Acclimatory gene expression of primed clams enhances robustness to elevated $p\text{CO}_2$

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INTRODUCTION

Climate change exerts environmental pressures on marine life and is projected to continue to intensify in the near future (IPCC, 2021; Lotze et al., 2006). In particular, ocean acidification (OA), or the reduction of ocean pH due to absorption of atmospheric $\text{CO}_2$, poses a major ecosystem and economic concern within highly eutrophic coastal estuarine regions (Ekstrom et al., 2015). OA affects essential
cellular processes (e.g., acid–base homeostasis and energy metabolism, Michaeldis et al., 2005; Dineshram et al., 2013) and shell formation and survival for calcifying organisms (Fabry et al., 2008; Kleypas et al., 2006), especially during early development and metamorphosis (Kurihara et al., 2007; Waldbusser et al., 2015; Kapsenberg et al., 2018). Exacerbation of low pH conditions in coastal systems (Cai et al., 2011; Melzner et al., 2013) presents a growing concern for aquaculture (Barton et al., 2012), prompting interventions through water quality buffering and selective breeding programmes (Barton et al., 2015). These actions optimize conditions for survival, but this can lead to domestication selection by propagating environmentally sensitive cohorts (Araki et al., 2007; Nascimento-Schulze et al., 2021). A growing body of study proposes moderate stress priming to increase adaptive plasticity (Costantini et al., 2010; Hackerott et al., 2021), such that repeated challenges initiate beneficial responses (Georgoulis et al., 2021; Hawkins & Warner, 2017) that may help marine organisms acclimatize to climate change.

Organismal environmental resistance depends on integration of predictable environmental cues into acclimatory phenotypes (Ghalambor et al., 2007; Snell-Rood et al., 2018). Although larvae of marine metazoans are highly susceptible to changes in the surrounding environment, early life presents an ideal window for developmental acclimatization due to the importance of environmental information in setting the stage for subsequent phenotypic outcomes (Burton & Metcalfe, 2014; Fawcett & Frankenhuiss, 2015). Environmental variation (both spatial and temporal) shapes phenotypes (Dowd et al., 2015) and numerous studies support an acclimatory capacity for marine invertebrates to cope with intermittent periods of thermal stress (Hraoui et al., 2021) and elevated CO2 within a generation (Détrée & Gallardo-Escárate, 2018; Gurr et al., 2020; Li et al., 2020; Suckling et al., 2015), as well as across a generation (Goncalves et al., 2016; Parker et al., 2015). Thus, the timing and magnitude of environmental change is likely to have a joint effect on plasticity (Donelson et al., 2018). Carryover effects of environmental history have physiological (Espinel-Velasco et al., 2021; Parker et al., 2012), ecological (Costantini, 2014; Hettinger et al., 2013) and evolutionary implications (Thomsen et al., 2017). In light of this, it is essential to understand how intermittent or repeated environmental signals, such as the challenges posed from climate change, are transduced to elicit acclimatory mechanisms.

Gene expression regulation is key to homeostasis and phenotypic plasticity. Both constitutive and inducible gene expression responses can enhance acclimatory capacity (Barshis et al., 2013; Georgoulis et al., 2021). Transcriptome profiling of clams and oysters exposed to changing environmental conditions has identified differential regulation of mitochondrial complexes, antioxidants and proteins related to lipid degradation (Chapman et al., 2011; Goncalves et al., 2017; López-Landavery et al., 2021; Teng et al., 2021), indicating that external abiotic conditions can affect metabolism and shift substrates for bioenergetics. Furthermore, enhanced constitutive expression, or gene frontloading, is a proposed mechanism to cope with challenging but predictable environmental signals (Barshis et al., 2013). For instance, limpets (Lottia sp.) occupying the high intertidal zone transcribe heat-shock proteins at a higher level relative to low-intertidal individuals, suggesting an acclimated response (Dong et al., 2008). Thus, transcriptomics provides a broad and sensitive means to assess the role of gene expression in environmental priming and the molecular underpinnings of important economic traits in aquaculture species (Chandhini & Kumar, 2019).

Geoduck clams (Panopea sp.) are long-lived molluscs of high economic value, and recent studies have corroborated their particular resilience to OA (Gurr et al., 2020, 2021; Spencer et al., 2019). Transcriptome profiles of geoduck clams reared under OA conditions found regulation of energy production and acid/base homeostasis in urchin-stage larvae of Mexican geoduck Panopea globosa (López-Landavery et al., 2021), whereas similar exposures delay metamorphosis in the Japanese geoduck P. japonica (Huo et al., 2019). Transcriptome profiling of P. generosa has provided critical molecular insight on effects of low-pH exposure, highlighting molecular metabolic shifts over larvae-to-juvenile development and the informative capacity of transcriptomics for examining responses to low pH in this species (Timmins-Schiffman et al., 2020). There is also evidence that repeated exposures of juvenile P. generosa under OA conditions elicits its compensatory growth and metabolism (Gurr et al., 2020) and differential DNA methylation (Putnam et al., 2017). In light of this, we postulated that pre-exposure, or priming, generates changes in transcriptome profiles under repeated encounters. Specifically, we hypothesized that there will be frontloading of distinct gene functions and pathways underpinning adaptive phenotypes from early-life priming. To this end, we examined gene expression patterns underlying high-pCO2 priming in postlarval P. generosa that resulted in larger (tissue biomass and shell length) juvenile clams with reduced total antioxidant capacity after repeat exposures (Gurr et al., 2021). Gene expression data were analysed for juvenile P. generosa acclimated at the pediveliger stage under ambient and moderately elevated pCO2 conditions for 110 days (day 0) and then were subsequently re-exposed in a reciprocal fashion to a second exposure of either ambient, moderately elevated pCO2, or severely elevated pCO2 for 7 days (day 7), a 7-day return to ambient pCO2 (day 14), and a third 7-day exposure to ambient or moderately elevated pCO2 (day 21; Figure 1). Gurr et al. (2021) review details regarding the rationale (i.e., timing and magnitude of pCO2 conditions) and limitations of this horneretic framework.

2 | METHODS

2.1 | pCO2 exposure experiment and tissue sampling

Larval Pacific geoduck clams were reared from gametes at the Jamestown Point Whitney Shellfish Hatchery following standard industry practice (i.e., live-algal feed regime, larvae runts culled periodically, etc.). Once animals reached settlement competency (30 days post-fertilization), pediveliger larvae were exposed to ambient and elevated pCO2 conditions (921 ± 41 and 2870 ± 65 μatm) for an initial 110-day conditioning period targeting the metamorphic transition
from pediveliger to the burrowing juvenile stage (N = 4 trays treatment−1 and N = 1.5 × 10^4 pediveliger geoduck per tray). The timing for primary exposure has naturalistic relevance, as postlarval development represents a transition from a free-swimming stage to a sedentary life in the benthos (Goodwin and Pease, 1989), where bacterial carbon mineralization and low buffering capacity elevate pCO₂ and decrease calcium carbonate saturation (Cai et al., 2011). Survivorship over the pediveliger-to-juvenile transition was ~30% regardless of pCO₂ condition (4–5 × 10^3 juvenile geoduck per tray; Gurr et al., 2021). Juveniles acclimated under ambient and elevated pCO₂, hereafter referred to as naïve and pre-exposed clams, were divided at equal density into 36 replicate vessels (N = 120 animals per vessel, N = 6 vessels per treatment), and subjected to a secondary 7-day period under three pCO₂ conditions (ambient pCO₂ = 754 ± 15 μatm, moderate pCO₂ = 2750 ± 31 μatm, severe pCO₂ = 4940 ± 45 μatm) followed by 7 days of ambient recovery (896 ± 11 μatm) before replicates were split into 72 vessels (N = 6 vessels per treatment) for a 7-day third exposure in two conditions (ambient pCO₂ = 967 ± 9 μatm and moderate pCO₂ = 3030 ± 23 μatm; Figure 1); the time to reach target treatments (i.e., ambient to moderately elevated pCO₂) occurred more rapidly (~3h) than the return to ambient conditions from elevated pCO₂ levels (~6–8h). Note these pCO₂ values are higher than pCO₂ in the open ocean because they are designed to be relevant to the native range of Panopea generosa, as they correspond to measurements at local estuarine sites and sediment conditions where the clams live (e.g., >2400 μatm and ΔΩarag < 0.4 in Hood Canal, WA: Feely et al., 2010; JCP et al., 2014; ΔΩarag 0.4–0.6 in subsurface sediments: Green et al., 2009). Geoduck were fed a live mixed-algae diet ad libitum with a programmable dosing pump (Jebao DP-4), targeting 5 × 10^4 cells ml⁻¹ in each vessel. Furthermore, marine bivalves can re-establish acid–base homeostasis 24–48h after exposure to acidified seawater (Michaelidis et al., 2005; Spicer et al., 2007), and therefore we considered a span of 7 days as sufficient to infer a stable state during exposure to elevated pCO₂ and ambient seawater. Additional details on geoduck rearing and experimental conditions are outlined in Gurr et al. (2021). As previously described in Gurr et al. (2021), the pre-exposed clams displayed a phenotype of reduced total antioxidant capacity and increased shell growth and tissue biomass under subsequent pCO₂ challenges, which provided evidence supporting the pediveliger-to-juvenile window for adaptive developmental plasticity. In this study, samples were sequenced at the same time points as physiological samples collected in Gurr et al. (2021) to investigate transcriptome profiles and their linkages to the differing phenotypic outcomes. Whole juveniles from each replicate tray and vessel were snap frozen in liquid nitrogen at ~10:00a.m. on the final day of the initial priming period (N = 8 per treatment; Figure 1 day 0; after 110 days of primary exposure), secondary exposure (N = 6 per treatment; Figure 1 day 7), ambient recovery (N = 6 per treatment except one instance where N = 5; Figure 1 day 14), and third exposure (N = 3–6 per treatment; Figure 1 day 21).

