

1 **Adult exposure to ocean acidification and warming**
2 **remains beneficial for oyster larvae following starvation**

3 Mitchell C. Gibbs^{1,2}, Laura M. Parker^{1,3}, Elliot Scanes^{1,2}, Maria Byrne^{1,2}, Wayne A. O'Connor⁴, Pauline M.
4 Ross^{1,2*}

¹The University of Sydney, School of Life and Environmental Sciences, Camperdown, New South Wales, 2006, Australia

²The Sydney Institute of Marine Science, Mosman, New South Wales, 2088, Australia

³The University of New South Wales, School of Biological, Earth and Environmental Sciences, Kensington, New South Wales, 2052, Australia

⁴New South Wales Department of Primary Industries, Port Stephens Fisheries Institute, Taylors Beach, New South Wales, 2316, Australia

*Corresponding author

Corresponding author mailing address: Carslaw Building, The University of Sydney, Camperdown, New South Wales, 2006, Australia, ph: +61 2 9351 5026, Fax; +61 2 9036 0000

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8 **ABSTRACT**

9 Climate change, is expected to warm and acidify oceans and alter the phenology of phytoplankton,
10 creating a mismatch between larvae and their food. Transgenerational plasticity may allow marine
11 species to acclimate to climate change, however, it is expected that this may come with elevated
12 energetic demands. This study used the oysters, *Saccostrea glomerata* and *Crassostrea gigas* to test the
13 effects of adult parental exposure to elevated $p\text{CO}_2$ and temperature on larvae during starvation and
14 recovery. It was anticipated that beneficial effects of transgenerational plasticity will be limited when
15 larvae oyster are starved. Transgenerational responses and lipid reserves of larvae were measured for
16 two weeks. Larvae of *C. gigas* and *S. glomerata* from parents exposed to elevated $p\text{CO}_2$ had greater
17 survival when exposed to elevated CO_2 but this differed between species and temperature. For *S.*
18 *glomerata*, survival of larvae was greatest when the conditions experienced by larvae matched the
19 condition of their parents. For *C. gigas* survival of larvae was greater when parents and larvae were
20 exposed to elevated $p\text{CO}_2$. Larvae of both species used lipids when starved. The total lipid content was
21 dependent on parental exposure and temperature. Against expectations, the beneficial
22 transgenerational plasticity responses of larvae remained, despite starvation.

23 **INTRODUCTION**

24 Climate change is occurring over multiple generations of marine species, potentially allowing for
25 responses to improve across generations (Donelson et al., 2012; Parker et al., 2012; 2015; 2017; Ross et
26 al., 2016; Byrne et al., 2020). Transgenerational plasticity (TGP) is a mechanism for rapid acclimation of
27 marine organisms, a form of phenotypic adjustment which results in climate change resilient offspring
28 when adult parents are exposed to climate change conditions (Donelson et al., 2012; Parker et al., 2012;
29 2015; 2017; Ross et al., 2016; Byrne et al., 2020). For example, Parker et al., (2012) found that exposure
30 of adult parents of the oyster *Saccostrea glomerata* to elevated CO_2 improved the response of their
31 offspring to elevated CO_2 . This positive transgenerational plasticity effect was found to persist into

32 adulthood and the next generation (Parker et al., 2015), but these advantages were extinguished when
33 larvae experienced multiple stressors (Parker et al., 2017). Putnam et al. (2019) found that exposure of
34 parents of the coral, *Pocillopora acuta* to elevated CO₂ during reproductive conditioning improved the
35 survival and settlement rate of the larval offspring and increased the growth rate of settled spat.
36 Further, Vehmaa et al. (2012) found that parental exposure of the copepod, *Acartia bifilosa* to elevated
37 temperature of +3 °C, improved nauplii production (Vehmaa et al. 2012). While transgenerational
38 plasticity has been shown to improve responses of marine organisms to climate change, improved
39 responses of offspring have been shown for some species to be accompanied by increased oxygen
40 consumption and metabolic rates (Parker et al., 2012; 2015). Increased metabolic rates infer a greater
41 energy requirement to support homeostasis (Pörtner, 2008) which for planktotrophic species needs to
42 be fuelled largely by energy from food (Thomsen et al. 2013). Not all studies report beneficial effects of
43 TGP, some studies report detrimental or neutral (no change) when adults are exposed (Uthicke et al.,
44 2013; Kong et al., 2019; Venkataraman et al., 2019).

