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

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ABSTRACT

Climate change, is expected to warm and acidify oceans and alter the phenology of phytoplankton, creating a mismatch between larvae and their food. Transgenerational plasticity may allow marine species to acclimate to climate change, however, it is expected that this may come with elevated energetic demands. This study used the oysters, *Saccostrea glomerata* and *Crassostrea gigas* to test the effects of adult parental exposure to elevated pCO_2 and temperature on larvae during starvation and recovery. It was anticipated that beneficial effects of transgenerational plasticity will be limited when larvae oyster are starved. Transgenerational responses and lipid reserves of larvae were measured for two weeks. Larvae of *C. gigas* and *S. glomerata* from parents exposed to elevated pCO_2 had greater survival when exposed to elevated CO_2 but this differed between species and temperature. For *S. glomerata*, survival of larvae was greatest when the conditions experienced by larvae matched the condition of their parents. For *C. gigas* survival of larvae was greater when parents and larvae were exposed to elevated pCO_2. Larvae of both species used lipids when starved. The total lipid content was dependent on parental exposure and temperature. Against expectations, the beneficial transgenerational plasticity responses of larvae remained, despite starvation.

INTRODUCTION

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

adulthood and the next generation (Parker et al., 2015), but these advantages were extinguished when larvae experienced multiple stressors (Parker et al., 2017). Putnam et al. (2019) found that exposure of parents of the coral, *Pocillopora acuta* to elevated CO_2 during reproductive conditioning improved the survival and settlement rate of the larval offspring and increased the growth rate of settled spat. Further, Vehmaa et al. (2012) found that parental exposure of the copepod, *Acartia bifilosa* to elevated temperature of +3 ˚C, improved nauplii production (Vehmaa et al. 2012). While transgenerational plasticity has been shown to improve responses of marine organisms to climate change, improved responses of offspring have been shown for some species to be accompanied by increased oxygen consumption and metabolic rates (Parker et al., 2012; 2015). Increased metabolic rates infer a greater energy requirement to support homeostasis (Pörtner, 2008) which for planktotrophic species needs to be fuelled largely by energy from food (Thomsen et al. 2013). Not all studies report beneficial effects of TGP, some studies report detrimental or neutral (no change) when adults are exposed (Uthicke et al., 2013; Kong et al., 2019; Venkataraman et al., 2019).

While positive effects of transgenerational plasticity have been observed in marine species, these are typically observed under ideal feeding conditions (Donelson et al., 2012; Hettinger et al., 2012, 2013; Parker et al., 2012; 2015; 2017; Ross et al., 2016). It is anticipated that over this century, climate-mediated alterations in currents and flow will shift the phenology of phytoplankton and zooplankton, creating temporal mismatches between marine species and the availability of their plankton food sources, further constraining larval energy budgets (Edwards and Richardson 2004; Poloczanska et al., 2013). The potential for such mismatches has already been observed, with spring phenology in the ocean advancing at different rates across taxonomic and functional groups (Poloczanska et al., 2013). In the only study to our knowledge to date to assess the impacts of reduced food supply following transgenerational exposure, larvae of the oyster *S. glomerata* from parents exposed to elevated CO_2 had greater mortality at elevated CO_2 when fed a reduced food diet, compared to larvae from non-exposed

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

Marine calcifying organisms such as molluscs display particular vulnerability to climate change. In coastal and estuarine environments where molluscs are commonly found, warming and acidification are manifesting more rapidly (Scanes et al., 2020; Cai et al., 2020). Here we test whether transgenerational responses native Sydney rock oyster *S. glomerata* and the invasive Pacific oyster *Crassostrea gigas* (alternatively named *Magallana gigas;* Salvi et al., 2014) are evident following periods of starvation and delayed access to food in larval offspring. Further, we test whether transgenerational plasticity will reduce the survival of larvae during starvation due to a faster depletion of their energy reserves. It was anticipated that larval performance improved by parental exposure to elevated pCO_2 and temperature will not be sustained when larvae experience starvation, due to an increase in metabolic rate (see Parker et al., 2017) and faster depletion of their endogenous energy reserves.

