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Restoring the flat oyster *Ostrea angasi* in the face of a changing climate

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ABSTRACT: Across the globe, restoration efforts are stemming the loss of native oyster reefs and the ecosystem services they provide, but these efforts will need to consider climate change in order to be sustainable. South-eastern Australia is the focus of restoring the once abundant oyster *Ostrea angasi*. This region is also a climate change 'hot spot' where the ocean is warming rapidly, with the potential to be exacerbated by marine heatwaves and coastal acidification. In this study, the impact of near-future (~2050) elevated temperature and pCO_2 on *O. angasi* was determined and considered in context with concerns for the long-term sustainability of oyster reef restoration efforts. Oysters were exposed to ambient and elevated pCO_2 concentrations (mean ± SE: 408 ± 19.8 and 1070 ± 53.4 µatm) and ambient and elevated temperatures (22.78 ± 0.17 and 25.73 ± 0.21°C) for 10 wk in outdoor flow-through mesocosms. Shell growth, condition index, standard metabolic rate (SMR), extracellular pH and survival were measured. Elevated temperature caused high mortality (36%) and decreased the condition of oysters (33%). Elevated pCO_2 increased SMR almost 4-fold and lowered the extracellular pH of *O. angasi* by a mean 0.29 pH units. In combination, elevated pCO_2 and temperature ameliorated effects on SMR and survivorship of oysters. *O. angasi* appears to be living near the limits of its thermal tolerance. Restoration projects will need to account for the temperature sensitivity of this species and its changing habitat to 'climate proof' long-term restoration efforts.

KEY WORDS: Reef restoration · *Ostrea angasi* · Ocean warming · Ocean acidification · Climate change · Flat oysters · Aquaculture · Physiology

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1. INTRODUCTION

Across the globe, native oyster reefs have declined, with estimated global losses of 85% (Beck et al. 2009, Zu Ermgassen et al. 2012, Gillies et al. 2018). This includes some locations where reefs have completely disappeared due to destructive fishing, overexploitation (Jackson et al. 2001, Kirby 2004), poor water quality, disease and parasite outbreaks, introduction of non-indigenous species and coastal development (Ruesink et al. 2005, Lotze et al. 2006, Beck et al. 2009, 2011). Restoration efforts worldwide are now aimed at stemming the loss of native oyster reefs (Schulte et al. 2009, Grabowski et al. 2012, Baggett et al. 2014, Gillies et al. 2017, Lipcius & Burke 2018). Oysters form aggregations and complex reef structures that perform vital ecosystem services, providing habitat and shelter for a diverse array of species and acting as a nursery ground for fish (Grabowski & Peterson 2007, Arkema et al. 2013, Baggett et al. 2014, Kellogg et al. 2014). Oysters also contribute to the health of coastal areas through their filter feeding

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activities, while increasing local biodiversity and improving water clarity and quality (Grabowski & Peterson 2007, Beck et al. 2009, Grabowski et al. 2012, Kellogg et al. 2014). They may also mitigate some effects of sea level rise and protect the coastline (Piazza et al. 2005, Arkema et al. 2013, Gittman et al. 2016). Efforts to restore oyster reefs have focused on restoring biodiversity to sustain the vital ecosystem services that they provide, including filtration and habitat (Lotze et al. 2006, Beck et al. 2011, Grabowski et al. 2012, Blomberg et al. 2018, Peterson & Lipcius 2003). For restoration actions to be sustainable in the face of reported oyster vulnerability (e.g. Gattuso et al. 2015), efforts must consider the response of oysters and their habitat to climate change (Harris et al. 2006).

It is well established that oysters and other molluscs are particularly vulnerable to climate-change driven ocean warming and acidification caused by anthropogenic CO_2 emissions; changes that will continue to accelerate into the future (Parker et al. 2013, 2015, Gattuso et al. 2015, Scanes et al. 2017). Exposure to elevated pCO_2 can weaken mollusc shells (Gazeau et al. 2013, Ivanina et al. 2013, Wright et al. 2018b), impair biomineralization and interfere with the acid–base balance (Beniash et al. 2010, Lannig et al. 2010, Kroeker et al. 2013, Matoo et al. 2013, Parker et al. 2015). Environmental acidification has a direct negative impact on shell crystallography of mussels and oysters with a more chaotic pattern in low pH conditions (Fitzer et al. 2014, 2018). Elevated pCO_2 has also been found to decrease size and increase abnormality in oyster larvae (Parker et al. 2009). Meta-analyses have reported higher vulnerability of bivalve larvae to climate change compared to later life stages; however, this was not found for other marine groups (Kroeker et al. 2013).

Exposure to elevated pCO_2 also significantly affects the physiology of adult oysters. For example, Parker et al. (2012) found that wild and a selectively bred line of the Sydney rock oyster *Saccostrea glomerata* (Gould, 1850) increased metabolic rates to cope with increased pCO_2. Elevated pCO_2 at levels commensurate with near-future (year 2100) ocean acidification (mean pH 7.68) decreased the haemolymph pH (extracellular pH; pH_e) in the Pacific oyster *Magallana gigas* (previously *Crassostrea gigas*; Thunberg, 1793) (Lannig et al. 2010) and *S. glomerata* (mean pH 7.90; Parker et al. 2015). Acidosis of the extracellular body fluids has to be compensated by energy-dependent mechanisms, such as buffering by accumulation of bicarbonate (HCO_3^-) (Lindinger et al. 1984, Michaelidis et al. 2005). Ocean acidification represents a major challenge for oyster restoration efforts; acidification-induced disturbance of energy budgets (Sokolova 2013, Parker et al. 2017) may lower the capacity of bivalves to perform ecosystem services such as habitat provision, water filtration and nutrient cycling in coastal areas (Grabowski & Peterson 2007).

