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Intraspecific differences in the transcriptional stress response of two populations of Sydney rock oyster increase with rising temperatures

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ABSTRACT: The vulnerability of sessile organisms to warming temperatures may depend on their capacity to adaptively alter their expression of genes associated with stress mitigation. We compared the transcriptional profile of 2 populations of Sydney rock oysters *Saccostrea glomerata* (one that had been selectively bred over 7 generations for fast growth and disease resistance and one wild type that had not been subjected to selection) following exposure to an artificial temperature gradient in the field. Oysters were attached to white, grey or black stone pavers that experienced mean maximum substrate temperatures of approximately 34, 37 and 40°C, respectively, at low tide. Across all pavers, selectively bred oysters suffered 12% higher mortality than wild-type oysters, although this difference was not significant. Expression profiles did not differ between oyster populations on the coolest (white) pavers. However, divergent transcriptional profiles of genes associated with the key intracellular stress mechanisms of antioxidant defence, heat shock response, energy metabolism and the cytoskeleton were detected in oysters on the hottest (black) pavers. Expression of these genes was upregulated at high temperatures by the selectively bred oysters but displayed little change, or was suppressed at high temperature among the non-selected wild-type oysters. One potential explanation is that the selectively bred oysters have traded off rapid growth for a lower thermal maximum. Complementary physiological and ecological studies are needed to confirm this hypothesis.

KEY WORDS: Climate adaptation · Global warming · Molecular mechanisms · *Saccostrea glomerata* · Selective breeding · Thermal stress

INTRODUCTION

Rising temperature is the greatest challenge climate change poses to biodiversity, as temperature affects all levels of biological function (Rosenzweig et al. 2008, Tomanek 2014). For all organisms, cellular function is constrained to a limited range of body temperatures, with functionality compromised at the extremes of this range (Pörtner & Farrell 2008). Ectothermic organisms are particularly vulnerable to climate warming as their body temperatures typically conform to the surrounding ambient temperature (Huey et al. 2012). Hence, to stay within the temperature range across which they can function, ectotherms must either migrate with the shifting climate (Parmesan & Yohe 2003), behaviourally thermoregulate (Ng et al. 2017) or adapt their physiology (Somero 2010). For species limited in their capacity for behavioural or migratory response, the ability to adaptively alter their gene expression to rising temperatures may determine their vulnerability to climate change (Somero 2005).

Warmer ocean waters have already altered the phenology and metabolic function of marine species

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(Edwards & Richardson 2004, Pörtner & Knust 2007), with only modest amounts of ocean warming (~2°C) expected to breach the physiological limits of thermally sensitive ectotherms (Pörtner et al. 2007). As the early embryonic and larval stages of organisms are generally more vulnerable to changes in temperature than juveniles and adults (Pankhurst & Munday 2011), research on marine invertebrates has focused on the sensitivity of these early life history stages to warming (Parker et al. 2009, Sheppard Brennand et al. 2010). Nevertheless, invertebrates recruiting to intertidal rocky shores must not only tolerate warming air and water temperatures, but also increasing radiant heat from the substrate to which they are attached (Somero 2002, Helmuth et al. 2006). This may result in diurnal fluctuations in the body temperatures of juveniles and adults by as much as 10–20°C (see Ivanina et al. 2009). Many intertidal species already live close to their thermal maxima, and for these species, increases of just 1–2°C above present-day maximum temperatures may lead to osmotic and oxidative stress, culminating in heart failure (Stillman 2003). For organisms that cannot exploit spatial refuges, metabolic depression and intracellular stress responses that maintain cellular homeostasis and prevent macromolecular damage are key mechanisms for providing periodic tolerance to extreme temperatures (Pörtner & Farrell 2008, Evans & Hofmann 2012). Adaptation to changing environmental conditions is dependent on mutation rates and adequate genetic variation within the population on which selection can act, as well as the absence of deleterious mutations or trade-offs with other essential functions (Stearns 1989, Barrett & Schluter 2008). An understanding of how temperature-adapted gene expression determines an organism's stress resilience is important in identifying which species are most vulnerable to temperature increases and, within species, which populations might display the greatest capacity to adapt to a changing environment (Crain & Bertness 2006, Keppel & Wardell-Johnson 2012).

Many marine invertebrates exhibit a generic intracellular stress response to cope with exposure to temperature fluctuations, as well as to other stressors, which is characterised by the production of molecular chaperones, upregulation of antioxidant defence and cytoskeletal activity, and altered energy metabolism (Feder & Hofmann 1999, Tomanek 2014, Anderson et al. 2015, Groh & Suter 2015). Increased expression of heat shock proteins, which prevent damage to proteins by providing structural stabilisation, is the most documented thermal stress response in marine invertebrates (Iwama et al. 1998, Fabbri et al. 2008, Tomanek 2014), and may be critical in determining an organism's thermal plasticity (Shamseldin et al. 1997, Hamdoun et al. 2003, Fangue et al. 2006). Cellular homeostasis is further maintained via increased expression of antioxidant enzymes (i.e. catalase, peroxidase and superoxide dismutase) that detoxify damaging reactive oxygen species (ROS) (Heise et al. 2006, Jo et al. 2008, Zhang et al. 2016). As body temperatures approach their critical limits, dysfunction of the antioxidant enzymatic system can occur, potentially setting an organism's maximum temperature tolerance (Lang et al. 2009, Tomanek & Zuzow 2010). However, in contrast to metabolic maintenance of cellular homeostasis, some invertebrates may instead suppress their metabolic activity to minimise tissue energy demand and the mitochondrial production of damaging ROS (Storey & Storey 2004, Tomanek 2014).

