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Author for correspondence:
Hannes Baumann
e-mail: hannes.baumann@uconn.edu

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Robust quantification of fish early life CO_2 sensitivities via serial experimentation

Hannes Baumann, Emma L. Cross and Chris S. Murray

Department of Marine Sciences, University of Connecticut, 1080 Shennecossett Road, 06340 Groton, CT, USA

HB, 0000-0002-4039-4230; ELC, 0000-0002-5855-2145; CSM, 0000-0001-8504-9054

Despite the remarkable expansion of laboratory studies, robust estimates of single species CO_2 sensitivities remain largely elusive. We conducted a meta-analysis of 20 CO_2 exposure experiments conducted over 6 years on offspring of wild Atlantic silversides (*Menidia menidia*) to robustly constrain CO_2 effects on early life survival and growth. We conclude that early stages of this species are generally tolerant to CO_2 levels of approximately 2000 µatm, likely because they already experience these conditions on diel to seasonal timescales. Still, high CO_2 conditions measurably reduced fitness in this species by significantly decreasing average embryo survival (−9%) and embryo+larval survival (−13%). Survival traits had much larger coefficients of variation (greater than 30%) than larval length or growth (3–11%). CO_2 sensitivities varied seasonally and were highest at the beginning and end of the species' spawning season (April–July), likely due to the combined effects of transgenerational plasticity and maternal provisioning. Our analyses suggest that serial experimentation is a powerful, yet underused tool for robustly estimating small but true CO_2 effects in fish early life stages.

1. Introduction

The challenge to understand marine species sensitivities to ocean acidification (OA) has fuelled a remarkable expansion of laboratory studies over recent decades [1]. The accumulating empirical evidence is critical for distinguishing CO_2-sensitive from CO_2-tolerant species, life stages and traits, and for elucidating mechanisms behind observed effects. Meta-analyses have revealed some general patterns across taxa [2,3], but robust estimates of CO_2 sensitivities within taxa have yet to emerge. This may partly be due to often divergent experimental findings between closely related species, populations or repeated studies [1]. Another methodological concern is the probability of detecting small CO_2 effects in fitness-relevant traits that are primarily controlled by other abiotic and biotic factors. To detect such effects, experimenters most commonly employ within-experiment replication. Unfortunately, logistical and pseudo-replication issues often limit feasible replication levels, hence most published CO_2 experiments have used three to five replicates. This suffices to detect major CO_2 effects, but other potentially important responses could go undetected for lack of statistical power. An alternative approach involves the replication of experiments themselves to strengthen statistical power [4] and quantify within- and among-experiment variability, including critical but so far lacking laboratory comparisons of same species CO_2 responses.

The Atlantic silverside (*Menidia menidia*, Atherinopsidae) is an ecologically important forage fish along the North American Atlantic coast and a long-term model for CO_2 sensitivity studies in our laboratory [5–9]. While research targeted different questions throughout the years, all experiments shared consistent rearing protocols, response variables and a standard contrast of CO_2 treatments. Here we synthesize all these standard experiments to derive

Table 1. Overview of 20 standard CO_2 exposure experiments (A: ambient, H: high CO_2) conducted between 2012 and 2017 on offspring of wild Atlantic silversides.[a]

experiment	year	source population	within-treatment replication	pCO_2 range (μatm)	source publication
1–5	2012	Poquot Cove, NY	3, 3, 5, 5, 5	A: 624–758 H: 2153–2380	[8]
6–10	2013		13, 5, 6, 5, 5	A: 459–560 H: 2145–2540	[5,8]
11–12	2014		5, 6	A: 447 H: 2881	[6]
13–16	2016	Mumford Cove, CT	5, 15, 5, 5	A: 345–427 H: 2157–2190	[6,9]
17–20	2017		3, 5, 5, 3	A: 368–389 H: 2155–2265	[6]

[a]Source data available at [10].

robustly constrained estimates for early life survival and growth responses to high CO_2 conditions in this species, including CO_2 effects on trait variability and trait correlations.