Habitat warming due to global climate change will also impact oysters (Pörtner 2001). Studies have already observed migration of bivalve populations towards the poles (Berge et al. 2005, Hofmann et al. 2018). Elevated temperature increases standard metabolic rate (SMR), leading to increased growth rates and stress, which may push organisms beyond their physiological limits (Somero 2002, Pörtner & Farrell 2008, Tomanek 2010) resulting in mortality (e.g. Pörtner & Knust 2007). For molluscs, thermal stress is energetically costly (e.g. upregulation of chaperone proteins; Somero 2002, Tomanek 2010), as more energy is needed to maintain basal processes and to repair damages caused by elevated temperature (Tomanek 2010). For example, exposure to elevated temperature decreased shell growth and condition index of the mussel *Mytilus edulis* (Linnaeus, 1758) and the clam *Arctica islandica* (Linnaeus, 1767) (Hiebenthal et al. 2013).

In combination, temperature and elevated pCO_2 may interact to either ameliorate or exacerbate the effects on oysters than either factor alone (Byrne & Przeslawski 2013). Previous studies reported mixed results when elevated pCO_2 and temperature were combined, perhaps due to species-specific responses (Parker et al. 2013). For example, the thermal window of the Sydney rock oyster *S. glomerata* narrowed in response to elevated pCO_2 (Parker et al. 2017). A synergistic impact of pCO_2 and warming was also detected on the metabolic rate of the Pacific oyster *M. gigas* (Lannig et al. 2010). Elevated temperature, however, ameliorated some of the effects of elevated pCO_2 on the calcification of the oyster *Crassostrea virginica* (Gmelin, 1791) (Waldbusser et al. 2011).

Oyster species that occur in southeast Australia will have less time to adapt to changes in temperature due to expected rapid habitat warming. This region is one of several ocean warming 'hot spots' across the globe where the ocean is warming 3–4 times the global average, with projections of an increase in surface sea temperatures (SSTs) by up to 4°C by 2050 (Fig. 1) (Ridgway 2007, Collins et al. 2013, Hobday & Pecl 2014). This is being exacerbated by marine heatwaves causing sustained, above average SSTs (Hobday et al. 2016), which are deleterious to local biota

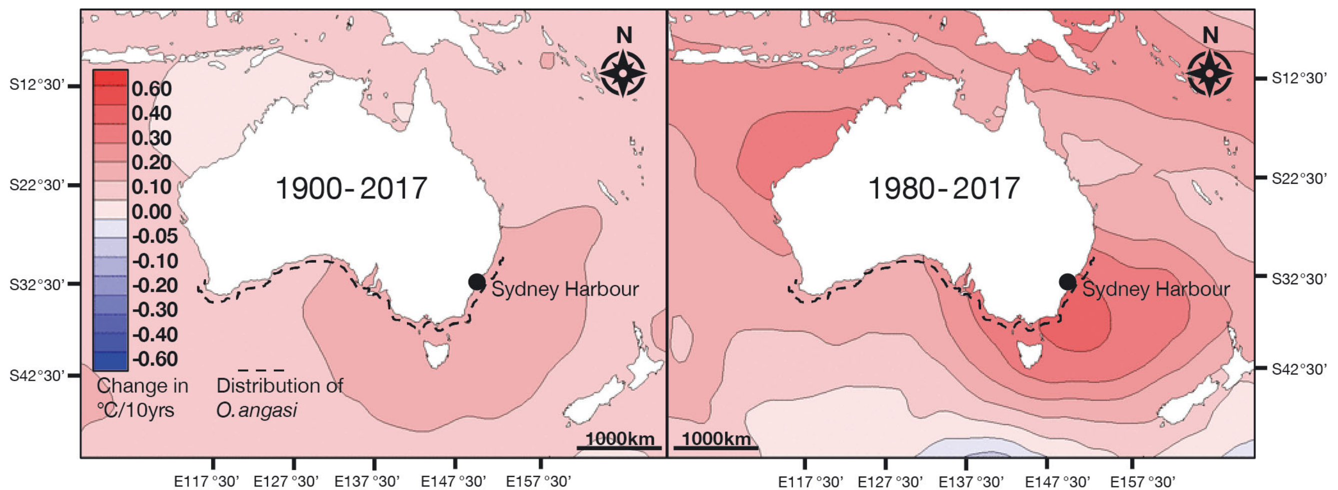

Fig. 1. Rate of change in Australian sea surface temperatures standardised to °C per 10 yr from 1900–2017 (left) and 1980–2017 (right). Black dot: Sydney Harbour; dashed line: historical distribution of *Ostrea angasi* (as described by Ogburn et al. 2007). Maps created using the Australian Bureau of Meteorology website (www.bom.gov.au) with NOAA Extended Reconstructed Sea Surface Temperature Version 5 (ERSST v5) data provided by the NOAA/ESRL PSD, Boulder, CO. Original images © Australian Bureau of Meteorology. Re-used here by the authors under the Creative Commons by Attribution (CC-BY) Licence 3.0

(Harianto et al. 2018). Coastal acidification due to climate-driven increases in precipitation is also problematic for the region (Fitzer et al. 2018). *Ostrea angasi* (Sowerby, 1871), commonly called the flat oyster, is native to southeast Australia. Historically, this species, together with the Sydney rock oyster *S. glomerata*, formed extensive subtidal and shallow intertidal reefs. On arrival in 1788, European colonists marvelled at the 'amazing' size of the reefs and the oysters that formed them in Sydney Harbour (Ogburn et al. 2007, Alleway & Connell 2015) (Fig. 1). By 1870, however, *O. angasi* had effectively disappeared from east Australian estuaries after dredging removed most of the oysters and compromised the integrity of remaining natural reefs (Ogburn et al. 2007, Gillies et al. 2017). The oysters were believed to be so exploited that in 1877 a Royal Commission was established to manage their harvesting. Overharvesting, combined with the introduction of pest species (*Polydora* sp.) from New Zealand, reduced wild stocks of *O. angasi* to a point of non-existence (Ogburn et al.2007). Having declined in so much of its native range, this species is now the focus of large-scale oyster reef restoration programs in southern and south-eastern Australia (Gillies et al. 2017).