2.2 | Gene expression

Individual whole juvenile geoduck samples were homogenized in DNA/RNA shield (1 ml) with 0.25 ml 0.5-mm glass beads by vortexing for 1 min. Total RNA was extracted from whole tissue homogenate using the Quick-DNA/RNA Kit (Zymo) according to the manufacturer’s instructions. RNA was quantified using the Qubit RNA Broad Range Assay Kit with fluorometer (ThermoFisher) and quality was ascertained using a 4200 TapeStation System (Agilent Technologies) to visualize ribosomal bands, keeping in mind that the bands comigrate in geoduck clams into a single peak. RNA samples (10 ng μl⁻¹) were used for TagSeq, a 3’ short transcript method that allows cost-effective and accurate estimation of transcript abundances relative to traditional RNAseq (Lohman et al., 2016). Library preparation and sequencing of the 141 samples was conducted at the University of Texas Austin, Genomic Sequencing and Analysis Facility. Sequencing was completed on two lanes of an Illumina NovaSeq 6000 SR100, targeting standard coverage of 3–5 million 100-bp single-end reads per sample. Raw TagSeq reads were trimmed of Illumina adapters, poly-A sequence and quality filtered with fastp (Chen et al., 2018); quality control for filter optimization was completed using multiqc (Ewels et al., 2016). Following quality control, reads were mapped to the P. generosa reference genome (Putnam et al., 2022) using hisat2...
(Kim et al., 2015) with a mapping efficiency of ~30%; the majority of unmapped reads aligned to ribosomal rRNAs (i.e., 18S and 28S) in ge- oduck clams (i.e., Panopea globosa). strongt2 (Pertea et al., 2015) was used to quantify reads and assemble a count matrix (using prepDE.py) for analysis in R version 3.5.1 (https://www.r-project.org). All code and data are publicly available (Gurr et al., 2022; doi:10.5281/zenodo.6908630).

2.3 | Gene expression analysis

We chose a combined analytical approach to first test for gene expression frontloading (sensu Barshis et al., 2013) and fine-tuning or responsiveness to pCO2 change by examining modules of co-expression via network analysis and their functional enrichment (Figure 2) and second to test for differential expression in pairwise treatment groups at each time point (i.e., differentially expressed genes; DEGs) and their functional enrichment.

First, Weighted Gene Co-expression Network Analysis (WGCNA; Zhang & Horvath, 2005; Langfelder & Horvath, 2008) was used to identify gene expression patterns and test for frontloading of transcripts (constitutive changes in expression, Figure 2c), or fine-tuning of transcripts under pCO2 change (dynamic changes across repeated exposures), due to conditioning history by calculating gene co-expression modules at each time point. Co-expression network construction allows an assessment of broad expression-level directionality and the influence of compounding treatment history (e.g., initial × second exposure × third exposure), as opposed to typical approaches of pairwise differential expression analysis. Second, we used DESeq2 to test for differential expression in pairwise group comparisons. While pairwise group comparisons are not able to examine the interactive effects, pairwise models investigated the effects of initial acclimation (ambient vs. moderate), second exposure (ambient vs. moderate, ambient vs. severe, and moderate vs. severe) and third exposure (ambient vs. moderate) and grouped contrasts to determine changes in gene expression due to cumulative (not interactive) pCO2 treatment history.

Gene expression in response to pre-exposure and repeated encounters was analysed with WGCNA using the bioconductor (version3.13) package “wgcna” (version 1.70–3; Langfelder & Horvath, 2008, Langfelder & Horvath, 2012) in R (R Core Team, 2021) to assess co-expression patterns (Zhang & Horvath, 2005). Following co-expression module assignment of each read matrix (days 0, 7, 14, 21; Figure 1), gene expression was transformed using variance-stabilizing transformation (“varianceStabilizingTransformation” DESeq2 version 1.12.3; Anders & Huber, 2010; discussed as vst-normalized gene expression) to visualize gene expression patterns associated with significant module–treatment associations. At each time point, the treatments represent the exposure history until that time point (e.g., Day 0 = A and M; Day 7 and Day 14 = AA, AM, AS, MA, MM, MS; and Day 21 = AAA, AAM, AMA, AMM, ASA, ASM, MAA, MAM, MMA, MMM, MSA, MSM). This full analysis identified that the primary exposure (A vs. M) was a major driver of the expression response. Thus we plotted heatmaps of module–treatment correlations for both the full analysis (all treatments) and based on the primary treatments only (the same gene set visualized and analysed with the main effect of primary history only). To determine the functional patterns associated with pCO2 treatment, co-expression modules significantly correlated with treatment variables were assessed for significantly enriched “molecular function” and “biological process” GO terms (p < .05) and GOslim assignment was used to place significantly enriched GO terms into hierarchical bins of broader function. Lastly, Kyoto Encyclopedia of Genes and Genomes (KEGG; Kanehisa & Goto, 2000) was applied to understand higher-level functional processes of co-expression modules and the web interface “KEGGmapper” (Kanehisa and Sato, 2020) was used to manually investigate genes involved in enriched pathways. Additional details regarding read filtration and gene expression analysis (WGCNA and DESeq2) are provided in the Supporting Information.

Co-expression module–treatment associations significantly correlated with the primary exposure period (Figure 2a) were categorized as “frontloaded” or “fine-tuned” with respect to their expression patterns. Gene sets were identified as either consistently higher in expression through time (Figure 2c) were identified as putatively frontloaded, and those that were dynamic across repeated exposures (Figure 2b) were identified as putatively fine-tuned. Quantitatively, gene frontloading was assessed as described in Barshis et al. (2013), where frontloaded genes are defined as those with a higher constitutive expression due to priming and less responsive to a subsequent environmental challenge. The term “frontloaded” was therefore assigned to those genes with higher expression under ambient pCO2 by pre-exposed clams (“control ratio”; Figure 2c y-axis) and lower response ratio by pre-exposed clams (“response ratio” = expression in elevated pCO2 / expression in ambient pCO2; Figure 2c x-axis). Frontloaded genes can then be defined as those with control ratio >1 and response ratio <1 (i.e., top left quadrant Figure 2c). A one-way ANOVA was used to test differences in vst-normalized gene expression of frontloaded genes between primary × second pCO2 treatments. In contrast to frontloaded genes, fine-tuned expression was defined by first identifying the genes in modules correlated with exposure to the primary exposure period, and then second by tracking these genes underlying the GO terms that were uniquely responsive to subsequent exposures (Figure 2b).

Raw sequence data are available on NCBI (BioProject: PRJNA740307). Analytical code and data files (i.e., gene lists and enrichment analysis) are publicly available in an open repository (Gurr et al., 2022; doi:10.5281/zenodo.6908630).

3 | RESULTS

3.1 | Co-expression network analysis overview

Network analysis using WGCNA (Langfelder & Horvath, 2008; Zhang & Horvath, 2005) resulted in groups of genes that shared expression at a time point, termed modules. These modules are each named
with a colour based on the convention of Horvath and Langfelder 
(Langfelder & Horvath, 2008; Zhang & Horvath, 2005). Given our 
goal to examine the interaction of repeated exposures, we analysed 
gene co-expression at each time point, resulting in modules named 
with colours at each point. To differentiate these, we have used 
the following nomenclature: “Day X module colour.” The modules 
were then tested for significant correlation with treatment groups 
via module eigengene–treatment correlations (using the Pearson 
method in the cor function in wgcna). These correlations quantify 
the strength and direction of the association between the gene ex-
pression profile of a module and a specific treatment. If a module 
eigengene–treatment correlation is positive, the expression of the 
module is greater within that treatment. Conversely if a module 
eigengene–treatment correlation is negative, the expression of the 
module is lower within that treatment.

3.1.1 | Day 0

Network analysis after the 110-day acclimation period (primary ex-
posure, day 0) resulted in six co-expression modules with between 
414 and 2818 genes (Figure S1), excluding the module containing 
only one gene (the module “grey” which contains unassigned genes). 
One module (Day 0 midnightblue) was significantly correlated with
the primary exposure treatment and represented genes with higher vst-normalized gene expression values in the naïve clams (Figure S1). Note that the authors of wgcna recommend larger sample matrices (e.g., >15 samples) to yield robust and biologically relevant data (Langfelder & Horvath, 2008); because eight samples were sequenced on day 0, these results must be interpreted with caution and are presented in Figure S1.

Genes within Day 0 module midnightblue were significantly enriched for “pentose phosphate pathway” (N = 8), “glycolysis/gluconeogenesis” (N = 14), “carbon metabolism” (N = 28), “foxO signaling pathway” (N = 16) and “ubiquitin mediated proteolysis” (N = 24; Figure S1). Gene families associated with enriched pathways included, but were not limited to, enzymes involved in glycolysis, citric acid cycle, immune response, protein ubiquitination, mitogen-activated protein kinase signalling and oxidative stress response.

3.1.2 | Day 7

**Modules correlated with initial treatment**

Network analysis of expression following second exposure (day 7) resulted in five co-expression modules containing between 304 and 3951 genes (Figure S2A). Of these five modules, two were significantly correlated with the primary exposure treatment (Day 7 module brown, and Day 7 module yellow Figure S2A). Day 7 module brown (N = 862 genes) showed higher vst-normalized gene expression values in the naïve clams relative to the pre-exposed clams, whereas Day 7 module yellow (N = 610 genes) had higher vst-normalized gene expression in the pre-exposed clams (Figure S2B).

Day 7 module brown, representing genes highly expressed by naïve clams, was enriched for pathways “fatty acid degradation” (N = 9), “fatty acid metabolism” (N = 9), “retinol metabolism” (N = 5), “peroxisome” (N = 12), “lysosome” (N = 18) and “endocytosis” (N = 16; Table S1 and Figure 3). Further, GO analysis of Day 7 module brown resulted in enriched terms in the following GOslim categories: ion binding, protein transport and transport, lipid binding, peptidase activity, immune response, protein ubiquitination, mitogen-activated protein kinase signalling and oxidative stress response.

Day 7 module yellow, representing genes highly expressed by pre-exposed clams, was enriched for the pathway “endocytosis” (N = 14; Table S2) and the following genes or gene families were associated with this pathway: E3 ubiquitin-protein ligases, receptor proteins, and protein trafficking and transport. Further, GO analysis of Day 7 module yellow resulted in enriched GO terms in the following GOslim categories: ion binding, cellular nitrogen compound metabolic process, methyltransferase activity, transcription factor and RNA binding, response to stress, immune system response, cell death, cell motility, cytoskeletal protein binding, enzyme binding/regulatory activity, and kinase activity (Figure 3; Figures S5B, S7 and S8A). Gene families associated with enriched GO terms included, but were not limited to, chromatin modifiers, transcription factors, stress response proteins, innate immune/antiviral response/signalling cascade, MAP kinases, ion transport, E3 ubiquitin-protein ligases and serine/threonine-protein kinases.

