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Wild Brown Trout Affected by Historical Mining in the Cévennes National Park, France

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Supporting Information

ABSTRACT: In the protected area of the Cévennes National Park (Southern France), 114 wild brown trout (*Salmo trutta fario*) were captured at six locations affected to different extents by historical mining and metallurgy dating from the Iron Age to Modern Times. Cadmium and lead in trout livers and muscles reflect high sediment contamination, although an age-related effect was also detected for hepatic metal concentrations. Lead isotope signatures confirm exposure to drainage from mining and metallurgical waste. Developmental instability, assessed by fluctuating asymmetry, is significantly correlated with cadmium and lead concentrations in trout tissues, suggesting that local contamination may have affected fish development. Nowadays, the area is among the least industrialized in France. However, our results show that 60% of the specimens at one site exceed EU maximum allowed cadmium or lead concentration in foodstuffs. The mining heritage should not be neglected when establishing strategies for long-term environmental management.

INTRODUCTION

Recent environmental policies have reduced atmospheric emissions of metals in industrialized countries. However, considerable amounts of metals have been incorporated in soils and sediments over centuries, even millennia, of ore- and metal-working.[1−5] In addition to atmospheric emissions, these activities also produced slag, dumps, and other industrial waste. Nowadays, mechanical erosion and leaching still introduce metals into streams and soils, which may durably contaminate ecosystems.[6−11] Even if the Parc National des Cévennes (PNC) is now one of the least industrialized areas of France, it has experienced intensive metallurgical and mining activity from the Iron Age until Modern Times.[12,13] The impact of these abandoned mines on aquatic ecosystems could be assessed by water and sediment analyses.[7,14] However, river water chemistry is particularly changeable,[15] and frequent flash floods modify streambed sediments in the region. Using the brown trout (*Salmo trutta fario*) as a biomonitor is a good choice.[16] This predatory fish, situated at the top of the food web, is relatively sedentary, ubiquist, and abundant in the PNC. Studies have already been undertaken on its tissues and bones in various natural conditions, providing a basis for comparison.[17,18] Isotopic compositions of lead (Pb) contained in trout tissues may also help to identify the origin of pollutants,[19−22] because isotopic ratios act as fingerprints of the ore from which they derive.[19] The instability of morphological development, assessed through fluctuating asymmetry (FA), is another parameter potentially sensitive to metallic contamination. It consists of small deviations from perfect symmetry, supposed to reflect the ideal state of

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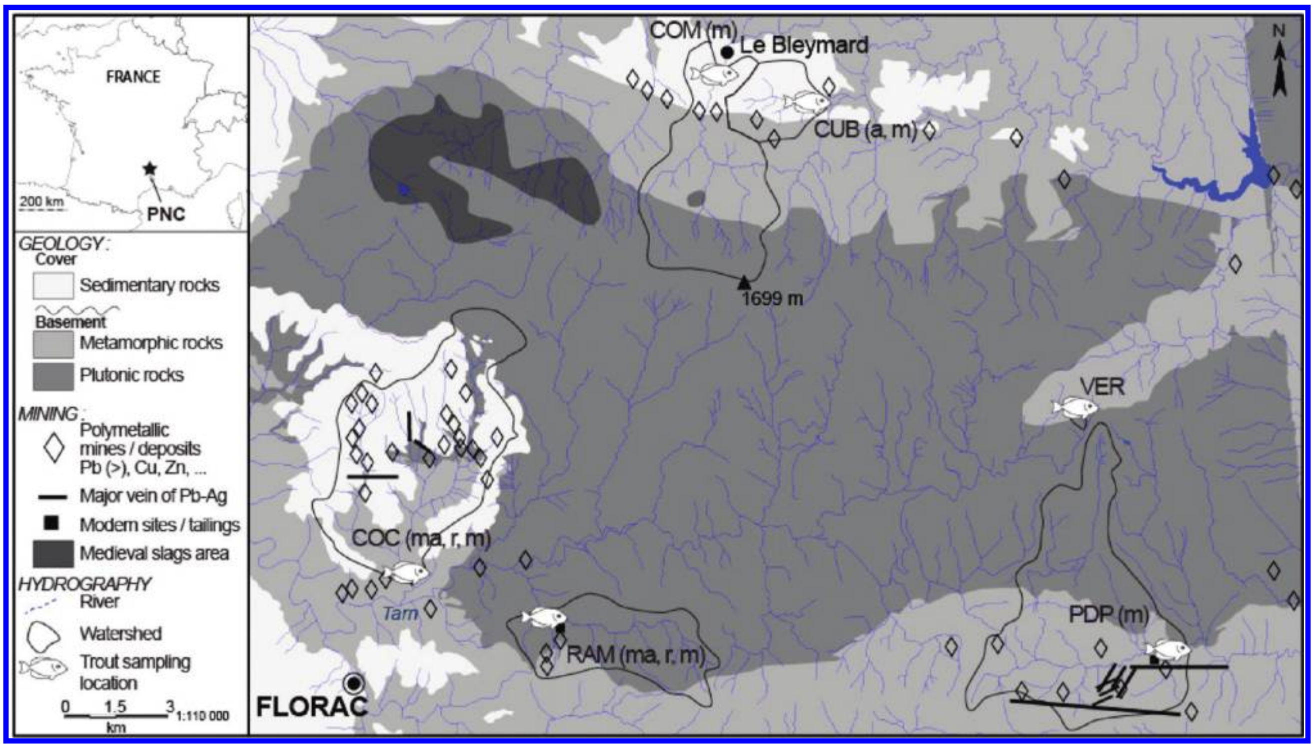

Figure 1. Simplified geology of the Cévennes. Trout sampling locations with associated watersheds. Mining and metallurgical sites are also indicated. Periods of exploitation are given between parentheses: a, ma, r, m for Antiquity, Middle Ages, Renaissance, and Modern works, respectively.

bilaterally paired traits.[23,24] Such instability is directly related to genetic and environmental stresses experienced by organisms.

