

High sensitivity of a keystone forage fish to elevated CO₂ and temperature

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Sand lances of the genus *Ammodytes* are keystone forage fish in coastal ecosystems across the northern hemisphere. Because they directly support populations of higher trophic organisms such as whales, seabirds or tuna, the current lack of empirical data and, therefore, understanding about the climate sensitivity of sand lances represent a serious knowledge gap. Sand lances could be particularly susceptible to ocean warming and acidification because, in contrast to other tested fish species, they reproduce during boreal winter months, and their offspring develop slowly under relatively low and stable $p\text{CO}_2$ conditions. Over the course of 2 years, we conducted factorial $p\text{CO}_2 \times$ temperature exposure experiments on offspring of the northern sand lance *Ammodytes dubius*, a key forage species on the northwest Atlantic shelf. Wild, spawning-ripe adults were collected from Stellwagen Bank National Marine Sanctuary (Cape Cod, USA), and fertilized embryos were reared at three $p\text{CO}_2$ conditions (400, 1000 and 2100 μatm) crossed with three temperatures (5, 7 and 10 °C). Exposure to future $p\text{CO}_2$ conditions consistently resulted in severely reduced embryo survival. Sensitivity to elevated $p\text{CO}_2$ was highest at 10 °C, resulting in up to an 89% reduction in hatching success between control and predicted end-of-century $p\text{CO}_2$ conditions. Moreover, elevated $p\text{CO}_2$ conditions delayed hatching, reduced remaining endogenous energy reserves at hatch and reduced embryonic growth. Our results suggest that the northern sand lance is exceptionally CO₂-sensitive compared to other fish species. Whether other sand lance species with similar life history characteristics are equally CO₂-sensitive is currently unknown. But the possibility is a conservation concern, because many boreal shelf ecosystems rely on sand lances and might therefore be more vulnerable to climate change than currently recognized. Our findings indicate that life history, spawning habitat, phenology and developmental rates mediate the divergent early life CO₂ sensitivities among fish species.

Key words: *Ammodytes dubius*, climate change, early life-history, ocean variability hypothesis, ocean acidification, multi-stressor experiment

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Introduction

Forage fish are essential trophic components of all marine ecosystems, because as small, schooling zooplanktivores they channel biological production from plankton to piscivorous fish, mammals and sea birds (Pikitch *et al.*, 2012). Forage

fish are also commercially exploited (Tacon and Metian, 2009), and populations under intense industrial fishing pressure have frequently collapsed (Essington *et al.*, 2015; Pinsky *et al.*, 2011). The severe ecosystem consequences of such collapses (Becker and Beissinger, 2006; Furness and Tasker, 2000; Hamre, 1994) underscore the importance of managing

forage fish populations with precaution (Pikitch et al., 2012). However, managing forage fish stocks is complicated by their tendency to respond strongly to environmental variability via rapid shifts in distribution or abundance (Alder et al., 2008). Marine climate change will therefore impact forage fish in ways that are complex and still highly uncertain (Shannon et al., 2009). Clearly, sustaining forage fish populations requires a greater understanding how these species will respond to concurrent climate stressors such as ocean warming and acidification.

Sand lances in the genus *Ammodytes* are a particularly interesting and important group of forage fish, because its eight species are all locally highly abundant in temperate to polar ecosystems in the northern hemisphere (Orr et al., 2015; Willson et al., 1999). The northern sand lance (*A. dubius*) occurs on sandbanks all along the western North Atlantic shelf, but it is especially important at the Stellwagen Bank National Marine Sanctuary (SBNMS) north of Cape Cod (Fig. 1), where it is the primary forage item for large populations of humpback whales, endangered sea birds, sharks and tunas (Willson et al., 1999) that attract millions of human visitors each year (Wiley et al., 2008). Despite their role in the ecosystem, very little is known about the sensitivity of any sand lance species to the concurrent stressors of marine climate change. Warmer winter temperatures have been shown to depress sand lance recruitment (Arnott and Ruxton, 2002; Lindegren et al., 2018), but detailed experimental work on their temperature sensitivity is scarce. Furthermore, the potential vulnerability of sand lances to future high CO₂ oceans remains completely unstudied.

As a group, sand lances may be particularly vulnerable to ocean acidification (OA). Like most of its congeners, the northern sand lance spawn during late fall, depositing demersal eggs onto offshore sandbanks where embryos develop slowly under cooling temperatures and relatively low and stable *p*CO₂ conditions (Salisbury and Jönsson, 2018; Smith et al., 1978). As a consequence, sand lance early life stages likely experience relatively low levels of ambient CO₂ variability during their development and therefore face weak selection for CO₂ tolerant phenotypes. This differs from other forage fish species (e.g. Atlantic silverside *Menidia menidia*) that spawn during spring and summer in highly productive, nearshore habitats, where *p*CO₂ fluctuations often reach maximum levels, which will not occur in the average open ocean for hundreds of years (Baumann and Smith, 2017; Duarte et al., 2013). Organism sensitivities likely decrease with increased environmental variability, as demonstrated, for example, for thermal sensitivities in marine ectotherms (i.e. Climate Variability Hypothesis; Compton et al., 2007; Stevens, 1989; Sunday et al., 2012; Woolsey et al., 2015). A similar framework should also apply to species' CO₂ sensitivities [Ocean Variability Hypothesis (OVH); Baumann, 2019] and is consistent with empirical data for some marine invertebrates (Hofmann et al., 2014; Kelly et al., 2013; Vargas et al., 2017) and fish (Hamilton et al., 2017; Jarrold et al., 2017; Jarrold and Munday, 2019).

We experimentally quantified the effects of current to future *p*CO₂ (400, 1000 and 2100 µatm) and temperature conditions (5, 7 and 10°C) on the northern sand lance offspring. We hypothesized that the slow development of the northern sand lance offspring in relatively CO₂ stable waters would make this species particularly CO₂-sensitive. We specifically examined whether exposure to future *p*CO₂ and temperature conditions affected hatching success, time to hatch, embryonic growth and endogenous energy consumption. Given the similarities in life history between sand lance species, our findings for the northern sand lance may have broad implications for the vulnerability of northern hemisphere marine food webs to the combined stressors of ocean warming and acidification.

Methods

Field sampling and fertilization

Spawning-ripe sand lance were collected from SBNMS (42° 9' 58.26" N, 70° 18' 44.19" W; Fig. 1) on 2 December 2016 and 22 November 2017, using a 1.3 × 0.7 m beam trawl (6 mm mesh) towed at 3 knots for 15 min. On deck, adults were checked for ripeness and sorted by sex. For the pilot trial in 2016, experimental embryos were fertilized at sea immediately after spawners were collected (2016: sea, Table S1). For the main experiment in 2017, experimental embryos were produced from two fertilizations (Table S1). The first was completed at sea immediately following the collection of spawners (2017: sea), while the second used adults transported to the Rankin Seawater Facility (University of Connecticut Avery Point) and strip-spawned after being held for 2 days in 400-l circular tanks at 10°C without food (2017: laboratory). In each case, we used at least 10 spawners per sex ranging 14–19 cm in total length (TL; mean, 16 cm; Table S1). Strip-spawning protocols were adapted from early experimental work on the congener *A. americanus* (Smigielski et al., 1984). For each fertilization event, gametes from all spawners were mixed together in plastic spawning trays for 1 hr at 10°C, which was the bottom water temperature at the time of adult collection. Embryos fertilized at sea were maintained in coolers at 10°C during transport to the laboratory. Embryos spawned in the laboratory were treated with 40 g l⁻¹ of diatomaceous earth (food grade, Root Naturally®) to better prevent the adhesive eggs from clumping (Smigielski et al., 1984).