**Modules correlated with interactions of treatments**

One module was only significantly correlated with the combined primary and second treatments (Day 7 module green; Figure S2) and suggests that primary acclimation treatments and second exposures under moderate and severe pCO2 lead to divergent expression patterns. Day 7 module green (N = 304 genes) showed low gene expression by pre-exposed clams re-exposed under moderate pCO2, whereas naïve clams had high expression values when exposed under severe pCO2 ("MM" < "AS"; Figure S2). This module was enriched for cellular nitrogen compound metabolic process and included transcription factors and other regulators of transcription. There were no pathways enriched for Day 7 module green.

**Frontloaded genes**

Of the full Day 7 module yellow (N = 610 genes), naïve clams increased expression of 346 and 184 genes under moderately elevated and severely elevated pCO2, of which 243 and 138 genes were assigned as “frontloaded” by pre-exposed clams; 106 genes were frontloaded under both moderately and severely elevated pCO2 (Figure 4). Frontloaded genes were attributed to, but not limited to, stress response and apoptosis/innate immune response (heat shock 70-kDa protein, toll-like receptors, caspase-10, antiviral innate immune response receptor RIG-I and MY88), homeostatic processes (sodium/calcium exchanger), protein ubiquitination/degradation (E3 ubiquitin ligases XIAP, TRAF6, and mfn168), transcription factors (i.e., Mafk, SNW domain-containing protein 1, Kruppel-like factor, TNF-receptor-associated factor and bile acid receptor), and histone and chromatin-binding proteins (i.e., histone-binding protein N1/N2, histone-lysine N-methyltransferase and chromatin remodelling ATPase INO80). Frontloaded genes were significantly affected by pCO2 treatment (one-way ANOVA; p < .001) and a posteriori pairwise differences reinforced the constitutive gene frontloading criteria (Figure 4).

3.1.3 | Day 14

**Modules correlated with initial treatment**

Network analysis of expression following ambient recovery (Day 14) resulted in nine co-expression modules containing between 391 and 2669 genes (Figure S3A). Of these nine modules, two were significantly correlated with primary exposure treatment (Day 14 module brown and Day 14 module black; Figure S3A). Day 14 module brown (N = 1164 genes) showed higher vst-normalized gene expression values in the naïve clams, whereas Day 14 module black (N = 415...
Day 14 module brown, representing genes highly expressed by naïve clams, was enriched for "pentose phosphate pathway" (N = 5), "proteasome" (N = 6), "glycolysis/gluconeogenesis" (N = 7), "biosynthesis of amino acids" (N = 9) and "carbon metabolism" (N = 12; Table S1 and Figure 3). Gene families associated with these pathways included glutathione components, proteases, endocytosis and trafficking-related proteins, and lipid catabolic proteins.

Day 14 module brown was also enriched for transmembrane transporter activity, lipid metabolic process, catabolic process, response to stress, ligase activity and enzyme regulatory activity (Figure 3; Figures S5A, S6 and S8B); these terms included genes for stress response, protein recycling and trypsin inhibition.

Day 14 module black, representing genes highly expressed by pre-exposed clams, was enriched for "pentose phosphate pathway" (N = 5), "proteasome" (N = 6), "glycolysis/gluconeogenesis" (N = 7), "biosynthesis of amino acids" (N = 9) and "carbon metabolism" (N = 12; Table S1 and Figure 3). Gene families associated with these pathways included glutathione components, proteases, endocytosis and trafficking-related proteins, and lipid catabolic proteins.

Day 14 module brown was also enriched for transmembrane transporter activity, lipid metabolic process, catabolic process, response to stress, ligase activity and enzyme regulatory activity (Figure 3; Figures S5A, S6 and S8B); these terms included genes for stress response, protein recycling and trypsin inhibition.

Day 14 module black, representing genes highly expressed by pre-exposed clams, was enriched for "pentose phosphate pathway" (N = 5), "proteasome" (N = 6), "glycolysis/gluconeogenesis" (N = 7), "biosynthesis of amino acids" (N = 9) and "carbon metabolism" (N = 12; Table S1 and Figure 3). Gene families associated with these pathways included glutathione components, proteases, endocytosis and trafficking-related proteins, and lipid catabolic proteins.
enriched pathways included, but were not limited to, glycolytic enzymes, citric acid cycle, protein recycling and aminotransferases. GO analysis of Day 14 module black included enriched terms in several GOslim categories also in Day 7 module yellow (also representing higher expression by pre-exposed clams; Figure 3; Figures S5B, S7 and S8) and additional transcriptional regulators and immune signalling proteins. Further, Day 14 module black was enriched for oxidoreductase activity and transmembrane transporter activity (Figure 3; Figures S5B, S7 and S8); these terms included proteins/complexes of the mitochondrial electron transport chain, iron-binding proteins, and regulation of sodium/bicarbonate and calcium ion channels.

**Modules correlated with interactions of treatments**

Four modules were significantly correlated with the combined primary and second treatments (Day 14 modules brown, black, pink and magenta; Figure S3A) and each suggests that primary acclimation treatments and second exposures under moderate and severe pCO₂ lead to divergent expression patterns. Of these four modules, two were not significantly correlated with the initial acclimation treatment (Day 14 module pink, and Day 14 module magenta; Figure S3A). Day 14 module magenta (N = 336 genes), representing genes with low expression values by pre-exposed clams re-exposed under severe pCO₂, was enriched for "spliceosome" (N = 8; Table S2) involving alternative splicing proteins. GO analysis of Day 14 module magenta showed enriched terms in GOslim categories for cellular nitrogen compound metabolic process and ion binding (Figure S8B) and included components of the spliceosome, E3 ubiquitin-protein ligases, tubulin proteins and Ras-related proteins. Day 14 module pink (N = 391 genes), representing genes highly expressed by pre-exposed clams re-exposed under severe pCO₂ (Figure S3), did not show pathway enrichment (Table S1), but GO terms were enriched in GOslim categories of cellular nitrogen compound metabolic process, DNA binding, enzyme regulator activity and oxidoreductase activity (Figure S8B), including homeodomain transcription factors, superoxide dismutase and Rho-GTPase activating proteins.

**3.1.4 Day 21**

**Modules correlated with initial treatment**

Network analysis of gene expression following the third exposure (Day 21) resulted in nine co-expression modules containing between 451 and 1753 genes (Figure S4). Of these nine modules, three were significantly correlated with the primary exposure treatment (Day 21 modules blue, magenta and yellow; Figure S4A). Day 21 modules blue (N = 1537 genes) and magenta (N = 241 genes) showed higher vst-normalized gene expression values in naïve clams, whereas Day 21 module yellow (N = 926 genes) showed higher expression values in the pre-exposed clams (Figure S4B).

Day 21 module blue, representing genes highly expressed by naïve clams, was enriched for "fatty acid degradation" (N = 11),
“fatty acid metabolism” (N = 13), “endocytosis” (N = 31), “peroxisome” (N = 15) and “lysosome” (N = 25; Table S1 and Figure 3), involving the same genes and gene families enriched in earlier modules with the same expression pattern (i.e., Day 7 module brown and Day 14 module brown). Day 21 module magenta was enriched for “protein processing in endoplasmic reticulum” (N = 10; Table S2) and contained genes for protein quality control and trafficking and stress of the endoplasmic reticulum. All enriched GO terms for Day 21 modules blue and magenta were in GOslim categories that were also enriched in earlier modules representing the same expression pattern (i.e., Day 7 module brown and Day 14 module brown) with the exception of enzyme binding and immune system response (Figures S5A, S6 and S9). These additional terms included genes for endosomal cargo trafficking and transport, regulation of ion channels, serine/threonine-protein kinases, E3 ubiquitin ligases and zinc finger proteins.

Day 21 module yellow, representing genes highly expressed by pre-exposed clams, was enriched for “mitophagy” (N = 7; Table S1 and Figure 3) and included PINK1-Parkin components, autophagy receptors, lysosomal degradation and forkhead box protein o transcription factor. All enriched GO terms in Day 21 module yellow were in GOslim categories that were also enriched in earlier modules representing the same expression pattern (i.e., Day 7 module yellow and Day 14 module black; Figures S5B, S7 and S9). Enriched terms included additional genes such as autophagy receptors, tyrosine-protein kinases, histone modifiers and transcriptional regulators.

**Modules correlated with interactions of treatments**

Six modules were significantly correlated with the combined primary, second and third treatments (Day 21 modules blue, magenta, red, black, pink, turquoise; Figure S4). Of these six modules, two were significantly correlated with the primary exposure treatment (Day 21 modules blue and yellow) and four were only correlated with the combined treatment history (Day 21 modules red, black, pink, turquoise; Figure S4A). Day 21 module black (N = 660), representing genes expressed at a low abundance by naïve animals under moderately elevated pCO₂ during the third exposure (Figure S4A), was enriched for “ribosome biogenesis in eukaryotes” (N = 15), “spliceosome” (N = 19), “RNA transport” (N = 19), and “protein processing in endoplasmic reticulum” (N = 13; Table S1). There were no pathways enriched for Day 21 modules pink (N = 451 genes), red (N = 713 genes) or turquoise (N = 1753 genes; Table S1).

### 3.2 Differential gene expression

Differential gene expression analysis on Day 0 resulted in 14 DEGs between treatments with higher expression level of 11 genes by naïve clams and three genes by pre-exposed clams (Table S2 and Figure 5). Only four DEGs contained gene name and functional annotation and included higher expression levels under ambient conditions for E3 ubiquitin-protein ligase rnf213-alpha and helicase with zinc finger domain and higher expression levels under elevated pCO₂ for putative isoforms for von Willebrand factor D protein.

Subsequent exposures on days 7, 14 and 21 showed greater differential expression due to pCO₂ priming than second or third pCO₂ treatments (Table S2). Pairwise comparison of primary ambient vs. moderately elevated pCO₂ yielded 108 DEGs on day 7 (62 upregulated and 49 downregulated), 429 DEGs on day 14 (317 upregulated and 112 downregulated) and 155 DEGs on day 21 (101 upregulated and 52 downregulated; Table S2 and Figure 5). In summary, the majority of DEGs resulting from primary exposure in this study were upregulated in the naïve phenotype (70%), especially following ambient recovery (85% of upregulated DEGs; Table S2 and Figure 5). Functional annotation of genes highly expressed by naïve clams involved glutathione components (dehydrogenase, peroxidase and transferase), endopeptidases, fatty-acid binding and lipid metabolism, and transmembrane regulatory activity, and genes highly expressed by pre-exposed clams were enriched for signalling, oxidoreductase activity, stress response (transforming growth factor beta binding) and metal ion binding (Figure 5).

