Wild brown trout affected by historical mining in the Cévennes National Park, France.

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Supporting Information S1

Extended details for morphometrics

Four bilateral morphometric traits were selected for FA determination: length of pectoral fins, length of pelvic fins, distance between snout and the anterior edge of the eye, distance between the posterior edge of the eye and the posterior edge of the operculum. All measurements were performed twice. Since a number of factors such as the presence of other forms of asymmetry, allometry or measurement error can lead to a biased estimation of FA,[1] a series of preliminary tests were conducted for all traits. These tests were performed for each sample on the distributions of either signed asymmetries (right-minus-left values [Ri-Li]) or absolute asymmetries (|Ri-Li|) of the four traits. Directional asymmetry (DA) and antisymmetry (AS) occur when one side of a bilateral character is consistently larger than the other but, in the case of AS, the side that is larger varies at random among individuals.[1] DA and AS were tested with conventional methods.[2] Absolute asymmetry values were also used to test for the presence of allometry by linear regression on character size defined as ([Ri+Li]/2). As numerous tests were repeatedly conducted on the four traits, the sequential Bonferroni test[3] was systematically applied (across the series of k=4 tests) in order to limit the occurrence of type-I error.

Finally, a two-way mixed model ANOVA (side-fixed × individual-random) was performed with repeated measurements on each side[2,4]. This approach, in addition to testing for DA, allows the measurement error variance to be partitioned out from the non-directional asymmetry variance.

Population FA levels were estimated for all traits using the means of the absolute asymmetry distributions (so-called index FA1)[2], as well as the variance component corresponding to the true between-sides variance obtained by partitioning measurement error out of the sides x individual mean squares of the two-way ANOVA results (so-called index FA10). Differences between samples were tested using ANOVA for FA1 and F-ratio for FA10.[2]

		COC						COM						CUB					
		df	MS	F	p	FA10	df(FA10)	df	MS	F	p	FA10	df(FA10)	df	MS	F	p	FA10	df(FA10)
Pectoral length	side	1	2.8E-03	0.05	0.83			1	0.1	0.12	0.73			1	1.0E-03	2.8E-03	0.96		
	individual	19	171.2	3034.7	3.9E-29			19	127.2	156.82	5.9E-17			17	51.5	145.0	4.8E-15		
	interaction	19	0.1	16.4	2.9E-13	0.03	16.7	19	0.8	74.87	2.5E-25	0.40	18.5	17	0.4	16.7	3.2E-12	0.17	15.0
	Meas. Error	40	3.4E-03					40	1.1E-02					36	2.1E-02				
Pelvic length	side	1	2.4E-03	0.04	0.84			1	0.8	1.19	0.29			1	4.8E-02	0.21	0.65		
	individual	19	108.8	1876.3	3.8E-27			19	65.5	97.88	4.9E-15			17	34.5	150.3	3.5E-15		
	interaction	19	0.1	11.0	1.7E-10	0.03	15.6	19	0.7	88.25	1.0E-26	0.33	18.6	17	0.2	9.3	1.5E-08	0.10	13.4
	Meas. Error	40	5.3E-03					40	7.6E-03					36	2.5E-02				
Snout-Eyes	side	1	9.4E-03	0.19	0.67			1	0.3	0.28	0.60			1	2.5E-02	0.09	0.77		
	individual	19	25.6	522.0	7.0E-22			19	38.5	34.10	7.9E-11			17	2.2	8.1	4.0E-05		
	interaction	19	4.9E-02	13.7	5.3E-12	0.02	16.3	19	1.1	149.43	0.0E+00	0.56	18.7	17	0.3	16.2	4.8E-12	0.13	14.9
	Meas. Error	40	3.6E-03					40	7.6E-03					36	1.7E-02				
Eyes-Operculum	side	1	0.2	3.61	0.07			1	0.9	2.46	0.13			1	0.1	0.94	0.35		
	individual	19	153.5	3304.8	1.7E-29			19	105.7	274.31	3.1E-19			17	19.4	132.9	9.9E-15		
	interaction	19	4.6E-02	9.1	3.4E-09	0.02	14.9	19	0.4	60.23	1.6E-23	0.19	18.4	17	0.1	4.3	1.2E-04	0.06	9.7
	Meas. Error	40	5.1E-03					40	6.4E-03					36	3.4E-02				

