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# 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 $CO_2$ problem', ocean acidification[1]. 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 fish[7]. Adult fish tolerate short-term exposures to $CO_2$ levels that exceed those predicted for the next 300 years (~2,000 ppm; ref. 8), but potential effects of increased $CO_2$ on growth and survival during the early life stages of fish remain poorly understood[7]. Here we show that the exposure of early life stages of a common estuarine fish (*Menidia beryllina*) to $CO_2$ concentrations expected in the world's oceans later this century caused severely reduced survival and growth rates. When compared with present-day $CO_2$ 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 $CO_2$-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 $CO_2$ 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 $CO_2$ 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 $CO_2$ by the world's oceans and the resulting decrease in ocean pH, carbonate ion concentration ($CO_3^{-2}$) and calcium carbonate ($CaCO_3$) saturation state ($\Omega$; 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 ($CaCO_3$; ref. 3). Experimentally increased $CO_2$ conditions have been shown to adversely affect many species of foraminifers[13], coccolithophores[14], corals[3], pteropodes[2], bivalves[5,6], crustaceans[15] and echinoderms[16].

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 $CO_2$ levels[4]. The high $CO_2$ 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 $CO_2$ (ref. 7; eight times the relevant level for future climate change scenarios). However, the susceptibility of the earliest life stages of fish to increased $CO_2$ levels has not yet been sufficiently examined[7], even though $CO_2$ sensitivity is highest during the larval stages in most other marine organisms[5,6,16]. Recent studies have documented that increased $CO_2$ 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 $CO_2$ were shown to grow abnormally heavy otoliths[21]. Despite such signs of $CO_2$ sensitivity in larval fish, however, direct $CO_2$ effects on larval-fish growth or survival have yet to be demonstrated[7,22].

Here we report on a series of controlled $CO_2$ 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 $CO_2$ concentrations ranging from ~390 to 1,100 ppm (Supplementary Tables S1–S5). $CO_2$ levels were carefully administered and monitored by adhering to established best practices for ocean acidification research (European Project on Ocean Acidification[23]). 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 $CO_2$ 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 $CO_2$ 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 $CO_2$ 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 $CO_2$ concentration. Average lengths (±1 s.e.m.) of survivors approximately one week post-hatch declined significantly ($F_{1,6} = 30.6$, $p = 0.001$)

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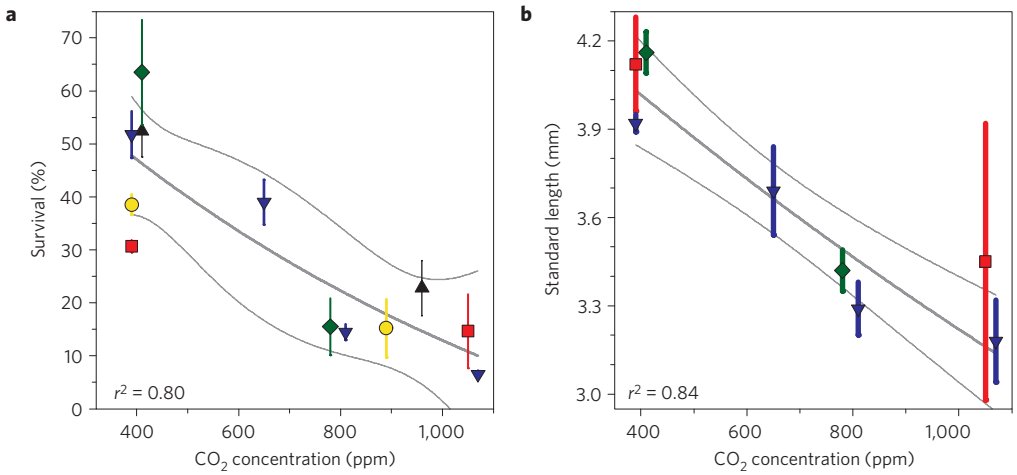


**Figure 1 | Effect of increased $CO_2$ on early life *M. beryllina* survival and length. a**, Survival was averaged across replicates (experiment 1, $n = 3$; experiments 2, 3, $n = 4$; experiment 4, $n = 6$; experiment 5, $n = 5$) for each experiment and $CO_2$ level. **b**, Weighted means (±1 s.e.m.) of standard length averaged across replicates per experiment and $CO_2$ 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 $CO_2$) to 3.3 ± 0.3 mm at 1,000 ppm $CO_2$ (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 $CO_2$ 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 epithelia[26]. The heightened $CO_2$ 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 adults[27]. Third, even if fish embryos and early larvae are capable of some level of physiological adaptation to increased $CO_2$, this would incur further metabolic costs[7] 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 conditions[28], the high levels of $CO_2$ 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 $CO_2$. To test this hypothesis, a further experiment was conducted where $CO_2$ 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 $CO_2$ 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 $CO_2$ 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