There were 22 DEGs (14 upregulated and eight downregulated) that occurred on all sampling days deemed as “persistent DEGs” (Figure 5). Thirteen of the 22 persistent DEGs had putative gene annotation; immune system response to bacteria was a common function among persistent DEGs (e.g., mucin-1, chitotriosidase-1 and defensin; Table S3). Persistent DEGs with higher expression level by naïve clams notably differed in their functional annotation for response to stress, lipid and calcium binding, and protease inhibition (apolipoprotein D, re-gucalcin, mucin-1, four-domain protease inhibition, etc.; Table S3). Persistent DEGs with higher expression level by pre-exposed clams, although fewer, were additionally associated with antimicrobial activity, cobalt transport (cobalamin) and protease inhibition (defensin, CD109 antigen, and BPTI/Kunitz domain-containing protein 4-like; Table S3).

Pairwise models addressing second and third pCO₂ exposures yielded minimal expression-level differences (0–13 total DEGs), with the exception of the second pCO₂ treatment on day 7 (106 total DEGs: 14 upregulated and 92 downregulated; Table S2). GO analysis of downregulated genes showed enrichment of cell adhesion, plasminogen activation and endopeptidase activity. Results of cumulative treatment histories on day 7 (primary x second; N = 16 pairwise models) found 168 total DEGs in the pairwise model MA vs. AM (31 upregulated and 137 downregulated; Table S4); upregulated genes were enriched for actin filament polymerization, cell migration and cilium assembly, and downregulated genes were primarily associated with plasminogen activation, cell adhesion and proteolysis. Results of cumulative treatment models for day 14 and 21 are provided in Tables S5 and S6.

### 4 DISCUSSION

Post-larval acclimation to elevated pCO₂ affected transcriptome profiles. Specifically, priming to moderately elevated pCO₂ involved
FIGURE 5 Effect of ambient and moderately elevated pCO₂ priming on differential gene expression. Venn diagrams show the number of genes upregulated (a, higher expression level in naïve clams) and downregulated (b, higher expression level in pre-exposed clams) and unique to and shared between experiment days; featured counts in bold represent persistent DEGs shared among days 7, 14 and 21. Segment charts represent significant “molecular function” GO terms (−log₁₀(p-value)) in greyscale for time points.
frontloaded and continuous expression of stress/innate immune response proteins, ubiquitin ligases, transcription factors and chromatin modifiers as well as transient expression of genes affecting cellular homeostasis (e.g., cellular quality control, mitophagy, immune signalling/ defence and energy metabolism) during periods of re-exposure to elevated pCO₂ and ambient recovery. Moreover, functional annotation suggests putative control via transcriptional modifiers (e.g., transcription factors and histone methyltransferases) in the pre-exposed phenotype. In contrast, the naïve phenotype showed higher overall gene expression (74% of genes in treatment-module correlations showing higher expression in naïve clams and >70% DEGs with higher expression level in naïve clams) with functional annotation for fatty acid metabolism, primarily by peroxisome β-oxidation, and glutathione metabolism. This transcriptomic pattern in naïve clams suggests increased oxidative stress and a catabolic shift (from carbohydrate metabolism; Figure S1B) favouring lipid degradation to putatively supply energy for a higher transcription under elevated pCO₂, at a cost for organismal physiology. Our results suggest beneficial gene-expression regulation via frontloading, but also the capacity for fine-tuned, or responsive, gene expression patterns. Fine-tuning, as expedient/dynamic gene expression, may bolster tolerance to external environmental changes. Altogether, this study corroborates physiological traits of emergent organismal and cellular phenotypes (Gurr et al., 2021) with transcriptomics, highlighting how early life priming events can rapidly induce transcriptional plasticity that have the potential to enhance resilience under subsequent environmental challenges later in life.

4.1 | Naïve profile: endogenous lipids supply high transcriptional demand

A growing body of research suggests that environmental changes, such as elevated pCO₂/low pH conditions, increase energy partitioning toward protein biosynthesis (Langenbuch & Pörtner, 2002; Pan et al., 2015), conferring energetic tradeoffs for somatic growth and storage retention (Sokolova, 2013; Stump et al., 2011). Our results support this, as a key difference between transcriptome profiles was a higher transcript abundance attributed to fatty acid degradation and glutathione components in the naïve phenotype (Figures 3 and S5). Persistently enriched pathways included peroxisome activity (β-oxidation), acetyltransferase to mitochondria (carnitines) and bioremediation of free radicals illustrating elevated use of endogenous metabolic fuel to satisfy greater transcriptional demand. Mobilization of endogenous reserves, primarily lipids, is essential to meet energetic requirements of early development (Liu et al., 2020; Waldbusser et al., 2013), but also plays a vital role in rapid energy provisioning during stress exposure (Sokolova et al., 2012) and may be advantageous to sustain acid-base homeostasis (Evans et al., 2017). Naïve juveniles were enriched for carbohydrate metabolism (i.e., pentose phosphate pathway, glycolysis and carbon metabolism) prior to pCO₂ challenge, further representing greater energetic requirements and the reliance for lipid sources to satisfy demand under pCO₂ change.

Across multiple marine calcifiers, exposure to elevated pCO₂ causes lipid loss and altered fatty acid metabolism coupled with shell malformations and delayed settlement competency (Dickinson et al., 2012; Liu et al., 2020; Talmage & Gobler, 2010; Timmins-Schiffman et al., 2020). For instance, elevated pCO₂ affects shell bio-mineralization and fatty acid metabolism in the pearl oyster Pinctada fucata (Li, Huang, et al., 2016; Li, Liu, et al., 2016) and reorganizes the lipid profile in purple-hinge rock scallop Crassadoma gigantea (Alma et al., 2020). Gurr et al. (2021) found naïve geoduck clams decreased organic biomass and shell length under elevated pCO₂, linking physiological responses with gene-expression patterns of naïve clams in this study. Similarly, marine invertebrates are known to increase lipid degradation and peroxisome activity under elevated pCO₂, such as higher expression levels of long-chain specific acyl-CoA dehydrogenase in the coral Acropora millepora and barnacle Balanus amphitrite (Wong et al., 2011; Kaniewska et al., 2012) and peroxiredoxins and carnitine O-acetyltransferase in larval oysters Crassostrea virginica and Crassostrea hongkongensis (Tomanek et al., 2011; Dineshram et al., 2015). In contrast, elevated pCO₂ may not affect fatty acid metabolism (Matson et al., 2012; Timmins-Schiffman et al., 2014), or may interact with multiple stressors on lipid use (e.g., dietary restriction; Gibbs et al., 2021), which is a testament to an array of contingencies affecting metabolic shifts (e.g., species, timing, stress type[s] and intensity). Analysis of the lipidome (totality of lipids in an organism) can assess the importance of lipid catabolism on physiological success (Laudicella et al., 2020) and future efforts should consider the tissue-specificity of proteomic and gene expression patterns (e.g., Elowitz, 2002; Wei et al., 2015), requiring fine-scale sampling in contrast to whole-tissue homogenates sequenced herein.

Altogether, the gene expression patterns of naïve Panopea generosa suggest that fatty acid degradation is a primary response to OA to ensure short-term survival and may compensate for the additional transcriptional requirements; however, depletion of endogenous storages confers an unsustainable mismatch between energy demand and supply if the pCO₂ challenge exacerbated or persisted (i.e., “pessimum” range; Sokolova et al., 2012; Sokolova, 2021). Thus, the transcriptomic profile of pre-exposed P. generosa illustrates the importance of early-life environmental cues for eliciting molecular resistance. Beyond the scope of this study, standing and cryptic genetic variation may underlie adaptive capacity and heritable plasticity to environmental change (Bitter et al., 2019). For example, survival of the Yesso scallop Patinopecten yessoensis (Yang et al., 2021) and normal development of the purple sea urchin Strongylocentrotus purpuratus (Pespeni et al., 2013) under elevated pCO₂ is linked to allele variation of candidate genes associated with energy and lipid metabolism, respectively. Future studies should further examine genomic markers affecting transcriptome profiles and selection.

A substantial increase in DEGs during repeat exposures, relative to immediate post-acclimation, suggests that phenotypic differences are most evident upon environmental change. In particular, a greater magnitude of change in gene expression occurred during ambient recovery (Figure 5 and Table S2). Similarly, mussels Mytilus galloprovincialis submitted to intermittent challenges increase transcriptional variation of candidate genes associated with energy and lipid metabolism, respectively. Future studies should further examine genomic markers affecting transcriptome profiles and selection.
during depuration (Détrée & Gallardo-Escárate, 2018). Expanded research should consider the relevant magnitude of elevated pCO₂ (i.e., P. generosa in Puget Sound, WA, USA; Feely et al., 2010; JCP et al., 2014) and the dynamic and intermittent behaviour of current and future OA projections. Moreover, the short timescale of this experiment relative to the lifespan of P. generosa (up to 168 years; Bureau, 2002) limits the selective implications of transcriptome profiles on fitness, as P. generosa have shown evidence of negative effects of elevated pCO₂ exposure (i.e., shell growth) precursor to compensatory responses later in life (Gurr et al., 2020).

