Ocean acidification alters the otoliths of a pantropical fish species with implications for sensory function

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Edited by George N. Somero, Stanford University, Pacific Grove, CA, and approved March 8, 2013 (received for review January 22, 2013)

Ocean acidification affects a wide diversity of marine organisms and is of particular concern for vulnerable larval stages critical to population replenishment and connectivity. Whereas it is well known that ocean acidification will negatively affect a range of calcareous taxa, the study of fishes is more limited in both depth of understanding and diversity of study species. We used new 3D microcomputed tomography to conduct in situ analysis of the impact of ocean acidification on otolith (ear stone) size and density of larval cobia (Rachycentron canadum), a large, economically important, pantropical fish species that shares many life history traits with other diversity of high-value, tropical pelagic fishes. We show that 2,100 μatm partial pressure of carbon dioxide (pCO2) significantly increased not only otolith size (up to 49% greater volume and 58% greater relative mass) but also otolith density (6% higher). Estimated relative mass in 800 μatm pCO2 treatments was 14% greater, and there was a similar but nonsignificant trend for otolith size. Using a modeling approach, we demonstrate that these changes could affect auditory sensitivity including a ~50% increase in hearing range at 2,100 μatm pCO2, which may alter the perception of auditory information by larval cobia in a high-CO2 ocean. Our results indicate that ocean acidification has a graded effect on cobia otoliths, with the potential to substantially influence the dispersal, survival, and recruitment of a pelagic fish species. These results have important implications for population maintenance/replenishment, connectivity, and conservation efforts for other valuable fish stocks that are already being deleteriously impacted by overfishing.

Results and Discussion

The sagittal and laparal otoliths of larval cobia raised for 20 d in acidified conditions expected for the years 2100 and 2300 [800 and 2,100 μatm partial pressure of carbon dioxide (pCO2), respectively] (5, 6) differed significantly from otoliths of larvae raised under control conditions (300 μatm pCO2; Table 1). Otoliths from larvae raised in seawater at 2,100 μatm pCO2 had significantly greater volume, surface area, and density relative to controls (Fig. 2A–C; see Table 2 for statistical summary). There was also a significant decrease in the surface area to volume ratio (SA:V) of otoliths in both elevated-CO2 treatments, as well as a significant increase in the estimated relative mass of sagittal otoliths under both elevated-CO2 treatments (Fig. 2D and E). Otoliths from larvae raised in seawater at 800 μatm pCO2 exhibited a trend of increased volume and surface area, but these patterns were not significant. Relative density of 800 μatm pCO2 treatment otoliths was also not significantly different from controls. No treatment effect was detected for the standard length (SL) of larvae at any treatment level (P = 0.809, n = 4). These results are unique direct measurements of otolith volume, surface area, and density impacts on otoliths and enabled modeling of the sensory consequences of those effects.

The study species we used, Rachycentron canadum (cobia), is one of the largest and most widely distributed tropical species studied to date and is also of significant ecological and economic value (23, 24). It is a eurytopic top predator and the target of recreational and commercial fisheries throughout a nearly circumglobal distribution in the continental shelf waters of tropical to warm temperate regions (23, 24). Global fishery landings were approximately 11,000 tons in the year 2000 and aquaculture production had a global value over USD 36 million in 2004 (25). Cobia life history traits are shared by several high-value, pelagic, tropical fishes; thus their use in these experiments provides a useful perspective of possible ocean acidification impacts to other pelagic species of high ecological and economic value.

