Cross-talk between tissues is critical for intergenerational acclimation to environmental change

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Abstract

An organism's reaction to environmental changes is mediated by coordinated responses of multiple tissues. Additionally, parental priming may increase offsprings' acclimation potential to changing environmental conditions. As acidification of oceans continues to intensify it is critical to assess the acclimation potential of species at the whole organismal scale. To do this we need to understand the cross-talk between tissues in regulating and responding to pH changes. Here by using a multi-tissue approach we determine the influence of 1) variation in parental behavioural tolerance and 2) parental environment, on molecular responses of their offspring in a coral reef fish. The gills and liver showed the highest transcriptional response to OA conditions in juvenile fish regardless of the parental environment, while the brain and liver showed the greatest signal of intergenerational acclimation. Key functional pathways that were altered in the brain and liver upon within-generational CO_2 exposure were restored to control levels when parents were exposure to OA conditions. Furthermore, the expression of a new complement of genes involved in key functions were altered in the offspring only when the parents were previously exposed to OA conditions. Therefore, previous parental conditioning to ocean acidification can reprogram tissue transcriptomic profiles of the offspring enabling them to better cope in an environment with elevated CO_2 levels. Overall, our results show that intergenerational plasticity is key in evolutionarily adaptation to global change and illustrates how transcriptional changes across multiple tissues integrate to facilitate organismal acclimation to OA.

Keywords: Climate change, multi-tissue, intergeneration, acclimation, ocean acidification, transcriptomics, spiny damselfish.

Significance statement

With the global climate changing rapidly, organisms need to acclimate to the new conditions to survive. Assessing the adaptive potential of complex organisms such as vertebrates is especially challenging as each tissue has its own unique function. However, acclimation of organisms to changes in their environment requires functional integration of all tissues which is usually overlooked in climate change research. Here we reveal that cross-communication between tissues is crucial in the adaptive response of organisms to future ocean conditions. Furthermore, both parental environment and parental behavioral variability influence the transcriptional reprograming of offspring tissues in response to elevated CO_2. Overall, it is the integration of transcriptional changes across multiple tissues that mediates intergenerational plasticity to future changes in ocean chemistry.

Introduction

With the global climate continuously shifting to more extreme conditions organisms need to acclimate and/or adapt to the changing environments in order to survive. The oceans are becoming increasingly acidified as they absorb a major portion of anthropogenic CO_2 emissions (Pörtner et al., 2022) leading to ocean acidification (OA) which is reported to negatively impact the physiology and behavior of various marine organisms including fish (Heuer & Grosell, 2014; Strader et al., 2020). However, increasing evidence suggests that multi-generational exposure to elevated CO_2 conditions could influence the adaptive capacity of future generations to OA conditions (Nagelkerken et al., 2023). In fact, several studies have reported transgenerational acclimation in a number of fish species as well as some invertebrates to OA (Strader et al., 2020). Specifically, transgenerational exposure to elevated CO_2 conditions has been shown to facilitate acclimation of metabolism, growth, survival, neuronal plasticity and behavior in independent studies (Allan et al., 2014; Miller et al., 2012; Monroe et al., 2021; Munday, 2014; Schade et al., 2014; Schunter et al., 2016, 2018; Stiasny et al., 2018) however, we are still learning about the underlying molecular mechanisms of such acclimation process.

Additionally, variation both within and across species in the biological responses to OA also exists due to differences in their evolutionary and environmental history. Studies examining the effect of elevated CO_2 on metabolism, growth, development, and reproduction in fish show variable results with some species being more affected than others (Heuer & Grosell, 2014). Variation in sensitivity to elevated CO_2 within a population could be crucial in long-term adaptation through selection of more tolerant individuals. Indeed, individual variation in behavioural tolerance to elevated CO_2 exposure has been reported to be heritable and hence could facilitate rapid selection of tolerant genotypes in the population (Lehmann et al., 2022; Welch & Munday, 2017). Such selection for CO_2 tolerance has been shown to occur

in nature, which could result in populations consisting of individuals with greater behavioural tolerance to elevated CO_2 (Munday et al., 2013). Furthermore, inter-individual variation in sensitivity to ocean acidification could have an epigenetic basis (Ryu et al., 2018; Turner, 2009) and in fact several studies have reported the expression levels of genes involved in epigenetic processes to be altered upon exposure to elevated CO_2 conditions (Huang et al., 2019; Schunter et al., 2018). Transfer of epigenetic factors from parents to offspring (epigenetic inheritance) could be one of the potential mechanisms of inter- and trans-generational acclimation and eventual adaptation to OA.

Adaptive processes to environmental changes at the organismal level requires integrated activity of various tissues, with each tissue undergoing changes in its transcriptional landscape resulting in the overall response of the organism. However, to date, research has mainly focused on individual tissue functional changes in response to OA with less emphasis on how these changes integrate to create a whole-body response. Several studies have examined the effects of OA on brain and neurosensory systems since the discovery of impaired behavioural responses in various fish species in elevated CO_2 conditions. The altered behavioural responses have been linked to changes in the functioning of the GABAergic signaling pathway (Schunter et al., 2019) and the circadian rhythm in the brain of fish exposed to elevated CO_2 (Lee et al., 2021; Schunter et al., 2016; Williams et al., 2019). Previous studies have also focused on the effects of OA conditions on the gill transcriptome due to it being the primary organ involved in acid-base regulation, immune defences, and stress response, and hence plays a vital role in maintaining cellular homeostasis under conditions of CO_2 stress (De Souza et al., 2014; Deigweiher et al., 2008, 2010). These processes are energetically expensive and indeed changes in the aerobic metabolic scope (Crespel et al., 2019; Gräns et al., 2014; Pimentel et al., 2014; Rummer et al., 2013) and expression levels of key metabolic genes (Frommel et al., 2020) have been reported in fish exposed to elevated CO_2. Therefore, exposure

to elevated CO_2 affects various aspects of fish physiology such as metabolism, cellular redox status, ion transport and acid-base homeostasis, neurological functioning and behavior thereby exerting a whole-body functional reprogramming (Grosell et al. 2019). Therefore, a systematic transcriptomic analysis is needed to determine how the biological processes associated with each tissue integrate together within the whole-organism to drive adaptive responses to elevated CO_2 environments.

In this study we conducted an intergenerational CO_2 exposure experiment and performed systematic analysis of gene expression changes in response to elevated CO_2 across three tissues, the brain, the gills, and the liver, in the spiny damselfish *Acanthochromis polyacanthus*. While *A. polyacanthus* can be sensitive to increases in water temperature and CO_2 levels, they have the potential to acclimate to the changing environmental conditions across multiple generations (Donelson et al., 2012; Schunter et al., 2016, 2018; Veilleux et al., 2015). *A. polyacanthus* has been used as a model to study the impacts of climate change, and also to investigate the molecular basis of intergenerational plasticity to environmental changes, due to its advantageous life-history traits for laboratory studies (Robertson, 1973), however past studies have only focused on single tissues (Ryu et al., 2018; Schunter et al., 2016, 2018). Here, by using a multi-tissue transcriptomic approach we aim to determine how dynamic cross-talk between tissues maintains whole-body homeostasis under future ocean acidification conditions. Additionally, we also assess how the acclimatory response of offspring mediated by transcriptional reprogramming across multiple tissues is influenced by variation in parental sensitivity to elevated CO_2 and parental environment. Through systemic characterization of the effects of OA we aim to identify how the adaptive processes within each tissue integrate to drive intergenerational acclimation to OA at the organismal level.

Methods

Sample collection, behavioural testing, and experimental design

Adult *Acanthochromis polyacanthus* were collected from the wild on the Great Barrier Reef, Australia (18°38'24.3"S, 146°29'31.8"E) and exposed to elevated CO_2 (754 ± 92 µatm) for seven days following which their behavioural sensitivity to conspecific chemical alarm cues (CAC) was tested using a two-chamber flume as described previously (Schunter et al., 2016). Briefly, the fish were classified as being behaviorally sensitive or tolerant to elevated CO_2 based on the amount of time spent in water containing the CAC. Sensitive individuals spent ≥ 70% time in CAC whereas tolerant individuals spent ≤ 30% time in CAC. Individuals of similar size displaying the same behavioural phenotype (sensitive or tolerant) were then grouped into breeding pairs and held in either control (414 ± 46 µatm) or elevated CO_2 conditions (754 ± 92 µatm) for three months prior to the breeding season. Offspring clutches from each breeding pair were placed into three different experimental treatments resulting in three combinations of parent-offspring conditions for each parental phenotype: (1) Control treatment – Parents and offspring held at control condition (414 ± 46 µatm); (2) Developmental treatment – Parents held at control condition and offspring exposed to elevated CO_2 (754 ± 92 µatm) immediately after hatching; and (3) Intergenerational treatment – Parents and offspring exposed to elevated CO_2 (754 ± 92 µatm). The offspring were held in their respective conditions until they were five months old after which nine fish from each parental phenotype, from each treatment condition (N = 27 from each parental phenotype; N = 54 total fish sampled) were euthanized and the brain, gills and liver were dissected, snap frozen in liquid nitrogen and stored at -80 ºC until further processing (Supplementary Figure S1).