The Sydney rock oyster *Saccostrea glomerata* (Gould 1850) is intensively farmed on the east Australian coastline (NSW DPI 2014), where it is also an important intertidal ecosystem engineer that supports biodiverse communities on rocky shorelines and in mangrove forests (McAfee et al. 2016). Laboratory studies have detected large intraspecific differences in the sub-cellular responses and individual fitness of *S. glomerata* when exposed to elevated temperatures and/or pCO_2 (Parker et al. 2011, 2012, Thompson et al. 2015, Goncalves et al. 2016). The larvae of *S. glomerata* selectively bred over 7 generations for fast growth and resistance to Queensland Unknown (QX) disease displayed greater survivorship and growth than wild-type oysters when exposed to elevated pCO_2 and/or temperature (Parker et al. 2012). Adults of the same generation of selectively bred oysters displayed differential expression of genes associated with antioxidant defence and energy metabolism (Goncalves et al. 2016) when exposed to elevated pCO_2. However, it remains unclear to what extent these differences represent generic responses, also evident following exposure of oysters to other stressors and that are applicable to field scenarios in which organisms may be simultaneously exposed to multiple stressors.

Here, we assessed transcriptional differences between selectively bred (for fast growth and disease resistance) and non-selected *S. glomerata* when exposed to an artificial temperature gradient in the field. We predicted that differences in the transcriptional profile of these 2 populations will increase with maximum temperatures, as will differences in mortality. Understanding the sub-cellular response of

oyster populations to rising temperatures will assist breeding programmes aiming to enhance the climate resilience of commercial aquaculture species, and may help identify populations with high environmental resilience that could benefit restoration projects targeting climate adaptation.

MATERIALS AND METHODS

Oysters were supplied by the New South Wales Department of Primary Industries (NSW DPI) and were 8.5 mo old (spawned mid-December 2014). Selectively bred oysters were from the B2 breeding line (mean shell length: 37.4 ± 4.5 [SD] mm; range: 30.7–45.5 mm), mass selected for fast growth and resistance to QX disease over 7 generations. Wild-type oysters (mean shell length: 34.9 ± 5.3 mm; range 26.7–43.7 mm) were hatchery spawned from wild oysters collected from Cromarty Bay. Both *Saccostrea glomerata* populations had overwintered on NSW DPI intertidal oyster farms in Cromarty Bay and were handled identically.

To test hypotheses about the transcriptomic response of these 2 populations of *S. glomerata* to different thermal regimes, a manipulative field experiment was conducted for 2 mo during the Austral spring (September and October) of 2015 at Cromarty Bay in Port Stephens estuary, New South Wales (NSW), Australia (32.71° S, 152.19° E). Port Stephens is a wave-dominated estuary, with semi-diurnal tides of ~1.5 m and mean salinities above 29.7 (Wolf & Collins 1979). Between September and October, sea surface temperatures range from 17.9–21.2°C (World Sea Temperatures 2017, Port Stephens: https://www.seatemperature.org/australia-pacific/australia/port-stephens.htm), and air temperature extremes range from 12.2–34.5°C (Bureau of Meteorology 2017).

Thermal gradient experiment

To expose oysters to different substrate temperatures, we produced an artificial thermal gradient by attaching oysters to white, grey or black stone pavers (300 × 300 × 17 mm; Fig. 1). When air-exposed in full sunlight for 30 min on a 27°C day, these white, grey and black pavers reached temperatures of 26, 30 and 39°C, respectively, when oysters were absent (infrared camera, Testo i80). Temperatures on white pavers were similar to those recorded from the neighbouring rocky shore (McAfee et al. 2017), while all temperatures were within the natural range that *S. glomerata*

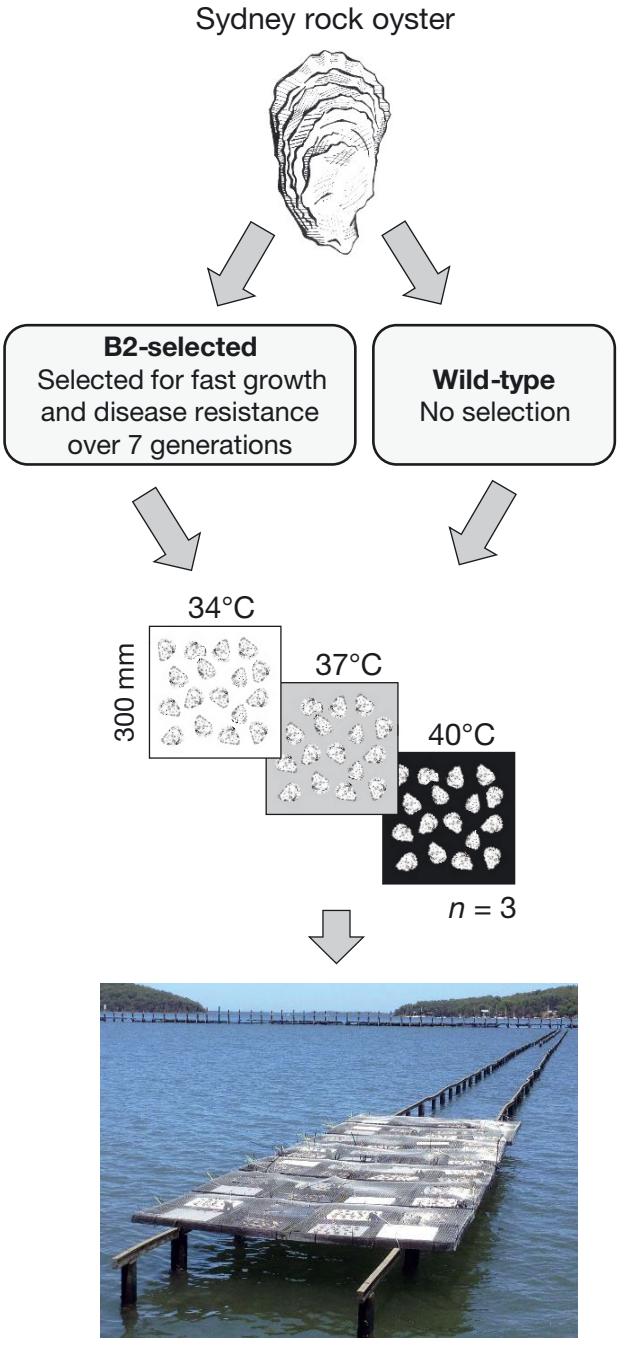

Fig. 1. Origin of the 2 hatchery-spawned Sydney rock oyster populations, and the experimental design separately exposing them to a passively warmed temperature gradient during aerial exposure. The average maximum temperature oysters experienced on each paver is provided above each treatment colour. During high tide, the oysters were covered in water

experiences during summer low tides (McAfee et al. 2016). We painted each paver with 2 coats of white, grey or black low-sheen paint (Dulux Weathershield). A homogenous surface chemistry among colours was

achieved by applying 3 coats of clear, non-toxic pond sealer (Crommelin Waterproofing) per paver.