MATERIALS AND METHODS

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

Experimental organisms, adult treatments and acclimation

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

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

To assess the effects of parental conditioning on larval responses, adult *S. glomerata* and *C. gigas* were exposed to orthogonal treatments of ambient (400 µatm) and elevated (856 µatm) pCO_2 selected to represent the CO_2 concentrations of Earth's atmosphere in the year 2100 based on multi-model averages (Collins et al., 2013). Adult oysters were also conditioned at two temperatures; 24 and 28 °C selected to represent the current and predicted temperatures for eastern Australia (Collins et al., 2013; Hobday and Pecl, 2014; Scanes et al., 2020). The mean (±SE) summer temperature of Port Stephens has

been measured at 24 ± 0.05 °C (calculated from data within Scanes et al., 2020), this temperature was selected to represent the current summer ambient temperature in Port Stephens (24 °C). The elevated temperature treatment of 28 °C was selected to represent the current upper temperature experienced in Port Stephens during summer, and a temperature that is predicted to become more common in the coming decades (Collins et al., 2013; Hobday and Pecl, 2014; CSIRO 2020; Scanes et al., 2020). Both species of oysters were split into twelve groups of twenty-five individuals (2 x pCO_2 treatments, 2 x temperature treatments, 3 x replicates), each group of 25 was placed into a 40 L tub of recirculating FSW. One 40 L tub containing *S. glomerata* and one tub containing *C. gigas* were both fed FSW from a single 750L header tank.

Six groups of oysters of each species were randomly assigned to an elevated pCO_2 treatment, and six were assigned to an ambient pCO_2 treatment. The CO_2 was slowly added to the tank to reduce pH to the desired level of 7.7 (856 µatm), over 8 days. From each pCO_2 level, three groups of 25 oysters of each species were assigned to the ambient temperature treatment (24 °C) and three groups were assigned to the elevated temperature (28 °C) treatment. Both ambient and elevated temperature treatments were warmed to the experimental temperature of 24 and 28 °C over a period of eight days using an individual 300W aquarium heater (Seabillion model:HL-388, HaiYi Electrical Appliance Factory, Zhongshan, Guangdong, China; accuracy ± 1°C) for each 750 L tank.

pCO_2 was manipulated in adult conditioning tanks by adding gaseous CO_2 to header tanks using a negative feedback system as described in detail by (Parker et al., 2012). The pH values of each tank (Table 1A) were monitored daily, and the pH electrode of each controlling system was checked daily against another calibrated pH probe (Tris buffers, WTW 3400*i*).

Spawning and larval populations

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

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

The sperm from each male was collected in a 1L container and a subsample was checked for motility under a light microscope (Leica 400x). Males with non-motile sperm were excluded. After 2h, the sperm of five males from the same replicate tank was pooled and then added to the pooled eggs of females from the same replicate tank. Sperm was added incrementally until 5-10 sperm were visible around each egg under a light microscope (*Leica* 400x). This created a 5 x 5 cross per conditioning tank. Fertilisation was confirmed as the presence of polar bodies in > 80% of eggs under a light microscope (*Leica* x200). Embryos from each conditioning tank were divided into ambient or elevated pCO_2 treatments similar to the design of Scanes et al., (2018; Figure 1A). This was done for each replicate tank (n=3) for each temperature (n=2), pCO_2 (n=2) treatments and species (n=2), resulting in 48 embryo populations.

Five million embryos from each of 48 embryo populations were transferred to a 200L (25 larvae mL^{-1}) 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 pCO_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 pCO_2
152 treatments (400 and 856µatm). 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 (± 1°C) in each tank.

155 The pCO_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 3400*i*). Three individual tanks of premixed gas supplied each of three replicate tanks per treatment.
158 Treatments that were exposed to ambient pCO_2 were continuously bubbled with air at the same rate as
159 the elevated pCO_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

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

Larval sampling and morphology

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

Larval metabolic rate (LMR)

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

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

Lipid extraction

To determine the impact of elevated pCO_2 and temperature on total lipid levels, egg and larval samples were freeze dried (Christ Alpha 1-4 LSC). Lipids were extracted using a modified Bligh and Dyer (1959) and quantified using an Iatroscan MK VI TH10 thin-layer chromatography-flame ionization detector (TLC-FID) analyser (Tokyo, Japan) following the methods described in Gibbs et al., (2020). Total lipid was determined as the sum of all lipid classes.