		PDP						RAM						VER					
		df	MS	F	p	FA10	df(FA10)	df	MS	F	p	FA10	df(FA10)	df	MS	F	p	FA10	df(FA10)
Pectoral length	side	1	0.1	0.17	0.69			1	0.6	2.6	0.13			1	0.0	0.07	0.80		
	individual	19	74.5	214.8	3.1E-18			15	54.9	253.6	2.9E-15			11	65.9	626.3	9.8E-14		
	interaction	19	0.3	36.1	2.4E-19	0.17	18.0	15	0.2	37.7	4.5E-16	0.11	14.2	11	0.1	8.8	5.3E-06	0.05	8.6
	Meas. Error	40	9.6E-03					32	5.7E-03					24	1.2E-02				
Pelvic length	side	1	0.2	0.43	0.52			1	0.3	0.60	0.45			1	0.2	2.4	0.15		
	individual	19	55.7	123.3	5.7E-16			15	31.1	66.8	5.6E-11			11	22.1	347.9	2.5E-12		
	interaction	19	0.5	21.7	2.3E-15	0.22	17.3	15	0.5	68.9	5.0E-20	0.23	14.6	11	0.1	2.9	1.4E-02	0.02	4.5
	Meas. Error	40	2.1E-02					32	6.8E-03					24	2.2E-02				
Snout-Eyes	side	1	0.1	0.16	0.69			1	0.2	1.2	0.29			1	1.4	5.7	0.04		
	individual	19	24.5	77.5	4.3E-14			15	19.1	107.6	1.7E-12			11	12.9	54.4	5.8E-08		
	interaction	19	0.3	11.8	5.7E-11	0.14	15.9	15	0.2	44.2	4.2E-17	0.09	14.3	11	0.2	15.0	3.5E-08	0.11	9.6
	Meas. Error	40	2.7E-02					32	4.0E-03					24	1.6E-02				
Eyes-Operculum	side	1	1.6E-03	0.01	0.93			1	3.7E-02	0.16	0.70			1	1.3E-04	0.00	0.98		
	individual	19	85.9	479.8	1.5E-21			15	45.4	192.6	2.3E-14			11	36.3	264.6	1.1E-11		
	interaction	19	0.2	22.1	1.6E-15	0.09	17.3	15	0.2	54.22	1.9E-18	0.12	14.4	11	0.1	13.8	7.7E-08	0.06	9.4
	Meas. Error	40	8.1E-03					32	4.3E-03					24	9.9E-03				

Table S2: Results of the two-way mixed model ANOVA (side-fixed × individual-random) with repeated measurements on each side. All interaction terms are highly significant meaning that FA is in all cases significant relative to measurement error. For each trait and each sample FA10, directly computed from variance component,[4] is given as well as its approximate degree of freedom. df: degree of freedom, MS: mean square, F: F ratio, p-value, see Palmer (1994) for complementary information.[2] COC for Cocurès, COM for Combe Sourde, CUB for Cubières, PDP for Pont-de-la-Planche, RAM for Ramponenche, and VER for Vérié.

Supporting Information S3

Concentration measurements.

For trout tissues, dried and powdered samples (about 50-70 mg) were dissolved overnight on a hot plate using 3 mL of Suprapure HNO_3 (Merck, Germany). The solutions were precisely diluted and measured by high resolution inductively coupled plasma mass spectrometer (HR-ICP-MS) Thermo Element XR at the University of Montpellier II using external and internal (indium) calibrations (5 replicates). Leachates of sediments were measured under the same conditions.

Isotopic measurements.

Solutions used for concentration measurements were diluted with MilliQ water to obtain an ion flow on the ^{208}Pb isotope of about 7.10^5 cps. Pb isotopes (^{206}Pb, ^{207}Pb, & ^{208}Pb) were measured using low resolution, analogue detection mode with peak hopping. Fifteen samples per peak were acquired close to the top of each peak. Two-fifths of the total time was consumed for the acquisition of each ^{206}Pb and ^{207}Pb, and one-fifth for the ^{208}Pb isotope, which is the more abundant isotope in lead. Ten runs of 400 passes each were performed for a total acquisition time of about 6 min.

Mass bias correction was operated by bracketing several NIST 981 lead standards every five samples. Further details about the complete procedure can be found elsewhere for Q-ICP-MS, which is the same for HR-ICP-MS.[5,6] Blank corrections were never required as they appeared negligible compared to the total amount of lead in the solutions. Errors for $^{206}Pb/^{207}Pb$ and $^{208}Pb/^{206}Pb$ ratios were typically 1-2 and 3-6 at the third decimal place, respectively (95% confidence intervals). Dead time correction was not needed as measurements were performed with the analogue detection mode.

	Cd ($\mu g.g^{-1}$)	Pb ($\mu g.g^{-1}$)	Cu ($\mu g.g^{-1}$)	Zn ($\mu g.g^{-1}$)
Determination limit ($ng.g^{-1}$)	0.001	0.03	0.04	1.9
A	0.026	0.74	3.3	18.8
B	0.030	0.70	3.5	18.5
C	0.029	0.90	4.0	15.5
D	0.026	0.74	3.0	20.3
Certified	0.026	0.87	3.7	17.9
Recovery (%)	99-115	80-103	81-107	86-113

Table S4: Determination limits (in $ng.g^{-1}$) assessed on the basis of 10 times the standard deviation of blanks, measured and certified values of the NIST-1547 (peach leaves) standard reference material, and recovery percentage.

	Cd ($\mu g.g^{-1}$)	Pb ($\mu g.g^{-1}$)	Cu ($\mu g.g^{-1}$)	Zn ($\mu g.g^{-1}$)
Determination limit ($ng.g^{-1}$)	0.001	0.03	0.04	1.9
A	0.49	0.180	235	112
B	0.53	0.186	239	127
C	0.56	0.191	260	143
D	0.54	0.187	244	137
E	0.54	0.206	261	146
F	0.59	0.170	284	175
G	0.51	0.236	257	132
H	0.53	0.203	280	138
I	0.58	0.182	271	172
Certified	0.54	0.172	277	139
Recovery (%)	90-109	99-137	85-102	80-126

Table S5: Determination limits (in $ng.g^{-1}$) assessed on the basis of 10 times the standard deviation of blanks, measured and certified values of the BCR 185-R (bovine liver) standard reference material, and recovery percentage.