The present study evaluates the impact of abandoned mines in the PNC by combining chemical and morphological analyses of 114 brown trout. The fishes were caught in six geologically distinct watersheds, generally in the vicinity of former industrial sites dating from different periods. As concentrations are reported in the literature for trout livers, and EU foodstuff levels exist for salmonid muscles,[25] both tissues were measured. The results, including FA of four bilateral characters, are juxtaposed with historical, geographical and geological data.

STUDY SITES

The geology of the PNC, southern Massif Central, includes a Hercynian basement covered in discordance by sedimentary Mesozoic formations (Figure 1). Polymetallic sulfide ore deposits of hydrothermal origin are abundant close to the granites and the discordance, or in association with faults. Ancient mining and smelting (Middle Ages, Renaissance, and perhaps Antiquity) have been identified far upstream from the site of Cocurès (COC). About one km upstream from the site of Combe Sourde (COM), the mines and cleansing factory of Bleymard formed a major center, which worked from the end of the 19th century to the 1950s for Pb(-Ag) and zinc (Zn). Sizeable lead-rich slag heaps, with some smaller ones dating from the Middle Ages, can be also found on the watershed.[26] The Cubières site (CUB) is located downstream from the Neyrac mines exploited during the 19th and 20th centuries, but where oil-lamp remains dating from Antiquity were also discovered.[27] Large mines of Vialas and a smelting factory, exploited from 1781 to 1895 for Pb and Ag, are located upstream from the sampling area of Pont-de-la-Planche (PDP). About 500 m upstream from the site of Ramponenche (RAM), the stream flows at the foot of impressive tailings produced by cleansing processes occurring from the mid-19th century to 1961, for Zn, Pb−Ag and Ba. Other mining works in the vicinity are presumed to date as early as the 15th or 16th centuries. The Vérié River (VER) was chosen as an "uncontaminated" site because no industrial activity existed on its granitic watershed.

EXPERIMENTAL SECTION

Sampling. It took place in May 2006, when fishes have access to abundant food, over a short two-day period to reduce the impact of possible hydrological or climatic changes. One hundred and fourteen trout were caught by electrofishing, sacrificed and frozen at −20 °C until further analyses. Between 12 and 22 trout were caught by site; this number may appear small for biological monitoring, but this was the maximum allowed by local fishing societies. Eleven commercial brown trout raised in fisheries were also analyzed for comparison. For each sampling site, streambed sediments were pooled and stored at 4 °C. The fraction finer than 250 μm was dried and ground in an agate mortar.

Biological Variables. The animals were weighed wet (W, in g) and measured from the tip of the snout to the fork of the tail (L, in cm) to the nearest 0.1 mm, using a digital caliper (Table 1). Livers and gonads were extracted and weighed wet: W_L and W_G, respectively. Fulton's condition factors, assumed to reflect the individual's energy state and overall quality, were calculated as FCF = $W \times 100/L^3$. The gonado-somatic index was calculated for females only, as GSI = $100 \times W_G/W$, and the hepato-somatic index as HSI = $100 \times W_L/W$. Ages were estimated by analyzing approximately 10 scales per fish. This technique consists of counting, under a binocular lens, the zones where growth slows

Table 1. Summary of Study Sites; Geographical and Chemical Settings; Main Biological Characteristics for Sampled Brown Trout; Range and Median in Parentheses for FCF, GSI, and HSI

site	Cocurès	Combe Sourde	Cubières	Pont de la Planche	Ramponenche	Vérié
simplified name	COC	COM	CUB	PDP	RAM	VER
coordinates (UTM-WSGS84)	549.2/4911	558.1/4926.5	560.7/4924.6	571/4908.5	553/4910	568.5/4914.9
water pH[a]	8.4	7.5	7.8	7.2	7.3	6.4
water hardness (mg/L)[a]	180	15	140	12	60	8
main mines on the watershed	Pb, Ba, (Zn.Cu), Ag	Pb, Zn	Pb, Ag, Zn	Pb, Zn, Ag, Ba	Pb, Zn, Ag, (Cu)	
number of fishes analyzed	20	22	20	20	20	12
weight (g)	35.1−291 (72.6)	36.5−255 (58.7)	25.3−85.7 (42.5)	47−273 (106)	48.4−211 (87.2)	16−84.5 (23.2)
length (mm)	139−281 (176)	141−269 (169)	123−190 (151)	159−281 (198)	158−250 (182)	112−195 (129)
age (yr)	1+ to 5+	1+ to 4+	1+ to 3+	2+ to 5+	2+ to 4+	1+ to 3+
FCF	1.11−1.45 (1.31)	1.04−1.57 (1.27)	1.12−1.48 (1.29)	1.10−1.49 (1.24)	1.21−1.49 (1.30)	1.01−1.31 (1.10)
GSI[b]	0.25−1.35 (0.83)	0.21−0.85 (0.76)	0.54−1.87 (0.73)	0.33−1.04 (0.66)	0.27−0.98 (0.66)	0.37−1.24 (0.67)
HSI	0.77−1.81 (1.29)	0.83−2.05 (1.43)	0.82−2.03 (1.23)	0.56−1.61 (1.20)	0.95−1.8 (1.42)	0.52−1.64 (1.29)

[a] Data provided by ONEMA (Pers. comm., 2007). [b] Calculated on females only (n = 10 for COC, n = 7 for CS, n = 8 for CUB, n = 14 for PDP, n = 11 for RAM, n = 4 for VER).

down. Results were confirmed by the use of the retro-measurement method.[28] 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,[29] a series of preliminary tests was conducted for all traits. Tests did not reveal directional asymmetry (DA), antisymmetry (AS) or significant relationships between asymmetry and trait size (see Supporting Information S1-2 for details).