We used 6 pavers of each colour treatment, 3 of which were randomly assigned to receive oysters of the wild-type and 3 received the B2 population. Each paver received 17 oysters of the assigned type, within the range of natural oyster densities observed on neighbouring rocky shorelines (Wilkie et al. 2012). Oysters were attached using non-toxic 2-part marine epoxy glue (Vivacity Engineering). Substrate temperatures on each paver were monitored using temperature dataloggers (DS1921G; Thermodata) programmed to record temperature every 20 min. Dataloggers were waterproofed using Plastidip rubber coating (Performix Brand: McAfee et al. 2016), and centrally positioned on each paver among the oysters. Pavers were deployed in Cromarty Bay on plastic commercial oyster trays (180 × 90 cm) that were secured to horizontal aquaculture racks at a height of mean low water of neap tides (Fig. 1). This ensured that all pavers were exposed to similar environmental conditions. Within each oyster tray, paver treatments were randomly arranged, and each tray was covered with a 12 × 12 mm wire mesh that excluded predators while minimising the shading of pavers. Every 2 wk, mud was gently washed from the pavers to maintain the colour treatments. After 2 mo, the pavers were returned to shore, and the number of oysters surviving was assessed by counting the gaping or missing valves.

RNA extraction and cDNA synthesis

Three surviving oysters from each of 3 replicate pavers (9 oysters treatment^{-1}) were randomly selected for gene expression analysis. Oysters were shucked and gill tissue (~2 × 2 mm), which is routinely used for transcriptomic analyses in *S. glomerata* because it is a high-quality source of RNA (Goncalves et al. 2016), was separately collected from each oyster and stored in 1 ml of RNA*later* (Sigma-Aldrich) at −20°C. All tissue samples were collected within 1 h of pavers emerging on the ebbing tide at the field site. Total RNA was isolated from approximately 100 mg of gill tissue, which was homogenised in 1 ml of Tri-reagent (Sigma-Aldrich) followed by phase separation with 1-bromo-3-chloropropane (Sigma-Aldrich). The upper clear phase was extracted and total RNA purified using an Illustra RNAspin Mini Isolation Kit (GE Healthcare Lifesciences) following the manufacturer's protocol (steps 2–8). We assessed the concentration and quality of each RNA sample using a NanoDrop spectrophotometer (Thermo Scientific NanoDrop 2000). Reverse transcription was performed for each oyster on 1 µg of total RNA using ImProm-II Reverse Transcription System (Promega) in 20 µl reactions with 0.5 µg Oligo(dT)$_{15}$, following the manufacturer's protocol.

qPCR and data analysis

Transcriptional responses of oysters to different thermal regimes were investigated using 12 genes associated with antioxidant defence, cellular stress, energy metabolism and the cytoskeleton (Table 1). These genes were chosen because their transcription is responsive to environmental stress (Zhang et al. 2012, Anderson et al. 2015) and varies between B2-selected and wild-type *S. glomerata* (Thompson et al. 2015, Goncalves et al. 2016).

qPCR assays were run on a LightCycler® 480 II (Roche). Each reaction (3 µl) consisted of 1.5 µl of KAPA SYBR® FAST qPCR Master Mix (Kapa Biosystems), 0.09 µl of forward and reverse primer, 0.32 µl PCR-grade water (ThermoFisher) and 1 µl of cDNA template (diluted 1:10). qPCR reactions (3 µl) were run in triplicate for each primer pair with the cDNA from individual oysters. qPCR cycling conditions consisted of 3 min at 95°C, followed by 45 cycles at 95°C for 10 s, 60°C for 20 s and 72°C for 30 s. At the end of the cycle, melting curve analysis was performed to collect fluorescence data between 65 and 95°C at 0.5°C increments. LightCycler® 480 II software analysed the amplification data to produce Cq values, with values averaged among the triplicate curves to provide a single value for each oyster, per gene.

Across all treatments, the most stable gene combination among our 4 reference and 12 target genes (Table 1), as determined by RefFinder (Xie et al. 2012), included 4 genes: β-actin, elongation factor 1 alpha, TATA-binding protein and ATP synthase alpha-subunit (combined geometric mean variance of 0.43 across all treatments). Changes in the relative expression of each target gene were calculated using the Livak & Schmittgen (2001) method, which normalises the Cq values of each gene relative to the combined geometric mean of the 4 reference genes.

To test for differences in the temperature of pavers and in the survivorship and the transcriptional response of oysters between populations and paver colours, 2-way permutational analyses of variance (PERMANOVAs; Anderson et al. 2008) were run. PERMANOVAs apply the traditional ANOVA parti-

Table 1. Genes and their primer sequences used for qPCR; Fw: forward, Rv: reverse

