

Table S1. Action and concentrations of the agents used to assay mitochondrial respiration of permeabilised heart fibres from the European cuttlefish

Agent	Action	Final concentration
Substrates		
Proline	Amino acid substrate	5 mmol l ⁻¹
Pyruvate	Carbohydrate substrate	5 mmol l ⁻¹
Succinate	Carbohydrate substrate	10 mmol l ⁻¹
ADP	Substrate for ATP generation	2.5 mmol l ⁻¹
Cytochrome <i>c</i>	Electron carrier, tests for outer membrane integrity	0.01 mmol l ⁻¹
TMPD	Complex IV substrate	5 mmol l ⁻¹
Ascorbate	Complex IV substrate	20 mmol l ⁻¹
Inhibitors		
Antimycin	Inhibits complex III, to test non-mitochondrial respiration	2.5 µmol l ⁻¹
Rotenone	Complex I inhibitor	2.5 µmol l ⁻¹
Oligomycin	Inhibits ATP synthase, to test proton leak	2 µg ml ⁻¹
Uncoupler		
FCCP	Uncouples the electron transport system electron flow from ATP generation by relieving the proton gradient	2.5 µmol l ⁻¹

Table S2. Measurements of the European cuttlefish *Sepia officinalis* used in acclimation experiments

Identifier	Origin	Acclimation temperature (°C)	Sex	Date of sampling	Total mass (g)	Mantle length (cm)	Systemic heart mass (mg)	Branchial heart #1 mass (mg)	Branchial heart #2 mass (mg)	Comments
A06	Adriatic	11	Male	7/07/2009	90.22	8.8	58.3	38.5	33.5	
A10	Adriatic	11	Male	14/07/2009	63.34	8.0	39.3	25.4	28.9	
A13	Adriatic	11	Male	16/07/2009	83.75	8.5	49.3	37.0	34.3	Animal was inking during capture
A30	Adriatic	11	Female	21/09/2009	99.20	9.1	58.6	38.0	37.4	Animal was inking during capture
A63	Adriatic	11	Female	14/07/2010	110.53	9.3	58.3	39.7	39.2	Animal was inking during capture
A64	Adriatic	11	Male	19/07/2010	78.47	8.2	40.1	27.8	30.9	Animal was inking during capture
A65	Adriatic	11	Female	20/07/2010	86.40	8.0	43.1	34.9	35.3	Animal was slightly inking during capture
A69*	Adriatic	11	Male	22/11/2010	48.20	–	24.6	19.9	18.4	
A07	Adriatic	16	Female	8/07/2009	46.90	6.8	30.5	23.7	22.4	
A09	Adriatic	16	Male	13/07/2009	40.57	7.0	27.0	11.6	19.5	Animal was inking during capture
A14	Adriatic	16	Male	20/07/2009	72.52	8.2	44.9	24.7	27.5	Animal was inking during capture
A19	Adriatic	16	Female	30/07/2009	77.88	7.9	46.4	42.2	38.5	Animal was inking during capture, very well developed gonads
A24	Adriatic	16	Female	10/08/2009	59.11	7.4	37.1	41.6	42.6	
A46*	Adriatic	16	Female	20/04/2010	50.10	7.1	27.3	18.3	18.7	Animal was inking during capture
A47*	Adriatic	16	Male	21/04/2010	49.87	7.0	27.1	15.0	16.5	Animal was inking during capture
A48*	Adriatic	16	Male	26/04/2010	59.67	7.5	33.7	24.5	21.8	Animal was inking during capture
A49*	Adriatic	16	Female	27/04/2010	62.66	7.2	33.0	20.9	24.7	Animal was inking during capture
A50*	Adriatic	16	Male	28/04/2010	52.05	6.9	29.1	22.1	24.3	Animal was inking during capture
A51*	Adriatic	16	Female	29/04/2010	41.34	6.2	25.6	13.6	16.7	Animal was inking during capture
A61	Adriatic	16	Male	12/07/2010	109.93	9.5	77.4	42.1	44.5	Animal was inking during capture
A62	Adriatic	16	Male	13/07/2010	144.00	10.5	101.1	56.3	56.2	
A52*	Adriatic	21	Female	26/05/2010	148.82	10.5	74.7	40.3	43.8	
A53*	Adriatic	21	Male	31/05/2010	128.71	10.0	68.7	40.0	39.8	
A54*	Adriatic	21	Female	2/06/2010	228.17	11.2	113.2	65.3	78.8	Female had proper developed gonads with yellowish transparent eggs
A55*	Adriatic	21	Male	3/06/2010	252.92	12.7	135.6	74.7	74.3	
A56*	Adriatic	21	Male	7/06/2010	149.30	10.4	75.7	55.3	47.6	
A57*	Adriatic	21	Male	9/06/2010	150.37	10.1	75.6	41.5	45.3	
A58	Adriatic	21	Female	7/07/2010	147.75	10.0	86.7	58.7	56.9	Female had eggs
A59	Adriatic	21	Female	8/07/2010	226.54	11.3	140.7	82.9	79.9	Animal was inking during capture, female had eggs
A60	Adriatic	21	Male	9/07/2010	198.63	11.6	109.0	69.4	74.8	Animal was inking during capture
A66	Adriatic	21	Female	21/07/2010	184.21	10.8	145.4	87.7	87.2	Female already mated as there were sucker marks on the head, had hardly ink in ink sac
A67	Adriatic	21	Female	23/07/2010	144.90	9.5	108.5	57.4	59.1	Female already mated as there were sucker marks on the head, had hardly ink in ink sac
A68	Adriatic	21	Female	26/07/2010	248.32	12.2	161.3	96.0	101.3	
A08	Oosterschelde	11	Male	10/07/2009	59.09	7.3	42.8	23.9	27.5	
A11	Oosterschelde	11	Male	15/07/2009	79.46	7.9	63.5	41.2	38.3	
A12	Oosterschelde	11	Female	21/07/2009	89.19	8.3	63.2	37.5	39.1	
A23	Oosterschelde	11	Male	6/08/2009	96.64	8.9	81.2	51.3	48.5	Animal was inking during capture
A25	Oosterschelde	11	Male	12/08/2009	112.12	9.5	70.1	47.0	42.0	
A26	Oosterschelde	11	Male	13/08/2009	77.84	8.5	60.1	37.0	38.5	
A27	Oosterschelde	11	Male	18/08/2009	92.99	8.6	73.0	43.4	45.8	Animal was inking during capture
A28	Oosterschelde	11	Male	8/09/2009	79.98	8.1	65.1	38.1	36.0	
A29	Oosterschelde	11	Male	17/09/2009	83.31	8.7	83.6	53.9	50.6	
A04	Oosterschelde	16	Female	4/06/2009	72.46	8.5	–	–	–	