### 4.2 Primed profile: fine-tuned response to intermittent OA

Pre-exposed P. generosa expressed fewer genes at a higher abundance relative to naïve geoducks, albeit functional annotation of these genes showed diverse regulatory processes in cellular quality control and homeostasis (Figure 3). A general decrease in transcription may be attributed to adaptive benefits under environmental change (Bultelle et al., 2021). For instance, mussels Mytilus californianus decrease gene expression when acclimated to dynamic thermal stress, as opposed to acute isothermal conditions, highlighting a lower transcriptional demand during intermittent exposures (Connor & Gracey, 2020). Moreover, environmental history can have positive carryover effects (Ross et al., 2016), especially when the current condition matches the perceived cue (Burggren, 2015; Zhao et al., 2018). For example, pre-exposed clams persistently downregulated genes involved in chitin degradation (i.e., regucalcin) and calcium homeostasis (i.e., regucalcin). Although critical for shell biomineralization, Mytilus edulis and Crassostrea gigas suppress expression of chitinases and regucalcin under high pCO₂ (Wei et al., 2015 and Hüning et al., 2013) suggesting an adjustment of energy and ion retention under chitin and calcium-demanding conditions. Pre-exposed P. generosa also showed distinct treatment-responsiveness when faced with subsequent pCO₂ exposures, both frontloading and heightening expression in cellular quality control, signalling, transcriptional modifiers, and stress and innate-immune response genes (Figure 3). Constitutive frontloading suggests that pre-exposed clams already expressed key proteins at an optimal abundance to cope with OA. For example, heat shock 70kDa is a common indicator of resilience and pre-emptive and acclimatory expression in marine invertebrates (Dong et al., 2008; Moya et al., 2015; Barshis et al., 2013) and was constitutively expressed by pre-exposed P. generosa under both moderate and severe pCO₂ change. Moreover, apoptotic and immune signalling genes (i.e., caspase-10, toll receptors, MyD88) were also frontloaded, whereas downregulation of these genes can compromise immune activity and development under elevated pCO₂ (Todgham & Hoffmann, 2009; Liu et al., 2016). Functional enrichment of gene sets expressed by pre-exposed P. generosa also linked to mitophagy during second and third exposures under elevated pCO₂ (Table S1), involving PINK1 protein kinase (Vives-Bauza et al., 2010; Wu et al., 2015), autophagy receptors (optineurin, sequestosome 1, tax1-binding protein 1; Moore & Holzbaur, 2016), amplification of autophagy signalling (TBK1; Manford & Rape, 2015) and regulation of autophagosomes (TBC1D15; Yamano et al., 2014). Similarly, offspring of Sydney rock oysters Saccostrea glomerata of pCO₂-conditioned broodstock upregulated PINK1 under elevated pCO₂ (Goncalves et al., 2017), an essential kinase of the PINK1-Parkin pathway for efficient clearance of mitochondria (Wu et al., 2015). Hypercapnia/acidosis affects mitochondrial integrity and can enhance free radical production (Lambert & Brand, 2004; Miwa & Brand, 2003; Tomanek et al., 2011), and thus removal of damaged mitochondria, or mitophagy, may regulate cellular homeostasis during repeated pCO₂ challenge. Pre-exposed P. generosa also expressed higher levels of genes essential for protein turnover, such as 26S proteasome, E3 ubiquitin ligases and caspase (Goldberg, 2003; Voges et al., 1999), that are otherwise unaffected by low pH in other species (i.e., C. virginica and Mercenaria mercenaria).
Götze et al., 2014) probably due to energetic limitations of exposure to environmental change (Ivanina et al., 2016).

Pre-exposed P. generosa increased expression of genes for energy metabolism (NADH dehydrogenase, cytochrome c oxidase and ATPase) and biosynthesis (pentose phosphate pathway) during ambient recovery, suggesting an opportunistic or pre-emptive increase in energy production under optimal conditions (Figures 3 and 6).

Stimulation of the electron transport chain increases energy production under low-pH conditions (Evans et al., 2017), but altered expression of mitochondrial complexes may also confer metabolic suppression. For example, under elevated pCO₂, geoduck P. globosa (López-Landavery et al., 2021), Pacific oyster C. gigas (Wei et al., 2015) and eastern oyster C. virginia (Chapman et al., 2011) upregulate NADH dehydrogenase, suggesting increased ATP production, whereas lower levels of gene expression for cytochrome c oxidase in C. gigas (Dineshram et al., 2012) and ATP synthase in C. hongkongensis (Dineshram et al., 2013) suggest metabolic suppression. Thus, increased expression of mitochondrial complexes by pre-exposed geoduck clams illustrates an opportunistic increase in energy production. Moreover, enrichment for glycolysis and the nonoxidative pentose phosphate pathway suggests the pre-exposed clams metabolized carbohydrate fuels and increased nucleotide biosynthesis during ambient recovery. This demonstrates expeditious rescue by primed clams, as naïve clams were enriched for carbohydrate metabolism under ambient priming but not post-challenge during ambient common garden conditions. GO term enrichment during ambient recovery also included iron binding proteins and phenoloxidases (*oxidoreductase activity*; Figure S6). Expression of ferric-chelate reductase may improve iron homeostasis and prevent excess iron-induced toxicity (Li et al., 2019), converting ferric iron to an “active” electron-donor state (ferrous iron) required for biological processes (Connolly et al., 2003). Since antioxidants were not abundantly expressed by pre-exposed clams, excess iron-induced toxicity (Fenton reaction enhancing free radicals) was probably negligible. In contrast, stressed mud snails Littorea littorea increase expression levels of antioxidants and ferritin (Larade & Storey, 2004) essential for storing iron, suggesting taxon-specific flux of iron constituents during stress. Lastly, laccase and tyrosinase are phenoloxidases of growing interest as biomarkers of immune response and detoxification during stress. Lastly, immunomodulation and signalling were key transcriptional components of pre-exposed P. generosa following the acclimatization period, including genes for activation and inhibition of NF-kappa β (Figures SSB and S6; e.g., mitogen-activated protein kinase, toll-like receptors 2, 3 and 4, TNF receptor-associated factor 6, MyDBB, B-cell lymphoma 3 protein, and death-associated inhibitor of apoptosis 2), a transcription factor involved in immune deficiency signalling cascade in defence of pathogens (Leuiler et al., 2006). Similarly, immune priming under abiotic (i.e., temperature) and biotic (i.e., synthetic and nonsynthetic viral exposure) challenges bolsters antiviral defences associated with upregulated expression of apoptotic, signalling and immune-related genes in the Pacific oyster C. gigas (Deilisile et al., 2020; Lafont et al., 2020) and Pacific abalone Haliotis discus hannai (Zhang et al., 2022). A growing body of research highlights the general importance of NF-kappa β for the innate immune response in bivalves (Huang et al., 2021; Li et al., 2015) and elevated pCO₂ may have synergistic and antagonistic effects on immunomodulation (Cao et al., 2018; Castillo et al., 2017). For example, increased levels of NF-kappa β in the mussel Mytilus coruscus may improve immune defences to compensate for weakened shell strength under low pH (Zhao et al., 2020). Moreover, the blood clam Tegillarca granosa decreases gene expression of NF-kappa β signalling during hypercapnia, rendering clams more susceptible to disease (Liu et al., 2016). After an initial stress encounter, Mytilus galloprovincialis reduces transcription of immune-related proteins, but insufficient to counteract decreased growth (Détrée & Gallardo-Escartá, 2018). Altogether, early-life experience heightened critical signalling and immune-related proteins potentially enhancing resilience to subsequent pCO₂ change in primed clams. Considering that pre-exposed P. generosa grew larger than naïve clams (Gurr et al., 2021), early-life priming to elevated pCO₂ probably affects organismal responses to subsequent encounters demonstrated herein from “fine-tuned” transcript abundance during repeated exposure.

### 4.3 | Transcriptional control suggests “memory” post-acclimation

Understanding how the environment triggers biological responses that lead to gene expression regulation is key to understanding environmental "memory." However, potentially transient and interdependent molecular mechanisms affecting regulation of gene expression remain poorly understood (Adrian-Kalchhauser et al., 2020). Growing evidence suggests that post-translational and nongenetic markers affect gene expression in marine taxa (e.g., oysters, coral and fish; Gavery & Roberts, 2013; Putnam et al., 2016; Ryu et al., 2018) and participate in phenotypic acclimatization to novel changes (Eirin-Lopez & Putnam, 2019; Liew et al., 2020). Stress “memory,” or stored/imprinted/regulatory information from initial stress enhancing robustness to future encounters, has largely been studied as a plant-based phenomenon (Bruce et al., 2007), with growing support in invertebrate models. Molecular mechanisms underpinning memory may manifest as nongenetic markers, transcription factors and key signalling metabolites with cascading implications for performance. For example, sustained expression of the transcription factor NrF2 co-occurs with improved antioxidant defence systems in cold-primed tunicates Ciona robusta (Li et al., 2020). In our study, pre-exposed P. generosa frontloaded several transcription factors, suggesting an influence of stress history on general regulatory transcription. Moreover, histone methylesterases (HMTs) were expressed at a higher abundance by pre-exposed clams (i.e., SETD5, ASH1L and NSD2) each affecting histone H3 tri/dimethyletion of lysine residue 36 (H3K36me3 and
4.4 | “Stress priming” to improve hatchery-propagated seed

This study highlights frontloaded and fine-tuned gene expression post-priming and under reciprocal encounters. We propose priming as an approach for ecological conservation and aquaculture enhancement. Applying concepts of developmental acclimatization (e.g., early-life programming “windows”; Fawcett & Frankenhuys, 2015) and mild hormesis (e.g., conditioning hormone and oxidative-stress hypothesis; Calabrese et al., 2007; Costantini, 2014) may help to minimize effects of domestication selection and improve environmental resilience in hatchery-propagated seed. Aquaculture is projected to surpass wild capture to satisfy global seafood demand (FAO, 2020); therefore, the irreversible nature of global acidification and rapid changes in coastal and benthic zones (Gruber et al., 2012) requires mitigation through societal actions (e.g., management interventions, policies and public awareness; Kelly et al., 2011) and aquaculture adaptations to improve food security (Nascimento-Schulze et al., 2021; Reid et al., 2019). Transcriptome profiling, as showcased in this study, can expand genomic resources for ecosystem conservation and aquaculture by identifying candidate genes and gene-expression patterns associated with stress-resilient or fast-growing economic traits (Chandhini & Kumar, 2019).

5 | CONCLUSION

In this study, we investigated the transcriptome profiles of juvenile geoduck clams post-acclimation and under intermittent pCO₂ change to test the hypothesis that priming during a developmental window elicits transcriptional frontloading and an expedient response to environmental challenges. In the absence of priming, naïve clams exhibited higher transcriptional demand during experimental exposures attributed to a putative catabolic shift favouring primarily lipids. In contrast, acclimatization conferred gene frontloading and gene-expression control, such that pre-exposed P. generosa contained lower levels of expression, but frontloaded and fine-tuned under subsequent pCO₂/pH changes. The pre-exposed transcriptome profile was attributed to heightened expression for cellular quality control, signalling, transcriptional modifiers, and stress and innate-immune response genes under subsequent pCO₂ challenge and putative markers of enhanced energy production during recovery. Altogether, this study demonstrates the importance of gene-expression regulation on positive developmental acclimatization.

AUTHOR CONTRIBUTIONS

Samuel J. Gurr, Brent Vadopalas, Steven B. Roberts and Hollie M. Putnam designed the experiments, Samuel J. Gurr conducted the experiments and analysed data, and Samuel J. Gurr, Shelly A. Trigg, Brent Vadopalas, Steven B. Roberts and Hollie M. Putnam drafted, revised, read and approved the final version of the manuscript for publication.

