

Supplementary information

Neurobiological and behavioural responses of cleaning mutualisms to ocean warming and acidification

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TABLE S1. Seawater physicochemical parameters in all experimental setups.

	Control	High CO ₂	Warming	Warming + High CO ₂
Measured				
Temperature(°C)	29.0 ± 0.3	29.0 ± 0.3	32.0 ± 0.6	32.0 ± 0.6
pH	8.04 ± 0.01	7.73 ± 0.01	8.04 ± 0.01	7.73 ± 0.02
AT (μmol/kgSW)	2388.4 ± 32.9	2398.9 ± 44.3	2392.0 ± 44.8	2392.3 ± 48.1
Salinity (ppt)	35	35	35	3.50E+01
Calculated				
CT (μmol/kgSW)	2043.0 ± 32.9	2224.1 ± 42.8	2017.2 ± 40.2	2197.6 ± 46.6
pCO ₂ (μatm)	409.0 ± 12.3	963.6 ± 37.0	404.8 ± 13.8	961.6 ± 45.7
Ω Arg	4.0 ± 0.1	2.3 ± 0.1	4.4 ± 0.1	2.5 ± 0.1

Salinity, pH and temperature were measured daily and averaged over the whole experimental period. The combination of total alkalinity (AT) and pHT (pH total scale) was used to calculate carbonate system parameters [pCO₂ (carbon dioxide partial pressure), CT (total inorganic carbon) and Ω Arg (aragonite saturation state)]. Values are represented as mean ± standard deviation

TABLE S2. Generalized Linear Models (GLM) analysis of the behavioural variables (number of interactions; proportion of interactions started by cleaners; client posing displays ratio; proportion of interactions with tactile stimulation; proportion of interactions with client jolts; interaction duration) measured during the behavioural trial between the cleaner fish *Labroides dimidiatus* and the client *Naso elegans*.

<i>Model: GLM (Negative Binomial) Response variable: Number of interactions</i>				
<i>Final model term(s): Principal effects of CO₂, temperature and interaction between stressors</i>				
	Estimate	Std. Error	Estimate/ Std. Error	p
(Intercept)	2.77	0.15	18.72	< 2e-16
Warming	-2.64	0.4	-6.54	6.40E-11
High CO ₂	-1.91	0.3	-6.41	1.50E-10
Warming × High CO ₂	3.22	0.52	6.16	7.20E-10
<i>Model: GLM (Binomial) Response variable: Proportion of interactions started by cleaners</i>				
<i>Final model term(s): Principal effects of CO₂</i>				
	Estimate	Std. Error	Estimate/ Std. Error	p
(Intercept)	0.67	0.18	3.7	2.10E-04
High CO ₂	-3.48	0.62	-5.61	2.10E-08
<i>Model: GLM (Gaussian) Response variable: Client posing displays ratio</i>				
<i>Final model term(s): Principal effects of CO₂, temperature and interaction between stressors</i>				
	Estimate	Std. Error	Estimate/ Std. Error	p
(Intercept)	0.06	0.11	0.56	0.58
Warming	0.16	0.16	0.99	0.33
High CO ₂	0.84	0.15	5.42	9.86E-06
Warming × High CO ₂	0.31	0.22	1.37	0.18
<i>Model: GLM (Binomial) Response variable: Proportion of interactions with tactile stimulation</i>				
<i>Final model term(s): Principal effects of CO₂</i>				
	Estimate	Std. Error	Estimate/ Std. Error	p
(Intercept)	-2.17	0.28	-7.67	1.70E-14
High CO ₂	1.24	0.42	2.97	2.90E-03
<i>Model: GLM (Binomial) Response variable: Proportion of interactions with client jolts</i>				
<i>Final model term(s): Principal effects of temperature</i>				
	Estimate	Std. Error	Estimate/ Std. Error	p
(Intercept)	-2.71	0.37	-7.42	1.20E-13
Warming	1.03	0.73	1.42	0.16
High CO ₂	1.61	0.89	1.80	0.07
Warming × High CO ₂	-1.95	1.22	-1.60	0.11
<i>Model: GLM (Gaussian) Response variable: Interaction duration</i>				
<i>Final model term(s): Principal effects of CO₂, temperature and interaction between stressors</i>				
	Estimate	Std. Error	Estimate/ Std. Error	p
(Intercept)	4.63	0.81	5.72	7.90E-06
Warming	-2.31	1.40	-1.65	0.11
High CO ₂	-1.05	1.14	-0.92	0.37
Warming × High CO ₂	2.34	1.83	1.28	0.22