A05	Oosterschelde	16	Female	17/06/2009	84.50	8.6	57.3	31.6	31.4	Animal was inking during capture
A15	Oosterschelde	16	Female	22/07/2009	97.70	8.9	61.6	38.4		
A18	Oosterschelde	16	Male	29/07/2009	108.29	9.1	111.4	46.4	43.8	Animal was inking during capture
A31	Oosterschelde	16	Male	22/09/2009	125.10	9.3	114.5	50.4	59.3	
A32	Oosterschelde	16	Male	23/09/2009	79.75	7.7	73.1	34.4	33.8	
A33	Oosterschelde	16	Female	24/09/2009	253.83	12.5	315.7	130.0	134.3	Female had proper developed gonads with yellowish transparent and white eggs
A34*	Oosterschelde	16	Female	28/08/2009	164.90	9.3	133.6	93.5	86.7	
A35	Oosterschelde	16	Male	29/09/2009	155.89	10.2	131.5	65.4	68.0	Animal was inking during capture
A36	Oosterschelde	16	Female	30/09/2009	128.38	9.1	97.3	54.7	56.1	Animal was inking during capture
A37	Oosterschelde	16	Female	1/10/2009	165.94	10.3	183.0	90.1	87.0	
A38	Oosterschelde	16	Male	5/10/2009	164.39	11.4	167.9	79.0	70.0	
A39*	Oosterschelde	16	Male	6/10/2009	278.93	12.6	214.8	127.8	133.0	
A40*	Oosterschelde	16	Male	7/10/2009	177.30	11.0	142.2	87.4	80.5	
A41*	Oosterschelde	16	Male	8/10/2009	178.59	10.5	123.9	80.8	89.8	Animal was slightly inking during capture
A42*	Oosterschelde	16	Female	12/10/2009	225.04	10.9	183.3	108.6	116.9	
A43*	Oosterschelde	16	Male	13/10/2009	258.39	12.3	210.0	131.8	138.1	Animal was slightly inking during capture
A44*	Oosterschelde	16	Female	14/10/2009	299.15	12.8	194.7	161.2	133.2	Female had proper developed gonads with yellowish transparent eggs
A45*	Oosterschelde	16	Female	15/10/2009	276.24	12.3	184.6	146.7	155.6	Female had proper developed gonads with yellowish transparent eggs
A16	Oosterschelde	21	Female	27/07/2009	71.33	7.9	56.8	36.2	32.7	
A17	Oosterschelde	21	Male	28/07/2009	83.59	8.1	77.7	41.4	39.5	Heart muscle was relatively tough
A20	Oosterschelde	21	Female	3/08/2009	102.13	8.5	75.5	45.7	44.5	
A21	Oosterschelde	21	Male	4/08/2009	124.66	9.9	106.3	62.3	67.9	
A22	Oosterschelde	21	Male	5/08/2009	123.77	8.9	110.7	65.3	60.4	

Individuals marked with asterisks were used for morphometric statistics but not for measurement of mitochondrial respiration.