Generally the measured certified reference materials are close to the certified values (except measurement G for Pb in BCR-185R). In any case, the concentration dynamic in brown trout is so high that uncertainties of ±30% do not modify the patterns in Figure 2 where metal concentrations are plotted along a logarithmic scale.

	Cd (μg.g^{-1})	Pb (μg.g^{-1})	Cu (μg.g^{-1})	Zn (μg.g^{-1})
COC04 A	0.72	0.89	278	107
COC04 B	0.78	0.89	304	127
COM07 A	9.4	12.8	18	209
COM07 B	7.0	11.9	15	172
COM20 A	18.2	31.0	72	184
COM20 B	18.6	31.9	78	197
PDP14 A	5.0	5.0	55	77
PDP14 B	6.5	6.8	93	106
PDP19 A	4.8	4.9	71	113
PDP19 B	4.3	5.1	70	98
PDP20 A	5.7	7.4	66	132
PDP20 B	5.4	7.1	66	120
RAM04 A	13.5	5.5	178	162
RAM04 B	12.5	6.2	172	125
RAM06 A	8.7	5.9	111	154
RAM06 B	9.1	7.6	112	156

Table S6: Replicated analyses (Cd, Pb, Cu, Zn) for eight livers (dry-based concentrations), COC for Cocurès, COM for Combe Sourde, PDP for Pont-de-la-Planche, RAM for Ramponenche. Analyses are less replicable with indigenous trout livers (especially for Pb in PDP14) than for CRMs, probably because liver powders present a greater heterogeneity. In any case, the concentration dynamic in brown trout is so high that uncertainties do not modify the patterns in Figure 2, where metal concentrations are plotted along a logarithmic scale.

	Cd ($\mu g.g^{-1}$)	Pb ($\mu g.g^{-1}$)	Cu ($\mu g.g^{-1}$)	Zn ($\mu g.g^{-1}$)
COC04 A	0.005	0.072	1.2	27.0
COC04 B	0.005	0.070	1.1	28.1
COC08 A	0.008	0.068	1.6	58.3
COC08 B	0.007	0.090	1.0	55.5
COC09 A	0.007	0.077	0.95	12.8
COC09 B	0.008	0.070	0.90	11.5
PDP14 A	0.054	0.160	1.6	48.6
PDP14 B	0.061	0.211	1.9	42.7

Table S7: Replicated analyses (Cd, Pb, Cu, Zn) for four muscles (dry-based concentrations), COC for Cocurès and PDP for Pont-de-la-Planche.

Sample	Cd (µg.g^{-1})	Pb (µg.g^{-1})	Cu (µg.g^{-1})	Zn (µg.g^{-1})	Sample	Cd (µg.g^{-1})	Pb (µg.g^{-1})	Cu (µg.g^{-1})	Zn (µg.g^{-1})
COC01	0.074	0.31	9.6	32	PDP01	0.50	0.41	10	11
COC02	0.10	0.16	16	29	PDP02	1.3	2.3	19	59
COC03	0.086	0.19	28	22	PDP03	1.3	2.3	48	61
COC04	0.16	0.19	60	25	PDP04	1.2	0.86	38	15
COC05	0.083	0.22	17	41	PDP05	1.1	0.57	31	31
COC06	0.12	0.20	11	21	PDP06	1.2	1.4	28	23
COC07	0.088	0.12	14	28	PDP07	0.70	0.87	33	18
COC08	0.092	0.29	53	24	PDP08	0.39	0.68	9,0	19
COC09	0.086	0.19	63	32	PDP09	0.95	1.0	40	48
COC10	0.089	0.076	11	32	PDP10	0.97	1.1	38	26
COC11	0.047	0.060	14	23	PDP11	1.1	1.7	36	24
COC12	0.053	0.058	6.2	26	PDP12	1.4	2.0	37	34
COC13	0.041	0.047	22	25	PDP13	0.38	1.2	31	29
COC14	0.046	0.057	14	26	PDP14	1.1	1.1	14	17
COC15	0.049	0.049	18	34	PDP15	1.0	2.9	41	31
COC16	0.038	0.069	8.6	14	PDP16	2.1	3.9	23	28
COC17	0.068	0.070	8.3	29	PDP17	0.78	0.98	21	27
COC18	0.039	0.068	5.8	23	PDP18	1.7	5.8	20	26
COC19	0.047	0.059	13	26	PDP19	0.98	1.1	16	23
COC20	0.037	0.061	5.1	18	PDP20	1.2	1.5	15	27
COM01	1.9	1.3	6.8	28	RAM01	2.0	4.1	93	39
COM02	3.5	4.6	6.9	35	RAM02	-	-	-	-
COM03	2.0	3.5	22	50	RAM03	2.3	2.0	69	55
COM04	2.0	4.8	4.7	53	RAM04	2.9	1.3	41	32
COM05	1.6	3.2	4.0	36	RAM05	-	-	-	-
COM06	8.3	21	19	100	RAM06	1.9	1.5	27	34
COM07	1.7	2.5	3.3	39	RAM07	-	-	-	-
COM08	1.9	5.4	3.0	45	RAM08	3.9	1.7	7.9	32
COM09	-	-	-	-	RAM09	0.72	0.90	37	49
COM10	-	-	-	-	RAM10	3.2	1.0	37	55
COM11	4.1	5.1	7.7	38	RAM11	1.6	0.74	29	38
COM12	0.4	3.3	6.2	31	RAM12	1.3	0.98	25	33
COM13	4.8	6.0	13	42	RAM13	0.40	0.26	9.4	14
COM14	2.6	7.5	7.0	50	RAM14	1.4	0.56	30	34
COM15	1.8	3.1	14	36	RAM15	1.1	0.57	20	25
COM16	3.1	8.4	7.1	48	RAM16	1.6	0.54	22	32
COM17	0.5	0.7	4.5	18	RAM17	1.1	0.57	48	46
COM18	2.2	24	14	50	RAM18	1.4	0.81	29	33
COM19	0.7	1.5	5.8	48	RAM19	1.5	0.67	19	49
COM20	3.8	6.4	15	39	RAM20	1.2	0.58	14	25
COM21	3.7	4.8	13	57					
COM22	2.0	2.5	4.5	29	VER01	0.80	2.1	6.7	21
					VER02	0.81	0.11	5.1	32
CUB01	0.59	0.16	17	41	VER03	1.5	0.23	11	68
CUB02	0.20	0.14	2.6	11	VER04	0.77	0.07	6.7	16
CUB03	0.27	0.10	7.0	17	VER05	1.7	0.12	11	63
CUB04	0.30	0.055	6.8	22	VER06	1.2	0.11	10	33
CUB05	0.20	0.079	8.0	23	VER07	1.1	0.11	4.8	38
CUB06	0.33	0.068	10	54	VER08	1.9	0.17	16	21
CUB07	0.36	0.11	6.9	28	VER09	0.82	0.17	9.1	15
CUB08	0.16	0.067	5.2	26	VER10	1.2	0.14	11	42
CUB09	0.42	0.065	17	30	VER11	0.95	0.10	6.6	28
CUB10	0.35	0.14	6.9	23	VER12	0.42	0.20	2.9	17
CUB11	-	-	-	-					
CUB12	0.39	0.089	18	17	SUPA	0.017	0.011	122	28
CUB13	0.36	0.098	10	13	SUPB	0.018	0.013	71	38
CUB14	1.12	0.15	16	50					
CUB15	0.67	0.097	15	34					
CUB16	0.17	0.079	4.6	19					
CUB17	0.23	0.085	1.6	21					
CUB18	0.45	0.13	9.8	27					
CUB19	-	-	-	-					
CUB20	0.51	0.064	7.2	21					