45 While positive effects of transgenerational plasticity have been observed in marine species, these are
46 typically observed under ideal feeding conditions (Donelson et al., 2012; Hettinger et al., 2012, 2013;
47 Parker et al., 2012; 2015; 2017; Ross et al., 2016). It is anticipated that over this century, climate-
48 mediated alterations in currents and flow will shift the phenology of phytoplankton and zooplankton,
49 creating temporal mismatches between marine species and the availability of their plankton food
50 sources, further constraining larval energy budgets (Edwards and Richardson 2004; Poloczanska et al.,
51 2013). The potential for such mismatches has already been observed, with spring phenology in the
52 ocean advancing at different rates across taxonomic and functional groups (Poloczanska et al., 2013). In
53 the only study to our knowledge to date to assess the impacts of reduced food supply following
54 transgenerational exposure, larvae of the oyster *S. glomerata* from parents exposed to elevated CO₂ had
55 greater mortality at elevated CO₂ when fed a reduced food diet, compared to larvae from non-exposed

56 parents (Parker et al., 2017). It remains untested whether parental conditioning to climate change
57 stressors can alter the use of energy in offspring via transgenerational plasticity when offspring are
58 starved.

59 Marine calcifying organisms such as molluscs display particular vulnerability to climate change. In coastal
60 and estuarine environments where molluscs are commonly found, warming and acidification are
61 manifesting more rapidly (Scanes et al., 2020; Cai et al., 2020). Here we test whether transgenerational
62 responses native Sydney rock oyster *S. glomerata* and the invasive Pacific oyster *Crassostrea gigas*
63 (alternatively named *Magallana gigas*; Salvi et al., 2014) are evident following periods of starvation and
64 delayed access to food in larval offspring. Further, we test whether transgenerational plasticity will
65 reduce the survival of larvae during starvation due to a faster depletion of their energy reserves. It was
66 anticipated that larval performance improved by parental exposure to elevated $p\text{CO}_2$ and temperature
67 will not be sustained when larvae experience starvation, due to an increase in metabolic rate (see Parker
68 et al., 2017) and faster depletion of their endogenous energy reserves.

69 **MATERIALS AND METHODS**

70 To determine whether TGP responses will be still evident following periods of starvation and delayed
71 access to food in larval offspring, adult *S. glomerata* and *C. gigas* were conditioned in ambient and
72 elevated CO_2 (340 μatm and 856 μatm , respectively) at ambient and elevated temperature (24°C and
73 28°C, respectively) during gamete development. Larvae were then reared at ambient and elevated CO_2
74 (340 μatm and 856 μatm , respectively) and ambient and elevated temperature (24°C and 28°C,
75 respectively) and food was made available after five, nine- or eleven-days starvation. Larval size,
76 mortality, metabolic rate, and lipid dynamics were measured following the different intervals of
77 starvation and to assess the response of larvae during starvation and subsequent recovery following
78 provision of food.

80 Experimental organisms, adult treatments and acclimation

81 Six hundred oysters, three hundred of each species, *S. glomerata* and *C. gigas*, were collected from
82 Holbert's Oyster Supplies (152° 4'0.69"E, 32°43'19.69"S) and Diemer's Oysters Supplies (152° 3'58.91"E,
83 32°43'27.49"S) respectively. Both suppliers grow oysters in Cromarty Bay, Port Stephens, NSW,
84 Australia. Inspection of the gonad both macroscopically and microscopically with gonad smears (Leica
85 400x) confirmed that the oysters were in a regressive-early ripening reproductive condition (Dinamani
86 1974). The oysters were transported to the Department of Primary Industries (DPI) Port Stephens
87 Fisheries Institute (PSFI), Taylors Beach, NSW, Australia (152° 3'20.16"E, 32°44'42.29"S). On arrival, the
88 oysters were scrubbed and cleaned from mud and any potentially fouling organisms then transferred
89 into 40L tubs filled with 1µm nominal filtered sea water (FSW, 16°C, 34.5 psu) supplied from a 750L
90 recirculating header tank where they were maintained for two weeks to acclimate to laboratory
91 conditions.

92 During acclimation and subsequent experiments, adult oysters received water changes every second day
93 using pre-equilibrated FSW. Throughout adult conditioning, oysters were fed an algal diet of 25 %
94 *Chaetoceros muelleri*, 25 % *Dicrateria lutheri*, 25 % *Tisochrysis lutea* and 25% *Tetraselmis chuii* at a
95 concentration of 1×10^9 cells oyster⁻¹ day⁻¹.

