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Global change biology

Adult exposure to ocean acidification is maladaptive for larvae of the Sydney rock oyster *Saccostrea glomerata* in the presence of multiple stressors

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Parental effects passed from adults to their offspring have been identified as a source of rapid acclimation that may allow marine populations to persist as our surface oceans continue to decrease in pH. Little is known, however, whether parental effects are beneficial for offspring in the presence of multiple stressors. We exposed adults of the oyster *Saccostrea glomerata* to elevated CO_2 and examined the impacts of elevated CO_2 (control = 392; 856 µatm) combined with elevated temperature (control = 24; 28°C), reduced salinity (control = 35; 25) and reduced food concentration (control = full; half diet) on their larvae. Adult exposure to elevated CO_2 had a positive impact on larvae reared at elevated CO_2 as a sole stressor, which were 8% larger and developed faster at elevated CO_2 compared with larvae from adults exposed to ambient CO_2. These larvae, however, had significantly reduced survival in all multistressor treatments. This was particularly evident for larvae reared at elevated CO_2 combined with elevated temperature or reduced food concentration, with no larvae surviving in some treatment combinations. Larvae from CO_2-exposed adults had a higher standard metabolic rate. Our results provide evidence that parental exposure to ocean acidification may be maladaptive when larvae experience multiple stressors.

1. Background

Parental effects have been identified as a source of rapid acclimation that may allow marine populations to persist as our surface oceans continue to decrease in pH [1,2]. For several marine invertebrates, exposure of parents to ocean acidification (OA) conditions promotes phenotypic changes in their larvae resulting in improved performance in those same acidified conditions [3–8]. It is not known, however, if there are limits to the parental effects induced by OA, especially in response to co-occurring multiple stressors.

Marine and estuarine larvae live in a multi-stressor world [9], characterized by a range of interacting abiotic and biotic conditions. Global change caused by increased atmospheric carbon dioxide (CO_2) is exacerbating the magnitude of

change in many of these stressors [10,11]. Sea-surface temperatures are rising, with an increase of 2–4.5°C projected by 2100 [11]. The prevalence of extreme weather events, freshwater intrusions and hypoxia will also increase [11]. In the presence of multiple stressors not previously conditioned to, the parental effects that enhance larval performance following parental exposure to OA, as a single stressor, may be of limited benefit or have negative repercussions [1,2].

Sydney rock oysters, *Saccostrea glomerata*, form the basis of a large aquaculture industry in coastal and estuarine locations in southeastern Australia. This region is an ocean warming hotspot, where increased poleward flow of the East Australian Current moves warm tropical water southwards along the coast [12,13]. An increase in the frequency of extreme rainfall events is also causing greater episodes of reduced salinity in the region [14]. In addition, the generally oligotrophic status of the region means that larval food supply is typically lower than in other temperate regions [12,15]. Previous transgenerational studies with *S. glomerata* have shown positive carryover effects following parental exposure to near-future OA conditions. Larvae generated from these parents were larger and developed faster in these same conditions compared with larvae from control parents [3,4]. Here we assessed the benefits of parental exposure to OA for the progeny of *S. glomerata* exposed to OA in the presence of a suite of stressors they would normally experience in nature: lower salinity, low food conditions and warming. Six performance traits—egg size and lipid content, larval development, growth, metabolic rate and survival—were quantified to determine whether the beneficial parental effects seen in response to OA as a single stressor also occur when larvae are exposed to OA in combination with these other stressors.

2. Methods

(a) Parental exposure

Adult *S. glomerata* were exposed to ambient (392 µatm) or elevated (856 µatm) CO_2 from the beginning of gametogenesis for eight weeks until reaching gravid stage (table 1) after Parker *et al*. [3,4]. There were three replicate tanks of each exposure type, making a total of six experimental units. Upon maturation, adults were spawned and gametes were collected from nine females and nine males (three per replicate) from both the ambient and elevated CO_2 parents and were used to create F_1 larvae. From each of the nine females, the sizes of 30 eggs and the total lipid content of 5000 eggs were determined.

