

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

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21 **Abstract**

22 An organism's reaction to environmental changes is mediated by coordinated responses of
23 multiple tissues. Additionally, parental priming may increase offsprings' acclimation potential
24 to changing environmental conditions. As acidification of oceans continues to intensify it is
25 critical to assess the acclimation potential of species at the whole organismal scale. To do this
26 we need to understand the cross-talk between tissues in regulating and responding to pH
27 changes. Here by using a multi-tissue approach we determine the influence of 1) variation in
28 parental behavioural tolerance and 2) parental environment, on molecular responses of their
29 offspring in a coral reef fish. The gills and liver showed the highest transcriptional response to
30 OA conditions in juvenile fish regardless of the parental environment, while the brain and liver
31 showed the greatest signal of intergenerational acclimation. Key functional pathways that were
32 altered in the brain and liver upon within-generational CO₂ exposure were restored to control
33 levels when parents were exposure to OA conditions. Furthermore, the expression of a new
34 complement of genes involved in key functions were altered in the offspring only when the
35 parents were previously exposed to OA conditions. Therefore, previous parental conditioning
36 to ocean acidification can reprogram tissue transcriptomic profiles of the offspring enabling
37 them to better cope in an environment with elevated CO₂ levels. Overall, our results show that
38 intergenerational plasticity is key in evolutionarily adaptation to global change and illustrates
39 how transcriptional changes across multiple tissues integrate to facilitate organismal
40 acclimation to OA.

41

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

44

45 **Significance statement**

46 With the global climate changing rapidly, organisms need to acclimate to the new
47 conditions to survive. Assessing the adaptive potential of complex organisms such as
48 vertebrates is especially challenging as each tissue has its own unique function. However,
49 acclimation of organisms to changes in their environment requires functional integration of all
50 tissues which is usually overlooked in climate change research. Here we reveal that cross-
51 communication between tissues is crucial in the adaptive response of organisms to future ocean
52 conditions. Furthermore, both parental environment and parental behavioral variability
53 influence the transcriptional reprogramming of offspring tissues in response to elevated CO₂.
54 Overall, it is the integration of transcriptional changes across multiple tissues that mediates
55 intergenerational plasticity to future changes in ocean chemistry.

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57

58 **Introduction**

59 With the global climate continuously shifting to more extreme conditions organisms
60 need to acclimate and/or adapt to the changing environments in order to survive. The oceans
61 are becoming increasingly acidified as they absorb a major portion of anthropogenic CO₂
62 emissions (Pörtner et al., 2022) leading to ocean acidification (OA) which is reported to
63 negatively impact the physiology and behavior of various marine organisms including fish
64 (Heuer & Grosell, 2014; Strader et al., 2020). However, increasing evidence suggests that
65 multi-generational exposure to elevated CO₂ conditions could influence the adaptive capacity
66 of future generations to OA conditions (Nagelkerken et al., 2023). In fact, several studies have
67 reported transgenerational acclimation in a number of fish species as well as some invertebrates
68 to OA (Strader et al., 2020). Specifically, transgenerational exposure to elevated CO₂
69 conditions has been shown to facilitate acclimation of metabolism, growth, survival, neuronal
70 plasticity and behavior in independent studies (Allan et al., 2014; Miller et al., 2012; Monroe
71 et al., 2021; Munday, 2014; Schade et al., 2014; Schunter et al., 2016, 2018; Stiasny et al.,
72 2018) however, we are still learning about the underlying molecular mechanisms of such
73 acclimation process.

74 Additionally, variation both within and across species in the biological responses to OA
75 also exists due to differences in their evolutionary and environmental history. Studies
76 examining the effect of elevated CO₂ on metabolism, growth, development, and reproduction
77 in fish show variable results with some species being more affected than others (Heuer &
78 Grosell, 2014). Variation in sensitivity to elevated CO₂ within a population could be crucial in
79 long-term adaptation through selection of more tolerant individuals. Indeed, individual
80 variation in behavioural tolerance to elevated CO₂ exposure has been reported to be heritable
81 and hence could facilitate rapid selection of tolerant genotypes in the population (Lehmann et
82 al., 2022; Welch & Munday, 2017). Such selection for CO₂ tolerance has been shown to occur

83 in nature, which could result in populations consisting of individuals with greater behavioural
84 tolerance to elevated CO₂ (Munday et al., 2013). Furthermore, inter-individual variation in
85 sensitivity to ocean acidification could have an epigenetic basis (Ryu et al., 2018; Turner, 2009)
86 and in fact several studies have reported the expression levels of genes involved in epigenetic
87 processes to be altered upon exposure to elevated CO₂ conditions (Huang et al., 2019; Schunter
88 et al., 2018). Transfer of epigenetic factors from parents to offspring (epigenetic inheritance)
89 could be one of the potential mechanisms of inter- and trans-generational acclimation and
90 eventual adaptation to OA.

