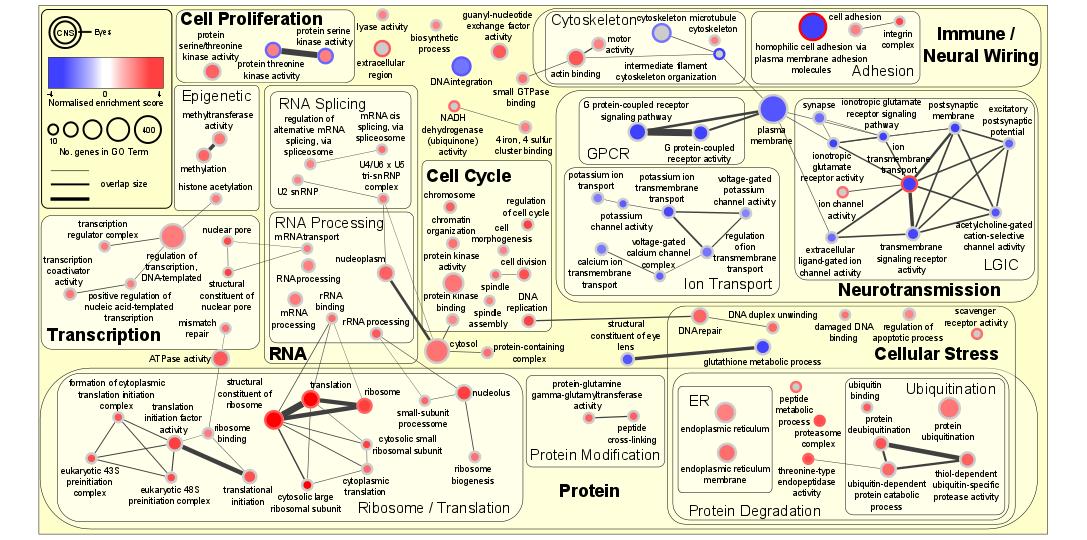
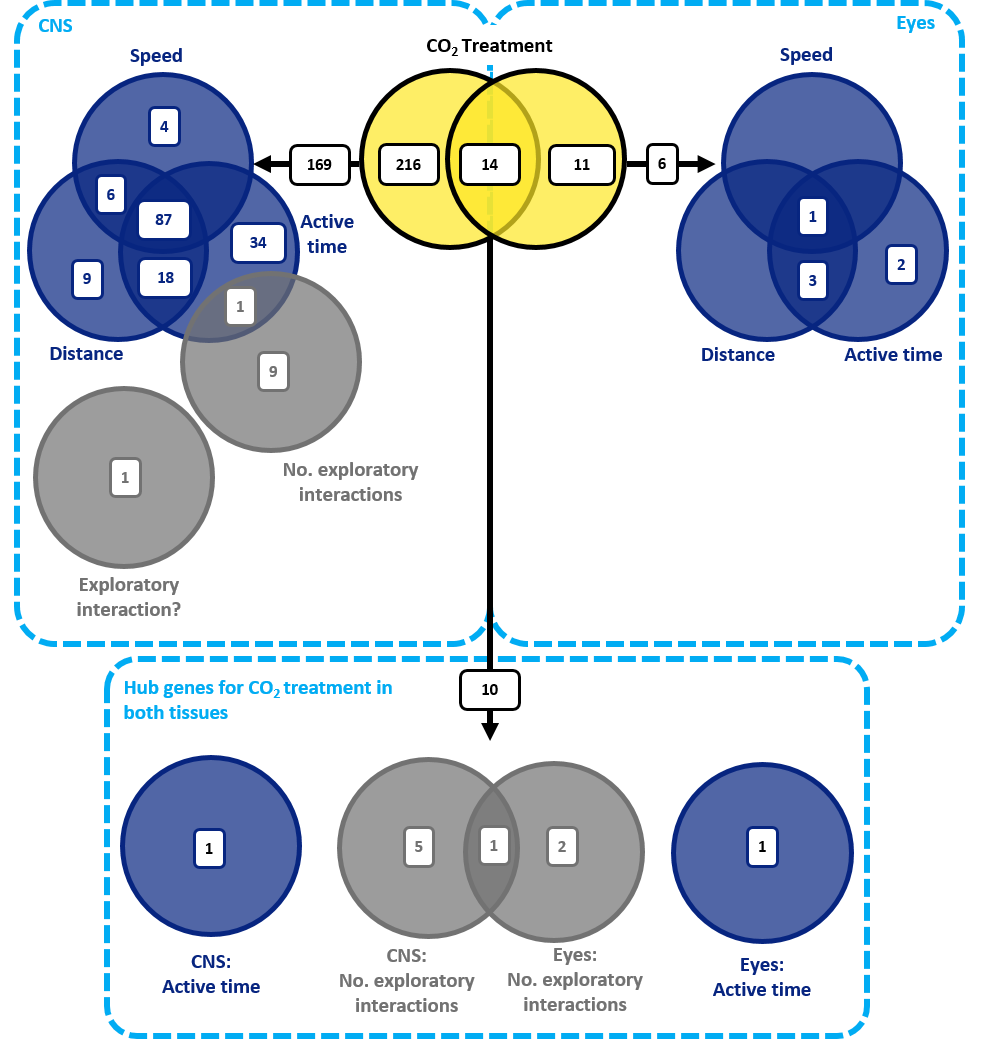


