Marine Biology (2018) 165:75
https://doi.org/10.1007/s00227-018-3335-x

ORIGINAL PAPER

Starvation rates in larval and juvenile Atlantic silversides (*Menidia menidia*) are unaffected by high CO_2 conditions

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Received: 13 December 2017 / Accepted: 23 March 2018 / Published online: 31 March 2018

Abstract

Over the past decade, laboratory experiments on fish early life stages have found many traits that are evidently sensitive to elevated CO_2 levels. With respect to larval growth, high CO_2 environments are commonly assumed to increase acid–base regulation and other plastic responses, thus incurring additional metabolic costs that reduce the scope for growth. This assumption is not well supported by empirical evidence. One reason might be that experiments often provide unrestricted feeding conditions, which could allow larvae to compensate for higher costs by increased food intake. To remove potentially confounding effects of larval feeding, we conducted a series of starvation trials on offspring of the Atlantic silverside (*Menidia menidia*), predicting faster starvation at high compared to ambient CO_2 treatments. We compiled observations from five separate experiments spanning different years, laboratories, temperatures (17–26 °C), life stages (newly hatched larvae, previously fed larvae, early juveniles), and CO_2 levels (300–6500 µatm). Contrary to expectation, we found that starvation rates were largely independent of the CO_2 environment in this fish species. The one exception occurred at the lowest temperature and most extreme CO_2 treatment, which resulted in slower not faster starvation in newly hatched larvae at high compared to ambient CO_2 treatments. The apparent failure of starvation rate as a proxy for CO_2 effects on larval fish metabolism may have several reasons, including potential CO_2 tolerance of offspring, observed large stochasticity in early life survival masking small metabolic costs of high CO_2, and the general depression and reconfiguration of fish metabolism in response to food deprivation.

Introduction

Recognition of "mankind's other CO_2 problem", ocean acidification (Doney et al. 2009), has led to a rapid and sustained expansion of CO_2 exposure experiments over the past two decades (Dupont and Pörtner 2013; Busch et al. 2015; Browman 2016). Two major goals of such experiments are to distinguish CO_2 sensitive from CO_2 tolerant species, life stages, and traits, and to elucidate the mechanisms behind detected CO_2 effects. Experimental approaches thus comprise an important first step towards inferring the vulnerability of marine life to the long-term threat of ocean acidification (McElhany 2017; Snyder et al. 2018). Reviews of the empirical evidence (Hendriks et al. 2010; Kroeker et al. 2010; Harvey et al. 2013) suggest that adverse effects of elevated CO_2 levels, as predicted for the next three centuries in the average open ocean, exist for many traits and across most taxa, particularly during early life stages and in calcifying marine invertebrates (Kleypas et al. 2006; Baumann et al. 2012; Waldbusser et al. 2013; Bednaršek et al. 2014). However, neutral or contrary experimental outcomes are also very common within most studied organism groups (Esbaugh 2018). The empirical complexity remains a formidable challenge, as it may reflect differences in methodology, parental exposure history (Munday 2014; Murray et al. 2014), or species- and population-specific responses due to different baselines of contemporary CO_2 fluctuations in the environment (Kelly et al. 2013).

For marine fish, CO_2 exposure experiments focus on early life stages, because they have yet to develop the acid/base competency typical for CO_2 tolerant juveniles and adults (Ishimatsu et al. 2008). In some but not most fish species studied to date (Esbaugh 2018), high CO_2 can directly

Responsible Editor: A.E. Todgham.

Reviewed by A. Frommel and undisclosed experts.