91 Adaptive processes to environmental changes at the organismal level requires
92 integrated activity of various tissues, with each tissue undergoing changes in its transcriptional
93 landscape resulting in the overall response of the organism. However, to date, research has
94 mainly focused on individual tissue functional changes in response to OA with less emphasis
95 on how these changes integrate to create a whole-body response. Several studies have
96 examined the effects of OA on brain and neurosensory systems since the discovery of impaired
97 behavioural responses in various fish species in elevated CO₂ conditions. The altered
98 behavioural responses have been linked to changes in the functioning of the GABAergic
99 signaling pathway (Schunter et al., 2019) and the circadian rhythm in the brain of fish exposed
100 to elevated CO₂ (Lee et al., 2021; Schunter et al., 2016; Williams et al., 2019). Previous studies
101 have also focused on the effects of OA conditions on the gill transcriptome due to it being the
102 primary organ involved in acid-base regulation, immune defences, and stress response, and
103 hence plays a vital role in maintaining cellular homeostasis under conditions of CO₂ stress (De
104 Souza et al., 2014; Deigweiher et al., 2008, 2010). These processes are energetically expensive
105 and indeed changes in the aerobic metabolic scope (Crespel et al., 2019; Gräns et al., 2014;
106 Pimentel et al., 2014; Rummer et al., 2013) and expression levels of key metabolic genes
107 (Frommel et al., 2020) have been reported in fish exposed to elevated CO₂. Therefore, exposure

108 to elevated CO₂ affects various aspects of fish physiology such as metabolism, cellular redox
109 status, ion transport and acid-base homeostasis, neurological functioning and behavior thereby
110 exerting a whole-body functional reprogramming (Grosell et al. 2019). Therefore, a systematic
111 transcriptomic analysis is needed to determine how the biological processes associated with
112 each tissue integrate together within the whole-organism to drive adaptive responses to
113 elevated CO₂ environments.

114 In this study we conducted an intergenerational CO₂ exposure experiment and
115 performed systematic analysis of gene expression changes in response to elevated CO₂ across
116 three tissues, the brain, the gills, and the liver, in the spiny damselfish *Acanthochromis*
117 *polyacanthus*. While *A. polyacanthus* can be sensitive to increases in water temperature and
118 CO₂ levels, they have the potential to acclimate to the changing environmental conditions
119 across multiple generations (Donelson et al., 2012; Schunter et al., 2016, 2018; Veilleux et al.,
120 2015). *A. polyacanthus* has been used as a model to study the impacts of climate change, and
121 also to investigate the molecular basis of intergenerational plasticity to environmental changes,
122 due to its advantageous life-history traits for laboratory studies (Robertson, 1973), however
123 past studies have only focused on single tissues (Ryu et al., 2018; Schunter et al., 2016, 2018).
124 Here, by using a multi-tissue transcriptomic approach we aim to determine how dynamic cross-
125 talk between tissues maintains whole-body homeostasis under future ocean acidification
126 conditions. Additionally, we also assess how the acclimatory response of offspring mediated
127 by transcriptional reprogramming across multiple tissues is influenced by variation in parental
128 sensitivity to elevated CO₂ and parental environment. Through systemic characterization of the
129 effects of OA we aim to identify how the adaptive processes within each tissue integrate to
130 drive intergenerational acclimation to OA at the organismal level.

131

132 **Methods**

133 **Sample collection, behavioural testing, and experimental design**

134 Adult *Acanthochromis polyacanthus* were collected from the wild on the Great Barrier
135 Reef, Australia (18°38'24.3"S, 146°29'31.8"E) and exposed to elevated CO₂ (754 ± 92 µatm)
136 for seven days following which their behavioural sensitivity to conspecific chemical alarm cues
137 (CAC) was tested using a two-chamber flume as described previously (Schunter et al., 2016).
138 Briefly, the fish were classified as being behaviorally sensitive or tolerant to elevated CO₂
139 based on the amount of time spent in water containing the CAC. Sensitive individuals spent ≥
140 70% time in CAC whereas tolerant individuals spent ≤ 30% time in CAC. Individuals of similar
141 size displaying the same behavioural phenotype (sensitive or tolerant) were then grouped into
142 breeding pairs and held in either control (414 ± 46 µatm) or elevated CO₂ conditions (754 ± 92
143 µatm) for three months prior to the breeding season. Offspring clutches from each breeding
144 pair were placed into three different experimental treatments resulting in three combinations of
145 parent-offspring conditions for each parental phenotype: (1) Control treatment – Parents and
146 offspring held at control condition (414 ± 46 µatm); (2) Developmental treatment – Parents
147 held at control condition and offspring exposed to elevated CO₂ (754 ± 92 µatm) immediately
148 after hatching; and (3) Intergenerational treatment – Parents and offspring exposed to elevated
149 CO₂ (754 ± 92 µatm). The offspring were held in their respective conditions until they were
150 five months old after which nine fish from each parental phenotype, from each treatment
151 condition (N = 27 from each parental phenotype; N = 54 total fish sampled) were euthanized
152 and the brain, gills and liver were dissected, snap frozen in liquid nitrogen and stored at -80 °C
153 until further processing (Supplementary Figure S1).