Numbers in bold p<0.05

TABLE S3. Generalized Linear Mixed Models (GLMM) analysis of the neurobiological variables (dopamine, serotonin, DOPAC and 5-HIAA concentration) measured in the cleaner fish *L. dimidiatus*.

<i>Model: GLMM (Gamma)</i>		<i>Response variable: Dopamine concentration</i>			
<i>Final model term(s): Principal effects of CO₂, temperature, brain region and interaction between CO₂ and temperature</i>					
<i>Reference level (Brain region): FB</i>	Estimate	Std. Error	Estimate/ Std. Error	p	
(Intercept)	1.14	0.27	4.22	2.50E-05	
High CO ₂	-0.89	0.30	-2.99	2.80E-03	
Warming	-0.56	0.30	-1.85	0.06	
Brain region (FBvsHB)	1.00	0.24	4.16	3.20E-05	
Brain region (FBvsMB)	1.26	0.24	5.17	2.30E-07	
High CO ₂ x Warming	1.13	0.42	2.71	0.01	
<i>Reference level (Brain region): MB</i>	Estimate	Std. Error	Estimate/ Std. Error	p	
(Intercept)	2.40	0.26	9.31	2.00E-16	
High CO ₂	-0.89	0.30	-2.99	2.80E-03	
Warming	-0.56	0.30	-1.85	0.06	
Brain region (MBvsFB)	-1.26	0.24	-5.17	2.30E-07	
Brain region (MBvsHB)	-0.27	0.24	-1.13	0.26	
High CO ₂ x Warming	1.13	0.42	2.71	0.01	
<i>Model: GLMM (Gamma)</i>		<i>Response variable: DOPAC concentration</i>			
<i>Final model term(s): Principal effects of CO₂, temperature, brain region and interaction between them.</i>					
<i>Reference level (Brain region): FB</i>	Estimate	Std. Error	Estimate/ Std. Error	p	
(Intercept)	1.92	0.58	3.30	9.60E-04	
High CO ₂	1.40	1.14	1.24	0.22	
Warming	-0.32	0.73	-0.44	0.66	
Brain region (FBvsHB)	0.06	0.38	0.15	0.88	
Brain region (FBvsMB)	1.69	1.06	1.60	0.11	
High CO ₂ x Warming	-1.59	1.32	-1.20	0.23	
High CO ₂ x Brain region (FBvsHB)	0.97	1.61	0.60	0.55	
High CO ₂ x Brain region (FBvsMB)	-0.36	1.96	-0.18	0.85	
Warming x Brain region (FBvsHB)	0.30	0.54	0.55	0.58	
Warming x Brain region (FBvsMB)	1.01	1.65	0.61	0.54	
High CO ₂ x Warming x Brain region (FBvsHB)	-1.39	1.68	-0.82	0.41	
High CO ₂ x Warming x Brain region (FBvsMB)	5.88	3.78	1.56	0.12	