(b) F_1 larval exposure

Seven treatments were selected for the F_1 larval exposure using a non-orthogonal design (table 1). Gametes from the ambient and elevated CO_2 parents were fertilized in 20 l buckets of filtered seawater set at the treatment conditions (three replicates per treatment, per parental exposure). Larvae were fed a mixed algal diet and water changes were made daily. Larvae remained in the treatments for 15 days. Mean shell length (15 days), percentage survival (15 days), percentage development to the umbonate stage (9 days) and standard metabolic rate (SMR; 15 days) of larvae were measured. Detailed methods have been uploaded as part of the electronic supplementary material.

(c) Data analysis

The effect of parental exposure on egg size and total lipid content was assessed with linear mixed effects models (REMLs). Parental

Table 1. Mean seawater physico-chemical conditions. TA, total alkalinity; DIC, dissolved inorganic carbon; CO_2, elevated CO_2; $\Omega_{calcite}$, calcite saturation state; Ω_{arag}, aragonite saturation state; T, elevated temperature; S, reduced salinity; F, half food diet (full food: 1×10^4–10^5 cells ml^{-1}; half food: 5×10^3–10^4 cells ml^{-1}; from beginning to end of experiment); ± indicates range.

condition	salinity	temperature (°C)	pH_{NIST}	TA (µmol kg^{-1})	pCO_2 (µatm)	DIC (µmol kg^{-1})	$\Omega_{calcite}$	Ω_{arag}
parents								
ambient	34.5 ± 0.3	24.0 ± 0.5	8.20 ± 0.01	2348 ± 18	392 ± 9	2048 ± 25	5.21 ± 0.12	3.42 ± 0.08
elevated	34.5 ± 0.3	24.0 ± 0.5	7.91 ± 0.01	2348 ± 18	856 ± 21	2194 ± 22	3.01 ± 0.02	1.98 ± 0.01
F_1 larvae								
control	34.5 ± 0.2	24.0 ± 0.5	8.19 ± 0.01	2339 ± 23	392 ± 10	2040 ± 21	5.18 ± 0.08	3.40 ± 0.06
CO_2	34.5 ± 0.2	24.0 ± 0.5	7.91 ± 0.02	2339 ± 23	854 ± 27	2185 ± 23	2.99 ± 0.06	1.97 ± 0.04
CO_2 + T	34.5 ± 0.3	28.0 ± 0.5	7.92 ± 0.01	2339 ± 23	855 ± 21	2160 ± 28	3.42 ± 0.07	2.27 ± 0.05
CO_2 + S	25.0 ± 0.1	24.0 ± 0.5	7.82 ± 0.01	1683 ± 11	855 ± 17	1622 ± 12	1.51 ± 0.04	0.96 ± 0.03
CO_2 + F	34.5 ± 0.2	24.0 ± 0.5	7.91 ± 0.01	2339 ± 23	854 ± 15	2185 ± 19	2.99 ± 0.09	1.97 ± 0.06
CO_2 + T + S	25.0 ± 0.1	28.0 ± 0.5	7.83 ± 0.02	1683 ± 11	855 ± 12	1607 ± 9	1.76 ± 0.06	1.14 ± 0.04
CO_2 + T + S + F	25.0 ± 0.1	28.0 ± 0.5	7.83 ± 0.02	1683 ± 11	856 ± 14	1607 ± 8	1.76 ± 0.05	1.13 ± 0.03

