Impact of nickel mining in New Caledonia on marbled eels *Anguilla marmorata*

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Abstract

New Caledonia is particularly affected by nickel open pit mining activities because of the presence of ultramafic soils rich in metal trace elements. The nickel particles dispersed by atmospheric transport and soil erosion during the excavation of nickel will end up by deposition or leaching in aquatic ecosystems where they may be bioaccumulated by living organisms in the rivers downstream the mines. Despite alarming freshwater metals concentrations, no study investigated the level of their bioaccumulation in eels living downstream mining sites, and if high bioaccumulation levels occur, the potential consequences on eel's health. The aim of this study was to determine how eels *Anguilla marmorata* are impacted *in situ* by metal concentrations issued from mining activity by measuring: morphometric parameters; metal concentrations in tissues and organs and transcription levels of target genes encoding proteins involved in several metabolic key functions. Among organs, liver was found to be the most affected by mining with average nickel concentrations of 5.14 mg/kg versus 1.63 mg/kg for eel liver away from mines leading to dysregulation of numerous genes involved in oxidative stress, DNA repair, apoptosis, reproduction and both lipid and mitochondrial metabolisms. This study should allow us to define in an integrated way if metals released by mining activities influence metals bioaccumulation in eels and induce biological effects.

Keywords: New Caledonia; Metals; Eels; Bioaccumulation; Gene transcription

1. Introduction

Aquatic ecosystems are particularly impacted by anthropogenic pollutants since many years, especially by metals coming from industrial, urban or agricultural sources, such as cadmium (Cd), mercury (Hg), zinc (Zn) or nickel (Ni) for instance, causing harmful impacts on species living in these environments. Some studies have already highlighted the toxic effects of water metal pollution on fish such as the yellow perch *Perca flavescens*, the spiny eel *Mastecembelus armatus,* or the European eel *Anguilla anguilla* [1-3].

Among the human activities responsible of this pollution, today, mining activities represent the second source of heavy metal soils contamination in the world [4]. In fact, soils are subjected to significant metal leaching due to the high concentration of heavy metals and the low capacity of the soil to retain water. Metals can then persist a long time in aquatic environment [5-9]. This type of contamination is particularly important in New Caledonia, one of the largest Ni producer in the world with resources concentrated in ultramafic formations covering 30 % of the subsoils [10]. These formations are particularly rich in metal trace elements including Ni, chromium (Cr), cobalt (Co), and manganese (Mn) [11]. Natural erosion, due to the laterite soils fragility and instability, especially under tropical climate conditions characterized by heavy rains [12], leads to mobilization of particles rich in metals from soils to freshwater [13-16]. Anthropic activities that destroy vegetation and reshape the soils like mining, increase this process by increasing soil leaching and exposing metals rich deep horizons. Runoff water is the main pathway of particles dispersion from soils to freshwater. Atmospheric dust issued from this soils can also contribute to a lesser extend to freshwater contamination. The dust concentration of Ni and Cr in Noumea are particularly elevated in comparison with other countries [17]. These particles finally end up by deposition or leaching in aquatic environment, and may affect living organisms in freshwaters downstream the mines.

In rivers under mining influence, high Ni concentrations are reported, around 96 µg/L, compared to those away from mining on the same type of lateritic soil, around 7 µg/L [15], confirming the high-water pollution level in this country. Indeed, these values are largely above the European regulation recommendations (< 4 µg/L). The increased use of Ni in industry may contribute to increase anthropic and natural emission of particles and, consequently, increase the risk of fish contamination. Some authors have demonstrated natural ecosystem and lagoon degradations due to Ni exploitations in New Caledonia [16, 18, 19] and recent studies have revealed that the contamination of aquatic organisms is directly linked to the enrichment of soils by Ni [20]. A significant bioaccumulation of Ni in gills and livers of five species (carp *Khulia rupestris*, white and black lochon *Awaous guamensis* and *Sicyopterus lagocephalus,* tilapia *Sarotherodon occidentalis* and marbled eel *Anguilla marmorata*) living in rivers under mining influence in the north of New Caledonia have been highlighted too (DMML project). Among these species, marbled eels, *Anguilla*

marmorata, are particularly interesting and not yet studied with regards to metals contamination in New Caledonia. Eels, as top predator, are good bio-indicators of water metal contamination [21-23] as they accumulate metals previously consumed by other species. Furthermore, with a complex biological life cycle, characterized by different stages of metamorphoses as previously described by [24], eels are particularly sensitive to water pollution. Indeed, during their juvenile growth stage, eels, living in freshwaters during several years, must accumulate energy reserves before starting their migration to reproduce themselves in open sea, tens of thousands kilometers away. During this migration, eels swim for several months, stop to feed and mature their gonads. Therefore, the quality of life in freshwaters is essential to ensure the success of migration and reproduction [25, 26] and prevent the marbled eel decline.

It is well known that other eel's species, like European eels, see their populations decrease due to metal contamination for instance by mercury [27]. This decline could be explained by morphometric alteration and metabolic parameters dysfunction especially due to oxidative stress, mitochondria disruption and carcinogenic factors activation [28-30].

Regarding the metals accumulated in eel's organs, previous studies suggested that metal concentration in organs can be used as a first proxy to assess metal toxicity, and the physiological consequences in each organ. Liver, kidneys and gills seems to be the main metals accumulating organs in European eels. It seems essential to know these parameters on marbled eels to clarify the consequences of water contamination in New Caledonia. Furthermore, determining if Ni and other associated metals accumulate in marbled eels muscles is essential to estimate the risk for human's consumption [31]. In New Caledonia, marbled eels living downstream mining sites seem to accumulate important Ni and other associated metals in their organs, but the consequences of this accumulation are unknown yet. The aim of this study was thus to determine the consequences of Ni and associated metals contamination in *Anguilla marmorata* by studying: (i) morphometric parameters; (ii) metal concentrations in liver, gills, kidneys, muscle and hearts; and (iii) transcription level of genes encoding proteins involved in lipid metabolism, oxidative stress, detoxification, cell cycle regulation, DNA repair and immunity in six different organs or tissues: liver, gills, kidneys, muscle, brain and spleen.

2. Materials and methods

2.1. Animals and biometric measurements

Twenty-one immature yellow marbled eels, *Anguilla marmorata*, were captured by electric fishing in May 2016 and July 2021 near the Koniambo Mount of New Caledonia in four different sites (with 4 to 6 individuals per site). Three sites were used for their high metal concentration levels due to the soil

composition (ultramafic soils) and the proximity of an open pit mine (Koniambo Nickel SAS, KNS) operating since 1997 along the Taléa river: left Taléa (LT) directly located downstream the mine; right Taléa (RT) not influenced by the mine; and downstream Taléa (DT), after the confluence between the two arms of the Taléa river (Fig. 1). One site was used as a reference located on a volcano-sedimentary soil, the downstream of the Tivoli river (Tiv). These sites are located on an intertropical climate zone, with an average of 1071.5 mm of water each year (MétéoNC), leading to important soil erosion and leaching in freshwaters.