2. Material and methods

(a) Overview of experiments

We used data collected over 6 years (2012–2017) from 20 published CO_2 exposure experiments [5,6,8,9] as summarized in table 1. Briefly, each experiment started with precisely counted, newly fertilized embryos that were obtained during the species' spawning season (April–July) by sampling and then strip-spawning wild adults (20+ per sex) from nearshore sites of Long Island Sound (41°N, 72.5°W). All standard experiments quantified CO_2 effects at the species' thermal optimum (24°C, [11]), used full strength seawater (28–32), a 15 L : 9 D light regime, and *ad libitum* post-hatch rations of *Artemia salina* nauplii. Within-treatment replication ranged from 3 to 15. The standard contrast was between ambient (approx. 400 μatm, range: 345–758 μatm) and high CO_2 conditions (approx. 2300 μatm, range: 2145–2881 μatm), the latter representing a common benchmark in OA research as well as extreme late summer conditions in the species habitat [12]. Realized pCO_2 levels were always calculated (CO2SYS) from water samples taken randomly from each treatment and measured for total alkalinity or DIC, pH, salinity and temperature. Each experiment counted and measured larvae (standard length, SL, nearest 0.01 mm) at 1 and 10 days post-hatch (dph), thus quantifying embryo survival (i.e. fertilization to 1 dph), larval survival (1–10 dph), overall survival (fertilization to 10 dph), SL at 1 dph (hereafter: hatch length), SL at 10 dph (hereafter: larval length) and larval growth rate. Please see the electronic supplementary material for further method details.

(b) Data analysis

We first computed treatment-specific means and coefficients of variation (CV = s.d./mean) for each trait and experiment, followed by averaging these values across experiments and calculating 95% confidence intervals (CI) using a bias-corrected accelerated bootstrap procedure (BCa, SPSS V20 IBM®). Second, we estimated the overall CO_2 effect size for each trait using log-transformed response ratios (RR). RRs are commonly employed in meta-analyses [2,3,13], because they quantify the proportional change resulting from experimental manipulations, have robust statistical properties and a straightforward biological interpretation [13]. We used treatment-specific replicate means for each trait (T_M) to first calculate the RR_M for each experiment [$RR_M = \ln(T_{M\ high}) - \ln(T_{M\ ambient})$], then averaged all RR_M's and calculated 95% CIs (BCa). Similarly, we estimated overall CO_2 effects on trait variability by calculating RR_{SD} [$=\ln(T_{SD\ High}) - \ln(T_{SD\ ambient})$] for each experiment, which were then averaged (±95% CI, BCa). We used unweighted RRs to avoid decreasing sample size and thus potential underestimation of effect sizes (following [13,14]). Overall CO_2 effects were considered significant, if their 95% CI did not include zero. We further examined whether trait-specific response ratios (RR_M) varied with the day of fertilization (April–July), given that wild spawners experience seasonal acidification that may influence offspring CO_2 sensitivity [8]. Last, we correlated embryo survival, larval survival, hatch length and larval length to evaluate whether the expected growth–mortality coupling during fish early life stages [15] was sensitive to high CO_2.

3. Results

Across experiments, silverside early life-history traits differed considerably in their CVs (table 2). Hatch (3%) and larval length (5%) were least variable, whereas larval and overall survival had CVs exceeding 30%. Embryo survival was more variable at high (16%) compared to ambient CO_2 treatments (10%).

Overall CO_2 effects (RR_M) were negative for all traits (figure 1*a*,*b*), but only for embryo and overall survival the 95% CI of excluded zero (figure 1*a*). The mean RR_M for embryo survival was −0.09 (95% CI: −0.17 to −0.03), hence, high CO_2 conditions reduced this trait by on average 9% (3–17%). Similarly, the negative RR_M of overall survival (embryo + larval stage) indicated an average CO_2 induced reduction by 13% (1.4–24%). Effects on hatch length (−0.1%), larval length (−1.4%) and growth (−3.4%) were not significant (figure 1*b*). High CO_2 conditions increased the variability in embryo survival ($RR_{SD} = 0.31$), but decreased the variability in hatch length ($RR_{SD} = -0.25$, figure 1*c*,*d*).

