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# Historical legacies of river pollution reconstructed from fish scales☆

Paloma Morán [a, 1], Laura Cal [b], Antonio Cobelo-García [b], Clara Almécija [b], Pablo Caballero [c], Carlos Garcia de Leaniz [d, *, 1]

[a] Departamento de Bioquímica, Genética e Inmunología, Facultad de Biología, Universidade de Vigo, Spain
[b] Instituto de Investigaciones Mariñas de Vigo (IIM-CSIC), Spain
[c] Servicio de Conservación de la Naturaleza de Pontevedra, Xunta de Galicia, Spain
[d] BioSciences, CSAR, Swansea University, Swansea SA2 8PP, UK

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ABSTRACT

Many rivers have been impacted by heavy metal pollution in the past but the long-term legacies on biodiversity are difficult to estimate. The River Ulla (NW Spain) was impacted by tailings from a copper mine during the 1970–1980s but absence of baseline values and lack of subsequent monitoring have prevented a full impact assessment. We used archived fish scales of Atlantic salmon to reconstruct levels of historical copper pollution and its effects on salmon fitness. Copper bioaccumulation significantly increased over baseline values during the operation of the mine, reaching sublethal levels for salmon survival. Juvenile growth and relative population abundance decreased during mining, but no such effects were observed in a neighbouring river unaffected by mining. Our results indicate that historical copper exposure has probably compromised the fitness of this Atlantic salmon population to the present day, and that fish scales are suitable biomarkers of past river pollution.



## 1. Introduction

Past industrial practices can leave a pollution legacy that is often difficult to assess, as there are seldom before and after measurements. Atlantic salmon (*Salmo salar*) is a locally adapted species (Garcia de Leaniz et al., 2007) which constitutes a good indicator of water quality. Juveniles require well oxygenated waters and their survival and growth are negatively affected by water pollution (Gibson, 1993; Coghlan and Ringler, 2005). For example, heavy metals can trigger osmotic imbalance and alter enzymatic processes that can impair growth (Jezierska et al., 2009; Heydarnejad et al., 2013) and also disrupt the sensory physiology and antipredatory behaviour of fish (Hecht et al., 2007; Sandahl et al., 2007; McIntyre et al., 2012), all of which can impair fitness and increase mortality.

The analysis of fish scales can be used to reconstruct individual growth profiles, as the spacing between adjacent growth rings is proportional to body size increments (Schröder and Garcia de Leaniz, 2011; Marco-Rius et al., 2013). In addition, the chemical composition of fish scales tends to reflect the composition of the waters in which the fish live (Pender and Griffin, 1996), as there is often a good correlation between trace element concentration in scales and the water they are exposed to (Sauer and Watabe, 1984, 1988; Wells et al., 2000). The analysis of fish scales, thus, could be used to simultaneously detect the presence of pollutants and their effects on fitness, as impaired growth is a typical consequence of water pollution (Clearwater et al., 2002).

Open pit mining has impacted many rivers throughout the world but the long-term effects are often difficult to quantify due to lack of historical baseline data and absence of long-term monitoring (Hudson-Edwards et al., 2011). The river Ulla (NW Spain) received elevated concentrations of sulphates and heavy metals from acidic runoff caused by open pit mining and copper flotation during a 14 year period (1974–1988; Fernandez et al., 2006). This resulted in substantial degradation of the surrounding environment (Otero et al., 2012). In 1988 the mine was closed due to falling copper prices and decreasing mineral quality (Cerqueira et al., 2012), and restoration measures were implemented in 2003 with the addition of technosols (Asensio et al., 2013). However, the extent to which open mining may have already compromised the local fish populations is unknown.

We took advantage of the availability of archived fish scales as

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* Corresponding author.
*E-mail address:* c.garciadeleaniz@swansea.ac.uk (C. Garcia de Leaniz).
[1] Joint first authors.

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indicators of fish growth and water chemistry to reconstruct levels of copper pollution and its effects on fitness of Atlantic salmon in the River Ulla during the operation of a copper mine. An analysis of several other heavy metals on a shorter time series has recently been provided by Cobelo-Garcia et al. (2017). Salmon scales from the neighbouring River Miño were used as controls. This river is located 200 km to the south and has been proposed as a reference site for ecotoxicological studies as it is unaffected by heavy metal pollution and has remained relatively pristine (Reis et al., 2009). Thus, the analysis of historical fish scales from an impacted and control river enabled us to capitalize on a natural disturbance experiment and assess the long-term effects of chronic heavy metal pollution on an endangered fish species.