<i>Reference level (Brain region): MB</i>	Estimate	Std. Error	Estimate/ Std. Error	p	
(Intercept)	3.61	1.07	3.36	7.70E-04	
High CO ₂	1.04	1.77	0.59	0.56	
Warming	0.69	1.68	0.41	0.68	
Brain region (MBvsFB)	-1.69	1.06	-1.60	0.11	
Brain region (MBvsHB)	-1.63	1.07	-1.53	0.13	
High CO ₂ x Warming	4.29	3.70	1.16	0.25	
High CO ₂ x Brain region (MBvsFB)	0.36	1.96	0.18	0.85	
High CO ₂ x Brain region (MBvsHB)	1.33	2.14	0.62	0.53	
Warming x Brain region (MBvsFB)	-1.01	1.65	-0.61	0.54	
Warming x Brain region (MBvsHB)	-0.72	1.67	-0.43	0.67	
High CO ₂ x Warming x Brain region (MBvsFB)	-5.88	3.78	-1.56	0.12	
High CO ₂ x Warming x Brain region (MBvsHB)	-7.26	3.89	-1.87	0.06	
<i>Model: GLMM (Gamma)</i>		<i>Response variable: Serotonin concentration</i>			
<i>Final model term(s): Principal effects of CO₂, brain region and interaction between them.</i>					
<i>Reference level (Brain region): FB</i>	Estimate	Std. Error	Estimate/ Std. Error	p	
(Intercept)	0.0896	0.0103	8.69	2.00E-16	
High CO ₂	0.01	0.02	0.86	0.39	
Brain region (FBvsHB)	0.31	0.05	6.32	2.50E-10	
Brain region (FBvsMB)	0.54	0.07	7.38	1.50E-13	
High CO ₂ x Brain region (FBvsHB)	-0.16	0.06	-2.75	0.01	
High CO ₂ x Brain region (FBvsMB)	-0.14	0.09	-1.46	0.14	
<i>Reference level (Brain region): MB</i>	Estimate	Std. Error	Estimate/ Std. Error	p	
(Intercept)	0.63	0.07	8.69	2.00E-16	
High CO ₂	-0.12	0.09	-1.33	0.18	
Brain region (MBvsFB)	-0.54	0.07	-7.38	1.50E-13	
Brain region (MBvsHB)	-0.23	0.09	-2.63	0.01	
High CO ₂ x Brain region (MBvsFB)	0.14	0.09	1.46	0.14	
High CO ₂ x Brain region (MBvsHB)	-0.02	0.11	-0.21	0.83	
<i>Model: GLMM (Gamma)</i>		<i>Response variable: 5-HIAA concentration</i>			
<i>Final model term(s): Principal effects of CO₂, temperature, brain region and interaction between them.</i>					
<i>Reference level (Brain region): FB</i>	Estimate	Std. Error	Estimate/ Std. Error	p	
(Intercept)	0.81	0.20	3.95	7.70E-05	
High CO ₂	-0.20	0.26	-0.77	0.44	
Warming	-0.09	0.28	-0.33	0.74	
Brain region (FBvsHB)	-0.13	0.13	-0.98	0.33	