96 To assess the effects of parental conditioning on larval responses, adult *S. glomerata* and *C. gigas* were
97 exposed to orthogonal treatments of ambient (400 µatm) and elevated (856 µatm) *p*CO₂ selected to
98 represent the CO₂ concentrations of Earth's atmosphere in the year 2100 based on multi-model
99 averages (Collins et al., 2013). Adult oysters were also conditioned at two temperatures; 24 and 28 °C
100 selected to represent the current and predicted temperatures for eastern Australia (Collins et al., 2013;
101 Hobday and Pecl, 2014; Scanes et al., 2020). The mean (±SE) summer temperature of Port Stephens has

102 been measured at 24 ± 0.05 °C (calculated from data within Scanes et al., 2020), this temperature was
103 selected to represent the current summer ambient temperature in Port Stephens (24 °C). The elevated
104 temperature treatment of 28 °C was selected to represent the current upper temperature experienced
105 in Port Stephens during summer, and a temperature that is predicted to become more common in the
106 coming decades (Collins et al., 2013; Hobday and Pecl, 2014; CSIRO 2020; Scanes et al., 2020). Both
107 species of oysters were split into twelve groups of twenty-five individuals (2 x $p\text{CO}_2$ treatments, 2 x
108 temperature treatments, 3 x replicates), each group of 25 was placed into a 40 L tub of recirculating
109 FSW. One 40 L tub containing *S. glomerata* and one tub containing *C. gigas* were both fed FSW from a
110 single 750L header tank.

111 Six groups of oysters of each species were randomly assigned to an elevated $p\text{CO}_2$ treatment, and six
112 were assigned to an ambient $p\text{CO}_2$ treatment. The CO_2 was slowly added to the tank to reduce pH to the
113 desired level of 7.7 (856 μatm), over 8 days. From each $p\text{CO}_2$ level, three groups of 25 oysters of each
114 species were assigned to the ambient temperature treatment (24 °C) and three groups were assigned to
115 the elevated temperature (28 °C) treatment. Both ambient and elevated temperature treatments were
116 warmed to the experimental temperature of 24 and 28 °C over a period of eight days using an individual
117 300W aquarium heater (Seabillion model:HL-388, HaiYi Electrical Appliance Factory, Zhongshan,
118 Guangdong, China; accuracy $\pm 1^\circ\text{C}$) for each 750 L tank.

119 $p\text{CO}_2$ was manipulated in adult conditioning tanks by adding gaseous CO_2 to header tanks using a
120 negative feedback system as described in detail by (Parker et al., 2012). The pH values of each tank
121 (Table 1A) were monitored daily, and the pH electrode of each controlling system was checked daily
122 against another calibrated pH probe (Tris buffers, WTW 3400*i*).

123 **Spawning and larval populations**

124 Adult oysters remained in their treatments for eight weeks to build reproductive condition. After eight
125 weeks of conditioning, oysters were gravid (Dinamani, 1974), confirmed by inspecting gonad smears of
126 three oysters from each treatment using a light microscope (Leica 200x). All oysters were then spawned
127 by lightly scoring the gonad with a scalpel blade and rinsing the gametes out with seawater.

128 To create larval cultures, a population of larvae from each species was produced from each adult
129 conditioning tank (12 total per species). Eggs of five females per replicate tank, and per species were
130 collected and pooled into a 1L container and allowed to rest for 2h. A 0.05 mL subsample of
131 concentrated egg mixture was taken, and eggs were counted on a Sedgewick-Rafter slide. Another
132 subsample was then taken from the mixture at the correct volume to sample 10,000 eggs. These were
133 placed in a 1 mL tube, centrifuged (ca. 5 mins at 500 g) to remove water and stored at -80 °C for lipid
134 analysis.

135 The sperm from each male was collected in a 1L container and a subsample was checked for motility
136 under a light microscope (Leica 400x). Males with non-motile sperm were excluded. After 2h, the sperm
137 of five males from the same replicate tank was pooled and then added to the pooled eggs of females
138 from the same replicate tank. Sperm was added incrementally until 5-10 sperm were visible around
139 each egg under a light microscope (*Leica* 400x). This created a 5 x 5 cross per conditioning tank.