## 2. Materials and methods

We used archived salmon scales from adult salmon returning to the rivers Ulla (impacted river) and Miño (control river; Fig. 1), caught over a 61 year period (1951–2012). Atlantic salmon has a generation time of c. 3 years in the Iberian peninsula (Consuegra et al., 2005), we therefore considered three periods for the purpose of analysis: pre-mining (scales from adult fish caught before 1977), the mining period (adult fish caught between 1977 and 1991), and post-mining (adult fish caught after 1991). Fitness-related data (growth and abundance) were collated for both rivers, but copper data was only available for the River Ulla. Thus, our study conformed to a BACI (Before-After-Control-Impact) design for the fitness data, and a BA design (Before-After) for the copper bio-accumulation data (Manly, 2008), as in other field studies of copper pollution on salmon (Sprague et al., 1965).

### 2.1. Scale growth analysis

Scales were available from 492 individuals caught between 1951 and 2012 which had been stored dry in paper envelopes. Between three and five scales with non-regenerated nuclei were selected per individual; from these, acetate impressions were made with the aid of a pressure roller, and these were scanned with a Canon 300 microfilm scanner at 23–50x magnification and saved as high resolution TIFF images (Kuparinen et al., 2009). The software Image-J v. 1.4.1 (Abramoff et al., 2004) was used to identify the scale annuli and age the fish (Shearer, 1992; Rifflart et al., 2006), and to estimate scale growth at the end of the first winter in freshwater, the point of entry into the sea (smolt size), and the end of the first summer at sea (post-smolt growth, PSG) as per Marco-Rius et al. (2013).

### 2.2. Reliability of scale growth analysis

We ascertained the precision of scale analysis by calculation the repeatability of two growth points (smolt size and end of first marine growing season) on a sample of 30 individuals repeated twice as per Kuparinen et al. (2009). Scale measurements were not converted to body size measurement to avoid additional errors, as variation in scale measurement (0.01 mm; CV: 13.9%) was lower than that of body size measurement (cm; CV: 15.3%). The use of a pressure roll to obtain acetate impressions of the salmon scales did not distort the size of the scales (paired $t$-test $t_{29} = 0.229$, $P = 0.41$).