Brain region (FBvsMB)	2.55	0.91	2.81	4.90E-03
High CO2 x Warming	0.26	0.39	0.65	0.51
High CO2 x Brain region (FBvsHB)	2.74	0.88	3.10	1.90E-03
High CO2 x Brain region (FBvsMB)	-1.10	1.07	-1.03	0.30
Warming x Brain region (FBvsHB)	2.21	0.78	2.85	4.40E-03
Warming x Brain region (FBvsMB)	-1.19	1.07	-1.11	0.27
High CO2 x Warming x Brain region (FBvsHB)	-4.76	1.19	-4.01	6.00E-05
High CO2 x Warming x Brain region (FBvsMB)	0.68	1.29	0.53	0.60
<i>Reference level (Brain region): MB</i>	Estimate	Std. Error	Estimate/ Std. Error	p
(Intercept)	3.36	0.90	3.73	1.90E-04
High CO2	-1.31	1.06	-1.23	0.22
Warming	-1.29	1.06	-1.21	0.23
Brain region (MBvsFB)	-2.55	0.91	-2.81	4.89E-03
Brain region (MBvsHB)	-2.68	0.90	-2.97	2.99E-03
High CO2 x Warming	0.94	1.28	0.73	0.46
High CO2 x Brain region (MBvsFB)	1.10	1.07	1.03	0.30
High CO2 x Brain region (MBvsHB)	3.84	1.36	2.82	4.84E-03
Warming x Brain region (MBvsFB)	1.19	1.07	1.11	0.27
Warming x Brain region (MBvsHB)	3.41	1.30	2.63	0.01
High CO2 x Warming x Brain region (MBvsFB)	-0.68	1.30	-0.53	0.60
High CO2 x Warming x Brain region (MBvsHB)	-5.44	1.71	-3.17	1.51E-03