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Author contributions: S.B., S.S., and R.K.C. designed research; S.B. performed research; I.C.E., D.P.M., and S.S. contributed new reagents/analytic tools; S.B. analyzed data; and S.B., I.C.E., D.P.M., S.S., and R.K.C. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.
Alteration of otolith size, density, and mass has direct impacts on otolith mechanics and influences sensory function (27–29). To simulate the mechanics of CO$_2$-altered otoliths, we applied the size and relative density data from sagittal otoliths in our experiment to a mathematical model of otolith motion in response to an 0.8-nm amplitude sinusoidal acoustic wave (27, 28). Our simulation demonstrated that when subjected to the same sound stimulus, the estimated CO$_2$-driven increase in relative otolith mass results in an increased displacement amplitude compared with control otoliths (Fig. 3A). Increased otolith displacement amplitude would enable larvae developing in high-CO$_2$ water to detect sounds that fish in low-CO$_2$ water cannot detect. For otolith displacement to reach the hearing threshold that was attained by control otoliths in response to a sound amplitude of 1 nm, 800 μatm pCO$_2$ treatment otoliths required 5% less sound amplitude (0.95 nm) and 2,100 μatm pCO$_2$ treatment otoliths required nearly 20% less sound amplitude (0.80 nm). As sound amplitude decreases with distance from the source (13), heightened auditory sensitivity leads to detection of sounds at a greater distance from the source. We calculated the relative hearing ranges for larval fish with the auditory sensitivities of high-CO$_2$ (0.80-nm sound amplitude threshold), intermediate-CO$_2$ (0.95-nm threshold), and control otoliths (1-nm threshold) from our mathematical model assuming cylindrical spreading of sound (13) and determined that the more massive otoliths from high-CO$_2$ larvae produced ~50% greater hearing range compared with control larvae, whereas otoliths from intermediate-CO$_2$ larvae produced ~10% greater hearing range (Fig. 3B).

Increased auditory or vestibular sensitivity has important implications for the utilization of these sensory functions by fishes: it could influence a fish’s ability to navigate to a desired habitat, detect predators or prey, perceive changes in water turbulence or current speeds, or maintain proper kinesthetic awareness. These changes would be most relevant near the periphery of hearing ability, such as at distance from a sound source or when otolith displacement amplitude approaches the threshold for detection. Altered sensory ability could prove to be beneficial or detrimental, depending on how a fish perceives this increased sensitivity. Improved detection of useful auditory information (e.g., distant nearshore sounds) would be advantageous to navigating coastal fishes; however, increased sensitivity to disruptive background noise (e.g., sea state) may mask useful auditory information. The need for auditory or vestibular sensitivity may also be life history specific. Many bottom dwelling fish species possess large otoliths relative to their body size, which may indicate an ecological need for high auditory and vestibular sensitivity (28). In contrast, highly mobile pelagic species often possess small otoliths relative to their body size, implying less sensitivity (28). Because these traits have likely evolved to suit the particular ecological needs of a species, benthic species may find increased otolith mass advantageous, whereas such changes may be detrimental for pelagic species. Of course, it is also conceivable that increased otolith size could impinge upon the closely associated sensory hair cells of the macula and be detrimental to the function of the otolith organ regardless of species. These effects should apply to fishes of all ages; however, younger fish have less sensitive hearing ability (30) and any sensory advantage or disadvantage during the larval stage could be particularly influential to survival, with cascading effects on recruitment, population connectivity, and stock replenishment. Although ocean acidification is typically considered a future threat, these implications already may influence the dispersal and distribution of fishes currently developing in high-CO$_2$ water in habitats such as fjords (31) and upwelling zones (3).

The mechanistic cause of increased otolith size with ocean acidification has not been determined empirically, but has been attributed to the physiological response of fish to high environmental CO$_2$ (i.e., HCO$_3^-$ retention) (32), which likely causes an increase in the aragonite saturation state of the endolymph fluid surrounding the otoliths (20). This physiological mechanism is sustained for the duration of high-CO$_2$ exposure (32), therefore it can be assumed that effects on otoliths will persist with age. Additionally, ocean acidification is known to alter neurological function in fishes (11) and there is evidence for neurological control of otolith mineralization (33). Therefore, CO$_2$-induced neurological disruption may indirectly contribute to increased otolith size and density, either by changing the chemical composition of endolymph fluid or by altering neurologically controlled expression of genes that influence the crystalline or lattice structure of otoliths (33). The results of either mechanism of change have important implications for the function of otoliths as sense organs, but there are also implications for their use as tools for fisheries biology research and conservation. Fisheries oceanographers and ecologists rely on otoliths to study fisheries stocks and the early life dynamics of fishes, often using the widths of daily otolith increments as a proxy for daily somatic growth (34). This method depends on a consistent correlation between otolith growth and somatic growth, but an increase in otolith size without a corresponding increase in somatic growth disrupts this relationship and may confound the use of this technique under conditions of elevated CO$_2$.