Functional group	Gene (abbreviation)	Sequence (Fw/Rv: 5′→ 3′)
Antioxidant defence	Catalase (Cat)	CGCTGACGTGGAGCAGATTG GGCGATGGGTGTCCGAATAA
	Superoxide dismutase (SOD)	AACTCTACCACGGCGAGCAT CCACGGTCGTCATCATGAAG
	Peroxiredoxin 6 (Perox)	GAAGGATGGAAGGACGGTGAT CACCTGTGGAAACACCTTCTC
	Glutathione peroxidase (Glut perox)	TGGTGGCCGAACTGGTTACA TCAGTACCACCAACTGAATGCA
	Glutathione S-transferase omega (Glut S-trans)	CGCTGGAGAAGGACGGAAAG TCCCGAGCTTGTTGGTATGG
Heat stress response	Heat shock protein70 (HSP70)	TGAATGGACACTCCTGGTTGG TGGGCATTGAAACTGCTGGA
	Heat shock protein 90 (HSP90)	CCCAGAGGATGAGGAGGAGA CAATACAGCAGGGCGATGTC
Energy metabolism	Cytochrome c oxidase I (COX1)	TTTCCTACCACGGGATGTG TGAGCTAATACCAGCCAAGTGA
	NADH dehydrogenase (NADH)	TCCTCCGGTACCCCAGTCAG TGCATCAAGGGGCTATTCCA
	ATP synthase alpha-subunit (used as reference)	CCTCCACTCTCGTCTGTTGG GAGATGACGTTGGTTGGGATG
Cytoskeleton	β-tubulin (TUB)	GCCATGACGAGGATCACAGG TGTCCCAGAACTCACACAGCAG
Protein folding	Peptidylprolyl isomerase A (PPIA)	CGGAGAAGACCACTTGGCTAGA ATCCATGCCCTCGACGACT
Reference genes	Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	ACCGCGCCAGTCTTTGTTG GGCATTGTTGAGGGTCTGATG
	β-actin	GCACCTGAATCGCTCGTTG CAGCAGCATCGTCATCATCC
	Elongation factor 1 alpha	CCATAGCGGCATCTCCACTC CCTTGATTGCCACACTGCTC
	TATA-binding protein	GGACTTTGGCTCCTGTAAGCAC AGAATGGTGAAGCCTCGTATTG

tioning procedure to a distance matrix, but use permutations to obtain p-values (Anderson et al. 2008). Consequently, unlike ANOVAs, PERMANOVAs do not have explicit assumptions about the underlying distributions of data and can use any distance matrix that is appropriate to the data. A multivariate analysis was run on a Bray Curtis dissimilarity matrix calculated using the relative expression of all target genes. Multivariate differences in the expression of target genes between oyster populations and paver colours were visualised using non-metric multidimensional scaling (nMDS). Separate univariate analyses were run on Euclidean distance matrices calculated from each of maximum temperature, oyster survivorship and the individual target genes (Table 1). Analyses of relative gene expression used as replicates the averages calculated from the 3 oysters sampled per paver. Univariate analyses detecting significant differences were followed by pairwise post hoc PERMANOVAs to detect the source of variation. Differences in gene expression between populations were further visualised with a heat map (MeV 4.9).

RESULTS

Temperature and oyster survivorship in the field

The hottest temperature, 43°C, was observed on a black paver, with black pavers, on average, display-

ing maximum temperatures that were significantly hotter, by 3.8°C, than grey pavers and by 7.3°C than white pavers (PERMANOVA; Table A1 in the Appendix; Fig. 2a). There was no difference in paver temperature between those with B2-selected and wild-type oysters (Table A1, Fig. 2a).

Overall, 50% of the wild-type and 38% of the B2-selected oysters survived the 2 mo experiment. Nevertheless, there was no significant effect of oyster population on survivorship on any of the 3 paver colours (Table A1, Fig. 2b). Instead, survivorship differed with the main effect of colour treatment, with significantly more oysters on white pavers surviving than on grey or black pavers, which did not differ (PERMANOVA; Table A1, Fig. 2b).

Transcriptional profiles of all target genes

Multivariate differences in the transcriptional expression profiles of wild-type and B2-selected oysters depended on the colour treatment (PERMANOVA, significant Population × Colour interaction; Table A2). Expression profiles significantly differed between oyster populations on the hotter (black) pavers, where B2-selected oysters had greater expression than wild-type oysters for 10 of the 12 genes (post hoc test, Fig. 3). In contrast, the oyster populations did not differ significantly in gene expression on grey or white pavers (Fig. 3). Within populations, expression profiles for both the wild-type and the B2-selected oysters differed significantly between grey and black pavers, while oysters on white pavers did not differ from those on grey or black pavers (Fig. 3a).

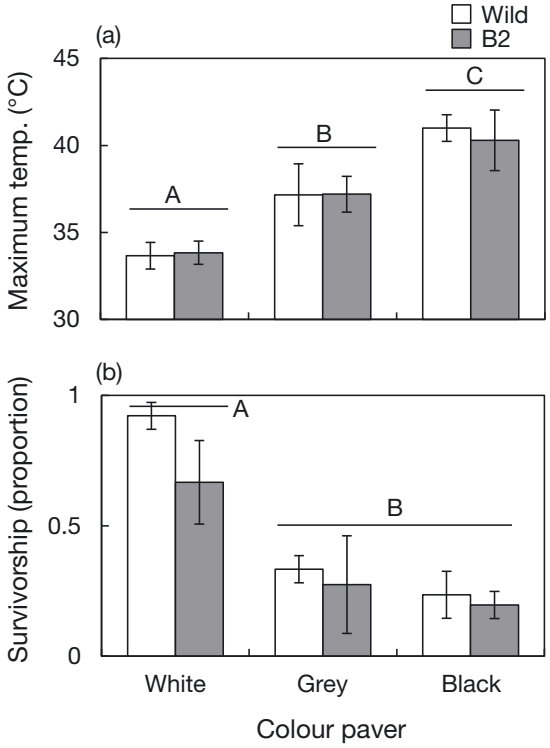

Fig. 2. Mean (±SE) (a) maximum temperature and (b) oyster survivorship recorded from white, grey and black stone pavers with wild-type (Wild; white bars) and B2-selected (B2; black bars) Sydney rock oysters attached. Significant differences (at $\alpha = 0.05$) between paver colours are denoted with letters above bars. n = 3 replicate pavers per colour treatment