Numbers in bold $p < 0.05$

TABLE S4. Generalized Linear Mixed Models (GLMM) analysis of the neurobiological variables (dopamine, serotonin, DOPAC and 5-HIAA concentration) measured in the client fish *Naso elegans*.

Model: GLMM (Gamma)		Response variable: Dopamine concentration			
Final model term(s): Principal effects of CO ₂ , temperature, brain region and interaction between CO ₂ and temperature					
Reference level (Brain region): FB	Estimate	Std. Error	Estimate/ Std. Error	p	
(Intercept)	0.18	0.05	3.73	1.90E-04	
High CO ₂	0.17	0.09	2.01	0.04	
Warming	0.06	0.07	0.89	0.37	
Brain region (FBvsHB)	1.87	0.22	8.43	2.00E-16	
Brain region (FBvsMB)	10.50	1.11	9.42	2.00E-16	
High CO ₂ x Warming	-0.16	0.11	-1.38	0.17	
Reference level (Brain region): MB	Estimate	Std. Error	Estimate/ Std. Error	p	
(Intercept)	10.68	1.12	9.58	<2E-16	
High CO ₂	0.17	0.09	2.01	0.04	
Warming	0.06	0.07	0.89	0.37	
Brain region (MBvsFB)	-10.50	1.11	-9.42	<2E-16	
Brain region (MBvsHB)	-8.63	1.14	-7.60	3.00E-14	
High CO ₂ x Warming	-0.16	0.11	-1.38	0.17	
Model: GLMM (Gamma)		Response variable: DOPAC concentration			
Final model term(s): Principal effects of CO ₂ , temperature, brain region and interaction between them.					
Reference level (Brain region): FB	Estimate	Std. Error	Estimate/ Std. Error	p	
(Intercept)	-3.77	0.49	-7.74	9.90E-15	
High CO ₂	0.62	0.66	0.94	0.35	
Warming	0.24	0.66	0.36	0.72	
Brain region (FBvsHB)	-0.08	0.66	-0.12	0.91	
Brain region (FBvsMB)	-2.73	0.66	-4.14	3.50E-05	
High CO ₂ x Warming	-0.85	0.91	-0.93	0.35	
High CO ₂ x Brain region (FBvsHB)	-0.06	0.91	-0.06	0.95	
High CO ₂ x Brain region (FBvsMB)	-0.36	0.91	-0.39	0.70	
Warming x Brain region (FBvsHB)	-0.40	0.91	-0.44	0.66	
Warming x Brain region (FBvsMB)	-0.79	0.91	-0.86	0.39	
High CO ₂ x Warming x Brain region (FBvsHB)	1.04	1.27	0.82	0.41	
High CO ₂ x Warming x Brain region (FBvsMB)	0.52	1.27	0.41	0.68	
Reference level (Brain region): MB	Estimate	Std. Error	Estimate/ Std. Error	p	
(Intercept)	-6.50	0.45	-14.61	2.00E-16	
High CO ₂	0.26	0.63	0.42	0.67	
Warming	-0.55	0.63	-0.88	0.38	
Brain region (MBvsFB)	2.73	0.66	4.14	3.50E-05	
Brain region (MBvsHB)	2.65	0.63	4.22	2.50E-05	
High CO ₂ x Warming	-0.33	0.89	-0.37	0.71	
High CO ₂ x Brain region (MBvsFB)	0.36	0.91	0.39	0.70	
High CO ₂ x Brain region (MBvsHB)	0.30	0.89	0.34	0.74	
Warming x Brain region (MBvsFB)	0.79	0.91	0.86	0.39	
Warming x Brain region (MBvsHB)	0.39	0.89	0.43	0.66	
High CO ₂ x Warming x Brain region (MBvsFB)	-0.52	1.27	-0.41	0.68	
High CO ₂ x Warming x Brain region (MBvsHB)	0.52	1.26	0.41	0.68	
Model: GLMM (Gamma)		Response variable: Serotonin concentration			
Final model term(s): Principal effects of CO ₂ , and brain region					
Reference level (Brain region): FB	Estimate	Std. Error	Estimate/ Std. Error	p	
(Intercept)	0.51	0.07	7.33	2.30E-13	
High CO ₂	-0.12	0.08	-1.60	0.11	
Brain region (FBvsHB)	-0.33	0.08	-4.27	2.00E-05	
Brain region (FBvsMB)	-1.24	0.08	-16.25	2.00E-16	
Reference level (Brain region): MB	Estimate	Std. Error	Estimate/ Std. Error	p	
(Intercept)	-0.73	0.07	-10.40	<2E-16	
High CO ₂	-0.12	0.08	-1.60	0.11	
Brain region (MBvsFB)	1.24	0.08	16.20	<2E-16	
Brain region (MBvsHB)	0.91	0.08	12.00	<2E-16	
Model: GLMM (Gamma)		Response variable: 5-HIAA concentration			
Final model term(s): Principal effects of CO ₂ , temperature, brain region and interaction between CO ₂ and brain region					
Reference level (Brain region): FB	Estimate	Std. Error	Estimate/ Std. Error	p	
(Intercept)	2.93	0.40	7.38	1.60E-13	
High CO ₂	-0.02	0.43	-0.05	0.96	
Warming	-0.61	0.41	-1.51	0.13	
Brain region (FBvsHB)	2.66	0.58	4.60	4.30E-06	
Brain region (FBvsMB)	4.44	0.75	5.92	3.20E-09	
High CO ₂ x Brain region (FBvsHB)	2.31	0.98	2.36	0.02	
High CO ₂ x Brain region (FBvsMB)	-0.18	1.04	-0.17	0.86	
Reference level (Brain region): MB	Estimate	Std. Error	Estimate/ Std. Error	p	

