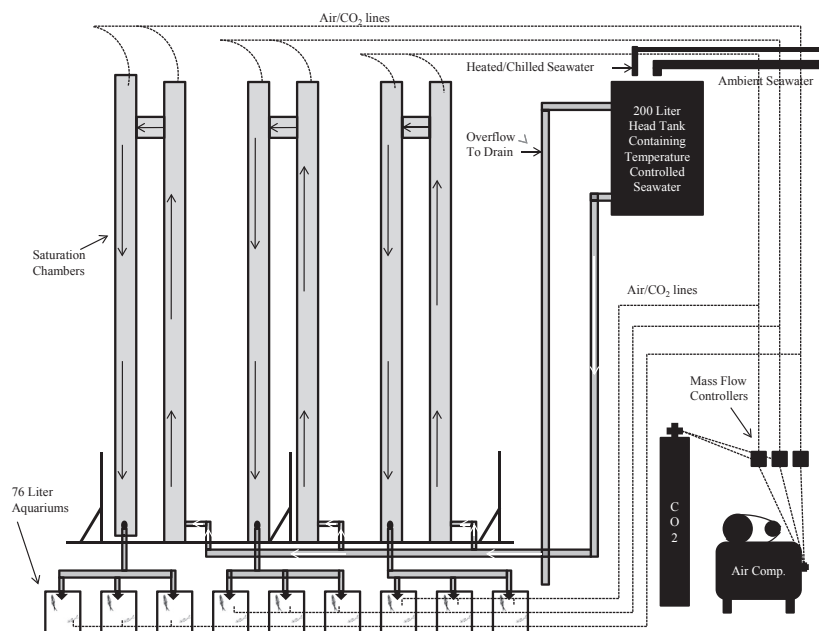

Figure 1. Schematic of flow-through seawater ocean acidification experimental system for conducting CO_2 exposure studies with marine fish.

Table 1. Summary of seawater parameters measured in control and ocean acidification treatments during experiments with scup. Values are means (±SE) of three replicate groups per treatment. Water quality values were measured daily, and carbonate system parameters (total pH, total alkalinity (TA) and nutrients were measured weekly and used to calculate pCO_2 using the program CO2SYS.

Treatment	Temperature °C	Salinity	O_2 mg/L	pH Total	TA μmol/kg	pCO2 μatm	Silicate μmol/kg	Phosphate μmol/kg
Control	21.4 ± 0.07	23.9 ± 0.08	6.83 ± 0.07	7.562 ± 0.02	1847 ± 9	1205 ± 63	20.24 ± 3.48	3.88 ± 0.37
Mid-level	21.3 ± 0.07	23.9 ± 0.08	6.83 ± 0.07	7.415 ± 0.02	1861 ± 14	1726 ± 72	19.76 ± 3.54	4.25 ± 0.40
High level	21.4 ± 0.07	23.9 ± 0.09	6.43 ± 0.08	7.235 ± 0.02	1854 ± 9	2614 ± 100	19.56 ± 3.47	4.28 ± 0.32

Furthermore, pH values from Long Island Sound collected since 2010 have ranged from 6.06 to 8.76, with a mean of 7.82 (CT DEEP, 2013). These values are dependent upon the time of year and the area sampled, and more typical values within Long Island Sound are 7.5–8.2 (G. Sennefelder, pers. comm.).

Growth, total length (TL) (mm), and wet weight (g) of all fish were measured weekly, and all aquaria and the large black tank were cleaned at that time. At the conclusion of the experiment, all fish were weighed, measured, and placed in an ice-water bath, then placed in labeled plastic bags and frozen for later removal of sagittal otoliths and X-ray analysis.

Otoliths and skeletal development

Left and right sagittal otoliths were removed from each fish from every replicate tank and stored dry inside labeled glass vials. Otoliths were photographed sulcus side down for analysis of symmetry, shape, and size using a Leica stereomicroscope (Leica Microsystems, Buffalo Grove, IL) with attached camera at 160×. Photographic images were analyzed using Image J software to determine otolith area in square mm. Otolith mass (0.1 mg) was obtained using a Metler-Toledo balance (Metler-Toledo, Columbus, OH).