Data Analyses

Size, mortality, total lipid of larvae *C. gigas* and *S. glomerata* were analysed separately for each species and time point to avoid co-dependence and allow for meaningful interpretation of results (Green 1979). Data was analysed at two meaningful time points; the end of starvation, being 5 days for Fed@5, 9 days for Fed@9 and 11 days for Fed@11 and for valid comparisons at 13 days of age for all treatments. A three-way analysis of variance (ANOVA) was used with "Temperature" (2 levels; 24°C and 28°C), "CO_2 treatment (parents; 2 levels, ambient and elevated)" and "CO_2 treatment (larvae; 2 levels, ambient and elevated)" as fixed factors. Post-Hoc Tukey Tests were used to determine significant (adjusted p value <0.05) differences among treatment levels for significant factors of interest. Residual normality for all analyses was checked using Pearson's-residual Plots. All analyses were done using R V3.5.4 statistical software (R core team) and significance was set as $\alpha < 0.05$. Cochran's test was used to detect any heterogeneity of variances. If necessary, the data were log transformed and re-checked with Cochran's test (Underwood 1997).

RESULTS

Lipid content in Eggs

The total lipid content of the eggs was generally greater in *C. gigas* compared to *S. glomerata* in all treatments. (Figure S1,Table S1). Lipid content of eggs of *S. glomerata* was significantly greater at elevated CO_2 at 28°C (Table S1).

Mortality and Size of Larvae

S. glomerata

Due to *S. glomerata* larval mortality, a complete set of statistical analysis of data on larval size and mortality is only available up to the Fed@5 day treatment. Larval mortality of *S. glomerata* was dependent on larval CO_2 exposure and temperature (Table 2). There was less mortality when parent and larval treatments were the same, either both ambient or elevated CO_2 (Figure 2a; Table 2). Temperature also affected mortality. Mortality of larvae of *S. glomerata* was greater at 28 compared to 24 ˚C with 100% mortality observed after nine days of starvation at 28 ˚C (Figure 2a; Tables 2 and S2). The maximum size of *S. glomerata* was at 28°C (Figure 2b). At 28 ˚C, larvae of *S. glomerata* were significantly larger when reared in ambient conditions from parents exposed to elevated pCO_2 (Figure 2b; Tables 2 and S2). At 24°C, the size of larvae was not dependent on parental exposure to elevated CO_2.

Crassostrea gigas

Larvae of *C. gigas* were present in most treatments for the duration of the experiments. Larval mortality of *C. gigas* was generally lower than that of *S. glomerata*. Mortality of larvae from parents exposed to elevated CO_2 was less and survival greater than larvae from ambient parents (compare Fed @9 and Fed@11 day Figure 3a; Table 2). Mortality of larvae was generally greater at the elevated temperature

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

Starvation affected the size of larvae of *C. gigas*. For *C. gigas*, parental and larval CO_2 exposure and temperature affected the size of larvae (Figure 3b; Table 2). When starved for five days larvae were greater in size when reared in ambient CO_2 from parents exposed to ambient conditions compared to larvae of these parents reared in elevated CO_2. This pattern persisted at 28 ˚C, although on day 13, larvae of *C. gigas* were larger in size from parents exposed to elevated CO_2 (Fed@5 Figure 3b; Table 2). When larvae were starved for nine and eleven days, larvae of *C. gigas* from parents exposed to elevated CO_2 were generally smaller than larvae from parents exposed to ambient CO_2 (Fed@9 and Fed@11 day Figure 3; Table S2).

Lipid Content in Larvae

S. glomerata

Larvae of *S. glomerata* used lipids when starved (Figure 2c). When larvae of *S. glomerata* were starved for 5 days, their total lipid content was significantly greater when they were exposed to ambient compared to elevated CO_2 (Fed@5 day: 5 day old). Following the commencement of feeding, this pattern was reversed, with larvae exposed to elevated CO_2 having greater total lipid content (Fed@5 day: 13 day old; Figure 2c; Tables 3 and S3). By 13 days the larvae reared at 28 °C had a greater lipid content than those reared at 24 °C as shown by the significant effect of temperature (Table 3).