(Intercept)	7.37	0.76	9.67	<2E-16
High CO2	-0.20	1.02	-0.20	0.84
Warming	-0.61	0.41	-1.51	0.13
Brain region (MBvsFB)	-4.44	0.75	-5.92	3.20E-09
Brain region (MBvsHB)	-1.78	0.88	-2.03	0.04
High CO2 x Brain region (MBvsFB)	0.18	1.04	0.17	0.86
High CO2 x Brain region (MBvsHB)	2.49	1.35	1.85	0.06

Numbers in bold p<0.05

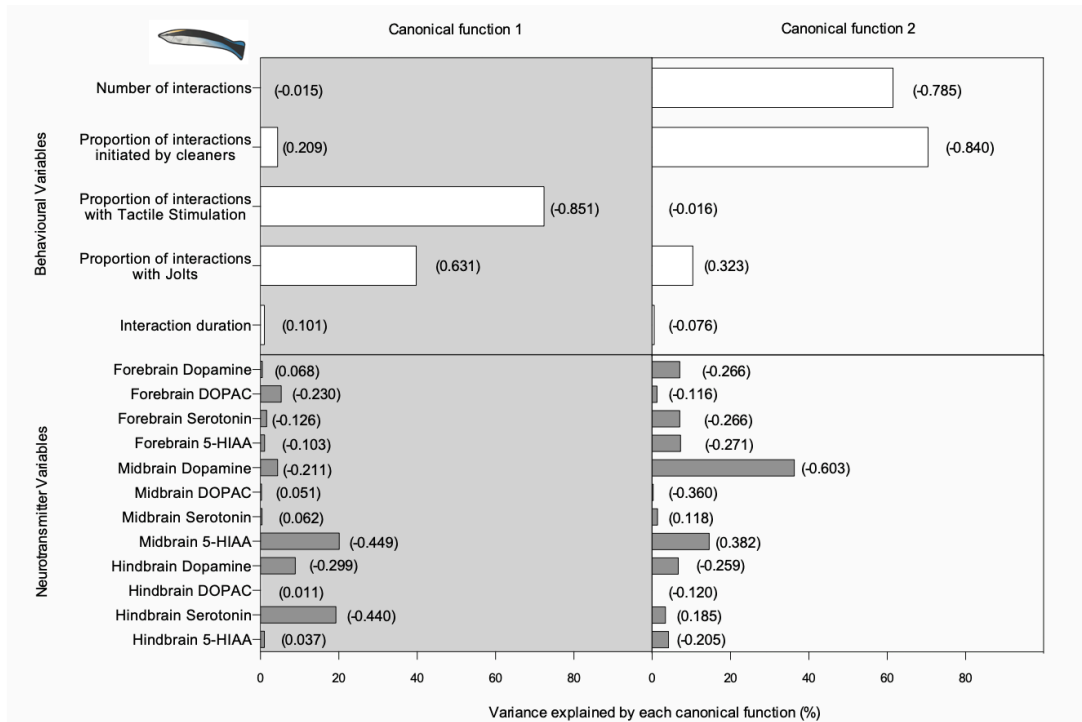


Figure S1. Canonical correlation analysis between behavioural and neurotransmitter variables related to cleaner fish *Labroides dimidiatus*. Percentage of variance explained each canonical function for analysed variables is shown in bars. Numbers in parenthesis indicate respective canonical cross-loading.

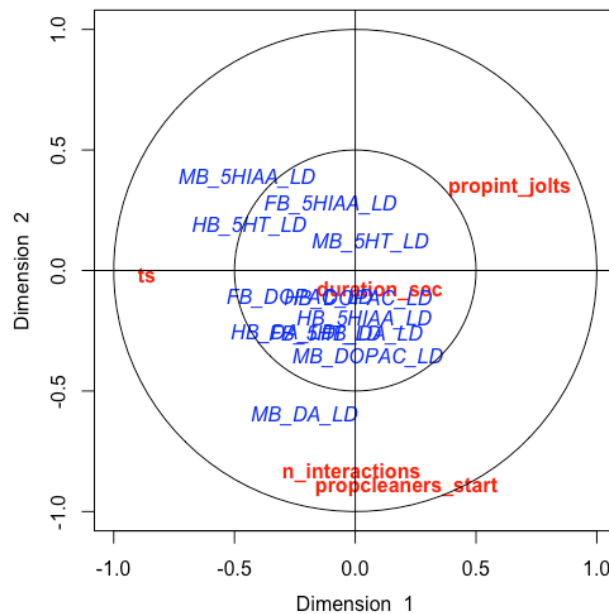


Figure S2. Biplot of the canonical correlation analysis between behavioural and neurotransmitter variables related to cleaner fish *Labroides dimidiatus*.