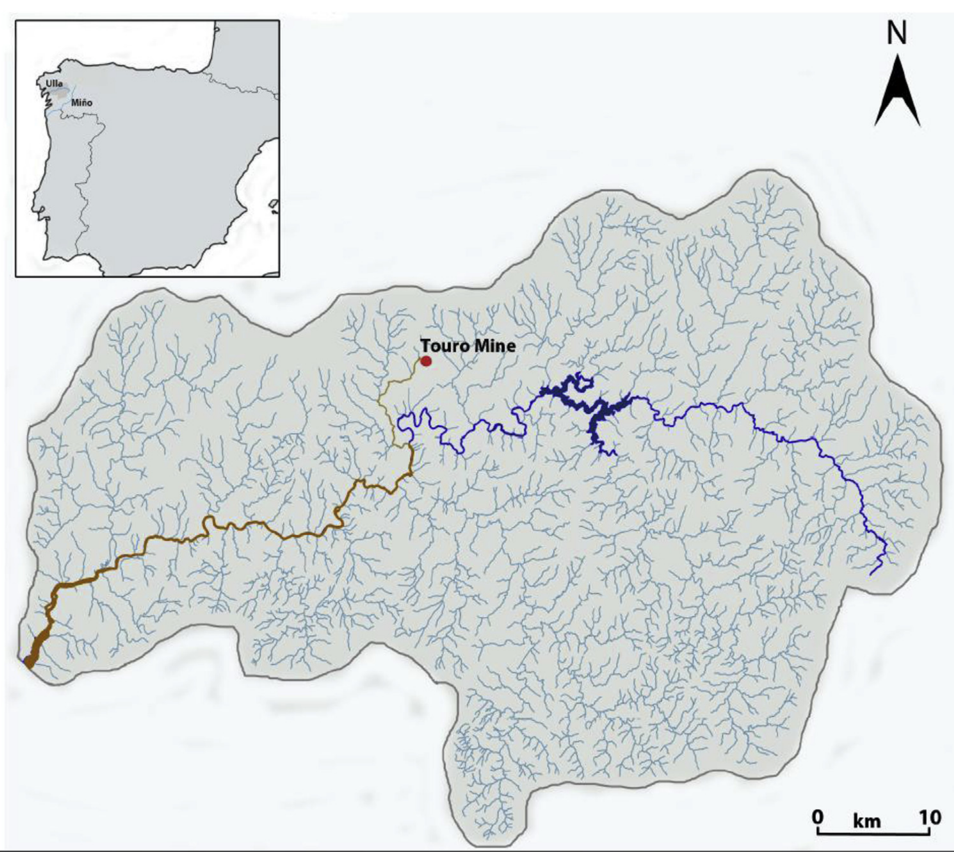


**Fig. 1.** Location of the study river impacted by open pit copper mining (R. Ulla), and of the reference control river unaffected by mining (R. Miño). Shown are the location of the Touro copper mine and the stream reach impacted by copper pollution (in red). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Repeatability in scale measurements was high (Cronbach's α, first winter growth = 0.963; total river growth = 0.981), indicating that our estimates of salmon growth based on scale measurements were precise.

### 2.3. Measures of salmon abundance

We used angling catches as a proxy of salmon abundance, as the study populations had been exploited by rod and line only during the study period, and all the salmon caught in the fishery are recorded (Garcia de Leaniz and Martinez, 1988; Álvarez et al., 2010). Most southern salmon populations have experienced pronounced declines in abundance in recent decades, so in order to compare the population trends of the impacted and reference populations we normalized their catches by calculating the proportional contribution of each river (excluding itself) to the total salmon catch in the region (Galicia, NW Spain) using data available in Álvarez et al. (2010). We used angling data from 1961 to 1999, as catches before 1961 were unreliable for the River Miño and catches after 1999 were subjected to catch quotas and other fishery restrictions that made direct comparisons among rivers difficult (Álvarez et al., 2010).

### 2.4. Analysis of barium and copper content of fish scales

Scales from the River Ulla collected between 1951 and 2007 were analysed for copper and barium; no scales were available from the River Miño for this purpose. Before digestion, scales were vigorously scrubbed and soaked in MQ water in order to remove surface contaminants. Between 50 and 100 mg (3–5 scales) were digested per individual with concentrated ultra-pure $HNO_3$ (Merck Suprapur). Analysis of scale digests (N = 23) was carried out by ICP-MS (X Series, Thermo Elemental) as described in Cobelo-Garcia et al. (2017). Analytical precision was checked using the DORM-2 reference standard (fish muscle powder, NRCC-Canada), obtaining a good agreement with the certified values (difference between observed and reference material, Ba = 2.9%, Cu = −9.3%). Because Ba bioaccumulation occurs mostly in freshwater (Adey et al., 2009), and fish from different periods differed in body size and age (Table 1), we used the Cu/Ba ratio to derive an index of freshwater Cu bioaccumulation that could be more readily related to the operation of the mine and compared across periods.

### 2.5. Statistical analysis

All analyses were carried out using R v. 3.3.2 (R Core Team, 2017). Temporal changes in Cu and Cu:Ba in the salmon scales were analysed via generalized additive modelling (GAM) using a penalized regression spline in the *mgcv* package to account for non-linearity (Wood, 2001). We employed a general linear model to analyse growth data at three life stages (first freshwater winter, entry into seawater, and end of the first marine summer) as a function of river of origin (impacted or control), period of mining (pre-mining, mining, post-mining) and freshwater age. To analyse changes in the relative abundance of salmon in the impacted and control river we employed a generalized linear model fitted with a quasibinomial link function to adequately model proportion data. In each case, we statistically compared models with and without a River × Period interaction to test whether salmon in the control and impacted rivers were responding differently to the operation of the mine, as one would expect from a BACI design if the mine was impacting one river but not the other.