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reduce early life survival (Baumann et al. 2012; Pimentel et al. 2014; Stiasny et al. 2016). The majority of documented CO_2 effects involve traits of inferred fitness relevance such as early life behavior (Munday et al. 2010), calcification (Bignami et al. 2013), morphometric and histological traits (Chambers et al. 2014; Frommel et al. 2016), as well as metabolism and growth (Munday et al. 2009; Murray et al. 2017). Experiments revealed that adverse behavioral effects are caused by high CO_2 interfering with the function of the GABA-A neurotransmitter (Nilsson et al. 2012). Observed over-calcification may result from increased bicarbonate levels in blood and endolymph due to buffering against lower pH levels (Bignami et al. 2013), whereas the mechanisms causing morphometric effects or tissue damages remain unresolved. With respect to metabolism and growth, one general hypothesis is that high CO_2 environments incur additional metabolic costs due to increased acid/base regulation, hyperventilation, and other plastic responses, which could reduce the scope for growth (Esbaugh et al. 2012; Heuer and Grosell 2016; Esbaugh 2018). However, empirical support for this hypothesis remains surprisingly elusive. Direct measurements of heart rate, metabolism and metabolic scope in fish early life stages have revealed positive, neutral, and negative CO_2 effects (Munday et al. 2009; Ern et al. 2017; Lonthair et al. 2017; Davis et al. 2018), while a majority of studies measuring growth have found neutral or positive effects at high compared to ambient CO_2 environments (Hurst et al. 2013; Murray et al. 2014; Kunz et al. 2016). One reason for these counterintuitive outcomes might be that additional metabolic costs due to high CO_2 are small and mostly undetectable as growth reductions in short-term studies spanning days to weeks (Murray et al. 2017). Another intriguing possibility relates to the fact that most CO_2 exposure experiments have typically administered *ad libitum* (i.e., excess) prey rations to larval fish, which is a practical if imperfect way to standardize food availability across replicate experimental units with often varying survivorship. It is, therefore, conceivable that offspring under high CO_2 conditions can compensate or overcompensate for higher metabolic costs by increased food consumption, thus rendering early life growth a flawed proxy for detecting CO_2 induced metabolic costs.

The rationale of the current study was to simply remove the likely confounding effects of fish early life feeding by conducting starvation trials. A similar approach by Bignami et al. (2016) showed faster starvation of cobia larvae (*Rachycentron canadum*) at high compared to ambient CO_2 conditions. We thus hypothesized that in the absence of food, higher CO_2 induced metabolic costs result in higher larval mortality at high compared to ambient CO_2 treatments. If so, starvation rates in fish early life stages might comprise a useful, straightforward proxy to corroborate the metabolic paradigm of high CO_2 effects. We compiled the results from a series of starvation experiments that were conducted in different years and with different source populations of the Atlantic silverside (*Menidia menidia*), an important forage fish species along the North-American Atlantic coast and common model in CO_2 exposure experiments (Murray et al. 2014; Malvezzi et al. 2015; Miller et al. 2016). We tested our prediction of faster CO_2 induced starvation rates across a range of temperatures, CO_2 levels, and life stages to examine the utility of this proxy.

Materials and methods

Field sampling and general rearing methods

We conducted five separate experiments (i.e., five different fertilizations) to quantify CO_2 dependent starvation rates in *M. menidia* offspring; two in 2014 and three in 2017. All experiments used offspring derived from wild spawners that were collected during the spawning season (May–June) from local sites with a 30 × 2 m beach seine (3 mm mesh). In 2014, spawners for experiments 1 and 2 were collected from Poquot Cove on the central north shore of Long Island (New York, 40.95°N, 73.10°W). In 2017, spawners for experiments 3–5 were collected from Mumford Cove (eastern Connecticut; 41.32°N, 72.02°W). The two sites are located on opposite shores of Long Island Sound approximately 100 km apart. Adults were transported to the laboratory (2014: Flax Pond Laboratory, Stony Brook University; 2017: Rankin Laboratory, University of Connecticut Avery Point) and held overnight in aerated tanks (60 L) at 20 °C to promote egg hydration. One day after field collection, a minimum of 20 females and 20 males were strip-spawned onto window screen (1 mm mesh), where fertilized eggs attach with their chorionic filaments. Within 2 h of fertilization, screens with attached embryos were randomly distributed into replicated 20 L rearing containers preconditioned for the intended CO_2 treatment and placed within temperature-controlled water baths (Aqualogic® thermostats connected to DeltaStar® chillers or commercial aquarium heaters). All experiments used seawater of 30 psu and a photoperiod of 15 h light:9 h dark. A summary of the five experiments and their methodology is provided in Table 1.