154 **RNA extraction, sequencing, and gene expression analyses**

155 Total RNA was extracted from the fish brains, livers and gills using the AllPrep
156 DNA/RNA Mini kit from Qiagen following the manufacturer's instructions. RNA quality was
157 determined using nanodrop and Agilent Bioanalyzer and samples having an RNA integrity
158 value (RIN) ≥ 8 were sequenced using Illumina HiSeq 2500 to get paired-end reads of 100 bp
159 at Macrogen Inc., South Korea. A total of $1,614.25 \pm 3.05$, $2,367.03 \pm 5.19$, and $2,227.23 \pm$
160 6.37 million raw paired-end reads were obtained from the 162 sequenced libraries from brain,
161 gills and liver respectively which included nine control, nine developmental and nine
162 intergenerational samples for each parental phenotype for each tissue (Supplementary Table
163 S1). The quality of the raw reads were examined using FastQC (Andrews, 2010) v0.11.8 and
164 adapters and low quality sequences were trimmed using Trimmomatic (Bolger et al., 2014)
165 v0.39 (ILLUMINACLIP: adapters.fa:2:30:15:8:true; SLIDINGWINDOW:4:20; MINLEN:32).
166 Only those sequences ≥ 32 bp in length with both the forward and reverse reads retained after
167 trimming were used for further analysis. Potential contaminant sequences were identified using
168 kraken (Wood & Salzberg, 2014) v2.0.8-beta, with a confidence score of 0.3, using the bacteria,
169 fungi and virus RefSeq genomic libraries as reference and removed from further analyses. A
170 total of $1,510.51 \pm 2.62$, $2,254.16 \pm 4.95$, and $2,116.25 \pm 6.04$ million high-quality sequences
171 were retained after the filtering process (Supplementary Table S1). These sequences were
172 mapped to the *Acanthochromis polyacanthus* reference genome (unpublished) using HISAT2
173 (Kim et al., 2019) v2.1.0. On average, $84 \pm 1.83\%$, $91.22 \pm 0.66\%$, and $93.33 \pm 0.81\%$ reads
174 mapped to the reference genome from the brain, gills, and liver respectively (Supplementary
175 Table S1). Raw read counts per gene were obtained using featureCounts (Liao et al., 2014)
176 v2.0.0 (parameters: -B -J -M --fraction), assigning fractional counts to multi-mapped reads.
177 Exploring the gene expression patterns across the whole dataset (162 samples) using principal
178 component analysis (PCA) revealed a clear clustering of samples by tissues indicating that
179 tissues vary greatly in their gene expression patterns (Supplementary Figure S2). Subsequent

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

182 Principal component analysis (PCA) using the regularized log transformed (rlog)
183 counts was done in R v4.2.1 to detect and remove outlier samples. A likelihood ratio test (LRT)
184 using a model comparison approach was then used to determine the effect of family line in
185 driving the gene expression patterns and to determine the best design formula for the final DE
186 analysis. First, significant differences in gene expression were measured by comparing a model
187 including treatment and family line against a reduced model without the family line factor
188 separately for each tissue. For a total of 924, 910, and 923 genes in the brain, gills, and liver
189 respectively, the model including family line better explained the observed differences in gene
190 expression compared to the reduced model excluding this factor (FDR corrected p-value < 0.05;
191 Supplementary Table S2). Pair-wise comparisons between the control, developmental and
192 intergenerational treatment was then carried out in DESeq2 (accounting for the family effect,
193 using Wald test) separately for each parental phenotype for each tissue to determine the effect
194 of parental environment and parental tolerance to CO₂ on the molecular responses of the
195 offspring to elevated CO₂. For each pair-wise comparison, the genes were considered to be
196 significantly differentially expressed (DE) if the False Discovery Rate (FDR) adjusted p-value
197 was less than 0.05, the absolute log 2-fold change in expression was greater than 0.3 and
198 baseMean was greater than 10. Functional enrichment analysis of the significant DE genes was
199 carried out in OmicsBox (<https://www.biobam.com/omicsbox>) v1.4.11 using Fisher's Exact
200 Test (FDR corrected p-value < 0.05) with the option of reducing to most specific GO terms to
201 reduce redundancy. GO terms that were identified to be over-represented among the DE genes
202 were also retained. The genes associated with the enriched and over-represented GO terms
203 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.