Antioxidant defence genes

Separate analyses of the individual genes associated with antioxidant defence detected significant interactions between populations and colour treatments for catalase and peroxiredoxin 6, with no interaction detected for the other 3 genes (PERMANOVAs, significant Population × Colour interaction, Table A3, Fig. 4). For catalase, *a posteriori* tests revealed no difference between populations on any colour treatment, or among colour treatments for B2-selected oysters. However, for wild-type oysters, catalase expression was significantly greater on white (by 1.8-fold) and black (by 1.9-fold) than on grey pavers (post hoc test; Fig. 4a). *A posteriori* tests for peroxiredoxin 6 expression showed no difference between populations on pavers of any colour. The only significant difference occurred within the B2-selected oysters, where oysters on black pavers had greater expression (by 1.7-fold) than those on grey pavers (post hoc test; Fig. 4b). For superoxide dismutase (SOD), gene expression differed with the main effects of population, and among colours (PERMANOVA; Table A3, Fig. 4c). Between populations, SOD expression was greater in B2-selected oysters (by 1.3-fold) than in wild-types, while among colours, SOD expression was greater on black pavers than on white or grey pavers (by 1.5-fold for both), while white and grey did not differ (post hoc test; Fig. 4c). Glutathione S-transferase omega expression differed with the main effect of colour treatment, with significantly greater expression on white (by 1.4-fold) than on grey pavers, and greater on black (by 1.3-fold) than on grey pavers, with no difference between white and black pavers (PERMANOVA; Table A3). By contrast, no significant difference was detected in the expression of glutathione peroxidase (Table A3).

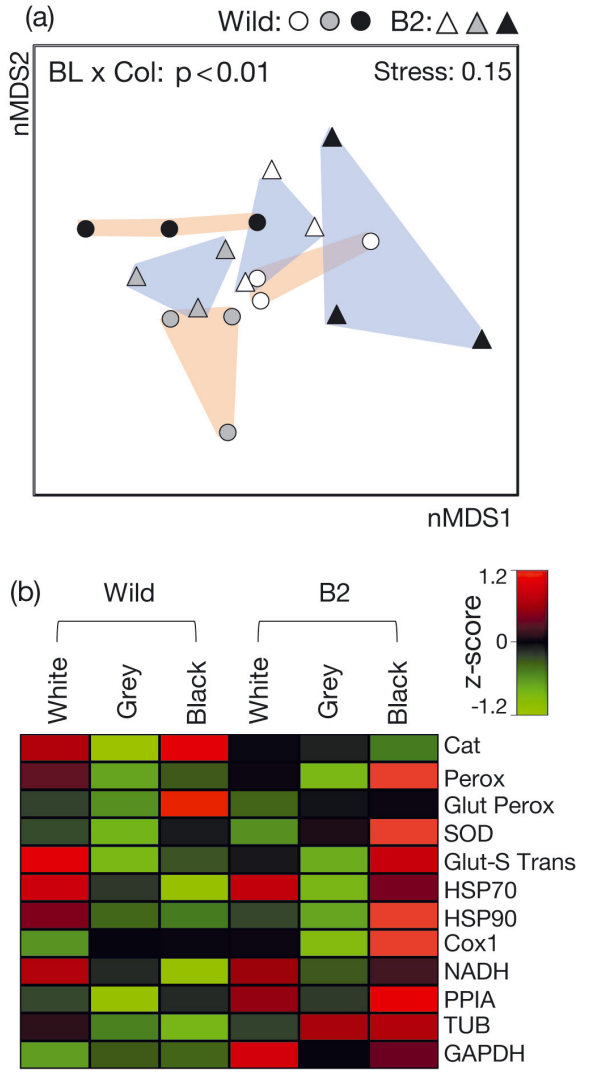

Fig. 3. Differences in the expression profiles for all target genes (a) between wild-type (circles and pink distribution markers) and B2-selected (triangles and blue distribution markers) Sydney rock oysters on white, grey and black colour treatments (depicted by the fill colour of data point symbols). Each point represents the mean expression of 3 oysters from 1 replicate paver. (b) Heat map of the mean expression of target genes (*z*-transformed). Scale reflects the *z*-score of individual gene expression: red indicates up-regulated expression; green indicates suppressed expression. Abbreviated gene names and their functional category provided in Table 1

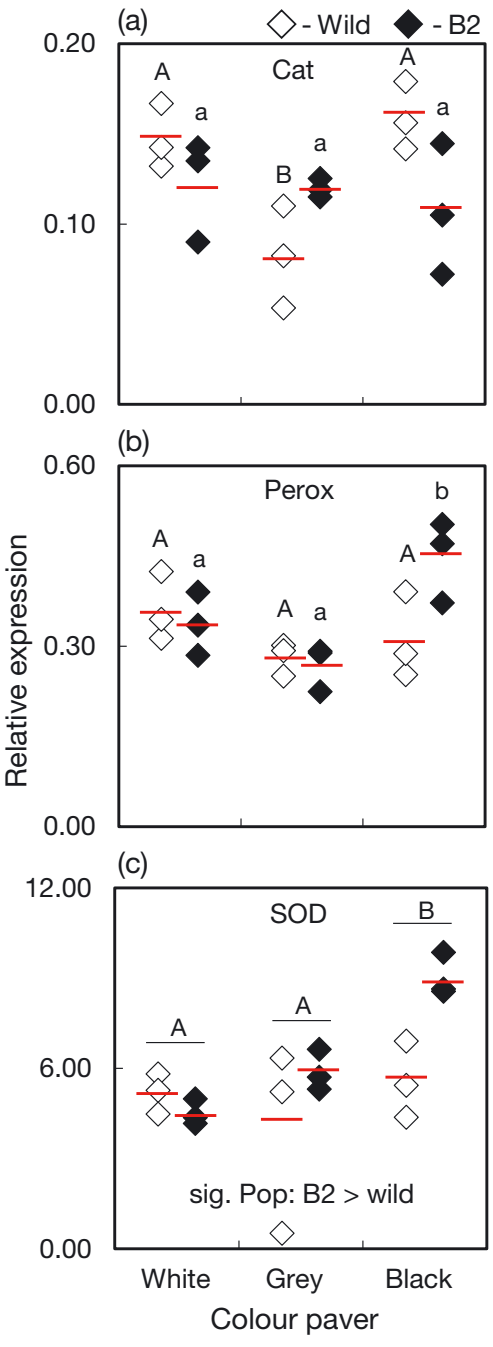

Fig. 4. Differences in the expression of 3 (catalase [cat], peroxiredoxin 6 [perox], superoxide dismutase [SOD]) of the 5 genes associated with antioxidant defence by wild-type (white diamonds; wild) and B2-selected (black diamonds; B2) oysters on white, grey and black pavers. Each point represents the mean expression of 3 oysters from 1 replicate paver. Horizontal red bars represent the treatment mean. For (a) cat and (b) perox 6, significant differences (at α = 0.05) within populations are denoted above treatments with upper-case letters for wild-type and lower-case letters for B2-selected oysters. For (c) SOD, significant differences (at α = 0.05) for the main effects of colour treatment are denoted with capital letters, and described for the main effects of population (Pop)