CO_2 manipulation and control

CO_2 levels were manipulated via gas proportioners (ColeParmer®) mixing ambient air with 100% bone-dry CO_2 and delivering gas mixes to the bottom of each rearing container via air stones. Target levels were controlled via daily pH measurements using hand-held pH probes (Orion ROSS Ultra pH/ATC Triode, and Orion Star A121 pH Portable Meter, Thermo Scientific; Hach HQ40d portable meter

Table 1 Method summary of 5 experiments conducted in 2014 and 2017 to quantify CO_2 dependent starvation rates in *M. menidia* offspring

Experiment	Source population	Date fertilized	Stage at experiment start	Temperature (s) (°C)	Ambient-High CO_2 levels (µatm)	# Replicates per treatment
1	Poquot cove (NY)	May 5, 2014	Newly hatched larvae	24	410–6555	7
2		May 14, 2014		17	379–5654	8
3	Mumford cove (CT)	June 14, 2017	(a) Newly hatched larvae, (b) previously fed larvae	20, 26	291–1942	3
4		July 11, 2017	(a) Newly hatched larvae, (b) previously fed larvae	20, 26	304–2226	3
5		June 30, 2017	Early juveniles (35 dph)	24	333–2261	3

with a PHC201 standard pH-probe) that were calibrated weekly with 3-point NIST buffers. Actual pCO_2 levels and related water chemistry parameters were determined from water samples taken from random subsets of rearing containers and analyzed for total alkalinity (A_T) via endpoint titration (Mettler Toledo™ G20 Potentiometric Titrator). The instrument has previously been shown to quantify A_T in Dr. Andrew Dickson's reference material (batch 147, $A_T = 2231.39$ µmol kg seawater^{-1}) with an average error of 0.6%. Actual levels of total dissolved inorganic carbon (C_T), partial pressure of CO_2 (pCO_2), and carbonate ion concentration were calculated in CO2SYS. (http://cdiac.ornl.gov/ftp/co2sys) based on measured A_T, pH (NIST), temperature, and salinity using K1 and K2 constants from Mehrbach et al. (1973) refit by Dickson and Millero (1987) and Dickson (1990) for $KHSO_4$ (Table 2).

Experiments 1 and 2

The first two experiments recorded daily larval mortality in the absence of feeding for two temperatures; 24 °C (experiment 1) and 17 °C (experiment 2), which encompassed optimal and lower end thermal conditions, respectively, conducive to silverside early life growth (Middaugh et al. 1987). These temperatures also encompass current conditions during the silverside spawning season from late April to early July. To contrast ambient CO_2 levels ($pH_{NIST} = 8.2$, 379–410 µatm CO_2), extreme CO_2 levels of 5654–6555 µatm ($pH_{NIST} = 7.15$) were chosen to represent the upper potential extreme of future conditions during the seasonal CO_2 peak in the most productive nearshore environments such as salt marshes. Within 2 h post fertilization, 100 embryos were placed into each of eight replicates per CO_2 treatment. After hatching, replicate rearing containers were examined twice daily and dead larvae counted and removed until complete mortality.

Experiments 3 and 4

Each of these experiments measured daily larval mortality at factorial combinations of two temperatures (20 and 26 °C) and two CO_2 levels (ambient: $pH_{NIST} = 8.2$, 291–304 µatm pCO_2, high CO_2: $pH_{NIST} = 7.50$, 1942–2226 µatm pCO_2). Temperatures were chosen to encompass current conditions typical of the peak silverside spawning season (20 °C) and in late summer (26 °C); the CO_2 treatment of ~2100 µatm

Table 2 Temperature (°C), target and actual pH (NIST) conditions, total alkalinity (A_T, µmol kg), dissolved inorganic carbon (C_T, µmol kg^{-1}), partial pressure of CO_2 (pCO_2, µatm), and carbonate ion concentrations (CO_3^{2-}, µmol kg^{-1}) during the five starvation experiments