RNA extraction, sequencing, and gene expression analyses

Total RNA was extracted from the fish brains, livers and gills using the AllPrep DNA/RNA Mini kit from Qiagen following the manufacturer's instructions. RNA quality was determined using nanodrop and Agilent Bioanalyzer and samples having an RNA integrity value (RIN) ≥ 8 were sequenced using Illumina HiSeq 2500 to get paired-end reads of 100 bp at Macrogen Inc., South Korea. A total of 1,614.25 ± 3.05, 2,367.03 ± 5.19, and 2,227.23 ± 6.37 million raw paired-end reads were obtained from the 162 sequenced libraries from brain, gills and liver respectively which included nine control, nine developmental and nine intergenerational samples for each parental phenotype for each tissue (Supplementary Table S1). The quality of the raw reads were examined using FastQC (Andrews, 2010) v0.11.8 and adapters and low quality sequences were trimmed using Trimmomatic (Bolger et al., 2014) v0.39 (ILLUMINACLIP: adapters.fa:2:30:15:8:true; SLIDINGWINDOW:4:20; MINLEN:32). Only those sequences ≥ 32 bp in length with both the forward and reverse reads retained after trimming were used for further analysis. Potential contaminant sequences were identified using kraken (Wood & Salzberg, 2014) v2.0.8-beta, with a confidence score of 0.3, using the bacteria, fungi and virus RefSeq genomic libraries as reference and removed from further analyses. A total of 1,510.51 ± 2.62, 2,254.16 ± 4.95, and 2,116.25 ± 6.04 million high-quality sequences were retained after the filtering process (Supplementary Table S1). These sequences were mapped to the *Acanthochromis polyacanthus* reference genome (unpublished) using HISAT2 (Kim et al., 2019) v2.1.0. On average, 84 ± 1.83%, 91.22 ± 0.66%, and 93.33 ± 0.81% reads mapped to the reference genome from the brain, gills, and liver respectively (Supplementary Table S1). Raw read counts per gene were obtained using featureCounts (Liao et al., 2014) v2.0.0 (parameters: -B -J -M --fraction), assigning fractional counts to multi-mapped reads. Exploring the gene expression patterns across the whole dataset (162 samples) using principal component analysis (PCA) revealed a clear clustering of samples by tissues indicating that tissues vary greatly in their gene expression patterns (Supplementary Figure S2). Subsequent

analysis of differences in gene expression levels was therefore carried out separately for each tissue using the DESeq2 (Love et al., 2014) v1.32.0 package in R (R Core Team, 2021) v4.2.1.

Principal component analysis (PCA) using the regularized log transformed (rlog) counts was done in R v4.2.1 to detect and remove outlier samples. A likelihood ratio test (LRT) using a model comparison approach was then used to determine the effect of family line in driving the gene expression patterns and to determine the best design formula for the final DE analysis. First, significant differences in gene expression were measured by comparing a model including treatment and family line against a reduced model without the family line factor separately for each tissue. For a total of 924, 910, and 923 genes in the brain, gills, and liver respectively, the model including family line better explained the observed differences in gene expression compared to the reduced model excluding this factor (FDR corrected p-value < 0.05; Supplementary Table S2). Pair-wise comparisons between the control, developmental and intergenerational treatment was then caried out in DESeq2 (accounting for the family effect, using Wald test) separately for each parental phenotype for each tissue to determine the effect of parental environment and parental tolerance to CO_2 on the molecular responses of the offspring to elevated CO_2. For each pair-wise comparison, the genes were considered to be significantly differentially expressed (DE) if the False Discovery Rate (FDR) adjusted p-value was less than 0.05, the absolute log 2-fold change in expression was greater than 0.3 and baseMean was greater than 10. Functional enrichment analysis of the significant DE genes was carried out in OmicsBox (https://www.biobam.com/omicsbox) v1.4.11 using Fisher's Exact Test (FDR corrected p-value < 0.05) with the option of reducing to most specific GO terms to reduce redundancy. GO terms that were identified to be over-represented among the DE genes were also retained. The genes associated with the enriched and over-represented GO terms were further categorized into broader functional groups based on their functional description

204 from the UniProt knowledgebase (UniProtKB; https://www.uniprot.org/). All figures are made

205 using ggplot in R v4.2.1.

206

Results

Molecular processes affected by all elevated CO_2 treatments

To understand a general effect of elevated CO_2 exposure regardless of time of exposure to elevated CO_2 we identified genes that were commonly differentially expressed (DE) in both the developmental and intergenerational treatments compared to control (Figure 1(a)(i)), which are considered the general "CO_2 response genes" (Supplementary Table S4). There was high tissue specificity in transcriptional response in both the elevated CO_2 treatments compared to control with no genes being commonly DE across the three tissues in the sensitive parental phenotype and only 3 and 81 genes being shared between the brain and gills, and liver and gills respectively in the tolerant parental phenotype (Supplementary Figure S3(a)). Liver had the greatest number of genes commonly differentially expressed in both the elevated CO_2 treatments compared to control, followed by the gills (Figure 1(b), Supplementary Table S3). Overall, offspring of tolerant parents had more differentially expressed (DE) genes in all tissues, with the difference in DE gene numbers between sensitive and tolerant phenotypes being more pronounced in the gills and liver than in the brain (Figure 1(b)).