Chemical Analyses. Livers were dried to constant weight at 60 °C (water content c.a. 75−80%) and powdered in an agate mortar. Approximately 60 mg of each sample was totally digested on a hot plate in a Savilex PTFE beaker using 3 mL of Suprapur HNO_3. Metals contained in about 100 mg of fine (<250 μm) sediment were extracted with 10 mL of HNO_3 10% on a hot plate overnight. Cadmium (Cd), copper (Cu), Pb, and Zn concentrations, as well as lead isotopic compositions were determined by high resolution inductively coupled plasma mass spectrometer (HR-ICP-MS) Thermo Element XR (see Supporting Information S3-7 for details about blanks, replicates, certified materials and mass bias correction for isotopic determination).

RESULTS AND DISCUSSION

Trout Biological Condition. All FCFs of the trout (aged from 1+ to 5+) are greater than 1 (median = 1.28, Table 1) and the slope of the $\log_{10}$(length) vs $\log_{10}$(weight) linear regression (not shown here) is 3.08. This indicates, at least with respect to size and weight, that animals are in good physical condition.[30] Values observed at VER, located at the top of Mont Lozère, are however significantly lower (Table 2). This can be explained by the altitude of the site, the temperature, and its granitic substratum. HSI and GSI do not show any clear statistical differences between sites (Table 2). If trout from PDP, which are significantly older, are excluded, no real difference in terms of age distribution can be noticed between the other sites (Fisher's exact test: $p < 0.05$ including PDP, and $p > 0.05$ without PDP). Because several waterfalls considerably limit fish movements (ONEMA, Pers. comm.), and small trout present low dispersal,[32] our animals are supposed to be relatively sedentary. For all these reasons, future between-site comparisons should be facilitated.

Table 2. Comparison between Sites for FCF, HSI, GSI, and Trace Elements in Trout Livers and Muscles

	KW test	DS test
FCF	$p < 0.0001$	COM^a RAM^a PDP^a CUB^a COC^a VER^b
HSI	$p = 0.020$	COM^a RAM^a PDP^a CUB^a COC^a VER^a
GSI[a]	$p = 0.813$	
Cd		
livers[b]	$p < 0.0001$	COM^a RAM^a PDP^b VER^b CUB^c COC^d
muscles[b]	$p < 0.0001$	COM^a VER^a RAM^b CUB^{bc} PDP^c COC^d
Pb		
livers[b]	$p < 0.0001$	COM^a PDP^b RAM^b VER^c CUB^d COC^d
muscles[b]	$p < 0.0001$	COM^a PDP^b RAM^{bc} VER^{cd} CUB^d COC^d
Cu		
livers[b]	$p < 0.0001$	PDP^a RAM^a COC^{ab} VER^b CUB^b COM^b
muscles[b]	$p = 0.422$	
Zn		
livers[b]	$p < 0.0001$	COM^a RAM^{ab} VER^{ab} PDP^{bc} COC^{bc} CUB^c
muscles[b]	$p = 0.199$	

[a] Calculated on females only. [b] Tests operated on wet tissue metal concentrations; A Kruskal−Wallis test (KW) was applied for each variable (a nonparametric approach was required at least for metal concentrations because they appear not to be normally distributed, even after a log-transformation). When KW tests appeared to be significant ($p < 0.05$), all pairwise comparisons between groups were then made using the nonparametric Dwass-Steel (DS) test adapted by Critchlow and Fligner (1991).[31] Sites are ranked from the highest to the lowest values. Sites with the same superscript letter are not significantly different for the considered variable. Note that significant differences appear with the KS test for HSI, but pairwise comparisons do not exhibit any significant differences. COC for Cocurès, COM for Combe Sourde, CUB for Cubières, PDP for Pont-de-la-Planche, RAM for Ramponenche, and VER for Vérié.

Trout and Metallic Pollution. Hepatic concentrations of Cd and Pb vary by more than 2 orders of magnitude, while Cu and Zn exhibit narrower ranges (Figure 2, Supporting Information