Experiment	Temp	Target pH	Mean pH	A_T	C_T	pCO_2	CO_3^{2-}
1	24	7.15	7.13	2567	2738	6555	21
		8.20	8.24	2567	2278	410	224
2	17	7.15	7.17	2567	2745	5654	19
		8.20	8.26	2567	2313	379	197
3	20	7.50	7.53	2038	2045	1942	39
		8.20	8.27	2050	1802	291	177
	26	7.50	7.55	2064	2050	1937	48
		8.20	8.22	2057	1794	341	186
4	20	7.50	7.48	2084	2108	2226	35
		8.20	8.24	2088	1856	325	168
	26	7.50	7.52	2129	2123	2154	47
		8.20	8.27	2114	1820	304	209
5	24	7.50	7.5	2180	2182	2261	45
		8.20	8.24	2109	1849	333	187

represents a common benchmark in CO_2 exposure studies. An additional goal of these experiments was to quantify mortality rates both in newly hatched and in previously fed larvae. Therefore, all embryos were first reared to hatch in a single 20 L container per temperature and CO_2 treatment, after which they were split into two groups. For the first group, 100 newly hatched larvae were counted and distributed into each of three replicates per temperature and CO_2 treatment, and then reared without food while recording mortalities twice daily until complete mortality. The second group of hatched larvae received *ad libitum* rations of newly hatched brine shrimp nauplii (San Francisco strain, brineshrimpdirect.com) for 6 days, after which an equal number (60–100) was randomly distributed in into each of three replicates per temperature and CO_2 treatment. These larvae were then reared further without feeding, while recording mortalities twice daily.

Experiment 5

The last experiment quantified the CO_2-dependent starvation rates in early juvenile *M. menidia*, which had been reared at 24 °C and ambient CO_2 conditions for 35 days post hatch. At the start of this experiment, 36 juveniles were gently netted and randomly distributed into each of three replicates per CO_2 treatment (ambient: $pH_{NIST} = 8.2$, 333 µatm CO_2, high CO_2: $pH_{NIST} = 7.50$, 2261 µatm CO_2, $N_{total} = 216$). Juveniles were then reared without food at 24 °C while recording and measuring (total length, TL, 0.1 mm) mortalities daily.

Data analysis

All statistical analyses were computed using SPSS (V20, IBM). For each experiment and replicate, recorded numbers of perished larvae were summed by day and then expressed as relative daily cumulative mortalities (M_{cum}: 0–1). These proportional data were then logit-transformed $[\log_{10}(M_{cum} \times (1 - M_{cum})^{-1})]$ prior to statistical analysis, with M_{cum} values of 0 and 1 replaced by 0.0001 and 0.9999, respectively (Warton and Hui 2011). Data were then analyzed separately for each experiment, life stage and temperature using a Repeated Measures General Linear Model (RM-GLM) with ‘Day’ as the within-subject factor and CO_2 treatment as the fixed between-subject factor. Significant day × CO_2 interactions would be interpreted as differential starvation responses of offspring at ambient vs. high CO_2 treatments. Because in all cases, the variance of M_{cum} expectedly decreased with increasing days of starvation, thus violating the sphericity assumption of RM-GLMs, the Greenhouse–Geisser procedure was applied to decrease degrees of freedom and thereby reduce the probability of type I errors. No statistics were computed for previously fed larvae in experiment 4, due to insufficient replication caused by high post-hatch mortality. In experiment 5, an additional RM-GLM was used to examine whether starvation progressed differently for juveniles of different sizes, thus testing for potential effects of day and day × CO_2 interactions on the average TL of perished fish.

Results

In experiment 1 (24 °C) mean ± SD of hatching success was not significantly different between ambient (74 ± 12%) and high CO_2 treatments (73 ± 9%, *t* test, *df* = 12, $P = 0.75$), whereas in experiment 2 (17 °C), mean ± SD of hatching success was slightly lower at ambient (69 ± 11%) compared to high CO_2 treatments (79 ± 7%, *t* test, *df* = 14, $P = 0.03$). Time to hatch was unaffected by CO_2. In experiment 1, newly hatched larvae took 7 days until complete mortality, and there was no significant difference between ambient and high CO_2 treatments (RM-GLM, $P = 0.8$, Table 3, Fig. 1b). In contrast, newly hatched larvae in experiment 2 took 2 days longer to complete mortality at high compared to ambient CO_2 treatments, resulting in a significant day × CO_2 interaction (RM-GLM, $P = 0.002$, Table 3, Fig. 1a). Newly hatched larvae in experiments 3 and 4 took 6 days to complete mortality at both 20 and 26 °C, and there were no significant day × CO_2 interactions (RM-GLM, Table 3, Fig. 2a, b). Previously fed larvae in experiment 3 took 6 and 7 days to complete mortality at 20 and 26 °C, respectively, and there were no significant day × CO_2 interactions (RM-GLM, Table 3, Fig. 2c, d).