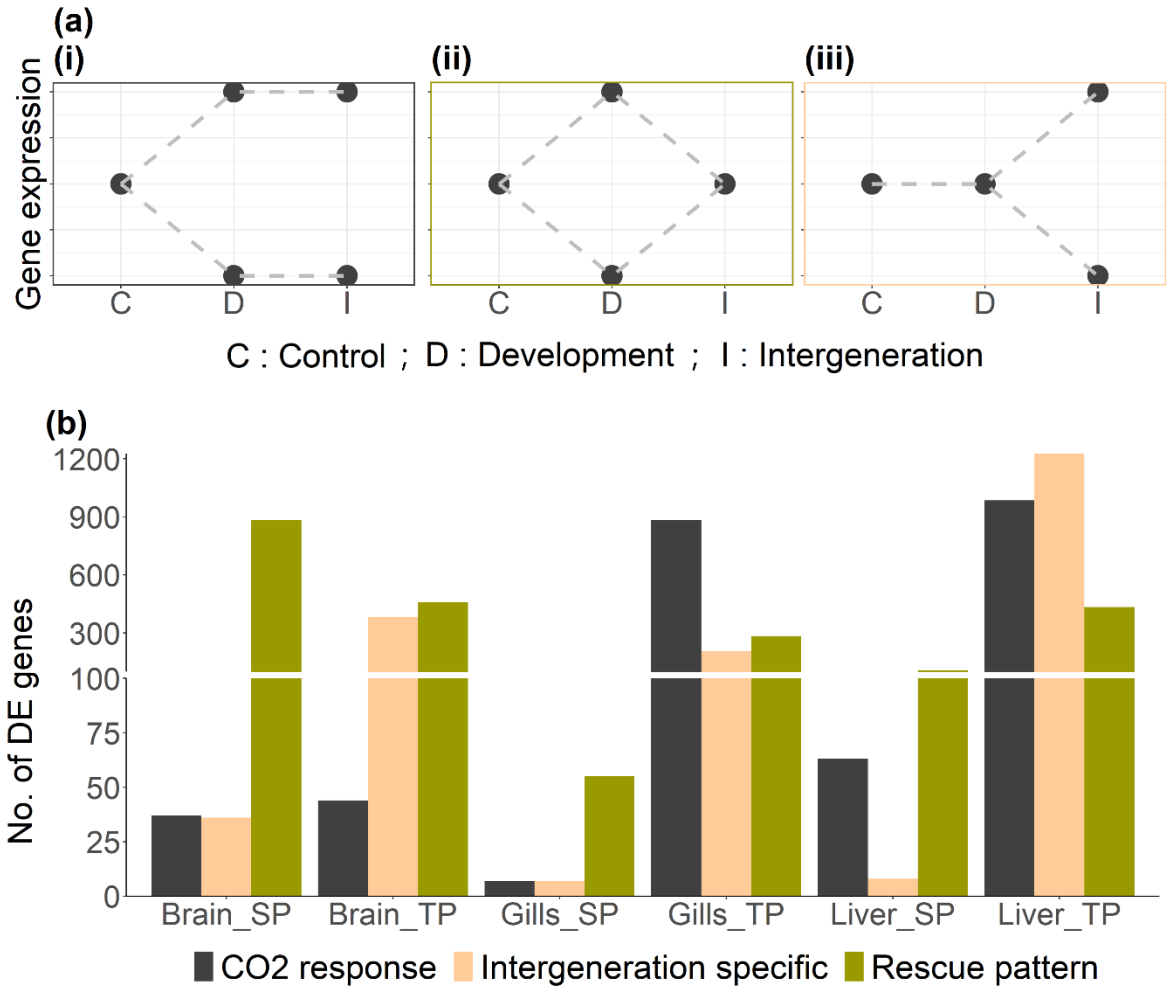

Figure 1: (a) Schematic graph representing the expression profile of (i) CO_2-response genes, (ii) genes showing a rescue pattern, and (iii) intergeneration-specific genes. (b) Number of differentially expressed genes across the three treatments in all the three tissues. SP indicates samples with a sensitive parental phenotype and TP indicates samples with a tolerant parental phenotype for each of the respective tissues. Note scale break in y-axis at 100 DE genes.

The three tissues also differed substantially in terms of functions associated with the CO_2-response genes. Only three functions, biosynthetic process, transport, and binding, were differentially regulated in all the three tissues. Gills showed the highest specificity in functional signatures with pentose-phosphate pathway, cytoskeleton, and protein folding/modification being primarily over-represented among the DE genes only in this tissue. Genes involved in cellular stress response were differentially regulated both in the brain and gills and genes involved in immune response, energy metabolism, and ubiquitin dependent protein catabolism were commonly differentially regulated in the gills and liver while transcription regulation was

primarily enriched only in the liver (Figure 2(a)). Interestingly, biosynthetic processes and immune response were downregulated only in the liver but upregulated in the brain and gills suggesting tissue specific regulation of these functional pathways (Supplementary Table S7, S10).

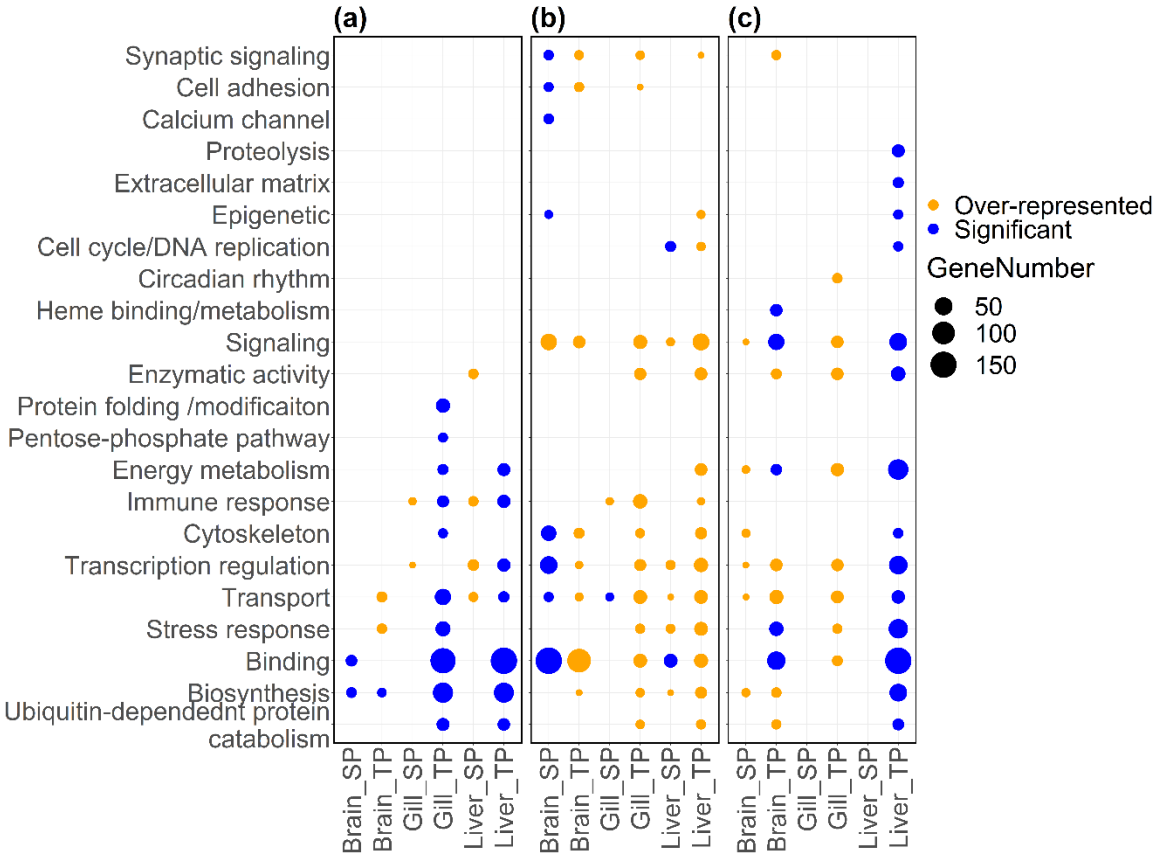

Figure 2: Functions that are significantly enriched (in blue; FDR <0.05) or over-represented (in orange; FDR > 0.05) among the DE genes involved in (a) overall CO2 response, (b) rescue pattern, and (c) intergenerational specific response. SP indicates samples with a sensitive parental phenotype and TP indicates samples with a tolerant parental phenotype.

Parental exposure to elevated CO_2 "rescues" developmental effects

A total of 1220, 328, and 542 genes that were DE in the developmental treatment (compared to control and intergeneration) in the brain, gills, and liver respectively returned to control levels in the offspring whose parents were previously exposed to OA conditions suggesting cross-generation plasticity resulting in "rescue" of gene expression levels (Figure 1(a)(ii); Supplementary Table S5). Parental conditioning had the largest effect on brain gene

expression followed by the liver (Figure 1(b), Table S3). Similar to the CO_2-affected genes, there were very few genes commonly DE across tissues (Supplementary Figure S3(b)). However, while the exact genes did not overlap, the majority of the underlying functional responses involved in intergenerational plasticity were commonly regulated across the three tissues (Figure 2(b)).

Functions such as transport (including transport of ions involved in pH homeostasis), synaptic signaling, signaling, RNA processing & transcription regulation, cytoskeleton-related, biosynthetic processes, and binding were commonly enriched or over-represented among the DE genes showing a "rescue" pattern. Liver showed the highest degree of specificity in functional regulation, with functions involved in cell cycle/ DNA replication and energy metabolism being primarily enriched or over-represented among the DE genes only in the liver. Additionally, epigenetic processes were commonly over-represented in the liver and brain, and immune response, stress response and ubiquitin dependent protein degradation processes were over-represented in both the liver and gills. Interestingly, although synaptic signaling was commonly over-represented in all tissues, calcium channel encoding genes, which are involved in neurotransmitter release, were differentially regulated mainly in the brain. This suggests that parental conditioning to elevated CO_2 selectively regulates certain specific functions in each tissue (Figure 2(b); Supplementary Table S8, S11).

Intergenerational specific response to elevated CO_2

We found a large transcriptional response to elevated CO_2 that was only seen in the intergenerationally exposed fish and not in fish with only developmental (within generation) exposure to elevated CO_2. This indicates plasticity of the offspring transcriptome due to parental conditioning to elevated CO_2 and was especially marked in offspring of tolerant parents (Figure 1(b)). These are genes that were only differentially expressed in the intergenerational treatment (compared to control and development) but were at control levels

in the developmental treatment (Figure 1(a)(iii)). Specifically, 383, 207, and 1,226 genes were DE in the brain, gills, and liver respectively in offspring with tolerant parents, while offspring with sensitive parents had 36, 7, and 8 DE genes in the brain, gills, and liver respectively that were specific to the intergenerational treatment (Supplementary Table S6). Offspring of both tolerant and sensitive parents showed high tissue specificity in the intergenerational specific transcriptional signature to elevated CO_2 with only one and five genes being commonly DE across all three tissues in the sensitive and tolerant parental phenotypes respectively (Supplementary Figure S3(c)).

