Elevated CO_2 Enhances Otolith Growth in Young Fish

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A large fraction (0.3 to 0.5) of the carbon dioxide (CO_2) added to the atmosphere by human burning of fossil fuels enters the ocean (*1*). This causes ocean acidification by increasing the concentrations of oceanic CO_2, bicarbonate (HCO_3^-) and hydrogen (H^+) ions and decreasing the concentration of carbonate (CO_3^{2-}) ion and hence the saturation state of calcium carbonate (Ω) (*1*). Addition of CO_2 to the atmosphere and ocean may thus influence the rates of formation and dissolution of aragonite and calcite, biominerals that are critical to diverse marine taxa. Although some recent studies have shown that elevated CO_2 enhances structural calcification in coccolithophores and invertebrates, most studies have shown a slowing of structural calcification (*2*). Otoliths are bony structures used by fish to sense orientation and acceleration and consist of aragonite-protein bilayers, which document fish age and growth. We hypothesized that otoliths in eggs and larvae reared in seawater with elevated CO_2 would grow more slowly than they do in seawater with normal CO_2. To test our hypothesis, we grew eggs and prefeeding larvae of white sea bass (*Atractoscion nobilis*) under a range of CO_2 concentrations and measured the size of their sagittal otoliths by using a scanning electron microscope (Fig. 1, A to C) (*3*).

In each experiment, we incubated eggs and larvae in seawater under control (380 μatm of CO_2, 1 atm = 101.325 kPa) and treatment (993 or 2558 μatm of CO_2) atmospheres. Initial experiments 1 and 2 used 2558 μatm of CO_2 to test whether elevated CO_2, resulting in aragonite undersaturation in the seawater, affected otolith size. Experiments 3 and 4 used 993 μatm of CO_2, an atmospheric concentration ~2.5 times the present concentration that may occur by 2100 (*4*). Contrary to expectations, the otoliths of fish grown in seawater with high CO_2, and hence lower pH and $\Omega_{aragonite}$, were significantly larger than those of fish grown under simulations of present-day conditions (Fig. 1D and table S1). For 7- to 8-day-old fish grown under 993 and 2558 μatm of CO_2, the areas of the otoliths were 7 to 9% and 15 to 17% larger, respectively, than those of control fish grown under 380 μatm of CO_2. Assuming otolith density is constant and that volume is proportional to area$^{1.5}$ (*3*), we estimate otolith masses were 10 to 14% and 24 to 26% greater, respectively, for fish under 993 and 2558 μatm of CO_2. The dry mass of fish did not vary with CO_2 (*3*), and thus fish of the same size had larger otoliths when grown under elevated CO_2.