Crassostrea gigas

Like *S. glomerata*, larvae of *C. gigas* used lipids when starved (Figure 3c). Larvae of *C. gigas* had greater total lipid content when they were from parents exposed to elevated CO_2 when starved for 5, 9 and 11 days at 24°C (Figure 3c; Table 3) compared to larvae at ambient CO_2 (5 day old Fed@5 day, 9 day old Fed@9 day and 11 day old Fed@11 P <0.01, Figure 3c; Tables 3 and S2). When feeding commenced, there was no effect of larval or parental CO_2 exposure by day 13 for larvae Fed@5, Fed@9 or Fed@11 day (Figure 3c; Table 3). There was an effect of temperature. The longer larvae were starved, the greater the depletion of total lipids. This depletion was generally greater at 24 compared to 28 °C (Fed@9 and Fed@11 day Figure 3; Table 3). Larvae of *C. gigas* had greater total lipid content at 28 compared to 24 °C at 13 days old when starved for 9 days (Fed@9 day) (Table 3).

LMR

At 24 h, the LMR of *S. glomerata* was greater when larvae were reared in the opposite CO_2 level to their parents (i.e. ambient parents: elevated larvae; elevated parents: ambient larvae; Figure 4a; Tables 4 and S3). After 5 days of starvation, this pattern was still observed at 24 but not at 28 ˚C. Instead, at 28 ˚C, LMR was reduced when larvae were reared at elevated CO_2 irrespective of the parental exposure. LMR of *S. glomerata* larvae was greater at 28 ˚C compared to 24 ˚C at 24 h but not after 5 days of starvation, where LMR at 28 ˚C became depressed (Figure 4a; Table 4). By day 9 only larvae in the 24 ˚C survived and there was no difference in LMR between offspring of adults from ambient or elevated CO_2 treatments (Figure 4a).

At 24 h, larvae of *C. gigas* reared at ambient CO_2 had a greater LMR if their parents were reared at elevated CO_2. When larvae were exposed to elevated CO_2, LMR increased in larvae from parents exposed to ambient CO_2 but not in larvae from parents exposed to elevated CO_2. Following starvation, the LMR of larvae of *C. gigas* was low regardless of parental source (Figure 4b). While there was no

effect of temperature on the LMR of *C. gigas* there was a significant interaction effect of temperature x CO_2 (Parent) x CO_2 (Larvae) (Figure 4b; Tables 4 and S3).

DISCUSSION

The majority of studies to date that have assessed the impacts of elevated CO_2 and temperature on oyster larvae, both acute and transgenerational, have assessed these impacts under optimal food concentrations (Gazeau et al., 2013; Parker et al., 2013). While these experiments provide essential information on how vulnerable marine species will respond over this century, they do not fully account for the natural environment, where food concentration is not always optimal. This study explored a knowledge gap by assessing the impacts of elevated CO_2 and temperature on the transgenerational response of larvae *S. glomerata* and *C. gigas*, when larvae were subjected to different levels of starvation due to delayed access to food. While it is unlikely that marine larvae will experience periods of no food in their natural environment, the incremental starvation and delayed provision of food in this study provided an opportunity to determine whether there were beneficial effects of transgenerational plasticity and tease apart the impacts of endogenous and exogenous energy supply on the transgenerational plasticity developmental responses and lipid dynamics of *S. glomerata* and *C. gigas* exposed to climate change.

Overall, the results of this study indicate that beneficial transgenerational plasticity in response to elevated pCO_2 can occur despite larval starvation. We found a clear positive effect of transgenerational plasticity on the survival of larvae despite starvation. For *C. gigas*, the only larvae remaining alive after 11 days of starvation were those from parents exposed to elevated CO_2. For *S. glomerata*, larval survival was greatest when the conditions experienced by larvae matched the condition of their parents. Parental exposure to elevated CO_2 did not improve the growth rate of larvae for either species when exposed to elevated CO_2. 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 CO_2 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 CO_2
315 in the Fed@5day treatment at 24 ˚C, or Sydney rock oyster larvae from ambient parents, exposed to
316 elevated CO_2 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 CO_2 – a result which coincided with greater survival in these larvae compared to
322 those from parents exposed to ambient CO_2. Previous analysis of the total lipid content of eggs of *C.*
323 *gigas* has shown that mothers exposed to elevated CO_2 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 CO_2-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.