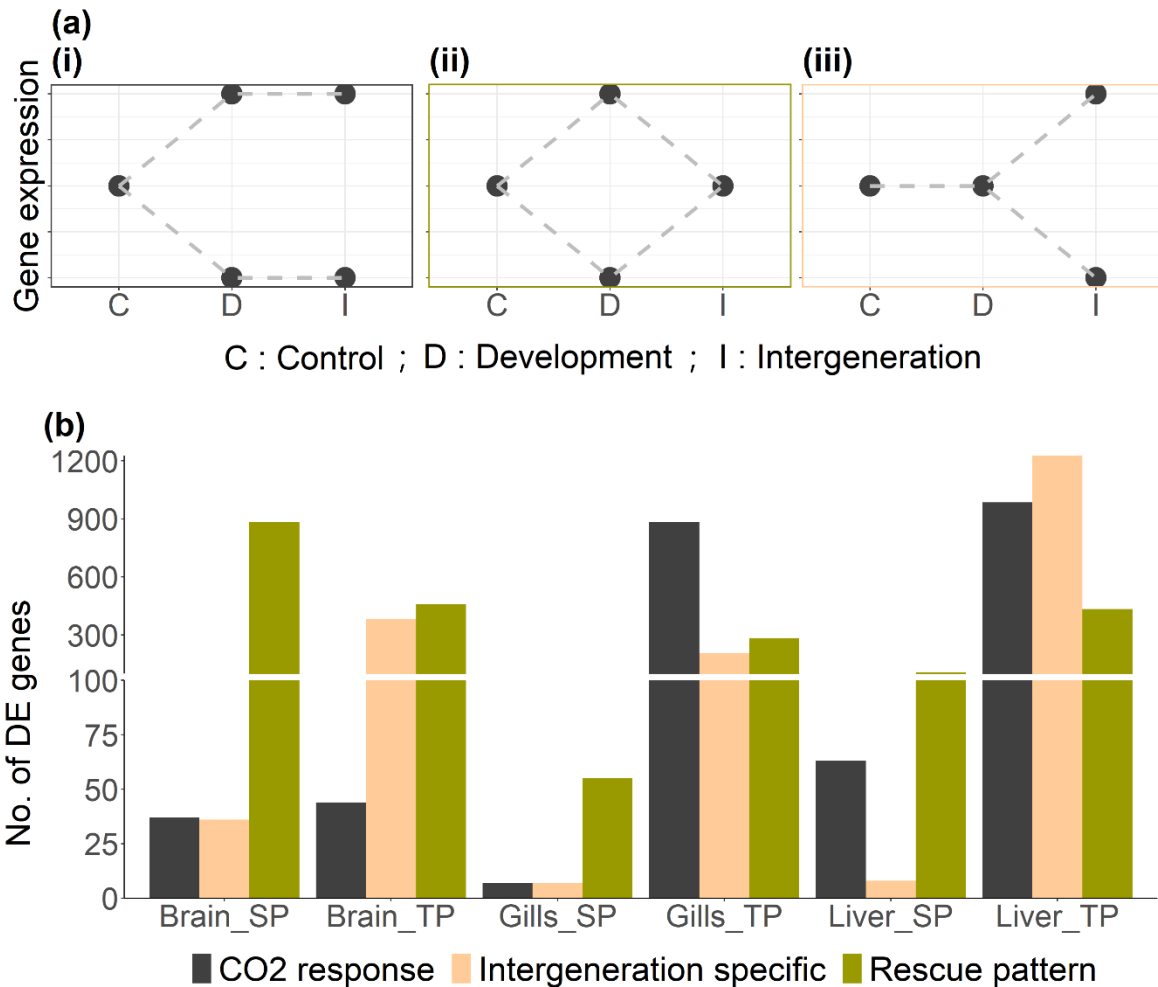
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207 **Results**

208 **Molecular processes affected by all elevated CO₂ treatments**

209 To understand a general effect of elevated CO₂ exposure regardless of time of exposure
210 to elevated CO₂ we identified genes that were commonly differentially expressed (DE) in both
211 the developmental and intergenerational treatments compared to control (Figure 1(a)(i)), which
212 are considered the general “CO₂ response genes” (Supplementary Table S4). There was high
213 tissue specificity in transcriptional response in both the elevated CO₂ treatments compared to
214 control with no genes being commonly DE across the three tissues in the sensitive parental
215 phenotype and only 3 and 81 genes being shared between the brain and gills, and liver and gills
216 respectively in the tolerant parental phenotype (Supplementary Figure S3(a)). Liver had the
217 greatest number of genes commonly differentially expressed in both the elevated CO₂
218 treatments compared to control, followed by the gills (Figure 1(b), Supplementary Table S3).
219 Overall, offspring of tolerant parents had more differentially expressed (DE) genes in all tissues,
220 with the difference in DE gene numbers between sensitive and tolerant phenotypes being more
221 pronounced in the gills and liver than in the brain (Figure 1(b)).