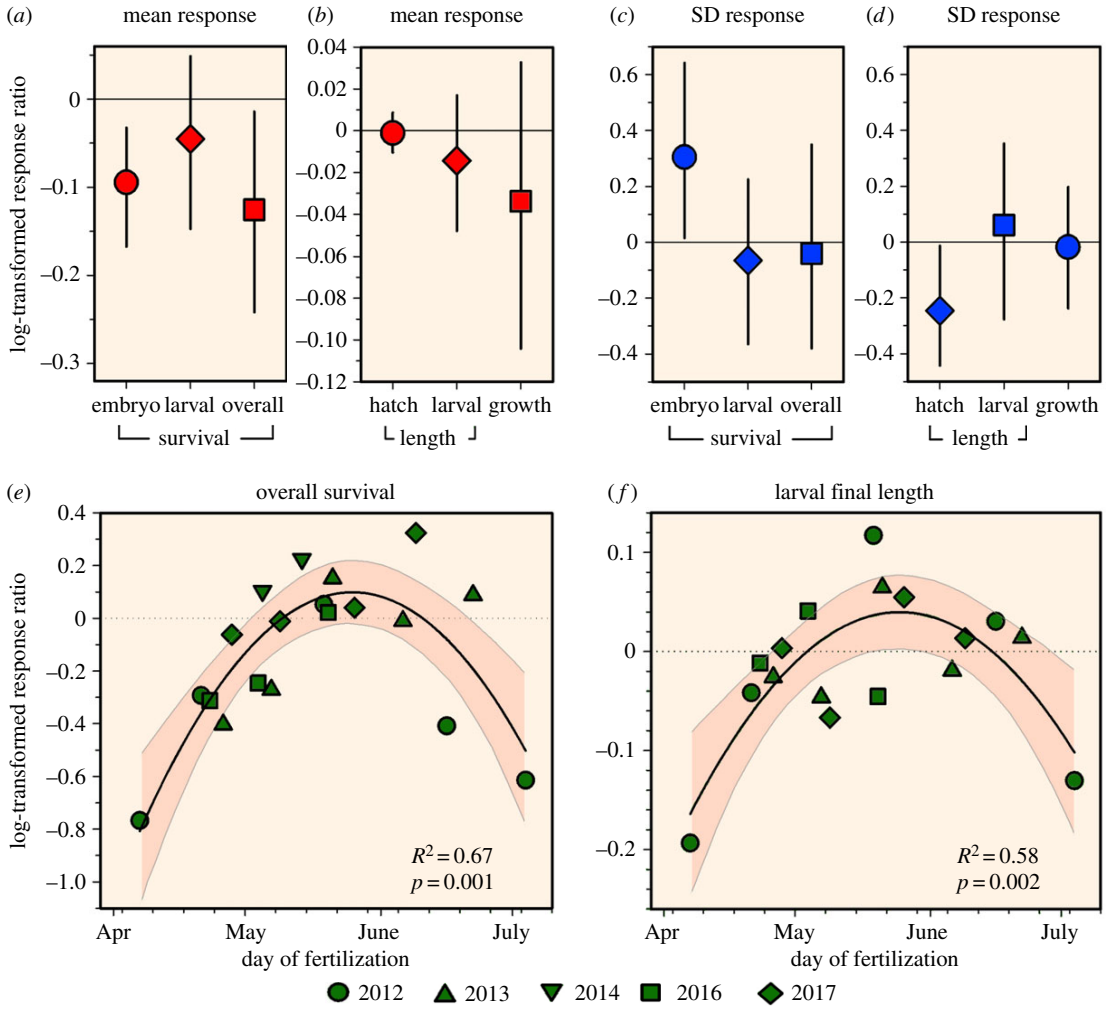

Figure 1. Mean (±95% CI) responses of *M. menidia* early life traits to high CO_2 conditions. (*a*,*b*) Trait means (RR_M); (*c*,*d*) trait variability (RR_{SD}). (*e*,*f*) Seasonal variability in CO_2 effects on (*e*) overall survival and (*f*) larval final length. Symbols represent effect sizes (RR_M) fitted with a quadratic regression (black lines and shaded 95% CI).