Specifically, we found molecular signatures indicating bicarbonate retention only in the intergenerationally treated fish such as downregulation of SLC4A1, CFTR, SLC12A2, and SLC9A3 in the gills and upregulation of SLC4A4 in the brain which can buffer pH changes caused be elevated environmental CO_2 levels. Other key functional pathways that showed intergeneration specific regulation were epigenetic processes and energy metabolism (Figure 2(c)). Therefore, fish whose parents also experience the same high CO_2 environment undergo rearrangements in their transcriptional landscape. This change in transcriptional signature could regulate the above mentioned "rescue" pattern and equip offspring to better cope with OA conditions (Supplementary Table S9, S12).

Parental variability in CO_2 sensitivity impacts the offspring transcriptome

Parental behavioural phenotype was found to have a substantial influence on the offspring transcriptional response. Across all three tissues, offspring with behaviorally tolerant parents when faced with elevated CO_2 had larger changes in gene expression levels (log2FC > 5; Figure 3) and a greater number of DE genes involved in the overall CO_2 response (common in developmental and intergenerational CO_2 exposure compared to control; Figure 1(b)). There were also very few genes involved in overall CO_2 response shared between the two parental phenotypes, specifically only six, three, and eleven common DE genes in the brain, gills, and

liver respectively (Supplementary Figure S3(a)). When considering genes involved in intergenerational plastic responses, there were more differentially expressed genes in the offspring of tolerant parents compared to those of sensitive parents, except for genes showing a rescue pattern in the brain (Figure 1(b). Additionally, none of the DE genes involved in intergenerational plasticity were shared in the liver tissue between the two parental phenotypes while the brain and gills had a small proportion of common DE genes (specifically, 121 and 11 common DE genes showing a rescue pattern in the brain and gills respectively and only two and one common DE genes in the intergeneration specific response in the brain and gills respectively (Supplementary Figure S3(b, c)).

The difference in transcriptional response between the two parental phenotypes was especially pronounced when considering genes showing an intergeneration-specific signature with offspring of tolerant phenotype having more DE genes with a much higher magnitude of gene expression changes (log2FC >5; Figure 3). Several functions such as acid-base regulation, signaling, transcription regulation, energy metabolism, and epigenetic processes were enriched in the intergeneration-specific treatment in all tissues only in the tolerant phenotype (Figure 2(c)). Similarly, several functions were uniquely regulated only in offspring with tolerant parents when considering the overall effect of elevated CO_2 exposure independent of the length of exposure (Figure 2(a)). However, when considering genes whose expression levels returned to control levels due to parental conditioning to elevated CO_2, there were more similarities in the underlying functional pathways between the two parental phenotypes for all three tissues (Figure 2(b)). Therefore, although there were some common transcriptional responses in offspring with sensitive and tolerant parents, overall offspring of tolerant parents showed a much stronger transcriptional response to elevated CO_2 in general and also had a stronger signature of intergenerational plasticity.

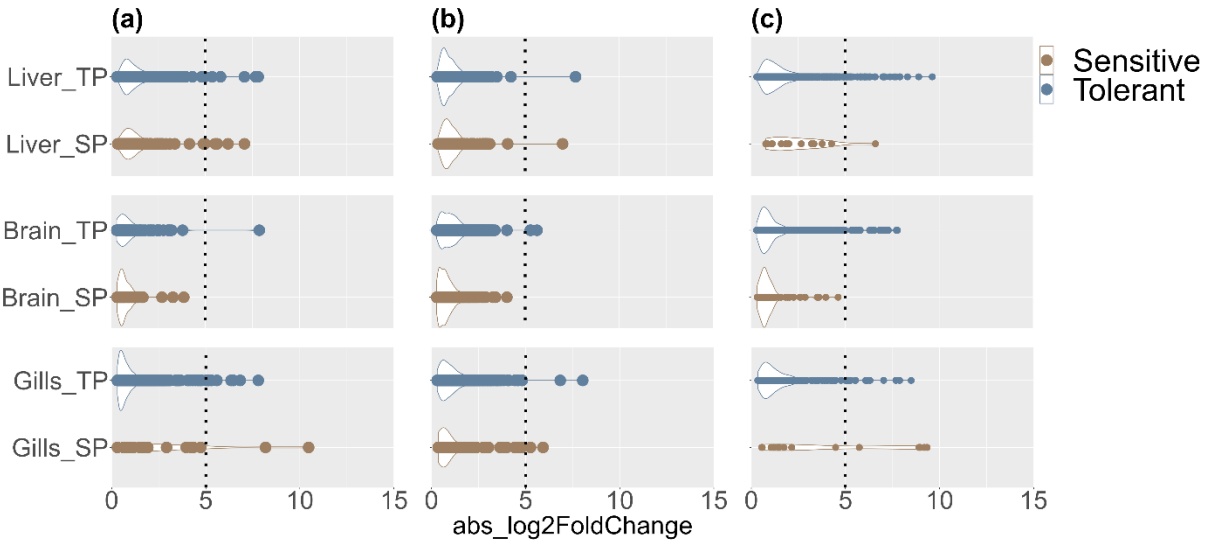

Figure 3: Log2 fold change in expression of genes involved in (a) overall CO_2 response, (b) rescue pattern, and (c) intergenerational specific response across all three tissues. SP indicates samples with a sensitive parental phenotype and TP indicates samples with a tolerant parental phenotype.

Discussion

The transcriptional landscape of three key tissues exhibited shared and tissue-specific signatures driving intergenerational acclimation to elevated CO_2 in a marine fish. Specifically, we found that gills are critical in maintaining overall cellular homeostasis in elevated CO_2 treatments and that the brain and liver had the greatest signal of intergenerational acclimatory response. In fact, intergenerationally treated fish no longer showed molecular signatures of altered neural signaling in the brain and reduced capacity for energy production in the liver that were seen in the developmental (within generation) treatment. Indeed, a new complement of genes involved in metabolism were upregulated only in offspring of parents who were previously exposed to elevated CO_2 indicating improved capability for energy production at a systemic level. *A. polyacanthus* is known to have a highly plastic genome enabling it to respond and acclimate to environmental changes (Bernal et al., 2020; Kang et al., 2022) and our results show that this persists across generations potentially enabling this species to rapidly acclimate to the changing ocean environment.

Genes that are always differentially expressed in elevated CO_2 conditions regardless of the type of exposure are key genes in the general response to ocean acidification (OA). The gills and liver exhibited a higher transcriptional response in all elevated CO_2 treatments suggesting that these tissues play an important role in the overall response of the fish to elevated CO_2. Genes involved in primary carbon metabolism, a process known to be altered under conditions of OA (Strader et al., 2020), were DE in both the gills and liver. This included genes in the tricarboxylic acid (TCA) cycle and the pentose phosphate pathway. Both these processes yield precursors for biosynthetic pathways and cofactors essential to maintain cellular homeostasis thereby influencing cellular processes beyond carbohydrate catabolism for energy (ATP) production (Gansemer et al., 2020). Therefore, the upregulation of these genes could indicate redirection of metabolic carbon fluxes to meet cellular demands for various cofactors

needed in the cellular stress response (CSR) pathways (Gansemer et al., 2020; Rokitta et al., 2012; Walsh & Milligan, 1993), which is often induced upon exposure to elevated CO_2 levels (Strader et al., 2020). In fact, various genes involved in CSR were found to be upregulated in the gills and brain. Therefore, there seems to be a systemic regulation of primary carbon metabolism in response to elevated CO_2 exposure to ensure sufficient production of precursors and cofactors needed for other biological processes.

Another key function we found to be required with elevated CO_2 is the immune response. Interestingly, genes involved in immune response were upregulated in the gills but downregulated in the liver indicating tissue-specific regulation of this function. Gills are one of the major surface tissues, which are continuously exposed to the external environment, and also serve as a first line of defense against potential infections (Harper & Wolf, 2009; Hu et al., 2023). Activation of immune responses has been previously observed under elevated CO_2 conditions which could be a preventive measure against opportunistic infections under CO_2 stress (De Souza et al., 2014; Machado et al., 2020). Hence the upregulation of genes involved in immune and stress response in the gills could be a protective mechanism to prevent cell damage caused by exposure to high CO_2.