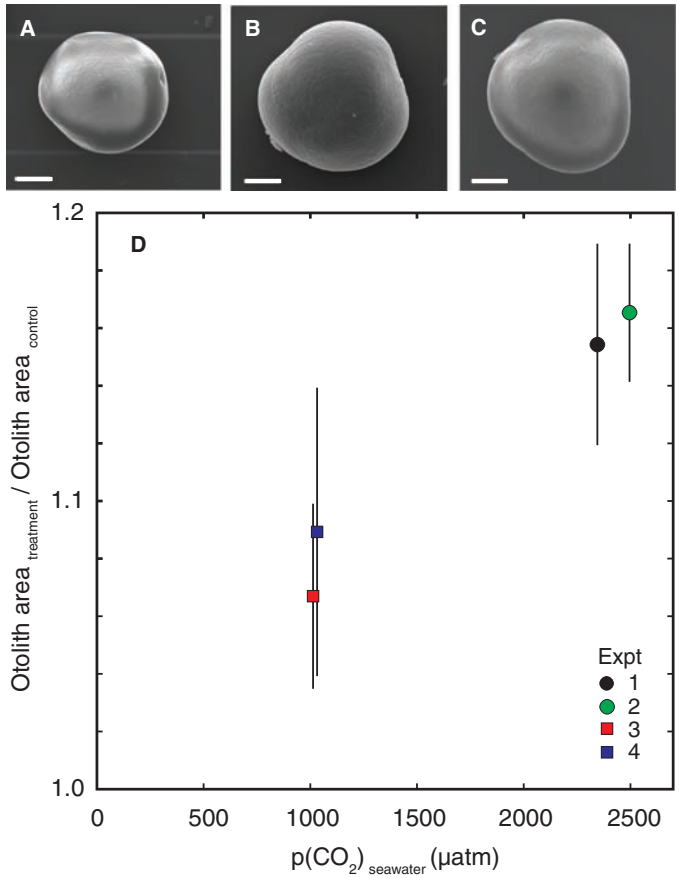

Fig. 1. Dorsal view of sagittal otoliths of 7-day-old white sea bass grown at (**A**) 430, (**B**) 1000, and (**C**) 2500 μatm $p(CO_2)_{seawater}$. Scale bars indicate 10 μm. (**D**) Ratio (treatment/control) of otolith area in relation to $p(CO_2)_{seawater}$. Mean ratios and their associated uncertainties (*3*) are plotted. The control level $p(CO_2)_{seawater}$ was ~430 μatm [$p(CO_2)_{atmosphere}$ ~ 380 μatm], for which otolith area ratio = 1.

Our results are consistent with young fish being able to control the concentration of ions (H^+ and Ca^{2+}), but not the neutral molecule CO_2, in the endolymph surrounding the otolith. Gases in tissues of fish eggs and larvae equilibrate rapidly with seawater by cutaneous exchange (*5*) but may also be affected by acid-base regulation (*6*). In the endolymph, with constant pH, elevated CO_2 increases CO_3^{2-} concentration and thus the $\Omega_{aragonite}$, accelerating formation of otolith aragonite. This is a fundamentally different effect of elevated CO_2 on marine biomineralization than those in previous reports on acidification (*1*, *2*).

We do not know whether our results apply to other taxa with aragonite sensory organs, such as squid and mysids (statoliths) or other fish species. Nor do we know whether larger otoliths have a deleterious effect, although we do know that asymmetry between otoliths can be harmful (*7*).

Our results indicate the need to understand the diverse effects of elevated CO_2 on biomineralization over taxa and developmental stages. The specific effects of elevated CO_2, not simply acidification, should be considered. Calcification and dissolution of calcium carbonate occur sequentially and often at different locations and under different conditions. Whatever the organism, to predict the effects of elevated CO_2, we need to know the mechanisms of production and dissolution and their relationships to changing seawater chemistry.

References and Notes

1. J. C. Orr *et al.*, *Nature* **437**, 681 (2005).
2. S. C. Doney *et al.*, *Annu. Rev. Mar. Sci.* **1**, 169 (2009).
3. Materials and methods are available as supporting material on *Science* Online.
4. Intergovernmental Panel on Climate Change (IPCC), *Climate Change 2007: Synthesis Report* (IPCC, Geneva, 2007).
5. B. Pelster, *Comp. Biochem. Physiol. A* **124**, 407 (1998).
6. H. O. Pörtner, A. P. Farrell, *Science* **322**, 690 (2008).
7. M. Gagliano *et al.*, *Proc. R. Soc. London Ser. B* **275**, 527 (2008).

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Supporting Online Material

www.sciencemag.org/cgi/content/full/324/5935/1683/DC1
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Supplementary Information

Methods

Oceanic seawater (33.7 salinity) collected WNW of San Diego, California (34.0°N, 121.1°W) was used in all experiments. The day before the start of an experiment, filtered seawater (FSW) was prepared using Whatman GF/F (0.6 μm nominal pore size) glass fiber filters.

Incubations were in two (control, treatment) water-jacketed, glass vessels with 4-L FSW. Incubation temperature was maintained at 18.0 ± 0.1°C with a water bath. A single fluorescent lamp illuminated both vessels 8am–8pm; the vessels were dark 8pm-8am. Each vessel was sealed with a lid of PVC with holes for the in- and outflow of gas. Inflow was regulated at 40–50 ml min^{-1} through a glass tube extending to the bottom of the vessel. The control gas was air with 380 ppm CO_2. The treatment gases were air with 993 (Expts. 3, 4) or 2558 (Expts. 1, 2, 5) ppm CO_2. The day before the start of an experiment, the experimental vessels were filled with FSW and equilibrated with temperature and gas overnight.