Table S8: Cd, Pb, Cu and Zn concentrations of trout livers (corrected to express the results in terms of concentrations in wet tissues). COC for Cocurès, COM for Combe Sourde, CUB for Cubières, PDP for Pont-de-la-Planche, RAM for Ramponenche, VER for Verié, and SUP for commercial trout (supermarket).

Sample	Cd ($\mu g.g^{-1}$)	Pb ($\mu g.g^{-1}$)	Cu ($\mu g.g^{-1}$)	Zn ($\mu g.g^{-1}$)	Sample	Cd ($\mu g.g^{-1}$)	Pb ($\mu g.g^{-1}$)	Cu ($\mu g.g^{-1}$)	Zn ($\mu g.g^{-1}$)
COC01	0.012	0.029	0.34	13	PDP01	0.008	0.035	0.31	15
COC02	0.002	0.035	0.41	4.1	PDP02	0.002	0.030	0.09	2.5
COC03	0.002	0.037	0.38	13	PDP03	0.010	0.044	0.39	15
COC04	0.001	0.019	0.32	6.4	PDP04	0.009	0.029	0.37	6.8
COC05	0.002	0.022	0.44	9.1	PDP05	0.012	0.059	0.30	5.2
COC06	0.002	0.019	0.50	12	PDP06	0.007	0.095	0.39	9.8
COC07	0.001	0.037	0.32	7.9	PDP07	0.018	0.078	0.32	8.9
COC08	0.002	0.017	0.43	15	PDP08	0.005	0.035	0.29	14
COC09	0.002	0.016	0.21	3.0	PDP09	0.008	0.062	0.58	14
COC10	0.002	0.029	0.28	3.7	PDP10	0.009	0.041	0.44	7.6
COC11	0.002	0.030	0.35	9.4	PDP11	0.008	0.052	0.43	9.0
COC12	0.003	0.063	0.62	7.1	PDP12	0.007	0.030	0.27	6.3
COC13	0.002	0.017	0.41	14	PDP13	0.006	0.046	0.41	9.1
COC14	0.003	0.022	0.24	19	PDP14	0.009	0.036	0.25	6.0
COC15	0.002	0.044	0.49	8.4	PDP15	0.007	0.112	0.37	4.8
COC16	0.002	0.025	0.19	7.0	PDP16	0.009	0.151	0.22	7.9
COC17	0.002	0.018	0.25	14	PDP17	0.007	0.066	0.41	6.7
COC18	0.002	0.027	0.51	8.4	PDP18	0.009	0.273	0.28	15
COC19	0.003	0.024	0.43	11	PDP19	0.008	0.088	0.33	18
COC20	0.002	0.034	0.66	10	PDP20	0.006	0.035	0.18	8.5
COM01	0.025	0.191	0.28	11	RAM01	0.009	0.069	0.46	4.2
COM02	0.036	0.147	0.55	8.5	RAM02	0.024	0.037	0.32	7.8
COM03	0.033	0.199	0.35	10	RAM03	0.007	0.021	0.33	9.9
COM04	0.118	0.091	0.42	9.2	RAM05	0.012	0.045	0.31	7.0
COM05	0.086	0.101	0.59	8.4	RAM06	0.008	0.146	0.24	7.8
COM06	0.080	0.650	0.32	10	RAM07	0.015	0.052	0.46	10
COM07	0.103	0.698	0.46	14	RAM08	0.021	0.118	0.34	7.4
COM08	0.045	0.222	0.47	11	RAM09	0.007	0.032	0.44	11
COM09	0.023	0.177	0.82	12	RAM10	0.016	0.046	0.37	11
COM10	0.054	0.259	0.23	7.1	RAM11	0.013	0.035	0.29	7.8
COM11	0.058	0.178	0.31	12	RAM12	0.011	0.048	0.37	5.5
COM12	0.011	0.089	0.37	8.4	RAM13	0.017	0.042	0.35	6.2
COM13	0.037	0.111	0.61	22	RAM14	0.021	0.052	0.55	15
COM14	0.056	0.364	0.25	23	RAM15	0.015	0.056	0.54	13
COM15	0.076	0.246	0.44	13	RAM16	0.015	0.057	0.44	20
COM16	0.171	0.491	0.42	9.7	RAM17	0.010	0.035	0.40	13
COM17	0.021	0.100	0.58	10	RAM18	0.011	0.067	0.36	6.5
COM18	0.071	0.395	0.33	25	RAM19	0.015	0.037	0.35	17
COM19	0.010	0.048	0.34	5.7	RAM20	0.010	0.025	0.35	14
COM20	0.060	0.221	0.37	14					
COM21	0.100	0.951	0.51	14	VER01	0.013	0.019	0.33	6.5
COM22	0.041	0.067	0.24	23	VER02	0.042	0.038	0.34	6.3
					VER03	0.035	0.067	0.59	9.4
CUB01	0.008	0.029	0.51	14	VER04	0.063	0.053	0.63	13
CUB02	0.008	0.023	0.35	6.1	VER05	0.013	0.021	0.24	13
CUB03	0.021	0.032	0.22	12	VER06	0.036	0.055	0.33	4.9
CUB04	0.013	0.035	1.49	24	VER07	0.066	0.085	0.50	22
CUB05	0.005	0.015	0.40	6.1	VER08	0.021	0.028	0.38	7.5
CUB06	0.005	0.021	0.35	8.4	VER09	0.012	0.020	0.26	11
CUB07	0.007	0.020	0.92	9.9	VER10	0.036	0.073	0.39	4.7
CUB08	0.008	0.045	0.47	8.6	VER11	0.037	0.067	0.55	11
CUB09	0.007	0.014	0.70	7.0	VER12	0.019	0.012	0.21	4.0
CUB10	0.004	0.018	0.39	10					
CUB11	0.007	0.017	0.39	7.6	SUP1	0.001	0.018	0.27	4.8
CUB12	0.005	0.018	0.27	5.3	SUP2	0.001	0.017	0.32	9.6
CUB13	0.034	0.030	0.27	4.7	SUP3	0.001	0.014	0.30	5.6
CUB14	0.007	0.010	0.32	8.0	SUP4	0.002	0.032	0.36	5.4
CUB15	0.010	0.032	0.30	5.8	SUP5	0.002	0.054	0.50	7.6
CUB16	0.010	0.074	0.48	7.1	SUP6	0.002	0.034	0.37	4.0
CUB17	0.014	0.139	0.61	44	SUP7	0.001	0.009	0.25	3.0
CUB18	0.032	0.016	0.37	8.6	SUP8	0.001	0.017	0.28	4.4
CUB19	0.016	0.041	0.42	18	SUP9	0.002	0.038	0.46	4.1
CUB20	0.006	0.017	0.26	5.4					

Table S9: Cd, Pb, Cu and Zn concentrations of trout muscles (corrected to express the results in terms of concentrations in wet tissues). COC for Cocurès, COM for Combe Sourde, CUB for Cubières, PDP for Pont-de-la-Planche, RAM for Ramponenche, VER for Verié, and SUP for commercial trout (supermarket).

	$r_{spearman}$ (liver)					$r_{spearman}$ (muscle)			
	Cd	Pb	Cu	Zn		Cd	Pb	Cu	Zn
COC	**0.807***	**0.821***	**0.485***	0.126		-0.386	-0.252	-0.100	-0.083
COM	**0.569***	**0.637***	**0.661***	0.314		-0.143	0.243	0.132	0.187
CUB	**0.752***	0.198	**0.774***	**0.509***		-0.258	**-0.528***	-0.366	-0.251
PDP	0.211	0.339	-0.111	-0.274		0.011	0.320	-0.078	0.064
RAM	**0.556***	**0.741***	0.212	0.232		-0.080	0.230	-0.322	-0.371
VER	**0.614***	0.183	**0.570***	0.016		-0.447	-0.096	0.032	0.319

Table S10: Spearman's correlation coefficients between age and hepatic and muscle contents of Cd, Pb, Cu and Zn (wet-based concentrations) for the 6 study sites. In bold and *: $p<0.05$. COC for Cocurès, COM for Combe Sourde, CUB for Cubières, PDP for Pont-de-la-Planche, RAM for Ramponenche, and VER for Vérié.