Juvenile scup from each treatment and replicate tank were placed on a tray, and digital X-ray images were taken of vertebral columns with an Xpert 80L cabinet system (Kubtec X-ray, Milford, CT). X-ray radiographs of all fish were examined for signs of skeletal abnormalities.

Water chemistry measurements

Measurements of dissolved oxygen (DO) concentration, percent oxygen saturation, temperature, and salinity were taken daily in each aquarium (YSI model 85; Yellow Springs Instruments, Inc., Yellow Springs, OH). The pH of the seawater in each aquarium was monitored daily with a portable pH meter (Hach HQ11D, Hach Company, Loveland, CO) calibrated daily with 4.01 and 10.01 buffers and a Tris-based buffer calibration solution (Dickson and Goyet 1994). Seawater samples were taken weekly from

each aquarium in dark polypropylene bottles and immediately analyzed for total pH, total alkalinity, and nutrients, using care to avoid bubbles and CO_2 exchange during sampling. Total pH was determined colorimetrically using a metacresol purple indicator dye (Sigma-Aldrich, St. Louis, MS; Dickson and Goyet 1994). Briefly, samples were placed in 10-cm glass cuvettes, warmed to 20 °C in a water bath, and absorbance measured before and after indicator dye addition using a Varian Cary dual-beam spectrophotometer equipped with jacketed temperature-controlled cell holders. Total alkalinity was measured using an open-cell titration system with a jacketed beaker (C. Langdon, University of Miami, FL) according to Dickson and Goyet (1994). This involved titrating a sample at 25 °C with 0.1 mol/L HCl to pH ~3, and using a Gran approach to calculate total alkalinity. Certified seawater reference material (Batch 98) from A. Dickson (Scripps Institution of Oceanography, La Jolla, CA) was used to check for accuracy of total alkalinity measurements. Seawater pCO_2 was calculated with the program CO2SYS (Lewis and Wallace 1998) using inputs of salinity, temperature, total pH, total alkalinity, phosphate, and silicic acid, and output was calculated using temperatures that were measured in the experimental aquaria.

Commercial diet

Scup were hand-fed four times daily with Otohime (Otohime, Reed Mariculture, Campbell, CA), a commercially prepared diet, at an initial ration of 10% body weight daily. Typical analysis of this feed is 51–52% protein/11–14% lipid. The pellet size was increased as the fish grew, at which time the feed ration was gradually decreased to a final amount of 4% body weight daily. The experiment was terminated after 8 weeks.

Calculations and statistical analyses

All data were analyzed for statistical differences among treatments with pCO_2 as the independent variable and instantaneous growth rate, weight (G_W), length (G_L), otolith area (Otoa), or otolith mass (Otom) as dependent variables. When our final data resulted in an unbalanced

design, due to an uneven amount of low mortality between treatments, we chose to avoid the uncertainty involved in estimating degrees of freedom for an F-test with unequal sample sizes (Pinheiro and Bates 2000; Quené and van den Bergh 2004; Kliegl et al. 2007). We used a likelihood ratio test and highest posterior density intervals (HPD intervals) to compare the final lengths and weights of the scup as well as the otolith area and mass. Statistical analyses were performed using the open source statistical software R (R Foundation for Statistical Computing 2009) and the lme4 package to fit linear mixed models (Bates et al. 2010). Data from skeletal images were analyzed using an exact contingency table test comparing expected values with results: http://www.physics.csbsju.edu/cgi-bin/stats/exact.