Heat stress response

Differences in the expression of HSP70 and HSP90 between populations were dependent on paver colour (PERMANOVAs, significant Population × Colour interaction, Table A4, Fig. 5). On black pavers, B2-selected oysters expressed significantly more HSP70 than wild-type oysters (by 1.5-fold;

Fig. 5a). However, on white and grey pavers, there was no difference in expression of HSP70 between the populations. Wild-type oysters displayed significantly greater expression of HSP70 on white than on black pavers (by 1.7-fold; Fig. 5a), with grey pavers not differing from either. B2-selected oysters expressed more HSP70 on white pavers than on grey pavers (by 1.5-fold; Fig. 5a), with black not differing from either. Expression of HSP90 did not differ between populations on pavers of any colour (Fig. 5b) and, for wild-type oysters, did not differ among paver colours (Fig. 5b). By contrast, HSP90 expression in B2-selected oysters was 1.4-fold greater on black than on grey pavers (Fig. 5b), with no other differences among treatments.

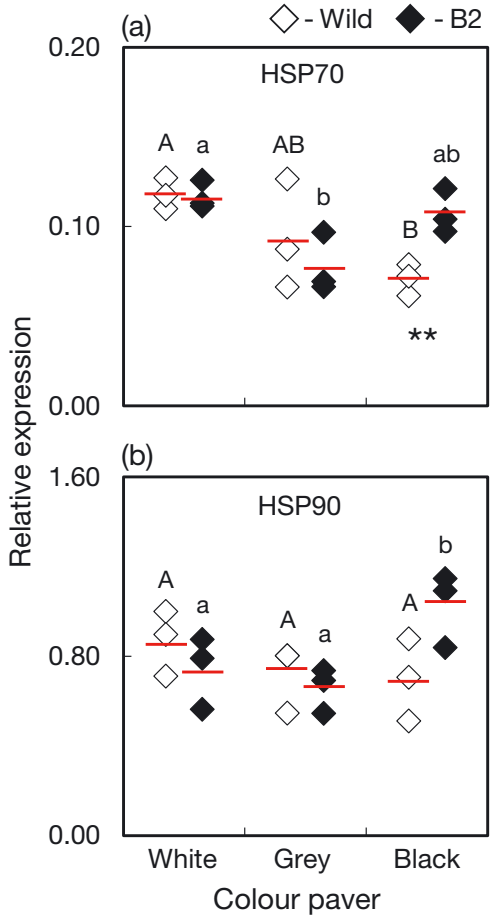

Fig. 5. Differences in the expression of heat-shock proteins (a) HSP70 and (b) HSP90 by wild-type (white diamonds) and B2-selected (black diamonds) oysters on white, grey and black colour treatments. Each point represents the mean expression of 3 oysters from 1 replicate paver. Horizontal red bars represent the treatment means. Significant post hoc differences (at $\alpha = 0.05$) within populations are denoted above treatments with upper-case letters for wild-type and lower-case letters for B2-selected oysters, and between populations are marked with asterisks (**) below the colour treatment

Energy metabolism

Expression of the genes associated with energy metabolism, cytochrome c oxidase 1 (COX1) and NADH dehydrogenase (NADH), did not display any significant difference between oyster populations, on pavers of any colour (Table A5). Instead, COX1 gene expression varied with the main effect of colour, with the expression of oysters significantly higher on black pavers than on grey (by 1.3-fold) or white (by 1.2-fold) pavers, while white and grey pavers did not differ (PERMANOVA; Table A5; Fig. 6a). In contrast, no significant differences among paver colours were detected for NADH expression (Fig. 6b).

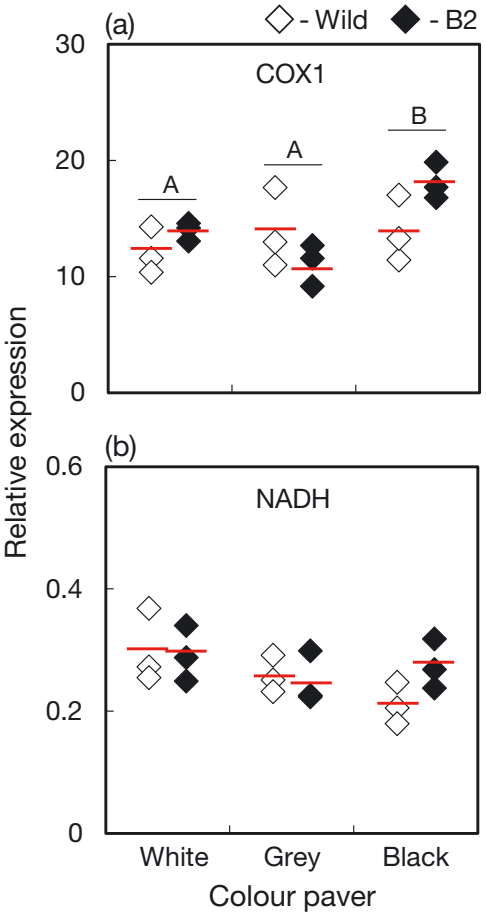

Fig. 6. Differences in the expression of the genes associated with energy metabolism, (a) cytochrome c oxidase I (COX1) and (b) NADH dehydrogenase (NADH), by wild-type (white diamonds) and B2-selected (black diamonds) oysters on white, grey and black pavers. Each point represents the mean expression of 3 oysters from 1 replicate paver. Horizontal red bars represent the treatment mean. For COX1, significant differences (at $\alpha = 0.05$) for the main effects of colour are denoted with letters above colour treatments. No significant differences were detected for NADH dehydrogenase

Expression of the gene associated with protein folding, peptidylproyl isomerase A (PPIA), did not show an interaction between population and colour (Table A6). Instead, PPIA expression differed with the main effect of population, with higher expression in B2-selected oysters (by 1.2-fold) than wild-type (PERMANOVA; Table A6). No significant differences were detected for the gene associated with the cytoskeleton (β-tubulin), or the anticipated reference gene, GAPDH (Table A6).