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**CONFLICT OF INTEREST**
The authors have declared no conflict of interest for this article.

**OPEN RESEARCH BADGES**

This article has earned an Open Data Badge for making publicly available the digitally-shareable data necessary to reproduce the reported results. The data is available at [https://doi.org/10.5281/zenodo.6908630](https://doi.org/10.5281/zenodo.6908630).

**DATA AVAILABILITY STATEMENT**
Raw sequence reads are deposited in the SRA (Accession: PRJNA740307; BioProject: Transcriptome profiles of Panopea generosa under hypercapnic seawater). All data have been submitted as a public Zenodo repository [https://doi.org/10.5281/zenodo.6908630](https://doi.org/10.5281/zenodo.6908630).

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**REFERENCES**
Adrian-Kalchhauser, I., Sultan, S. E., Shama, L. N., Spence-Jones, H., Tiso, S., Valsecchi, C. I. K., & Weissing, F. J. (2020). Understanding 'non-genetic' inheritance: Insights from molecular-evolutionary cross-talk. Trends in Ecology & Evolution, 35, 1078–1089.
Alma, L., Kram, K. E., Holtgrieve, G. W., Barbarino, A., Fiamengo, C. J., & Padilla-Gamino, J. L. (2020). Ocean acidification and warming effects on the physiology, skeletal properties, and microbiome of the purple-hinge rock scallop. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 240, 110579.
Anastasiadi, D., Díaz, N., & Pferrer, F. (2017). Small ocean temperature increases elicit stage-dependent changes in DNA methylation and gene expression in a fish, the European sea bass. Scientific Reports, 7, 1–12.
Anders, S., & Huber, W. (2010). Differential expression analysis for sequence count data. Genome Biology, 11, 106.
Araki, H., Cooper, B., & Blouin, M. S. (2007). Genetic effects of captive breeding cause a rapid, cumulative fitness decline in the wild. Science, 318, 100–103.
Barshis, D. J., Ladner, J. T., Oliver, T. A., Seneca, F. O., Traylor-Knowles, N., & Palumbi, S. R. (2013). Genomic basis for coral resilience to temperature: Current status and applications. Reviews in Aquaculture, 11, 1379–1397.
Chen, S., Zhou, Y., Chen, Y., & Gu, J. (2018). fastp: An ultra-fast all-in-one FASTQ preprocessor. Bioinformatics, 34, 1884–1890.
Connor, K., & Gracey, A. Y. (2020). Cycles of heat and aerial-exposure induce changes in the transcriptome related to cell regulation and metabolism in Mytilus californianus. Marine Biology, 167, 1–12.
Costantini, D. (2014). Does hormesis foster organism resistance to extreme events? Frontiers in Ecology and the Environment, 12, 209–210.
Costantini, D., Metcalfe, N. B., & Monaghan, P. (2010). Ecological processes in a hormetic framework. Ecology Letters, 13, 1435–1447.
de Almeida, S. F., Grosso, A. R., Koch, F., Fenouil, R., Carvalho, S., Andrade, J., Levezinho, H., Gut, M., Eick, D., Gut, I., & Andrau, J. C. (2015). Impacts of coastal acidification on the Pacific Northwest shellfish industry and adaptation strategies implemented in response. Oceanography, 28, 146–159.
Bitter, M. C., Kapsenberg, L., Gattuso, J.-P., & Pfister, C. A. (2019). Standing genetic variation fuels rapid adaptation to ocean acidification. Nature Communications, 10, 5821.
Bruce, T. J. A., Matthes, M. C., Napier, J. A., & Pickett, J. A. (2007). Stressful ‘memories’ of plants: Evidence and possible mechanisms. Plant Science, 173, 603–608.
Bultelle, F., Boutet, I., Devin, S., Caza, F., St-Pierre, Y., Pédéon, R., Brousseau, P., Chan, P., Vaudry, D., Le Foll, F., & Fournier, M. (2021). Molecular response of a sub-Antarctic population of the blue mussel (Mytilus edulis platensis) to a moderate thermal stress. Marine Environmental Research, 169, 105393.
Bureau, D. (2002). Age, size structure and growth parameters of geoducks (Panopea abrupta, Conrad1849) from 34 locations in British Columbia sampled between 1993 and 2000. Canadian Technical Report of Fisheries and Aquatic Sciences, 2413, 1–84.
Burggren, W. W. (2015). Dynamics of epigenetic phenomena: Intergenerational and intragenerational phenotype ‘washout’. Journal of Experimental Biology, 218, 80–87.
Burton, T., & Metcalfe, N. B. (2014). Can environmental conditions experienced in early life influence future generations? Proceedings of the Biological Sciences, 281, 20140311.
Cai, W.-J., Hu, X., Huang, W.-J., Murrell, M. C., Lehrer, J. C., Lohrenz, S. E., Chou, W.-C., Zhai, W., Hollibaugh, J. T., Wang, Y., & Zhao, P. (2011). Acidification of subsurface coastal waters enhanced by eutrophication. Nature Geoscience, 4, 766–770.
Calabrese, E. J., Bachmann, K. A., Baller, A. J., Bolger, P. M., Borak, J., Cai, L., Cedergreen, N., Cherian, M. G., Chieueh, C. C., Clarkson, T. W., & Cook, R. R. (2007). Biological stress response terminology: Integrating the concepts of adaptive response and preconditioning stress within a hormetic dose-response framework. Toxicology and Applied Pharmacology, 222, 122–128.
Cao, R., Wang, Q., Yang, D., Liu, Y., Ran, W., Qu, Y., Wu, H., Cong, M., Li, F., Ji, C., & Zhao, J. (2018). CO₂-induced ocean acidification impairs the immune function of the Pacific oyster against Vibrio splendidus challenge: An integrated study from a cellular and proteomic perspective. Science of The Total Environment, 625, 1574–1583.
Castillo, N., Saavedra, L. M., Vargas, C. A., Gallardo-Escárte, C., & Détrée, C. (2017). Ocean acidification and pathogen exposure modulate the immune response of the edible mussel Mytilus chilensis. Fish & Shellfish Immunology, 70, 149–155.
Chandhini, S., & Kumar, V. J. R. (2019). Transcriptomics in aquaculture: Current status and applications. Reviews in Aquaculture, 11, 1379–1397.
Chapman, R. W., Mancia, A., Beal, M., Veloso, A., Rathburn, C., Blair, A., Holland, A. F., Warr, G. W., Didinato, G., Sokolova, I. M., & Wirth, E. F. (2011). The transcriptomic responses of the eastern oyster, Crassostrea virginica, to environmental conditions. Molecular Ecology, 20, 1431–1449.
Chen, S., Zhou, Y., Chen, Y., & Gu, J. (2018). fastp: An ultra-fast all-in-one FASTQ preprocessor. Bioinformatics, 34, 1884–1890.
Connolly, E. L., Campbell, N. H., Grotz, N., Prichard, C. L., & Lou Guerinot, M. (2003). Overexpression of the FRO2 ferric chelate reductase confers tolerance to growth on low iron and uncovers posttranscriptional control. Plant Physiology, 133, 1102–1110.
Connor, K., & Gracey, A. Y. (2020). Temperature and aero-exposure influence changes in the transcriptome related to cell regulation and metabolism in Mytilus californianus. Marine Biology, 167, 1–12.
Costantini, D. (2014). Does hormesis foster organism resistance to extreme events? Frontiers in Ecology and the Environment, 12, 209–210.
Costantini, D., Metcalfe, N. B., & Monaghan, P. (2010). Ecological processes in a hormetic framework. Ecology Letters, 13, 1435–1447.
Splicing enhances recruitment of methyltransferase HYPB/Setd2 and methylation of histone H3 Lys36. *Nature Structural & Molecular Biology*, 18, 977–983.

Delisle, L., Pauletto, M., Vidal-Dupiol, J., Petton, B., Bargelloni, L., Montagnani, C., Pernet, F., Corporeau, C., & Fleuré, E. (2020). High temperature induces transcriptomic changes in *Crassostrea gigas* that hinder progress of oestrid herpesvirus (OsHV-1) and promote survival. *The Journal of Experimental Biology*, 223, jeb226233.

Détrée, C., & Gallardo-Scárate, C. (2018). Single and repetitive microplastics exposures induce immune system modulation and homeostasis alteration in the edible mussel *Mytilus galloprovincialis*. *Fish & Shellfish Immunology*, 83, 52–60.

Dhayalan, A., Rajavelu, A., Rathert, P., Tamas, R., Jurkowska, R. Z., Ragozin, S., & Jeltsch, A. (2010). The Dtnm3a PWWP domain reads histone 3 lysine 36 trimethylation and guides DNA methylation. *The Journal of Biological Chemistry*, 285, 26114–26120.

Dickinson, G. H., Ivanina, A. V., Matoo, O. B., Pörtner, H. O., Lannig, G., Bock, C., Beniash, E., & Sokolova, I. M. (2012). Interactive effects of salinity and elevated CO₂ levels on juvenile eastern oysters, *Crassostrea virginica*. *Journal of Experimental Biology*, 215, 29–43.

Dineshram, R., Quan, Q., Sharma, R., Chandramouli, K., Yalamanchili, H. K., Chu, I., & Thiagarajan, V. (2015). Comparative and quantitative proteomics reveal the adaptive strategies of oyster larvae to ocean acidification. *Proteomics*, 15, 4120–4134.

Dineshram, R., Thiagarajan, V., Lane, A., Ziniu, Y., Xiao, S., & PTY, L. (2013). Elevated CO₂ alters larval proteome and its phosphorylation status in the commercial oyster, *Crassostrea hongkongensis*. *Marine Biology*, 160, 2189–2205.

Dineshram, R., Wong, K. K. W., Xiao, S., Yu, Z., Qian, P. Y., & Thiagarajan, V. (2012). Analysis of Pacific oyster larval proteome and its response to high-CO₂, *Marine Pollution Bulletin*, 64, 2160–2167.

Donelson, J. M., Salinas, S., Munday, P. L., & Shama, L. N. S. (2018). Transgenerational plasticity and climate change experiments: Where do we go from here? *Global Change Biology*, 24, 13–34.

Dong, Y., Miller, L. P., Sanders, J. G., & Somero, G. N. (2008). Heat-shock protein 70 (Hsp70) expression in four limpets of the genus *Lottia*: mal extremes and the physiological performance of individuals. *The Journal of Biological Chemistry*, 293, 29–43.