140 Fertilisation was confirmed as the presence of polar bodies in > 80% of eggs under a light microscope
141 (*Leica* x200). Embryos from each conditioning tank were divided into ambient or elevated $p\text{CO}_2$
142 treatments similar to the design of Scanes et al., (2018; Figure 1A). This was done for each replicate tank
143 (n=3) for each temperature (n=2), $p\text{CO}_2$ (n=2) treatments and species (n=2), resulting in 48 embryo
144 populations.

145 Five million embryos from each of 48 embryo populations were transferred to a 200L (25 larvae mL^{-1})
146 polyethylene larval rearing tank (48 x 200L tanks). Each tank was drained after 24 hours through a 45 μm

147 mesh to catch D-veliger larvae. The number of D-Veliger larvae in a subsample of known volume was
148 determined for each tank on a Sedgewick rafter slide under a light microscope (*Leica* 200x). New 200L
149 tanks were restocked with one million D-veliger larvae giving a 5 mL⁻¹ stocking density.

150 To determine the impact of elevated $p\text{CO}_2$ and temperature on the size, mortality and lipid content of
151 larvae, embryos from each parental tank were reared in orthogonal combinations of two $p\text{CO}_2$
152 treatments (400 and 856 μatm). Larvae were also reared at the same temperature as their parents were
153 conditioned (24 and 28°C) for each species (Figure 1B). The temperatures were maintained using
154 individual 300W aquarium heaters ($\pm 1^\circ\text{C}$) in each tank.

155 The $p\text{CO}_2$ of tanks containing larvae were manipulated via the constant delivery of premixed CO_2 in air at
156 856 μatm (BOC gas co. Tighes Hill, NSW) to give a pH_{NIST} of 7.8 (checked by calibrated pH probe; WTW
157 3400i). Three individual tanks of premixed gas supplied each of three replicate tanks per treatment.
158 Treatments that were exposed to ambient $p\text{CO}_2$ were continuously bubbled with air at the same rate as
159 the elevated $p\text{CO}_2$ tanks. Water samples were collected for total alkalinity (TA) 12 times through
160 experiment and analysed by potentiometric titration and the data were input into CO_2SYS with other
161 parameters to determine the carbonate chemistry (Table 1B).

162 **Feeding Treatments**

163 The feeding treatments were designed to determine how larvae respond to periods of starvation (see
164 Moran & Manahan 2004; Gibbs et al., 2020; Figure 1B). Larvae of *S. glomerata* and *C. gigas* were
165 maintained without food from fertilisation to the end of starvation treatments. On days 5, 9 and 11 post
166 fertilisation, 100,000 larvae were removed from each 200L tank and transferred to a 20L tank where
167 they were fed a diet of 25 % *C. muelleri*, 25 % *D. lutheri*, 25 % *T. lutea* and 25% *C. calcitrans* at a rate at
168 concentration of 1×10^4 – 10^5 cells mL⁻¹. These feeding delays were determined by a pilot study that
169 indicated three days of starvation did not elicit an effect in larvae, therefore, a minimum of 5 days

170 starvation was selected. Starvation treatments were then selected to occur every second day (except
171 day 7) to coincide with water changes. These feeding delays also matched closely with those used by
172 Moran and Manahan (2004). This created the feeding treatments Fed@5 days, Fed@9day and Fed@11
173 day. In the treatments Fed@5 and Fed@9 day and Fed@11 day, the larvae were starved for four, eight
174 and ten days after fertilisation respectively (depending on survival; Figure 1A). After feeding
175 commenced, Fed@5 larvae were reared for nine days with measurements at 1, 5, 7, 9, 11 and 13 days
176 old and Fed@9day larvae were reared for nine days with measurements at 1, 5, 9, 11, 13, 15 and 17
177 days old or until death. Fed@11 day larvae were reared for seven days with measurements at 1, 5, 9,
178 11, 13, 15 and 17 days old or until death (Figure 1b; Gibbs et al. 2020).

179 **Larval sampling and morphology**

180 To determine the size, survival, and energetics of larvae, following transfer to the 20L tank larvae were
181 sampled every second day. Survival was determined by counting the number of live larvae in a
182 subsample of the first 30 encountered in a sub sample under a light microscope (*Leica* x200). The size of
183 30 larvae was measured along their posterior anterior axis using an ocular micrometer under a light
184 microscope (Parker et al. 2010) (*Leica* x200). For lipid analysis 5000 larvae from each replicate were
185 transferred to a 1.5mL centrifuge tube, centrifuged to allow removal of the sea water (ca. 5 mins at 500
186 g) and immediately frozen at -80°C.