Parental exposure to altered environmental conditions can pre-acclimate the offspring transcriptome to these new conditions via intergenerational plasticity. The expression of genes involved in key functional pathways were altered in juvenile *A. polyacanthus* upon developmental exposure to elevated CO_2 but were restored to control levels in the intergenerationally treated fish. These included genes involved in metabolism, synaptic plasticity and signaling, and RNA processing and transcription regulation. Intergenerationally exposed fish did not show transcriptional signatures of metabolic depression that was observed upon developmental CO_2 exposure in the liver, the major organ for metabolism. Specifically, fish exposed to elevated CO_2 during development, but not intergenerationally, exhibited

reduced capacity for energy production marked by downregulation of lipid and glucose metabolism, and genes involved in iron-sulfur (Fe-S) cluster assembly, which function as cofactors in the mitochondrial respiratory chain. In addition, there was upregulation of PCK1, a gluconeogenic enzyme (Yu et al., 2021) and AGL, involved in glycogen breakdown (Ni et al., 2022), which could indicate glucose shortage for energy production when the offspring are only exposed to elevated CO_2 within their lifetime. Metabolic suppression is a commonly observed physiological response to CO_2 stress (Strader et al., 2020), however, the expression levels of the above-mentioned genes returned to control levels with previous parental exposure, revealing that parental conditioning to elevated CO_2 restores the metabolic capacity of the offspring.

Furthermore, the expression of genes involved in synaptic plasticity and calcium channel activity were altered in the developmental treatment, predominantly in the brain, but were similar to control levels in the intergenerationally treated fish. Exposure to OA conditions has been shown to affect neural plasticity and neurogenesis in some fish species, which could result in changes in neural circuitry and signaling (Costa et al., 2022; Lai et al., 2017). While neural plasticity could facilitate increased flexibility to environmental changes (Ebbesson & Braithwaite, 2012), it could result in behavioural alterations that have been observed in fish exposed to OA conditions (Schunter et al., 2019). Increased GABAergic signaling is a commonly observed within-generation response to elevated CO_2 in *A. polyacanthus* (Schunter et al., 2018) and a similar increase in neural signaling pathways was found in the olfactory epithelium of *D. labrax* even after prolonged transgenerational OA exposure for two generations (Cohen-Rengifo et al. 2022). The restoration of OA induced changes in neural signaling processes with previous parental exposure to elevated CO_2 could indicate elevated intergenerational plasticity of *A. polyacanthus* compared to other species. Synaptic plasticity is regulated by the cytoskeleton (Gordon-Weeks & Fournier, 2014; Zapara et al., 2000) and

intergenerationally treated fish did not show changes in expression of various cytoskeleton and cell adhesion genes seen in the developmental treatment. Therefore, intergenerational CO_2 exposure restores the dynamic equilibrium of cytoskeletal proteins, thereby re-establishing synaptic signaling processes to control levels.

Transcriptional regulatory elements were identified to be key in regulating the response to elevated CO_2. Transcription factors and RNA-mediated gene silencers mainly mediated plasticity in the within-generation response to elevated CO_2. Changes in the external environment can trigger reprogramming of transcriptional networks resulting in dynamic regulation of gene expression (Swift & Coruzzi, 2017) as also suggested in wild fish populations naturally exposed to elevated CO_2 (Petit-Marty et al., 2021). Therefore, these regulatory genes could play a key role in developmental plastic responses to elevated environmental CO_2 levels, however, these are no longer needed in intergenerationally treated fish. The overall "rescue" of various key functional pathways upon intergenerational exposure to elevated CO_2 suggests parental priming of the offspring transcriptome enabling acclimation of future generations to OA.

We also found a new complement of genes to be differentially expressed only when the parents are exposed to the same elevated CO_2 condition as their offspring, which could further facilitate acclimation of the offspring enabling them to better cope with an elevated CO_2 environment. Such intergenerational specific transcriptional signature was observed in genes involved in ion transport resulting in bicarbonate retention in the brain and gills. Increasing internal bicarbonate ion concentrations is a commonly observed compensatory response in fish to buffer acid-base disturbance caused by exposure to elevated CO_2 levels (Heuer & Grosell, 2014). Differential expression (DE) of bicarbonate transporters in the intergenerationally treated fish suggests that parental conditioning enables the offspring to more effectively buffer pH changes (Brauner et al., 2019; Chen et al., 2009; Choi, 2012.; Esbaugh, 2017). Furthermore,

changes in potassium channel activity enables the fish to sense pH disturbances (Hibino et al., 2010; Qin et al., 2010) and in turn initiate compensatory responses to maintain homeostasis. Therefore, intergenerational CO_2 exposure could have resulted in transcriptional rearrangements of ion transporters in the offspring, especially in the brain and gills, to maintain pH homeostasis primarily by bicarbonate retention rather that acid extrusion. While changes in ion channel activity can be an efficient mechanism to buffer pH changes and prevent acidosis in elevated CO_2 conditions, it is an energy demanding process (Ishimatsu et al., 2005; Auffret et al., 2023). However, parental conditioning to elevated CO_2 also increased the offspring's capacity of energy metabolism. Transcriptional signatures indicating metabolic suppression observed in the developmental treatment was no longer observed in the intergenerationally treated fish. In fact, when considering only the intergeneration-specific transcriptional response, metabolic processes including mitochondrial electron transport were upregulated in all three tissues, but particularly in the liver. Exposure to elevated CO_2 for prolonged periods results in increased energetic costs (Araújo et al. 2018; Schunter et al. 2016, 2021; Tsang et al. 2020) and while liver is the main tissue involved in metabolism (Auffret et al., 2023), the shared upregulation of metabolic genes in all three tissues suggests an increase in metabolic processes in a whole-body context. Therefore, parental conditioning to elevated CO_2 may enable the offspring to meet the energetic demands associated with living in a high CO_2 environment.

One of the mechanisms by which parental experiences influence the next generation is by the transfer of epigenetic factors (Perez & Lehner, 2019). Here, we found genes involved in chromatin remodeling to be DE exclusively in the liver. These genes are known to affect chromatin compaction and accessibility thereby controlling fundamental cellular processes such as transcription, DNA damage response and repair, cellular proliferation, and apoptosis (Allen et al., 2013; Jacquet et al., 2016; Kalakonda et al., 2008; Nady et al., 2012; Torchy et al., 2015). Epigenetic mechanisms have been reported to play a role in plasticity and

460 acclimation to environmental changes including elevated temperature and CO_2 levels
461 (Anastasiadi et al., 2017; Fuxjäger et al., 2019; Lighten et al., 2016; Ryu et al., 2018; Veilleux
462 et al., 2015). This could be the case here with parental exposure to elevated CO_2 conditions
463 influencing the offsprings' liver epigenome via remodeling the chromatin thereby regulating
464 the expression of genes involved in diverse biological processes.

465 Parental behavioural phenotype has been shown to influence the offspring brain
466 transcriptional response to elevated CO_2 in *A. polyacanthus* (Monroe et al., 2021), with
467 behavioural tolerance being heritable (Welch & Munday, 2017). We observed a stronger
468 transcriptional response to elevated CO_2 exposure with higher number of DE genes and larger
469 fold changes in the offspring of tolerant parental phenotype across all three tissues. Selection
470 experiments have indicated a genetic basis for individual variation in OA induced responses in
471 a variety of animals (Langer et al. 2009; Parker et al., 2011; Pistevos et al. 2011; Sunday et al.
472 2011), including the behavioural phenotype to chemical alarm cues in elevated CO_2 in *A.*
473 *polyacanthus* (Lehmann et al. 2022). This intraspecific variation in organismal response is key
474 in driving future adaptive evolution. Here, we found that offspring of parents with a tolerant
475 behavioural phenotype had an increased capacity for intergenerational plasticity in their
476 transcriptome, which in turn suggests greater adaptive potential to future ocean acidification
477 conditions.