Sample	Cd ($\mu g.g^{-1}$)	Pb ($\mu g.g^{-1}$)	Cu ($\mu g.g^{-1}$)	Zn ($\mu g.g^{-1}$)
COC A	1.4	420	20	160
COC B	1.7	250	19	260
COC C	2.1	280	18	330
COM A	190	25000	53	31000
COM B	160	8100	40	29000
COM C	120	11000	24	25000
CUB A	5.2	2200	17	1200
CUB B	5.4	250	16	1000
CUB C	2.4	290	14	830
PDP A	3.0	880	25	260
PDP B	2.0	860	46	200
PDP C	1.5	200	20	150
RAM A	9.0	2100	61	1400
RAM B	7.5	550	57	1300
VER A	0.31	67	15	140
VER B	0.41	70	14	130

Table S11: Cd, Pb, Cu and Zn concentrations in streambed sediments (fraction < 250 µm). COC for Cocurès, COM for Combe Sourde, CUB for Cubières, PDP for Pont-de-la-Planche, RAM for Ramponenche, and VER for Vérié.

Sample	$^{206}Pb/^{207}Pb$	±	$^{208}Pb/^{206}Pb$	±
COC01	1.178	0.001	2.093	0.004
COC04	1.178	0.002	2.093	0.004
COC06	1.176	0.001	2.096	0.003
COC08	1.178	0.002	2.096	0.003
COM03	1.182	0.002	2.087	0.004
COM06	1.183	0.002	2.084	0.003
COM12	1.183	0.001	2.089	0.005
COM14	1.183	0.002	2.087	0.005
COM22	1.181	0.001	2.085	0.004
CUB01	1.179	0.002	2.091	0.004
CUB01dupl	*1.178*	*0.002*	*2.092*	*0.007*
CUB14	1.180	0.002	2.089	0.003
PDP11	1.172	0.002	2.094	0.006
PDP12	1.172	0.001	2.096	0.006
PDP15	1.173	0.001	2.092	0.005
PDP16	1.173	0.002	2.094	0.005
PDP20	1.173	0.002	2.096	0.004
RAM01	1.175	0.002	2.094	0.004
RAM04	1.176	0.002	2.092	0.004
RAM06	1.176	0.002	2.095	0.003
RAM18	1.173	0.001	2.098	0.005
RAM19	1.175	0.002	2.098	0.003

Table S12: $^{206}Pb/^{207}Pb$ and $^{208}Pb/^{206}Pb$ ratios in trout livers. COC for Cocurès, COM for Combe Sourde, CUB for Cubières, PDP for Pont-de-la-Planche, RAM for Ramponenche, and VER for Vérié. Errors are given at 95% confidence level. *dupl*. for duplicate.