187 **Larval metabolic rate (LMR)**

188 Larval metabolic rate (LMR) was measured when larvae were transferred from the 200 L to 20 L tanks.
189 This occurred at 1 day (24 h), 5 days, 9 days and 11 days (*C. gigas* only). This meant that LMR was always
190 measured on starved larvae. Larvae from each 200 L tank were placed into 5 mL oxygen monitoring
191 sensor vials (PreSens SV-PSt5) at a concentration of 25000 larvae vial⁻¹ including a control vial containing

192 no larvae, to allow larval respiration rates to be corrected for background bacterial respiration. Oxygen
193 concentrations were monitored and LMR was calculated as per Parker et al., (2017).

194 **Lipid extraction**

195 To determine the impact of elevated pCO₂ and temperature on total lipid levels, egg and larval samples
196 were freeze dried (Christ Alpha 1-4 LSC). Lipids were extracted using a modified Bligh and Dyer (1959)
197 and quantified using an Iatroscan MK VI TH10 thin-layer chromatography-flame ionization detector (TLC-
198 FID) analyser (Tokyo, Japan) following the methods described in Gibbs et al., (2020). Total lipid was
199 determined as the sum of all lipid classes.

200 **Data Analyses**

201 Size, mortality, total lipid of larvae *C. gigas* and *S. glomerata* were analysed separately for each species
202 and time point to avoid co-dependence and allow for meaningful interpretation of results (Green 1979).
203 Data was analysed at two meaningful time points; the end of starvation, being 5 days for Fed@5, 9 days
204 for Fed@9 and 11 days for Fed@11 and for valid comparisons at 13 days of age for all treatments. A
205 three-way analysis of variance (ANOVA) was used with “Temperature” (2 levels; 24°C and 28°C), “CO₂
206 treatment (parents; 2 levels, ambient and elevated)” and “CO₂ treatment (larvae; 2 levels, ambient and
207 elevated)” as fixed factors. Post-Hoc Tukey Tests were used to determine significant (adjusted p value
208 <0.05) differences among treatment levels for significant factors of interest. Residual normality for all
209 analyses was checked using Pearson’s-residual Plots. All analyses were done using R V3.5.4 statistical
210 software (R core team) and significance was set as $\alpha < 0.05$. Cochran’s test was used to detect any
211 heterogeneity of variances. If necessary, the data were log transformed and re-checked with Cochran’s
212 test (Underwood 1997).

213 **RESULTS**

214

215 **Lipid content in Eggs**

216 The total lipid content of the eggs was generally greater in *C. gigas* compared to *S. glomerata* in all
217 treatments. (Figure S1, Table S1). Lipid content of eggs of *S. glomerata* was significantly greater at
218 elevated CO₂ at 28°C (Table S1).

219

220 **Mortality and Size of Larvae**

221 *S. glomerata*

222 Due to *S. glomerata* larval mortality, a complete set of statistical analysis of data on larval size and
223 mortality is only available up to the Fed@5 day treatment. Larval mortality of *S. glomerata* was
224 dependent on larval CO₂ exposure and temperature (Table 2). There was less mortality when parent
225 and larval treatments were the same, either both ambient or elevated CO₂ (Figure 2a; Table 2).

226 Temperature also affected mortality. Mortality of larvae of *S. glomerata* was greater at 28 compared to
227 24 °C with 100% mortality observed after nine days of starvation at 28 °C (Figure 2a; Tables 2 and S2).

228 The maximum size of *S. glomerata* was at 28°C (Figure 2b). At 28 °C, larvae of *S. glomerata* were
229 significantly larger when reared in ambient conditions from parents exposed to elevated pCO₂ (Figure
230 2b; Tables 2 and S2). At 24°C, the size of larvae was not dependent on parental exposure to elevated
231 CO₂.

232 *Crassostrea gigas*

233 Larvae of *C. gigas* were present in most treatments for the duration of the experiments. Larval mortality
234 of *C. gigas* was generally lower than that of *S. glomerata*. Mortality of larvae from parents exposed to
235 elevated CO₂ was less and survival greater than larvae from ambient parents (compare Fed @9 and
236 Fed@11 day Figure 3a; Table 2). Mortality of larvae was generally greater at the elevated temperature

237 of 28 °C compared to 24 °C except when larvae had been starved for 11 days, when the only survivors
238 where larvae from elevated parents at 28 °C (Figure 3a; Tables 2 and S2).