478 In this study we used a systematic approach by incorporating multiple factors including
479 parental behavioral variability, parental environment, and multiple tissues to provide greater
480 predictive power in estimating the adaptive potential of species to future ocean conditions. We
481 determine the extent of transcriptional reprogramming induced by elevated CO_2 in three major
482 tissue groups and reveal cross-tissue communication facilitating acclimation to future OA
483 conditions. The gills were especially sensitive to all elevated CO_2 treatments regardless of the
484 length of exposure. Parental exposure to elevated CO_2 conditions had a substantial influence

485 on the offspring transcriptome, with key functional pathways that were altered in the
486 developmental treatment being "rescued" in the intergenerationally exposed fish in the brain
487 and liver. Furthermore, intergeneration specific regulation revealed how previous parental
488 conditioning to OA can mediate reprogramming of the offspring transcriptome, including
489 energy metabolism and acid-base homeostasis in all tissues and epigenetic-related genes in the
490 liver. Overall, we found that both parental behavioural phenotype as well as the parental
491 environment influence offspring transcriptome in response to elevated CO_2. Our study reveals
492 how intergenerational plasticity is facilitated from a whole-organism perspective and illustrates
493 how transcriptional changes across multiple tissues integrate to drive potential adaptation to
494 the changing ocean chemistry.
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496

Author contributions

The experiment was designed and run by MJW and PLM. Molecular lab work was performed by CS and sequenced by TR. SS carried out the transcriptome expression analysis with input from CS. SS lead the writing of the manuscript with input from CS and all authors read, edited and approved the final manuscript.

Ethics

Sample collection was carried out following all institutional and national law guidelines. The experiment was completed under James Cook University ethics approval A1828.

Competing financial interests

All authors declare they have no competing interests.

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Data availability

The brain RNA-Seq raw sequences are deposited in NCBI under BioProject ID PRJNA311159. The gills and liver RNA-Seq raw sequences are deposited in NCBI under BioProject ID PRJNA989422 (https://dataview.ncbi.nlm.nih.gov/object/PRJNA989422?reviewer=q3n0q75hbbf2p1v85n0he3uv86).

References

Allan, B. J. M., Miller, G. M., Mccormick, M. I., Domenici, P., & Munday, P. L. (2014). Parental effects improve escape performance of juvenile reef fish in a high-CO_2 world. *Proceedings of the Royal Society B: Biological Sciences*, 281(1777). https://doi.org/10.1098/RSPB.2013.2179

Allen, H. F., Wade, P. A., & Kutateladze, T. G. (2013). The NuRD architecture. *Cellular and Molecular Life Sciences*, 70, 3513-3524.

Anastasiadi, D., Díaz, N., & Piferrer, 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), 12401. https://doi.org/10.1038/s41598-017-10861-6

Andrews, S. (2010). FastQC: a quality control tool for high throughput sequence data.

Araújo, J. E., Madeira, D., Vitorino, R., Repolho, T., Rosa, R., & Diniz, M. (2018). Negative synergistic impacts of ocean warming and acidification on the survival and proteome of the commercial sea bream, Sparus aurata. *Journal of Sea Research*, 139, 50–61. https://doi.org/10.1016/j.seares.2018.06.011

Auffret, P., Servili, A., Gonzalez, A. A., Fleury, M. L., Mark, F. C., & Mazurais, D. (2023). Transgenerational exposure to ocean acidification impacts the hepatic transcriptome of European sea bass (*Dicentrarchus labrax*). *BMC genomics*, 24(1), 1-11.

Bernal, M. A., Schunter, C., Lehmann, R., Lightfoot, D. J., Allan, B. J. M., Veilleux, H. D., Rummer, J. L., Munday, P. L., & Ravasi, T. (2020). Species-specific molecular responses of wild coral reef fishes during a marine heatwave. *Science Advances*, 6(12). https://doi.org/10.1126/sciadv.aay3423

Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*, 30(15), 2114–2120. https://doi.org/10.1093/BIOINFORMATICS/BTU170

Brauner, C. J., Shartau, R. B., Damsgaard, C., Esbaugh, A. J., Wilson, R. W., & Grosell, M. (2019). Acid-base physiology and CO_2 homeostasis: Regulation and compensation in response to elevated environmental CO_2. In *Fish Physiology* (Vol. 37, pp. 69–132). Academic Press. https://doi.org/10.1016/BS.FP.2019.08.003

Chen, J. H., Cai, Z., & Sheppard, D. N. (2009). Direct Sensing of Intracellular pH by the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) Cl Channel*♦. *Journal of Biological Chemistry*, 284(51), 35495-35506. https://doi.org/10.1074/jbc.M109.072678

Choi, I. (2012). SLC4A transporters. In *Current topics in membranes* (Vol. 70, pp. 77-103). Academic Press.

Costa, R. A., Olvera, A., Power, D. M., & Velez, Z. (2022). Ocean acidification affects the expression of neuroplasticity and neuromodulation markers in seabream. *Biology Open*, 11(3). https://doi.org/10.1242/bio.059073

Crespel, A., Anttila, K., Lelièvre, P., Quazuguel, P., Le Bayon, N., Zambonino-Infante, J. L., Chabot, D., & Claireaux, G. (2019). Long-term effects of ocean acidification upon energetics and oxygen transport in the European sea bass (*Dicentrarchus labrax*, Linnaeus). *Marine Biology*, 166(9), 116. https://doi.org/10.1007/s00227-019-3562-9

De Souza, K. B., Jutfelt, F., Kling, P., Förlin, L., & Sturve, J. (2014). Effects of Increased CO_2 on Fish Gill and Plasma Proteome. *PLOS ONE*, 9(7), e102901. https://doi.org/10.1371/JOURNAL.PONE.0102901

Deigweiher, K., Hirse, T., Bock, C., Lucassen, M., & Pörtner, H. O. (2010). Hypercapnia induced shifts in gill energy budgets of Antarctic notothenioids. *Journal of Comparative Physiology B*, 180(3), 347–359. https://doi.org/10.1007/s00360-009-0413-x

Deigweiher, K., Koschnick, N., Pörtner, H. O., & Lucassen, M. (2008). Acclimation of ion regulatory capacities in gills of marine fish under environmental hypercapnia. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 295(5), R1660–R1670. https://doi.org/10.1152/ajpregu.90403.2008

Donelson, J. M., Munday, P. L., McCormick, M. I., & Pitcher, C. R. (2012). Rapid transgenerational acclimation of a tropical reef fish to climate change. *Nature Climate Change*, 2(1), 30–32. https://doi.org/10.1038/nclimate1323

Ebbesson, L. O. E., & Braithwaite, V. A. (2012). Environmental effects on fish neural plasticity and cognition. *Journal of Fish Biology*, 81(7), 2151–2174. https://doi.org/10.1111/j.1095-8649.2012.03486.x

Esbaugh, A. J. (2017). Physiological implications of ocean acidification for marine fish: emerging patterns and new insights. *Journal of Comparative Physiology B*, 188(1), 1–13. https://doi.org/10.1007/S00360-017-1105-6

Frommel, A. Y., Hermann, B. T., Michael, K., Lucassen, M., Clemmesen, C., Hanel, R., & Reusch, T. B. H. (2020). Differential gene expression patterns related to lipid metabolism in response to ocean acidification in larvae and juveniles of Atlantic cod. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 247, 110740. https://doi.org/10.1016/j.cbpa.2020.110740

Fuxjäger, L., Wanzenböck, S., Ringler, E., Wegner, K. M., Ahnelt, H., & Shama, L. N. S. (2019). Within-generation and transgenerational plasticity of mate choice in oceanic stickleback under climate change. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 374(1768), 20180183. https://doi.org/10.1098/rstb.2018.0183

Gansemer, E. R., McCommis, K. S., Martino, M., King-McAlpin, A. Q., Potthoff, M. J., Finck, B. N., Taylor, E. B., & Rutkowski, D. T. (2020). NADPH and Glutathione Redox Link TCA Cycle Activity to Endoplasmic Reticulum Homeostasis. *IScience*, 23(5), 101116. https://doi.org/10.1016/J.ISCI.2020.101116

Gordon-Weeks, P. R., & Fournier, A. E. (2014). Neuronal cytoskeleton in synaptic plasticity and regeneration. *Journal of Neurochemistry*, 129(2), 206–212. https://doi.org/10.1111/JNC.12502

Gräns, A., Jutfelt, F., Sandblom, E., Jönsson, E., Wiklander, K., Seth, H., Olsson, C., Dupont, S., Ortega-Martinez, O., Einarsdottir, I., Björnsson, B. T., Sundell, K., & Axelsson, M. (2014). Aerobic scope fails to explain the detrimental effects on growth resulting from warming and elevated CO_2 in Atlantic halibut. *Journal of Experimental Biology*, 217(5), 711–717. https://doi.org/10.1242/jeb.096743

Grosell M., Munday P. L., Farrell A. P., Brauner C. J. (2019). Fish Physiology Volume 37: Carbon Dioxide (Amsterdam: Elsevier).