Table 2. Cross-experimental means (±95% CI) and coefficients of variation (CV ± 95% CI) of early life-history traits in *M. menidia* offspring reared at 24°C and contrasting CO_2 conditions.

	ambient CO_2		high CO_2	
trait	average (95% CI)	CV (95% CI)	average (95% CI)	CV (95% CI)
embryo survival	84% (80–86)	10% (8–12)	76% (73–80)	16% (11–21)
larval survival	53% (48–57)	33% (23–44)	53% (49–56)	32% (23–41)
overall survival	44% (40–48)	35% (26–46)	40% (36–44)	38% (29–49)
hatch length	5.3 mm (5.2–5.3)	3% (2–3)	5.3 mm (5.2–5.3)	2% (2–2)
larval length	10.9 mm (10.5–11.4)	5% (4–6)	10.8 mm (10.4–11.3)	5% (4–6)
growth	0.50 mm d^{-1} (0.47–0.54)	11% (9–13)	0.50 mm d^{-1} (0.46–0.53)	11% (9–14)

In four of six traits (larval and overall survival, final length, growth), CO_2 effects were significantly ($p < 0.004$) related to the fertilization day of each experiment, with quadratic regressions explaining up to 67% of the variability in RR_M's. Hence, negative CO_2 effects on overall survival or larval length occurred mostly in offspring produced at the beginning or end of the silverside spawning season (April–July, figure 1*e*,*f*). Embryo survival was unrelated to larval survival or larval length irrespective of CO_2 treatments. Positive correlations ($p < 0.05$) occurred in both CO_2 treatments between hatch length versus larval length, hatch length versus embryo survival and larval length versus larval survial. Only at high CO_2 conditions, however, hatch length and larval survival were positively correlated ($p = 0.02$, electronic supplementary material, table S1).

4. Discussion

Years of sustained experimental work on the CO_2 sensitivity of wild Atlantic silverside offspring [5,6,8,9] produced the most robustly constrained estimates of trait responses to high CO_2 for a marine organism to date. Our analyses suggest that early stages of this forage fish are generally tolerant of CO_2 levels of approximately 2000 µatm. This may confirm the default expectation for nearshore marine organisms, which often already experience high CO_2 conditions on diel to seasonal timescales [12,16]. And yet, even in this species high CO_2 conditions measurably reduced fitness via decreasing embryo survival by 3–17%. Silversides are therefore similar to other taxa, where the earliest life stages generally show highest CO_2 sensitivities [2,3]. However, important carry-over effects may occur [7], given that the survival reduction of silverside embryos and larvae combined was greater (−13%) than during each stage alone. We note that these CO_2 effects seem small but still correspond to considerable productivity losses if scaled to population level [17].

Our comparisons revealed that individual experiments differed often markedly from the robust, overall picture. Significant CO_2 effects on hatch length, for example, occurred in both directions in few experiments, but were overall not evident. For other effects, high inter-experimental variability was clearly seasonal and was likely increased further by parental effects and trait stochasticity. Seasonal plasticity in offspring CO_2 sensitivity has been described before and linked to transgenerational adaptation to a gradually acidifying environment throughout the species' spawning season [8,18]. Seasonal acidification is typical for many coastal habitats and may similarly affect other coastal organisms [12]. In silversides, CO_2 sensitivities increased again at the end of their spawning season (July), which was likely due to decreased egg quality, given that absolute egg survival also declined during this period [8]. Moreover, maternal egg provisioning with fatty acids varies in this species and may influence offspring CO_2 sensitivity [9]. In contrast to wild populations, laboratory brood stocks experience less parental and environmental variability, which should be taken into account when predicting population CO_2 sensitivities from experiments.