Experimental CO₂ and temperature conditions:

We tested factorial combinations of three *p*CO₂ × three temperature levels. The target for *p*CO₂ controls was 400 µatm (~8.15 pH_{NIST}), a level characteristic of the average open ocean and of the *A. dubius* spawning habitat in late fall (Salisbury and Jönsson, 2018). As elevated *p*CO₂ contrasts, we chose 1000 µatm (~7.78 pH) and 2100 µatm (~7.48 pH), which correspond to predicted average ocean *p*CO₂

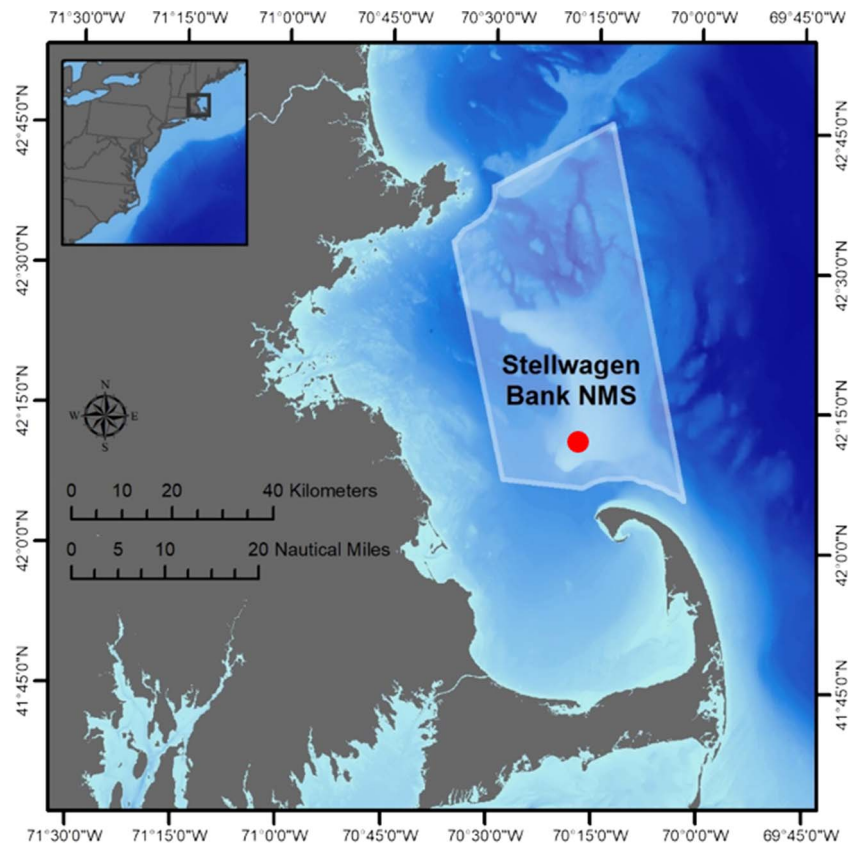


Fig. 1: Map of SBNMS and the surrounding region. The red circle denotes the sampling location for spawning-ripe *A. dubius*.

levels by the years 2100 and 2300, respectively (Caldeira and Wickett, 2005). The three experimental temperatures were 5, 7 and 10°C, which encompass the current range of decreasing temperature conditions on Stellwagen Bank during the estimated spawning period of northern sand lance in late fall and early winter (Salisbury and Jönsson, 2018; Smith et al., 1978). Given the rapid warming already observed in the Gulf of Maine (Pershing et al., 2015) and potential warming of 2–3°C over the next century (Alexander et al., 2018), the warm 10°C treatment represents a thermal condition likely to become more common during the reproductive period of *A. dubius*.

We developed a LabView (National Instruments®) program to automate $p\text{CO}_2$ manipulations in treatment tanks as described in detail by Murray & Baumann (2018). The pH and dissolved oxygen (DO) conditions of each tank were monitored by a central pH electrode (Hach pH_D®, calibrated weekly using 2-point pH_{NIST} references to nearest 0.01) and an optical DO probe (Hach LDO® Model 2). DO levels were maintained at ~100% saturation. Temperature was monitored by an embedded sensor in the LDO probe and was controlled in each tank by thermostats (Aqualogic®) that powered in-line chillers (DeltaStar®). Realized treatment $p\text{CO}_2$ conditions were calculated based on measurements of

pH, temperature, salinity and total alkalinity (A_T). For measurements of A_T ($\mu\text{mol kg}^{-1}$), treatment tanks were sampled three times during each experiment by filtering (to 10 μm) treatment seawater into 300-ml borosilicate bottles. Salinity was measured at the time of sampling using a refractometer. Bottles were stored at 3°C and measured for A_T within two weeks of sampling using an endpoint titration (Mettler Toledo® G20 Potentiometric Titrator). Methodological accuracy (within $\pm 1\%$) of alkalinity titrations were verified and calibrated using Dr Andrew Dickson's certified reference material for A_T in seawater (University of California San Diego, Scripps Institution of Oceanography, Batch Nr. 162 & 164). Unmeasured carbonate parameters were calculated in CO2SYS (V2.1, <http://cdiac.ornl.gov/ftp/co2sys>) as described by Murray & Baumann (2018). Treatment levels and measurements of carbonate chemistry for this study are reported in Table 1.

Experimental design

Sand lance embryos and larvae were reared in a purpose-built system consisting of nine recirculating units that each housed up to five replicate rearing containers (20-l polyethylene buckets). Embryos developed in customized baskets (meshed, 2-l polyethylene cups) floating inside each rearing container

Table 1: Carbon chemistry and temperature measurements from CO₂ × temperature factorial experiments (pilot and main experiment) on *A. dubius* offspring

Exp.	Target temp	Temp	Target pCO ₂	pH	pCO ₂	A _T	C _T	fCO ₂	CO ₃ ²⁻
Pilot	5	5.4 ± 0.1	400	8.08 ± 0.02	446 ± 3	2137 ± 17	2032 ± 17	444 ± 3	23 ± 0.2
	5	5.4 ± 0.1	1000	7.80 ± 0.01	890 ± 3	2132 ± 8	2108 ± 8	886 ± 3	46 ± 0.1
	5	5.3 ± 0.2	2100	7.50 ± 0.03	1828 ± 8	2137 ± 10	2197 ± 10	1821 ± 8	96 ± 0.4
	10	10.0 ± 0.8	400	8.09 ± 0.01	453 ± 4	2143 ± 19	2013 ± 18	451 ± 4	20 ± 0.1
	10	10.3 ± 0.8	1000	7.81 ± 0.01	909 ± 6	2125 ± 16	2082 ± 15	905 ± 6	40 ± 0.3
	10	10.3 ± 0.8	2100	7.51 ± 0.03	1881 ± 2	2131 ± 3	2171 ± 3	1873 ± 2	83 ± 0.1
Main	5	4.9 ± 0.2	400	8.15 ± 0.06	385 ± 11	2198 ± 69	2074 ± 65	384 ± 11	97 ± 4
	5	4.9 ± 0.2	1000	7.72 ± 0.04	1103 ± 49	2175 ± 103	2177 ± 103	1099 ± 49	38 ± 2
	5	4.8 ± 0.3	2100	7.43 ± 0.07	2180 ± 68	2159 ± 76	2246 ± 79	2171 ± 68	19 ± 1
	7	7.1 ± 0.3	400	8.16 ± 0.05	379 ± 9	2177 ± 64	2039 ± 59	377 ± 9	105 ± 4
	7	7.0 ± 0.3	1000	7.74 ± 0.07	1076 ± 29	2185 ± 71	2174 ± 69	1072 ± 28	43 ± 2
	7	7.2 ± 0.3	2100	7.45 ± 0.08	2155 ± 74	2177 ± 82	2250 ± 84	2146 ± 74	22 ± 1
	10	10.0 ± 0.4	400	8.15 ± 0.04	404 ± 9	2219 ± 41	2066 ± 41	402 ± 9	116 ± 1
	10	9.7 ± 0.4	1000	7.76 ± 0.04	1051 ± 10	2180 ± 22	2153 ± 22	1047 ± 10	50 ± 1
	10	9.5 ± 0.4	2100	7.48 ± 0.03	2056 ± 23	2173 ± 34	2228 ± 33	2048 ± 23	26 ± 1

Mean (±SD) pH (NIST) and temperature (°C) are from daily measurements. Mean (±SD) salinity, total alkalinity (A_T; μmol kg⁻¹), dissolved inorganic carbon (C_T; μmol kg⁻¹), partial pressure and fugacity of CO₂ (pCO₂; fCO₂; μatm) and carbonate ion concentration (CO₃²⁻; μmol kg⁻¹) quantified from replicated seawater samples. Salinity was measured via refractometer and A_T from endpoint titrations while pCO₂, C_T, fCO₂ and CO₃²⁻ were calculated in CO2SYS.