222



223

224 **Figure 1:** (a) Schematic graph representing the expression profile of (i) CO₂-response genes,
 225 (ii) genes showing a rescue pattern, and (iii) intergeneration-specific genes. (b) Number of
 226 differentially expressed genes across the three treatments in all the three tissues. SP indicates
 227 samples with a sensitive parental phenotype and TP indicates samples with a tolerant parental
 228 phenotype for each of the respective tissues. Note scale break in y-axis at 100 DE genes.
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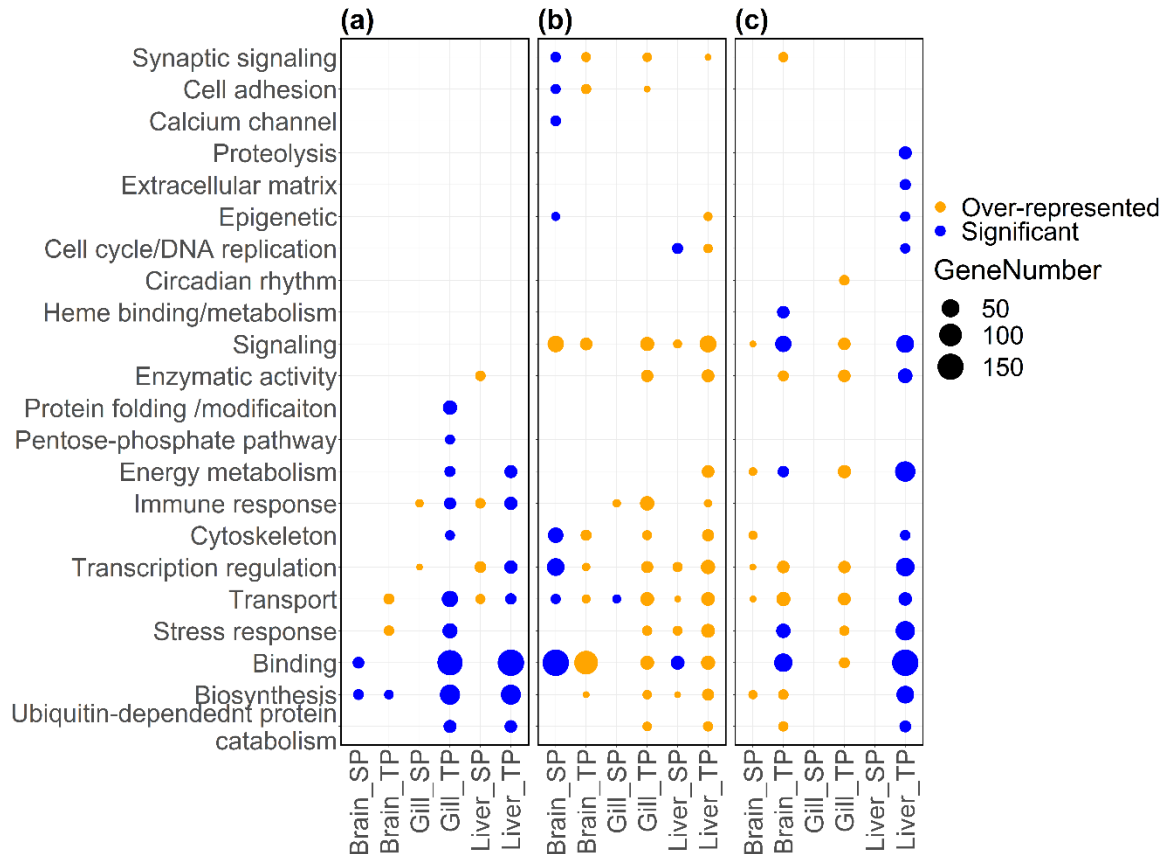
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The three tissues also differed substantially in terms of functions associated with the
 CO₂-response genes. Only three functions, biosynthetic process, transport, and binding, were
 differentially regulated in all the three tissues. Gills showed the 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

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



242
 243 **Figure 2:** Functions that are significantly enriched (in blue; FDR < 0.05) or over-represented
 244 (in orange; FDR > 0.05) among the DE genes involved in (a) overall CO₂ response, (b) rescue
 245 pattern, and (c) intergenerational specific response. SP indicates samples with a sensitive
 246 parental phenotype and TP indicates samples with a tolerant parental phenotype.
 247

248 Parental exposure to elevated CO₂ “rescues” developmental effects

249 A total of 1220, 328, and 542 genes that were DE in the developmental treatment
 250 (compared to control and intergeneration) in the brain, gills, and liver respectively returned to
 251 control levels in the offspring whose parents were previously exposed to OA conditions
 252 suggesting cross-generation plasticity resulting in “rescue” of gene expression levels (Figure
 253 1(a)(ii); Supplementary Table S5). Parental conditioning had the largest effect on brain gene

254 expression followed by the liver (Figure 1(b), Table S3). Similar to the CO₂-affected genes,
255 there were very few genes commonly DE across tissues (Supplementary Figure S3(b)).
256 However, while the exact genes did not overlap, the majority of the underlying functional
257 responses involved in intergenerational plasticity were commonly regulated across the three
258 tissues (Figure 2(b)).

259 Functions such as transport (including transport of ions involved in pH homeostasis),
260 synaptic signaling, signaling, RNA processing & transcription regulation, cytoskeleton-related,
261 biosynthetic processes, and binding were commonly enriched or over-represented among the
262 DE genes showing a “rescue” pattern. Liver showed the highest degree of specificity in
263 functional regulation, with functions involved in cell cycle/ DNA replication and energy
264 metabolism being primarily enriched or over-represented among the DE genes only in the liver.
265 Additionally, epigenetic processes were commonly over-represented in the liver and brain, and
266 immune response, stress response and ubiquitin dependent protein degradation processes were
267 over-represented in both the liver and gills. Interestingly, although synaptic signaling was
268 commonly over-represented in all tissues, calcium channel encoding genes, which are involved
269 in neurotransmitter release, were differentially regulated mainly in the brain. This suggests that
270 parental conditioning to elevated CO₂ selectively regulates certain specific functions in each
271 tissue (Figure 2(b); Supplementary Table S8, S11).

272 **Intergenerational specific response to elevated CO₂**

273 We found a large transcriptional response to elevated CO₂ that was only seen in the
274 intergenerationally exposed fish and not in fish with only developmental (within generation)
275 exposure to elevated CO₂. This indicates plasticity of the offspring transcriptome due to
276 parental conditioning to elevated CO₂ and was especially marked in offspring of tolerant
277 parents (Figure 1(b)). These are genes that were only differentially expressed in the
278 intergenerational treatment (compared to control and development) but were at control levels

279 in the developmental treatment (Figure 1(a)(iii)). Specifically, 383, 207, and 1,226 genes were
280 DE in the brain, gills, and liver respectively in offspring with tolerant parents, while offspring
281 with sensitive parents had 36, 7, and 8 DE genes in the brain, gills, and liver respectively that
282 were specific to the intergenerational treatment (Supplementary Table S6). Offspring of both
283 tolerant and sensitive parents showed high tissue specificity in the intergenerational specific
284 transcriptional signature to elevated CO₂ with only one and five genes being commonly DE
285 across all three tissues in the sensitive and tolerant parental phenotypes respectively
286 (Supplementary Figure S3(c)).

