

Reduced early life growth and survival in a fish in direct response to increased carbon dioxide

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Absorption of anthropogenic carbon dioxide by the world's oceans is causing mankind's 'other CO₂ problem', ocean acidification¹. Although this process will challenge marine organisms that synthesize calcareous exoskeletons or shells^{2–6}, it is unclear how it will affect internally calcifying organisms, such as marine fish⁷. Adult fish tolerate short-term exposures to CO₂ levels that exceed those predicted for the next 300 years (~2,000 ppm; ref. 8), but potential effects of increased CO₂ on growth and survival during the early life stages of fish remain poorly understood⁷. Here we show that the exposure of early life stages of a common estuarine fish (*Menidia beryllina*) to CO₂ concentrations expected in the world's oceans later this century caused severely reduced survival and growth rates. When compared with present-day CO₂ levels (~400 ppm), exposure of *M. beryllina* embryos to ~1,000 ppm until one week post-hatch reduced average survival and length by 74% and 18%, respectively. The egg stage was significantly more vulnerable to high CO₂-induced mortality than the post-hatch larval stage. These findings challenge the belief that ocean acidification will not affect fish populations, because even small changes in early life survival can generate large fluctuations in adult-fish abundance^{9,10}.

Since the industrial revolution, average atmospheric and oceanic CO₂ concentrations have risen by 40% to 393 ppm (ref. 11; in 2011); levels that now far exceed those of the past one million years (180–280 ppm; ref. 12). Current emission scenarios predict that CO₂ concentrations will increase further and reach ~800 ppm during this century and potentially 2,000 ppm by the year 2300 (ref. 8). Apart from accelerating global climate change, another major concern is the absorption of CO₂ by the world's oceans and the resulting decrease in ocean pH, carbonate ion concentration (CO₃²⁻) and calcium carbonate (CaCO₃) saturation state (Ω ; refs 1,2,4). Collectively known as ocean acidification, these shifts in marine chemistry will probably alter the phenotypes and hence the fitness of many marine organisms, particularly those with exoskeletons and shells made from calcium carbonate (CaCO₃; ref. 3). Experimentally increased CO₂ conditions have been shown to adversely affect many species of foraminifers¹³, coccolithophores¹⁴, corals³, pteropods², bivalves^{5,6}, crustaceans¹⁵ and echinoderms¹⁶.

In contrast, direct effects of ocean acidification on fish, the world's most important marine resource, are at present assumed to be negligible^{4,7,17}. Fish calcify internal (bones, otoliths) rather than external skeletal elements, and as highly mobile vertebrates have evolved effective acid–base and osmoregulatory mechanisms to overcome high metabolic CO₂ levels⁴. The high CO₂ tolerance of juvenile and adult fish has been extensively documented for decades, suggesting no measureable growth or survival effects even at exposures of up to 16,000 ppm CO₂ (ref. 7; eight times the relevant level for future climate change scenarios). However, the

susceptibility of the earliest life stages of fish to increased CO₂ levels has not yet been sufficiently examined⁷, even though CO₂ sensitivity is highest during the larval stages in most other marine organisms^{5,6,16}. Recent studies have documented that increased CO₂ levels (>700 ppm) can have detrimental behavioural effects on larval reef fish by impairing their ability to detect olfactory cues of predators or nearby reefs^{18–20}. White sea bass larvae reared at 1,000–2,500 ppm CO₂ were shown to grow abnormally heavy otoliths²¹. Despite such signs of CO₂ sensitivity in larval fish, however, direct CO₂ effects on larval-fish growth or survival have yet to be demonstrated^{7,22}.

Here we report on a series of controlled CO₂ exposure experiments with embryos of the inland silverside *Menidia beryllina*. This small, schooling fish occurs naturally in estuaries along the North American Atlantic coast, where it is of ecological importance as both a zooplanktivore and prey for higher trophic animals. In addition, *M. beryllina* is commonly used as a model species in environmental risk assessments. In five separate experiments, we exposed a known number of newly fertilized eggs (<24 h old) to replicated CO₂ concentrations ranging from ~390 to 1,100 ppm (Supplementary Tables S1–S5). CO₂ levels were carefully administered and monitored by adhering to established best practices for ocean acidification research (European Project on Ocean Acidification²³). Approximately one week after hatching, the surviving larvae were counted, photographed and measured for length.