Salinity was 31 psu for all treatments.

and received a continuous flow of 4 l h⁻¹. All offspring were reared under a light cycle of 11L:13D and a salinity of 31. For the pilot trial, equal amounts of embryos were randomly distributed into replicate rearing containers per pCO₂ treatment (n = 2 for 400; 2100 μatm; n = 1 for 1000 μatm) and temperature (5 and 10°C) within 9 hrs post-fertilization. The exact number of embryos allotted to each rearing basket was confirmed at 130 degree days post-fertilization (dpf) (degree days = rearing temperature × days, ddpf) after examination via dissecting microscope (8× mag). Thereafter, embryo baskets were monitored daily, and hatched larvae were counted and immediately preserved in a 5% formaldehyde/freshwater solution saturated with sodium tetraborate buffer. Standard length at hatch (SL, nearest 0.01 mm) was measured via calibrated microscope images using Image Pro Premier (V9.0, Media Cybernetics®). The pilot trial was terminated after 400 ddpf to fully encapsulate the potential hatching period (40 days at 10°C and 80 days at 5°C).

For the main experiment, equal amounts of embryos (2.5 ml or ~3000 embryos) from the fertilization at sea were randomly placed into each of two replicate rearing containers per factorial pCO₂ (400; 1000; 2100 μatm) and temperature combination (5, 7 10°C) within 9 hrs of fertilization. Two days later, 300 embryos from the fertilization in the laboratory were distributed into three additional replicate rearing containers in six factorial pCO₂

(400; 1000; 2100 μatm) and temperature combinations (5 and 10°C) within 2 hrs post-fertilization. Low availability of laboratory fertilized embryos precluded additional replicates for all 7°C pCO₂ treatments (see Fig. S1 for a schematic of the experimental design).

Daily checks for hatchlings started at 100 ddpf, and hatchlings were counted and then preserved. For embryos fertilized at sea, subsamples (n ≥ 12) for morphometric measurements were taken on the first day when 10 or more larvae hatched in a given replicate, whereas all hatchlings from the laboratory fertilization were preserved for morphometric measurements (Table S2). Daily monitoring of 7°C treatments was discontinued after initial subsampling, but daily counts of hatchlings continued for all 5 and 10°C treatments until 400 ddpf.

Response traits

Replicate-specific hatching success (%) was calculated for the pilot trial and main experiment as the proportion of hatchlings relative to the number of starting embryos (Pilot: ~350; Main_{sea}: 3000 Main_{lab}: 300). Treatment-specific daily hatch frequencies (=daily treatment hatch/total hatch within temperature treatment) and replicate-specific average time to hatch (dpf = summed age of all larvae at hatch/total hatch count) were calculated for the main experiment. Hatching success and frequencies were not calculated for main

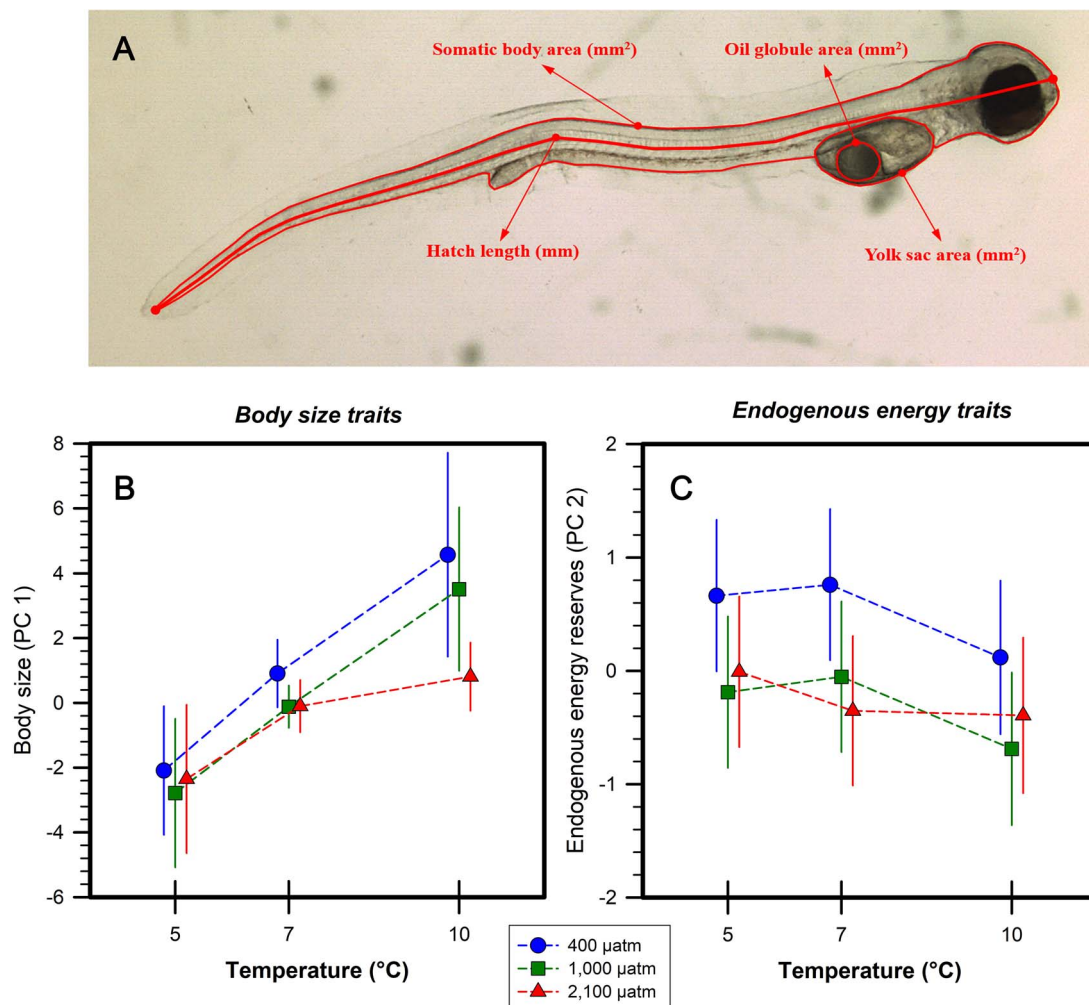


Fig. 2: *A. dubius*. (A) Schematic of morphometric measurements made on newly hatched larvae from the main experiment. Measurements include hatch length (SL, nearest 0.01 mm), somatic body area (nearest 0.01 mm²), yolk sac area (nearest 0.001 mm²) and oil globule area (nearest 0.001 mm²). (B, C) Sea fertilization. Model-adjusted ($\pm 95\%$ confidence interval) treatment PC scores of body size traits (B; PC 1; SL and somatic body area) and endogenous energy reserves (C; PC 2; yolk sac and oil globule area) per temperature and pCO₂ treatment (blue circles, 400 μ atm; green squares, 1000 μ atm; red triangles, 2100 μ atm).

experiment treatments at 7°C. During the main experiment, subsampled hatchlings were measured for two body size metrics [SL and somatic body area (nearest 0.01 mm²)] and two measures of remaining endogenous energy reserves, i.e. yolk sac area (nearest 0.001 mm²) and oil globule area (nearest 0.001 mm²) as illustrated in Fig. 2A. All experimental procedures complied with the ethical guidelines under Institutional Animal Care and Use Committee protocol #A17–043.

Statistical analysis

We tested for significant treatment effects on offspring hatching success, age at hatch and growth for the main experiment, while simply reporting the pilot trial data for comparison.