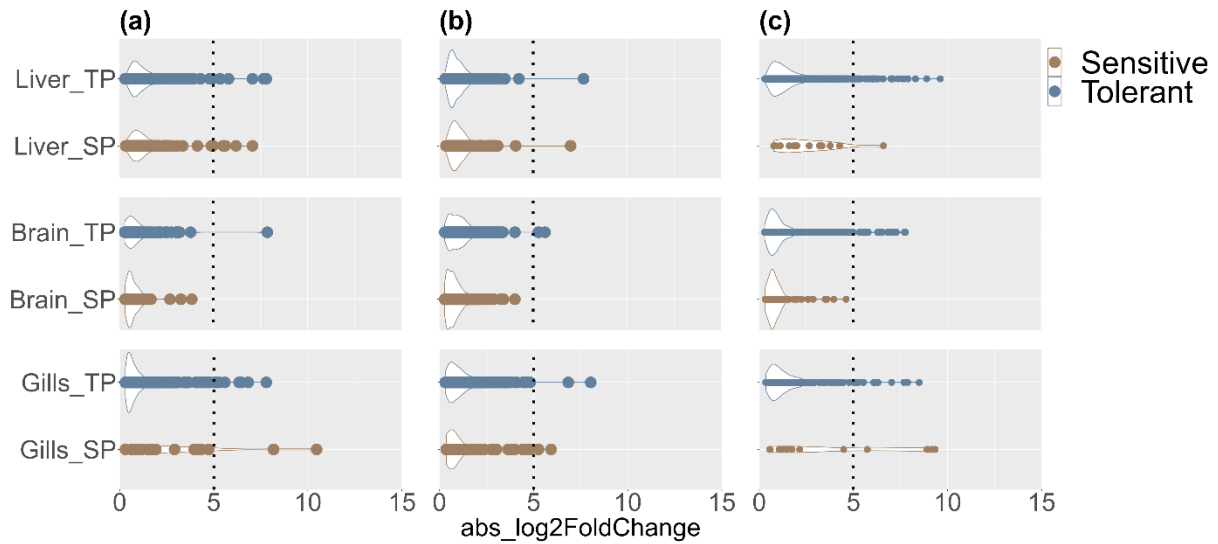
287 Specifically, we found molecular signatures indicating bicarbonate retention only in the
288 intergenerationally treated fish such as downregulation of SLC4A1, CFTR, SLC12A2, and
289 SLC9A3 in the gills and upregulation of SLC4A4 in the brain which can buffer pH changes
290 caused by elevated environmental CO₂ levels. Other key functional pathways that showed
291 intergenerational specific regulation were epigenetic processes and energy metabolism (Figure
292 2(c)). Therefore, fish whose parents also experience the same high CO₂ environment undergo
293 rearrangements in their transcriptional landscape. This change in transcriptional signature
294 could regulate the above mentioned “rescue” pattern and equip offspring to better cope with
295 OA conditions (Supplementary Table S9, S12).

296 **Parental variability in CO₂ sensitivity impacts the offspring transcriptome**

297 Parental behavioural phenotype was found to have a substantial influence on the
298 offspring transcriptional response. Across all three tissues, offspring with behaviorally tolerant
299 parents when faced with elevated CO₂ had larger changes in gene expression levels ($\log_2FC >$
300 5; Figure 3) and a greater number of DE genes involved in the overall CO₂ response (common
301 in developmental and intergenerational CO₂ exposure compared to control; Figure 1(b)). There
302 were also very few genes involved in overall CO₂ response shared between the two parental
303 phenotypes, specifically only six, three, and eleven common DE genes in the brain, gills, and

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

313 The difference in transcriptional response between the two parental phenotypes was
314 especially pronounced when considering genes showing an intergeneration-specific signature
315 with offspring of tolerant phenotype having more DE genes with a much higher magnitude of
316 gene expression changes ($\log_2FC > 5$; Figure 3). Several functions such as acid-base regulation,
317 signaling, transcription regulation, energy metabolism, and epigenetic processes were enriched
318 in the intergeneration-specific treatment in all tissues only in the tolerant phenotype (Figure
319 2(c)). Similarly, several functions were uniquely regulated only in offspring with tolerant
320 parents when considering the overall effect of elevated CO₂ exposure independent of the length
321 of exposure (Figure 2(a)). However, when considering genes whose expression levels returned
322 to control levels due to parental conditioning to elevated CO₂, there were more similarities in
323 the underlying functional pathways between the two parental phenotypes for all three tissues
324 (Figure 2(b)). Therefore, although there were some common transcriptional responses in
325 offspring with sensitive and tolerant parents, overall offspring of tolerant parents showed a
326 much stronger transcriptional response to elevated CO₂ in general and also had a stronger
327 signature of intergenerational plasticity.



328

329 **Figure 3:** Log₂ fold change in expression of genes involved in (a) overall CO₂ response, (b)
330 rescue pattern, and (c) intergenerational specific response across all three tissues. SP indicates
331 samples with a sensitive parental phenotype and TP indicates samples with a tolerant parental
332 phenotype.