**Table 1**
Demographic data (mean ± SE) for adult Atlantic salmon in the impacted and control rivers.

| River | Period | N | River age (yr) | Fork length (mm) | Weight (gr) |
|---|---|---|---|---|---|
| R. Ulla (Impacted) | | | | | |
| | Pre-mining | 96 | 1.32 (0.05) | 827 (9.9) | 6555 (198) |
| | Mining | 96 | 1.39 (0.05) | 798 (6.1) | 5011 (136) |
| | Post-mining | 96 | 1.17 (0.04) | 722 (11.8) | 4106 (229) |
| R. Miño (Control) | | | | | |
| | Pre-mining | 44 | 1.43 (0.08) | 872 (17.0) | 6702 (355) |
| | Mining | 57 | 1.30 (0.06) | 802 (8.0) | 5631 (205) |
| | Post-mining | 103 | 1.45 (0.05) | 799 (4.4) | 5354 (94) |

## 3. Results

### 3.1. Copper concentration in salmon scales

The influence of the open pit mine is clearly seen by an increase in the deposition of copper on the scales of Atlantic salmon in the River Ulla (Fig. 2a). GAM analysis indicates that 77% of the deviance in copper in salmon scales can be explained by a strong temporal trend (smooth component of year class, $F_{edf\ 4.039,\ ref\ 4.894} = 11.65$, $P < 0.001$).

The concentration of copper in salmon scales changed significantly during the three periods considered (ANOVA, $F_{2,20} = 45.741$, $P < 0.001$). Mean copper concentration during the operation of the

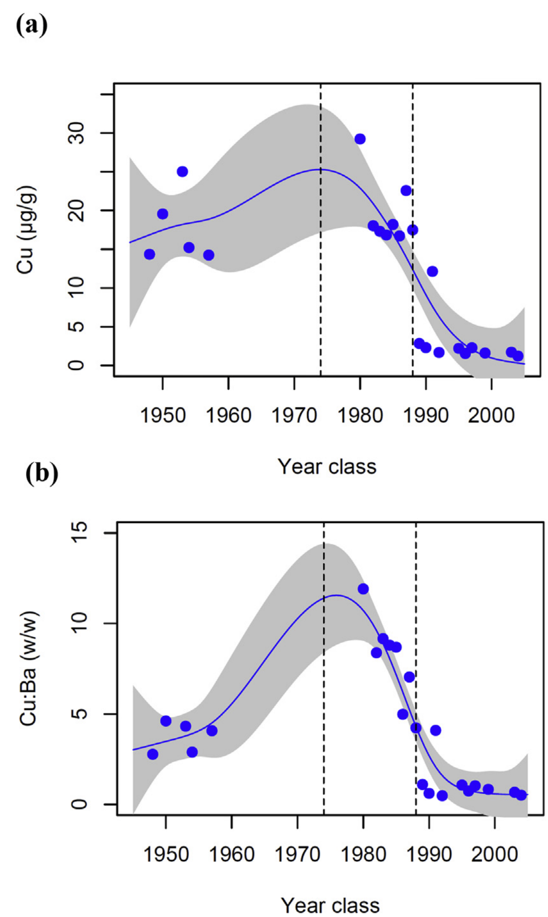


**Fig. 2.** Temporal trends obtained by generalized additive modelling (GAM, 95 CI intervals) in (a) copper concentration (µg/g) and (b) copper to barium ratio (w/w) in fish scales of adult Atlantic salmon returning to the River Ulla. Dotted lines indicate year classes that would have been affected by heavy metal pollution from the open pit mine.

mine (19.56 ± 1.13 μg/g) exceeded about 7 times the values found after the mine was closed (2.96 ± 1.03 μg/g; Tukey Honest Significant Difference test; $P < 0.001$) and was also higher, but not significantly so (Tukey HSD $P = 0.69$) than the mean during the pre-mining period (17.68 ± 2.08 μg/g; Table 2). An even stronger signal for the operation of the mine is obtained when copper values are normalized by the concentration of barium to account for variation in the size and age of salmon in different periods, as barium is deposited mostly in freshwater (Adey et al., 2009). Using this approach, a strongly humped temporal trend in Cu:Ba is revealed, which coincides with the mining period (Fig. 2b). GAM analysis indicates that 89% of the deviance in Cu:Ba (w/w) is explained by the temporal trend (smooth component of year class, $F_{\mathrm{edf}\ 5.134,\ \mathrm{ref}\ 6.102} = 21.14$, $P < 0.001$). Cu:Ba values differed significantly during the three study periods (ANOVA, $F_{2,20} = 37.161$, $P < 0.001$) and mean Cu:Ba during mining (7.90 ± 0.87) showed a 7-fold enrichment compared to the post-mining period (1.11 ± 0.34), and two-fold enrichment compared to the pre-mining period (3.73 ± 0.38). Post-hoc pairwise comparisons were all statistically different (Tukey HSD; Pre-mining vs. mining, $P < 0.001$; mining vs. post-mining, $P < 0.001$; pre-mining vs. post-mining, $P = 0.02$).