Eggs of white seabass (*Atractoscion nobilis*) were obtained from the Hubbs-SeaWorld Research Institute the morning after being spawned and fertilized. For each experiment, six groups of 50 fertilized eggs, each with a single oil globule, were rinsed three times in FSW. Each experiment was started by placing three, randomly selected groups of 50

fertilized eggs in each of the control and treatment vessels, covering each vessel with its lid, and continuing gas infusion. Each experiment continued until either 7 or 8 days post-fertilization (dpf).

At termination, samples were first taken of water and then of larvae. Replicate seawater samples were analyzed for total alkalinity and dissolved inorganic carbon. Salinity was also measured. From these data, seawater $p(CO_2)$, pH, and $\Omega_{aragonite}$ were calculated using the program CO2SYS (http://cdiac.ornl.gov/oceans/co2rprt.html). Live larvae were removed individually by pipette and placed either in 95% EtOH (for SEM, Expts. 1–4) or on Teflon (for weighing, Expt. 5).

SEM –The sagittal otoliths of each larva were removed and transferred to an SEM stub, coated with platinum, and imaged at 4000× magnification. The area (μm^2) and circularity ($4\pi \times \text{area}/\text{perimeter}^2$) of each otolith were measured using NIH ImageJ. Only data from otoliths oriented with a full view of the dorsal or ventral surface were used.

Mass – Larvae were dried on Teflon at 60°C for 24 h. Individual larvae were removed from the Teflon and their dry mass measured to the nearest μg.

Results

The otoliths of treatment (~1000, ~2500 μatm CO_2) fish were significantly larger in area than the otoliths of control (~430 μatm CO_2) fish in each experiment (Table S1). Otoliths of fish 8 dpf were significantly larger than those of fish 7 dpf. A 2-way ANOVA showed significant effects of both CO_2 and age, but no interaction. To account for age differences, we present the ratio of the areas of otoliths of treatment to those of control fish (Fig. 1, Table S1).

There was no significant effect of CO_2 on the shape (circularity) of otoliths viewed laterally and, thus, volume was proportional to area$^{1.5}$. Otoliths are greater than 99% aragonite, by mass (*1*), and thus aragonite, not protein, comprised the observed increase in otolith size.

The dry mass of fish in Expt. 5 did not vary significantly between control (438 μatm CO_2, 69 ± 1 μg dry mass fish^{-1} [n = 30]) and treatment (2498 μatm CO_2, 68 ± 1 μg dry mass fish^{-1} [n = 29]).

Discussion

Prior studies (*2*,*3*) relating carbonate formation by fish to elevated CO_2 used juveniles and adults, whereas we used eggs and larvae. Much less is known of the effects of elevated CO_2 on eggs and larvae than juveniles and adults. Gas exchange is by cutaneous diffusive transport in eggs and larvae and by gills and blood in juveniles and adults and hemoglobin appears only at metamorphosis (*4*,*5*).

One-year old freshwater trout (*Oncorhynchus mykiss*) stressed with chlorine gas (Cl_2) had higher endolymph CO_2 but reduced growth on the proximal edge of the otolith viewed in the sagittal plane (*2*). The higher endolymph CO_2 was hypothesized to result from the sequestration of Ca^{2+} by endolymph protein, which increased 2.6× under Cl_2 stress, causing a decrease otolith growth and an accumulation of HCO_3^-. We reared eggs and larvae of a marine fish in seawater with elevated CO_2 but no other stress. The differing stage of fish, type of stress, and lack of comparable data preclude easy comparison of the results of these two studies. Future investigation of the effects of elevated seawater CO_2 on otolith formation by marine fish would benefit from direct measurements of endolymph and plasma chemistry.

Carbonate precipitates in the guts of fish may contribute 3-15% of total oceanic carbonate production (*3*). Marine fish produce carbonates in the gut as a by-product of their osmoregulation in calcium-rich seawater. Rising CO_2 is hypothesized to elevate CO_2 in the blood of marine fish, stimulate HCO_3^- production by intestinal cells and, thus, enhance intestinal secretion of precipitated carbonates. We used eggs and larvae whereas post-metamorphic fish were considered in the study of gut carbonates. Both studies predict enhanced biomineralization by marine fish with elevated CO_2, albeit by different mechanisms.