239 Starvation affected the size of larvae of *C. gigas*. For *C. gigas*, parental and larval CO₂ exposure and
240 temperature affected the size of larvae (Figure 3b; Table 2). When starved for five days larvae were
241 greater in size when reared in ambient CO₂ from parents exposed to ambient conditions compared to
242 larvae of these parents reared in elevated CO₂. This pattern persisted at 28 °C, although on day 13,
243 larvae of *C. gigas* were larger in size from parents exposed to elevated CO₂ (Fed@5 Figure 3b; Table 2).
244 When larvae were starved for nine and eleven days, larvae of *C. gigas* from parents exposed to elevated
245 CO₂ were generally smaller than larvae from parents exposed to ambient CO₂ (Fed@9 and Fed@11 day
246 Figure 3; Table S2).

247

248 **Lipid Content in Larvae**

249 *S. glomerata*

250 Larvae of *S. glomerata* used lipids when starved (Figure 2c). When larvae of *S. glomerata* were starved
251 for 5 days, their total lipid content was significantly greater when they were exposed to ambient
252 compared to elevated CO₂ (Fed@5 day: 5 day old). Following the commencement of feeding, this
253 pattern was reversed, with larvae exposed to elevated CO₂ having greater total lipid content (Fed@5
254 day: 13 day old; Figure 2c; Tables 3 and S3). By 13 days the larvae reared at 28 °C had a greater lipid
255 content than those reared at 24 °C as shown by the significant effect of temperature (Table 3).

256

257 *Crassostrea gigas*

258 Like *S. glomerata*, larvae of *C. gigas* used lipids when starved (Figure 3c). Larvae of *C. gigas* had greater
259 total lipid content when they were from parents exposed to elevated CO₂ when starved for 5, 9 and 11
260 days at 24°C (Figure 3c; Table 3) compared to larvae at ambient CO₂ (5 day old Fed@5 day, 9 day old
261 Fed@9 day and 11 day old Fed@11 $P < 0.01$, Figure 3c; Tables 3 and S2). When feeding commenced,
262 there was no effect of larval or parental CO₂ exposure by day 13 for larvae Fed@5, Fed@9 or Fed@11
263 day (Figure 3c; Table 3). There was an effect of temperature. The longer larvae were starved, the greater
264 the depletion of total lipids. This depletion was generally greater at 24 compared to 28 °C (Fed@9 and
265 Fed@11 day Figure 3; Table 3). Larvae of *C. gigas* had greater total lipid content at 28 compared to 24 °C
266 at 13 days old when starved for 9 days (Fed@9 day) (Table 3).

267 **LMR**

268 At 24 h, the LMR of *S. glomerata* was greater when larvae were reared in the opposite CO₂ level to their
269 parents (i.e. ambient parents: elevated larvae; elevated parents: ambient larvae; Figure 4a; Tables 4 and
270 S3). After 5 days of starvation, this pattern was still observed at 24 but not at 28 °C. Instead, at 28 °C,
271 LMR was reduced when larvae were reared at elevated CO₂ irrespective of the parental exposure. LMR
272 of *S. glomerata* larvae was greater at 28 °C compared to 24 °C at 24 h but not after 5 days of starvation,
273 where LMR at 28 °C became depressed (Figure 4a; Table 4). By day 9 only larvae in the 24 °C survived
274 and there was no difference in LMR between offspring of adults from ambient or elevated CO₂
275 treatments (Figure 4a).

276 At 24 h, larvae of *C. gigas* reared at ambient CO₂ had a greater LMR if their parents were reared at
277 elevated CO₂. When larvae were exposed to elevated CO₂, LMR increased in larvae from parents
278 exposed to ambient CO₂ but not in larvae from parents exposed to elevated CO₂. Following starvation,
279 the LMR of larvae of *C. gigas* was low regardless of parental source (Figure 4b). While there was no

280 effect of temperature on the LMR of *C. gigas* there was a significant interaction effect of temperature x
281 CO₂ (Parent) x CO₂ (Larvae) (Figure 4b; Tables 4 and S3).