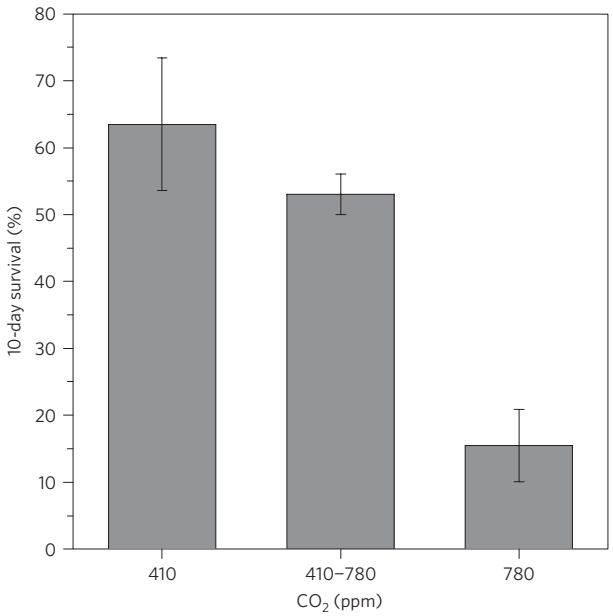


**Figure 2 | $CO_2$ sensitivity of the egg versus early post-hatch stage in *M. beryllina*.** Bars depict average survival (±1 s.e.m.) 10 days after fertilization in control (410 ppm), increased (780 ppm) and 'switch', where $CO_2$ concentration was increased only after eggs hatched (5 days post-fertilization), treatments. Precise $CO_2$ levels and complete carbonate chemistry from experiments appear in Supplementary Tables S1–S5.

compared with control $CO_2$ 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 $CO_2$ levels that are expected in the world's oceans later this century. Together with other emerging evidence[18–21], such apparently high $CO_2$ sensitivity contradicts the notion that ocean acidification will have no direct consequences for marine fish populations. Furthermore, the $CO_2$ levels used in our experiments already occasionally occur in temperate coastal waters, often coinciding with the spawning season of fish[29,30]. Hence, $CO_2$-induced offspring mortality may already be influencing patterns of adult-fish abundance in the ocean. We

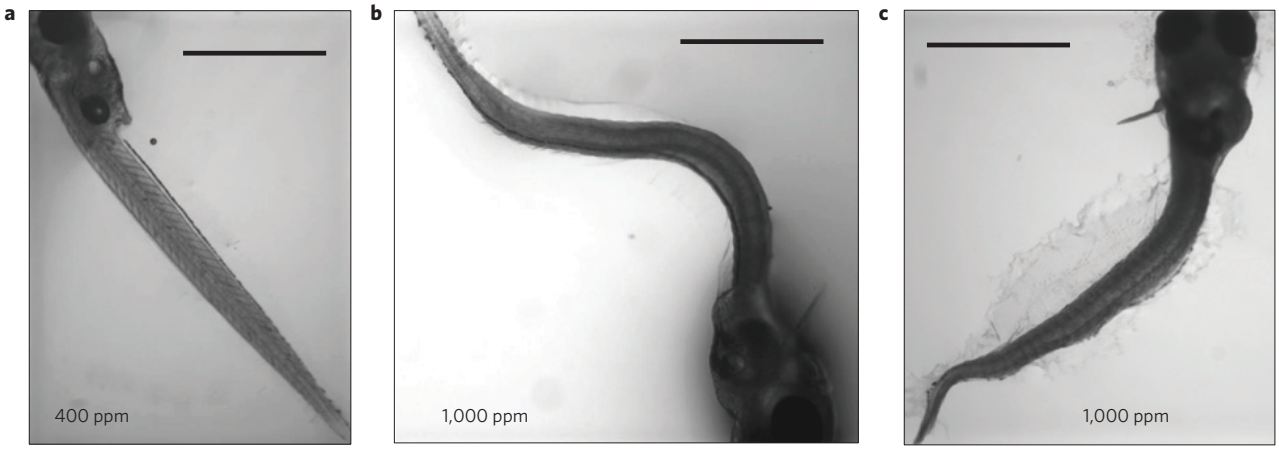


**Figure 3 | *M. berylina* larvae exposed to normal and elevated levels of $CO_2$. a–c**, Larvae with curved or curled bodies were significantly more common at increased (**b**,**c**) when compared with control (**a**) $CO_2$ levels. Scale bar = 1 mm.

expect, however, in a manner similar to what is now emerging from studies on invertebrates[1,3–5], that responses to increased $CO_2$ levels in fish will be highly species specific. For example, oceanic fish species that spawn pelagic eggs might be more susceptible to $CO_2$ increases than benthic spawners[22], where eggs may be more adapted to natural $CO_2$ 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 $CO_2$ sensitivity in fish, but also the general potential of marine organisms to adapt to the $CO_2$ levels projected for future oceans. Our study indicates that future work should focus on the earliest life stages, as the effects of increased $CO_2$ 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 (4 l) per $CO_2$ level (390–1,060 ppm, Supplementary Tables S1–S5). An air: $CO_2$ mix, adjusted to desired levels by a gas proportionator system (Cole Parmer Flowmeter), was continuously delivered to each rearing container. Attained $CO_2$ 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 $7 \pm 2$ 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 $CO_2$, 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 $CO_2$ treatments (PASW Statistics 18). Further details regarding methods are available as Supplementary Methods.

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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.