Anguilla marmorata total body lengths and weights were first recorded to determine the Fulton condition factor [weight of eel (g) / (total length of the fish)^3 (cm^3)]. Eels were then euthanized and kidneys, brain, muscle, liver, gills, heart and spleen were recovered for further analyses. The total weight of liver was measured to calculate the hepato-somatic index (weight of the liver / total weight of the fish x 100). Samples for RT-qPCR analyses were immediately fixed in RNA later before to be stored at -20°C until analyses. Samples for metal analyses were stored as soon as possible at -20°C until analyses.

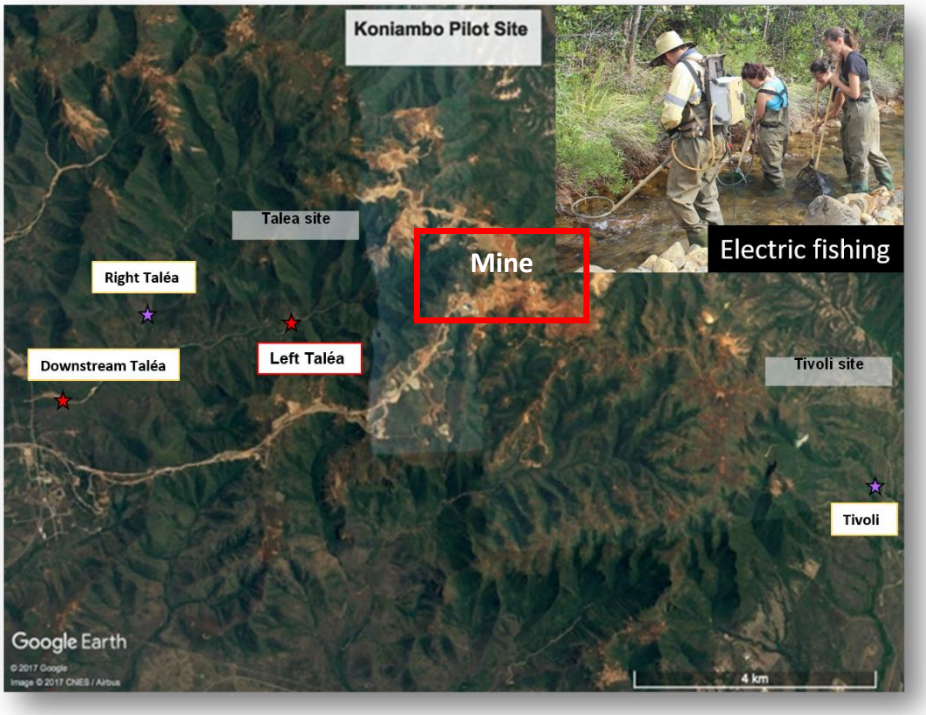


Figure 2 : Map of fishing sites, Koniambo Pilot Site, New-Caledonia

2.2. Metal concentration determination

Biological samples: kidneys, muscles, gills, liver and heart, were oven dried (72 h at 50 °C), grounded into a finely homogenized powder using an agate mortar, weighed and then digested with 1.5 mL of nitric acid 20 % at 200°C for 15 min (Microwave Acid Digestion - MARS 6, CEM, “Fish tissue” method).

After the mineralization, 1.5 mL of ultra-pure water (Milli-Q,Bedford, MA, USA) was added to each sample. Six metals were analyzed simultaneously by Inductively Coupled Plasma Optical Emission Spectrometer (700 Series ICP-OES, Agilent): Ni, Co, Cu, Cr and Mn. Certified reference materials DOLT-5 (dogfish (*Squalus acanthias* liver), TORT-3 (Lobster Hepatopancreas), and IAEA-413 (Algae) were analyzed using the same methodology as the eel samples. Their recovery rates, limit of detection and quantification are presented in table 1. Metal concentrations are expressed in $mg.kg^{-1}$. For values below the instrument detection limit, theoretical minimum concentration values were calculated (the detection limit of the instrument (in $mg.kg^{-1}$) multiplied by the volume of the sample (in L) divided by the sample weight (in g)).

Table 1 : Limit of detection and quantification with the recovery rates obtained from the analyses of certified reference materials (TORT-3, DOLT-5 and IAEA-413).

	Ni	Cr	Co	Mn
Limit of Detection (µg/L ± SD)	1.91 ± 0.70	0.46 ± 0.09	0.97 ± 0.31	0.045 ± 0.018
Limit of Quantification at 10 % (µg/L ± SD)	9.55 ± 3.30	2.32 ± 0.46	4.89 ± 1.53	0.23 ± 0.09
DOLT-5 recovery rate (% SD)				81.5 ± 3.10
TORT-3 recovery rate (% SD)	73.1 ± 3.30			78.5 ± 3.80
IAEA-413 recovery rate (% SD)	91.1 ± 1.30	87.9 ± 0.90	100.3 ± 5.10	87.4 ± 0.80

2.3. Gene transcription

Quantitative reverse transcription PCR (RT-qPCR) was used to determine mRNA transcription levels in fish sampled in 2021. In order to obtain the coding sequences of target genes, cDNA sequences from the assembled transcriptome of *A. anguilla* [32, 33] were aligned with the assembled genome of *A. marmorata* (GCA_901111315.1) using the Blast program. Sequences with high homologies were then used to design specific primers for *A. marmorata* by means of the primer3plus software (Table 1; [34]). Total mRNA extraction was performed from liver, kidneys, gills, brain, spleen and muscles samples using a commercially available NucleoZOL (RNA isolation, nucleoZOL, Marcherey-nagel®, USA), according to the manufacturer's recommendations. Reverse transcription was done by M-MLV (Moloney Murine Leukemia Virus) Reverse Transcriptase kit (Promega). Finally, PCR reactions were performed using a LC480 (Roche), 95°C for 2 min, followed by 45 cycles of 95°C for 15 sec and 60°C for 1 min. Each 15 µl reaction contained 7.5 µl GoTaq qPCR Master mix (Promega), 5 µl template and the specific primer pairs at a final concentration of 200 nM each. The reaction specificity was determined for each reaction from the dissociation curve of the PCR product. The ribosomal protein L7 (*rpl7*) and ß-actin (*ß-act*) genes were used as reference genes. Relative quantification of each gene expression was normalized according to the reference genes and generated using the $2^{-\Delta\Delta Ct}$ method described by [35]. Amplification efficiencies for all primer sets were calculated; all values

proved to be sufficient to allow direct comparison of amplification plots according to the $2^{-\Delta\Delta Ct}$ method.