333

334

335 Discussion

336 The transcriptional landscape of three key tissues exhibited shared and tissue-specific
337 signatures driving intergenerational acclimation to elevated CO₂ in a marine fish. Specifically,
338 we found that gills are critical in maintaining overall cellular homeostasis in elevated CO₂
339 treatments and that the brain and liver had the greatest signal of intergenerational acclimatory
340 response. In fact, intergenerationally treated fish no longer showed molecular signatures of
341 altered neural signaling in the brain and reduced capacity for energy production in the liver that
342 were seen in the developmental (within generation) treatment. Indeed, a new complement of
343 genes involved in metabolism were upregulated only in offspring of parents who were
344 previously exposed to elevated CO₂ indicating improved capability for energy production at a
345 systemic level. *A. polyacanthus* is known to have a highly plastic genome enabling it to respond
346 and acclimate to environmental changes (Bernal et al., 2020; Kang et al., 2022) and our results
347 show that this persists across generations potentially enabling this species to rapidly acclimate
348 to the changing ocean environment.

349 Genes that are always differentially expressed in elevated CO₂ conditions regardless of
350 the type of exposure are key genes in the general response to ocean acidification (OA). The
351 gills and liver exhibited a higher transcriptional response in all elevated CO₂ treatments
352 suggesting that these tissues play an important role in the overall response of the fish to elevated
353 CO₂. Genes involved in primary carbon metabolism, a process known to be altered under
354 conditions of OA (Strader et al., 2020), were DE in both the gills and liver. This included genes
355 in the tricarboxylic acid (TCA) cycle and the pentose phosphate pathway. Both these processes
356 yield precursors for biosynthetic pathways and cofactors essential to maintain cellular
357 homeostasis thereby influencing cellular processes beyond carbohydrate catabolism for energy
358 (ATP) production (Gansemer et al., 2020). Therefore, the upregulation of these genes could
359 indicate redirection of metabolic carbon fluxes to meet cellular demands for various cofactors

360 needed in the cellular stress response (CSR) pathways (Gansemer et al., 2020; Rokitta et al.,
361 2012; Walsh & Milligan, 1993), which is often induced upon exposure to elevated CO₂ levels
362 (Strader et al., 2020). In fact, various genes involved in CSR were found to be upregulated in
363 the gills and brain. Therefore, there seems to be a systemic regulation of primary carbon
364 metabolism in response to elevated CO₂ exposure to ensure sufficient production of precursors
365 and cofactors needed for other biological processes.

366 Another key function we found to be required with elevated CO₂ is the immune
367 response. Interestingly, genes involved in immune response were upregulated in the gills but
368 downregulated in the liver indicating tissue-specific regulation of this function. Gills are one
369 of the major surface tissues, which are continuously exposed to the external environment, and
370 also serve as a first line of defense against potential infections (Harper & Wolf, 2009; Hu et al.,
371 2023). Activation of immune responses has been previously observed under elevated CO₂
372 conditions which could be a preventive measure against opportunistic infections under CO₂
373 stress (De Souza et al., 2014; Machado et al., 2020). Hence the upregulation of genes involved
374 in immune and stress response in the gills could be a protective mechanism to prevent cell
375 damage caused by exposure to high CO₂.

376 Parental exposure to altered environmental conditions can pre-acclimate the offspring
377 transcriptome to these new conditions via intergenerational plasticity. The expression of genes
378 involved in key functional pathways were altered in juvenile *A. polyacanthus* upon
379 developmental exposure to elevated CO₂ but were restored to control levels in the
380 intergenerationally treated fish. These included genes involved in metabolism, synaptic
381 plasticity and signaling, and RNA processing and transcription regulation. Intergenerationally
382 exposed fish did not show transcriptional signatures of metabolic depression that was observed
383 upon developmental CO₂ exposure in the liver, the major organ for metabolism. Specifically,
384 fish exposed to elevated CO₂ during development, but not intergenerationally, exhibited

385 reduced capacity for energy production marked by downregulation of lipid and glucose
386 metabolism, and genes involved in iron-sulfur (Fe-S) cluster assembly, which function as
387 cofactors in the mitochondrial respiratory chain. In addition, there was upregulation of PCK1,
388 a gluconeogenic enzyme (Yu et al., 2021) and AGL, involved in glycogen breakdown (Ni et
389 al., 2022), which could indicate glucose shortage for energy production when the offspring are
390 only exposed to elevated CO₂ within their lifetime. Metabolic suppression is a commonly
391 observed physiological response to CO₂ stress (Strader et al., 2020), however, the expression
392 levels of the above-mentioned genes returned to control levels with previous parental exposure,
393 revealing that parental conditioning to elevated CO₂ restores the metabolic capacity of the
394 offspring.