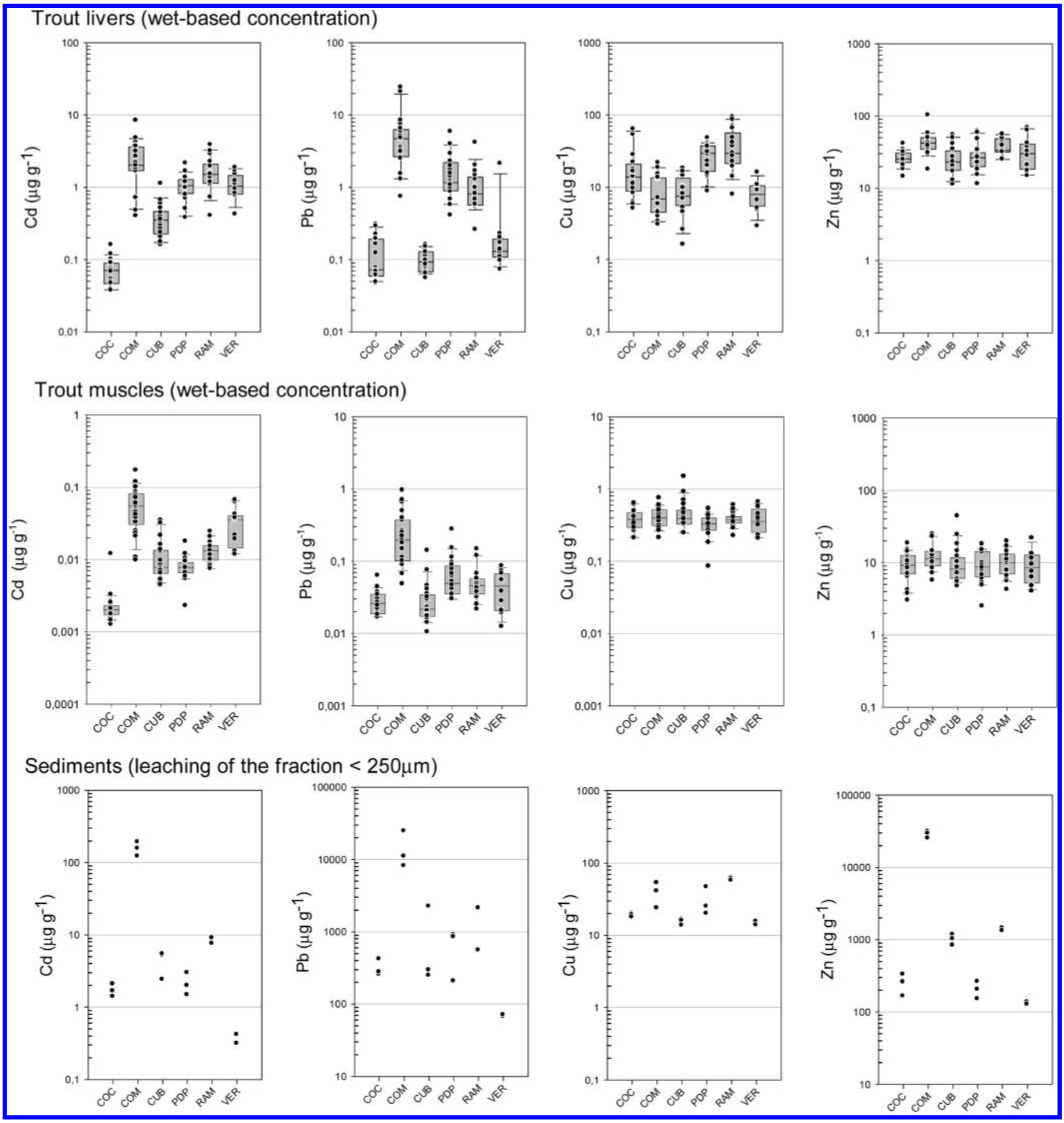

Figure 2. Cd, Pb, Cu, and Zn concentrations (logarithmic scale) in trout livers (wet-based concentrations), muscles (wet-based concentrations), and streambed sediments (fraction <250 μm). COC for Cocurès, COM for Combe Sourde, CUB for Cubières, PDP for Pont-de-la-Planche, RAM for Ramponenche, and VER for Vérié. Median, 25th and 75th percentiles are represented as vertical boxes. Vertical lines range from 10th to 90th percentiles. See Table 2 for comparison tests.

S8-9). In livers, the highest Pb concentrations are noticed at COM, followed by PDP and RAM, VER and finally by CUB and COC (Table 2). For Cd content, the sequence is slightly different due to the position of VER. In muscles, the patterns for Cd and Pb are almost the same but with lower concentrations, so that some marginal intersite differences are no longer recognized. Minor but statistically significant interpopulation differences are identified for Cu and Zn in livers but not in muscles.

Among biotic variables playing a role in metal accumulation, the species and sex of animals may have some influence. Here, the study was undertaken on a single species, and the sex of individuals proved to be an insignificant variable for metal accumulation (Mann−Whitney U-test for each site, all $p > 0.05$), except for Cu in livers (p = 0.003), for reasons unknown. Significant relationships between metal concentrations and age have been reported elsewhere.[33] Spearman's correlation coefficients between age and Cd, Pb, Cu, and Zn concentrations in tissues were computed for each site (Supporting Information S10). Hepatic concentrations of Cd (for 5 sites out of 6) and, to a lesser extent, those of Pb and Cu (for three and four sites out of six, respectively) are significantly and positively correlated to trout age. Muscle concentrations do not show any significant relationship with the age of specimens, except for Pb concentrations at CUB, which exhibit a moderate, negative correlation. Metals therefore continuously accumulate in trout liver but not in muscle. However, in the present case, the liver constitutes a suitable organ because age distribution is approximately the same among sites, and intersite variations are much higher than for muscle.