Table 2: Specific primer pairs used for RT-qPCR

Gene name	Gene code	Function	Forward primer	Reverse primer	Efficiency (mean ± SEM)
ribosomal protein l7	*rpl7*	Ribosomal components (reference gene)	5′-GCATCTGGGCAATTACCATT -3′	5′-CAAGGAACTGGCTACCAAGC -3′	98.2 ± 8 %
ß-actin	*ß-actin*	Cell structure (reference gene)	5′-CTCTATCGTCCACCGCAAAT -3′	5′- CACCTTCACCGTTTTCCAGT-3′	93.4 ± 5 %
clathrin b	*cltb*	Internalization	5′-GCAGAAGTCCCATAGGTCCA -3′	5′- AACACCGGAAGCGTAATGTC-3′	91.2 ± 8 %
superoxide dismutase zinc/copper	$sod_{zn/cu}$	Oxidative stress (copper/zinc)	5′-TCAAGGACAGAATGCTCACG -3′	5′-TGCTTTTGGGACACCCTTAC -3′	90.8 ± 5 %
superoxide dismutase manganese	sod_{mn}	Oxidative stress (manganese)	5′- CGCCACATATGTCAACAACC-3′	5'- TAGGGGACAGGTTTGTCCAG-3'	106.2 ± 8 %
metallothioneine	*mt*	Metal homeostasis detoxification	5′-CGAGCTGCTGTCAGTGAAGA -3′	5′- GCTCTGCATGGATGACAAAA-3′	88.7 ± 4 %
ribosomal rna 12s	*12S*	Mitochondrial metabolism	5′-CTCACCATCCCTTGCCTAAA -3′	5′- CTCAGAGCCGGTTTCAAAAG-3′	91.7 ± 1 %
Cytochrome c oxidase subunit 1	*cox1*	Mitochondrial metabolism	5′-CCAGATGCATACACCCTGTG -3′	5′-CGAATGTGTGGTATGGTGGA -3′	91.3 ± 3 %
atp synthase membrane subunit 6-8	*atp6-8*	Mitochondrial metabolism	5′- TGCAGTCCCAATATGACTCG-3′	5′- ATGGCCTGCTGTTAGGTTTG-3′	90 ± 4 %
acetyl-coa carboxylase	*acc*	Lipid metabolism	5′-GAGCAGTACAAGCCCGACAT -3′	5′-AGCATGGTCACCAGGATGTT -3′	97.4 ± 8 %
triglyceride lipase	*tgl*	Lipid metabolism	5′-GACGCTGTTGTTCCAGATGA -3′	5′- GGGAACACGTACGAAGCCTA-3′	95.8 ± 10 %
glyceraldehyde-3-phosphate dehydrogenase	*gapdh*	Lipid metabolism	5′-TCTTTTGGGGGATCAAACTG -3′	5′-TGGCCTCAAAGGAGTAGAGC -3′	99.3 ± 9 %
vitellogenin	*vtg*	precursor of egg yolk	5′- CAGCTGCTTCTCAGCACAAG-3′	5′- AGCCTGAAGAACTGGCAGAA-3′	91.7 ± 2 %
bcl-2–associated x protein	*bax*	Cell cycle arrest/apoptosis	5′- GCGACCCTAACCACAAGAAG-3′	5′- GTCAAGCTCGTCTCCGATTT-3′	91.2 ± 11 %
tumor protein 53	*p53*	Cell cycle arrest/apoptosis	5′-GAGCCCAAGAAAGGGAAAAG -3′	5′- GACATGGAACTGACCCCAAC-3′	98.9 ± 9 %
growth arrest and dna damage-inducible 45	*gadd45*	DNA repair	5′- CCGTTGTTGTCCACTGTGTC-3′	5′-GATGAGGAAGACGAGGACGA -3′	98.4 ± 12 %
nlr family card domain containing 3	*nlrc3*	Immune system	5′- CCTCCAGAGTCCGTGTTAGC-3′	5′- TGCGCTTTCTGTCTGGTATG-3′	98.7 ± 13 %

2.4. Statistical analysis

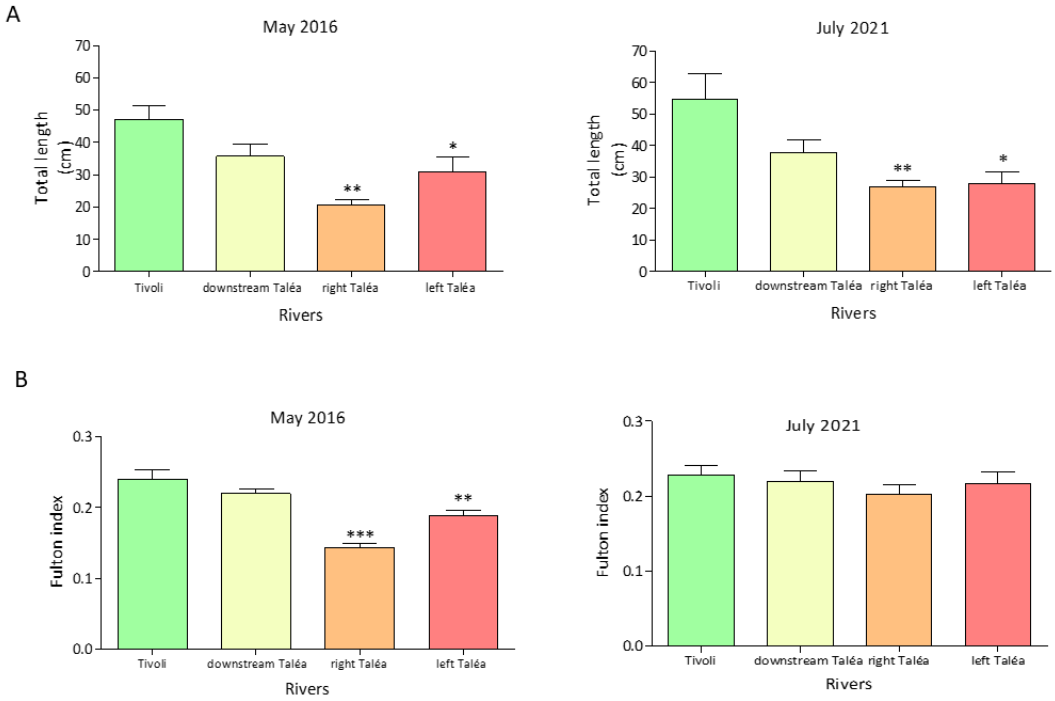
Data are expressed as mean ± Standard Error of the Mean (SEM) for n independent samples with five and four sampled eels in Left Taléa in 2016 and 2021 respectively, three in 2016 and five in 2021 in Right Taléa, five in 2016 and six in 2021 in Downstream Taléa and five in Tivoli in 2016 and 2021.

