

Supplementary Information

Table S1. List of GenBank from Cyt *b* sequences used to design the two assays specific to *Huso huso* and *Acipenser naccarii*. Each sequence was trimmed to match only the region targeted by the assay. Species, GenBank accession number (GenBank) and reference are indicated. The sequences were downloaded from NCBI-GenBank (Nucleotide Archive) on 2023-05-26.

Species	GenBank	Reference
<i>Acipenser naccarii</i>	AF006150; AF006161	[1]
	AF217208	[2]
	AF238659; AF238660	[1]
	AF283730	[3]
	AF404783	[1]
	AJ245833; AJ245834	[4]
<i>Huso huso</i>	FJ974044	[5]
	KC130110 - KC130117	[6]
<i>Acipenser baerii</i>	AF217206; AF217207	[2]
	AF283727; AF283728	[3]
	AF308887 - AF308892	[7]
	AJ245825	[4]
	EU733253	[8]
	JX238385 - JX238395	[9]
	JX238422; JX238423	[9]
	OM320999	[10]
<i>Acipenser baerii baerii</i>	X95054	[1]
	AF238644 - AF238654	[1]
<i>Acipenser baerii baicalensis</i>	FJ010860 - FJ010862	[11]
	AF238625 - AF238631	[1]
<i>Acipenser baerii baicalensis</i>	AF404779	[1]
	KT232131 - KT232133	[12]
<i>Acipenser gueldenstaedtii</i>	MG648475	[13]
<i>Acipenser ruthenus</i>	AY846680 - AY846688	[9]
<i>Acipenser stellatus donensis</i>	AY846689 - AY846700	[14]
<i>Acipenser stellatus ponticus</i>	AY846701	[14]
<i>Acipenser stellatus stellatus</i>	AF184107	[15]
	AF308911	[7]
	AF404784	[1]
	JX238412 - JX238421	[9]
	KF013247	[16]
	X14944	[17]
<i>Acipenser sturio</i>	AF006134	[1]
	AF217209	[2]
	AF283742	[3]
	EU733248 - EU733250	[18]
	FJ974043	[5]
	FN256390 - FN256394	[19]

Table S2. Detailed results obtained for each environmental sample regarding the detection of *Acipenser naccarii* and *Huso huso*. Sample name (ID), sample type (column – benthic water), cycle thresholds (C_t) for each replicate, number of the cycle indicated, (Rep) and the average cycle thresholds with the standard deviation (C_t ± s.d.) values are also reported.

<i>Acipenser naccarii</i>									<i>Huso huso</i>						
ID	Type	Rep1	Rep2	Rep3	Rep4	Rep5	Rep6	C _t ± s.d.	Rep1	Rep2	Rep3	Rep4	Rep5	Rep6	C _t ± s.d.
S1	Column	33	-	32	-	32	31	32 ± 0.69	30	30	-	31	-	-	30 ± 0.40
	Benthic	30	31	30	32	31	31	31 ± 0.65	30	30	31	31	30	30	30 ± 0.50
S2	Column	32	31	-	-	31	32	31 ± 0.60	33	-	33	33	-	32	32 ± 0.40
	Benthic	30	29	30	31	30	30	30 ± 0.51	30	32	31	32	32	32	31 ± 0.75
15c	Column	32	31	32	31	31	31	31 ± 0.52	33	32	32	32	32	32	32 ± 0.65
15b	Benthic	30	30	30	32	31	31	31 ± 0.72	33	32	32	32	32	32	32 ± 0.65
16c	Column	-	-	-	-	-	-	-	33	33	32	33	33	32	33 ± 0.71
16b	Benthic	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17c	Column	40	40	41	29	30	30	35 ± 5.86	33	33	32	32	32	33	33 ± 0.62
17b	Benthic	39	40	41	29	30	30	35 ± 2.20	35	35	33	34	35	35	34 ± 0.62
1c	Column	34	35	35	35	35	35	35 ± 0.66	40	41	41	37	38	-	39 ± 1.93
1b	Benthic	35	36	35	36	35	36	35 ± 0.53	41	-	-	-	-	-	41 ± 0.00
2c	Column	35	36	-	36	37	-	36 ± 0.73	34	34	34	37	36	36	35 ± 1.33
2b	Benthic	34	35	36	36	36	41	36 ± 2.48	36	34	35	35	37	36	35 ± 1.02
3c	Column	34	34	34	36	39	39	36 ± 2.26	37	39	39	-	-	-	38 ± 1.21
3b	Benthic	36	36	-	37	-	-	36 ± 0.72	-	-	-	-	-	-	-
4c	Column	35	33	35	35	34	35	34 ± 0.63	36	34	35	40	36	-	36 ± 2.19
4b	Benthic	34	35	34	34	34	33	34 ± 0.65	36	35	36	35	35	34	35 ± 0.71
5c	Column	35	35	36	38	37	38	37 ± 1.43	34	38	37	35	36	36	36 ± 1.30
5b	Benthic	34	35	34	32	36	33	34 ± 1.12	35	35	34	35	36	35	35 ± 0.62
6c	Column	34	34	35	35	33	34	35 ± 0.62	36	34	35	34	35	-	35 ± 0.73
6b	Benthic	34	35	35	33	33	33	34 ± 1.16	34	35	33	36	35	36	35 ± 0.99
7c	Column	34	33	35	38	38	38	36 ± 1.97	35	37	-	34	-	37	36 ± 1.41
7b	Benthic	33	34	33	36	35	34	34 ± 1.22	39	-	39	39	38	-	39 ± 0.56
8c	Column	31	32	32	31	32	31	32 ± 0.62	34	-	-	-	-	-	34 ± 0.00
8b	Benthic	34	35	33	37	37	37	36 ± 1.75	36	-	-	37	-	-	36 ± 0.20
9c	Column	33	36	33	32	33	32	33 ± 1.20	-	-	-	-	-	-	-
9b	Benthic	34	37	33	33	33	33	34 ± 1.39	-	-	-	-	-	-	-