O. angasi is mostly found in the subtidal (up to 30 m) but can be also found in intertidal areas with past distribution ranging from Western Australia to New South Wales, including the Tasmanian coast (Ogburn et al. 2007, Edgar 2012, Crawford 2016) (Fig. 1). *O. angasi* is a brooding species; females rear larvae in their brood chamber (Dix 1976). Larvae can experience low pH naturally within the mother's brood chamber and this may increase their capacity to withstand elevated pCO_2; Cole et al. (2016) found that larvae of *O. angasi* were less impacted compared to larvae from other oyster species that developed free in the water column.

To predict species responses to changing climate, experiments need to approximate conditions relevant to the region and incorporate multi-stressor treatments (Przeslawski et al. 2015). The aim of this study was to assess the prospects for long-term sustainability of restoring *O. angasi* in southeast Australia, specifically in an important estuary of New South Wales (Sydney Harbour), by investigating the effect of ocean warming and acidification (elevated temperature and pCO_2) on key physiological traits and survival of adult *O. angasi.* A recent study on the larvae of *O. angasi* revealed that they are tolerant of elevated pCO_2; however, combining elevated pCO_2 with elevated temperature impaired larval development (Cole et al. 2016). Adult responses of *O. angasi* remain unexplored. We exposed adult *O. angasi* to elevated temperature and pCO_2 conditions in a multi-stressor study incorporating predicted climatic changes estimated by year 2050 for the Australian coast (Collins et al. 2013, Hobday & Pecl 2014) for 10 wk in outdoor flow-through mesocosms supplied by water from their habitat (Sydney Harbour) throughout spring and summer. We hypothesised that warming and acidification would significantly

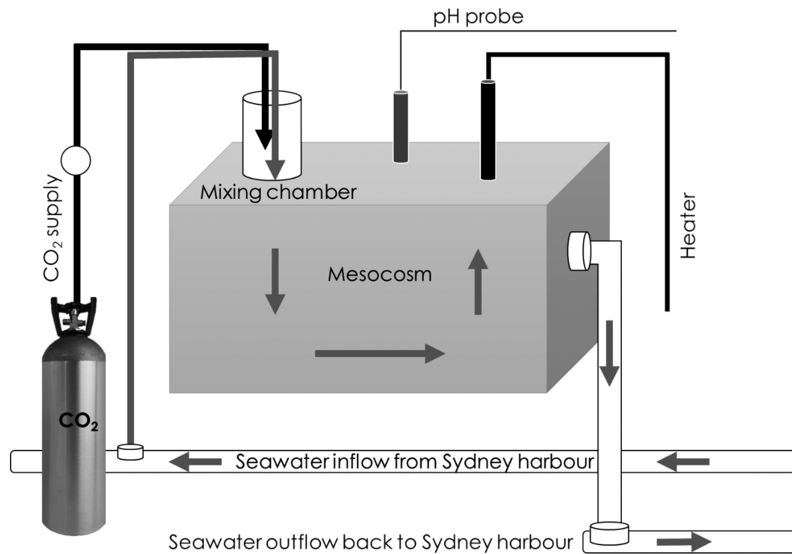

Fig. 2. Mesocosm setup showing 1 of 12 mesocosms, with dimensions 37 × 62.5 × 40 cm; total volume: 70 l. Each mesocosm held 20 flat oysters *Ostrea angasi*. Each elevated temperature mesocosm was heated independently, and each elevated pCO_2 mesocosm had an independent pH computer (controlling CO_2 delivery), CO_2 supply and mixing chamber

impact the physiology of *O. angasi.* We consider our results with respect to present-day conditions and the long-term sustainability of regional oyster reef restoration efforts in the face of a changing climate.

2. MATERIALS AND METHODS

2.1. Animals and mesocosm setup

Adult oysters *Ostrea angasi* were obtained from an oyster farm in the Georges River south of Sydney (34° 02′ 10.0″ S, 151° 08′ 51.9″ E) and were transported to Chowder Bay (33° 50′ 24.6″ S, 151° 15′ 15.9″ E), Port Jackson (commonly called Sydney Harbour). Oysters had a mean (±SE) shell length of 49.18 ± 0.57 mm and mean wet weight of 9.32 ± 0.32 g (N = 189). In total, 240 *O. angasi* were allocated to 12 independent 70 l mesocosms (Fig. 2) supplied with unfiltered seawater pumped directly from the harbour where *O. angasi* once naturally occurred. Mesocosms were located outdoors, where they received ambient light and experienced day/night and seasonal temperature flux. The oysters were acclimated to flow-through mesocosm conditions for 8 wk, feeding on natural plankton. Following this acclimation period, oysters were exposed to treatments for a further 10 wk. This length of exposure was chosen based on previous studies of ocean acidification and warming on oysters and other bivalves (Parker et al. 2012, 2015, Scanes et al. 2014, 2017). Mesocosms received a full change of seawater every 2 h and 20 min. The treatments consisted of an orthogonal design with 2 pH treatments: control (ambient pH) and elevated pCO_2 (minus 0.4 pH_{NBS} units); and 2 temperature treatments: control (ambient) and elevated (4°C above ambient). The flow rate in the mesocosms was 0.5 l min^{-1}, which allowed incoming seawater to mix with CO_2 in the mixing chamber. Elevated pCO_2 and temperature treatments were commensurate with predictions for 2050 based on 'business as usual' CO_2 emissions (Collins et al. 2013, Hobday & Pecl 2014). CO_2 concentrations were manipulated by the use of a negative feedback system (Aqua Medic, Aqacenta; accuracy ±0.01 pH units), which consisted of a CO_2 cylinder (food grade; BOC Australia) connected to a mixing chamber, with gas flux controlled by a solenoid valve (Aqua Medic Electronic shut off valve for CO_2-Standard) and a pH probe (Aqua Medic pH electrode with BNC connection) attached to the mesocosm. Once the desired pCO_2 concentration was reached (~1000 µatm) by the bubbling of gas into a mixing chamber, the solenoid valve closed to shut off the gas supply. Each of the 6 elevated pCO_2 mesocosms (out of a total of 12) was controlled by an independent CO_2 control system. The pH probe above was calibrated weekly with fresh buffers (pH 4.0 and 7.0; Aqua Medic). The pH was also measured weekly using the *m*-cresol spectrophotometric method and calculated in the total scale (pH_{total}) (Liu et al. 2011), following recommendations from Dickson et al. (2007) for reporting ocean CO_2.