Statistical analyses were performed using analysis of variance: one-way ANOVA followed by Tukey's post-hoc test for multiple comparisons, and t-test to compare each sites to reference site, Tivoli (*P < 0.05, **P < 0.01 and ***P < 0.001) with GraphPad statistics software [36]. When assumptions necessary for using a parametric test were not met (normality, independence and homoscedasticity of the error term), we used non-parametric test. P values < 0.05 were considered statistically significant. Correlations between measured parameters on livers were revealed by principal component analysis using Statistica 13.3 software.

3. Results

3.1. Morphometric parameters

The analyses of *A. marmorata* morphometric parameters showed that eels living in the rivers downstream the mines are significantly smaller (**P < 0.01 and *P < 0.05 respectively for RT and LT rivers) than eels from the reference site (Tiv), and this for the two periods (Fig.2A). Furthermore, eels taken from the same rivers had approximately the same size in 2016 and 2021, for example in LT they measured 31.02 ± 4.58 cm in 2016 and 27.75 ± 3.1 cm in 2021. The Fulton condition index did not vary among sites in July 2021, but in May 2016, eels living in Taléa rivers had a lower Fulton index as compared to those from Tivoli (**P < 0.01). However, the hepato-somatic index varied in the same way for the two periods, with a significant lower value for eels living in Taléa rivers in 2016 (**P < 0.01) and 2021 (*P < 0.05) compared to those collected in Tivoli (Fig. 3).



187 **Figure 2:** A. Total length (cm, mean ± SEM) of eels in May 2016 (n = 4-6) and July 2021 (n = 5). B. Fulton
188 condition index (mean ± SEM) of eels in May 2016 (n = 4-6) and July 2021 (n = 5). Means designated with star
189 (*) are significantly different as compared to the reference Tivoli site (one-way analysis ANOVA followed by
190 Tukey's post-test, * P < 0.05; ** P < 0.01).

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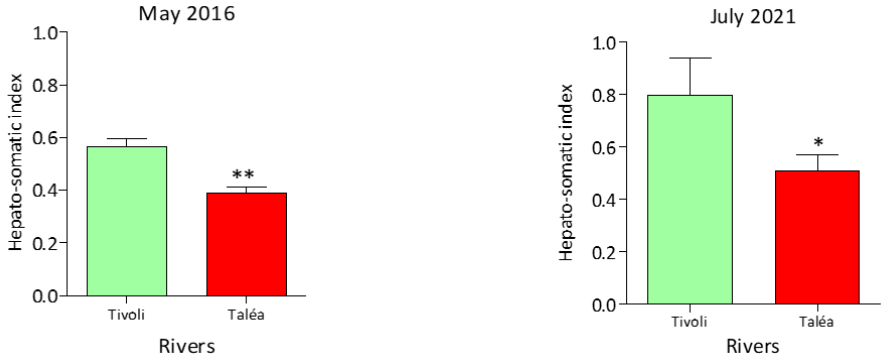

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193 **Figure 3:** Hepato-somatic index (mean ± SEM) of eels sampled in May 2016 (n = 5-13) and July 2021 (n = 5-9).
194 Means designated with star (* or **) are significantly different as compared to the reference Tivoli site (t-test,
195 * P < 0.05; ** P < 0.01).

196 ### 3.2. Metals bioaccumulation in fish organs

197 Metals concentrations were determined for Ni, Cr, Co and Mn in liver, gills and muscle at both
198 periods, 2016 and 2021. Kidneys and hearts were only analyzed in 2021.

For Ni, the results showed hepatic concentrations significantly higher for eels living in Taléa river, particularly in LT, than those living in Tivoli, either in 2016 or 2021 (Fig. 4). For muscle in 2016 and 2021, and for kidneys and hearts in 2021, the same patterns were observed, with highest concentrations measured in eels from LT. The same tendencies were thus observed on both periods except for gills, for which Ni concentrations were largely higher in 2016 than in 2021 (28.34 mg/kg and only 2.58 mg/kg in LT respectively for 2016 and 2021).

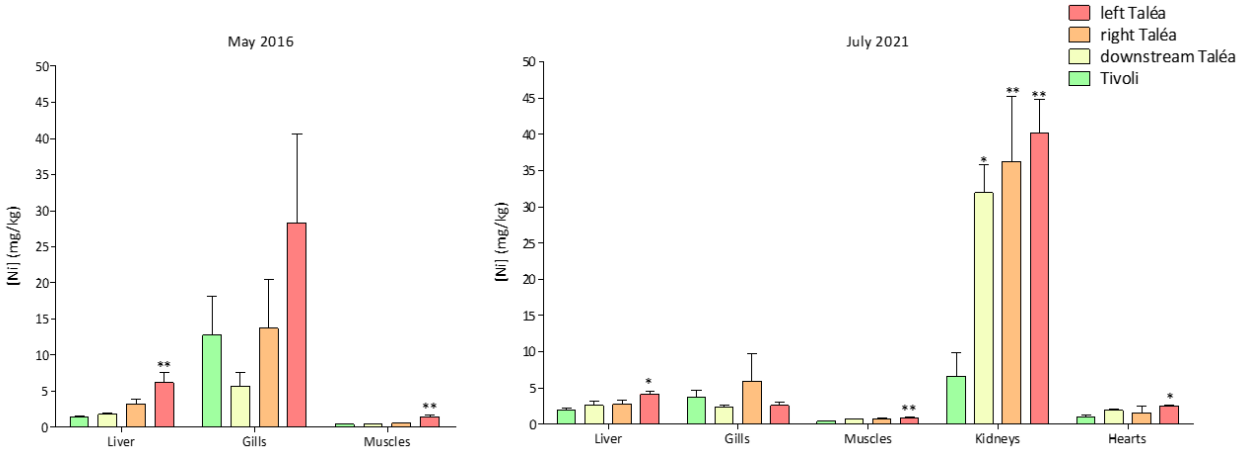

Figure 4: Ni concentrations (mg/kg) in liver, gills, muscles, kidneys and hearts of eels sampled in Koniambo Mount in New Caledonia (mean ± SEM, n = 3-5 in 2016 and n = 4-6 in 2021). Means designated with star (*) are significantly different as compared to the reference Tivoli site (one-way analysis ANOVA followed by Tukey's post-test, * $P < 0.05$; ** $P < 0.01$).