![Fig. 1. Lateral view micro-CT imagery of a 22-d posthatch larval cobia head. Three-dimensional data were filtered to produce imagery of (A) the complete skeletal structure of the cobia skull and (B) only more dense material, such as otoliths (marked with arrow).](image-url)

### Table 1. Summary of water chemistry results

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Temperature, °C</th>
<th>pH</th>
<th>total scale TA, μmol·kg$^{-1}$</th>
<th>pCO$_2$, μatm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>27.0 (±0.1)</td>
<td>8.13 (±0.01)</td>
<td>2291 (±32)</td>
<td>305 (±8)</td>
</tr>
<tr>
<td>Year 2100</td>
<td>26.9 (±0.1)</td>
<td>7.79 (±0.02)</td>
<td>2291 (±37)</td>
<td>796 (±37)</td>
</tr>
<tr>
<td>Year 2300</td>
<td>27.0 (±0.1)</td>
<td>7.40 (±0.03)</td>
<td>2285 (±34)</td>
<td>2123 (±113)</td>
</tr>
</tbody>
</table>

Temperature, pH, and total alkalinity measured during larval rearing and mean pCO$_2$ calculated with the software CO2SYS (26). Values are means (±SEM).
variable CO₂ conditions. Also, otoliths formed in high-CO₂ water may have a different mineralogical composition, thereby interfering with stock identification methodologies such as those using otolith microchemistry analysis (35). Similar to the ecological effects discussed above, present day occurrence of high-CO₂ water in fjords (31) and upwelling zones (3) makes this a current problem, and may already influence the interpretation of data collected using these techniques.

Our results indicate a graded impact of ocean acidification on cobia otoliths, similar to previously reported effects on 2D otolith surface area under identical treatment conditions (22). This is evident in the end-of-century 800 μatm pCO₂ acidification treatment, where effects on otolith size followed a similar but nonsignificant trend. This is a potentially optimistic result, indicating some resistance to acidification and suggesting that under near-future scenarios these impacts may be most relevant in habitats already experiencing high pCO₂ levels. However, the trend for larger otoliths with increased CO₂ still produced an ~10% increase in hearing range and it is not yet clear at what point these effects will become ecologically significant. Empirically, it is also unclear if natural exposure to variable environmental conditions leads to preadaptation, and thus resistance, to acidification in fishes (36). Because cobia is eurytopic, inhabiting environments ranging from epipelagic to estuarine waters, this may affect the intensity of their response to ocean acidification, whereas species restricted to more constant environments (e.g., entirely pelagic species) may respond differently to similar acidification scenarios.

It is widely accepted that the impact of ocean acidification on marine organisms varies along a gradient from obvious to subtle effects. Our observation of CO₂-induced increases in otolith size and relative density is an unexpected subtle effect with important implications for the sensory abilities of fishes. Whether these sensory changes are ultimately positive or negative will depend on the species, but they have the potential to influence the survival, dispersal, and recruitment of a diversity of marine fishes, with subsequent population consequences. Because many ecologically and economically important species have characteristics similar to cobia, such population changes are expected to produce substantial ecological and economic effects. These results contribute to a fuller understanding of the complex suite of direct and indirect ocean acidification effects on fishes as well as the broader ecological and economic consequences that may challenge fishery populations and conservation efforts in the future.