Specific growth rate (SGR) was calculated using the following formula, where wt_1 = mean weight (g) at the initial time point, wt_2 = mean weight (g) at the final time point, and ET = elapsed time (d) between measures of weight.

$$SGR = (\log_e(wt_2) - \log_e(wt_1))/ET \times 100$$

Results

Water chemistry

All seawater carbonate parameters and water quality measurements are shown in Table 1. Calculated mean pCO_2 levels were 1205, 1726, and 2614 μatm for the control, mid-level, and high-level treatments, respectively. Mea-

sured seawater parameters were very similar between treatments with the exception of pCO_2 and pH levels. Mean seawater temperatures remained within 0.1°C, salinity values were consistent, and measurements of dissolved oxygen were within 0.4 mg/L among all treatments throughout the experiment.

Growth and survival

Survival was high throughout the experiment. Mean survival for all three replicates in each treatment was 95.7%, 93.3%, and 91.4% for scup in the control, mid-level, and high-level treatments, respectively. Survival for all treatments was 93.2% over the study period. There were only 19 mortalities among the fish used in the experiment, and these were not grouped in any specific treatment tank.

Although there was an observable trend toward a greater weight and length gain in the scup from the higher CO_2, low-pH treatments, these results were not statistically significant (HPD interval, $P > 0.05$, Figs 2 and 3). Specific growth rates (%/day ± SE) were 5.37 ± 0.07, 5.23 ± 0.05, and 5.27 ± 0.03 for fish from the high-level, mid-level, and control treatments, respectively. Scup exposed to both the high- and mid-level treatments of CO_2 gained the most weight over the course of the experiment (Fig. 2). Juvenile scup from the high exposure treatments increased in mean (±SE) weight from 1.03 ± 0.03 g to 20.90 ± 0.3 g, a gain of 19.87 g. Fish from the mid-level treatments began with an initial mean (±SE) weight of 1.06 ± 0.03 g and grew to a final
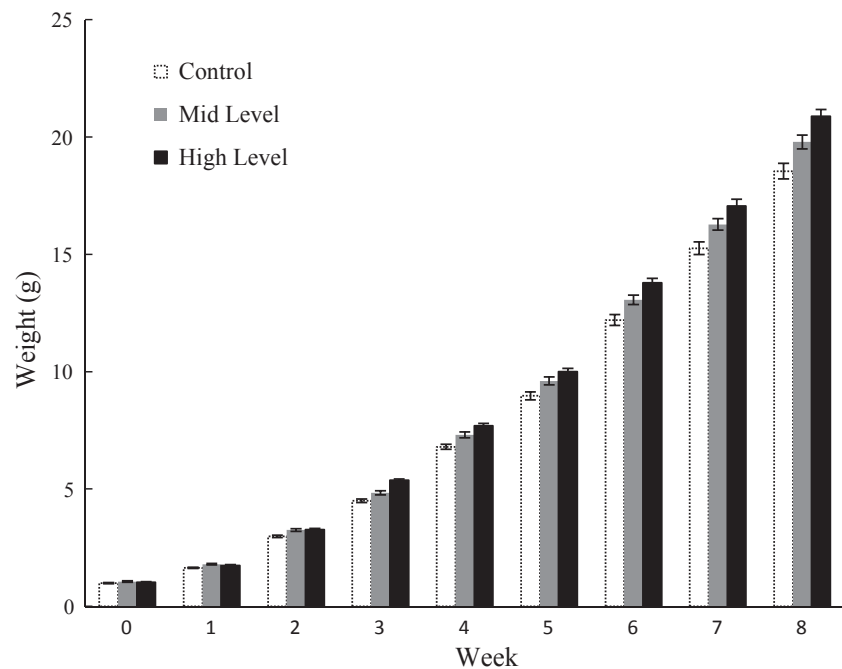