Eels collected from Taléa rivers accumulated also more Cr in their liver than eels living in Tivoli ($p = 0.003$ in 2016 and $p = 0.024$ in 2021) for the two periods of sampling (Fig. 5). Gills and muscle in 2016 bioaccumulated Cr to a greater extent in LT and kidneys in 2021 in all Taléa sites compared to Tiv.

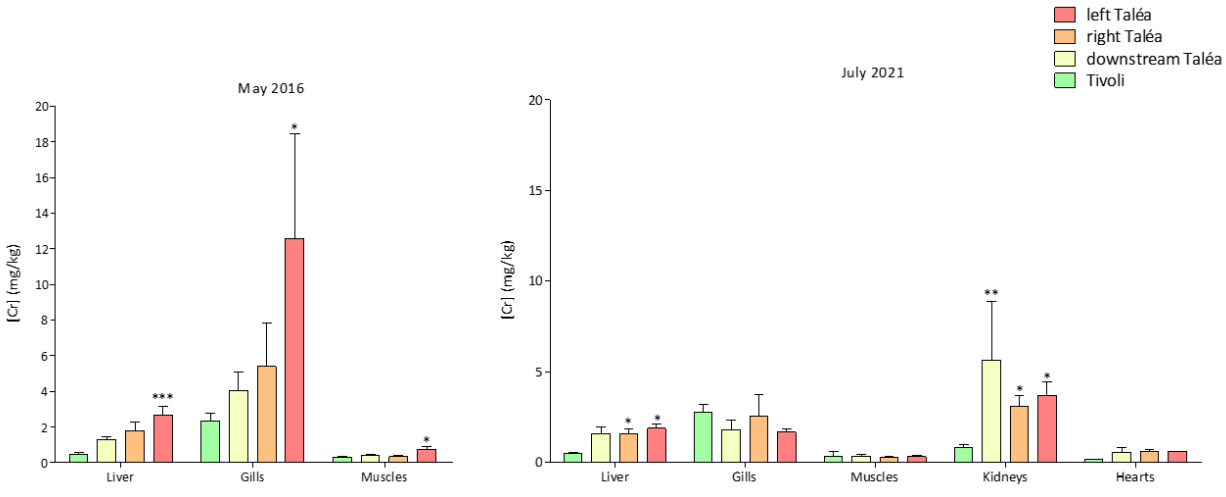

Figure 5: Cr concentrations (mg/kg) in liver, gills, muscle, kidneys and heart of eels sampled in Koniambo Mount in New Caledonia (mean ± SEM, n = 3-5 in 2016 and n = 4-6 in 2021). Means designated with star (*) are significantly different as compared to the reference Tivoli site (one-way analysis ANOVA followed by Tukey's post-test, * P < 0.05; ** P < 0.01).

For Co, results showed concentrations significantly higher for eels living in Taléa rivers than those living in Tivoli for liver in 2016 and 2021 and for kidneys and heart in 2021 (Fig. 6). In 2016, the Co concentrations in muscle were higher in Taléa compared to Tivoli (p = 0.0034). For gills, only the concentrations measured in DT were significantly higher than the other sites in 2016, but in 2021, also those from RT bioaccumulated more Co than eels from Tiv.

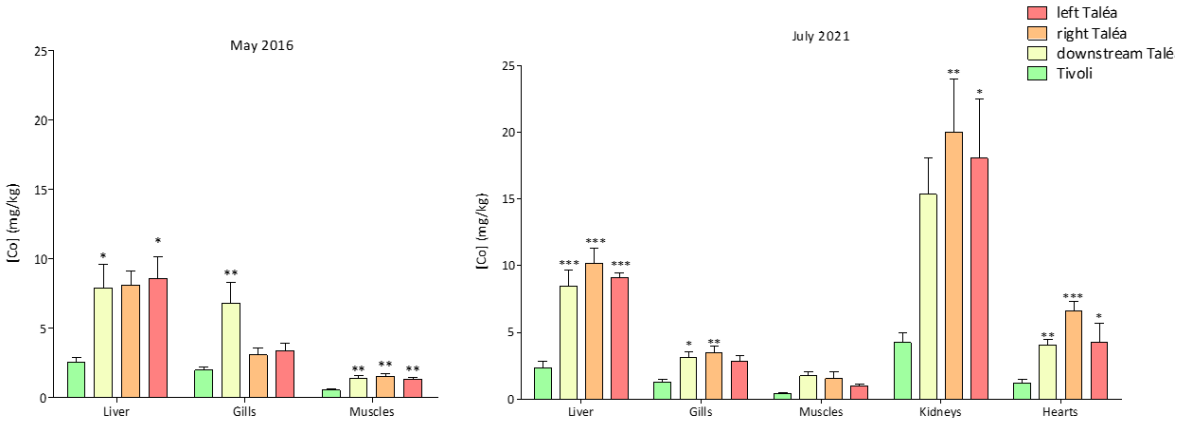

Figure 6: Co concentrations (mg/kg) in liver, gills, muscle, kidneys and heart of eels sampled in Koniambo Mount in New Caledonia (mean ± SEM, n = 3-5 in 2016 and n = 4-6 in 2021). Means designated with star (*) are significantly different as compared to reference site Tivoli (one-way analysis ANOVA followed by Tukey's post-test, * P < 0.05; ** P < 0.01).

For Mn in 2016, no significant difference in concentrations was observed among sites, but in 2021 for heart and gills, Mn concentrations were higher in LT or RT respectively as compared to Tiv (p = 0.02 and p = 0.05 respectively) (Fig. 7).