Materials and Methods

Study System. Cobia (R. canadum) is a highly mobile marine fish that reaches sizes >1.5 m and over 60 kg (23, 24). Pelagic spawning occurs during warmer
months (25–30 °C) and planktonic larvae hatch at ~3 mm SL, undergo flexion at 5–10 mm SL, and develop via a gradual transition into the juvenile stage within 30 d at 15–30 mm SL (23). Cobia eggs and larvae for this experiment were produced at the University of Miami Experimental Hatchery from a population of 10 F₁-generation broodstock (six females, four males). Eggs were collected and allowed to hatch and develop until 2 d posthatch (dph), then stocked into 12 replicated 400 L flow-through experimental tanks at a density of 9–10 larvae L⁻¹ and raised according to established methods (22, 27). Treatments were applied upon stocking and reached full effect within 24 h. Larvae were sampled at 22 dph, preserved in 95% ethanol, and the SL of each larva was measured to the nearest 0.1 mm using digital calipers (MC0006; Avenger). All live animal use was conducted with approval of the University of Miami Institutional Animal Care and Use Committee (Protocol #MC0006; Avenger).

**Water Chemistry.** Treatments represented ocean acidification scenarios for the years 2100 (800 μatm pCO₂) (6) and 2300 (2,100 μatm pCO₂) (5), but are also found presently in fjords (31) and upwelling zones (Table 1) (3). Sea-water carbonate chemistry was manipulated via the addition of equimolar HCl and NaHCO₃ before introduction into tanks (38). Tank pH was monitored daily using a handheld pH meter (pH 11; Oakton) and Ross Electrode (Orion 9102BWN; Thermo Scientific) calibrated daily with Tris buffer. Water samples were collected every 5 d in 250 mL polyethylene terephthalate (PET) bottles and fixed with 1% formaldehyde. Total alkalinity (TA) and total pH (pHT) were measured using automated Gran titration checked for accuracy with Dickson standards ( Scripps Institution of Oceanography, La Jolla, CA). The software CO2SYS was used to solve the carbonate system using the two measured parameters (pHT and TA) (26). Temperature and dissolved oxygen were measured with a combination meter (550A; YSI) twice and once d⁻¹, respectively, and salinity was measured once daily using a refractometer (RHS-10 ATC; Premium Aquatic). See Table 1 for summary of water chemistry results.

**Micro-CT Procedures.** Three larvae per tank (12 per treatment) were randomly selected and individually scanned in the micro-CT scanner (SkyScan 1174v2, 13-μm resolution, 0.3° step, 180° total rotation). X-ray attenuation was standardized across scans using hydroxyapatite bone mineral density (BMD) standards. Two-dimensional X-ray images were reconstructed into 3D image stacks using NRecon (v1.6.6.0) (Bruker-microCT) and analyzed using CTan (v1.12.4.3) (Bruker-microCT). Gray-scale X-ray images were thresholded to isolate regions of interest (ROIs) containing otoliths. Mean BMD was recorded from within these regions, 3D volumes were interpolated across image stacks using a “shrink-wrap” function, and both volume and surface area were measured. Relative density was determined by comparing the mean X-ray attenuation coefficients from micro-CT scans between control and treatment otoliths.

**Mathematical Modeling.** The mathematical model adapted to simulate otolith displacement is based on an elliptical otolith oscillating in response to a plane sinusoidal wave (27, 28). Otolith displacement, Δx, relative to the closely associated bed of sensory hair cells (macula) is described by

\[ \Delta x = a_x \omega^2 (m - \rho_g V) \cos(\omega t + \tan^{-1}(\frac{-\rho_g V}{A})) \left[ \frac{1}{A^2} + \left(\frac{\rho_g V}{A}\right)^2 \right]^{0.5}, \]

where

\[ A = -k_x + \omega^2 (m - \rho_g V + \delta_g) \]

and \( a_x \) is amplitude of water particle displacement, \( \omega \) is angular frequency of the wave (\( \omega = 2\pi \nu \)) and \( \nu \) is frequency, \( m \) is otolith mass in milligrams, \( \rho_g \) is density of the endolymph fluid (assumed to be 1,000 mg·cm⁻³), \( V \) is otolith volume in cubic centimeters, \( \tau \) is time in seconds, \( \gamma_x \) is a coefficient of friction (\( \gamma_x = 0.0029535 \times 10^5 \mu m kg^{-1} s^{-1} \)), \( k_x \) is a stiffness coefficient of hair cell bundles (\( k_x = 21.2652 \times 10^5 \mu m N m^{-1} \)), and \( \delta_g \) is the apparent additional mass of an object moving irregularly in liquid (\( \delta_g = 0.0241m \)) (27, 28). Control otolith density was set at a standard value of 2,900 mg·cm⁻³ (27) and used along with micro-CT volume and relative density data to calculate simulated otolith density and mass.