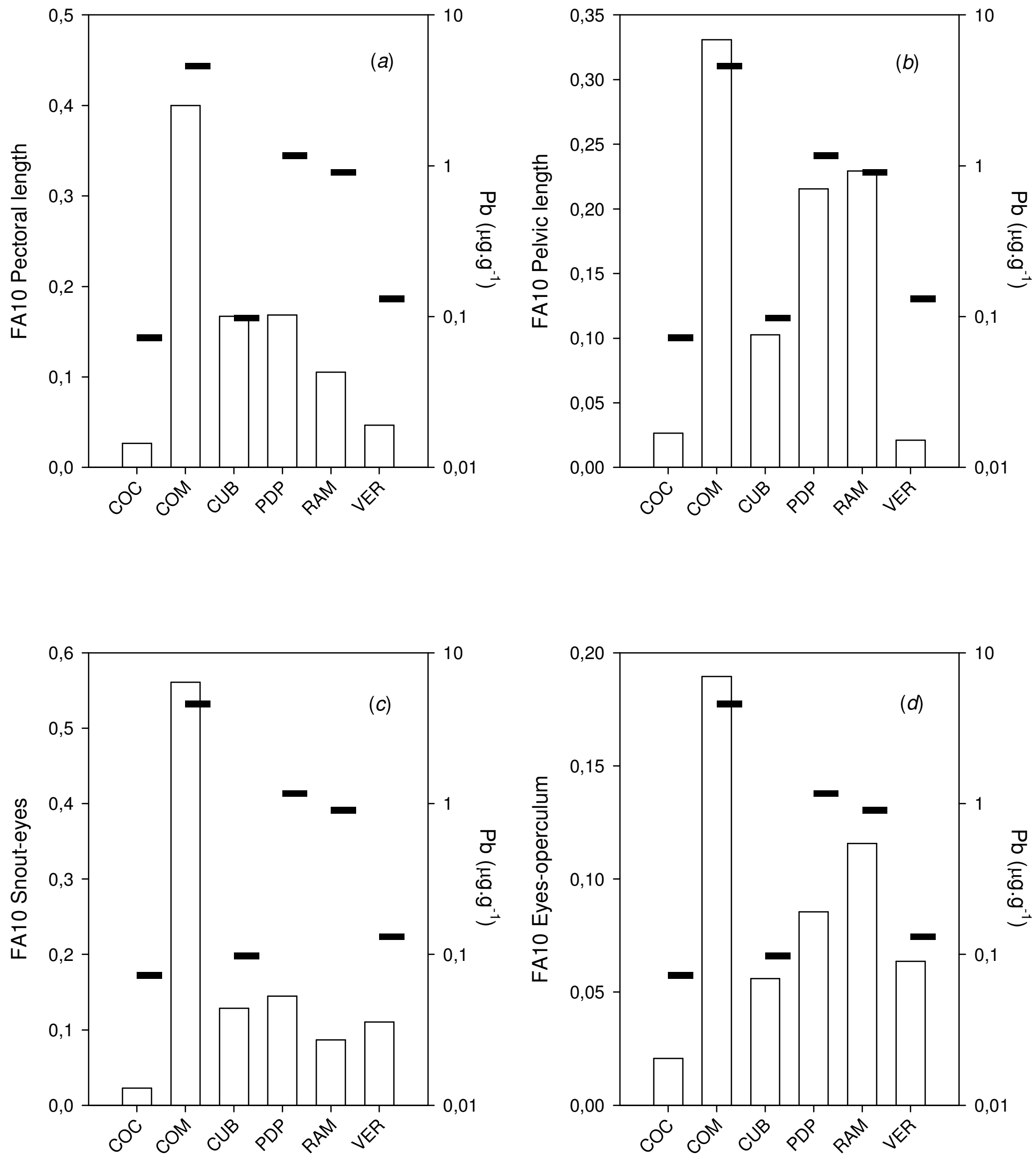

Figure S13: FA10 for pectoral length (a), pelvic length (b), snout-eye distance (c), operculum-eye distance (d) represented by white boxes. Median Pb in livers (wet-based concentration) for each site is plotted as a bold black line for comparison. COC for Cocurès, COM for Combe Sourde, CUB for Cubières, PDP for Pont-de-la-Planche, RAM for Ramponenche, and VER for Vérié.

