

Element composition of shark vertebrae shows promise as a natural tag

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Supplementary material

Table S1. Analysis of variance of rearing conditions in the laboratory and mesocosm experiments over the duration of the experimental period

	d.f.	MS	pH		d.f.	Temperature		
			F	P		MS	F	P
Egg – Laboratory stage								
CO ₂	1	21.22	2986.8	< 0.001	508	0.84	3.107	< 0.001
Temperature	1	0.146	20.584	< 0.001	1	2927.4	10826.1	< 0.001
Temperature × CO ₂	1	0.265	37.357	< 0.001	303	0.21	0.779	0.995
Residual	1730	0.007			829	0.27		
Shark – Laboratory stage								
CO ₂	1	8.52	416.06	< 0.001	1	0.28	0.319	0.572
Temperature	1	0.081	3.959	0.055	1	2137.6	2404.215	< 0.001
Temperature × CO ₂	1	0.003	0.16	0.689	1	3.69	4.147	0.042
Residual	1022	0.021			1022	0.89		
Shark – Meso stage								
CO ₂	1	1.784	198.69	< 0.001	1	1.21	0.571	0.45
Temperature	1	0.11	12.229	0.001	1	956.91	450.192	< 0.001
Temperature × CO ₂	1	0.022	2.411	0.121	1	0.37	0.174	0.677
Residual	643	0.009			1387	2.13		

Table S2. Overall mean (± standard errors) limits of detection (LOD) for each element (in ppm) for each day of vertebral element analyses

Sampling day	n	⁷ Li	¹¹ B	²⁴ Mg	⁵⁵ M	⁶⁵ C	⁸⁸ Sr	¹¹⁵ In	¹³⁸ Ba	²³⁸ U
Day 1	63	0.505 (0.157)	2.142 (0.338)	862.760 (149.074)	8.292 (1.891)	3.412 (0.871)	247.467 (37.795)	0.358 (0.094)	0.788 (0.176)	0.041 (0.010)
Day 2	41	0.508 (0.207)	0.885 (0.062)	383.999 (25.754)	4.249 (1.508)	4.713 (0.743)	98.460 (18.132)	1.721 (0.249)	0.922 (0.199)	0.014 (0.005)

Table S3. Parameter estimates of top ranked linear models of elemental composition in Port Jackson shark *Heterodontus portusjacksoni* vertebrae (using the dredge function)

In total, 40 models were ranked for each element, but only the top five ranked models are shown (based on AICc). Note for elements marked in bold, the highest ranking models contained the covariate ‘experiment-type’. For these elements, their data were partitioned into laboratory and mesocosm datasets and the suite of models re-run. The best ranked individual laboratory and mesocosm linear models were run to see how element incorporation differed between experiment-types (refer to Table S4). In categorical variables EXP (experiment type) and Sex, a plus (+) symbol means they were included in the model

Element	Model	df	EXP	Condition	pH	Sex	Temp	pH x Temp	r ²	AICc
Li:Ca	8	5	+	0.02	0.13				0.31	-225.24
	2	3	+						0.28	-225.05
	6	4	+		0.12				0.30	-224.90
	4	4	+	0.02					0.30	-224.85
	24	6	+	0.02	0.14		0.00		0.32	-223.79
B:Ca**	18	4	+				0.03		0.43	-161.65
	26	5	+			+	0.03		0.44	-160.52
	22	5	+		-0.05		0.03		0.43	-159.63
	20	5	+	0.00			0.03		0.43	-159.51
	28	6	+	-0.01		+	0.03		0.44	-158.58
Mg:Ca	7	4		-0.03	0.14				0.10	-171.30
	4	4	+	-0.03					0.10	-171.19
	23	5		-0.02	0.16		0.01		0.12	-171.06
	21	4			0.19		0.01		0.09	-171.01
	20	5	+	-0.02			0.01		0.11	-170.88
Mn:Ca	19	4		-0.08			0.10		0.22	38.63
	17	3					0.10		0.20	39.71
	27	5		-0.09		+	0.10		0.23	39.80
	20	5	+	-0.07			0.10		0.23	40.30
	23	5		-0.07	0.13		0.10		0.23	40.47
Cu:Ca	17	3					0.00		0.11	-473.65
	21	4			-0.02		0.00		0.13	-473.44
	18	4	+				0.00		0.13	-473.17
	53	5			0.24		0.12	-0.01	0.15	-473.04
	23	5		0.00	-0.02		0.00		0.13	-471.84
Sr:Ca**	18	4	+				0.09		0.60	55.90
	22	5	+		-0.43		0.09		0.60	56.73
	54	6	+		-4.33		-1.68	0.22	0.61	57.42
	20	5	+	0.02			0.09		0.60	57.91
	26	5	+			+	0.09		0.60	58.13
Ba:Ca	17	3					0.03		0.14	-138.44
	18	4	+				0.03		0.15	-137.58
	21	4			-0.08		0.03		0.15	-137.30
	19	4		0.00			0.03		0.14	-136.28
	25	4				+	0.03		0.14	-136.27
U:Ca**	2	3	+						0.35	-576.63
	10	4	+			+			0.36	-576.39
	12	5	+	0.00		+			0.37	-575.52
	18	4	+				0.00		0.36	-575.52
	26	5	+			+	0.00		0.37	-575.37