This experiment took place between Spring and Summer (November–February) 2016–2017. Temperature within the mesocosms was manipulated using aquarium heaters (AquaOne 200 W). Heaters were set in warming treatments to warm the seawater +4°C above ambient seawater temperature. This allowed mesocosms to vary in temperature in a way that is comparable with the variation in summer temperature measured within the habitats of *O. angasi* (Fig. 3). Long-term temperature data in Sydney Harbour has shown that temperatures of 25°C were regularly experienced during summer between the years of 2015–2017, with an average of approximately 22°C (Harianto et al. 2018). Therefore, we can confirm that the

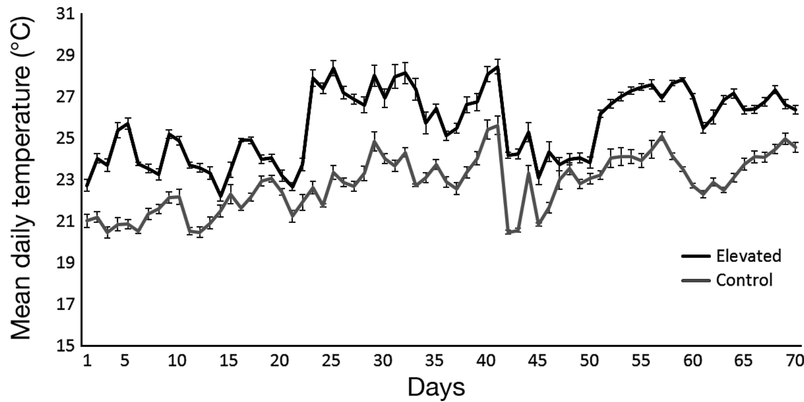

Fig. 3. Mean (±SE) daily temperature in control and elevated temperature mesocosms measured by 4 loggers (1 per treatment) for the 10 wk of experimental exposure with *Ostrea angasi* during November 2016–January 2017

temperatures experienced within ambient mesocosms were comparable to 'normal' summer conditions in Sydney Harbour. One logger per treatment was placed within mesocosms to record temperature (Hobo Pendant; Onset loggers), logging data every 2 h. Temperature within each mesocosm was also measured twice daily with a handheld digital thermometer to verify logger measurements (Fig. 3, Table 1).

2.2. Seawater chemistry

Samples of seawater were taken at Weeks 1, 3, 5, 7 and 10 (minimum 4 samples; 2 from ambient pCO_2 and 2 from elevated pCO_2) and immediately fixed with mercuric chloride for total alkalinity (TA) measurements. TA was determined potentiometrically in at least duplicates and at maximum triplicates using an automatic titration system connected to a calibrated pH_{NBS} meter (Model 888 Titrando; Metrohm). TA was calculated as described by Dickson et al. (2007) against a certified reference standard (Dickson standard, Batch 139). The pCO_2 values and other parameters of the carbonate chemistry system were determined from measured values of pH, TA, temperature and salinity using CO_2SYS (Lewis et al. 1998) and the dissociation constants of Mehrbach et al. (1973) refitted by Dickson & Millero (1987). Salinity in Chowder Bay (where the mesocosms were located) was monitored at a depth of 1 m by a calibrated monitoring mooring operated as part of the Australian Integrated Marine Observing System (IMOS 2019) (Tables 1 & 2).

Table 1. Weekly mean (±SE) water temperature (°C) in control and elevated temperature treatments with *Ostrea angasi.* Water temperature was logged continuously every 2 h by 4 loggers (1 per treatment) for 10 wk from November 2016–January 2017

Week	Temperature treatment	
	Control	Elevated
1	20.90 ± 0.12	24.13 ± 0.39
2	21.33 ± 0.27	23.75 ± 0.39
3	22.25 ± 0.24	23.87 ± 0.32
4	22.65 ± 0.25	27.02 ± 0.59
5	23.81 ± 0.29	27.70 ± 0.34
6	23.32 ± 0.60	26.54 ± 0.59
7	22.14 ± 0.43	24.48 ± 0.30
8	23.96 ± 0.28	26.49 ± 0.44
9	23.31 ± 0.38	26.79 ± 0.42
10	24.14 ± 0.23	26.58 ± 0.14

2.3. Measurements

2.3.1. Shell growth

Oysters were individually labelled with a reference number on UV-resistant tags (Hallprint) prior to acclimation. Measurements of shell height were taken with digital callipers at the beginning of the experiment and at the end of 8 wk (linear extension perpendicular to the hinge, along the dorsal–ventral axis (see Wright et al. 2018a for explanation of shell axis). Shell height growth was calculated as: $(X_2 - X_1) / (t_2 - t_1) \times 100$, where X_1 is the initial shell height (in mm), X_2 is the final height (in mm), t_1 is equal to 0 (as is the day the experiment started) and t_2 is the day measurements were taken.