Figure 2. Mean weight (±SE) versus time for juvenile scup exposed to elevated levels of CO_2. Error bars are SE of three replicate groups per treatment.
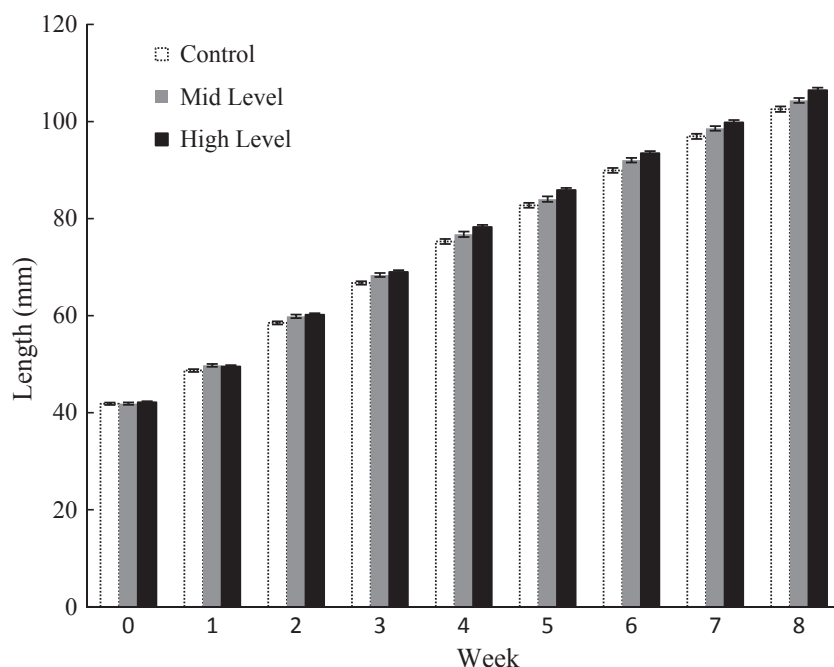
Figure 3. Mean length ($\pm$SE) versus time for juvenile scup exposed to elevated levels of CO_2. Error bars are SE of three replicate groups per treatment.

mean ($\pm$SE) weight of 19.80 $\pm$ 0.3 g, a gain of 18.7 g. Scup from the control treatments gained the least weight with an initial mean ($\pm$SE) weight of 0.99 $\pm$ 0.03 g and a final mean ($\pm$SE) weight of 18.83 $\pm$ 0.3 g, a gain of 17.84 g (Fig. 2).

Scup reared in high- and mid-levels of CO_2 were also longer than those fish exposed to the control level; however, these results were not statistically significant (HPD interval, $P > 0.05$, Fig. 3). Scup from the highest CO_2 treatments grew from an initial mean ($\pm$SE) length of 42.2 $\pm$ 0.3 mm to a final mean ($\pm$SE) length of 106.5 $\pm$ 0.5 mm, an increase of 64.3 mm. Those exposed to the mid-level of CO_2 had an initial mean ($\pm$SE) length of 41.9 $\pm$ 0.3 mm and grew to a final mean ($\pm$SE) length of 104.4 $\pm$ 0.5 mm, an increase of 62.5 mm. Scup in the control treatments had an initial mean ($\pm$SE) length of 41.9 $\pm$ 0.3 mm and a final mean ($\pm$SE) length of 103.0 $\pm$ 0.6 mm, a gain of 61.1 mm.

Otoliths and skeletal development

The size and mass of otoliths from the juvenile scup in the treatment aquaria were not significantly different from the otoliths of scup taken from fish in the control aquaria (HPD interval $P > 0.05$). Mean otolith area ($\pm$SE) for scup in the control treatments was 3.35 $\pm$ 0.04 mm, 3.43 $\pm$ 0.03 mm for scup in the mid-level treatments and 3.45 $\pm$ 0.03 mm for scup in the high-CO_2 treatments. Mean otolith mass ($\pm$SE) was 0.97 $\pm$ 0.01 mg, 1.04 $\pm$ 0.01 mg, and 1.40 $\pm$ 0.01 mg for scup in the

control, mid-level, and high-CO_2 treatments, respectively. Additionally, no asymmetries or differences in shape were found by visual observations of the otoliths from fish exposed to any of the treatments, including the controls.