**Figure 1.** Experimental design overview. CCA = canonical correlation analysis. *Idiosepius pygmaeus* photograph by Jodi Thomas.

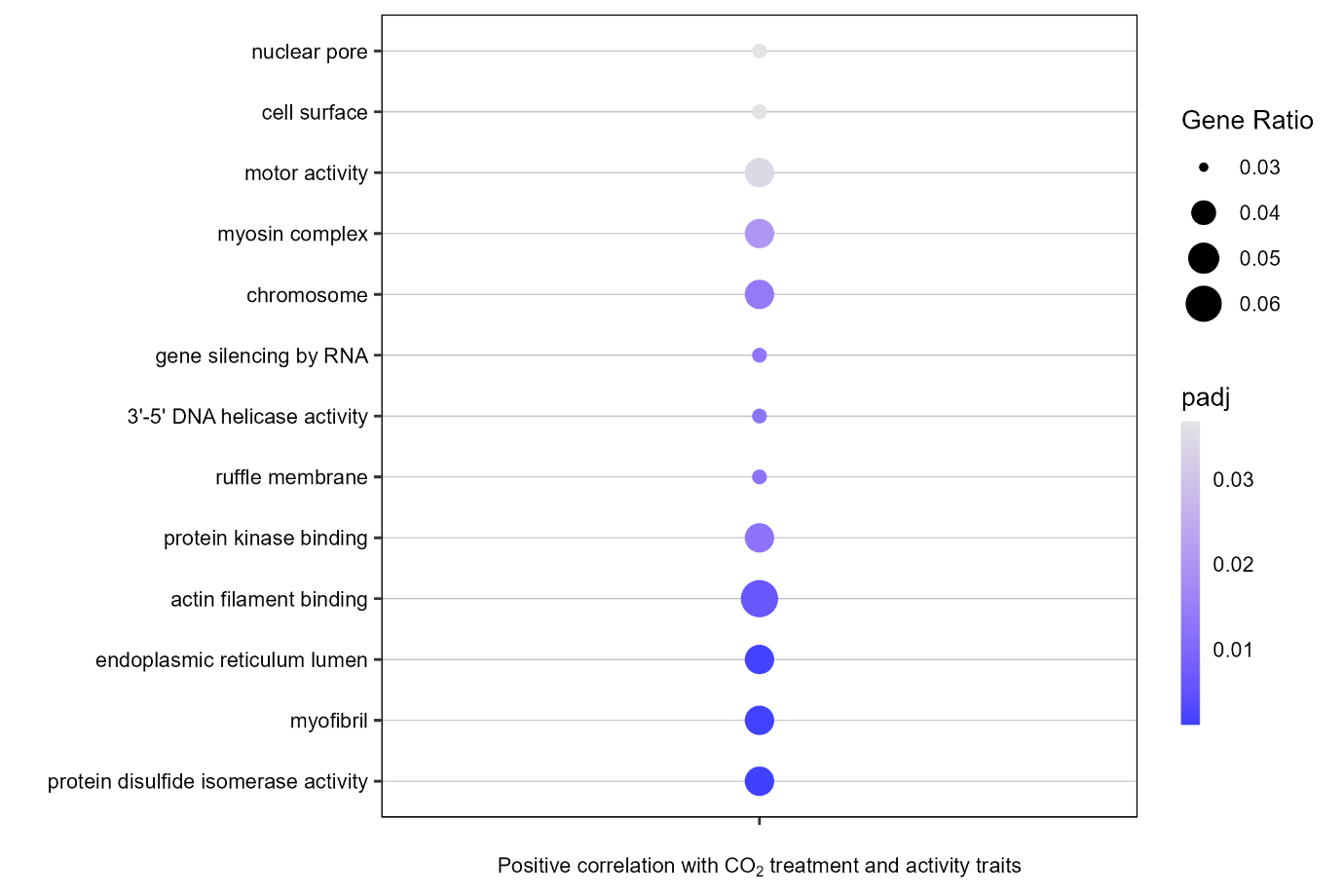
**Figure 2.** Differential expression and GSEA results. **A** PC1 and PC2 axes from the principal components analysis of all genes for the 40 samples. **B** Venn diagram comparing the DEGs between current-day and elevated CO2 levels in the CNS and eyes. **C** Venn diagram comparing the GO terms / functional categories found to be significantly different between current-day and elevated CO2 levels in the CNS and eyes by GSEA. ⭘ = CNS current-day, 🞎 = CNS elevated CO2, ◇ = Eyes current-day, △ = Eyes elevated CO2, ↑ = upregulated at elevated CO2 conditions, ↓ = downregulated at elevated CO2 conditions.