395 Furthermore, the expression of genes involved in synaptic plasticity and calcium
396 channel activity were altered in the developmental treatment, predominantly in the brain, but
397 were similar to control levels in the intergenerationally treated fish. Exposure to OA conditions
398 has been shown to affect neural plasticity and neurogenesis in some fish species, which could
399 result in changes in neural circuitry and signaling (Costa et al., 2022; Lai et al., 2017). While
400 neural plasticity could facilitate increased flexibility to environmental changes (Ebbesson &
401 Braithwaite, 2012), it could result in behavioural alterations that have been observed in fish
402 exposed to OA conditions (Schunter et al., 2019). Increased GABAergic signaling is a
403 commonly observed within-generation response to elevated CO₂ in *A. polyacanthus* (Schunter
404 et al., 2018) and a similar increase in neural signaling pathways was found in the olfactory
405 epithelium of *D. labrax* even after prolonged transgenerational OA exposure for two
406 generations (Cohen-Rengifo et al. 2022). The restoration of OA induced changes in neural
407 signaling processes with previous parental exposure to elevated CO₂ could indicate elevated
408 intergenerational plasticity of *A. polyacanthus* compared to other species. Synaptic plasticity
409 is regulated by the cytoskeleton (Gordon-Weeks & Fournier, 2014; Zapara et al., 2000) and

410 intergenerationally treated fish did not show changes in expression of various cytoskeleton and
411 cell adhesion genes seen in the developmental treatment. Therefore, intergenerational CO₂
412 exposure restores the dynamic equilibrium of cytoskeletal proteins, thereby re-establishing
413 synaptic signaling processes to control levels.

414 Transcriptional regulatory elements were identified to be key in regulating the response
415 to elevated CO₂. Transcription factors and RNA-mediated gene silencers mainly mediated
416 plasticity in the within-generation response to elevated CO₂. Changes in the external
417 environment can trigger reprogramming of transcriptional networks resulting in dynamic
418 regulation of gene expression (Swift & Coruzzi, 2017) as also suggested in wild fish
419 populations naturally exposed to elevated CO₂ (Petit-Marty et al., 2021). Therefore, these
420 regulatory genes could play a key role in developmental plastic responses to elevated
421 environmental CO₂ levels, however, these are no longer needed in intergenerationally treated
422 fish. The overall “rescue” of various key functional pathways upon intergenerational exposure
423 to elevated CO₂ suggests parental priming of the offspring transcriptome enabling acclimation
424 of future generations to OA.

425 We also found a new complement of genes to be differentially expressed only when the
426 parents are exposed to the same elevated CO₂ condition as their offspring, which could further
427 facilitate acclimation of the offspring enabling them to better cope with an elevated CO₂
428 environment. Such intergenerational specific transcriptional signature was observed in genes
429 involved in ion transport resulting in bicarbonate retention in the brain and gills. Increasing
430 internal bicarbonate ion concentrations is a commonly observed compensatory response in fish
431 to buffer acid-base disturbance caused by exposure to elevated CO₂ levels (Heuer & Grosell,
432 2014). Differential expression (DE) of bicarbonate transporters in the intergenerationally
433 treated fish suggests that parental conditioning enables the offspring to more effectively buffer
434 pH changes (Brauner et al., 2019; Chen et al., 2009; Choi, 2012.; Esbaugh, 2017). Furthermore,

435 changes in potassium channel activity enables the fish to sense pH disturbances (Hibino et al.,
436 2010; Qin et al., 2010) and in turn initiate compensatory responses to maintain homeostasis.
437 Therefore, intergenerational CO₂ exposure could have resulted in transcriptional
438 rearrangements of ion transporters in the offspring, especially in the brain and gills, to maintain
439 pH homeostasis primarily by bicarbonate retention rather than acid extrusion. While changes in
440 ion channel activity can be an efficient mechanism to buffer pH changes and prevent acidosis
441 in elevated CO₂ conditions, it is an energy demanding process (Ishimatsu et al., 2005; Auffret
442 et al., 2023). However, parental conditioning to elevated CO₂ also increased the offspring's
443 capacity of energy metabolism. Transcriptional signatures indicating metabolic suppression
444 observed in the developmental treatment was no longer observed in the intergenerationally
445 treated fish. In fact, when considering only the intergeneration-specific transcriptional response,
446 metabolic processes including mitochondrial electron transport were upregulated in all three
447 tissues, but particularly in the liver. Exposure to elevated CO₂ for prolonged periods results in
448 increased energetic costs (Araújo et al. 2018; Schunter et al. 2016, 2021; Tsang et al. 2020)
449 and while liver is the main tissue involved in metabolism (Auffret et al., 2023), the shared
450 upregulation of metabolic genes in all three tissues suggests an increase in metabolic processes
451 in a whole-body context. Therefore, parental conditioning to elevated CO₂ may enable the
452 offspring to meet the energetic demands associated with living in a high CO₂ environment.

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

460 acclimation to environmental changes including elevated temperature and CO₂ 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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂ treatments regardless of the
484 length of exposure. Parental exposure to elevated CO₂ 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 CO₂. 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.

495

496

497 **Author contributions**

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

502 **Ethics**

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

505 **Competing financial interests**

506 All authors declare they have no competing interests.

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511 **Data availability**

512 The brain RNA-Seq raw sequences are deposited in NCBI under BioProject ID PRJNA311159.
513 The gills and liver RNA-Seq raw sequences are deposited in NCBI under BioProject ID
514 PRJNA989422
515 ([https://dataview.ncbi.nlm.nih.gov/object/PRJNA989422?reviewer=q3n0q75hbbf2p1v85n0h
516 e3uv86](https://dataview.ncbi.nlm.nih.gov/object/PRJNA989422?reviewer=q3n0q75hbbf2p1v85n0he3uv86)).

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