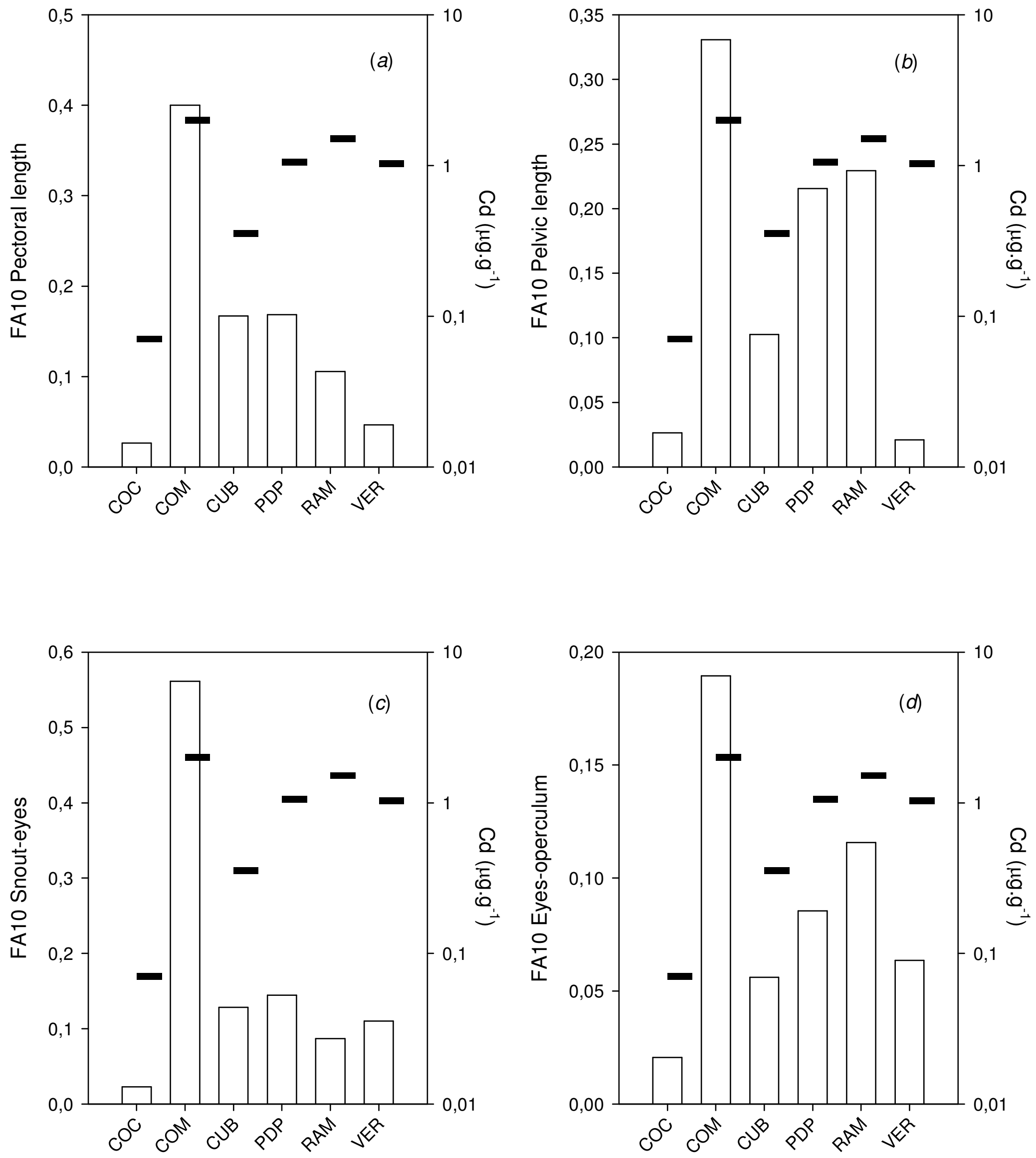

Figure S14: FA10 for pectoral length (a), pelvic length (b), snout-eye distance (c), operculum-eye distance (d) represented by white boxes. Median Cd in livers (wet-based concentration) for each site is plotted as a bold black line for comparison. COC for Cocurès, COM for Combe Sourde, CUB for Cubières, PDP for Pont-de-la-Planche, RAM for Ramponenche, and VER for Vérié.