It is well-known that metal accumulation in fishes is also governed by abiotic variables, including the nature and intensity of pollution that could contaminate water, sediment, and hence the food web.[6,10,16−18] Water chemistry (pH, Ca content, etc.) also tends to make metals more or less bioavailable (and thus more or less toxic) for animals. In the study area, and more particularly at COM, Cd, Pb, and Zn concentrations measured in fine streambed sediments (Figure 2, Supporting Information S11) are far higher than the levels usually noted in uncontaminated environments: ~0.3, 40, 240 $\mu g\ g^{-1}$, respectively,[34] while Cu contents are similar to pristine areas: 50 $\mu g\ g^{-1}$.[34] Although the chemical procedure applied to sediments is overaggressive when compared to real-life processes, it is interesting to note the resemblance between Pb patterns in sediments and those observed in fish livers. Isotopic compositions may help to confirm the pathway from mines through sediment to trout. The $^{206}Pb/^{207}Pb$ and $^{208}Pb/^{206}Pb$ ratios of trout livers vary within 1.170−1.185 and 2.082−2.102, respectively (Figure 3a and b, Supporting Information S12). Except for the trout from VER, which were not measured for their Pb isotopic compositions, each site appears clustered and distinct from the others (Figure 3b). Such signatures are incompatible with liquid urban waste, atmospherically deposited Pb previously estimated locally using lichens as receptors, lead naturally present at trace level in local granite and in Jurassic sedimentary rocks, or with Pb recently used as an antiknock compound in gasoline (Figure 3a and references cited therein). A multicomponent mixing, involving some of these sources in adequate proportions could theoretically produce the same isotopic composition as that found in fishes. Nonetheless, ore deposits located upstream from the sampling areas[35] are an excellent match, and are therefore more obvious candidates than any hypothetical mixing. Lead from mining and metallurgical wastes must have entered the aquatic ecosystem by mechanical erosion and/or natural weathering of lead-rich particles, and was further homogenized in trout tissues. As a consequence, trout livers from each site present a lower isotopic heterogeneity than the local ore deposits where the Pb originated (Figure 3a). Cadmium, which is closely correlated to Pb in sediments, must have followed the same pathway from mining wastes, through sediments, to fish tissues. Within this general scheme, given the low Cd concentrations in the sediments and substratum (under 1 $\mu g\ g^{-1}$ in local granites, n = 6), the Cd levels in trout tissues at VER are much higher than expected. This watershed is exclusively granitic, so that pH and hardness are the lowest measured in the study: ~6.4 and 8 $mg\ L^{-1}$, respectively (Table 1). Ca^{2+} is a powerful competitor for waterborne metal assimilation in organisms,[37] so the absence of calcareous rocks may have facilitated the incorporation of Cd in biota. Moreover, the solubility of metal salts increases in acid waters, especially Cd which is highly labile.[38] The dynamics of Zn and Cu concentrations in fish tissues appear to be much lower than those of Cd and Pb, because there is a homeostatic control of internal concentrations due to their essentiality.[10,39] No clear patterns are observed for these elements in fish tissues (Figure 2, Table 2).

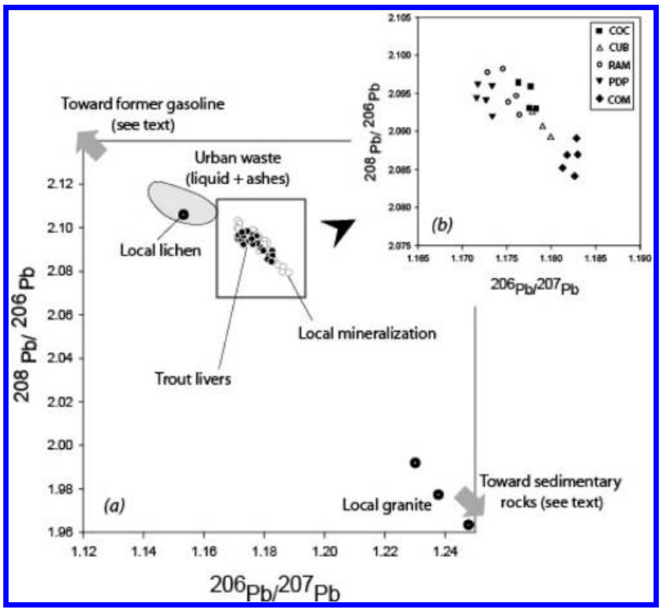

Figure 3. (a): $^{208}Pb/^{206}Pb$ vs $^{206}Pb/^{207}Pb$ ratios in trout livers from the PNC (closed circles, this study) and for local mineralizations (open circles,[35]). Other potential sources: local granite,[35] sedimentary rocks,[36] leaded gasoline and urban waste,[21] and indigenous lichens,[26] are also plotted for comparison. (b): close-up for trout livers.

Interpopulational variations are clearly established at least for Pb and Cd using animals of various ages, although age structure might explain at least a part of the intrasite variations in metal concentrations. The trout livers from COM, PDP, and RAM are the most contaminated in Pb. These sites correspond to major mining which recently ceased, located in the near fishing area (less than 700 m). Lower Cd and Pb concentrations in trout livers from CUB could be related to a minor mining network located from 1.4 km upstream, which probably operated from Antiquity until Modern Times. As expected, the COC site, which is further downstream (>3.4 km) from mines dating from the Middle Ages and the Renaissance (perhaps even the Iron Age), yields trout with lower Pb and Cd contents. Although the chronology of mining and metallurgy may at times be incomplete or uncertain, trout contamination is nevertheless related to the distance between the sampling sites and the mines or dumps, and to the nature, intensity and duration of the works.