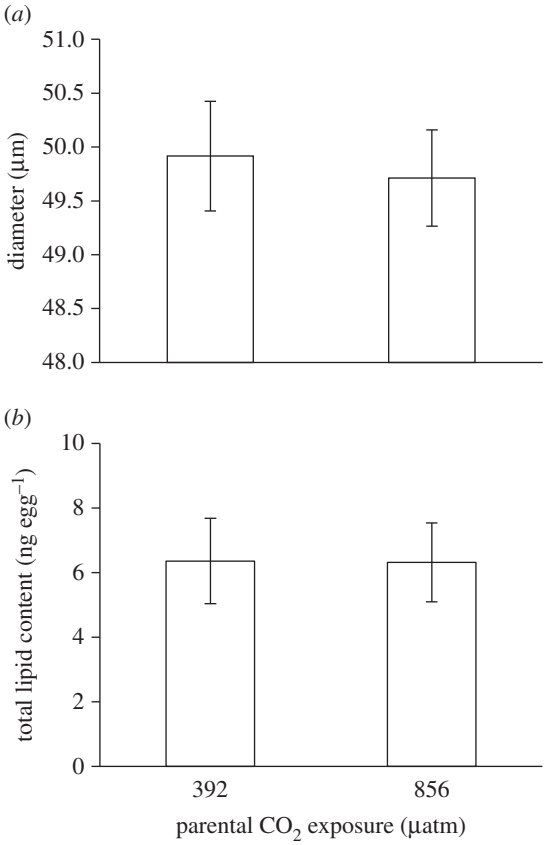

Figure 1. Impact of parental exposure of *Saccostrea glomerata* to ambient (392 µatm) and elevated (856 µatm) CO_2 for 8 weeks on (*a*) diameter ($n = 3$) and (*b*) total lipid content of eggs ($n = 3$) with standard error bars.

exposure, F_1 larvae treatment effects and their interaction were also tested for the larval data. The treatment structure for SMR and shell length data was unbalanced owing to missing data (larvae died in all replicates of the elevated parent, F_1 treatments $CO_2 + T$ and $CO_2 + T + S + F$) requiring a REML analysis. Larval survival data were analysed using unbalanced ANOVA, weighted by the number of larvae present on 0 days. The proportion of umbonate larvae data was analysed using a binomial generalized linear model with logit link, and predicted means were compared on the logit scale using approximate pairwise least significant differences and back transformed for presentation. All tests were performed with α set at 0.05, using GENSTAT.

3. Results

Egg size and total lipid content were similar for parents exposed to ambient or elevated CO_2 (figure 1*a*, $F_{1,4} = 0.54$, $p = 0.502$; figure 1*b*, $F_{1,4} = 0.0005$, $p = 0.983$, respectively).

Mean size (15 days) and proportion of umbonate larvae (9 days) from CO_2-exposed parents (143.35 µm, 0.38) were greater than that of larvae from ambient parents (132.57 µm, 0.26) (figure 2*a*, $F_{1,24} = 44.36$, $p < 0.001$; figure 2*b*, deviance ratio = 9.37, approx. $F_{1,28}$ probability = 0.005). Further, mean size and proportion of umbonate larvae were greatest in the F_1 larval treatments with increased temperature, regardless of parental exposure. For the remaining treatments, the size and proportion of umbonate larvae were reduced compared with the control treatment (figure 2*a*, $F_{6,24} = 71.99$, $p < 0.001$; figure 2*b*, deviance ratio = 11.86, approx. $F_{6,28}$ probability < 0.001). Total survival of larvae from CO_2-exposed parents (0.24) was less than survival of larvae from ambient parents (0.30; $F_{1,28} = 5.73$, $p < 0.05$). In the $CO_2 + T$ and $CO_2 + T + S + F$ treatments, there was no survival of larvae from CO_2-exposed parents after 15 days (0% survival at days 13 and 15, respectively; see electronic supplementary material, data for full survivorship curves; electronic supplementary material, figures S1 and S2). Across larval lines, highest mean survival was found in the control treatment (0.61), whereas lowest survival was found in the $CO_2 + T$, $CO_2 + T + S + F$ and $CO_2 + T + S$ treatments (0.02, 0.07 and 0.14, respectively; figure 2*c*, $F_{6,28} = 40.18$, $p < 0.001$). When salinity was combined with elevated CO_2, there was no additive effect on survival.

The SMR of larvae from CO_2-exposed parents was higher than that of larvae from ambient parents, with the exception of the $CO_2 + T + S$ treatment, where there was no difference in the SMR of both larval lines (figure 2*d*, $F_{4,24} = 3.90$, $p < 0.05$).

4. Discussion

Parental exposure to elevated CO_2 had a positive impact on larvae reared at elevated CO_2 as a sole stressor. Larvae from CO_2-exposed parents were 8% larger (larvae from CO_2-exposed parents = 143.5 ± s.e. 1.5 µm, larvae from ambient parents = 132.5 ± s.e. 3.0 µm) and developed faster at elevated CO_2 compared with larvae from ambient parents. However, these larvae had significantly reduced survival. This was particularly evident when larvae were reared at elevated CO_2 combined with elevated temperature or reduced food concentration, with no larvae surviving in some treatment combinations.