Our results were surprisingly unambiguous. Despite variable control survival rates, each experiment revealed a consistent decline in larval survival with increasing CO₂ concentration (experiment 1, analysis of variance $F_{1,4} = 5.2$, $p = 0.08$; experiments 2–5, four analyses of variance, $F_{1-3,6-12} = 14.5-38.9$, $p < 0.009$; Fig. 1a). When averaged across replicate means, an exponential decay model best fitted the entire data set ($r^2 = 0.80$) and indicated a 74% reduction in average survival at increased (~1,000 ppm) when compared with control levels (~400 ppm).

An alternative interpretation of the patterns in Fig. 1a is that CO₂ sensitivity in *M. beryllina* remained essentially stable until reaching a threshold (650–800 ppm, consistent with refs 18,20), beyond which survival and growth (Fig. 1a,b) sharply declined. Regardless of the functional relationship, *M. beryllina* seems to show a direct link between early life mortality and the levels of oceanic CO₂ predicted for the twenty-first century. Whether this is common among fish is at present unknown. However, the question has important implications for future fish stock abundance, because larval survival mediates the strength of new year classes in fish populations, including those of commercial importance^{9,10}.

Compounding these concerns, we also observed a consistent decrease in larval length with increasing CO₂ concentration. Average lengths (± 1 s.e.m.) of survivors approximately one week post-hatch declined significantly ($F_{1,6} = 30.6$, $p = 0.001$)

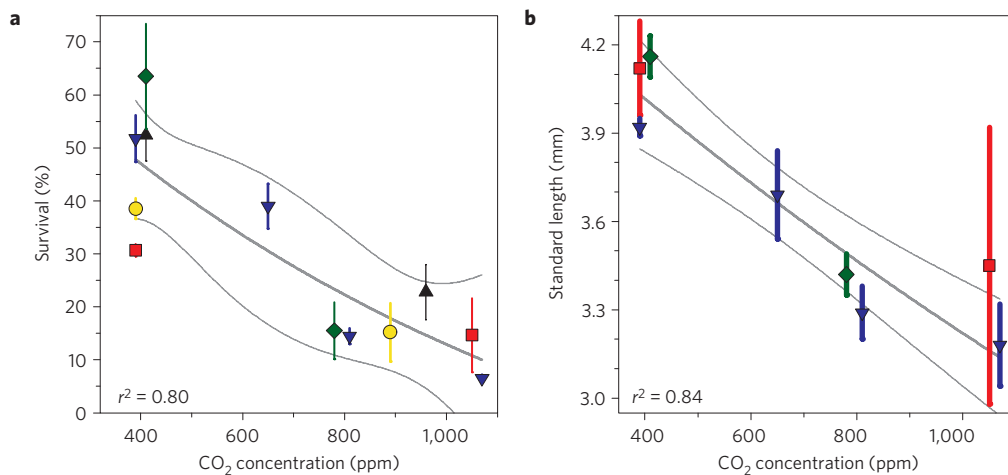


Figure 1 | Effect of increased CO₂ on early life *M. beryllina* survival and length. **a**, Survival was averaged across replicates (experiment 1, $n = 3$; experiments 2, 3, $n = 4$; experiment 4, $n = 6$; experiment 5, $n = 5$) for each experiment and CO₂ level. **b**, Weighted means (± 1 s.e.m.) of standard length averaged across replicates per experiment and CO₂ level. Pooled data in **a** and **b** were fitted with an exponential decay model (thick grey line) with 95% confidence intervals (thin grey lines). Experiment 1, red squares; experiment 2, blue down triangles; experiment 3, green diamonds; experiment 4, yellow circles; experiment 5, black up triangles. Points represent means ± 1 s.d.

by 18% from 4.0 ± 0.07 mm at control levels (400 ppm CO₂) to 3.3 ± 0.3 mm at 1,000 ppm CO₂ (Fig. 1b). In most fish species, larval growth and mortality rates are inversely related, because slower-growing larval cohorts are vulnerable for longer to the suite of ichthyoplankton predators and therefore experience higher cumulative mortalities^{24,25}. Reduced early life growth may therefore further reduce the productivity of fish stocks in future acidified oceans.