X-ray analysis of the skeletons from all of the juvenile scup revealed hyperossification in the vertebrae of 3 fish from the highest CO_2 treatments, compared to only one fish from the control treatments. These results were not statistically different ($P = 0.30$).

Discussion

This study revealed no statistically significant differences in weight and length in scup exposed to the mid-level (1726 μatm) and the high-level (2614 μatm) treatments of pCO_2 when compared to the fish in the control (1205 μatm) treatments. It is possible that significant differences would occur, had the experiment been continued over a longer period of time. There were no negative effects of OA on the growth, survival, otolith condition, or on the skeletal development of juvenile scup. This suggests that juvenile scup have well-developed mechanisms for acid–base regulation. Adults and juvenile fish are thought to be more resilient to the effects of OA because they can compensate for acidosis and alkalosis in their tissues, or blood by controlling the acid–base transport of ions through the ion and pH regulatory mechanisms of epithelial tissues of the gills, kidney, and intestine (Heisler 1986; Clairborne et al. 2002; Evans et al. 2005; Michaelidis et al. 2007).

In this study, we found that higher levels of CO_2 did not have a detrimental effect on survival, consistent with findings from other studies. However, there are conflicting results in growth rates of juvenile marine fish exposed to high levels of CO_2. Our results agree with those of Hurst et al. (2012) who found juvenile walleye pollock (*Theragra chalcogramma*) also had high survival and exhibited no significant effect on growth when exposed to elevated levels of CO_2, but subyearling pollock grew faster in two higher CO_2 treatments during laboratory experiments. Reduced growth effects were only observed in juvenile Atlantic cod (*Gadus morhua*) when the pH was lowered to 7.4 (Foss et al. 2006) and were only found in juvenile spotted wolfish (*Anarhichas minor*) at pH levels below 6.5 (Foss et al. 2003). However, Moran and Stottrup (2011) found weight gain and growth rate were substantially reduced in juvenile Atlantic cod with increasing levels of CO_2. In a study with juvenile European sea bass, growth of fish reared at pH 6.1 was comparable to the growth in the control group (pH 7.9) and growth was enhanced when the pH was reduced from 7.9 to 7.0 (Lemarié et al. 2000). The experimental pH values in the present study with juvenile scup (7.42, 7.24) were similar to pH values that others have reported to have caused effects of increased growth. Another study showed elevated levels of pCO_2 had no effect on growth, or survival in juvenile spiny damselfish, *Acanthochromis polyacanthus*, a tropical marine fish (Munday et al. 2011b). Results thus far on effects of elevated levels of CO_2 on the growth of juvenile marine fish are mixed and appear to be species specific. It is apparent that OA will not affect the growth rate of different species of juvenile marine fish in the same manner.

Juvenile scup may be more tolerant of OA due to exposure to natural fluctuations in ambient CO_2 in an estuary, such as Long Island Sound. Our results may indicate an adaptational response to elevated levels of CO_2, as field data from Long Island Sound collected from June to October 2013, have indicated calculated in situ levels of pCO_2 at 25°C to range from 689 to 1828 ppm (S. Meseck, pers. comm.). It has been proposed that species sensitivity to OA may be related to the degree of exposure to natural variation in the levels of CO_2 in the habitats occupied by different life stages (Munday et al. 2011b; Hurst et al. 2012). It is expected that species that inhabit near-shore coastal waters, which exhibit natural variation in CO_2 levels, have adapted to these conditions and those that inhabit more stable pelagic waters may be more sensitive to increases in CO_2 levels (Hofmann et al. 2011; Munday et al. 2011b; McElhany and Busch 2013). However, in a study by Hurst et al. (2012), juvenile walleye pollock, a species that inhabits midwater pelagic waters, was resilient to the effects of ocean acidification and