Table 3 Results of repeated measures GLMs testing for interactions between CO_2 treatment and day-of-the-experiment on logit-transformed, relative cumulative mortalities of food-deprived *M. menidia* offspring at different experiments, temperatures (*T*), and life stages

Experiment	Life stage	*T* (°C)	*df*	*F*	*P*
1	Newly hatched larvae	24	1.94	0.21	0.80
2		17	2.45	7.00	**0.002**
3		20	1.29	0.21	0.73
		26	1.11	0.71	0.46
4		20	1.44	0.96	0.40
		26	1.50	3.29	0.11
3	Previously fed larvae	20	1.38	3.05	0.13
		26	1.28	0.33	0.65
4		20[a]			
		26[a]			
5	Early juveniles	24	1.66	0.45	0.62

The Greenhouse–Geisser procedure was used to adjust *df*'s in each analysis

[a] No statistics computed due to insufficient replication

Significant effects are bolded

Fig. 1 *M. menidia*. Relative cumulative starvation mortalities of newly hatched larvae reared at 17 °C (**a**, experiment 2) and 24 °C (**b**, experiment 1) under ambient (grey line, diamonds) vs. high CO_2 conditions (black line, circles). Circles depict individual replicates, lines represent treatment means

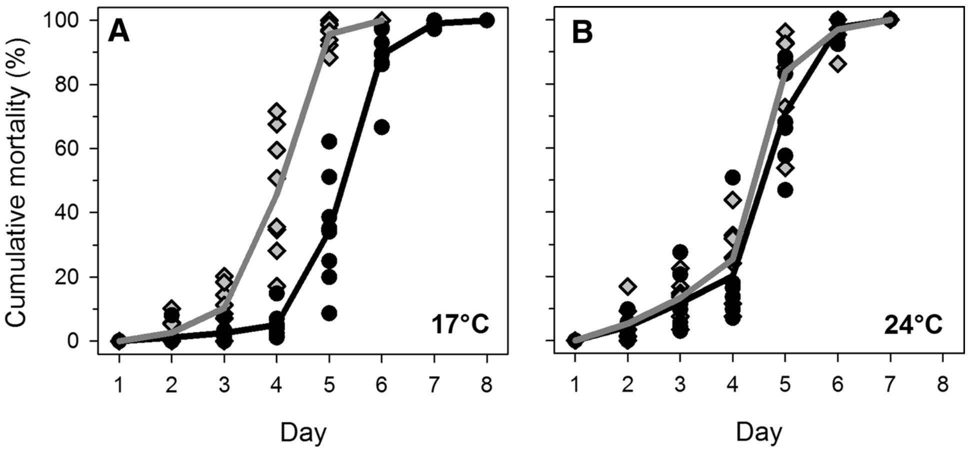
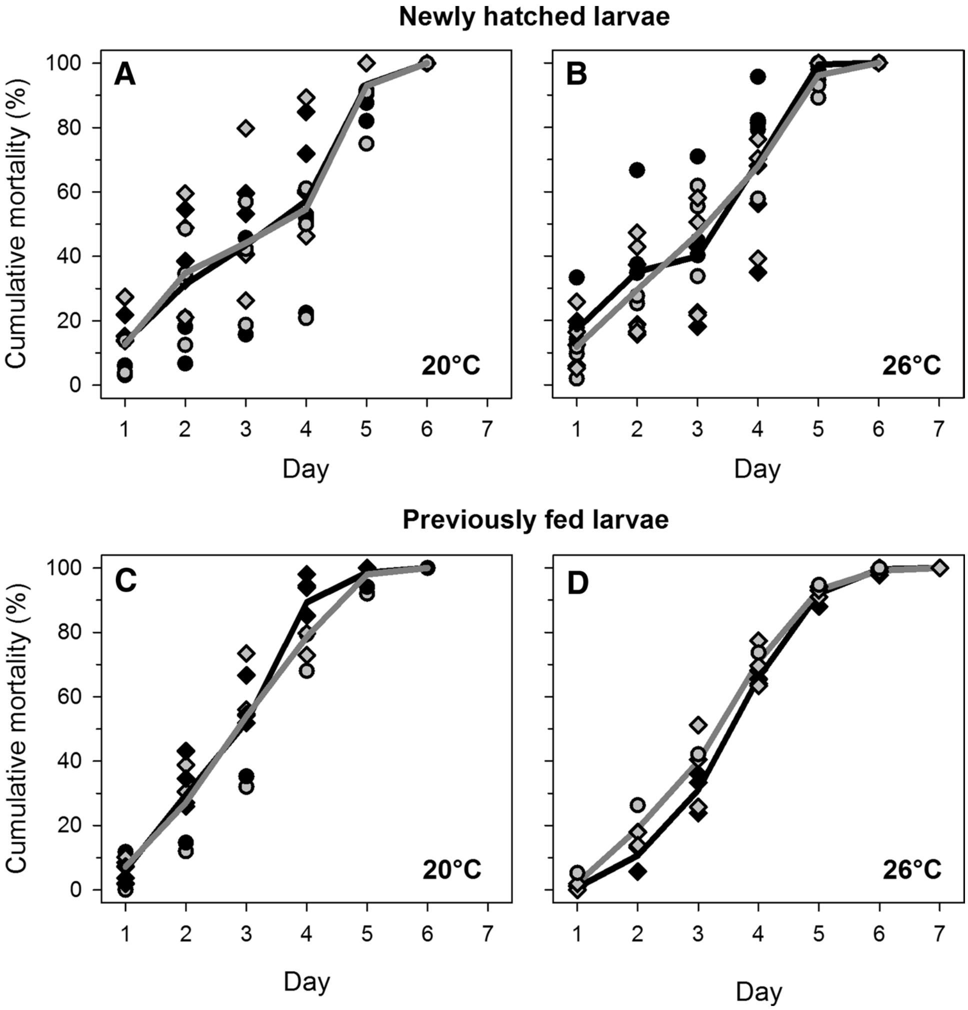