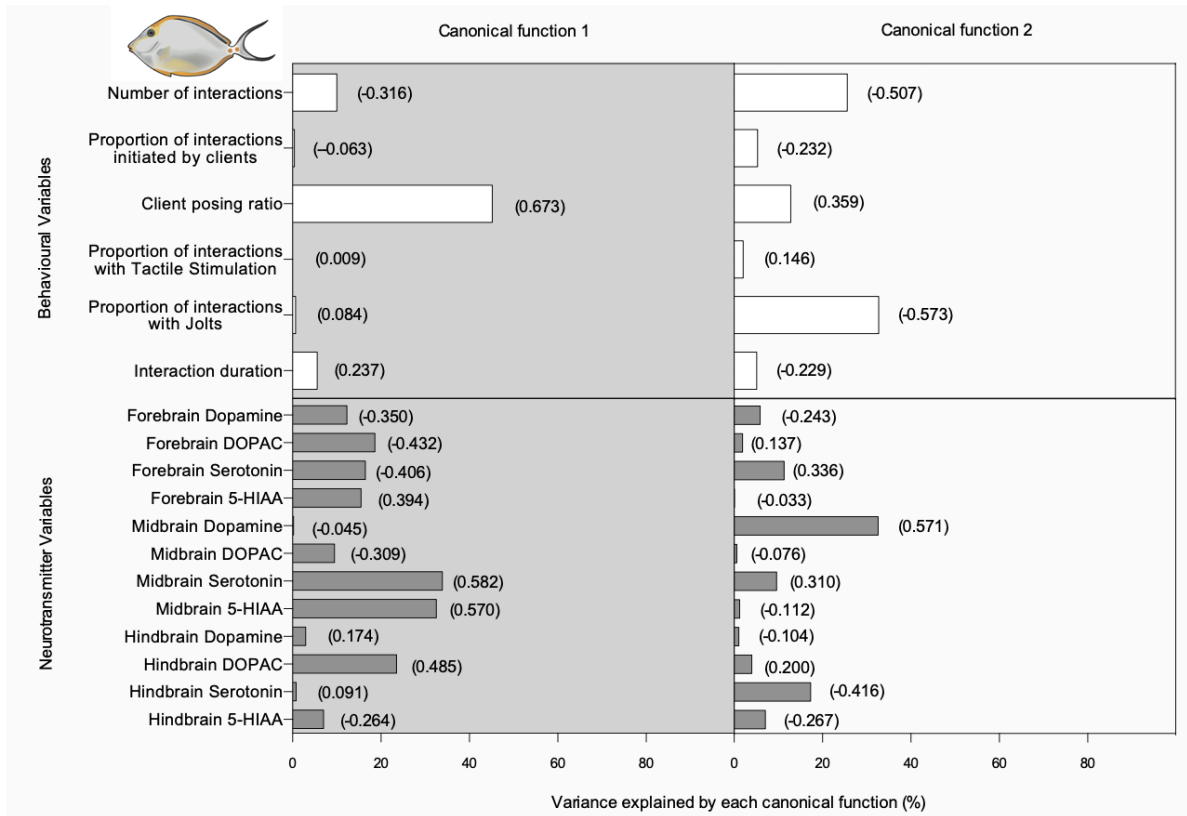


Figure S3. Canonical correlation analysis between behavioural and neurotransmitter variables related to client fish *Naso elegans*. Percentage of variance explained by each canonical function for analysed variables is shown in bars. Numbers in parenthesis indicate respective canonical cross-loading.

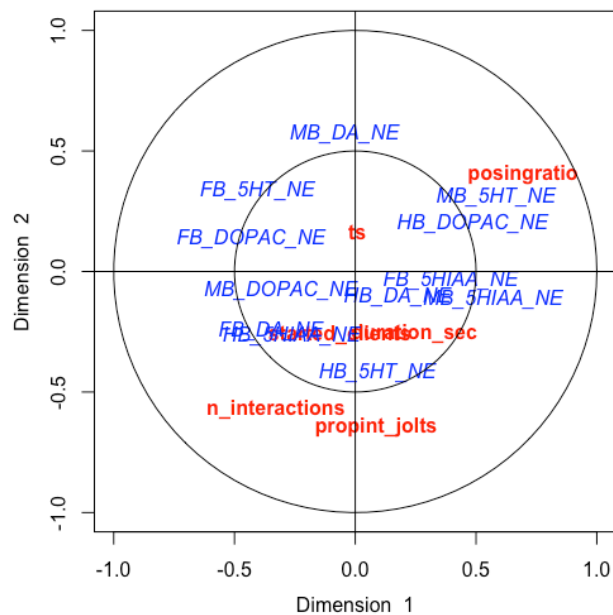


Figure S4. Biplot of the canonical correlation analysis between behavioural and neurotransmitter variables related to client fish *Naso elegans*.