The altered performance of *S. glomerata* larvae, both positive and negative, following parental exposure to elevated CO_2, most likely occurred owing to their significantly higher SMR. A high SMR has previously been associated with the resilience of marine organisms to OA [3,4,8], providing benefits such as higher ion and acid–base regulation, protein synthesis and/or growth [9,10]. In our study, the higher SMR of larvae from CO_2-exposed parents presumably led to faster growth and development. This is beneficial for larvae in the presence of elevated CO_2 as a sole stressor as it reduces the time to metamorphosis. When, however, larvae were exposed to elevated CO_2 and other stressors that impact their energy budget, a higher SMR may lead to a faster depletion in energy reserves that pushes larvae past their tolerance limits sooner [10,16].

No increase in maternal energy investment was observed in eggs following parental exposure to elevated CO_2. Egg size and total lipid content did not differ between mothers exposed to ambient or elevated CO_2. A longer parental exposure period may have yielded different results, as shown for a sea urchin [6]. Further, the parental effects passed to offspring of *S. glomerata* may have differed if parents were exposed to all four stressors that were experienced by the larvae, rather than CO_2 only. Parental exposure of the urchin *Sterechinus neumayeri* to both elevated CO_2 and temperature led to an improvement in larval survival and size in those same conditions, although an increase in the number of deformed larvae suggested that the parental effects were not beneficial for all traits [7]. Additionally, parental exposure of the polychaete *Ophryotrocha labronica* to elevated CO_2 led to reduced egg volume but these effects were ameliorated following parental exposure to elevated CO_2 and temperature [17].

Finally, given that the impacts of elevated CO_2 on the carbonate system became more pronounced when salinity was reduced (e.g. lower TA, pH and $CaCO_3$ saturation), it