Trout Morphometry. Contrasting degrees of mean FA are observed between sites (Figure 4, FA10 data in Supporting Information S2, all traits and Pb in S13, all traits and Cd in S14). Whatever the trait considered, the highest levels of developmental instability are noticed in areas highly polluted in Pb or Cd, especially COM. Correlations between FA and metal concentrations in trout livers are also significant when they are computed for individuals (Supporting Information S15−16). Although correlation does not necessarily imply causality, such

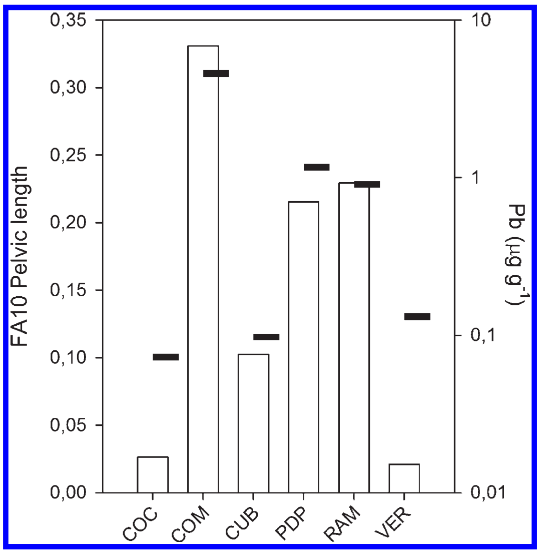

Figure 4. FA10 for pelvic length represented by white boxes. Median Pb in livers (wet-based concentration) for each site is plotted as a bold black line for comparison. COC for Cocurès, COM for Combe Sourde, CUB for Cubières, PDP for Pont-dela-Planche, RAM for Ramponenche, and VER for Vérié. FA10 of the other traits can be seen in Supporting Information S1, 13−14.

a finding is consistent with numerous studies demonstrating that environmental stress increases FA.[24,40] However, Linde et al. (1998) reported no clear association between Cu, Pb, and Cd concentrations and FA levels of three meristic traits in brown trout collected in Spain.[16] These interesting results do not necessarily contradict ours, since their study was performed on fewer specimens collected from fewer locations, characterized by lower levels of pollution. Moreover, meristic traits which are determined early in development may be less or not affected by Pb and Cd because established before accumulation within the organisms.

Necessary Environmental Management. In the present study, both hepatic and muscle concentrations of Cd and Pb are among the highest ever reported in natural environments for brown trout, and ∼3 orders of magnitude greater than those of commercial trout produced by fish farming and sold in supermarkets (Supporting Information S17). At the COM site, 60% of the trout exceed the EU maximum allowed concentrations for human consumption for Pb (0.3 $\mu g\ g^{-1}$, fresh muscles) and/or for Cd (0.05 $\mu g\ g^{-1}$, fresh muscles).[25] The streambed sediments are comparable to highly contaminated sediments sampled in the North Pennines, formerly one of the most productive Pb and Zn mining areas in Britain.[41] It is clear that direct runoff and mechanical erosions from slag heaps, workshop grounds and mining and cleansing dumps still contaminate aquatic environments in the PNC, long after mining has ceased. Although fishes may have acquired tolerance to prolonged metal exposure, adverse environmental stress is related to measurable developmental disturbances and may well have a deleterious effect on animals. At first sight, such a situation may seem surprising in a protected area. However, environmental policies mainly address current human activities, and do not really take into account the persistent pollution caused by historical mining. Dealing with such contamination is a complex task because of the size of the area involved. This state of affairs is not uncommon in such a seemingly pristine environment. In France, it was recently demonstrated from a peat bog core from the Celtic site of Bibracte in the Morvan (also located in the Massif Central) that about 20% of the total anthropogenic Pb was atmospherically deposited before our era, and about 50% before the 18th century; these pollutants also originated from historical mining.[4] Metal contamination has been identified in areas of the Vosges mountains which were intensively mined in the past.[42] Such pollution hot spots are likely to have a deleterious effect on biodiversity, and could be dangerous for human health. Unfortunately, the industrial history of these middle altitude areas has either been forgotten or has remained so familiar that it is no longer considered to represent a danger. Furthermore, research into metal contamination has generally focused on urban and industrialized areas, and on the largest mining sites of the recent past. For all these reasons, the environmental impact of minor centers where historical mining took place has often been disregarded. Yet this troublesome environmental heritage remains of primary importance, particularly for the unwary human local population.

■ ASSOCIATED CONTENT

S Supporting Information. Fluctuating asymmetry for the four measured traits, analytical procedure, determination limits, results obtained on certified reference materials, replicates, metal concentrations in sediments, trout livers and muscles, lead isotopic compositions, Spearman's correlation coefficients between age and metal concentrations in tissues, relationships between FA10 for the four measured traits and Pb or Cd in trout livers, literature values. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ REFERENCES

(1) Ambert, P.; Coularou, J.; Cert, C.; Guendon, J.-L.; Bourgarit, D.; Mille, B.; Dainat, D.; Houles, N.; Baumes, B. The oldest settlement of metalworkers in France (3rd millennium BC): Peret (Herault, France). *Comptes Rendus Palevol.* **2002**, *1* (1), 67–74.

(2) Ettler, V.; Vanďk, A.; Mihaljevič, M.; Bezdička, P. Contrasting lead speciation in forest and tilled soils heavily polluted by lead metallurgy. *Chemosphere* **2005**, *58* (10), 1449–1459.

(3) Mighall, T. M.; Abrahams, P. W.; Grattan, J. P.; Hayes, D.; Timberlake, S.; Forsyth, S. Geochemical evidence for atmospheric pollution derived from prehistoric copper mining at Copa Hill, Cwmystwyth, mid-Wales, UK. *Sci. Total Environ.* **2002**, *292* (1−2), 69–80.

(4) Monna, F.; Petit, C.; Guillaumet, J.-P.; Jouffroy-Bapicot, I.; Blanchot, C.; Dominik, J.; Losno, R.; Richard, H.; Leveque, J.; Chateau, C. History and environmental impact of mining activity in Celtic Aeduan

territory recorded in a peat bog (Morvan, France). *Environ. Sci. Technol.* **2004**, *38* (3), 665–673.