	Pb (µg.g^{-1})	Cd (µg.g^{-1})
Pectoral length	0.423 ($p < 10^{-5}$)	0.431 ($p < 10^{-5}$)
Pelvic length	0.435 ($p < 10^{-5}$)	0.404 ($p < 10^{-4}$)
Snout-Eyes length	0.335 ($p < 10^{-3}$)	0.380 ($p < 10^{-4}$)
Eyes-Operculum length	0.304 ($p < 10^{-3}$)	0.305 ($p < 10^{-3}$)

Table S15: Spearman's correlation coefficient between R-L (absolute value) of each trait and Pb or Cd in liver (dry-based concentration).

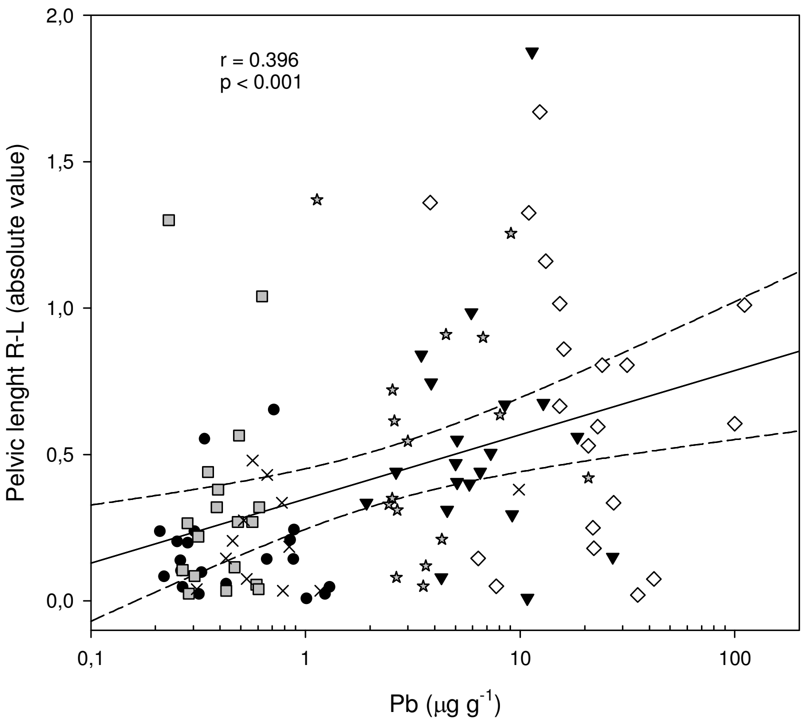

Figure S16: Pelvic length (right minus left, in absolute value) *vs* Pb in liver (dry-based concentration). Closed circles for COC, grey boxes for CUB, white diamonds for COM, closed triangles for PDP, grey stars for RAM, and crosses for VER. Significant linear regression is plotted together with its 99% confidence interval (Pearson's correlation coefficient = 0.396, p<0.001). A non-parametric approach using Spearman's correlation coefficient provides $r_{spearman}$ = 0.44, which is also significant ($p < 10^{-5}$). This graph is representative of the results obtained with the other three traits. The specimens appear to be clustered by site, so that at least a part of the significant but moderate correlation is linked to the fact that populations differ in terms of Pb concentrations and asymmetry levels.

	Ref	wet/dry	*n*	Cd (μg.g^{-1})	Pb (μg.g^{-1})	Cu (μg.g^{-1})	Zn (μg.g^{-1})
Liver							
PNC - France	This study	wet	107	0.037-8.3[a]	0.047-24.0[a]	1.6-92.9[a]	11.3-102[a]
Commercial trout	This study	wet	4	0.014-0.015[a]	0.002-0.017[a]	50.4-104.8[a]	22.8-30.6[a]
Spain	[7]	wet	45	0.1 ± 0.2[b]	0.2 ± 0.3[b]	12 ± 10[b]	28 ± 8[b]
Buško Blato -Bosnia	[8]	wet	10	0.069-0.687[a]	0.694-1.797[a]	38.4-51.5[a]	39.4-88.4[a]
Kola Region, Russia	[9]	dry	23	4.3-7.6[a]	0.1-0.73[a]	72-490[a]	92-239[a]
Muscle							
PNC	This study	wet	113	0.001-0.17[a]	0.01-0.95[a]	0.048-1.48[a]	2.48-43.7[a]
Commercial trout	This study	wet	9	0.001-0.002[a]	0.010-0.054[a]	0.25-0.54[a]	3.0-9.6[a]
Buško Blato-Bosnia	[8]	wet	10	0.025-0.52[a]	0.671-1.106[a]	0.418-0.652[a]	22.4-63.7[a]
Kola Region, Russia	[9]	dry	23	0.006-0.071[a]	-	1-5.2[a]	10-65[a]
UE guidelines	[10]	wet		0.05	0.3		

Table S17: Metal concentrations measured in livers and muscles of brown trout from the PNC and values found in the literature. n: number of individuals, [a]:range, [b]: mean and standard deviation.

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