Elowitz, M. B. (2002). Stochastic gene expression in a single cell. *Science*, 297, 1183–1186.

Espinel-Velasco, N., Lamare, M., Kluibenscheld, A., Moss, G., & Cummings, V. (2021). Ocean acidification induces carry-over effects on the larval settlement of the New Zealand abalone, *Haliotis iris*. *ICES Journal of Marine Science*, 78, 340–348.

Evans, T. G., Pespeni, M. H., Hofmann, G. E., Palumbi, S. R., & Sanford, E. (2017). Transcriptomic responses to seawater acidification among sea urchin populations inhabiting a natural pH mosaic. *Molecular Ecology*, 26, 2257–2275.

Ewels, P., Magnnusson, M., Lundin, S., & Käller, M. (2016). MultiQC: Summarize analysis results for multiple tools and samples in a single report. *Bioinformatics*, 32, 3047–3048.

Fabry, V. J., Seibel, B. A., Feely, R. A., & Orr, J. C. (2008). Impacts of ocean acidification on marine fauna and ecosystem processes. *ICES Journal of Marine Science*, 65, 414–432.

FAO. (2020) The State of World Fisheries and Aquaculture 2020. Sustainability in action. Rome. https://doi.org/10.4060/ca9229en

Fawcett, T. W., & Frankenhus, W. E. (2015). Adaptive explanations for sensitive windows in development. *Frontiers in Zoology*, 12(Suppl 1), S3.

Feely, R. A., Alin, S. R., Newton, J., Sabine, C. L., Warner, M., Devol, A., Krembs, C., & Maloy, C. (2010). The combined effects of ocean acidification, mixing, and respiration on pH and carbonate saturation in an urbanized estuary. *Estuaries, Coastal and Shelf Science*, 88, 442–449.

Fox, R. J., Donelson, J. M., Schunter, C., Ravasi, T., & Gaitán-Espitia, J. D. (2019). Beyond buying time: The role of plasticity in phenotypic adaptation to rapid environmental change. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, 374, 20180174.

Gavery, M. R., & Roberts, S. B. (2013). Predominant intragenic methylation is associated with gene expression characteristics in a bivalve mollusc. *PeerJ*, 1, e215.

Georgoulis, I., Feidantsis, K., Giantis, I. A., Kakale, A., Bock, C., Pörtner, H. O., Sokolova, I. M., & Michaelidis, B. (2021). Heat hardening enhances mitochondrial potential for respiration and oxidative defence capacity in the mantle of thermally stressed *Mytilus galloprovincialis*. *Scientific Reports*, 11, 1–8.

Ghalambor, C. K., McKay, J. K., Carroll, S. P., & Reznick, D. N. (2007). Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Functional Ecology*, 21, 394–407.

Gibbs, M. C., Parker, L. M., Scanes, E., Byrne, M., O’Connor, W. A., & Ross, P. M. (2021). Energetic lipid responses of larval oysters to ocean acidification. *Marine Pollution Bulletin*, 162, 112441.

Goldberg, A. L. (2003). Protein degradation and protection against misfolded or damaged proteins. *Nature*, 26, 895–899.

Goncalves, P., Anderson, K., Thompson, E. L., Melwani, A., Parker, L. M., Ross, P. M., & Raftos, D. A. (2016). Rapid transcriptional acclimation following transgenerational exposure of oysters to ocean acidification. *Molecular Ecology*, 25, 4836–4849.

Goncalves, P., Jones, D. B., Thompson, E. L., Parker, L. M., Ross, P. M., & Raftos, D. A. (2017). Transcriptomic profiling of adaptive responses to ocean acidification. *Molecular Ecology*, 26, 5974–5988.

Goodwin, C. L., & Pease, B. (1989). Species profiles: Life histories and environmental requirements of coastal fishes and invertebrates (Pacific Northwest). Pacific geoduck clam. *Biological Report 82*. U.S. Army Corps of Engineers and U.S. Department of the Interior

Götze, S., Matoo, O. B., Beniash, E., Saborowski, R., & Sokolova, I. M. (2014). Interactive effects of CO₂ and trace metals on the proteasome activity and cellular stress response of marine bivalves *Crassostrea virginica* and *Mercenaria mercenaria*. *Aquatic Toxicology*, 149, 65–82.

Green, M. A., Waldbusser, G. G., Reilly, S. L., Emerson, K., & O’Donnell, S. (2009). Death by dissolution: Sediment saturation state as a mortality factor for juvenile bivalves. *Limnology and Oceanography*, 54, 1037–1047.

Greer, E. L., & Shi, Y. (2012). Histone methylation: A dynamic mark in health, disease and inheritance. *Nature Reviews Genetics*, 13, 343–357.

Gruber, N., Hauri, C., Lachkar, Z., Loher, D., Frölicher, T. L., & Plattner, G.-K. (2012). Rapid progression of ocean acidification in the California Current System. *Science*, 337, 220–223.

Gurr, S. J., Trigg, S. A., Vadopalas, B., Roberts, S. B., & Putnam, H. M. (2021). Repeat exposure to hypercapnic seawater modifies growth and oxidative status in a tolerant burrowing clam. *Journal of Experimental Biology*, 224. https://doi.org/10.1242/jeb.233932
(dataset) Gurr SJ, Trigg SA, Vadopalas B, Roberts SB, Putnam HM. 2022. Acclimatory gene expression of primed clams enhances robustness to elevated pCO₂; Zenodo: v1.2. https://doi.org/10.5281/zenodo.6908630

Gurr, S. J., Vadopalas, B., Roberts, S. B., & Putnam, H. M. (2020). Metabolic recovery and compensatory shell growth of juvenile Pacific geoduck following short-term exposure to acidified seawater. Conservation Physiology, 8, coaa024.

Hackerott, S., Martell, H. A., & Eirin-Lopez, J. M. (2021). Coral environmental memory: Causes, mechanisms, and consequences for future reefs. Trends in Ecology & Evolution, 36, 1011–1023.

Hawks, T. D., & Warner, M. E. (2017). Warm preconditioning protects against acute heat-induced respiratory dysfunction and delays bleaching in a symbiotic sea anemone. The Journal of Experimental Biology, 220, 969–983.

Hettinger, A., Sanford, E., Hill, T. M., Lenz, E. A., Russell, A. D., & Gaylord, B. (2013). Larval carry-over effects from ocean acidification persist in the natural environment. Global Change Biology, 19, 3317–3326.

Hraoui, G., Breton, S., Miron, G., Boudreau, L. H., Hunter-Manseau, F., & Pichaud, N. (2021). Mitochondrial responses towards intermittent heat shocks in the eastern oyster, Crassostrea virginica. Journal of Experimental Biology, 17, jeb242745.

Huang, B., Dong, J., Sang, X., Li, L., Li, F., Ma, J., Wang, X., Wang, X., & Liu, Y. (2021). A review on marine mollusk NF-xB/Rel studies in immunity and the characterization of a Chlamys farrei Rel gene. Aquaculture, 544, 730746.

Hüning, A. K., Melzner, F., Thomsen, J., Gutowska, M. A., Krämer, L., Frickenhuisen, S., Rosenstiel, P., Pörtner, H. O., Philipp, E. E., & Lucassen, M. (2013). Impacts of seawater acidification on mantle gene expression patterns of the Baltic Sea blue mussel: Implications for shell formation and energy metabolism. Marine Biology, 8, 1845–1861.

Huo, Z., Ribbani, M. G., Cui, H., Xu, L., Yan, X., Fang, L., Wang, Y., & Yang, F. (2019). Larval development, juvenile survival, and burrowing rate of geoduck clams (Panopea japonica) under different pH conditions. Aquaculture International, 27, 1331–1342.

IPCC. (2021). Climate Change 2021: The physical science basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press.

Ivanina, A. V., Nesmelova, I., Leamy, L., Sokolov, E. P., & Sokolova, I. M. (2016). Intermittent hypoxia leads to functional reorganization of mitochondria and affects cellular bioenergetics in marine molluscs. The Journal of Experimental Biology, 219, 1659–1674.

JCP, R., Alin, S. R., Feely, R. A., Newton, J., Warner, M., & McElhany, P. (2014). Seasonal carbonate chemistry covariation with temperature and salinity in a fjord estuary: Implications for the design of ocean acidification experiments. PLoS One, 9, e89619.

Kanehisa, M., & Goto, S. (2000). KEGG: Kyoto encyclopedia of genes and genomes. Nucleic Acids Research, 28, 27–30.

Kanehisa, M., & Sato, Y. (2020). KEGG Mapper for inferring cellular functions from protein sequences. Protein Science, 29, 28–35.

Kaniewska, P., Campbell, P. R., Kline, D. I., Rodríguez-Lanetty, M., Miller, D. J., Dove, S., & Hoegh-Guldberg, O. (2012). Major cellular and physiological impacts of ocean acidification on a reef building coral. PLoS One, 7, e34659.

Kapsenberg, L., Miglioli, A., Bitter, M. C., Tambutté, E., Dumolland, R., & Gattuso, J.-P. (2018). Ocean pH fluctuations affect mussel larvae at key developmental transitions. Proceedings of the Biological Sciences, 285, 20182381.

Kelly, R. P., Foley, M. M., Fisher, W. S., Feely, R. A., Halpern, B. S., Waldbusser, G. G., & Caldwell, M. R. (2011). Oceans. Mitigating local causes of ocean acidification with existing laws. Science, 332, 1036–1037.

Kim, D., Langmead, B., & Salzberg, S. L. (2015). HISAT: A fast spliced aligner with low memory requirements. Nature Methods, 12, 357–360.

Kleypas JA, Feely RA, Langdon C, Sabine CL, Robbins LL. (2006). Impacts of ocean acidification on coral reefs and other marine calcifiers: A guide for future research (p. 88). Report of a workshop sponsored by NSF, NOAA, and the U.S. Geological Survey. St. Petersburg, Florida.

Kurihara, H., Kato, S., & Ishimatsu, A. (2007). Effects of increased seawater pCO₂ on early development of the oyster Crassostrea gigas. Aquatic Biology, 1, 91–98.

Lafort, M., Vergnes, A., Vidal-Dupiol, J., de Lorgeril, J., Gueguen, Y., Haffner, P., Potton, B., Chaparro, C., Barrachina, C., Destoumieux-Garzon, D., & Mitta, G. (2020). A sustained immune response supports long-term antiviral immune priming in the Pacific oyster, Crassostrea gigas. Mbio, 11(2), e02777-19.