In contrast to *C. gigas*, mothers of *S. glomerata* have previously been found to increase total lipid investment per egg following exposure to elevated CO_2 (Gibbs et al., 2020). Yet here, we found that this increase in maternal investment did not lead to an increase in the total lipid content in the larvae. Following starvation, the total lipid content of larvae of *S. glomerata* was greater when larvae were reared at ambient compared to elevated CO_2. This result was like that found by Talmage and Gobler (2011) who showed that the lipid index of larvae of the clam, *Mercenaria mercenaria* and scallop, *Argopecten irradians* decreased during exposure to elevated CO_2. But, when starved larvae of *S. glomerata* were given food, the pattern of total lipid content was reversed, with larvae exposed to elevated CO_2 having greater total lipid content than those exposed to ambient CO_2. This suggests differences in the mechanisms involved in the accrual of lipid in the two groups of larvae. However, in contrast to the larvae of *C. gigas,* an increase in total lipid did not appear to be closely linked to survival in *S. glomerata*. For *S. glomerata*, larval survival was greatest when the conditions experienced by larvae matched the conditions that their parents experienced. For both species, the total lipid content of larvae was greater at the elevated temperature of 28 compared to 24 ˚C. This result was surprising given that the LMR of larvae was similar at 24 and 28 ˚C during periods of starvation.

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

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

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS The study was conceived and designed by M.G, P.M.R, L.M.P and W.A.O. Data analysis and presentation was done by M.G, E.S and P.M.R. All authors were involved in the writing, editing and revising of the manuscript.

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REFERENCES

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Canadian journal of biochemistry and physiology 37, 911–917.

Byrne, M., Foo, S.A., Ross, P.M., Putnam, H.M., 2020. Limitations of cross-and multigenerational plasticity for marine invertebrates faced with global climate change. Global change biology 26, 80–102. https://doi.org/10/ghcdd7

Cai W.-J., Feely R. A., Testa J. M., Li M., Evans W., Alin S. R., Xu Y.-Y., Pelletier G., Ahmed A., Greeley D. J., Newton J. A. & Bednaršek N., in press. Natural and anthropogenic drivers of acidification in large estuaries. Annual Review of Marine Science.

Collins, M, Knutti, R, Arblaster, J, et al. 2013. Long-term climate change: projections, commitments and irreversibility. In: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change

[Stocker, T.F., D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley (eds.)]. Cambridge University Press, Cambridge, UK and New York, NY, USA.

Commonwealth Science and Industry Research Organisation (CSIRO) 2020. State of the climate report, 2020. Australian Government, Canberra, ACT, Australia.

Dinamani, P., 1974. Reproductive cycle and gonadial changes in the New Zealand rock oyster *Crassostrea glomerata*. New Zealand Journal of Marine and Freshwater Research 8, 39–65. https://doi.org/10/b8ffm9

Donelson, J.M., Munday, P.L., McCormick, M.I., Pitcher, C.R., 2012. Rapid transgenerational acclimation of a tropical reef fish to climate change. Nature Climate Change 2, 30–32. https://doi.org/10/cp6nxd

Edwards, M., Richardson, A.J., 2004. Impact of climate change on marine pelagic phenology and trophic mismatch. Nature 430, 881–884. https://doi.org/10/dswfg2

Gazeau, F., Parker, L.M., Comeau, S., Gattuso, J.-P., O'Connor, W.A., Martin, S., Pörtner, H.-O., Ross, P.M., 2013. Impacts of ocean acidification on marine shelled molluscs. Marine Biology 1–39.

Gibbs, M., Scanes, E., Parker, L., Byrne, M., O'Connor, W.A., Virtue, P., Ross, P.M., 2020. Larval energetics of the Sydney rock oyster (*Saccostrea glomerata*) and Pacific oyster (*Magallana gigas*) Marine Ecology Progress Series 656:51-64 .

