Supplementary Information for

The past, present and future of cleaner fish cognitive performance as a function of CO2 levels

José Ricardo Paula[1*], Miguel Baptista[1], Francisco Carvalho[1], Tiago Repolho[1], Redouan Bshary[2], Rui Rosa[1]

Corresponding author: José Ricardo Paula

Email: jrpaula@fc.ul.pt

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Supplementary methods

Acclimation and monitoring

Upon arrival to the facilities of Laboratorio Marítimo da Guia, Fish were acclimated for five days at seawater conditions similar to the collection site: salinity = 35±0.5, temperature 26°C, pH 8.1 and pCO_2 404 µatm. Fish were provided with a PVC tube for refuge (2 cm diameter; 10 cm length). Cleaners were fed *ad libitum* once a day during the acclimation period. In each treatment, we used flow-through systems to maintain correct levels of alkalinity. Natural seawater (NSW) was pumped from the sea into a 5 m3 storage tank. From the tank, NSW was filtered (0.35 µm) and UV-irradiated (Vecton 300, TMC, Portugal) before being supplied to a mixing reservoir (4 mixing reservoir per treatment). In this, CO_2 was regulated according to each treatment. From each reservoir the water was pumped to 3 experimental tanks. These were kept under a photoperiod of 12h/12h. pH was monitored and automatically adjusted (Profilux 3.1N, GLH, Germany), downregulated by direct injection of certified CO_2 gas (Air Liquide, Portugal) and upregulated through scrubbing air with soda lime (Sofnolime, soda lime, Molecular Products Ltd) in mixing tanks. Temperature was regulated using heaters (300W, TMC, Portugal) in an temperature-controlled room. We complemented the automatic systems with a manual daily monitoring of seawater temperature (TFX 430, Germany), salinity (V2 refractometer, TMC, Portugal) and pH (826 pH, Metrohm, Germany). We quantified pH using a pH meter connected to a glass electrode (Si analytics, ±0.001), calibrated with TRIS-HCl (TRIS) and 2- aminopyridine-HCl (AMP) seawater buffers. Seawater carbonate system speciation was calculated twice a week from total alkalinity (spectrophotometrically at 595nm) and pH [S1]. Bicarbonate and pCO_2 values were calculated using CO2SYS software (constants from Mehrbach et al., 1973 [S2] refit by Dickson and Millero, 1987 [S3]).

References:

S1. Sarazin G, Michard G, Prevot F. 1999 A rapid and accurate spectroscopic method for alkalinity measurements in sea water samples. Water Res. 33, 290–294. (doi:10.1016/S0043-1354(98)00168-7)

S2. Mehrbach, C., Culberson, C. H., Hawley, J. E., Pytkowicx, R. M., 1973 Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure, Limnology and Oceanography, 18, doi: 10.4319/lo.1973.18.6.0897.

S3. Dickson, A. G., & Millero, F. J. 1987. A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. Deep Sea Research Part A, Oceanographic Research Papers, 34, 1733-1743. https://doi.org/10.1016/0198-0149(87)90021-5

Table S1. Seawater physicochemical parameters in all experimental setups.

Treatment	Sal.	T (°C)	pH	TA (µmol/kgSW)	TC (µmol/kgSW)	pCO_2 (µatm)	Ω Arg
275	35	25.96 ± 0.07	8.2 ± 0.01	2459 ± 24	2019 ± 25	263.5 ± 10	4.96 ± 0.05
315	35	25.87 ± 0.1	8.15 ± 0.01	2440 ± 27	2041 ± 25	305.2 ± 11.1	4.51 ± 0.1
404	35	25.65 ± 0.05	8.06 ± 0.01	2485 ± 17	2146 ± 19	405.5 ± 13.1	3.91 ± 0.06
750	35	25.61 ± 0.09	7.84 ± 0.01	2461 ± 25	2249 ± 30	734.2 ± 31.7	2.6 ± 0.05
980	35	25.63 ± 0.04	7.77 ± 0.02	2502 ± 28	2316 ± 37	933.5 ± 41.5	2.38 ± 0.14

Salinity, pH and temperature were measured daily and averaged over the whole experimental period. The combination of total alkalinity (TA) and pHT (pH total scale) was used to calculate carbonate system parameters [pCO_2 (carbon dioxide partial pressure), TC (total inorganic carbon) and Ω Arg (aragonite saturation state)]. Values are represented as mean ± standard deviation

Table S2. Bayes Generalized Linear Model (bayesGLM) of cleaner fish cognitive performance in function of pCO_2 treatment and plate colour.

Model: bayesGLM (Binomial)	***Response variable*: Cognitive performance**			
Final model term(s): Principal effects of pCO_2 and plate colour				
Reference level: pCO_2 404				
	Estimate	Std. Error	Z value	P
(Intercept)	0.197	0.622	0.317	0.752
pCO_2 276	0.202	0.815	0.248	0.804
pCO_2 315	0.305	0.815	0.374	0.709
pCO_2 750	-1.168	0.803	-1.455	0.146
pCO_2 980	-1.908	0.834	-2.289	**0.022**
Plate Green	1.334	0.590	2.61	**0.024**

Table S3. Bayes Generalized Linear Model (bayesGLM) of cleaner fish cognitive performance in function of pCO_2 treatment and plate colour, without fast performers.

Model: bayesGLM (Binomial)	***Response variable*: Cognitive performance**			
Final model term(s): Principal effects of pCO_2 and plate colour				
Reference level: pCO_2 404				
	Estimate	Std. Error	Z value	P
(Intercept)	0.380	0.636	0.598	0.550
pCO_2 276	-0.112	0.830	-0.135	0.893
pCO_2 315	0.052	0.836	0.062	0.950
pCO_2 750	-1.154	0.790	-1.462	0.144
pCO_2 980	-4.044	1.671	-2.420	**0.016**
Plate Green	0.848	0.642	1.321	0.186