282

283 **DISCUSSION**

284 The majority of studies to date that have assessed the impacts of elevated CO₂ and temperature on
285 oyster larvae, both acute and transgenerational, have assessed these impacts under optimal food
286 concentrations (Gazeau et al., 2013; Parker et al., 2013). While these experiments provide essential
287 information on how vulnerable marine species will respond over this century, they do not fully account
288 for the natural environment, where food concentration is not always optimal. This study explored a
289 knowledge gap by assessing the impacts of elevated CO₂ and temperature on the transgenerational
290 response of larvae *S. glomerata* and *C. gigas*, when larvae were subjected to different levels of
291 starvation due to delayed access to food. While it is unlikely that marine larvae will experience periods
292 of no food in their natural environment, the incremental starvation and delayed provision of food in this
293 study provided an opportunity to determine whether there were beneficial effects of transgenerational
294 plasticity and tease apart the impacts of endogenous and exogenous energy supply on the
295 transgenerational plasticity developmental responses and lipid dynamics of *S. glomerata* and *C. gigas*
296 exposed to climate change.

297 Overall, the results of this study indicate that beneficial transgenerational plasticity in response to
298 elevated pCO₂ can occur despite larval starvation. We found a clear positive effect of transgenerational
299 plasticity on the survival of larvae despite starvation. For *C. gigas*, the only larvae remaining alive after
300 11 days of starvation were those from parents exposed to elevated CO₂. For *S. glomerata*, larval survival
301 was greatest when the conditions experienced by larvae matched the condition of their parents.
302 Parental exposure to elevated CO₂ did not improve the growth rate of larvae for either species when
303 exposed to elevated CO₂. This result contrasts with previous transgenerational studies done on *S.*

304 *glomerata* under optimal feeding conditions (Parker et al. 2012; 2015; 2017), however, for this study is
305 likely due to the slow grow of larvae during starvation across all treatments.

306 It was hypothesised that the beneficial transgenerational effects passed from parents to their larval
307 offspring during exposure to elevated CO₂ and temperature would become maladaptive during periods
308 of starvation, as transgenerational larvae would have an inherently increased LMR and therefore
309 deplete their energy reserves sooner (Parker et al. 2017). Instead, across nearly all treatments, starved
310 larvae of *S. glomerata* and *C. gigas* had a reduced LMR, presumably conserving energy and prolonging
311 survival until food became available (Pörtner 2012; Sokolova 2013). Such reductions in metabolic rate
312 during episodes of starvation have previously been observed in other animals (Storey and Storey 1990,
313 2004; Hahn and Denlinger 2007; McCue et al. 2017). Interestingly, in the few treatments where LMR was
314 not reduced during starvation (i.e. Pacific oyster larvae from ambient parents, exposed to ambient CO₂
315 in the Fed@5day treatment at 24 °C, or Sydney rock oyster larvae from ambient parents, exposed to
316 elevated CO₂ in the Fed@5day treatment at 24 °C), larvae experienced a greater depletion of total lipids,
317 greater mortality but maintained a faster rate of growth, compared to larvae from treatments which
318 experienced a reduction in LMR. This further supports the notion that larvae reduce their LMR during
319 periods of starvation to conserve energy and prolong survival.

320 The total lipid content of larvae of *C. gigas* during starvation was greatest in larvae from parents
321 exposed to elevated CO₂ – a result which coincided with greater survival in these larvae compared to
322 those from parents exposed to ambient CO₂. Previous analysis of the total lipid content of eggs of *C.*
323 *gigas* has shown that mothers exposed to elevated CO₂ do not increase the total lipids which they invest
324 per egg (Figure S1). This suggests that the increased total lipid content of larvae from CO₂-exposed
325 parents during starvation occurred due to differences in the usage of endogenous lipid reserves
326 between larvae. Following the commencement of feeding the effect of parental exposure on total lipid
327 content was no longer present, however, lasting effects on survival were still observed.

328 In contrast to *C. gigas*, mothers of *S. glomerata* have previously been found to increase total lipid
329 investment per egg following exposure to elevated CO₂ (Gibbs et al., 2020). Yet here, we found that this
330 increase in maternal investment did not lead to an increase in the total lipid content in the larvae.
331 Following starvation, the total lipid content of larvae of *S. glomerata* was greater when larvae were
332 reared at ambient compared to elevated CO₂. This result was like that found by Talmage and Gobler
333 (2011) who showed that the lipid index of larvae of the clam, *Mercenaria mercenaria* and scallop,
334 *Argopecten irradians* decreased during exposure to elevated CO₂. But, when starved larvae of *S.*
335 *glomerata* were given food, the pattern of total lipid content was reversed, with larvae exposed to
336 elevated CO₂ having greater total lipid content than those exposed to ambient CO₂. This suggests
337 differences in the mechanisms involved in the accrual of lipid in the two groups of larvae. However, in
338 contrast to the larvae of *C. gigas*, an increase in total lipid did not appear to be closely linked to survival
339 in *S. glomerata*. For *S. glomerata*, larval survival was greatest when the conditions experienced by larvae
340 matched the conditions that their parents experienced. For both species, the total lipid content of larvae
341 was greater at the elevated temperature of 28 compared to 24 °C. This result was surprising given that
342 the LMR of larvae was similar at 24 and 28 °C during periods of starvation.