2.3.2. SMR

The SMR of 9 individuals (3 per mesocosm) from each treatment was measured following 10 wk of experimental exposure using the methods of Parker et al. (2012). To calculate SMR, oxygen consumption was measured by a closed respirometry system (OXY-10; PreSens, AS1). One individual was placed in a sealed container (370 ml volume) containing filtered seawater (20 µm) adjusted to the treatment conditions in which that oyster was kept. Each container then had a fibre optic probe attached. The probe was previously calibrated using 2 O_2 concen-

Table 2. Mean (±SE) seawater chemistry parameters for the entire 10 wk exposure period (November 2016–January 2017). Salinity was measured every second day, and water samples for seawater chemistry were taken at Weeks 1, 3, 5, 7 and 10 (minimum 4 samples; 2 from control pCO_2 and 2 from elevated pCO_2; at Week 10 only elevated pCO_2 samples). TA: total alkalinity; Ω: saturation state

	Salinity	pCO_2 (µatm)	TA (µmol kg^{-1})	pH$_{total}$	$\Omega_{calcite}$	$\Omega_{aragonite}$
Control (n = 4)	35.08 ± 0.07	423.03 ± 21.72	2307.33 ± 6.14	8.03 ± 0.02	4.84 ±0.16	3.18 ± 0.10
Elevated temperature (n = 4)	35.08 ± 0.07	392.97 ± 34.82	2304.43 ± 4.95	8.05 ± 0.03	5.09 ± 0.30	3.34 ± 0.20
Elevated pCO_2 (n = 5)	35.08 ± 0.07	1016.13 ± 91.07	2307.06 ± 7.08	7.70 ± 0.03	2.62 ± 0.18	1.73 ± 0.12
Elevated temperature and pCO_2 (n = 6)	35.08 ± 0.07	1114.49 ± 63.69	2312.12 ± 6.21	7.66 ± 0.02	2.41 ± 0.11	1.59 ± 0.07

tration points (0 and 100% oxygen saturation of seawater). Oysters were gently cleaned of any fouling organisms and then placed in filtered seawater (20 µm; adjusted to the corresponding treatment levels) for 24 h before respiration trials to avoid noise from digestion processes. The time that individuals took to lower the oxygen concentration by 20% (~1.2 O_2 mg l^{-1}) was recorded. Following the procedure of Parker et al. (2012), only the time that the oyster was open and actively respiring (determined by observed decreasing oxygen) was used to calculate SMR. This was done to guard against the oyster remaining closed from handling stress. Oysters took about 2–3 h to reduced oxygen in the chambers by 20%. After each trial, each container was rinsed clean with filtered seawater (20 µm) and wiped clean with paper towel. Each oyster was then removed from the container and shucked to separate body tissues and shell. The tissue was then dried in an oven at 60°C for 3 d to measure constant dry body tissue and shell weight (±0.0001 g; Analytical Balance Sartorius Research). The following calculation for SMR was used:

$$\mathrm{SMR} = \frac{V_r(\mathrm{l}) \times \Delta C_w O_2 (\mathrm{mg\,O_2\,l^{-1}})}{\Delta t(\mathrm{h}) \times b_w(\mathrm{g})} \quad (1)$$

where SMR is the oxygen consumption normalized to 1 g of dry tissue mass (mg O_2 g^{-1} dry tissue mass h^{-1}), V_r is the volume of the respirometry chamber minus the volume of the oyster (in l), C_wO_2 is the change in water oxygen concentration measured (in mg O_2 l^{-1}), t is measuring time (in h) and b_w is the dry tissue mass (in g) of the oyster.

2.3.3. pH$_e$

At the end of the 10 wk experiment, extracellular fluid was carefully extracted from 36 individuals (3 ind. mesocosm^{-1}) with a 1 ml sterile syringe and placed into an Eppendorf tube. Fluid pH was determined using a micro pH probe (Orion 9110DJWP Double Junction pH Electrode) following methods in Parker et al. (2015). The probe was calibrated prior to use with fresh buffers (pH 4, 7 and 10); measurements were made at 21°C for oysters from the control temperature treatments and at 25°C for oysters from the elevated temperature treatments (temperatures chosen to represent Sydney Harbour current and future conditions).

2.3.4. Condition index and survival

At the end of the experiment (10 wk), the condition index (C_i) of oysters was determined. In total, 4 oysters mesocosm^{-1} were used (12 oysters treatment^{-1}; N = 48) to calculate averages. The body mass and shell of oysters were dried separately (60°C for 3 d) and weighed using a digital scale (±0.0001 g; Analytical Balance Sartorius Research). C_i was calculated by the formula (Lucas & Beninger 1985, Mann 1992):

$$C_i = \frac{[\text{Dry body weight (g)}]}{[\text{Dry shell weight (g)}]} \times 100 \quad (2)$$

Mortality in each treatment was recorded daily over the 10 wk experimental period.

2.4. Data analysis

Data analyses were done with GMAV5 for Windows (Underwood et al. 2002). Prior to each analysis, homogeneity of variances were checked using Cochran's test, and all data were found to meet this assumption ($p < 0.05$; Underwood et al. 2002). A 3-way ANOVA, with CO_2 and temperature as fixed factors

with 2 levels each (control and elevated) and mesocosm as a random factor with 3 levels (nested in the other 2 factors), was used to determine significant differences for all data except survival. Survival data were analysed as a 2-way ANOVA because data were collected as a percentage, and mesocosms were replicates (n = 3). When an interaction of factors was detected, Student-Newman-Keuls (SNKs; Sokal & Rohlf 1995) tests were performed to determine the source of variation. Results were considered significantly different when $p < 0.05$.