To determine when copper was most likely to have been deposited in the scales, we compared model fits with year of sampling (i.e. year of return to freshwater from the sea) and with year class (i.e. cohort or year of hatching, three years before adults were sampled following their return from the sea). Using year class resulted in models with significantly lower AIC values (and thus provided a more plausible fit) than with year of return for both Cu (AIC year class = 133.38; AIC year of return = 157.64; ΔAIC = −24.25) and Cu:Ba (AIC year class = 93.42; AIC year of return = 98.34; ΔAIC = −4.92). This suggests that copper bioaccumulation most likely occurred during the juvenile phase in freshwater, rather than during the adult phase in the estuary or the sea, or when adults returned to the river.

### 3.2. Changes on salmon growth

Comparisons between the unaffected (control) river (R. Miño) and the river impacted by mining (R. Ulla) indicates the existence of a significant interaction between river and period for all metrics of salmon growth (Fig. 3), as one would expect from a BACI design. Thus, growth during the juvenile phase in freshwater declined sharply during the mining period in the impacted river, but not in the control river, where it remained stable over the whole period.

**Table 2**
Copper bioaccumulation in adult salmon scales (mean ± SE) from the impacted river Ulla at different periods during the operation of the copper mine.

| Parameter | Period | Adult salmon scales | | | | |
|---|---|---|---|---|---|---|
| | | Year of sampling | Impacted year classes | N | Mean | SE |
| **Copper (Cu, μg/g)** | | | | | | |
| | Pre-mining (before 1974) | 1951–1960 | 1948–1957 | 5 | 17.68 | 2.079 |
| | Mining (1974–1988) | 1983–1991 | 1980–1988 | 8 | 19.56 | 1.533 |
| | Post-mining (after 1988) | 1992–2007 | 1989–2004 | 10 | 2.96 | 1.031 |
| **Copper/Barium (Cu:Ba w/w)** | | | | | | |
| | Pre-mining (before 1974) | 1951–1960 | 1948–1957 | 5 | 3.73 | 0.378 |
| | Mining (1974–1988) | 1983–1991 | 1980–1988 | 8 | 7.90 | 0.867 |
| | Post-mining (after 1988) | 1992–2007 | 1989–2004 | 10 | 1.11 | 0.338 |