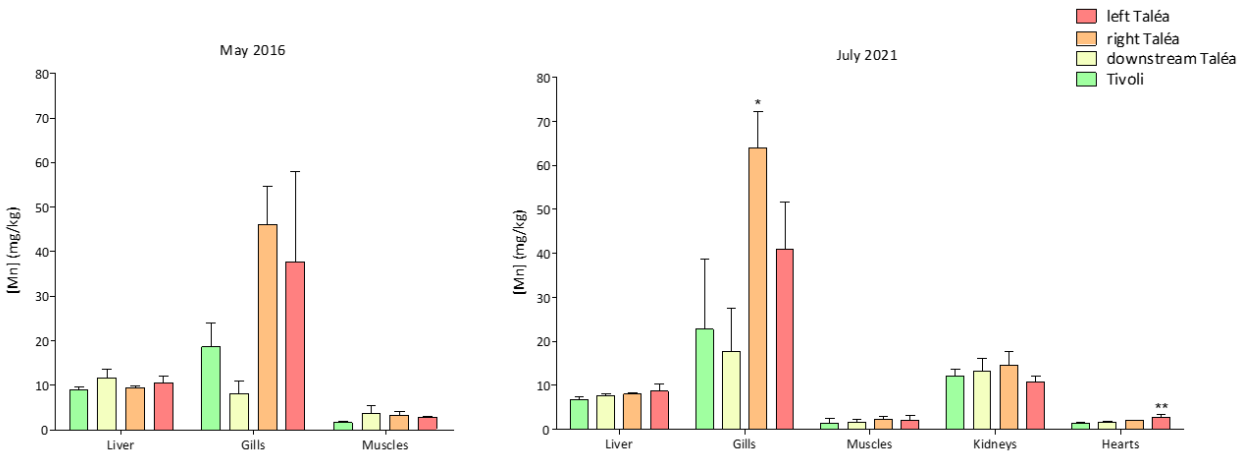

Figure 7: Mn concentrations (mg/kg) in liver, gills, muscle, kidneys and heart of eels sampled in Koniambo Mount in New Caledonia (mean ± SEM, n = 3-5 in 2016 and n = 4-6 in 2021). Means designated with star (*) are significantly different as compared to the reference Tivoli site (one-way analysis ANOVA followed by Tukey's post-test, * $P < 0.05$; ** $P < 0.01$).

We can observe that the metal that is the most concentrated in the organs is Mn, then Ni. For Cr and Co the concentrations are of the same order of magnitude in the organs, whereas the concentration of Cr in the freshwaters is always higher than that of Co.

3.3. Gene transcription levels

To assess the impact of Ni and other associated metals from mining activity in organs of eels, RT-qPCR analyses were carried out on six organs: liver, kidneys, gills, brain, spleen and muscle from eels sampled in 2021. Fifteen genes were analyzed to explore the internalization (*cltb*); the oxidative stress ($sod_{zn/cu}$ sod_{mn}); the metal detoxification (*mt*); the mitochondrial metabolism (*12s*; *cox1* and *atp6-8*); the lipid metabolism (*acc*; *tgl* and *gapdh*); the reproductive function (*vtg*); the apoptosis (*p53* and *bax*); the DNA repair (*gadd45*) and the immune system (*nlrc3*). The results showed that liver was the most impacted organ, as the transcription level of almost 12 genes on the 16 studied was differentially regulated in eels from one or several Taléa sites compared to Tivoli (up-regulation for oxidative stress and lipid metabolism and down-regulation for mitochondrial metabolism, DNA repair, apoptosis, reproduction and immunity) (Table 3).

Table 3: Fold changes in gene transcription levels in eels collected from Taléa river sites (n = 4-6 per site) as compared to eels from the reference Tivoli site. Only significant changes are reported (one-way analysis ANOVA followed by Tukey's post-test, * P < 0.05; ** P < 0.01 as compared to Tivoli) (*e.g*. red = down regulation and green = up regulation).

Ultramafics rivers	Organs	Genes and function															
		Oxidative stress			Metal detoxification	Mitochondrial metabolism			Lipid metabolism			DNA repair	Apoptosis		Endocytosis	Reproduction	Immunity
		sod_{cu}	*sod_{mn}*	*cat*	*mt*	*12S*	*cox*	*atp6-8*	*gapdh*	*tgl*	*AcCoa*	*gadd45*	*p53*	*bax*	*cltb*	*Vtg*	*nlrc3*
Taléa downstream	Liver	982.0* ± 243.5	14.27* ± 4.11	/	/	/	/		16.85* ± 3.55	/	/	/	0.33* ± 0.22	/	/	0.34* ± 0.2	0.2* ± 0.15
Taléa right		1189* ± 752.5	21.98* ± 8.62	/	/	0.20** ± 0.06	/	0.14** ± 0.053	24.4* ± 6.22	/	0.096* ± 0.03	0.34* ± 0.14	0.10* ± 0.045	0.15* ± 0.044	/	0.093* ± 0.04	0.052* ± 0.03
Taléa left		1636* ± 513.6	20.95* ± 4.1	/	/	0.18* ± 0.045	/	0.22* ± 0.06	36.38* ± 11.4	62.02* ± 42.4	0.046* ± 0.016	0.14* ± 0.03	0.078* ± 0.035	0.13* ± 0.032	/	0.074* ± 0.052	0.03* ± 0.018
Taléa downstream	Kidney	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
Taléa right		/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
Taléa left		/	/	/	/	/	/	/	/	3.03* ± 0.97	3.32* ± 0.9	3.92* ± 1.72	/	2.59* ± 0.68	6.29* ± 0.85	/	2.06* ± 0.46
Taléa downstream	Gills	/	/	/	/	/	/	/	/	/	/	/	/	0.32* ± 0.13	/	/	/
Taléa right		/	/	/	/	/	/	/	/	/	/	/	/	0.45* ± 0.12	/	/	/
Taléa left		24.15* ± 4.89	9.97* ± 1.46	/	3.35* ± 1.43	/	/	/	/	/	/	/	0.36* ± 0.07	0.29* ± 0.035	/	/	/
Taléa downstream	Brain	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
Taléa right		/	/	/	/	/	/	/	/	/	/	3.8* ± 1.12	/	/	/	/	/
Taléa left		/	/	/	/	/	/	/	/	/	/	5.27* ± 1.3	/	/	/	/	/
Taléa downstream	Spleen	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
Taléa right		/	/	/	/	/	/	0.36* ± 0.12	/	/	/	/	/	/	/	/	/
Taléa left		/	/	/	/	/	/	0.52* ± 0.09	/	/	/	/	/	/	/	/	/

250

The kidneys showed only up-regulated genes involved in lipid metabolism, DNA repair, apoptosis, endocytosis and immunity.

The gills were on the one hand up-regulated for genes involved in oxidative stress and metal detoxification and on the other hand down-regulated for genes involved in apoptosis.

Finally, brain and spleen are the less affected organs with only one gene that was differentially regulated (down-regulation of *atp6-8* for spleen and up-regulation of *gadd45* for brain).

No difference in gene transcription level was observed in muscles.