Green, R. H., 1979. Sampling design and statistical methods for environmental biologists. Wiley – Interscience, New York.

Hahn, D.A. and Denlinger, D.L., 2007. Meeting the energetic demands of insect diapause: nutrient storage and utilization. Journal of Insect Physiology, 53(8), pp.760-773.

Hettinger, A., Sanford, E., Hill, T.M., Hosfelt, J.D., Russell, A.D. and Gaylord, B., 2013. The influence of food supply on the response of Olympia oyster larvae to ocean acidification. Biogeosciences, *10*(10).

Hettinger, A., Sanford, E., Hill, T.M., Russell, A.D., Sato, K.N., Hoey, J., Forsch, M., Page, H.N. and Gaylord, B., 2012. Persistent carry-over effects of planktonic exposure to ocean acidification in the Olympia oyster. Ecology, 93(12), pp.2758-2768.

Hobday, A.J., Pecl, G.T., 2014. Identification of global marine hotspots: sentinels for change and vanguards for adaptation action. Reviews in Fish Biology and Fisheries 24, 415–425. https://doi.org/10/gdxwzp

Kong, H., Jiang, X., Clements, J.C., Wang, T., Huang, X., Shang, Y., …and Wang, Y., 2019. Transgenerational effects of short-term exposure to acidification and hypoxia on early developmental traits of the mussel *Mytilus edulis*. Marine Environmental Research. https://doi.org/10.1016/j.marenvres.2019.02.011

McCue, M.D., Terblanche, J.S. and Benoit, J.B., 2017. Learning to starve: impacts of food limitation beyond the stress period. Journal of Experimental Biology, 220(23), pp.4330-4338.

Moran, A.L., Manahan, D.T., 2004. Physiological recovery from prolonged 'starvation'in larvae of the Pacific oyster *Crassostrea gigas*. Journal of Experimental Marine Biology and Ecology 306, 17–36. https://doi.org/10/c7q725

Parker, L.M., Ross, P.M., O'Connor, W.A., Pörtner, H.O., Scanes, E., Wright, J.M., 2013. Predicting the response of molluscs to the impact of ocean acidification. Biology 2, 651–692. https://doi.org/10/gb9hkx

Parker, L.M., Ross, P.M. and O'Connor, W.A., 2010. Comparing the effect of elevated pCO_2 and temperature on the fertilization and early development of two species of oysters. Marine Biology, 157(11), pp.2435-2452.

Parker, L.M., Ross, P.M., O'Connor, W.A., Borysko, L., Raftos, D.A., Pörtner, H.O., 2012. Adult exposure influences offspring response to ocean acidification in oysters. Global Change Biology 18, 82–92. https://doi.org/10/cq55q3

Parker, L.M., O'Connor, W.A., Byrne, M., Coleman, R.A., Virtue, P., Dove, M., Gibbs, M., Spohr, L., Scanes, E., Ross, P.M., 2017. Adult exposure to ocean acidification is maladaptive for larvae of the Sydney rock oyster *Saccostrea glomerata* in the presence of multiple stressors. Biology Letters 13, 20160798. https://doi.org/10/gdxwzn

Parker, L.M., O'Connor, W.A., Raftos, D.A., Pörtner, H.-O., Ross, P.M., 2015. Persistence of positive carryover effects in the oyster, *Saccostrea glomerata*, following transgenerational exposure to ocean acidification. PloS one 10, e0132276. https://doi.org/10/gd57bc

Poloczanska, E.S., Brown, C.J., Sydeman, W.J., Kiessling, W., Schoeman, D.S., Moore, P.J., Brander, K., Bruno, J.F., Buckley, L.B., Burrows, M.T. and Duarte, C.M., 2013. Global imprint of climate change on marine life. Nature Climate Change, *3*(10), pp.919-925.

Pörtner, H.O., 2008. Ecosystem effects of ocean acidification in times of ocean warming: a physiologist's view. Marine Ecology Progress Series 373, 203–217. https://doi.org/10/cfzmqh

Pörtner, H.O., 2012. Integrating climate-related stressor effects on marine organisms: unifying principles linking molecule to ecosystem-level changes. Marine Ecology Progress Series, 470, pp.273-290.