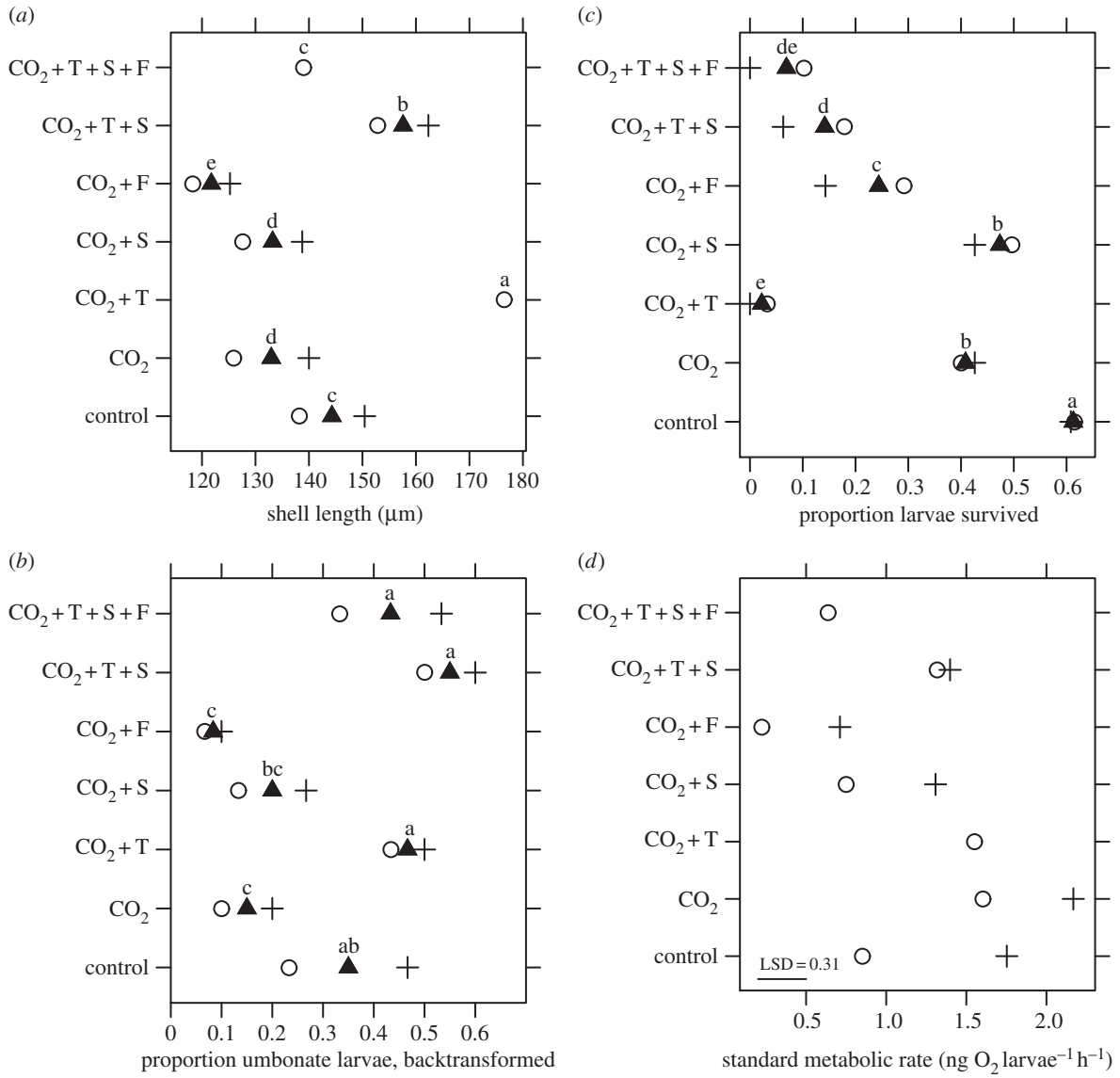

Figure 2. Impact of parental exposure and F_1 larval treatment on (*a*) shell length (15 days), (*b*) proportion umbonate larvae (9 days), (*c*) proportion survival (15 days) and (*d*) standard metabolic rate (15 days) of larvae of *Saccostrea glomerata*. Means for ambient parents (circles) and elevated parents (plus symbol) are shown. F_1 treatment means (filled triangles) with different letters are significantly different at $p = 0.05$. Missing symbols in (*a*) and (*d*) represent treatments with zero survival of larvae. CO_2, elevated CO_2; T, elevated temperature; S, reduced salinity; F, half food diet; LSD, least significant difference.

was expected that reduced salinity would exacerbate the negative impacts of elevated CO_2. Reduced salinity, however, had no additive impacts on larvae of *S. glomerata* reared at elevated CO_2. Larval shell length, survival and development rate were similar in the 'elevated CO_2' and the 'elevated CO_2 and reduced salinity' treatments. This response may be because reduced salinity decreased the SMR of *S. glomerata* larvae. A reduction in SMR may benefit oysters over this century by reducing the strain that other environmental stressors place on their energy budget. Exposure of larvae to a salinity level lower than that used in this study (salinity 25), however, may create a greater osmotic challenge for the larvae, with negative consequences [18,19,20].

This study suggests that the benefits of parental exposure to OA are context-dependent and more complicated than previously thought. We predict that the ability of *S. glomerata* and potentially other marine species to acclimate to OA will be influenced by other stressors in their environment. This will likely lead to a narrowing of the distribution of *S. glomerata* in their natural habitat with downstream consequences for the ecosystem and aquaculture. Future studies are needed to address whether this maladaptive response would be reversed if adults were also conditioned in the same multi-stressor environment.

Ethics. Organisms used in this study are not subject to ethics approval.

Data accessibility. Data are available from the Dryad Digital repository: http://dx.doi.org/10.5061/dryad.1h2ft [21].

Authors' contributions. L.M.P., P.M.R., W.A.O., M.B., R.A.C. and M.D. designed the study; L.M.P., P.V., M.G. and E.S. conducted the study; L.S. and L.M.P. analysed the data; all authors were involved in writing the manuscript. All authors approve the final version of the manuscript and agree to be held accountable for the content therein.

Competing interests. We have no competing interests

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