**Figure 3.** Enrichment map displaying the gene set enrichment analysis (GSEA) results in both the CNS and eyes. Significant GO terms/functional categories are represented by a circular node. Results from the CNS and eyes are represented by colouration of the inner node area and node border, respectively. Red represents functional categories upregulated at elevated CO2 and blue represents functional categories downregulated at elevated CO2. Colour intensity represents the normalised enrichment score and node size the number of core enrichment genes in each functional category. Functional categories found not significant (padj > 0.05) are coloured grey for the corresponding tissue type. The nodes from functional categories with a similarity > 0.25 are connected by an edge, with edge width increasing with increasing similarity (increasing number of genes shared by the functional categories). Similar functional categories were manually grouped into clusters and assigned a label.



**Figure 4.** Venn diagram depicting the number of hub genes identified for CO2 treatment and behavioural traits in the CNS and eyes. The yellow Venn diagram in the centre depicts the number of hub genes for CO2 treatment that are CNS-specific (left) and eyes-specific (right), and the overlap represents the number of CO2 treatment hub genes shared by both tissues. CNS-specific and eyes-specific CO2 treatment hub genes also identified as a hub gene for one or more behavioural traits in the CNS or eyes are on the left and right, respectively. Hub genes for CO2 treatment found in both tissues, that are also a hub gene for a behavioural trait in one or both tissues, are shown at the bottom centre. CO2 treatment hub genes shared with activity traits (active time, distance and speed) and exploratory conspecific-directed behaviours (number of exploratory interactions and whether any exploratory interactions occurred) are in blue and grey, respectively. Exploratory interaction? = whether any exploratory interactions occurred (yes/no).



**Figure 5.** GO Terms/functional categories significantly enriched from the 87 genes positively correlated with CO2 treatment and all three activity traits in the CNS. padj = adjusted p-value, blue indicates higher significance. Gene Ratio = the number of genes represented in the genes positively correlated with CO2 treatment and all three activity traits in the CNS, in comparison to all of the genes in the CNS.