Putnam, H.M., Ritson-Williams, R., Cruz, J.A., Davidson, J.M. and Gates, R.D., 2020. Environmentally-induced parental or developmental conditioning influences coral offspring ecological performance. Scientific reports, 10(1), pp.1-14.

Ross, P.M., Parker, L., Byrne, M., 2016. Transgenerational responses of molluscs and echinoderms to changing ocean conditions. ICES Journal of Marine Science: Journal du Conseil fsv254. https://doi.org/10/f8brgx

Ruesink, J.L., Lenihan, H.S., Trimble, A.C., Heiman, K.W., Micheli, F., Byers, J.E. and Kay, M.C., 2005. Introduction of non-native oysters: ecosystem effects and restoration implications. Annual review of ecology, evolution, and systematics, 36.

Salvi, D., Macali, A., Mariottini, P., 2014. Molecular phylogenetics and systematics of the bivalve family Ostreidae based on rRNA sequence-structure models and multilocus species tree. PLoS One 9. https://doi.org/10/ghcdh3

Scanes, E., Parker, L. M., O'Connor, W. A., Gibbs, M. C., & Ross, P. M. 2018. Copper and ocean acidification interact to lower maternal investment but have little effect on adult physiology of the Sydney rock oyster *Saccostrea glomerata*. Aquatic Toxicology, 203, 51-60.

Scanes, E., Scanes, P.R., Ross, P.M., 2020. Climate change rapidly warms and acidifies Australian estuaries. Nature Communications 11, 1803. https://doi.org/10/ghcddx

Scanes, E., Johnston, E.L., Cole, V.J., O'Connor, W.A., Parker, L.M. and Ross, P.M., 2016. Quantifying abundance and distribution of native and invasive oysters in an urbanised estuary. Aquatic Invasions 11(4), pp.425-436.

Sokolova, I.M., 2013. Energy-limited tolerance to stress as a conceptual framework to integrate the effects of multiple stressors. Integrative and comparative biology, 53(4), pp.597-608.

Storey, K.B. and Storey, J.M., 1990. Metabolic rate depression and biochemical adaptation in anaerobiosis, hibernation and estivation. The Quarterly Review of Biology, 65(2), pp.145-174.

Storey, K.B. and Storey, J.M., 2004. Metabolic rate depression in animals: transcriptional and translational controls. Biological Reviews, 79(1), pp.207-233.

Talmage, S.C., Gobler, C.J., 2011. Effects of elevated temperature and carbon dioxide on the growth and survival of larvae and juveniles of three species of Northwest Atlantic bivalves. PLoS One 6, e26941. https://doi.org/10/cdhgr9

Thomsen, J., Casties, I., Pansch, C., Körtzinger, A. and Melzner, F., 2013. Food availability outweighs ocean acidification effects in juvenile Mytilus edulis: laboratory and field experiments. Global Change Biology, 19(4), pp.1017-1027.

Underwood, A.J., 1997. Experiments in ecology: their logical design and interpretation using analysis of variance. Cambridge Univ Pr.

Uthicke, S., Soars, N., Foo, S. and Byrne M., 2013. Effects of elevated pCO_2 and the effect of parent acclimation on development in the tropical Pacific sea urchin *Echinometra mathaei*. Marine Biology. https://doi.org/10.1007/s00227-012-2023-5

Vehmaa, A., Brutemark, A. and Engström-Öst, J., 2012. Maternal effects may act as an adaptation mechanism for copepods facing pH and temperature changes. PLoS one, 7(10), p.e48538.

Venkataraman, V.R., Spencer, L.H. and Roberts S.B., 2019. Larval response to parental low pH exposure in Pacific oysters (*Crassostrea gigas*)**.** Journal of Shellfish Research. https://doi.org/10.2983/035.038.0325

481

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 pCO_2. **B)** Conceptual
486 diagram of the feeding delay experimental design.

487 **Figure 2.** Mean (±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) pCO_2at 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 (±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) pCO_2at 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, ±S.E, n=3) Larval Metabolic Rate (ng O_2 $larva^{-1}$ 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) pCO_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.