Precisely how increased CO₂ levels affect survival and growth in fish early life stages is at present unknown. For some fish species, the explanation may involve the high surface-to-volume ratio of eggs and larvae, which may make individuals more vulnerable to diffusive processes across epithelia²⁶. The heightened CO₂ sensitivity of the earliest life stages may further reflect poorly developed mechanisms of acid–base regulation and cardiorespiratory control, as both are probably linked to increased gill function and muscle activity due to swimming in later-stage larvae, juveniles and adults²⁷. Third, even if fish embryos and early larvae are capable of some level of physiological adaptation to increased CO₂, this would incur further metabolic costs⁷ and thus reduce energy available for tissue synthesis (growth reduction) or post-hatch survival on diminished yolk reserves. As some fish eggs, including those of *M. beryllina*, seem to be tolerant of low-pH conditions²⁸, the high levels of CO₂ or associated changes in carbonate chemistry may be more important to larval-fish survival than hydrogen ion concentrations.

It is possible that the earliest life stages of fish (that is, embryos in eggs) are most susceptible to CO₂. To test this hypothesis, a further experiment was conducted where CO₂ exposure of *M. beryllina* was switched from control (410 ppm) to increased (780 ppm) levels only after eggs had hatched (5 days after fertilization, Fig. 2). Survivorship five days later was then compared with constant-control and constant-increased CO₂ treatments (10 days of 410 or 780 ppm, respectively). As survival in the ‘switch’ group was only marginally lower than in the constant-control group (t -test, $df = 6$, $p = 0.5$), but significantly higher than in the constant-increased group (t -test, $df = 6$, $p < 0.01$), the main CO₂ impact evidently occurred during the egg stage (Fig. 2). This may partly explain why studies so far that have carried out experiments on fish larvae, but not on eggs, have not observed the negative effects of ocean acidification^{21,22}. Furthermore, one day post-hatch *M. beryllina* showed a significantly (t -test, $df = 10$, $p < 0.01$) higher percentage of malformations in increased (37%, 960 ppm) when

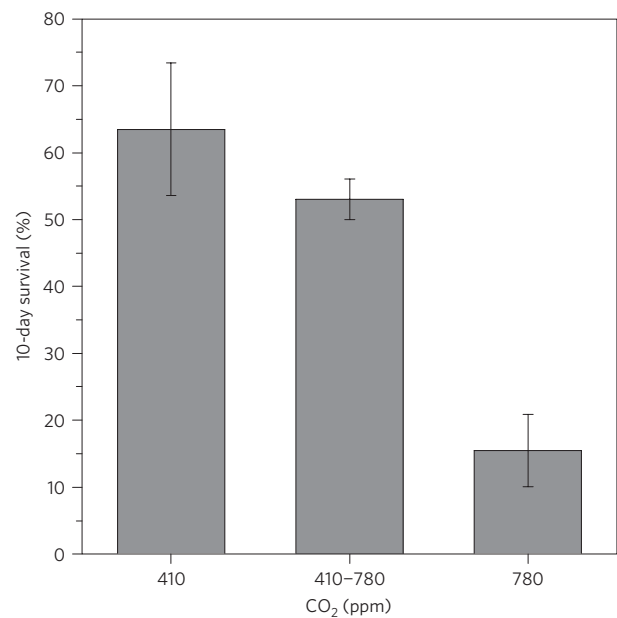


Figure 2 | CO₂ sensitivity of the egg versus early post-hatch stage in *M. beryllina*. Bars depict average survival (± 1 s.e.m.) 10 days after fertilization in control (410 ppm), increased (780 ppm) and ‘switch’, where CO₂ concentration was increased only after eggs hatched (5 days post-fertilization), treatments. Precise CO₂ levels and complete carbonate chemistry from experiments appear in Supplementary Tables S1–S5.

compared with control CO₂ levels (7%, 410 ppm), supporting the particular sensitivity of the egg stage (Fig. 3).

In summary, we present evidence of direct adverse growth and survival effects in the early life stages of fish due to exposure to CO₂ levels that are expected in the world’s oceans later this century. Together with other emerging evidence^{18–21}, such apparently high CO₂ sensitivity contradicts the notion that ocean acidification will have no direct consequences for marine fish populations. Furthermore, the CO₂ levels used in our experiments already occasionally occur in temperate coastal waters, often coinciding with the spawning season of fish^{29,30}. Hence, CO₂-induced offspring mortality may already be influencing patterns of adult-fish abundance in the ocean. We



Figure 3 | *M. beryllina* larvae exposed to normal and elevated levels of CO₂. **a–c**, Larvae with curved or curled bodies were significantly more common at increased (**b,c**) when compared with control (**a**) CO₂ levels. Scale bar = 1 mm.

expect, however, in a manner similar to what is now emerging from studies on invertebrates^{1,3–5}, that responses to increased CO₂ levels in fish will be highly species specific. For example, oceanic fish species that spawn pelagic eggs might be more susceptible to CO₂ increases than benthic spawners²², where eggs may be more adapted to natural CO₂ fluctuations due to elevated rates of microbial respiration.