3.4. Multivariate analysis on the liver

As the liver appeared as the most impacted organ in eels living on ultramafic soils impacted by mining activities, a Principal Component Analysis (PCA) was done between the several studied parameters - morphometric parameters, metals bioaccumulation and transcriptomic responses (Fig. 8). The PCA explained a total of 97.48% of the variability of the data, with the axis 1 the more discriminant (89.23%) and corresponding to the most contaminated and disruptive stations on the right (especially Left Taléa) and the less contaminated one on the left (Tivoli). Significant correlations are observed between Ni, Mn, Cr and to a lesser extent Co with genes implicated in oxidative stress and lipid metabolism on the most contaminated site (LT). On the contrary, morphological parameters are correlated to genes implied in reproduction, in mitochondrial metabolism, in immunity, in cell cycle regulation and correspond to the reference site Tiv.

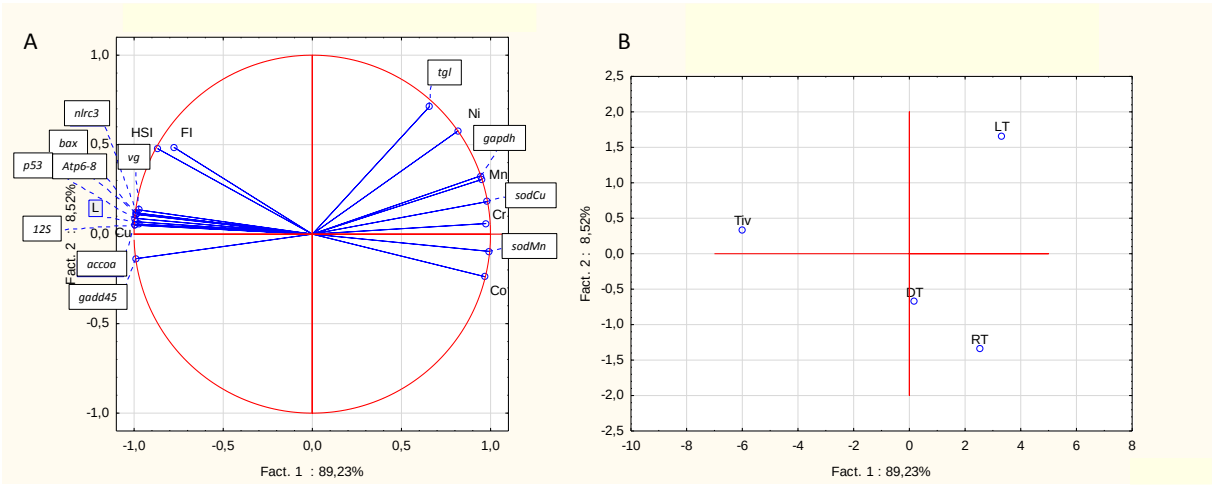

Figure 8: Principal component analysis of morphometric parameters, metals bioaccumulation and gene transcription levels in liver of *A. marmorata*, after collection in July 2021 on the 4 sites: Tiv (Tivoli), LT (Left Taléa), RT (Right Taléa) and DT (Downstream Taléa). A. Correlation circle of the different studied parameters. B. Illustration of the sites.

4. Discussion

This study aimed to assess the consequences for *Anguilla marmorata* to live in freshwaters downstream Ni mines in New Caledonia, region particularly affected by mining activities due to the composition of soils particularly rich in Ni. Eels are known to be sensitive to water metal contamination [23] that could affect their migration and reproduction leading to a decline of their populations [29, 30]. Until now, no study was conducted in New Caledonia on this species, whereas eels are present in freshwaters as top fish predators and are potentially collected by local populations to be eaten. Thus in this project, we collected eels in four sites (three on ultramafic soils along the Taléa river, with one arm directly under mining influence, and one as a reference site, Tivoli river) to assess the bioaccumulation of metals and their toxicity. The fish were sampled at two different periods, in May 2016 and July 2021, in order to assess the influence of time on parameters of interest and to verify whether the observed effects could be maintained over time.

4.1. Morphometric parameters

First of all, we noticed that eels living in rivers downstream the mines were smaller than eels living in the reference site, with a lower hepato-somatic index at both different periods, as it has been already observed in eels exposed to other metals such as Hg and Cd [22]. These results seemed to indicate that eels, in particular their liver function, are probably impacted by physico-chemical or nutrient conditions occurring in Taléa river compared to Tivoli, whatever the period and season. Interestingly, the Fulton index differed between the two periods. Indeed, no differences were observed in 2021, whereas, in 2016, eels living in Taléa presented a smaller Fulton index compared to Tivoli. In 2021, the sampling campaign was done after a long episode of rain which could explain at least in part the low dissolved metal concentration in the rivers. For example, the concentrations of dissolved Ni was 48 µg/L in 2016 compared to 8 µg/L few weeks before the fisheries in 2021. Indeed, in 2016 the cumulative rainfall in the 6 months preceding the fisheries (December 2015 to May 2016) was 930 mm of rain (an average of 5.2 mm of rain per day) but 2910 mm of rain (an average of 16.2 mm of rain per day) in 2021 (February 2021 to July 2021) (MeteoNC, 2016 ; MeteoNC, 2021). The higher dissolved metal levels in 2016, could further affect the food chain and eel nutrient quality, but also eels themselves. As the Fulton index reflects the physiological state of the fish [37], it is possible that in 2016, rivers under mining influence offered poor condition for growth and that eels inhabiting these sites were more impacted by metals, and therefore, had a lower condition index.

4.2. Metals bioaccumulation

Due to the composition of laterite soils [12, 14], four metals were analyzed in eel organs: Ni, Cr, Co and Mn in 2016 and 2021. This kind of research has never been conducted in New Caledonia on *Anguilla marmorata*. At first, for all metals in 2016 and 2021, a dose-dependent contamination was observed according to the sites under mining influence or not, with eels from Taléa being significantly more contaminated than those from Tivoli. Those results are in line with the metal concentrations in the freshwater, which are much higher in Taléa river compared to Tivoli river.

The same trends were noticed in 2016 and 2021 but some variations especially for metal concentrations in livers and gills were nevertheless observed. In fact, for Ni and Cr, in 2016, eels appeared to be mainly contaminated by the direct route of exposure (*i.e*. dissolved metals in water) whereas in 2021 the main route of contamination of eels in Taléa seemed to be the trophic route. This was highlighted by higher metal concentrations in the gills compared to the livers for all sites in 2016 in contrast to 2021 where Ni and Cr concentrations in gills were lower than in livers. Indeed, metals bioaccumulation in the gills is expected to be higher following waterborne exposure, while higher bioaccumulation levels in the liver is followed by absorption of metals from the gut [38]. It seems that few dissolved metals were assimilated by the gills in 2021, that could also be explained by the moment of the fisheries, which were realized after heavy rain episodes in 2021. As already evoked, these hydrologic conditions in 2021 would have probably diluted the metals in the freshwaters by high leaching of soils during several months before sampling and modified their chemical speciation, with a highest proportion of them adsorbed to particulate matter in 2021 compared to 2016 [39, 40]. All these findings could suggest that the contamination of eels is dependent on the weather conditions and the fishing season. However, the livers are in all cases impacted by higher concentrations of metals measured in eels living under mining influence compared to Tivoli.