Harper, C., & Wolf, J. C. (2009). Morphologic Effects of the Stress Response in Fish. *ILAR*

Journal, 50(4), 387–396. https://doi.org/10.1093/ILAR.50.4.387

Heuer, R. M., & Grosell, M. (2014). Physiological impacts of elevated carbon dioxide and ocean acidification on fish. *American Journal of Physiology - Regulatory Integrative and Comparative Physiology*, 307(9), R1061–R1084. https://doi.org/10.1152/ajpregu.00064.2014

Hibino, H., Inanobe, A., Furutani, K., Murakami, S., Findlay, I., & Kurachi, Y. (2010). Inwardly rectifying potassium channels: Their structure, function, and physiological roles. *Physiological Reviews*, 90(1), 291–366. https://doi.org/10.1152/physrev.00021.2009

Hu, X., Bai, J., Liu, R., & Lv, A. (2023). Comprehensive transcriptomics and proteomics analysis of *Carassius auratus* gills in response to *Aeromonas hydrophila*. *Fish and Shellfish Immunology Reports*, 4, 100077. https://doi.org/10.1016/J.FSIREP.2022.100077

Huang, R., Ding, J., Gao, K., De Carvalho, M. H. C., Tirichine, L., Bowler, C., & Lin, X. (2019). A potential role for epigenetic processes in the acclimation response to elevated pCO_2 in the model diatom phaeodactylum tricornutum. *Frontiers in Microbiology*, 9, 3342. https://doi.org/10.3389/fmicb.2018.03342

Ishimatsu, A., Hayashi, M., Lee, K. S., Kikkawa, T., & Kita, J. (2005). Physiological effects on fishes in a high-CO_2 world. *Journal of Geophysical Research: Oceans*, 110(C9), 1–8. https://doi.org/10.1029/2004JC002564

Kang, J., Nagelkerken, I., Rummer, J. L., Rodolfo-Metalpa, R., Munday, P. L., Ravasi, T., & Schunter, C. (2022). Rapid evolution fuels transcriptional plasticity to ocean acidification. *Global Change Biology*, 28(9), 3007-3022. https://doi.org/10.1111/GCB.16119

Kim, D., Paggi, J. M., Park, C., Bennett, C., & Salzberg, S. L. (2019). Graph-based genome alignment and genotyping with HISAT2 and HISAT-genotype. *Nature Biotechnology* 37(8), 907-915. https://doi.org/10.1038/s41587-019-0201-4

Lai, F., Fagernes, C. E., Bernier, N. J., Miller, G. M., Munday, P. L., Jutfelt, F., & Nilsson, G. E. (2017). Responses of neurogenesis and neuroplasticity related genes to elevated CO_2 levels in the brain of three teleost species. *Biology Letters*, 13(8). https://doi.org/10.1098/rsbl.2017.0240

Lee, D. W., Song, J. A., Park, H.-S., & Choi, C. Y. (2021). Circadian Rhythm Disturbances Due to Exposure to Acidified Conditions and Different Photoperiods in Juvenile Olive Flounder (*Paralichthys olivaceus*). *Ocean Science Journal*, 56(2), 198–206. https://doi.org/10.1007/s12601-021-00018-y

Lee, Y. H., Kang, H. M., Kim, M. S., Lee, J. S., Wang, M., Hagiwara, A., Jeong, C. B., & Lee, J. S. (2020). Multigenerational Mitigating Effects of Ocean Acidification on *In Vivo* Endpoints, Antioxidant Defense, DNA Damage Response, and Epigenetic Modification in an Asexual Monogonont Rotifer. *Environmental Science & Technology*, 54(13), 7858–7869. https://doi.org/10.1021/acs.est.0c01438

Lee, Y. H., Kim, M. S., Wang, M., Bhandari, R. K., Park, H. G., Wu, R. S. S., & Lee, J. S. (2022). Epigenetic plasticity enables copepods to cope with ocean acidification. *Nature Climate Change*, 12(10), 918–927. https://doi.org/10.1038/s41558-022-01477-4

Lehmann, R., Schunter, C., Welch, M. J., Arold, S. T., Nilsson, G. E., Tegner, J. N., Munday, P. L., & Ravasi, T. (2022). Genetic architecture of behavioural resilience to ocean acidification. *BioRxiv*, 2022.10.18.512656. https://doi.org/10.1101/2022.10.18.512656

Liao, Y., Smyth, G. K., & Shi, W. (2014). featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics*, 30(7), 923–930. https://doi.org/10.1093/BIOINFORMATICS/BTT656

Lighten, J., Incarnato, D., Ward, B. J., van Oosterhout, C., Bradbury, I., Hanson, M., & Bentzen, P. (2016). Adaptive phenotypic response to climate enabled by epigenetics in a K-strategy species, the fish *Leucoraja ocellata* (Rajidae). *Royal Society Open Science*, 3(10), 160299. https://doi.org/10.1098/rsos.160299

Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*, 15(12), 1–21. https://doi.org/10.1186/s13059-014-0550-8

Machado, M., Arenas, F., Svendsen, J. C., Azeredo, R., Pfeifer, L. J., Wilson, J. M., & Costas, B. (2020). Effects of Water Acidification on Senegalese Sole Solea senegalensis Health Status and Metabolic Rate: Implications for Immune Responses and Energy Use. *Frontiers in Physiology*, 11, 26. https://doi.org/10.3389/FPHYS.2020.00026/BIBTEX

Miller, G. M., Watson, S. A., Donelson, J. M., McCormick, M. I., & Munday, P. L. (2012). Parental environment mediates impacts of increased carbon dioxide on a coral reef fish. *Nature Climate Change*, 2(12), 858-861. https://doi.org/10.1038/NCLIMATE1599

Monroe, A. A., Schunter, C., Welch, M. J., Munday, P. L., & Ravasi, T. (2021). Molecular basis of parental contributions to the behavioural tolerance of elevated pCO_2 in a coral reef fish. *Proceedings of the Royal Society B: Biological Sciences*, 288(1964). https://doi.org/10.1098/rspb.2021.1931

Munday, P. L. (2014). Transgenerational acclimation of fishes to climate change and ocean acidification. *F1000Prime Reports*, 6. https://doi.org/10.12703/P6-99

Munday, P. L., McCormick, M. I., Meekan, M., Dixson, D. L., Watson, S. A., Chivers, D. P., & Ferrari, M. C. O. (2013). Selective mortality associated with variation in CO_2 tolerance in a marine fish. *Ocean Acidification*, 1, 1–5. https://doi.org/10.2478/OAC-2012-0001

Nagelkerken, I., Allan, B. J., Booth, D. J., Donelson, J. M., Edgar, G. J., Ravasi, T., Rummer, J. L., Vergés, A., & Mellin, C. (2023). The effects of climate change on the ecology of fishes. *PLOS Climate*, 2(8), e0000258.

Ni, J., Zhu, P., Mo, Q., Luo, W., Du, Z., Jiang, J., Yang, S., Zhao, L., Gong, Q., & Wang, Y. (2022). Dynamic Transcriptomic Profiling During Liver Development in Schizothorax Prenanti. *Frontiers in Physiology*, 13, 1397. https://doi.org/10.3389/FPHYS.2022.928858/BIBTEX

OmicsBox – Bioinformatics Made Easy, BioBam Bioinformatics, March 3, 2019, https://www.biobam.com/omicsbox

Parker, L. M., Ross, P. M., & O'Connor, W. A. (2011). Populations of the Sydney rock oyster, *Saccostrea glomerata*, vary in response to ocean acidification. *Marine Biology*, 158, 689-697.

Perez, M. F., & Lehner, B. (2019). Intergenerational and transgenerational epigenetic

689 inheritance in animals. *Nature Cell Biology*, 21(2), 143–151.
690 https://doi.org/10.1038/s41556-018-0242-9

691 Petit-Marty, N., Nagelkerken, I., Connell, S. D., & Schunter, C. (2021). Natural CO_2 seeps
692 reveal adaptive potential to ocean acidification in fish. *Evolutionary Applications*, 14(7),
693 1794–1806. https://doi.org/10.1111/eva.13239

694 Pimentel, M., Pegado, M., Repolho, T., & Rosa, R. (2014). Impact of ocean acidification in
695 the metabolism and swimming behavior of the dolphinfish (*Coryphaena hippurus*) early
696 larvae. *Marine Biology*, 161(3), 725–729. https://doi.org/10.1007/s00227-013-2365-7

697 Pörtner, H. O., Roberts, D. C., Masson-Delmotte, V., Zhai, P., Tignor, M., Poloczanska, E.,
698 & Weyer, N. M. (2019). The ocean and cryosphere in a changing climate. *IPCC special*
699 *report on the ocean and cryosphere in a changing climate*, 1155.