Proportional data were logit transformed [$\log_{10}(\text{value}/(1 - \text{value}))$] prior to analysis, with zero values replaced with 0.001 (Warton and Hui, 2011). A two-way linear mixed-effect model (LMM) was used to test for significant effects ($\alpha < 0.05$) of pCO₂, temperature, their interaction (fixed factors) and fertilization event (random factor) on hatching success and time to hatch using the model:

$$\text{Hatching trait} = p\text{CO}_2 + \text{temperature} + p\text{CO}_2 \times \text{temperature} + \text{fertilization} + \text{error}.$$

Statistical testing of morphometric measurements was limited to newly hatched subsamples taken from sea fertilized replicates of the main experiment. Because morphometric traits were intercorrelated, we performed principal compo-

Table 2: *A. dubius*. Summary statistics of LMMs evaluating $p\text{CO}_2$, temperature and age effects on morphometric PC scores from the main experiment sea fertilization

PC	Factor	Num. df	Den. df	F	P
Body size	$p\text{CO}_2$	2	8.264	6.295	0.022
	Temp	2	8.116	10.469	0.006
	$p\text{CO}_2 \times \text{temp}$	4	8.220	3.542	0.059
	Age	1	7.900	9.867	0.014
Endogenous energy	$p\text{CO}_2$	2	9.836	7.045	0.013
	Temp	1	9.796	2.278	0.154
	$p\text{CO}_2 \times \text{temp}$	2	9.792	0.070	0.825

Numerator (Num.) and denominator (Den.) degrees of freedom (df) are shown for each factor. Significant effects are denoted in bold.

ment (PC) analyses preceded by evaluating sampling adequacy (Kaiser–Meyer–Olkin measure, >0.5) and Bartlett's test of sphericity ($P < 0.001$). We extracted two rotated PCs (oblimin procedure) with eigenvalues >1 that explained 43 and 39% of the total variance, respectively (82% cumulatively). Component scores were assigned to each larva. Body size metrics loaded positively on PC1 (>0.89), while endogenous energy metrics loaded positively on PC2 (>0.89). A two-way LMM was used to test for significant effects of $p\text{CO}_2$, temperature, their interaction (fixed factors), age_{days} (covariate) and rearing vessel (random factor) on body size and endogenous energy PC scores using the model:

$$\text{PC} = p\text{CO}_2 + \text{temperature} + p\text{CO}_2 \times \text{temperature} + \text{rearing vessel (tank)} + \text{age} + \text{error}.$$

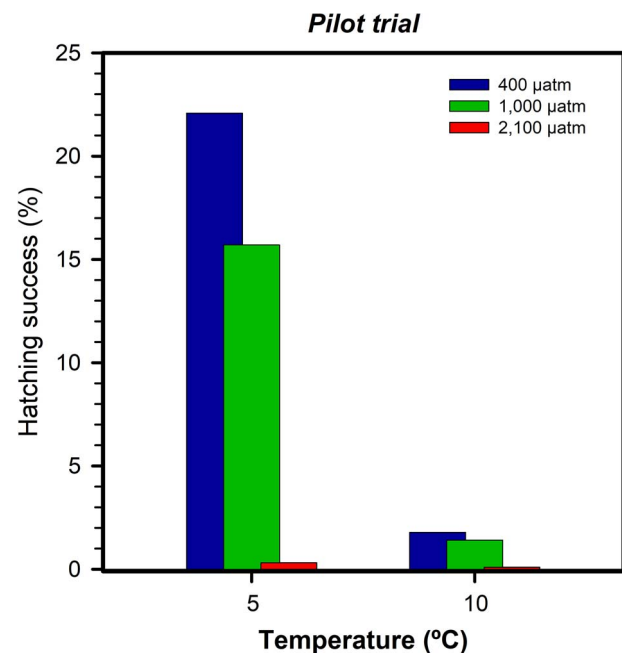
Sample age significantly influenced body size scores (Table 2) and the LMM evaluated treatment effects on scores normalized to a sample age of 29.6 days. Sample age did not significantly affect endogenous energy scores and was not included in the final model. All statistical tests were conducted in SPSS (V20, IBM). For all tests, model residuals were checked for normality by visual inspection of Q-Q plots and for variance homogeneity at each level of fixed and random effects via Levene's test. Bonferroni corrected post hoc tests were applied for multiple comparisons.

Results

Hatching success

Across years, we found a consistent reduction in hatching success with increasing $p\text{CO}_2$ and temperature conditions. In the pilot trial, hatching success at 5°C was 23% at 400 μatm , 16% at 1000 μatm and 1% under 2100 μatm . At 10°C, hatching success was 2%, 1% and 0% at 400, 1000 and 2100 μatm $p\text{CO}_2$, respectively (Fig. 3).

During the main experiment, mean hatching success varied between and within treatments (Table S3) but was significantly affected by a $p\text{CO}_2 \times \text{temperature}$ interaction (LMM,

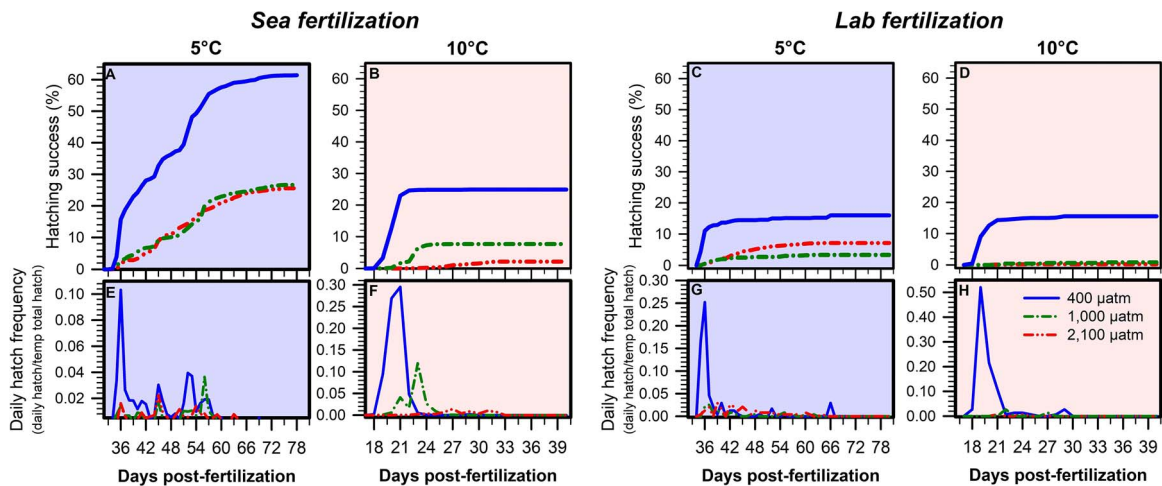
**Fig. 3:** *A. dubius*. Mean embryo survival (%) of offspring reared at 400 μatm (blue, left bar), 1000 μatm (green, central bar) and 2100 μatm $p\text{CO}_2$ (red, right bar) and two temperatures from the pilot trial.

$P = 0.001$; Table 3, Fig. 4A–D). We found that the warm rearing temperature exacerbated the negative effect of elevated $p\text{CO}_2$ on hatching success. Within the 5°C treatments, mean hatching success under both elevated $p\text{CO}_2$ conditions declined by 62% relative to embryos reared under 400 μatm (Bonferroni, $P < 0.01$). At 10°C, the percent reduction in hatching success under elevated $p\text{CO}_2$ conditions increased to 89% (Bonferroni, $P < 0.001$). For offspring reared under 400 μatm $p\text{CO}_2$, hatching did not vary between temperature treatments, but both elevated $p\text{CO}_2$ treatments significantly reduced hatching success at 10°C relative to 5°C (Bonferroni, $P < 0.001$). Within temperature treatments, hatching rates

Table 3: *A. dubius*. Summary statistics from a LMMs testing $p\text{CO}_2 \times \text{temperature}$ effects on hatching success (logit transformed) and age at hatch from the main experiment

Trait	Factor	Num. df	Den. df	F	P
Hatching success	$p\text{CO}_2$	2	23	51.942	<0.001
	Temp	1	23	73.835	<0.001
	$p\text{CO}_2 \times \text{temp}$	2	23	9.964	0.001
Age at hatch	$p\text{CO}_2$	2	22	7.553	0.004
	Temp	1	22	388.048	<0.001
	$p\text{CO}_2 \times \text{temp}$	2	22	0.086	0.918

Numerator (Num.) and denominator (Den.) degrees of freedom (df) are shown for each factor. Significant effects are denoted in bold.