Lambert, A. J., & Brand, M. D. (2004). Superoxide production by NADH:ubiquinone oxidoreductase (complex I) depends on the pH gradient across the mitochondrial inner membrane. Biochemical Journal, 382, 511–517.

Langenbuch, M., & Pörtner, H. O. (2002). Changes in metabolic rate and N excretion in the marine invertebrate Sipunculus nudus under conditions of environmental hypercapnia. Journal of Experimental Biology, 205, 1153–1160.

Langfelder, P., & Horvath, S. (2008). WGCNA: An R package for weighted correlation network analysis. BMC Bioinformatics, 559, 1–13.

Langfelder, P., & Horvath, S. (2012). Fast R functions for robust correlations and hierarchical clustering. Journal of Statistical Software, 46, 1–17.

Larade, K., & Storey, K. B. (2004). Accumulation and translation of ferritin heavy chain transcripts following anoxia exposure in a marine invertebrate. Journal of Experimental Biology, 207, 1353–1360.

Laucidella, V. A., Whitfield, P. D., Carboni, S., Doherty, M. K., & Hughes, A. D. (2020). Application of lipodomics in bivalve aquaculture, a review. Reviews in Aquaculture, 12, 678–702.

Leulier, F., Lhocine, N., Lemaître, B., & Meier, P. (2006). The Drosophila inhibitor of apoptosis protein DIAP2 functions in innate immunity and is essential to resist gram-negative bacterial infection. Molecular and Cellular Biology, 26, 7821–7831.

Li, H., Huang, X., & Zhan, A. (2020). Stress memory of recurrent environmental challenges in marine invasive species: Ciona robusta as a case study. Frontiers in Physiology, 11, 94.

Li, L., Ye, L., Kong, Q., & Shou, H. (2019). A vacuolar membrane ferrichelate reductase, OsFRO1, alleviates Fe toxicity in rice (Oryza sativa L.). Frontiers in Plant Science, 10, 700.

Li, R., Zhang, R., Zhang, L., Zou, J., Xing, Q., Hou, H., Xu, Z., Zhang, L., Wang, R., & Bao, Z. (2015). Characterizations and expression analyses of NF-xB and Rel genes in the Yesso scallop (Pinctada yesoensis) suggest specific response patterns against Gram-negative bacterial infection. Fish & Shellfish Immunology, 44, 611–621.

Li, S., Huang, J., Liu, C., Liu, Y., Zheng, G., Xie, L., & Zhang, R. (2016). Interactive effects of seawater acidification and elevated temperature on the transcriptome and biominaleralization in the pearl oyster Pinctada fucata. Environmental Science & Technology, 50, 1157–1165.

Li, S., Liu, C., Huang, J., Liu, Y., Zhang, S., Zheng, G., Xie, L., & Zhang, R. (2016). Transcriptome and biominaleralization responses of the pearl oyster Pinctada fucata to elevated CO₂ and temperature. Scientific Reports, 6, 18943.

Liew, Y. J., Howells, E. J., Wang, X., Michell, C. T., Burt, J. A., Idaghdour, Y., & Aranda, M. (2020). Intergenerational epigenetic inheritance in reef-building corals. Nature Climate Change, 10, 254–259.

Liew, Y. J., Zoccola, D., Li, Y., Tambutté, E., Venn, A. A., Michell, C. T., Cui, G., Deutekom, E. S., Kaandorp, J. A., Voolstra, C. R., & Forêt, S. (2018). Epigenome-associated phenotypic acclimatization to ocean acidification in a reef-building coral. Science Advances, 4, eaar8028.

Lim, Y. K., Cheung, K., Dang, X., Roberts, S. B., Wang, X., & Thyagarajan, V. (2021). DNA methylation changes in response to ocean acidification at the time of larval metamorphosis in the edible oyster,
Crasostrea hongkongensis. Marine Environmental Research, 163, 105214.

Liu, S., Shi, W., Guo, C., Zhao, X., Han, Y., Peng, C., Chai, X., & Liu, G. (2016). Ocean acidification weakens the immune response of blood clam through hampering the NF-kappa β and toll-like receptor pathways. Fish & Shellfish Immunology, 54, 322–327.

Liu, Z., Zhang, Y., Zhou, Z., Zong, Y., Zheng, Y., Liu, C., Kong, N., Gao, Q., Wang, L., & Song, L. (2020). Metabolomic and transcriptomic profiling reveals the alteration of energy metabolism in oyster larvae during initial shell formation and under experimental ocean acidification. Scientific Reports, 10, 6111.

Lohman, B. K., Weber, J. N., & Bolnick, D. I. (2016). Evaluation of TagSeq, a reliable low-cost alternative for RNA seq. Molecular Ecology Resources, 6, 1315–1321.

López-Landavery, E. A., Carpio-Iturarte, E. J., Pérez-Carrasco, L., Díaz, F., la Cruz, F. L.-D., García-Esquivel, Z., Hernández-Ayón, J. M., & Galindo-Sánchez, C. E. (2021). Acidification stress effect on umberate veliger larval development in Panopea globosa. Marine Pollution Bulletin, 163, 111945.

Lotze, H. K., Lenihan, H. S., Bourque, B. J., Bradshaw, R. H., Cooke, R. G., Kay, M. C., Kidwell, S. M., Kirby, M. X., Peterson, C. H., & Jackson, J. B. C. (2006). Depletion, degradation, and recovery potential of estuaries and coastal seas. Science, 312, 1806–1809.

Luna-Acosta, A., Breitwieser, M., Renault, T., & Thomas-Guyon, H. (2017). Recent findings on phenoloxidases in bivalves. Marine Pollution Bulletin, 122, 5–16.

Manford, A. G., & Rape, M. (2015). Better safe than sorry: Interlinked feedback loops for robust mitophagy. Molecular Cell, 60, 1–2.

Matson, P. G., Yu, P. C., Sewell, M. A., & Hofmann, G. E. (2012). Development under elevated p CO₂ conditions does not affect lipid utilization and protein content in early-life-history stages of the purple sea urchin, Stronglylocentrotus purpuratus. The Biological Bulletin, 223, 312–327.

Melzner, F., Thomsen, J., Koeve, W., Oschlies, A., Gutowska, M. A., Bange, H. W., Hansen, H. P., & Körtzinger, A. (2013). Future ocean acidification will be amplified by hypoxia in coastal habitats. Marine Biology, 160, 1875–1888.

Michaelidis, B., Ouzounis, C., Paleras, A., & Pörtner, H. O. (2005). Effects of long-term moderate hypercapnia on acid-base balance and growth rate in marine mussels Mytilus galloprovincialis. Marine Ecology Progress Series, 293, 109–118.

Miwa, S., & Brand, M. D. (2003). Mitochondrial matrix reactive oxygen species production is very sensitive to mild uncoupling. Biochemical Society Transactions, 31, 1300–1301.

Moore, A. S., & Holzbaur, E. L. F. (2016). Dynamic recruitment and activation of ALS-associated TBK1 with its target optineurin are required for efficient mitophagy. Proceedings of the National Academy of Sciences of the United States of America, 113, E3349–E3358.

Moya, A., Huisman, L., Foret, S., Gattuso, J. P., Hayward, D. C., Ball, E. E., & Miller, D. J. (2015). Rapid acclimation of juvenile corals to CO2-mediated acidification by upregulation of heat shock protein and Bcl-2 genes. Molecular Ecology, 24, 438–452.

Mozgova, I., Mikulski, P., Pecinka, A., & Farrona, S. (2019). Epigenetic mechanisms of abiotic stress response and memory in plants. In Epigenetics in plants of agronomic importance: Fundamentals and applications (pp. 1–64). Springer.

Nanty, L., Carbajosa, G., Heap, G. A., Ratniesks, F., van Heel, D. A., Down, T. A., & Raky, V. K. (2011). Comparative myelohemics reveals gene-body H3K36me3 in Dro sophila predicts DNA methylation and Cpg landscapes in other invertebrates. Genome Research, 21, 1841–1850.

Nascimento-Schulze, J. C., Bean, T. P., Houston, R. D., Santos, E. M., Sanders, M. B., Lewis, C., & Ellis, R. P. (2021). Optimizing hatchery practices for genetic improvement of marine bivalves. Reviews in Aquaculture, 13, 2289–2304.

Osipovich, A. B., Gangula, R., Vianna, P. G., & Magnuson, M. A. (2016). Setd5 is essential for mammalian development and the co-transcriptional regulation of histone acetylation. Development, 143, 4595–4607.

Pan, T.-C. F., Applebaum, S. L., & Manahan, D. T. (2015). Experimental ocean acidification alters the allocation of metabolic energy. Proceedings of the National Academy of Sciences of the United States of America, 112, 4696–4701.

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.

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.

Pertea, M., Pertea, G. M., Antonescu, C. M., Chang, T. C., Mendell, J. T., & Salzberg, S. L. (2015). StringTie enables improved reconstruction of a transcriptome from RNA-seq reads. Nature Biotechnology, 33, 290–295.

Pespeni, M. H., Sanford, E., Gaylord, B., Hill, T. M., Hosfelt, J. D., Jaris, H. K., LaVigne, M., Lenz, E. A., Russell, A. D., Young, M. K., Sanford, E., Gaylord, B., Hill, T. M., Hosfelt, J. D., Jaris, H. K., LaVigne, M., Lenz, E. A., Russell, A. D., Young, M. K., & Palumbi, S. R. (2013). Evolutionary change during experimental ocean acidification. Proceedings of the National Academy of Sciences of the United States of America, 110, 6937–6942.

Putnam, H. M., Davidson, J. M., & Gates, R. D. (2016). Ocean acidification influences host DNA methylation and phenotypic plasticity in environmentally susceptible corals. Evolutionary Applications, 9, 1165–1178.

Putnam H. M., Roberts S, Spencer LH. (2017) Capacity for adaptation and acclimatization to ocean acidification in geoduck through epigenetic mechanisms. Poster, Figshare. https://doi.org/10.6084/m9.Figshare.490889v

Putnam, H. M., Trigg, S. A., White, S. J., Spencer, L. H., Vadopalsa, B., Natarajan, A., Hetzel, J., Jaeger, E., Soohoo, J., Escárate, C. G., Goetz, F. W., & Roberts, S. (2022). Dynamic DNA methylation contributes to carryover effects and beneficial acclimatization in geoduck clams. bioRxiv. https://doi.org/10.1101/2022.06.24.497506

R Core Team. (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing. https://www.R-proje ct.org/