700 Qin, Z., Lewis, J. E., & Perry, S. F. (2010). Zebrafish (*Danio rerio*) gill neuroepithelial cells
701 are sensitive chemoreceptors for environmental CO_2. *The Journal of Physiology*, 588(5),
702 861-872. https://doi.org/10.1113/JPHYSIOL.2009.184739

703 R Core Team. (2021). *R:* A language and environment for statistical computing. R
704 Foundation for Statistical Computing, Vienna, Austria. https://www.r-project.org/.

705 Robertson, D. R. (1973). Field Observations on the Reproductive Behaviour of a Pomacentrid
706 Fish, *Acanthochromis polyacanthus*. *Zeitschrift Für Tierpsychologie*, 32(3), 319–324.
707 https://doi.org/10.1111/j.1439-0310.1973.tb01108.x

708 Rokitta, S. D., John, U., & Rost, B. (2012). Ocean Acidification Affects Redox-Balance and
709 Ion-Homeostasis in the Life-Cycle Stages of *Emiliania huxleyi*. *PLOS ONE*, 7(12),
710 e52212. https://doi.org/10.1371/JOURNAL.PONE.0052212

711 Rummer, J. L., Stecyk, J. A. W., Couturier, C. S., Watson, S.-A., Nilsson, G. E., & Munday,
712 P. L. (2013). Elevated CO_2 enhances aerobic scope of a coral reef fish. *Conservation*
713 *Physiology*, 1(1), cot023. https://doi.org/10.1093/conphys/cot023

714 Ryu, T., Veilleux, H. D., Donelson, J. M., Munday, P. L., & Ravasi, T. (2018). The
715 epigenetic landscape of transgenerational acclimation to ocean warming. *Nature Climate*
716 *Change*, 8(6), 504–509. https://doi.org/10.1038/s41558-018-0159-0

717 Schade, F. M., Clemmesen, C., Wegner, M., & Wegener, A. (2014). Within-and
718 transgenerational effects of ocean acidification on life history of marine three-spined
719 stickleback (*Gasterosteus aculeatus*). *Marine Biology*, 161(7), 1667–1676.
720 https://doi.org/10.1007/s00227-014-2450-6

721 Schunter, C., Jarrold, M. D., Munday, P. L., & Ravasi, T. (2021). Diel pCO_2 fluctuations alter
722 the molecular response of coral reef fishes to ocean acidification conditions. *Molecular*
723 *Ecology*, 30(20), 5105–5118. https://doi.org/10.1111/mec.16124

724 Schunter, C., Ravasi, T., Munday, P. L., & Nilsson, G. E. (2019). Neural effects of elevated
725 CO_2 in fish may be amplified by a vicious cycle. *Conservation Physiology*, 7(1).
726 https://doi.org/10.1093/CONPHYS/COZ100

727 Schunter, C., Welch, M. J., Nilsson, G. E., Rummer, J. L., Munday, P. L., & Ravasi, T.
728 (2018). An interplay between plasticity and parental phenotype determines impacts of
729 ocean acidification on a reef fish. *Nature Ecology & Evolution*, 2(2), 334–342.
730 https://doi.org/10.1038/s41559-017-0428-8

731 Schunter, C., Welch, M. J., Ryu, T., Zhang, H., Berumen, M. L., Nilsson, G. E., Munday, P.
732 L., & Ravasi, T. (2016). Molecular signatures of transgenerational response to ocean
733 acidification in a species of reef fish. *Nature Climate Change*, 6(11), 1014–1018.
734 https://doi.org/10.1038/nclimate3087

735 Stiasny, M. H., Mittermayer, F. H., Göttler, G., Bridges, C. R., Falk-Petersen, I. B.,
736 Puvanendran, V., Mortensen, A., Reusch, T. B. H., & Clemmesen, C. (2018). Effects of
737 parental acclimation and energy limitation in response to high CO_2 exposure in Atlantic
738 cod. *Scientific Reports*, 8(1), 1–8. https://doi.org/10.1038/s41598-018-26711-y

739 Strader, M. E., Wong, J. M., & Hofmann, G. E. (2020). Ocean acidification promotes broad
740 transcriptomic responses in marine metazoans: A literature survey. *Frontiers in Zoology*,
741 17(1), 1–23. https://doi.org/10.1186/s12983-020-0350-9

742 Swift, J., & Coruzzi, G. M. (2017). A matter of time — How transient transcription factor
743 interactions create dynamic gene regulatory networks. *Biochimica et Biophysica Acta*
744 *(BBA) - Gene Regulatory Mechanisms*, 1860(1), 75–83.
745 https://doi.org/10.1016/j.bbagrm.2016.08.007

746 Torchy, M. P., Hamiche, A., & Klaholz, B. P. (2015). Structure and function insights into the
747 NuRD chromatin remodeling complex. *Cellular and Molecular Life Sciences*, 72, 2491-
748 2507.

749 Tsang, H. H., Welch, M. J., Munday, P. L., Ravasi, T., & Schunter, C. (2020). Proteomic
750 Responses to Ocean Acidification in the Brain of Juvenile Coral Reef Fish. *Frontiers in*
751 *Marine Science*, 7, 605. https://doi.org/10.3389/FMARS.2020.00605/BIBTEX

752 Turner, B. M. (2009). Epigenetic responses to environmental change and their evolutionary
753 implications. *Philosophical Transactions of the Royal Society B: Biological Sciences*,
754 364(1534), 3403–3418. https://doi.org/10.1098/rstb.2009.0125

755 Veilleux, H. D., Ryu, T., Donelson, J. M., van Herwerden, L., Seridi, L., Ghosheh, Y.,
756 Berumen, M. L., Leggat, W., Ravasi, T., & Munday, P. L. (2015). Molecular processes
757 of transgenerational acclimation to a warming ocean. *Nature Climate Change*, 5(12),
758 1074–1078. https://doi.org/10.1038/nclimate2724

759 Walsh, P. J., & Milligan, C. L. (1993). Roles of Buffering Capacity and Pentose Phosphate
760 Pathway Activity in the Gas Gland of the Gulf Toadfish Opsanus Beta. *Journal of*
761 *Experimental Biology*, 176(1), 311–316. https://doi.org/10.1242/JEB.176.1.311

762 Welch, M. J., & Munday, P. L. (2017). Heritability of behavioural tolerance to high CO_2 in a
763 coral reef fish is masked by nonadaptive phenotypic plasticity. *Evolutionary*
764 *Applications*, 10(7), 682–693. https://doi.org/10.1111/eva.12483

765 Williams, C. R., Dittman, A. H., McElhany, P., Busch, D. S., Maher, M. T., Bammler, T. K.,
766 MacDonald, J. W., & Gallagher, E. P. (2019). Elevated CO_2 impairs olfactory-mediated
767 neural and behavioral responses and gene expression in ocean-phase coho salmon
768 (*Oncorhynchus kisutch*). *Global Change Biology*, 25(3), 963–977.
769 https://doi.org/10.1111/gcb.14532

770 Wood, D. E., & Salzberg, S. L. (2014). Kraken: Ultrafast metagenomic sequence
771 classification using exact alignments. *Genome Biology*, 15(3).
772 https://doi.org/10.1186/gb-2014-15-3-r46

773 Yu, S., Meng, S., Xiang, M., & Ma, H. (2021). Phosphoenolpyruvate carboxykinase in cell

774 metabolism: Roles and mechanisms beyond gluconeogenesis. *Molecular Metabolism*,
775 53. https://doi.org/10.1016/J.MOLMET.2021.101257

776 Zapara, T. A., Simonova, O. G., Zharkikh, A. A., & Ratushnyak, A. S. (2000). The Effects of
777 the Dynamic State of the Cytoskeleton on Neuronal Plasticity. *Translated from*
778 *Rossiiskii Fiziologicheskii Zhurnal Imeni I. M. Sechenova, I/Ol*, 30(3), 128–138.

779