343 In summary, transgenerational plasticity may be a beneficial mechanism for mollusc larvae to survive
344 climate change despite potential food shortages. The capacity for transgenerational plasticity may prove
345 decisive for marine mollusc populations living in oceans that are rapidly changing with little sign of
346 slowing in the coming decades. From this study, it appears that the invasive *C. gigas* may have a
347 competitive advantage over the native *S. glomerata* as our oceans continue to acidify and warm, as they
348 survived considerably longer through periods of starvation. *C. gigas* has proven to be a major invasive
349 species across the globe (Ruesink et al., 2005), yet despite its faster rates of growth and filtration and
350 greater metabolic efficiency, has failed to widely displace the native *S. glomerata* in Australia (Scanes et

351 al., 2016). Climate change may provide *C. gigas* with an advantage that could further displace *S.*
352 *glomerata* from Australian estuaries over this century.

353 **DATA AVAILABILITY STATEMENT**

354 The data underlying this article will be shared on reasonable request to the corresponding author.

355 **AUTHOR CONTRIBUTIONS** The study was conceived and designed by M.G, P.M.R, L.M.P and W.A.O.

356 Data analysis and presentation was done by M.G, E.S and P.M.R. All authors were involved in the

357 writing, editing and revising of the manuscript.

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482 **FIGURE LEGENDS**

483 **Figure 1. A)** Experimental design used for larval experiments. The first row are tanks which represent the
484 adult exposure treatment. Pooled eggs and sperm from five male and five female oysters in each
485 replicate tank were then stocked into two 200 L tanks set at ambient or elevated $p\text{CO}_2$. **B)** Conceptual
486 diagram of the feeding delay experimental design.

487 **Figure 2. Mean (\pm S.E, n=3) A)** larval mortality (%) **B)** larval size (shell length μm) and **C)** total lipid content
488 (ng larva^{-1}) of the larvae of *Saccostrea glomerata* exposed to experimental treatments of ambient (400
489 μatm) and elevated (856 μatm) $p\text{CO}_2$ at 24 °C and 28 °C. Larvae were measured every second day from
490 day 5 and for a maximum of nine days following the introduction food, larvae in some treatments died
491 before nine days. Arrows on the x-axis indicate the day at which food was introduced following the
492 feeding delay.

493 **Figure 3. Mean (\pm S.E, n=3) A)** larval mortality (%) **B)** larval size (shell length μm) and **C)** total lipid
494 content (ng larva^{-1}) of the larvae of *Crassostrea gigas* exposed to experimental treatments of ambient
495 (400 μatm) and elevated (856 μatm) $p\text{CO}_2$ at 24 °C and 28 °C. Larvae were measured every second day
496 from day 5 and for a maximum of nine days following the introduction food, larvae in some treatments
497 died before nine days. Arrows on the x-axis indicate the day at which food was introduced following the
498 feeding delay.

499

500 **Figure 4. Mean (large symbols, \pm S.E, n=3) Larval Metabolic Rate ($\text{ng O}_2 \text{larva}^{-1} \text{h}^{-1}$) (LMR) of the larvae of**
501 *oysters (a) Saccostrea glomerata* and *(b) Crassostrea gigas* exposed to orthogonal combinations of
502 ambient (400 μatm) and elevated (856 μatm) $p\text{CO}_2$ as both parents (Ambient=blue circles, Elevated= red
503 squares) and larvae, as well as 24 °C and 28 °C temperature treatments and feeding delay treatments of
504 five days starvation (Fed@5), nine days starvation (Fed@9) and eleven days starvation (Fed@11). LMR
505 was measured on starved larvae at one, five, nine and eleven days following metamorphosis. The raw
506 data for each replicate are also shown as smaller symbols.