DISCUSSION

The capacity of marine ectotherms to adaptively shift their gene expression in response to rising temperature is key for their climate adaption, particularly for sessile intertidal organisms exposed to rising atmospheric, water and radiant temperatures (Somero 2010). Here, we found that intraspecific differences in the intracellular stress response between 2 populations of *Saccostrea glomerata* increased with maximum temperature. No difference in the expression profile of the 12 genes was detected between populations on the coolest (white) pavers, or on the grey pavers of intermediate temperature. However, on the hotter (black) pavers, expression profiles differed significantly between populations: B2-selected oysters displayed greater expression of genes associated with heat shock response, antioxidant defence and energy metabolism than on the cooler pavers, while wild-type oysters displayed similar or suppressed expression.

There are several potential explanations for the differential expression of stress response by the 2 oyster populations at high temperatures. On the one hand, upregulated expression may indicate that an organism is metabolically capable of mitigating extreme temperatures, while suppressed expression may indicate the compromised metabolism of a stressed organism (Feder & Hofmann 1999). For example, Zhang et al. (2012) attributed the ability of Pacific oysters to withstand extreme summer temperatures to their upregulation of HSP expression. On the other hand, upregulated expression may signify that an organism is experiencing temperatures approaching its thermal maxima, while suppressed expression may indicate that temperatures are not stressful enough to warrant metabolic thermoregulation (Fangue et al. 2006, Tomanek 2014). For example, *Collisella* limpets living at mid-intertidal elevations up-regulate HSP expression at lower temperatures than congenerics living in the upper intertidal that regularly experience maximum temperatures up to 5°C hotter (Sanders et al. 1991). Similarly, the highest temperatures at which intertidal *Tegula* sea snails synthesise HSPs closely relates to their upper temperature threshold (Tomanek & Somero 1999). Metabolic suppression is a common periodic stress response for intertidal ectotherms to avoid production of dangerous ROS, and may be followed by sudden metabolic upregulation if adverse conditions persist or strengthen (Hand & Hardewig 1996).

Here, 2 lines of evidence suggest that the second explanation – i.e. that the B2-selected oysters have a lower thermal maximum than the wild-type oysters — is the more likely cause of the differential stress response of the 2 oyster populations. (1) Although in the present study there was no difference in the survivorship of the 2 oyster populations across the thermal gradient, in a previous year-long study using the same 2 oyster populations, and in which much higher maximum temperatures of ~48°C were recorded on black pavers, we found much greater survivorship of the wild-type oysters (McAfee et al. 2017). This difference in survivorship between the 2 populations increased from white to grey to black pavers, so was interpreted as evidence for a greater thermal tolerance of the wild-type than the B2-selected oysters (McAfee et al. 2017). (2) The maximum temperature of 43°C recorded on the black pavers in the present study was just shy of the median lethal temperature (LT_{50}) of 45.4°C recorded for 2 yr old hatchery-reared *S. glomerata* exposed to elevated air temperatures for 6 h (Krassoi 2001). This suggests that the temperatures that the oysters experienced in this experiment are indeed approaching their thermal maximum. If the suppression of gene expression by the wild-type oysters was, alternatively, due to metabolic inhibition at high temperatures, greater mortality of the wild-type than B2-selected oysters would, to the contrary, be expected.

A lower thermal maximum of the B2-selected than the wild-type oysters would be consistent with the hypothesis of a trade-off between thermal tolerance and growth rate (Stearns 1989). Thermal tolerance comes at a high energetic cost to an organism (Pörtner et al. 2004), lowering the capacity for energetic investment in other fundamental metabolic activities (see Stearns 1989, Angeilletta et al. 2003). For example, increased resistance to stressful environmental temperatures is associated with a shorter life span and smaller body size among teleost fish (Martinez et al. 2016). Previous studies suggest that the faster rate of growth of the B2-selected than wild-type oysters is underpinned by a higher standard metabolic rate of the former (Parker et al. 2012), which may reduce

energy available for other stress responses. Additionally, contrary to the expectation that the mass-selection of the B2-selected oysters may reduce their genetic diversity (Zhong et al. 2016), and in turn reduce their capacity to adapt to environmental change (Barrett & Schluter 2008), B2-selected oysters have higher genetic diversity than wild *S. glomerata* populations (Thompson et al. 2017).

Other studies have also detected differential responses of the B2-selected and wild-type *S. glomerata* to other environmental stressors, but these have been variable in direction (e.g. Parker et al. 2011, Thompson et al. 2015, Goncalves et al. 2016). Larvae of the B2-selected oysters displayed greater fertilization success and decreased developmental abnormalities than wild-type oysters under ocean acidification scenarios (Parker et al. 2011). Among juveniles, similar to our findings, 50% of the genes upregulated by B2-selected oysters following exposure to high CO_2 conditions were downregulated in wild-type oysters (Goncalves et al. 2016). However, among adults (1.5–2 yr old), Thompson et al. (2015) found that the concentrations of proteins involved in antioxidant defence and energy metabolism were downregulated in B2-selected adults at elevated CO_2, and upregulated among wild-type adult oysters. The authors concluded that the higher standard metabolism emergent from the selection for fast growth and disease resistance (Parker et al. 2012) may enhance the developmental potential of B2-selected larvae at the cost of the adults' environmental resilience to additional stressors in natural settings (Thompson et al. 2015). However, in order for the physiological and ecological ramifications of these differences in sub-cellular stress responses to be interpreted, such transcriptional studies need to be coupled with measurements of key performance traits such as survivorship, growth and reproduction.