3. RESULTS

3.1. Shell growth and C_i

Oyster shell growth, measured by change in shell height, did not differ significantly among treatments (mean ± SE: 0.08 ± 0.01 mm d^{-1}, n = 4; Fig. 4a). C_i was significantly lower in the elevated temperature and combined elevated temperature and pCO_2 treatments (1.10 ± 0.13) compared to the elevated pCO_2 (1.66 ± 0.09, n = 12) and control treatments. This was a decrease of 33% ($F_{1,8}$ = 9.57, p = 0.0148; Table 3, Fig. 4b).

3.2. SMR

Oyster SMR was significantly greater at elevated pCO_2 compared to the control and other treatments ($F_{1,8}$ = 6.44, p = 0.03; Table 3, Fig. 5a). The SMR of oysters exposed to elevated pCO_2 was 0.04 ± 0.006 mg O_2 g^{-1} dry tissue h^{-1} (n = 9), double that of oysters exposed to combined elevated pCO_2 and temperature (0.02 ± 0.004, n = 9) and those in control conditions (0.015 ± 0.002 mg O_2 g^{-1} dry tissue h^{-1}, n = 9; Fig. 5a).

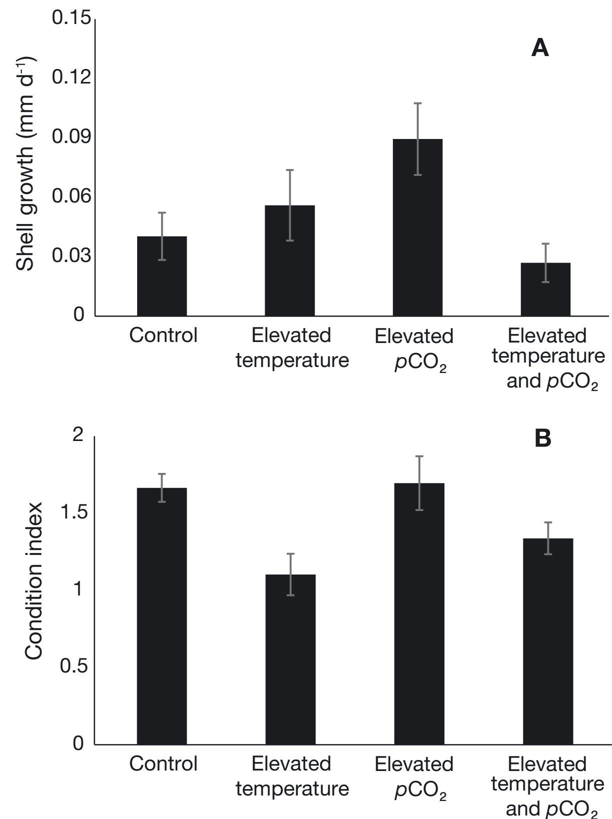

Fig. 4. Mean (±SE) (A) shell growth and (B) condition index of *Ostrea angasi* after exposure to experimental treatments (n = 12; N = 48)

3.3. pH_e

Oyster pH_e was significantly lower (6.96 ± 0.06, n = 9) at elevated pCO_2 ($F_{1,8}$ = 6.64, p = 0.03; Fig. 5b), compared to the control (7.26 ± 0.03, n = 9), elevated temperature (7.25 ± 0.08, n = 9) and combined elevated temperature and pCO_2 treatments (7.16 ± 0.04, n = 9).

Table 3. ANOVA results for *Ostrea angasi* shell growth, condition index, standard metabolic rate (SMR), extracellular pH (3-way ANOVA) and survival (2-way ANOVA) in control and elevated pCO_2 treatments (mean ± SE: 408 ± 19.8 and 1070 ± 53.4 µatm, respectively) and control and elevated temperature treatments (22.68 ± 1.92 and 25.33 ± 2.24°C, respectively) in an orthogonal experimental design. Significant differences are in **bold** (p < 0.05). Shell growth and condition index (n = 4, N = 48); SMR and extracellular pH (n = 3, N = 36); survival (n = 3, N = 12)

Source of variation	Shell height growth				Condition index				SMR				Extracellular pH				Survival			
	df	MS	*F*	p	df	MS	*F*	p	df	MS	*F*	p	df	MS	*F*	p	df	MS	*F*	p
pCO_2 (C)	1	0.001	0.28	0.611	1	0.210	0.80	0.398	1	0.002	17.33	**0.003**	1	0.327	6.64	**0.032**	1	252.083	5.76	**0.043**
Temperature (T)	1	0.0067	1.53	0.251	1	2.532	9.57	**0.015**	1	0.001	7.71	**0.024**	1	0.070	1.43	0.266	1	752.083	17.19	**0.003**
Mesocosm (T × C)	8	0.004	1.90	0.091	8	0.265	1.41	0.225	8	0.000	0.68	0.705	8	0.049	1.78	0.132				
pCO_2 × T	1	0.018	4.25	0.073	1	0.125	0.47	0.511	1	0.000	6.44	**0.035**	1	0.093	1.89	0.206	1	752.083	17.19	**0.0032**
Residual	36	0.002			36	0.187			24	0.000			24	0.028			8	43.750		
Total	47				47				35				35				11			