Table S3. Detailed results obtained for tissue sample of *Acipenser naccarii* and *Huso huso* used to generate the standard curve. Eight dilutions were tested, concentration of DNA ([DNA] ng/μl), cycle thresholds (C_t) for each replicate, number of the cycle indicated, (Rep), average cycle thresholds (C_t) value are reported.

Dilutions	[DNA]	<i>Acipenser naccarii</i>			<i>Huso huso</i>		
		Rep1	Rep2	C_t	Rep1	Rep2	C_t
0	340	17	17	17	16	16	16
1	340 × 10 ⁻¹	20	20	20	20	20	20
2	340 × 10 ⁻²	25	25	25	24	24	24
3	340 × 10 ⁻³	30	30	30	28	28	28
4	340 × 10 ⁻⁴	34	33	33	32	32	32
5	340 × 10 ⁻⁵	38	38	38	36	35	35
6	340 × 10 ⁻⁶	42	42	42	39	39	39
7	340 × 10 ⁻⁷	-	-	-	-	-	-
8	340 × 10 ⁻⁸	-	-	-	-	-	-

Table S4. qPCR outcome summary of *Acipenser naccarii* and *Huso huso* environmental samples analysis. Sample name (ID), sample type (column – benthic water), average cycle thresholds (C_t), concentration of DNA (ng/ μ l), DNA counts/ μ l and DNA counts/L are indicated.

ID	Type	<i>Acipenser naccarii</i>				<i>Huso huso</i>			
		C_t	[DNA] ng/ μ l	DNA counts/ μ l	DNA counts/L	C_t	[DNA] ng/ μ l	DNA counts/ μ l	DNA counts/L
S1	Column	32	0.103	3.79E+25	3.79E+31	30	0.0809	9.28E+28	9.28E+34
	Benthic	31	0.179	4.12E+27	4.12E+33	30	0.0885	1.82E+29	1.82E+35
S2	Column	31	0.127	2.25E+26	2.25E+32	32	0.0214	4.43E+24	4.43E+30
	Benthic	30	0.262	1.06E+29	1.06E+35	31	0.0410	5.80E+26	5.80E+32
15c	Column	31	0.130	2.76E+26	2.76E+32	32	0.0427	7.81E+26	7.81E+32
15b	Benthic	31	0.174	3.29E+27	3.29E+33	32	0.0267	2.32E+25	2.32E+31
16c	Column	-	-	-	-	33	0.0182	1.33E+24	1.33E+30
16b	Benthic	-	-	-	-	-	-	-	-
17c	Column	35	0.019	2.55E+19	2.55E+25	33	0.0193	2.08E+24	2.08E+30
17b	Benthic	35	0.011	3.19E+17	3.19E+23	34	0.0064	5.27E+20	5.27E+26
1c	Column	35	0.021	5.74E+19	5.74E+25	39	0.0004	2.29E+11	2.29E+17
1b	Benthic	35	0.016	4.99E+18	4.99E+24	41	0.0001	7.33E+07	7.33E+13
2c	Column	36	0.011	1.84E+17	1.84E+23	35	0.0044	3.30E+19	3.30E+25
2b	Benthic	36	0.009	4.67E+16	4.67E+22	35	0.0037	8.25E+18	8.25E+24
3c	Column	36	0.011	2.77E+17	2.77E+23	38	0.0007	2.08E+13	2.08E+19
3b	Benthic	36	0.010	9.87E+16	9.87E+22	-	-	-	-
4c	Column	34	0.026	3.44E+20	3.44E+26	36	0.0025	4.90E+17	4.90E+23
4b	Benthic	34	0.032	1.85E+21	1.85E+27	35	0.0038	1.11E+19	1.11E+25
5c	Column	37	0.008	2.23E+16	2.23E+22	36	0.0028	1.03E+18	1.03E+24
5b	Benthic	34	0.031	1.67E+21	1.67E+27	35	0.0047	5.61E+19	5.61E+25
6c	Column	35	0.021	6.06E+19	6.06E+25	35	0.0044	3.05E+19	3.05E+25
6b	Benthic	34	0.037	5.94E+21	5.94E+27	35	0.0047	5.62E+19	5.62E+25
7c	Column	36	0.120	3.50E+17	3.50E+23	36	0.0026	6.41E+17	6.41E+23
7b	Benthic	34	0.027	4.06E+20	4.06E+26	39	0.0005	4.48E+12	4.48E+18
8c	Column	32	0.106	5.00E+25	5.00E+31	34	0.0067	7.78E+20	7.78E+26
8b	Benthic	36	0.013	1.27E+18	1.27E+24	36	0.0019	5.42E+16	5.42E+22
9c	Column	33	0.045	3.73E+22	3.73E+28	-	-	-	-
9b	Benthic	34	0.035	3.87E+21	3.87E+27	-	-	-	-

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