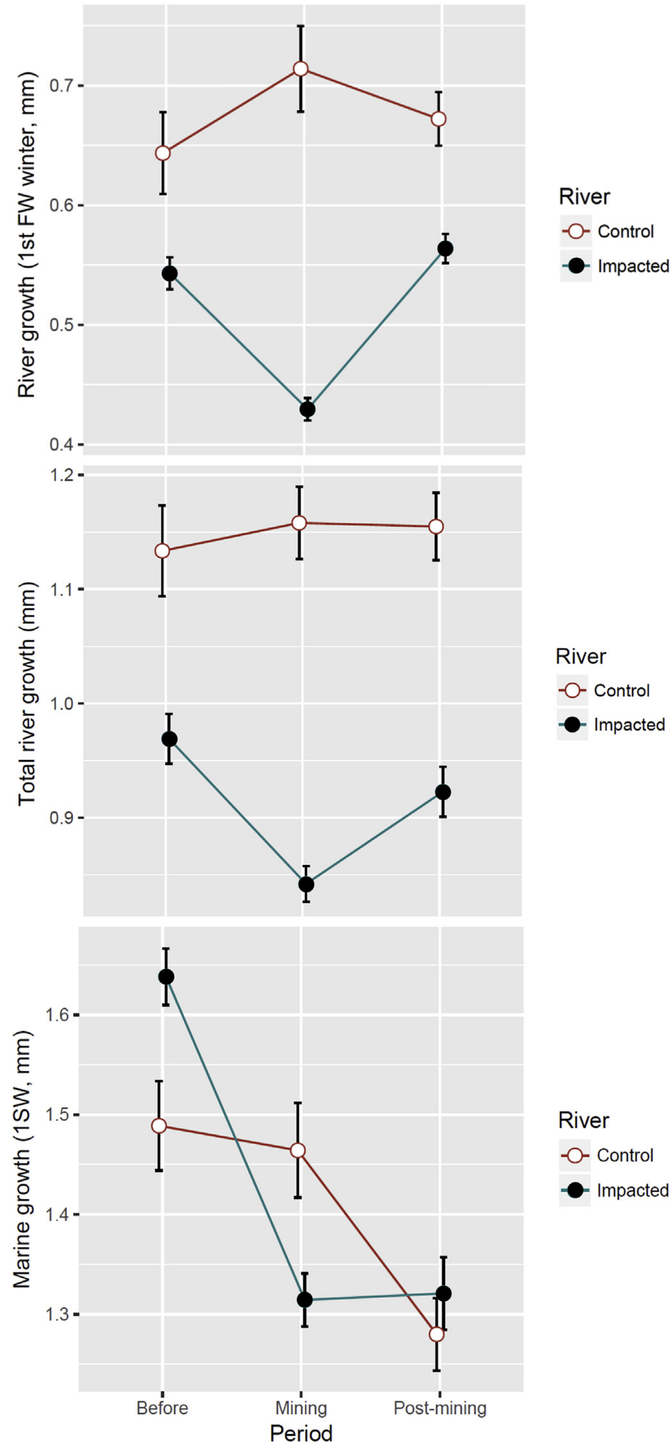


**Fig. 3.** Changes in mean scale growth (±95 CI) of adult Atlantic salmon in the control river unaffected by mining (R. Miño) and the impacted river (R. Ulla) before, during and after the operation of the copper mine.

After the operation of the mine ceased, freshwater growth recovered in the River Ulla, reaching or approaching pre-mining values (Fig. 3a and b). A reduction in freshwater growth in the impacted river was evident both during the first year of life (Fig. 3a; ANOVA $F_{7,484} = 37.8$, $P < 0.001$; River $F_{1,484} = 123.8$, $P < 0.001$; Period $F_{2,484} = 4.7$, $P = 0.010$; Freshwater age $F_{1,484} = 85.8$, $P < 0.001$; River x Period $F_{2,484} = 10.4$, $P < 0.001$; River x Freshwater age $F_{1,484} = 24.9$, $P < 0.001$) and over the entire juvenile phase in freshwater (Fig. 3b; ANOVA $F_{8,483} = 25.3$, $P < 0.001$; River $F_{1,483} = 139.2$, $P < 0.001$; Period $F_{2,483} = 3.3$, $P = 0.038$; Freshwater age $F_{1,483} = 33.2$, $P < 0.001$; River x Period $F_{2,483} = 8.3$, $P < 0.001$; Period x Freshwater age $F_{2,483} = 4.5$, $P = 0.011$). Marine growth during the first summer at sea (Fig. 3c) declined significantly for both rivers during the study period (ANOVA $F_{6,485} = 17.2$, $P < 0.001$; Period $F_{2,485} = 33.3$, $P < 0.001$) but did so differently for the impacted river and the control (River x Period $F_{2,485} = 4.3$, $P = 0.014$), once the effects of variation in freshwater age are taken into account (River age $F_{1,485} = 18.2$, $P < 0.001$). Thus, migrating smolts in the impacted river experienced a very sharp decline in marine growth during the mining period, something that was not observed in the control fish (Fig. 3c).

### 3.3. Changes in relative salmon abundance

As with the metrics of growth, a significant River × Period interaction existed for the index of relative salmon abundance, indicating that the abundance of the salmon populations in the control and impacted rivers had changed differently during the study period. The generalized linear model with the River × Period interaction term explained 43.4% of deviance compared to 36.4% for the model with only main effects (model comparison by ANOVA, df = −2, deviance = −440.67, $P = 0.024$). Thus, relative abundance in the impacted river decreased sharply during the years of mining activity and continued to decrease thereafter with no evidence of recovery, while in the reference river abundance did not change significantly during the mining period, and then increased post-mining (Fig. 4).