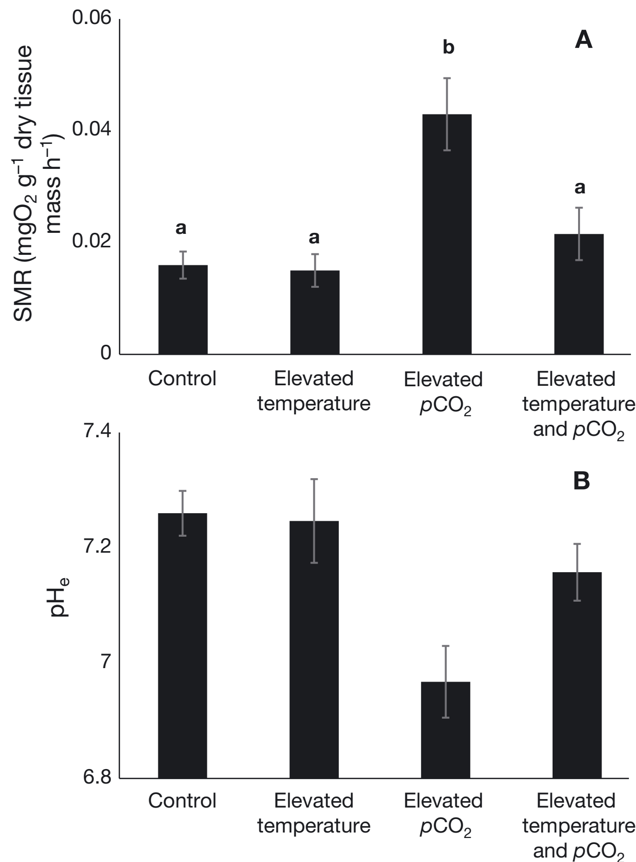

Fig. 5. Mean (±SE) (A) standard metabolic rate (SMR) and (B) extracellular pH values after 10 wk of exposure (n = 9; N = 36). Different lowercase letters above bars represent significant differences among treatments ($p \leq 0.05$) determined by post hoc Student-Newman-Keuls tests

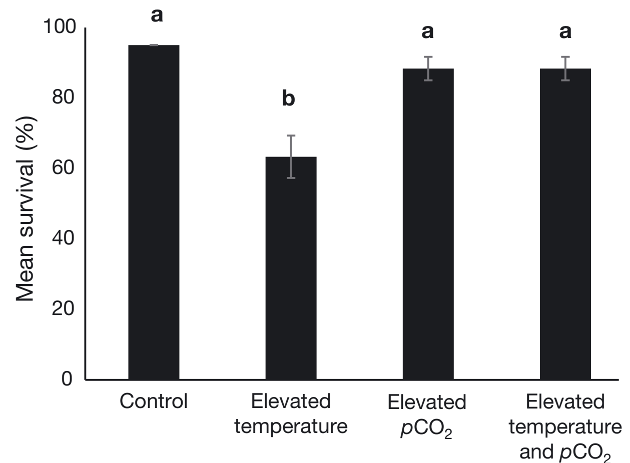

Fig. 6. Mean (±SE) survival rates of *Ostrea angasi* oysters in experimental treatments. Diffrent lower-case letters above bars represent significant differences among treatments ($p \leq 0.05$) determined by post hoc Student-Newman-Keuls tests

3.4. Survival

Oyster survival was significantly lower in the elevated temperature treatment (63% ± 6.0, n = 3; $F_{1,8}$ = 17.19, p = 0.0032) than in the elevated pCO_2 treatment (88 ± 3.33%, n = 3), the combined elevated temperature and pCO_2 treatment (88 ± 3.33%, n = 3) and the control treatment (95%, n = 3) (Fig. 6, Table 3).

4. DISCUSSION

The elevated temperature treatment had the greatest impact on *Ostrea angasi*, resulting in a lower C_i and survival. When elevated pCO_2 and temperature were combined, however, there was an antagonistic effect, with elevated pCO_2 acting to ameliorate the negative effect of temperature on survival.

Oysters exposed to elevated pCO_2 had greater SMR and lower extracellular pH compared to those in ambient conditions. Furthermore, there was an antagonistic effect on SMR. When elevated temperature and CO_2 were combined, this ameliorated the effects of elevated pCO_2 on SMR.

4.1. Responses of *O. angasi* to elevated pCO_2 and temperature

Molluscs must function inside a thermal window with critical lower and upper limits of temperature that, if surpassed, can be lethal (Pörtner 2008, Pörtner & Farrell 2008). Ivanina et al. (2013) argued that long-term exposure to elevated temperature can be deleterious for some oyster populations and cause significant mortality because they are unable to store energy (as glycogen) in their tissues. High mortality of *O. angasi* in the elevated temperature treatment used in this study indicates that oysters may have been placed under stress and that their thermal limits were exceeded. The lower C_i observed in the elevated temperature treatment indicates that the oysters may have experienced an increased energy demand (Lucas & Beninger 1985). To address this energetic demand, a trade-off between investment in somatic growth and protective responses against temperature may have occurred and resulted in the lower C_i. C_i is an important and simple parameter for assessing the growth and status of individuals in both natural populations and restoration efforts. It is commonly used to measure performance in restoration management (Baggett et al. 2014, Lipcius & Burke 2018).

Elevated pCO_2 is reported to have variable impacts on the SMR of bivalves, with some studies reporting

an increase (Beniash et al. 2010, Lannig et al. 2010, Parker et al. 2015), decrease (Beniash et al. 2010, Lannig et al. 2010, Parker et al. 2015) or no effect (Scanes et al. 2014). The increase in SMR of *O. angasi* in response to elevated pCO_2 in this study is in accordance with findings for *Saccostrea glomerata* and *Crassostrea virginica* (Beniash et al. 2010, Parker et al. 2015). Parker et al. (2011, 2015) found that the SMR of wild and selectively bred *S. glomerata* increased after a 5 wk exposure to elevated pCO_2. Beniash et al. (2010) found a similar increase for juvenile *C. virginica* after 20 wk. An increase in SMR may occur as oysters attempt to allocate more energy to maintain homeostasis and protect body fluids from acidosis (Melzner et al. 2009), or as they allocate more energy towards protein production (Pörtner 2008), including protective chaperone proteins. This increase in SMR may facilitate survival of *O. angasi* under elevated pCO_2.