**Fig. 4:** *A. dubius*. Hatching timelines from each fertilization event (2017: sea; 2017: laboratory) tested during the main experiment. Embryos were reared at 5°C (blue background) and 10°C (red background) crossed with three $p\text{CO}_2$ levels: 400 μatm (blue lines), 1000 μatm (green lines) and 2100 μatm (red lines). Traits presented are hatching success (A–D, %) and daily hatch frequencies (E–H, daily hatch/total final hatch within temperature treatment).

did not vary between 1000 and 2100 μatm $p\text{CO}_2$. Overall, hatching success was higher in embryos fertilized at sea (5–62% across treatments) compared to embryos fertilized in the laboratory (0–16% across treatments; Fig. 4, Table S3).

The cold rearing temperature and exposure to elevated $p\text{CO}_2$ significantly increased the average time to hatch, but no interaction was found (LMM, $P < 0.005$; Table 3, Table S3). Embryos reared at 400 μatm $p\text{CO}_2$ hatched in one major peak shortly after first hatch, whereas embryos developing at 1000 and 2100 μatm hatched gradually over a protracted period of time (Fig. 4E–H). This was most pronounced at 10°C, where 99% of embryos at 400 μatm emerged within the first 3 days of hatching compared to only 5% of embryos in 2100 μatm $p\text{CO}_2$ treatment. In the latter, it took 8–12 days for the majority of hatchlings to emerge (Fig. 4F).

Morphometrics

Treatment means ($\pm\text{SD}$) for all morphometric measurements can be found in Table S2. An LMM found a significant

effect of $p\text{CO}_2$ on body size scores ($P = 0.022$; Table 2, Fig. 2B). Larvae reared under 2100 μatm were significantly smaller at hatch than those exposed to 400 μatm $p\text{CO}_2$ (Bonferroni, $P = 0.022$). Rearing temperature had a significant influence on hatchling body size (LMM, $P = 0.006$; Table 2, Fig. 2B). Offspring reared at 10°C were significantly larger than samples from 5 and 7°C (Bonferroni, $P < 0.05$). A $p\text{CO}_2 \times \text{temperature}$ interaction was not detected (Table 2).

Endogenous energy PC scores were significantly influenced by $p\text{CO}_2$ level (LMM, $P = 0.013$; Table 2, Fig. 2C). Offspring reared under 400 μatm had significantly more endogenous energy reserves at hatch compared to larvae from 1000 and 2100 μatm $p\text{CO}_2$ (Bonferroni, $P < 0.05$). Temperature did not independently affect endogenous energy size, nor did it influence the effect of $p\text{CO}_2$ (Table 2).

Discussion

Sand lances represent a group of globally important forage fishes, but their vulnerability to marine climate change

remains understudied. We conducted the first comprehensive evaluation of early life $\text{CO}_2 \times$ temperature sensitivities in a sand lance species (northern sand lance, *A. dubius*) and found that exposure to future $p\text{CO}_2$ and temperature conditions severely reduced hatching success. The lethality of elevated $p\text{CO}_2$ increased with increasing temperature. To date, lethal effects of 1000–2100 $\mu\text{atm } p\text{CO}_2$ in fish early life stages have been documented in some, but not most species (Cattano *et al.*, 2018). Notable examples include reductions in survival of up to 74% in inland silversides (*Menidia beryllina*) (Baumann *et al.*, 2012; Gobler *et al.*, 2018), 48% in summer flounder (*Paralichthys dentatus*) (Chambers *et al.*, 2014) and 47% in Atlantic cod (*Gadus morhua*) (Dahlke *et al.*, 2017). In *A. dubius*, the reductions in embryo survival ranged from 60 to 89% under 1000–2100 $\mu\text{atm } p\text{CO}_2$ (69% on average) relative to contemporary $p\text{CO}_2$ conditions, hence making it one of the most CO_2 sensitive fish species documented to date (Cattano *et al.*, 2018).

In addition, exposure to acidified conditions reduced the remaining endogenous reserves of yolk-sac hatchlings. Exposure to elevated $p\text{CO}_2$ may have increased energetic demands through increased rates of ion regulation and protein synthesis and turnover (Melzner *et al.*, 2009; Pan *et al.*, 2015). Embryos exposed to 2100 $\mu\text{atm } p\text{CO}_2$ were also significantly smaller at hatch, suggesting that yolk utilization had already been maximized, and further CO_2 acclimation required metabolic tradeoffs prioritizing energy for homeostasis over somatic growth (Wieser and Krumschnabel, 2001). These effects would likely be detrimental to *A. dubius* larvae in the wild, because smaller larvae with less endogenous energy generally experience higher cumulative mortality rates (Miller *et al.*, 1988). The observed delay in bulk hatching under acidified conditions may further compound these effects. A CO_2 -induced decoupling of embryonic duration with temperature and other drivers of phenology means that the timing of bulk hatch could be offset from optimal resources and predation pressure (Cushing, 1990).

We found that the warm rearing temperature compounded the negative effects of elevated CO_2 on survival, which is consistent with a growing number of studies reporting negative synergistic effects of elevated $p\text{CO}_2$ and sub-optimal temperatures in fish early life stages (Dahlke *et al.*, 2017; Davis *et al.*, 2018; Flynn *et al.*, 2015; Gobler *et al.*, 2018; Pimentel *et al.*, 2014). Furthermore, the apparent high sensitivity of *A. dubius* embryo survival to elevated $p\text{CO}_2$, and temperature is consistent with findings across other marine taxa (Przeslawski *et al.*, 2015). However, not all fish early life stages have demonstrated synergistic $\text{CO}_2 \times$ temperature effects (Lefevre, 2016; Murray and Baumann, 2018); hence, a more detailed mechanistic understanding of $\text{CO}_2 \times$ temperature effects in marine fish is required (Jutfelt *et al.*, 2018; Pörtner *et al.*, 2017). Nevertheless, the negative synergistic effect on hatching success is particularly concerning for a species that spawns throughout the Gulf of Maine, one of the fastest warming

regions of the ocean (Alexander *et al.*, 2018; Pershing *et al.*, 2015). Warming temperatures have already been linked to depressed sand lance recruitment in the North Sea (Arnott and Ruxton, 2002; Lindegren *et al.*, 2018). Further investigation into the underlying mechanisms of $\text{CO}_2 \times$ temperature sensitivity in sand lance are urgently needed as rapid warming in the northwest Atlantic may already be affecting sand lance early life stages.

What exactly caused the $p\text{CO}_2$ lethality in *A. dubius* embryos is unknown. Mortality could have been due to uncompensated acidosis (Kikkawa *et al.*, 2004) impairing pH-sensitive vital processes (Pörtner *et al.*, 2005). Intriguingly, post-experiment observations of rearing baskets showed that dead embryos from acidified treatments appeared pigmented and near full development, but were seemingly unable to hatch. Thus, an alternative explanation could be that elevated $p\text{CO}_2$ disrupted the hatching process itself. This is supported by our observation of prolonged, intermittent hatching under acidified conditions. Hatching in fish is largely dependent on the activity of hatching enzymes, which are pH sensitive and generally perform best in more alkaline conditions (Korwin-Kossakowski, 2012). Therefore, high $p\text{CO}_2$ levels may acidify the perivitelline fluid thereby reducing the proteolytic properties of hatching enzymes and leading to delayed or unsuccessful hatching (Havas and Rosseland, 1995). Hatching enzyme expression or activity has not yet been evaluated in the context of OA, but potentially represents a critical knowledge gap in marine fishes.

During the main experiment of this study, hatching success varied substantially between fertilization events. Relative to the adults spawned in the laboratory, the adults that were strip-spawned at sea produced four-fold more experimental embryos per female, and when averaged across treatments their offspring had better hatching success. This may indicate that the stress of transport combined with a two-day holding period reduced the reproductive quality of the laboratory-spawned adults. Embryos of poor quality likely experience elevated rates of mortality during development (Bobe and Labbé, 2010) and may be more sensitive to abiotic stressors like acidification (Baumann *et al.*, 2018). Therefore, we cannot fully discount that reduced egg quality influenced the effects of elevated $p\text{CO}_2$ in laboratory-fertilized offspring. Low-hatching success of laboratory-fertilized embryos could also have been due to poor fertilization rates. However, fertilization success is not a good proxy for later developmental success in fish early life-stages (Shields *et al.*, 1997) and thus would not influence embryonic $p\text{CO}_2$ sensitivities. Nevertheless, research efforts devoted to improving the spawning and rearing protocols of non-model species like sand lance will improve estimates of their potential climate sensitivity.