References

1. G. Borelli, N. Mayer-Gostan, *et al*., *Calcif. Tiss. Int.* **72**, 717 (2003).

2. P. Payan, H. De Pontual, *et al*., *Can. J. Fish. Aq. Sci.* **61**, 1247-1255 (2004).

3. R.W. Wilson, F.J. Millero *et al*., *Science* **323**, 359-362 (2009).

4. B. Pelster, *Comp. Biochem. Physiol.*, **A124**, 407-412 (1999).

5. P.J. Rombough, Chapter 2. In Hoar, W.S., and D.J. Randall (eds.), *Fish Physiology. XI. The Physiology of Developing Fish.* Academic Press, San Diego, 59-161 (1988).

Table S1. Experimental conditions and results. Experiments 1–4 investigated the effects of CO_2 on otolith area. Experiment 5 investigated the effect of CO_2 on fish dry mass. Dates are from fertilization to termination of experiment. Age is days from fertilization to termination. $p(CO_2)_{atm}$ is the partial pressure of CO_2 in air in the gas infusion. $p(CO_2)_{sw}$ is the partial pressure of CO_2 in seawater at the termination of the experiment. Otolith area mean ($\bar{x}$) and standard error [$\sigma(\bar{x})$] are for otoliths of fish (N, number of fish) in control and treatment conditions. Treatment to control area ratios are the ratios of mean values. 'nd', no data.

Expt. No.	Dates	Age	$p(CO_2)_{atm}$	Salinity	Total Alkalinity	Dissolved Inorganic Carbon	Ω_{arag}*	pH	$p(CO_2)_{sw}$	Otolith Area				
										$\bar{x}$	$\sigma(\bar{x})$	N	Treatment to Control Area Ratio	Treatment to Control Area Ratio Uncertainty□
		days	µatm		µmol kg^{-1}	µmol kg^{-1}			µatm	µm^2	µm^2			
1	26 Apr–3 May 2007	7	380	33.66	2258	2043	2.44	8.01	432	1330	34	14	1.155	0.035
			2558	33.65	2269	2293	0.61	7.34	2345	1536	25	16		
2	24–31 May 2007	7	380	33.67	2251	2043	2.37	8.00	448	1341	22	15	1.166	0.024
			2558	33.68	2257	2289	0.57	7.32	2496	1563	19	15		
3	20–28 Sept 2007	8	380	33.67	2260	2043	2.46	8.02	428	2133	43	16	1.067	0.032
			993	33.69	2260	2176	1.26	7.69	1013	2276	51	16		
4	16–24 Jan 2008	7	380	33.71	2262	2049	2.42	8.01	438	1419	64	8	1.089	0.050
			993	33.74	2260	2179	1.24	7.68	1032	1546	15	15		
5	23–30 May 2008	7	380	33.72	2256	2043	2.41	8.01	438	nd	nd	nd	nd	nd
			2558	33.72	2256	2289	0.56	7.32	2498	nd	nd	nd		

* $\Omega_{arag} = [Ca^{2+}][CO_3^{2-}] / K_{sp(arag)}$, where $K_{sp(arag)}$ is the stoichiometric solubility product of aragonite at the measured salinity and a temperature of 18.0°C, $[Ca^{2+}]$ is estimated from the salinity and $[CO_3^{2-}]$ is calculated from the total alkalinity and total dissolved inorganic carbon.

□ Calculated using the expression $u(\bar{x}_T / \bar{x}_C) = (\bar{x}_T / \bar{x}_C) \times \sqrt{(\sigma(\bar{x}_T)/\bar{x}_T)^2 + (\sigma(\bar{x}_C)/\bar{x}_C)^2}$, where u is the uncertainty, $\bar{x}_T$ is the mean value of the area of otoliths subjected to the treatment, and $\bar{x}_C$ the mean area of otoliths in the corresponding control experiment.