Elevated pCO_2 also caused a decrease in the pH_e of *O. angasi* in our study, which has also been reported in other studies (e.g. Lannig et al. 2010, Parker et al. 2015). Marine organisms use mechanisms such as ion exchange to buffer high levels of environmental pCO_2 (Pörtner 2008). Oysters in general are not as effective at buffering compared to other more active molluscs such as cephalopods (Melzner et al. 2009). Disturbances in the acid–base balance are also known to place greater energy demands on oysters as homeostatic processes become more energy intensive (Pörtner et al. 2004). This process may have caused the greater SMR in the *O. angasi* exposed to elevated pCO_2. Periods of lower pH_e can cause a significant reduction in protein synthesis (Kwast & Hand 1996, Reid et al. 1997), which can ultimately lead to decreased somatic growth in oysters (Michaelidis et al. 2005). There was a non-significant trend for elevated temperature and CO_2 combined to ameliorate some effects of pCO_2 on pH_e. This trend may be due to the effects of temperature buffering extracellular pH (Pörtner et al. 2004); however, because this was a trend, the result should be treated cautiously. Here, we observed that *O. angasi* C_i was affected by temperature alone, but not elevated pCO_2.

The lack of a decrease in shell growth of *O. angasi* with elevated pCO_2 contrasts with other studies where ocean acidification has been found to have a negative effect on calcification and growth of oysters (Gazeau et al. 2013, Parker et al. 2013). Previous work on *O. angasi* indicates this species may be more tolerant to elevated pCO_2 than other oysters (Cole et al. 2016). During development in the gill brood space, *O. angasi* larvae experience pH as low as 7.46; considerably lower than that predicted for climate change scenarios (Cole et al. 2016). This pre-exposure to low pH as larvae may provide offspring (and perhaps adults) with some acclimatization to elevated pCO_2, and thereby resilience (Cole et al. 2016). As our experiment was longer than most other studies examining the effects of ocean acidification on molluscs (longer than 85% of studies identified in a review by Parker et al. 2013), there may have been acclimation of flat oysters to experimental conditions. Low pH environments are known to select for tolerant bivalves (Thomsen et al. 2017). While most studies report a negative effect of elevated pCO_2 on shell growth, a trend for greater shell growth observed here for *O. angasi* may be linked to increased SMR; where the increased costs of shell growth were met by greater energy investment. While our result for shell growth was not significant, we suggest that extra energy investment may have negative consequences for other processes, e.g. investment in gametes.

Our multi-stressor pCO_2 and temperature study with *O. angasi* in outdoor mesocosms was designed to evaluate the combined effect of these stressors on the physiology of this important native oyster. Meta-analyses indicate that synergistic interactions are most common when these stressors are combined (Harvey et al. 2013, Przeslawski et al. 2015), resulting in greater negative consequences for marine species (Parker et al. 2009, Lannig et al. 2010, Matoo et al. 2013, Ivanina et al. 2013, Kroeker et al. 2017). In contrast, we found an antagonistic effect for *O. angasi*, with pCO_2 ameliorating the negative effects of temperature on mortality, and elevated temperature lowering metabolic demands of oysters exposed to elevated pCO_2. Few studies have investigated the capacity of brooding oysters, including *O. angasi* (Cole et al. 2016) and *O. edulis*, to withstand climate change. Studies performing a direct comparison of this genus with broadcast spawning genera e.g. *Crassostrea, Magallana* and *Saccostrea* may be beneficial.

The reasons for the antagonistic effects observed in this study are unclear. Unlike many previous studies, we used unfiltered seawater in mesocosms that closely mimicked the natural conditions that the oysters would experience with respect to photoperiod, diurnal temperature flux and phytoplanktonic food. The elevated pCO_2 condition may have affected the phytoplankton communities (i.e. food) within the mesocosms. Changes in food availability could have affected the response of oysters. Studies that include environmental variability (e.g. diurnal and seasonal

changes in temperature) are important (Andersson et al. 2015) as this can alter the physiological responses of marine organisms.

4.2. Implications for restoration under climate change

Ostrea angasi populations have declined in Sydney Harbour and throughout south-eastern Australia (Ogburn et al. 2007, Alleway & Connell 2015). In this region, *O. angasi* are living on the edge of their thermal limits, and this habitat is predicted to rapidly warm in the future, with an increased risk of marine heatwaves. In certain localities, the sea water chemistry is also being influenced by environmental acidification from climate-driven sea water uptake of CO_2, precipitation events and sea level rise (Fitzer et al. 2018). These are key concerns for the restoration of *O. angasi* reefs and are likely to hamper the recovery of this species on the Australian coast.

Ocean warming 'hot spots' are a global phenomenon, therefore managers need to be aware of the future habitat in areas where reefs are to be restored, including the local chemistry and pollutants. Previous work has shown that common pollutants in Sydney Harbour can interact with elevated CO_2 to affect oysters, reducing their capacity to reproduce (Scanes et al. 2018). The carbonate chemistry and local pH of seawater also needs to be considered. The carbonate chemistry and local pH of seawater also needs to be considered. In Wallis Lake, NSW, current coastal acidification (from acid soil drainage) affects the shell biomineralization of *S. glomerata* and would likely hamper any efforts to restore oyster reefs (Fitzer et al. 2018). We have shown that adult *O. angasi* have a capacity to withstand low pH. However, *O. angasi* is vulnerable to warming, a factor that needs to be considered.

Climate change has the potential to exacerbate local processes with consequences for the long-term sustainability and survival of restored oyster reefs. More studies on the influence of other factors (such as salinity interacting with warming and elevated pCO_2) on the physiology of *O. angasi* are needed to 'climate-proof' future reef restoration programs in Australia. Our results suggest that *O. angasi* will be vulnerable to warming, but that these effects may be ameliorated by elevated pCO_2. These findings reinforce that managers need to consider the current and future habitat and inherent resilience of species when considering the best species and locations for restoration of oyster reefs. The warming of the southeast Australian coast is likely to threaten efforts to restore *O. angasi*.

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