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 CO2 and examined the impacts of elevated CO2 (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 CO2 had a positive impact on larvae reared at elevated CO2 as a sole stressor, which were 8% larger and developed faster at elevated CO2 compared with larvae from adults exposed to ambient CO2. These larvae, however, had significantly reduced survival in all multistressor treatments. This was particularly evident for larvae reared at elevated CO2 combined with elevated temperature or reduced food concentration, with no larvae surviving in some treatment combinations. Larvae from CO2-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 (CO2) 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 supply is typically lower than in other temperate regions [12,15]. Previous trans-generational 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 (836 μatm) CO2 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 CO2 parents and were used to create F1 larvae. From each of the nine females, the sizes of 30 eggs and the total lipid content of 5000 eggs were determined.

(b) F1 larval exposure
Seven treatments were selected for the F1 larval exposure using a non-orthogonal design (table 1). Gametes from the ambient and elevated CO2 parents were fertilized in 201 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
exposure, F₁ 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, F₁ treatments and shell length data was unbalanced owing to missing data also tested for the larval data. The treatment structure for SMR was reduced (e.g. lower TA, pH and CaCO₃ saturation), it may have differed if parents were exposed to all four stressors that were experienced by the larvae, rather than CO₂ only. Parental exposure of the urchin S. glomerata to ambient CO₂-exposed parents were 8% larger (larvae from CO₂-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 CO₂ compared with larvae from ambient parents. However, these larvae had significantly reduced survival. This was particularly evident when larvae were reared at elevated CO₂ combined with elevated temperature or reduced food concentration, with no larvae surviving in some treatment combinations.

The SMR of larvae from CO₂-exposed parents was higher than that of larvae from ambient parents, with the exception of the CO₂ + T + S treatment, where there was no difference in the SMR of both larval lines (figure 2d, F₄₂₄ = 3.90, p < 0.05).

4. Discussion
Parental exposure to elevated CO₂ had a positive impact on larvae reared at elevated CO₂ as a sole stressor. Larvae from CO₂-exposed parents were 8% larger (larvae from CO₂-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 CO₂ compared with larvae from ambient parents. However, these larvae had significantly reduced survival. This was particularly evident when larvae were reared at elevated CO₂ 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 CO₂, 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 CO₂-exposed parents presumably led to faster growth and development. This is beneficial for larvae in the presence of elevated CO₂ as a sole stressor as it reduces the time to metamorphosis. When, however, larvae were exposed to elevated CO₂ 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 CO₂. Egg size and total lipid content did not differ between mothers exposed to ambient or elevated CO₂. 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 CO₂ only. Parental exposure of the urchin Sterechinus neumayeri to both elevated CO₂ 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 CO₂ led to reduced egg volume but these effects were ameliorated following parental exposure to elevated CO₂ and temperature [17].

Finally, given that the impacts of elevated CO₂ on the carbonate system became more pronounced when salinity was reduced (e.g. lower TA, pH and CaCO₃ saturation), it

3. Results
Egg size and total lipid content were similar for parents exposed to ambient or elevated CO₂ (figure 1a, F₁,₄ = 0.54, p = 0.502; figure 1b, F₁,₄ = 0.0005, p = 0.983, respectively).

Mean size (15 days) and proportion of umbonate larvae (9 days) from CO₂-exposed parents (143.35 μm, 0.38) were greater than that of larvae from ambient parents (132.57 μm, 0.26) (figure 2a, F₁,₂₄ = 44.36, p < 0.001; figure 2b, deviance ratio = 9.37, approx. F₁,₂₄ probability = 0.005). Further, mean size and proportion of umbonate larvae were greatest in the F₁ 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 2a, F₀,₂₄ = 71.99, p < 0.001; figure 2b, deviance ratio = 11.86, approx. F₀,₂₄ probability < 0.001). Total survival of larvae from CO₂-exposed parents (0.24) was less than survival of larvae from ambient parents (0.30; F₁,₂₄ = 5.73, p < 0.05). In the CO₂ + T and CO₂ + T + S + F treatments, there was no survival of larvae from CO₂-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 CO₂ + T, CO₂ + T + S + F and CO₂ + T + S + F treatments (0.02, 0.07 and 0.14, respectively; figure 2c, F₆₂₈ = 40.18, p < 0.001). When salinity was combined with elevated CO₂ there was no additive effect on survival.

The SMR of larvae from CO₂-exposed parents was higher than that of larvae from ambient parents, with the exception of the CO₂ + T + S treatment, where there was no difference in the SMR of both larval lines (figure 2d, F₄₂₄ = 3.90, p < 0.05).

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Finally, given that the impacts of elevated CO₂ on the carbonate system became more pronounced when salinity was reduced (e.g. lower TA, pH and CaCO₃ saturation), it
was expected that reduced salinity would exacerbate the negative impacts of elevated CO₂. Reduced salinity, however, had no additive impacts on larvae of *S. glomerata* reared at elevated CO₂. Larval shell length, survival and development rate were similar in the ‘elevated CO₂’ and the ‘elevated CO₂ 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.

**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. CO₂, elevated CO₂; T, elevated temperature; S, reduced salinity; F, half food diet; LSD, least significant difference.

**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.lh2ft [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.

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