Table S4. Parameter estimates of top ranked linear models of elemental composition in Port Jackson shark *Heterodontus portusjacksoni* vertebrae (using the dredge function) for elements where data were partitioned by ‘experiment type’

In total, 20 models were ranked for each element, but only the top five ranked models are shown (based on AICc). Experiment-type is defined as either laboratory (LABORATORY) or mesocosm (MESO). In categorical variable Sex, a plus (+) symbol means it was included in the model

Element	Model	df	Condition	pH	Sex	Temp	pH x Temp	r ²	AICc
Li:Ca _{LABORATORY}	2	3	0.03					0.05	-138.20
	1	2						0.00	-137.20
	4	4	0.03	0.11				0.07	-137.10
	10	4	0.03			0.00		0.06	-136.30
	6	4	0.03		+			0.05	-136.10
Li:Ca _{MESO}	3	3		0.26				0.10	-91.40
	1	2						0.00	-90.50
	11	4		0.31	0.01	0.14			-90.20
	7	4		0.26	+			0.10	-88.80
	4	4	0.00	0.26				0.10	-88.80
B:Ca _{LABORATORY}	9	3				0.02		0.10	-95.30
	10	4	-0.03			0.02		0.12	-94.10
	13	4			+	0.02		0.11	-93.50
	11	4		-0.09		0.02		0.11	-93.40
	14	5	-0.03		+	0.02		0.13	-92.80
B:Ca _{MESO}	9	3				0.04		0.16	-66.30
	10	4	0.02			0.04		0.19	-64.90
	13	4			+	0.04		0.18	-64.40
	11	4		0.17		0.04		0.18	-64.30
	1	2						0.00	-63.30
Sr:Ca _{LABORATORY}	9	3				0.08		0.14	39.10
	11	4		-0.57		0.08		0.17	39.40
	27	5		-5.99		-2.33	0.31	0.19	40.50
	13	4			+	0.08		0.15	40.60
	15	5		-0.59	+	0.08		0.18	40.80
Sr:Ca _{MESO}	9	3				0.13		0.13	21.10
	13	4			+	0.12		0.17	22.20
	1	2						0.00	23.20
	11	4		0.33		0.13		0.14	23.50
	10	4	0.01			0.13		0.13	23.70
U:Ca _{LABORATORY}	1	2						0.00	-373.30
	9	3				0.00		0.01	-371.70
	2	3	0.00					0.01	-371.50
	5	3			+			0.00	-371.30
	3	3		-0.01				0.00	-371.30
U:Ca _{MESO}	5	3			+			0.13	-205.60
	6	4	0.00		+			0.16	-204.20
	1	2						0.00	-203.60
	13	4			+	0.00		0.14	-203.40
	7	4		0.01	+			0.14	-203.30