We designated a water particle displacement amplitude (\( a_x \)) of 1.0 mm at 100 Hz as the threshold sound level for control fish. This is reported to be the behavioral threshold for detection of particle motion in some adult fishes without gas bladders (39) and is a frequency representative of sounds produced by coastal fishes (40). This was chosen in contrast to thresholds measured using the pressure component of sound and neuronal response, which may not accurately describe the detection of particle motion (39) and can underestimate behavioral response thresholds (13). Because thresholds have been shown to decrease with age (30), 1.0 mm is likely a conservative estimate for a larval fish. The otolith displacement magnitude (\( \Delta x \)) needed for auditory detection (hearing threshold) was obtained by calculating otolith displacement (~0.002 mm) under modeled conditions. Modeling was then repeated with particle displacement reduced to 0.8 nm, where the simulated control otolith no longer reached the hearing threshold but the 2,100 μatm pCO₂ treatment otolith did. Hearing range was calculated assuming cylindrical spreading of sound with distance (r) from the source and amplitude decreasing as \( 1/r \) (13).

**Data Analysis.** Data were analyzed in SYSTAT software (version 11; SYSTAT) using tank means, following verification of normality and homoscedasticity by Shapiro–Wilk and Bartlett tests. Data from the right or left sagittal and lapillar otoliths were randomly selected from each fish to test volume and surface area using analysis of covariance (ANCOVA), for which surface area or volume was the response variable, pCO₂ a fixed factor, and SL the continuous covariate. Adjusted means of surface area and volume data calculated during ANCOVA were subsequently used to produce SA:V and relative mass data, using mean mass of control treatments as a reference. Relative densities of all otoliths were calculated in reference to mean control treatment otolith density. Larval SL, relative otolith density, SA:V, and relative mass were tested using analysis of variance (ANOVA), with each as the response variable and pCO₂ as a fixed factor. SA:V and relative otolith mass data were log transformed to correct for lack of homoscedasticity. See Table 2 for summary statistics.

Fig. 3. Simulated otolith displacement amplitude and hearing range for larval cobia under elevated-pCO₂ conditions. (A) When exposed to a simulated 0.8-nm amplitude 100 Hz sound wave, otoliths at 2,100 μatm pCO₂ (red line) had greater otolith displacement than those at 800 μatm pCO₂ (orange line) or controls (300 μatm pCO₂, blue line), thereby reaching the hearing threshold (dotted horizontal line) when 800 μatm and control otoliths did not. (B) Assuming loss of sound amplitude by cylindrical spreading (13), 300 μatm pCO₂ fish (blue) had hearing range 3 and 800 μatm pCO₂ fish (orange) had a 10% greater range, but those at 2,100 μatm pCO₂ (red) had 50% greater range due to the lower sound amplitude necessary for threshold otolith displacement.
ACKNOWLEDGMENTS. We thank D. Gledhill and L. Jewett of the National Oceanic and Atmospheric Administration's Ocean Acidification Program for providing the micro-CT scanner; T. Capo, D. Benetti, C. Langdon, E. Bryant, and K. Ternus for facility use and logistical assistance; M. Grosell, J. Dallman, Z. Lu, and E. Staaterman for discussions; and the University of Miami Experimental Hatchery. This study was supported by grants from the National Science Foundation GK-12 program, University of Miami Maytag Ichthyology Chair, Guy Harvey Ocean Foundation, Florida Sea Grant, International Light Tackle Tournament Association, Manasquan River Marlin and Tuna Club, and the Yamaha Condé/Miami Billfish Tournament.