With less than 0.5% of all marine fish species tested, CO_2 effects appear to be highly species- or even population specific (Cattano *et al.*, 2018), which still precludes generalizations for extrapolating findings to untested taxa. One promising

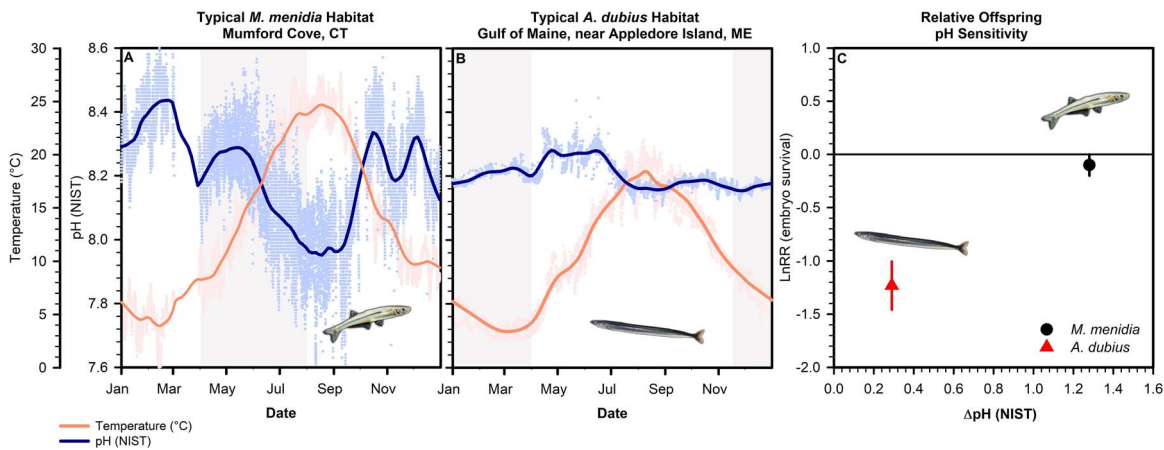


Fig. 5: Seasonal pH (NIST, blue) and temperature (°C, red) variability from spawning habitats of *M. menidia* (A; Mumford Cove, CT; 41° 19' 25'' N 72° 01' 07'' W; Baumann, 2016) and *A. dubius* (B; Coastal Western Gulf of Maine Mooring; 43° 1' 12'' N, 70° 32' 24'' W; Sutton et al., 2014). The Gulf of Maine mooring is located ~ 100 km north of the sand lance collection site (~10 km offshore, 65 m depth). Faded circles, individual measurements; solid lines, smoothed data; shading, seasonal window of early life stages. (C) pH variability vs. pH/CO₂ sensitivity in *M. menidia* vs. *A. dubius*; average CO₂ effect (~2000 μatm) on embryo survival (LnRR±95% CI) plotted against the average pH range experienced during spawning season (ΔpH). Data for *M. menidia* (red circle) was taken from Baumann et al. (2018). In both species, CO₂-driven reductions in embryo survival (negative LnRRs) are considered significant because 95% confidence intervals do not include 0 (Hedges et al., 1999).

hypothesis is that the CO₂ tolerance of species and populations increases with the level of *p*CO₂ variability experienced in their natural habitats (OVH; Baumann, 2019). Consider the well-studied Atlantic silverside (*M. menidia*), another coastal forage fish that spawns in nearshore, sub-tropical to temperate habitats during spring and summer (Conover and Kynard, 1984). These habitats typically undergo seasonal, metabolic acidification with diel pH fluctuations exceeding 0.60 units (7.43–8.10 pH; Fig. 5A). Years of serial experimentation on the species revealed that wild silverside embryos are weakly affected by elevated *p*CO₂ early in the season, but become progressively more CO₂ tolerant (Baumann et al., 2018) until by early summer they are unaffected by even extreme *p*CO₂ conditions (>4000 μatm) (Murray and Baumann, 2018).

Mechanisms that rapidly enhance offspring CO₂ tolerance are likely adaptive for nearshore species that spawn embryos into highly variable environments (Hoffmann and Hercus, 2000) and therefore appear tolerant of future *p*CO₂ conditions in experimental settings (Hamilton et al., 2017; Hofmann et al., 2014; Maas et al., 2012; Vargas et al., 2017). In the Gulf of Maine, *A. dubius* spawn in late fall and deposit embryos onto offshore sandbanks, where *p*CO₂ conditions generally resemble stable surface ocean levels (Salisbury and Jönsson, 2018). Since pH values never fall below 8.00 (Fig. 5B), *A. dubius* offspring may face little selection for CO₂ tolerant phenotypes, assuming that such tolerance is metabolically costly and thus not maintained if not needed (Sunday et al., 2014). As a result, the sensitivity of *A. dubius* embryos to 2100 μatm *p*CO₂ appears to be one order of magnitude higher than in silversides (Fig. 5C).

Some empirical assessments of fish early life CO₂ sensitivities appear to contradict the OVH, e.g. in yellowtail kingfish (*Seriola lalandi*), which spawns in subtropical, pelagic waters with little *p*CO₂ variability but produces largely CO₂ resilient offspring (Munday et al., 2016; Watson et al., 2018). However, offspring of *S. lalandi* and other tropical piscivores develop very rapidly, and larvae become active predators within a week of fertilization (Chen et al., 2006). The early onset of rapid growth and mobility likely requires advanced acid-base regulatory capabilities to develop early in ontogeny (Melzner et al., 2009). By contrast, offspring of species like *A. dubius* with protracted early development may have a lower buffering capacity for metabolic CO₂ and are consequently more sensitive to acidification (Baumann, 2019; Melzner et al., 2009). Variability in stage duration may thus explain why CO₂ effects observed in tropical species are generally sub-lethal (Bignami et al., 2014; Munday et al., 2009; Welch and Munday, 2016, but see Frommel et al., 2016), whereas examples of direct CO₂-induced mortality are mostly represented by higher latitude species (Chambers et al., 2014; Dahlke et al., 2017; Flynn et al., 2015; Pimentel et al., 2014).

The OVH framework incorporates natural *p*CO₂ variability and stage duration and may thus prove useful for predicting early life climate sensitivities and for refocusing research efforts on species likely affected first by OA. The present study provides evidence to support this framework. However, slow-developing fish that spawn in relatively stable CO₂ environments are severely understudied with respect to CO₂ × temperature impacts, likely because working with offshore and winter spawners from temperate to polar regions is logistically challenging. To date, experimenters often choose study species based on availability or familiarity. However,

robust evaluations of OA $\times$ warming sensitivity in the ocean will only be achieved by choosing study species based on concrete theoretical frameworks rather than logistical convenience.

Our discovery that wild embryos of *A. dubius* are exceptionally sensitive to near-future $p\text{CO}_2$ and temperature conditions has direct conservation implications for marine ecosystems. First, many sand lance species share reproductive and early life history characteristics with *A. dubius*, hence there is the potential that other sand lances are equally sensitive to warming and acidifying oceans. Given the central importance of these forage fishes, their elevated climate sensitivity may pose a greater risk to the productivity and resiliency of coastal shelf ecosystems across the northern hemisphere than currently acknowledged. If so, iconic higher trophic predators reliant on sand lance may be challenged. Second, this also means that existing plans for large-scale exploitation of sand lance populations by industrial fisheries should be viewed more skeptically or at least should be proceed with extra precaution due to their heightened sensitivity to marine climate change (Lindgren et al., 2018). Our study thus provides a clear mandate for follow-up research to understand the mechanisms and prevalence of near-future $p\text{CO}_2$ lethality in this ecologically important group of forage fish.