subyearling pollock had enhanced growth in the higher CO_2 treatments. In a study with anemone fish, *Amphiprion melanopus*, elevated levels of CO_2 caused an increase in metabolic rate and decreases in length, weight, condition, and survival of juvenile fish; however, these effects were absent, or reversed when the parent fish also experienced the same CO_2 concentrations (Miller et al. 2012). Recently, Murray et al. (2014) found that offspring from Atlantic silverside (*Menidia menidia*) became increasingly tolerant to the effects of elevated levels of CO_2 as the spawning season progressed. This may be related to parental conditioning, or transgenerational plasticity (TGP), when parents experience elevated levels of CO_2 prior to fertilization resulting in offspring that are tolerant to the effects of OA. Seawater in Long Island Sound has been shown to exhibit pH declines (Connecticut Department of Energy and Environmental Protection (CT DEEP) 2013), and it is possible that adult scup may have experienced lower pH values, resulting in transgenerational acclimation to increasing CO_2 levels in their offspring. Further experiments utilizing fish species with differing life histories and habitats are needed to identify conditions relating the species sensitivity to the effects of ocean acidification.

Fish have been shown to compensate for increased metabolic demands through increased feeding (Hurst and Conover 2001). In our study, the scup that exhibited the highest growth rates were held in the higher CO_2 treatments. These fish could not have increased consumption rates to compensate for energetic stress because they were not fed to apparent satiation. Instead, fish in all treatments were fed a daily ration based on body weight measurements taken weekly.

While the present study showed no effects of elevated levels of CO_2 on otolith shape, size, symmetry, or mass from juvenile scup, sensitivity varies among species. Our results are similar to those of Munday et al. (2011b), who found no effect of exposure to elevated levels of CO_2 on otolith size, shape, or symmetry in juvenile spiny damselfish; however, the pCO_2 treatments (up to ~850 μatm) were lower than those used in the present study. In another study, otoliths from juvenile walleye pollock exposed to elevated CO_2 levels (>450 μatm) for six weeks showed an increase in deposition rates (Hurst et al. 2012). None of the studies found any effect on otolith shape, roundness, or symmetry, the same results obtained in the present study. The effects of elevated levels of CO_2 on otoliths appear different for diverse fish species and for distinct developmental stages and may relate to a species physiological adaptation to certain environments.

The skeletal tissue of marine fish is primarily composed of calcium phosphate minerals (hydroxylapatite), instead of calcium carbonate, and is not thought to be susceptible

to reduced ocean pH (Ishimatsu et al. 2008; Munday et al. 2011b). X-ray radiographs of the juvenile scup from this study did not show any considerable skeletal abnormalities, which is in agreement with findings from other studies. Atlantic salmon exposed to increasing levels of pCO_2 for 135 days had higher calcium and phosphate contents in vertebral bones, but showed no internal abnormalities when analyzed by X-ray radiography (Gil Martens et al. 2006). Munday et al. (2011b) found no observed effects from exposure to elevated levels of CO_2 on the size of skeletal elements throughout the body in the spiny damselfish. However, Munday et al. (2011b) state that acid–base regulation in fish exposed to elevated levels of CO_2 may affect skeletal development through changes in extracellular concentrations of bicarbonate and nonbicarbonate ions. While we observed no significant abnormalities of hyperossification in vertebrae of fish exposed to the highest levels of CO_2, more studies are needed specifically addressing this issue with larger numbers of fish and earlier, and potentially more susceptible, developmental stages.

Our results suggest that the growth, otolith condition, and the skeletal bones of juvenile scup were not negatively affected by OA. This study reveals that ocean acidification is not likely to have detrimental effects on every species of marine fish. This may be related to parental conditioning prior to fertilization, or result from physiological adaptations from residing in a variable pH environment, such as an estuary. Additionally, juvenile and adult fish have well-developed intracellular and extracellular pH regulatory mechanisms to compensate for the effects of ocean acidification. Areas for future research should be focused on the potential for selection of tolerant genotypes to ocean acidification over future generations, effects on early life history stages, and impacts on food webs.

Acknowledgments

Conflict of Interest

None declared.

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