Studies investigating the transcriptional response of marine ectotherms to rising temperatures in the field are rare, and to our knowledge, this is the first study to investigate the sub-cellular stress response of oysters to an artificial temperature gradient in the wild. We found that intraspecific differences between *S. glomerata* populations increased with maximum temperatures, and that selectively bred oysters generally displayed greater gene expression in the hottest treatments. Knowledge of the sub-cellular mechanisms by which species respond to rising temperatures, and how these link to key performance traits, will assist breeding programmes focused on safeguarding the production of aquaculture species, and conservation projects aiming to build the resilience of ecologically important species.

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Appendix. Data output for PERMANOVA analyses

Table A1. Two-way univariate PERMANOVAs examining sources of variation in maximum temperature and oyster survivorship recorded from pavers that differed in the oyster population received (Pop: B2-selected, wild-type) and their colour (Col: white, grey, black) over 2 months. p-*F* = pseudo-*F*. Res = residual. Significant results (at α = 0.05) are highlighted in **bold**. n = 3

Source	df	Max. temperature			Oyster survivorship		
		MS	p-*F*	p	MS	p-*F*	p
Pop	1	0.6	0.2	0.661	802.4	1.1	0.308
Col	2	66.9	23.1	**0.002**	4761.1	6.7	**0.004**
Pop × Col	2	0.7	0.2	0.761	741.2	1.1	0.408
Res	12						

Table A2. Two-way multivariate PERMANOVA examining sources of variation in the transcriptional expression of 12 genes by Sydney rock oyster populations (Pop: B2-selected, wild-type) that were attached to pavers that differed in colour (Col: white, grey, black) over 2 months. p-*F* = pseudo-*F*. Res = residual. Significant results (at α = 0.05) are highlighted in **bold**. n = 3

Source	df	MS	p-*F*	p
Pop	1	16.6	1.9	0.064
Col	2	22.3	2.5	**0.005**
Pop × Col	2	18.1	2.0	**0.015**
Res	12			

Table A3. Two-way univariate PERMANOVAs examining sources of variation in the transcriptional expression of genes associated with antioxidant defence (catalase, superoxide dismutase [SOD], peroxiredoxin 6 [Perox], glutathione peroxidase [Glut perox], glutathione S-transferase omega [Glut S-trans]) by 2 Sydney rock oyster populations (Pop: B2-selected, wild-type) that were attached to pavers that differed in colour (Col: white, grey, black) over 2 months. p-*F* = pseudo-*F*. Res = residual. Significant results (at α = 0.05) are highlighted in **bold**. n = 3

Source	df	Catalase			SOD			Perox		
		MS	p-*F*	p	MS	p-*F*	p	MS	p-*F*	p
Pop	1	0.7	1.2	0.265	2.5	5.0	**0.036**	0.9	1.8	0.185
Col	2	1.9	3.6	0.067	2.7	5.4	**0.010**	2.9	5.7	**0.017**
Pop × Col	2	2.9	5.3	**0.030**	1.5	3.0	0.070	2.1	4.0	**0.042**
Res	12									
		Glut S-trans			Glut perox					
Pop	1	<0.1	<0.1	0.921	0.3	0.3	0.621			
Col	2	3.0	5.1	**0.031**	1.6	1.7	0.241			
Pop × Col	2	1.9	3.3	0.080	1.0	1.1	0.399			
Res	12									

Table A4. Two-way univariate PERMANOVAs examining sources of variation in the transcriptional expression of genes associated with cellular stress (heat shock protein 70 [HSP70], heat shock protein 90 [HSP90]) by 2 Sydney rock oyster populations (Pop: B2-selected, wild-type) that were attached to pavers that differed in colour (Col: white, grey, black) over 2 months. p-*F* = pseudo-*F*. Res = residual. Significant results (at α = 0.05) are highlighted in **bold**. n = 3

Source	df	HSP70			HSP90		
		MS	p-*F*	p	MS	p-*F*	p
Pop	1	0.3	0.7	0.394	0.3	0.4	0.503
Col	2	3.8	6.9	**0.013**	1.4	2.1	0.192
Pop × Col	2	2.0	4.2	**0.044**	2.7	3.9	**0.048**
Res	12						

Table A5. Two-way univariate PERMANOVAs examining sources of variation in the transcriptional expression of genes associated with energy metabolism (cytochrome c oxidase I [COX1], NADH dehydrogenase [NADH]) by 2 Sydney rock oyster populations (Pop: B2-selected, wild-type) that were attached to pavers that differed in colour (Col: white, grey, black) over 2 months. p-*F* = pseudo-*F*. Res = residual. Significant results (at α = 0.05) are highlighted in **bold**. n = 3

Source	df	COX1			NADH		
		MS	p-*F*	p	MS	p-*F*	p
Pop	1	0.6	1.1	0.308	0.5	0.6	0.432
Col	2	2.5	4.3	**0.042**	2.1	2.4	0.099
Pop × Col	2	2.2	3.7	0.055	1.1	1.3	0.290
Res	12						

Table A6. Two-way univariate PERMANOVAs examining sources of variation in the transcriptional expression of genes associated with protein folding (peptidylprolyl isomerase A [PPIA]), cytoskeletal structure (β-tubulin [TUB]), and a potential reference gene (GAPDH) by 2 Sydney rock oyster populations (Pop: B2-selected, wild-type) that were attached to pavers that differed in colour (Col: white, grey, black) over 2 months. p-*F* = pseudo-*F*. Res = residual. Significant results (at α = 0.05) are highlighted in **bold**. n = 3

Source	df	PPIA			TUB			GAPDH		
		MS	p-*F*	p	MS	p-*F*	p	MS	p-*F*	p
Pop	1	4.5	6.7	**0.033**	2.9	3.1	0.089	3.6	3.6	0.079
Col	2	2.1	3.2	0.080	<0.1	<0.1	0.955	0.1	0.1	0.872
Pop × Col	2	<0.1	0.1	0.888	1.6	1.7	0.215	0.6	0.6	0.530
Res	12									

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