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References

- Alder J, Campbell B, Karpouzi V, Kaschner K, Pauly D (2008) Forage fish: from ecosystems to markets. *Annu Rev Env Resour* 33: 153–116.
- Alexander MA, Scott JD, Friedland KD, Mills KE, Nye JA, Pershing AJ, Thomas AC (2018) Projected sea surface temperatures over the 21 st century: changes in the mean, variability and extremes for large marine ecosystem regions of northern oceans. *Elementa Science of the Anthropocene* 6: 9.
- Arnott SA, Ruxton GD (2002) Sandeel recruitment in the North Sea: demographic, climatic and trophic effects. *Mar Ecol Prog Ser* 238: 199–210.
- Baumann H (2016) Temperature, pH, DO, and salinity data from Mumford cove in Connecticut, USA starting in April 2015. *Biological and Chemical Oceanography Data Management Office (BCO-DMO) Dataset version 2016-09-27*.
- Baumann H (2019) Experimental assessments of marine species sensitivities to ocean acidification and co-stressors: how far have we come? *Can J Zool* 97: 399–408.
- Baumann H, Cross E, Murray CS (2018) Robust quantification of fish early life CO_2 sensitivities via serial experimentation. *Biol Lett* 14: 20180408.
- Baumann H, Smith EM (2017) Quantifying metabolically driven pH and oxygen fluctuations in US nearshore habitats at diel to interannual time scales. *Estuaries Coast* 41: 1102–1117.
- Baumann H, Talmage SC, Gobler CJ (2012) Reduced early life growth and survival in a fish in direct response to increased carbon dioxide. *Nature Climate Change* 2: 38–41.
- Becker BH, Beissinger SR (2006) Centennial decline in the trophic level of an endangered seabird after fisheries decline. *Conserv Biol* 20: 470–479.
- Bignami S, Sponaugle S, Cowen RK (2014) Effects of ocean acidification on the larvae of a high-value pelagic fisheries species, mahi-mahi *Coryphaena hippurus*. *Aquat Biol* 21: 249–260.
- Bobe J, Labbé C (2010) Egg and sperm quality in fish. *Gen Comp Endocrinol* 165: 535–548.
- Caldeira K, Wickett ME (2005) Ocean model predictions of chemistry changes from carbon dioxide emissions to the atmosphere and ocean. *J Geophys Res* 110: C09S04.
- Cattano C, Claudet J, Domenici P, Milazzo M (2018) Living in a high CO_2 world: a global meta-analysis shows multiple trait-mediated responses of fish to ocean acidification. *Ecological Monographs* 88: 320–335.
- Chambers R, Candelmo A, Habeck E, Poach M, Wicczorek D, Cooper K, Greenfield C, Phelan B (2014) Ocean acidification effects in the early life-stages of summer flounder, *Paralichthys dentatus*. *Biogeosciences* 10: 13897–13929.
- Chen BN, Qin JG, Kumar MS, Hutchinson W, Clarke S (2006) Ontogenetic development of the digestive system in yellowtail kingfish *Seriola lalandi* larvae. *Aquaculture* 256: 489–501.
- Compton TJ, Rijkenberg MJ, Drent J, Piersma T (2007) Thermal tolerance ranges and climate variability: a comparison between bivalves from differing climates. *J Exp Mar Biol Ecol* 352: 200–211.
- Conover DO, Kynard BE (1984) Field and laboratory observations of spawning periodicity and behavior of a northern population of the Atlantic silverside, *Menidia menidia* (pisces: Atherinidae). *Environ Biol Fishes* 11: 161–171.
- Cushing D (1990) Plankton production and year-class strength in fish populations: an update of the match/mismatch hypothesis, advances in marine biology. *Elsevier Vol26*: 249–293.

- Dahlke FT, Leo E, Mark FC, Pörtner HO, Bickmeyer U, Frickenhaus S, Storch D (2017) Effects of ocean acidification increase embryonic sensitivity to thermal extremes in Atlantic cod, *Gadus morhua*. *Glob Chang Biol* 23: 1499–1510.
- Davis BE, Flynn EE, Miller NA, Nelson FA, Fanguie NA, Todgham AE (2018) Antarctic emerald rockcod have the capacity to compensate for warming when uncoupled from CO₂-acidification. *Glob Chang Biol* 24: e655–e670.
- Duarte CM, Hendriks IE, Moore TS, Olsen YS, Steckbauer A, Ramajo L, Carstensen J, Trotter JA, McCulloch M (2013) Is ocean acidification an open-ocean syndrome? Understanding anthropogenic impacts on seawater pH. *Estuaries Coast* 36: 221–236.
- Essington TE, Moriarty PE, Froehlich HE, Hodgson EE, Koehn LE, Oken KL, Siple MC, Stawitz CC (2015) Fishing amplifies forage fish population collapses. *Proc Natl Acad Sci* 112: 6648–6652.
- Flynn EE, Bjelde BE, Miller NA, Todgham AE (2015) Ocean acidification exerts negative effects during warming conditions in a developing Antarctic fish. *Conservation Physiology* 3: cov033.
- Frommel AY, Margulies D, Wexler JB, Stein MS, Scholey VP, Williamson JE, Bromhead D, Nicol S, Havenhand J (2016) Ocean acidification has lethal and sub-lethal effects on larval development of yellowfin tuna, *Thunnus albacares*. *J Exp Mar Bio Ecol* 482: 18–24.
- Furness WR, Tasker LM (2000) Seabird-fishery interactions: quantifying the sensitivity of seabirds to reductions in sandeel abundance, and identification of key areas for sensitive seabirds in the North Sea. *Mar Ecol Prog Ser* 202: 253–264.
- Gobler CJ, Merlo LR, Morell BK, Griffith AW (2018) Temperature, acidification, and food supply interact to negatively affect the growth and survival of the forage fish, *Menidia beryllina* (inland silverside) and *Cyprinodon variegatus* (sheepshead minnow). *Front Mar Sci* 5: 86.
- Hamilton SL, Logan CA, Fennie HW, Sogard SM, Barry JP, Makukhov AD, Tobosa LR, Boyer K, Lovera CF, Bernardi G (2017) Species-specific responses of juvenile rockfish to elevated pCO₂: from behavior to genomics. *PLoS One* 12: e0169670.
- Hamre J (1994) Biodiversity and exploitation of the main fish stocks in the Norwegian-Barents Sea ecosystem. *Biodivers Conserv* 3: 473–492.
- Havas M, Rosseland BO (1995) Response of zooplankton, benthos, and fish to acidification: an overview. *Water Air Soil Pollut* 85: 51–62.
- Hedges LV, Gurevitch J, Curtis PS (1999) The meta-analysis of response ratios in experimental ecology. *Ecology* 80: 1150–1156.
- Hoffmann AA, Hercus MJ (2000) Environmental stress as an evolutionary force. *Bioscience* 50: 217–226.
- Hofmann G, Evans T, Kelly M, Padilla-Gamiño J, Blanchette C, Washburn L, Chan F, McManus M, Menge B, Gaylord B (2014) Exploring local adaptation and the ocean acidification seascape—studies in the California current large marine ecosystem. *Biogeosciences* 11: 1053–1064.
- Jarrold MD, Humphrey C, McCormick MI, Munday PL (2017) Diel CO₂ cycles reduce severity of behavioural abnormalities in coral reef fish under ocean acidification. *Sci Rep* 7: 10153.
- Jarrold MD, Munday PL (2019) Diel CO₂ cycles and parental effects have similar benefits to growth of a coral reef fish under ocean acidification. *Biol Lett* 15: 2018072.
- Jutfelt F, Norin T, Ern R, Overgaard J, Wang T, McKenzie DJ, Lefevre S, Nilsson GE, Metcalfe NB, Hickey AJ (2018) Oxygen- and capacity-limited thermal tolerance: blurring ecology and physiology. *J Exp Biol* 221: jeb169615.
- Kelly MW, Padilla-Gamiño JL, Hofmann GE (2013) Natural variation and the capacity to adapt to ocean acidification in the keystone sea urchin *Strongylocentrotus purpuratus*. *Glob Chang Biol* 19: 2536–2546.
- Kikkawa T, Kita J, Ishimatsu A (2004) Comparison of the lethal effect of CO₂ and acidification on red sea bream (*Pagrus major*) during the early developmental stages. *Mar Pollut Bull* 48: 108–110.
- Korwin-Kossakowski M (2012) Fish hatching strategies: a review. *Rev Fish Biol Fisher* 22: 225–240.
- Lefevre S (2016) Are global warming and ocean acidification conspiring against marine ectotherms? A meta-analysis of the respiratory effects of elevated temperature, high CO₂ and their interaction. *Conserv Physiol* 4: cow009.
- Lindgren M, Van Deurs M, MacKenzie BR, Worsoe Clausen L, Christensen A, Rindorf A (2018) Productivity and recovery of forage fish under climate change and fishing: North Sea sandeel as a case study. *Fish Oceanogr* 27: 212–221.
- Maas AE, Wishner KF, Seibel BA (2012) The metabolic response of pteropods to acidification reflects natural CO₂-exposure in oxygen minimum zones. *Biogeosciences* 9: 747–757.
- Melzner F, Gutowska M, Langenbuch M, Dupont S, Lucassen M, Thorndyke MC, Bleich M, Pörtner HO (2009) Physiological basis for high CO₂ tolerance in marine ectothermic animals: pre-adaptation through lifestyle and ontogeny? *Biogeosciences* 6: 2313–2331.
- Miller TJ, Crowder LB, Rice JA, Marschall EA (1988) Larval size and recruitment mechanisms in fishes: toward a conceptual framework. *Can J Fish Aquat Sci* 45: 1657–1670.
- Munday PL, Donelson JM, Dixon DL, Endo GKG (2009) Effects of ocean acidification on the early life history of a tropical marine fish. *Proc Biol Sci* 276: 3275–3283.
- Munday PL, Watson S-A, Parsons DM, King A, Barr NG, Mcleod IM, Allan BJ, Pether SM (2016) Effects of elevated CO₂ on early life history development of the yellowtail kingfish, *Seriola lalandi*, a large pelagic fish. *ICES J Mar Sci* 73: 641–649.
- Murray CS, Baumann H (2018) You better repeat it: complex CO₂ × temperature effects in Atlantic silverside offspring revealed by serial experimentation. *Diversity* 10: 69.