(5) Pyatt, F. B.; Gilmore, G.; Grattan, J. P.; Hunt, C. O.; McLaren, S. An Imperial Legacy? An exploration of the environmental impact of ancient metal mining and smelting in southern Jordan. *J. Archaeol. Sci.* **2000**, *27*, 771–778.

(6) Besser, J. M.; Brumbaugh, W. G.; May, T. W.; Church, S. E.; Kimball, B. A. Bioavailability of metals in stream food webs and hazards to brook trout (*Salvelinus fontinalis*) in the Upper Animas River Watershed, Colorado. *Arch. Environ. Contam. Toxicol.* **2001**, *40* (1), 48–59.

(7) Casiot, C.; Egal, M.; Elbaz-Poulichet, F.; Bruneel, O.; Bancon-Montigny, C.; Cordier, M.-A.; Gomez, E.; Aliaume, C. Hydrological and geochemical control of metals and arsenic in a Mediterranean river contaminated by acid mine drainage (the Amous River, France); preliminary assessment of impacts on fish (*Leuciscus cephalus*). *Appl. Geochem.* **2009**, *24* (5), 787–799.

(8) Ettler, V.; Mihaljevič, M.; Šebek, O.; Molek, M.; Grygar, T.; Zeman, J. Geochemical and Pb isotopic evidence for sources and dispersal of metal contamination in stream sediments from the mining and smelting district of Pribram, Czech Republic. *Environ. Pollut.* **2006**, *142* (3), 409–417.

(9) Leblanc, M.; Morales, J.-A.; Borrego, J.; Elbaz-Poulichet, F. 4,500-year-old mining pollution in southwestern Spain: long-term implications for modern mining pollution. *Econ. Geol.* **2000**, *95* (3), 655.

(10) Moiseenko, T. I.; Kudryavtseva, L. P. Trace metal accumulation and fish pathologies in areas affected by mining and metallurgical enterprises in the Kola Region, Russia. *Environ. Pollut.* **2001**, *114* (2), 285–297.

(11) Piatak, N. M.; Seal, R. R., II; Hammarstrom, J. M. Mineralogical and geochemical controls on the release of trace elements from slag produced by base-and precious-metal smelting at abandoned mine sites. *Appl. Geochem.* **2004**, *19* (7), 1039–1064.

(12) Ploquin, A.; Allee, P.; Bailly-Maître, M.-C.; Baron, S.; de Beaulieu, J.-L.; Carignan, J.; Laurent, S.; Lavoie, M.; Mahe-Le Carlier, C.; Peytavin, J.; Pulido, M. Medieval lead smelting on the Mont Lozère, Southern France. In *International Conference: Archaeometallurgy in Europe*; Associazione Italiana Metallurgia: Milan, Italy, 2003; Vol. 1, ; p 635−644.

(13) Baron, S.; Lavoie, M.; Ploquin, A.; Carignan, J.; Pulido, M.; de Beaulieu, J.-L. Record of metal workshops in peat deposits/: history and environmental impact on the Mont Lozère Massif, France. *Environ. Sci. Technol.* **2005**, *39* (14), 5131–5140.

(14) Balistrieri, L. S.; Seal, R. R., II; Piatak, N. M.; Paul, B. Assessing the concentration, speciation, and toxicity of dissolved metals during mixing of acid-mine drainage and ambient river water downstream of the Elizabeth Copper Mine, Vermont, USA. *Appl. Geochem.* **2007**, *22* (5), 930–952.

(15) Braungardt, C. B.; Achterberg, E. P.; Elbaz-Poulichet, F.; Morley, N. H. Metal geochemistry in a mine-polluted estuarine system in Spain. *Appl. Geochem.* **2003**, *18* (11), 1757–1771.

(16) Linde, A. R.; Sanchez-Galan, S.; Izquierdo, J. I.; Arribas, P.; Maranon, E.; García-Vázquez, E. Brown trout as biomonitor of heavy metal pollution: effect of age on the reliability of the assessment. *Ecotoxicol. Environ. Saf.* **1998**, *40* (1−2), 120–125.

(17) Lamas, S.; Fernandez, J. A.; Aboal, J. R.; Carballeira, A. Testing the use of juvenile *Salmo trutta L.* as biomonitors of heavy metal pollution in freshwater. *Chemosphere* **2007**, *67* (2), 221–228.

(18) Has-Schön, E.; Bogut, I.; Kralik, G.; Bogut, S.; Horvatić, J.; Čačić, I. Heavy metal concentration in fish tissues inhabiting waters of ≪ Buško Blato ≫ reservoar (Bosnia and Herzegovina). *Environ. Monit. Assess.* **2008**, *144* (1), 15–22.

(19) Komárek, M.; Ettler, V.; Chrastný, V.; Mihaljevič, M. Lead isotopes in environmental sciences: A review. *Environ. Int.* **2008**, *34* (4), 562–577.

(20) Ettler, V.; Mihaljevič, M.; Komárek, M. ICP-MS measurements of lead isotopic ratios in soils heavily contaminated by lead smelting: tracing the sources of pollution. *Anal. Bioanal. Chem* **2004**, *378* (2), 311–317.

(21) Monna, F.; Lancelot, J.; Croudace, I. W.; Cundy, A. B.; Lewis, J. T. Pb isotopic composition of airborne particulate material from France and the southern United Kingdom: Implications for Pb pollution sources in urban areas. *Environ. Sci. Technol.* **1997**, *31* (8), 2277–2286.