Fig. 2 *M. menidia*. Relative cumulative starvation mortalities of newly hatched larvae (**a**, **b**) and previously fed larvae (**c**, **d**) reared during experiments 3 (diamonds) and 4 (circles) at 20 °C (**a**, **c**) and 26 °C (**b**, **d**) under ambient (grey line and symbols) vs. high CO_2 conditions (black line and symbols). Symbols depict individual replicates, lines represent treatment means across both experiments



In experiment 5, early juvenile *M. menidia* took 16 days to starve (Fig. 3a), although two outlier individuals (one in ambient, one in high CO_2 treatments) did not perish until day 25 and 22 of the experiment, respectively. There was no significant day × CO_2 interaction in relative cumulative mortalities, indicating similar starvation rates in ambient and high CO_2 treatments (RM-GLM, $P = 0.6$, Table 3). When compared to the TL distribution of a random subsample of juveniles taken at the start of the experiment, juveniles from the lower end of the distribution started to perish first in both ambient and high CO_2 treatments (Fig. 3b). TL of perished individuals increased over the course of the experiment,

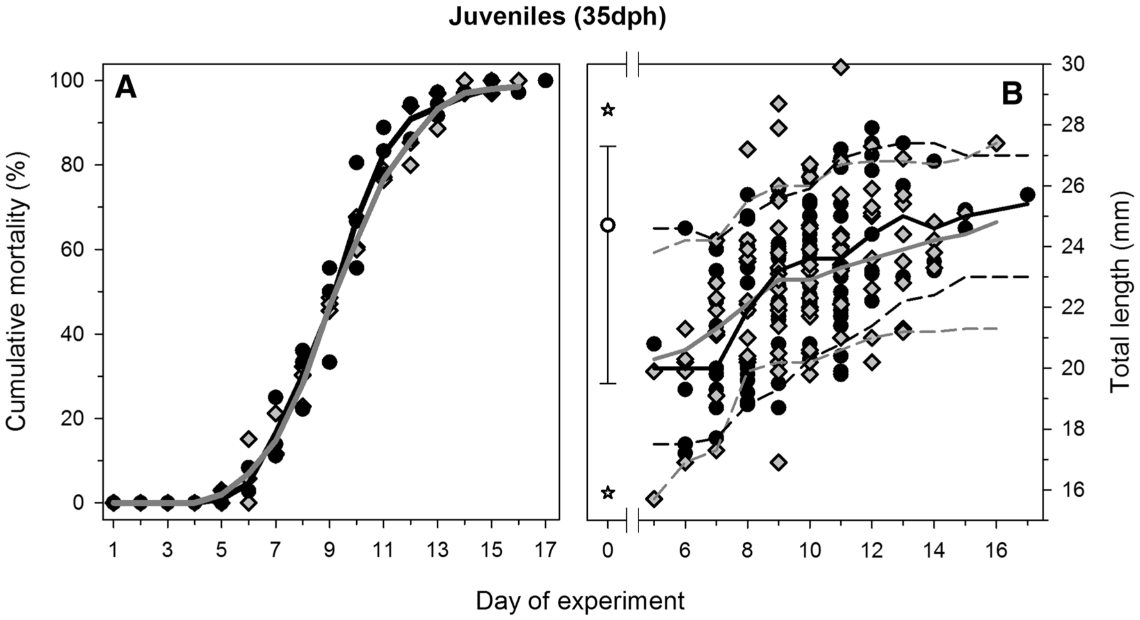


Fig. 3 *M. menidia*. **a** Relative cumulative starvation mortalities of early juveniles reared at 24 °C under ambient (grey line, diamonds) vs. high CO_2 conditions (black line, circles). Symbols depict individual replicates, lines represent treatment means. **b** Total length of juveniles perishing during the experiment at ambient (grey diamonds) vs. high CO_2 conditions (black circles). Lines represent the median (solid lines), 5th and 95th percentiles (dashed lines) of TL estimated with locally weighted, non-parametric density estimators with variable bandwidths, a method described in detail by Evans (2000). The initial TL distribution at the beginning of the experiment is depicted on day 0 as the median (white circle), 5th/95th percentiles (whiskers) and the minimum and maximum (white stars)