In contrast, for Co and Mn the same trends were observed for the two seasons, with contamination mainly via the trophic way for Co and by the direct route for Mn. In 2016, the eels seemed to less accumulate Mn than in 2021. Furthermore, in 2021, two other organs were analyzed: heart and kidneys. Kidneys are the organs that accumulate the highest levels of metals, as they represent the main site of detoxification and excretion [41, 42]. Moreover, numerous studies have shown that for the majority of metals, the highest concentrations in fish are found in the liver and kidneys [43-45]. These observations are also reported in our study because for Ni, Co and Cr, kidneys are the organs that bioaccumulated the most. On average, metal concentrations in the kidneys of Taléa eels were 12 to 16 times higher than those in Tivoli and more important than in gills, confirming the hypothesis of trophic way of contamination. In order to ensure a good migration, eels need an optimal heart capacity, thus, any cardiotoxicity could affect their migration [46, 47]. In 2021, we observed that

hearts were also affected, to a lesser extent because only eels of the LT site had significantly higher Ni and Mn concentrations than eels living in the reference site, except for Co, for which all eels from Taléa were impacted. Finally, concerning muscle, the accumulations remain very low compared to the other organs, with generally higher values on the most impacted site (LT). These results are in line with the literature which demonstrated that metals, including Ni, accumulates poorly in the fish muscle [48]. Also in muscle, the concentrations are under the European recommendation threshold for food, so the consumption for humans of *Anguilla marmorata* in New Caledonia seems totally safe, at least for the metals analyzed in the present study.

4.3. Genes transcription

A clear link has been established between metals bioaccumulation in fish organs and alterations in gene transcription levels, especially those involved in the fight against oxidative stress [49]. Oxidative stress is known to play a key role in the physiopathology of organs, notably liver, by altering the calcium homeostasis and the mitochondrial function of hepatocytes [50, 51]. The qPCR analysis was conducted only on the 2021 samples. The results suggest that livers and gills could be submitted to an important oxidative stress (sod_{Cu} and sod_{Mn} inductions), biomarkers reflecting pollution of freshwater ecosystems [52]. Oxidative stress could then be responsible of the mitochondrial dysfunction observed with an alteration of the mitochondrial respiratory chain (MRC) in liver and spleen (*12S* and *atp6-8* depletions). These results are highlighted by the PCA analysis conducted on the liver (Fig. 8), which demonstrated a clear link between oxidative stress, lipid metabolism and bioaccumulation of Ni, Mn, Cr and to a lesser extent Co. In New Caledonia, just one study noted an alteration of mitochondrial metabolism on goldfish after Ni exposition [53], such as in Pacific eels in the present study. These effects were associated to a dysregulation of lipid metabolism [54, 55], as can be illustrated in our study by an induction of *gapdh* and *tgl* in livers and kidneys, markers of an increased utilization of glucose and triglycerides, which has previously been demonstrated in the European eel *Anguilla anguilla* after Cd exposure [28]. This alteration could compromise the efficiency of lipid storage necessary for their migration, and then, their reproductive success. Furthermore, studies also suggested that triglyceride catabolism is a biomarker of oxidative stress in eels [56] and could explain the lower hepato-somatic index of eels living under mining influence [57]. In addition, we observed a decrease in the precursor of egg yolk, *vtg*, in the liver, which could be a consequence of the lipid metabolism disruption and the oxidative stress observed [58]. Reproductive impairment in eels living in polluted waters has already been reported in previous studies [26]. We have to note that despite our sampling efforts, we failed to obtain fish of similar sizes among sampling sites. This can be explained either by an effect of metals on fish growth rate or by variations in fish age among sites [59]. However, even if we cannot exclude an effect of age or sex, we observed

a significant link between metal concentrations in liver, hepato-somatic index and *vtg* transcription levels, which suggests that contamination has probably an impact on reproduction and/or sex determination [60, 61].

Another important fact is that eels living in contaminated freshwaters seem to have an important inflammation with *nlrc3* depletion in livers. On the contrary, an induction of *nlrc3* observed in kidneys indicate a decrease of pro-inflammatory response leading to a decrease of response if eels are in contact with virus, parasites or bacteria with potentially serious consequences about their health condition [62, 63].

Finally, the transcriptomic studies showed in liver and gills, a potential initiation of carcinogenic pathways by down-regulating the transcription levels of genes involved in apoptosis and DNA repair (*gadd45*, *p53* and *bax*). Indeed, the carcinogenic effects of metals in fish liver were already reported in *Solea vulgaris*, *Anguilla anguilla* and *Liza aurata* [64, 65]. In contrast, the apoptosis pathway seemed activated in kidneys (*bax* induction) with DNA repair, also reported in brain (*gadd45* induction). The induction of apoptosis can remove potentially harmful cells, thereby blocking tumor growth [66]. The kidneys may have activated this cell death process to protect against toxics effects of metal contamination. Muscle were less impacted than other organs, accordingly to bioaccumulation observations.

In New Caledonia, the mining activities seem then to alter the quality of life of eels living in the rivers downstream the mines, by organ function alteration, especially the liver. Due to the importance of liver function for eels, metallic contamination could contribute to hepatic dysfunctions leading to impairment of lipid metabolism and could then alter the success of their migration and maturation and, potentially their reproduction.

5. Conclusion

The current study shows that metal concentrations observed in freshwater downstream mining sites may induce alterations of *Anguilla marmorata* organs in New Caledonia. Disruption of Fulton and hepato-somatic index might be explained by the high metals levels found in kidneys and livers of eels. In addition, metals triggered an important liver, kidneys and gills toxicity through the induction of mitochondrial dysfunction, oxidative stress, inflammation, immunotoxicity and apoptosis or carcinogenic effects. Therefore, the exposition of *Anguilla marmorata* to metals such as Ni, Cr, Co and Mn in New Caledonia because of open pit mining activities on ultramafic basin catchments may put the fish at risk of developing impaired migration and reproduction.

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Statement of contributions

Germande Ophélie: Conceptualization, Formal Analysis, Methodology, Visualization, Writing – original draft

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Baudrimont Isabelle: Project administration, Conceptualization, Funding acquisition, Methodology, Supervision, Validation, Writing – review & editing

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