## 4. Discussion

Our analysis of a natural disturbance experiment demonstrates the value of using archived fish scales for reconstructing historical levels of river pollution and for assessing the long-term effects of heavy metals on fish fitness. Analysis of Atlantic salmon scales collected over a 61 year period revealed a significant increase in copper bioaccumulation that coincided with the operation of an open pit copper mine, and which returned to near basal levels after the mine was closed. Using a powerful BACI design we also show that exposure to copper was accompanied by a marked decrease in juvenile growth and in the relative abundance of salmon in the impacted river, something that was not seen in salmon from a nearby river unaffected by mining. Given that metal concentrations in fish scales are proportional to metal concentrations in the water the fish live in (Sauer and Watabe, 1984; Wells et al., 2000), it is possible to reconstruct the approximate concentration of copper in the impacted river. Thus, taking a ‘pristine’ value of dissolved Cu of 1–5 nM for neighbouring rivers in the present day (Prego and Cobelo-Garcia, 2003), we may estimate copper concentrations of up to 75–100 nM (4.8–6.4 ppb) and an average of 7–35 nM (0.45–2.2 ppb) during the operation of the copper mine in the 1980's. Although this is a mere estimate subject to several sources of error, such copper concentrations have been shown to impair fitness and diminish the sensory capabilities of salmonids (Scott and Sloman, 2004; Hecht et al., 2007), and our study shows that there were significant decreases in juvenile growth and relative abundance.

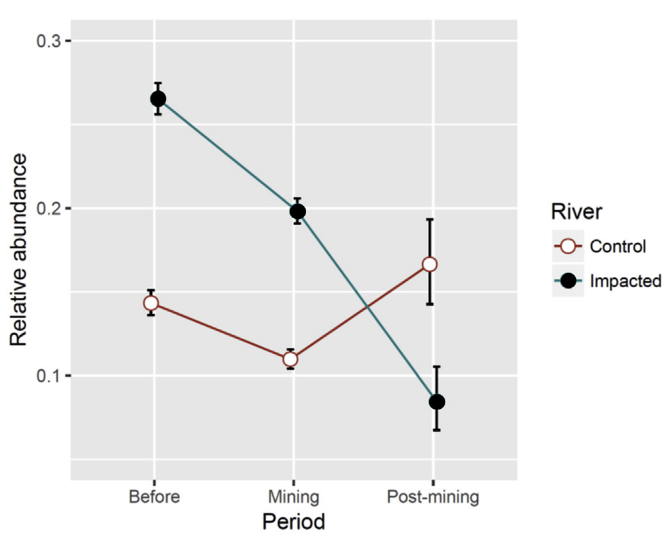


**Fig. 4.** Changes in the relative abundance (mean ± 95 binomial CI) of adult Atlantic salmon in the control river unaffected by mining (R. Miño) and the impacted river (R. Ulla) before, during and after the operation of the copper mine. Shown is the relative contribution of each river (excluding itself) to the rod and line catch of Atlantic salmon in the region of Galicia (NW Spain).

Copper is an essential trace metal for aquatic organisms, acting as a cofactor for several enzymes that play a central role in cellular metabolism (Wood et al., 2012). Fish take up copper from the diet and also from the water through the gills. Copper-rich water can be toxic to fish because copper directly affects the structural integrity of the gill epithelium and impairs osmoregulation (Heath, 1995; Wood et al., 2012), which will reduce survival, particularly during the juvenile stages when fish have a small body size (Grosell et al., 2007; Shaw et al., 2012) or when fish migrate from freshwater to the marine environment (Heath, 1995); copper exposure has also many other impacts on fish (reviewed in Clearwater et al., 2002), a reduction in condition factor and specific growth rates being some of the most common impacts (McKim and Benoit, 1974; Heydarnejad et al., 2013). This may explain why juvenile salmon in the impacted river did not grow as well as those from the undisturbed control river and did not appear to have survived as well, as inferred from the dramatic decrease in relative abundance.

Analysis of scales of Atlantic salmon in southern rivers indicates that growth occurs in the river and in the sea, with limited or no growth occurring in the estuaries (Consuegra et al., 2005). As ocean waters are naturally poor in dissolved copper (<3.5 nmol/kg, Boyle et al., 1981), copper bio-accumulation in salmon scales must have taken place mostly in freshwater. This is also supported by our analysis of Cu:Ba values, which suggests that copper deposition occurred mostly in the freshwater part of the fish scales, and by model comparisons, which indicates that year class (i.e. hatch year) is a better predictor of copper in the scales than year of return, again indicating the copper deposition must have occurred during the juvenile phase in the river, rather than in the estuary or the sea.