indicating continuing faster starvation of smaller over larger fish. While the increase in TL appeared to be slightly steeper at high compared to low CO_2 treatments, the day × CO_2 interaction term was not significant (RM-GLM, $df_{adj} = 1.48$, $P = 0.242$).

Discussion

We tested whether starvation rates in Atlantic silverside offspring differ between contrasting CO_2 conditions, based on the hypothesis that acidified environments incur additional metabolic costs in fish early life stages. We compiled observations from five separate starvation experiments spanning different years, laboratories, temperatures, life stages, and CO_2 levels, finding that—contrary to expectation—mortality patterns were largely independent of the CO_2 environment the fish experienced. The one exception to this was experiment 2 (17 °C), which resulted in slower not faster mortality rates in newly hatched larvae at high compared to ambient CO_2 treatments. Developmental differences are unlikely to account for that, given that time to hatching was similar between treatments.

Experimental methods evolved between years and laboratories and limit some comparisons. In this case, however, the variance also strengthens our overall conclusion that starvation rates were unaffected by CO_2, in contrast to recent findings in cobia larvae (Bignami et al. 2016). High CO_2 treatments in 2017 administered levels of ~2100 µatm, whereas experiments in 2014 used high CO_2 levels of >5500 µatm. These values were chosen to represent current as well as potential short-term pH extremes, respectively, in nearshore temperate habitats during the most productive summer months. In 2014, temperature treatments comprised sequential experiments, covering both the optimal (24 °C) and the lower end temperature (17 °C) of this species. In 2017, temperature × CO_2 treatments were factorial, using two temperatures at the mid- (20 °C) and upper thermal range of this species (26 °C). The two laboratories used water sources of similar salinity (30psu) but differing alkalinity (Flax Pond: ~2500 µmol kg^{-1}, Rankin Lab: ~2100 µmol kg^{-1}). Adult strip-spawning and general embryo and larval rearing procedures, on the other hand, were highly consistent between years.