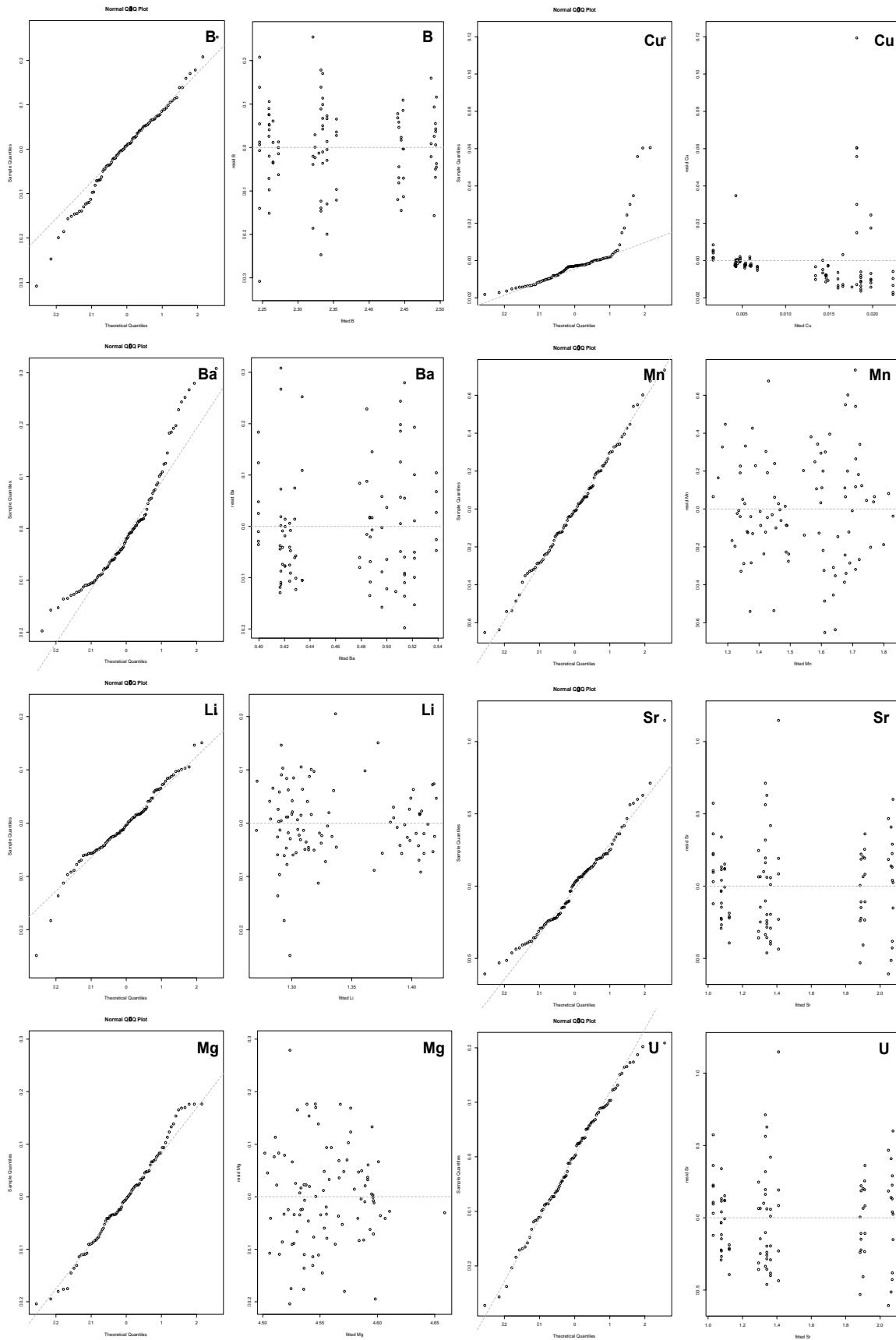


Fig. S2. Composite residuals and Q-Q plots for each element.

Fig. S3. Summary plot of the significant main effects of temperature (a–g, in green) and pH (h, i, in cyan) on vertebrae elemental chemistry of Port Jackson shark *Heterodontus portusjacksoni* in aquaria and mesocosm conditions – adapted from Fig. 2 in MS. Shaded ribbons represent the 95% confidence interval. Experiment-type is defined as laboratory (LABORATORY) or mesocosm (MESO). When the best ranked model contained the term ‘experiment’ the model was re-run with the data partitioned by ‘experiment-type’. Note, all element : Ca values are on a log scale. Circles represent raw data. See next page.