Salmon scales consists of a well-mineralized external bioapatite layer overlying a thicker, collagen-rich basal plate that makes up 70% of the scale mass (Hutchinson and Trueman, 2006; Adey et al., 2009). Scale growth is achieved by deposition of successive layers of collagen, followed by mineralization of the external layer, which grows centrifugally (Hutchinson and Trueman, 2006). Our study cannot distinguish between dietary and waterborne copper uptake in the salmon scales, but given the extent of copper pollution in the study river, it is likely that both mechanisms were involved. In rainbow trout, dietary copper uptake appears to be more important than waterborne uptake in the gills, liver and gut (Kamunde et al., 2002), and it is likely that the same happens in fish scales.

Most studies on copper toxicity in fish have been carried out under laboratory conditions during short periods of time (Berntssen et al., 1999; Shaw et al., 2012), or are derived from benthic species living among polluted sediments (Oliva et al., 2012). Long-term studies on the impacts of heavy metals on natural fish populations living in rivers are rare. The juvenile stages of freshwater fish are particularly vulnerable to pollution by heavy metals but studies in the wild are difficult to monitor due to lack of baseline values and the challenge of finding suitable metrics of fitness. The novelty of our approach lies in the fact that we were able to implement a robust experimental design that included controls (for fitness measures), as well as before and after samples, to simultaneously detect the presence of a contaminant (copper) and monitor its effects on fitness (growth) using fish scales collected over several decades. This enabled us to demonstrate growth inhibition, most likely due to copper exposure, during the juvenile phase of Atlantic salmon in freshwater. Although our study was ecological - not experimental, and did not establish a causal relationship between copper exposure and copper in the scales, previous studies have shown that the concentration of trace metals in fish scales is positively correlated to that of the surrounding water in several fish species (mummichog, Sauer and Watabe, 1984; barramundi, Pender and Griffin, 1996; Norfolk spot, Wells et al., 2000). Moreover, in Atlantic salmon, scales of juveniles collected from different rivers display significantly different concentrations of trace metals (including copper; Flem et al., 2005; Adey et al., 2009), which are thought to reflect geological differences in the bedrock (and hence in the water) the fish lived in.

With the above caveat in mind, our results suggest that chronic exposure to sublethal levels of dissolved copper may have increased juvenile salmon mortality, although the long-term effects of copper pollution on salmon survival are difficult to assess. While all salmon rivers in the study region have experienced a marked decline in abundance over the last few decades (Garcia de Leaniz et al., 2001; Álvarez et al., 2010), our analysis indicates that salmon catches in the river impacted by copper pollution decreased to a much larger extent than in neighbouring rivers. Thus, the relative abundance of salmon in the impacted river (measured as the river's contribution to total salmon catches in the region excluding itself) decreased from 38% (pre-mining) to 25% (mining) and then to 7% (post-mining), while in the control river they remained stable at 11–12% and then increased to 24% in more recent years. We acknowledge that using angling catches to infer changes in salmon survival has inherent limitations (Bielak and Power, 1988), but our study populations are small, and all fish caught in the fishery are recorded, which probably increases the reliability of using catch data for monitoring trends in abundance (Garcia de Leaniz et al., 2001). Indeed, we have previously shown that salmon catches provide valid proxies for year class strength under these conditions (Consuegra et al., 2005).

Taken together, our study indicates that copper pollution caused by run-off from an open pit mine seems to have compromised the fitness of Atlantic salmon in the River Ulla to the present day, and that fish scales are suitable biomarkers of past river pollution. Although the closure of the mine may have brought levels of dissolved copper back to basal levels (as judged by the observed decrease in copper bioaccumulation in fish scales), this was not enough to prevent the continuing decline of the impacted salmon population, whose relative abundance decreased more than threefold since mining stopped.

Copper pollution caused by mining was found to prevent the upstream migration of Atlantic salmon in the Miramichi River in Canada, with no evidence of habituation over successive year-classes (Saunders and Sprague, 1967), and was also predicted to delay recovery time and to increase the risk of extinction in endangered Chinook salmon (Mebane and Arthaud, 2010). Many Atlantic salmon populations in the Iberian peninsula (including the study river) are endangered or critically endangered (Garcia de Leaniz et al., 2001; WWF, 2001). They have few age classes, low resilience, and small effective population sizes (Consuegra et al., 2005; Kuparinen et al., 2010), which will make their recovery difficult or even impossible after prolonged heavy metal pollution. Thus, current proposals to reopen the Touro copper mine (Prieto, 2017; DOG, 2017) will likely place the salmon population in the River Ulla at the brink of extinction.

## Acknowledgments

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.envpol.2017.11.057.

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