Growth and survival are generally coupled in fish early life stages [15] which was corroborated by the positive relationship between e.g. larval length and survival. However, only at high CO_2 conditions did greater length at hatch confer a survival advantage into the larval stage; implying that this natural selection process could strengthen in future high CO_2 oceans.

While this analysis robustly characterized CO_2 sensitivities at the species' thermal optimum, patterns across its full thermal range could differ [3]. For silversides, however, we have so far found only limited evidence for increased sensitivities at higher (28°C) or lower temperatures (17, 20°C), and more observations are needed to confidently establish these functional responses [6]. In addition, long-term and whole-life cycle responses (e.g. fecundity) of high CO_2 environments are still uncertain, but have now received increasing attention [7].

The inherent variability of early life traits suggests that serial experimentation could be required in some cases to robustly estimate CO_2 effects or to even detect them. We depict this in figure 2, where we plotted the degrees of freedom for each experiment (i.e. its replication level) against absolute treatment differences for embryo survival (%). We then used the average treatment-specific variances to derive and overlay the probability surface of a *t*-distribution, illustrating that only two of our experiments had high enough replication and/or response levels to outright reject the null hypothesis, while most experiments had insufficient replication ($n = 3$–5) for observed treatment differences (figure 2). In combination, however, the significant 9% reduction in embryo survival became evident.

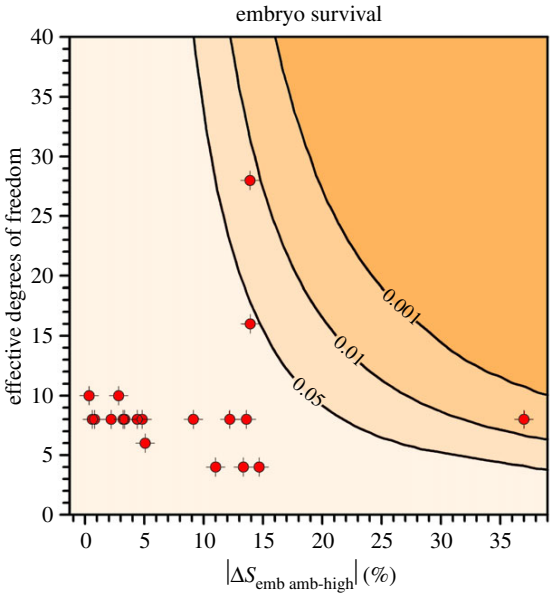

Figure 2. Detecting significant effects in CO_2 exposure experiments. Symbols represent average absolute treatment differences (%) in embryo survival (ΔS_{emb}) plotted against the degrees of freedom (DF) for each experiment. Probability isolines ($p = 0.05$, $p = 0.01$, $p = 0.001$) were derived from a two-tailed *t*-distribution with *t*-values calculated as

$$t = \frac{\Delta S_{\text{embryo}}}{\sqrt{\frac{SD^2_{amb}}{N} + \frac{SD^2_{amb}}{N}}}$$

using overall treatment averages for SD ($SD_{amb} = 13\%$, $SD_{high} = 17\%$) and applied to a matrix of theoretical ΔS_{emb} and effective DF values (Welch–Satterthwaite equation).

Several research groups likely have similar repetitive data for other model species, and a synthesis of such data would be equally valuable to inform predictive frameworks. Going forward, refined experiments will continue to contribute important data toward anticipating the impacts of ocean acidification, particularly when they include the earliest life stages, examine potential carry-over effects and are focused on traits of known fitness relevance.

Ethics. Approved by Institutional Animal Care and Use Committees (IACUC) of Stony Brook University (no. 2010–1842) and University of Connecticut (no. A14-032).

Data accessibility. Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.4573j74 [10].

Authors' contributions. H.B. and C.S.M. designed and performed experiments; H.B., E.L.C., and C.S.M. analysed data. All authors interpreted the results, contributed to writing the manuscript and approved the final version, for which they agree to be held accountable.

Competing interests. We declare we have no competing interests.

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