- Orr JW, Wildes S, Kai Y, Raring N, Nakabo T, Katugin O, Guyon J (2015) Systematics of North Pacific sand lances of the genus *Ammodytes* based on molecular and morphological evidence, with the description of a new species from Japan. *Fish Bull* 113: 129–156.
- Pan T-CF, Applebaum SL, Manahan DT (2015) Experimental ocean acidification alters the allocation of metabolic energy. *Proc Natl Acad Sci USA* 112: 4696–4701.
- Pershing AJ, Alexander MA, Hernandez CM, Kerr LA, Le Bris A, Mills KE, Nye JA, Record NR, Scannell HA, Scott JD (2015) Slow adaptation in the face of rapid warming leads to collapse of the Gulf of Maine cod fishery. *Science* 350: 809–812.
- Pikitch E, Boersma PD, Boyd I, Conover D, Cury P, Essington T, Heppell S, Houde E, Mangel M, Pauly D (2012) *Little Fish, Big Impact: Managing a Crucial Link in Ocean Food Webs*. Washington, DC
- Pimentel MS, Faleiro F, Dionísio G, Repolho T, Pousão-Ferreira P, Machado J, Rosa R (2014) Defective skeletogenesis and oversized otoliths in fish early stages in a changing ocean. *J Exp Biol* 217: 2062–2070.
- Pinsky ML, Jensen OP, Ricard D, Palumbi SR (2011) Unexpected patterns of fisheries collapse in the World's oceans. *Proc Natl Acad Sci* 108: 8317–8322.
- Pörtner H-O, Bock C, Mark FC (2017) Oxygen- and capacity-limited thermal tolerance: bridging ecology and physiology. *J Exp Biol* 220: 2685–2696.
- Pörtner HO, Langenbuch M, Michaelidis B (2005) Synergistic effects of temperature extremes, hypoxia, and increases in CO₂ on marine animals: from earth history to global change. *J Geophys Res Oceans* 110: 1–15.
- Przeslawski R, Byrne M, Mellin C (2015) A review and meta-analysis of the effects of multiple abiotic stressors on marine embryos and larvae. *Glob Chang Biol* 21: 2122–2140.
- Salisbury JE, Jönsson BF (2018) Rapid warming and salinity changes in the Gulf of Maine alter surface ocean carbonate parameters and hide ocean acidification. *Biogeochemistry* 141: 401–418.
- Shannon L, Coll M, Neira S, Cury P, Roux J-P (2009) Impacts of fishing and climate change explored using trophic models. In C Roy, D Checkley, J Alheit, Y Oozeki, eds, *Climate Change and Small Pelagic Fish*. Cambridge University Press, Cambridge, pp. 158–190
- Shields RJ, Brown NP, Bromage NR (1997) Blastomere morphology as a predictive measure of fish egg viability. *Aquaculture* 155: 1–12.
- Smigielski AS, Halavik TA, Buckley LJ, Drew SM, Laurence GC (1984) Spawning, embryo development and growth of the American sand lance *Ammodytes americanus* in the laboratory. *Mar Ecol Prog Ser* 14: 287–292.
- Smith W, Sullivan L, Berrien P (1978) Fluctuations in production of sand lance larvae in coastal waters off the northeastern United States, 1974 to 1977. International Council for the Exploration of the Sea. 50: 30.
- Stevens GC (1989) The latitudinal gradient in geographical range: how so many species coexist in the tropics. *Am Nat* 133: 240–256.
- Sunday JM, Bates AE, Dulvy NK (2012) Thermal tolerance and the global redistribution of animals. *Nat Clim Change* 2: 686.
- Sunday JM, Calosi P, Dupont S, Munday PL, Stillman JH, Reusch TB (2014) Evolution in an acidifying ocean. *Trends Ecol Evol* 29: 117–125.
- Sutton AJS, Christopher L, Salisbury JE, Vandemark D, Musielewicz S, Stacy MJ, Dietrich C, Bott R, Osborne J (2014) High-Resolution Ocean and atmosphere pCO₂ time-series measurements from mooring NH_70W_43N (National Oceanographic Data Center). NOAA Version 7.7.
- Tacon AGJ, Metian M (2009) Fishing for aquaculture: non-food use of small pelagic forage fish—a global perspective. *Rev Fish Sci* 17: 305–317.
- Vargas CA, Lagos NA, Lardies MA, Duarte C, Manríquez PH, Aguilera VM, Broitman B, Widdicombe S, Dupont S (2017) Species-specific responses to ocean acidification should account for local adaptation and adaptive plasticity. *Nat Ecol Evol* 1: 0084.
- Warton DI, Hui FK (2011) The arcsine is asinine: the analysis of proportions in ecology. *Ecology* 92: 3–10.
- Watson S-A *et al.* (2018) Ocean warming has a greater effect than acidification on the early life history development and swimming performance of a large circumglobal pelagic fish. *Glob Chang Biol* 24: 4368–4385.
- Welch MJ, Munday PL (2016) Contrasting effects of ocean acidification on reproduction in reef fishes. *Coral Reefs* 35: 485–493.
- Wieser W, Krumschnabel G (2001) Hierarchies of ATP-consuming processes: direct compared with indirect measurements and comparative aspects. *Biochem J* 355: 389–395.
- Wiley DN, Moller JC, Pace IIRM, Carlson C (2008) Effectiveness of voluntary conservation agreements: case study of endangered whales and commercial whale watching. *Conserv Biol* 22: 450–457.
- Willson MF, Armstrong RH, Robards MD, Piatt JF (1999) *Sand Lance as Cornerstone Prey for Predator Populations*. USDA Forest Service, Pacific Northwest Research Station, Portland, OR.
- Woolsey E, Keith SA, Byrne M, Schmidt-Roach S, Baird A (2015) Latitudinal variation in thermal tolerance thresholds of early life stages of corals. *Coral Reefs* 34: 471–478.