In light of the broad implications of our findings, we believe that there is now a need to comprehensively investigate not only the incidence, the physiological causes and the form of the functional response of early life CO₂ sensitivity in fish, but also the general potential of marine organisms to adapt to the CO₂ levels projected for future oceans. Our study indicates that future work should focus on the earliest life stages, as the effects of increased CO₂ may be especially acute during this phase of development.

Methods

All five experiments were conducted between July 2010 and January 2011, using <24-h-old *M. beryllina* embryos obtained from a large, commercial brood stock (Aquatic Research Organisms). A known number of eggs (experiments 1–3, $n = 100$; experiments 4, 5, $n = 50$) was randomly placed in each of three to five replicate rearing containers (41 per CO₂ level (390–1,060 ppm, Supplementary Tables S1–S5). An air: CO₂ mix, adjusted to desired levels by a gas proportionator system (Cole Parmer Flowmeter), was continuously delivered to each rearing container. Attained CO₂ levels were calculated with the program CO2SYS on the basis of measured total inorganic carbon (EGM-4 Environmental Gas Analyser, PP Systems), pH, temperature and salinity. Fish were reared at constant temperature (24 °C), salinity (30) and photoperiod levels (15L:9D) and were provided with live rotifers and brine shrimp nauplii after hatching. Survival was measured 7 ± 2 days post-hatch as the percentage of live larvae relative to the number of seeded eggs. Our protocols ensured that potential mortality sources unrelated to CO₂, for example due to varying egg quality, transport and handling, minor fluctuations in food and water quality, or the shape of our rearing containers, were random between replicated treatments and experiments. Standard lengths of survivors were measured using calibrated digital pictures and image analysis software (ImagePro 4.5.1, Media Cybernetics). All reported p and F values were derived from analyses of variance to compare survival (arc-sine transformed) and length between CO₂ treatments (PASW Statistics 18). Further details regarding methods are available as Supplementary Methods.