The apparent failure of starvation rate as a proxy for CO_2 effects on larval fish metabolism may have several explanations. A first one is the large stochasticity in early larval survival and thus the large variance observed between replicates. Among all replicates larval cumulative mortalities on day 4 ranged from 2 to 95% (mean ± SD = 41 ± 28%). It appears unlikely that all perished larvae in these trials succumbed strictly to starvation, instead large and highly variable mortality rates of larvae immediately after hatch are common in this and most other marine fish species (Killen et al. 2007; Snyder et al. 2018). This inherently large trait variance decreases the probability of detecting effects, particularly if such effects and the level of replication are small. This may also explain why starvation times of newly hatched larvae in the current study were surprisingly similar across temperature treatments. As expected, within replicate variability markedly decreased from early larval, to larval, to juvenile life stages, thereby increasing confidence in the utility of the proxy while strengthening the null hypothesis of no CO_2 effects.

How the starvation process itself affects fish metabolism is another important consideration. Starvation in larval and juvenile fish has been extensively studied for aquaculture purposes, recruitment determinants (Lasker et al. 1970; Leggett and Deblois 1994) or to infer the nutritional status of individuals sampled in the field (Gadomski and Petersen 1988; Huwer et al. 2011). Physiological studies revealed that starving fish not only decrease locomotor and overall metabolic activities (Wieser 1991; Méndez and Wieser 1993), but also modify metabolic capacities of certain tissues (Guderley et al. 2003) and prioritize the degradation of liver lipids over glycogen and muscle proteins (Black and Love 1986; Guderley et al. 2003). Overall metabolic depression and decreased activity may reduce the need of starving larvae for acid–base regulation and diffusive CO_2 offloading via the gills, thus rendering high CO_2 levels in the environment largely inconsequential. Alternatively, acid–base regulation may simply comprise a very small component of the standard metabolism in silverside early life stages, hence making changes difficult to detect against high inherent variability (Ern and Esbaugh 2016; Lefevre 2016). Numerous other metabolic abnormalities may develop during starvation and disguise any CO_2 related costs in fish early life stages. It is even possible that under extreme conditions, such as the very high CO_2 concentrations and low temperatures in experiment 2, metabolic depression is more pronounced at high CO_2, and therefore, results in longer, not shorter starvation compared to control fish.

Plastic physiological responses to high CO_2 environments may comprise a third main reason why starvation patterns in Atlantic silversides did not differ between CO_2 treatments. For example, experiments demonstrated that fish can respond to high CO_2 levels in the environment by reducing the branchial diffusion distance and thereby ease CO_2 offgasing at the gills. They may also increase the mRNA expression of CO_2 channels or acid–base relevant enzymes (Esbaugh et al. 2012; Esbaugh 2018). These plastic responses arguably counteract or even cancel the hypothesized additional metabolic costs of high CO_2 environments in fish.

For the above reasons, we conclude that starvation trials are not a useful proxy to demonstrate CO_2 related metabolic costs in larval and early juvenile silversides. Potential increases in acid–base related metabolism may not exist or may be too small to be detectable during fish early life stages (Hurst et al. 2017). Over longer time-scales, however, even small metabolic CO_2 effects may accrue and become detectable. For example, a previous study rearing Atlantic silversides for 4.5 months (approximately one third of the species life span) under meticulously controlled and restricted feeding ratios, found significant growth reductions in high compared to ambient CO_2 treatments (Murray et al. 2017). These negative growth effects only became apparent late during the juvenile stage, but were consistent with the small additional energy demands of high CO_2 environments (Esbaugh et al. 2016) as modeled or theoretically estimated by independent approaches (Heuer and Grosell 2016).

Experimental approaches play an integral role in elucidating the mechanistic basis of CO_2 sensitivities in marine organisms. While no single experiment can predict the fate of future populations, the accumulation of continuously improving empirical evidence will lead to robust estimates of CO_2 × temperature-dependent reaction norms in contemporary species. Experiments will also be critical for quantifying intra-specific variability and the potential of species to adapt to a high CO_2 world (Sunday et al. 2014; Malvezzi et al. 2015).

Acknowledgements We are grateful to C. Woods, J. Pringle, J. Snyder, and J. Harrington for assistance in the lab.

Funding This study was funded by a Directorate for Biological Sciences, National Science Foundation Grant to H. B. (NSF-OCE 1536165) and the NSF-REU Grant (1658663).

Compliance with ethical standards

Conflict of interest All authors declare no competing or financial interests.

Ethical approval Experiments were performed under IACUC protocol #A14-032.

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