(22) Véron, A.; Flament, P.; Bertho, M.-L.; Alleman, L.; Flegal, R.; Hamelin, B. Isotopic evidence of pollutant lead sources in Northwestern France. *Atmos. Environ.* **1999**, *33*, 3377–3388.

(23) Graham, J. H.; Raz, S.; Hel-Or, H.; Nevo, E. Fluctuating asymmetry: Methods, theory, and applications. *Symmetry* **2010**, *2* (2), 466–540.

(24) Polak, M. Developmental Instability: Causes and Consequences, Oxford University Press, New York, 2003.

(25) Official Journal of the European Union. Commission regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. 2006; http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:364:0005:0024:EN:PDF (accessed 2009).

(26) Baron, S. Traçabilité et évolution d'une pollution métallurgique médiévale de Plomb Argentifère sur le Mont-Lozère. Ph.D. Dissertation; University of Montpellier II: France, 2005.

(27) Prassl, J.-A. Exploitation antique dans le secteur minier du Bleymard (Lozère). *Archéologie Languedoc* **1997**, *21*, 217–223.

(28) Baglinière, J.-L.; Bomassi, P.; Bousquet, B.; Chancerel, F.; De Pontual, H.; Dumas, J.; Euzenat, G.; Fontenelle, G.; Fournel, F.; Gayou, F.; Luquet, J.-F.; Maisse, G.; Martin Ventura, J.-A.; Marty, A.; Nihouarn, A.; Porcher, J.-P.; Prevost, E.; Prouzet, P.; Pustelnik, G.; Richard, A.; Troadec, H. La détermination de l'âge par scalimétrie chez le Saumon atlantique (*Salmo salar*) dans son aire de répartition méridionale: utilisation pratique et difficultés de la méthode. *Bull. Fr. Pêche Piscic.* **1985**, *298*, 69–105.

(29) Palmer, A. R.; Strobeck, C. Fluctuating asymmetry analyses revisited. In *Developmental Instability: Causes and Consequences*; Polak, M., Ed.; Oxford University Press: Oxford, UK, 2003; p 279−319.

(30) Fulton, T. W.The rate of growth of fishes. In *Fisheries Board of Scotland Annual Report*, Part 3, 1904; Vol. *22*, pp 141−241.

(31) Critchlow, D. E.; Fligner, M. A. On distribution-free multiple comparisons in the one-way analysis of variance. *Commun. Stat.-Theory Meth.* **1991**, *20* (1), 127–139.

(32) Norris, D. O.; Camp, J. M.; Maldonado, T. A.; Woodling, J. D. Some aspects of hepatic function in feral brown trout, *Salmo trutta*, living in metal contaminated water. *Comp. Biochem. Physiol., Part C: Pharmacol., Toxicol. Endocrinol.* **2000**, *127* (1), 71–78.

(33) Webb, M. A. H.; Feist, G. W.; Fitzpatrick, M. S.; Foster, E. P.; Schreck, C. B.; Plumlee, M.; Wong, C.; Gundersen, D. T. Mercury concentrations in gonad, liver, and muscle of white sturgeon *Acipenser transmontanus* in the lower Columbia River. *Arch. Environ. Contam. Toxicol.* **2006**, *50* (3), 443–451.

(34) Martin, J.-M.; Meybeck, M. Elemental mass-balance of material carried by major world rivers. *Mar. Chem.* **1979**, *7* (3), 173–206.

(35) Baron, S.; Carignan, J.; Laurent, S.; Ploquin, A. Medieval lead making on Mont-Lozère Massif (Cévennes-France)/: Tracing ore sources using Pb isotopes. *Appl. Geochem.* **2006**, *21*, 241–252.

(36) Monna, F.; Clauer, N.; Toulkeridis, T.; Lancelot, J.-R. Influence of anthropogenic activity on the lead isotope signature of Thau Lake sediments (Southern France): origin and temporal evolution. *Appl. Geochem.* **2000**, *15* (9), 1291–1305.

(37) Rogers, J. T.; Wood, C. M. Characterization of branchial lead-calcium interaction in the freshwater rainbow trout *Oncorhynchus mykiss*. *J. Exp. Biol.* **2004**, *207* (5), 813–825.

(38) Moiseenko, T. I.; Sharova, O. Physiological mechanisms of degradation of fish populations in acidified water bodies. *Russ. J. Ecol.* **2006**, *37* (4), 257–263.

(39) Olsvik, P. A.; Gundersen, P.; Andersen, R. A.; Zachariassen, K. E. Metal accumulation and metallothionein in two populations of brown trout, *Salmo trutta*, exposed to different natural water environments during a run-off episode. *Aquat. Toxicol.* **2000**, *50* (4), 301–316.

(40) Leary, R. F.; Allendorf, F. W. Fluctuating asymmetry as an indicator of stress: Implications for conservation biology. *TREE* **1989**, *4* (7), 214–217.

(41) Macklin, M. G.; Hudson-Edwards, K. A.; Dawson, E. J. The significance of pollution from historic metal mining in the Pennine orefields on river sediment contaminant fluxes to North Sea. *Sci. Total Environ.* **1997**, *194/195*, 391–397.

(42) Forel, B.; Monna, F.; Petit, C.; Bruguier, O.; Losno, R.; Fluck, P.; Begeot, C.; Richard, H.; Bichet, V.; Chateau, C. Historical mining and smelting in the Vosges Mountains (France) recorded in two ombrotrophic peat bogs. *J. Geochem. Explor.* **2010**, *107* (1), 9–20.