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References

- Doney, S. C., Fabry, V. J., Feely, R. A. & Kleypas, J. A. Ocean acidification: The other CO₂ problem. *Annu. Rev. Mar. Sci.* **1**, 169–192 (2009).
- Orr, J. C. *et al.* Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature* **437**, 681–686 (2005).
- Kleypas, J. A. *et al.* *Impacts of Ocean Acidification on Coral Reefs and Other Marine Calcifiers: A Guide for Future Research* (Report of a workshop held 18–20 April 2005, St. Petersburg, FL, Sponsored by NSF, NOAA, and the US Geological Survey, 2006).
- Fabry, V. J., Seibel, B. A., Feely, R. A. & Orr, J. C. Impacts of ocean acidification on marine fauna and ecosystem processes. *ICES J. Mar. Res.* **65**, 414–432 (2008).
- Talmage, S. C. & Gobler, C. J. Effects of past, present, and future ocean carbon dioxide concentrations on the growth and survival of larval shellfish. *Proc. Natl Acad. Sci. USA* **107**, 17246–17251 (2010).
- Kurihara, H., Kato, S. & Ishimatsu, A. Effects of increased seawater p_{CO_2} on early development of the oyster *Crassostrea gigas*. *Aquat. Biol.* **1**, 91–98 (2007).
- Ishimatsu, A., Hayashi, M. & Kikkawa, T. Fishes in high-CO₂, acidified oceans. *Mar. Ecol. Prog. Ser.* **373**, 295–302 (2008).
- Caldeira, K. & Wickett, M. E. Anthropogenic carbon and ocean pH. *Nature* **425**, 365–365 (2003).
- Sissenwine, M. P. in *Exploitation of Marine Communities* (ed. May, R.) 59–94 (Springer, 1984).
- Trippel, E. A. & Chambers, R. C. in *Early Life History and Recruitment in Fish Populations* (eds Chambers, R. C. & Trippel, E. A.) xxi–xxxii (Chapman & Hall, 1997).
- www.esrl.noaa.gov/gmd/ccgg/trends/.
- Tripathi, A. K., Roberts, C. D. & Eagle, R. A. Coupling of CO₂ and ice sheet stability over major climate transitions of the last 20 million years. *Science* **326**, 1394–1397 (2009).
- Spero, H. J., Bijma, J., Lea, D. W. & Bemis, B. E. Effect of seawater carbonate concentration on foraminiferal carbon and oxygen isotopes. *Nature* **390**, 497–500 (1997).
- Riebesell, U. *et al.* Reduced calcification of marine plankton in response to increased atmospheric CO₂. *Nature* **407**, 364–367 (2000).
- Kurihara, H., Matsui, M., Furukawa, H., Hayashi, M. & Ishimatsu, A. Long-term effects of predicted future seawater CO₂ conditions on the survival and growth of the marine shrimp *Palaemon pacificus*. *J. Exp. Mar. Biol. Ecol.* **367**, 41–46 (2008).
- Dupont, S., Havenhand, J., Thorndyke, W., Peck, L. & Thorndyke, M. C. Near-future level of CO₂-driven ocean acidification radically affects larval survival and development in the brittlestar *Ophiothrix fragilis*. *Mar. Ecol. Prog. Ser.* **373**, 285–294 (2008).
- Hayashi, M., Kita, J. & Ishimatsu, A. Acid–base responses to lethal aquatic hypercapnia in three marine fishes. *Mar. Biol.* **144**, 153–160 (2004).
- Munday, P. L. *et al.* Ocean acidification impairs olfactory discrimination and homing ability of a marine fish. *Proc. Natl Acad. Sci. USA* **106**, 1848–1852 (2009).
- Munday, P. L. *et al.* Replenishment of fish populations is threatened by ocean acidification. *Proc. Natl Acad. Sci. USA* **107**, 12930–12934 (2010).
- Dixon, D. L., Munday, P. L. & Jones, G. P. Ocean acidification disrupts the innate ability of fish to detect predator olfactory cues. *Ecol. Lett.* **13**, 68–75 (2010).
- Checkley, D. M. *et al.* Elevated CO₂ enhances otolith growth in young fish. *Science* **324**, 1683–1683 (2009).
- Munday, P. L., Gagliano, M., Donelson, J. M., Dixon, D. L. & Thorrold, S. R. Ocean acidification does not affect the early life history development of a tropical marine fish. *Mar. Ecol. Prog. Ser.* **423**, 211–221 (2011).
- Riebesell, U., Fabry, V. J., Hansson, L. & Gattuso, J. P. in *Guide to Best Practices for Ocean Acidification Research and Data Reporting* (Publications Office of the European Union, 2010).
- Anderson, J. T. A review of size dependent survival during pre-recruit stages of fishes in relation to recruitment. *J. Northw. Atl. Fish. Sci.* **8**, 55–66 (1988).

25. Leggett, W. C. & DeBlois, E. Recruitment in marine fishes: Is it regulated by starvation and predation in the egg and larval stages? *Neth. J. Sea Res.* **32**, 119–134 (1994).
26. Mangor-Jensen, A. Water balance in developing eggs of the cod *Gadus morhua* L. *Fish Physiol. Biochem.* **3**, 17–24 (1987).
27. Perry, S. F. & Gilmour, K. M. Acid–base balance and CO₂ excretion in fish: Unanswered questions and emerging models. *Respir. Physiol. Neurobiol.* **154**, 199–215 (2006).
28. Hemmer, M. J., Middaugh, D. P. & Moore, J. C. Effects of temperature and salinity on *Menidia beryllina* embryos exposed to terbufos. *Dis. Aquat. Org.* **8**, 127–136 (1990).
29. Feely, R. A., Sabine, C. L., Hernandez-Ayon, J. M., Ianson, D. & Hales, B. Evidence for upwelling of corrosive ‘acidified’ water onto the continental shelf. *Science* **320**, 1490–1492 (2008).
30. Salisbury, J., Green, M., Hunt, C. & Campbell, J. Coastal acidification by rivers: A threat to shellfish? *EOS Trans. AGU* **89** <http://dx.doi.org/10.1029/2008EO500001> (2008).

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Author contributions

H.B., S.C.T. and C.J.G. designed the experiments, conducted the experiments, generated the data, analysed samples, analysed the data and wrote the manuscript.

Additional information

The authors declare no competing financial interests. Supplementary information accompanies this paper on www.nature.com/natureclimatechange. Reprints and permissions information is available online at <http://www.nature.com/reprints>. Correspondence and requests for materials should be addressed to C.J.G.