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# 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

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

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

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

68 *marmorata*, are particularly interesting and not yet studied with regards to metals contamination in  
69 New Caledonia. Eels, as top predator, are good bio-indicators of water metal contamination [21-23]  
70 as they accumulate metals previously consumed by other species. Furthermore, with a complex  
71 biological life cycle, characterized by different stages of metamorphoses as previously described by  
72 [24], eels are particularly sensitive to water pollution. Indeed, during their juvenile growth stage,  
73 eels, living in freshwaters during several years, must accumulate energy reserves before starting their  
74 migration to reproduce themselves in open sea, tens of thousands kilometers away. During this  
75 migration, eels swim for several months, stop to feed and mature their gonads. Therefore, the  
76 quality of life in freshwaters is essential to ensure the success of migration and reproduction [25, 26]  
77 and prevent the marbled eel decline.

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

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

## 96 **2. Materials and methods**

### 97 **2.1. Animals and biometric measurements**

98 Twenty-one immature yellow marbled eels, *Anguilla marmorata*, were captured by electric fishing in  
99 May 2016 and July 2021 near the Koniambo Mount of New Caledonia in four different sites (with 4 to  
100 6 individuals per site). Three sites were used for their high metal concentration levels due to the soil

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

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

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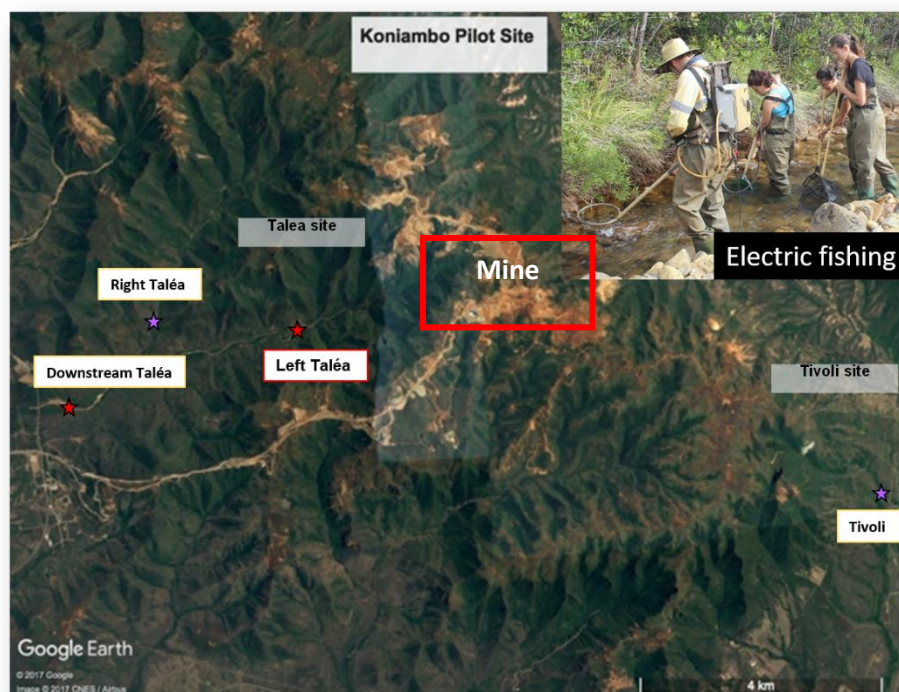
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**Figure 2** : Map of fishing sites, Koniambo Pilot Site, New-Caledonia

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## 2.2. Metal concentration determination

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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).

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

139 **Table 1** : Limit of detection and quantification with the recovery rates obtained from the analyses of certified  
 140 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   |

141

### 142 **2.3. Gene transcription**

143 Quantitative reverse transcription PCR (RT-qPCR) was used to determine mRNA transcription levels in  
 144 fish sampled in 2021. In order to obtain the coding sequences of target genes, cDNA sequences from  
 145 the assembled transcriptome of *A. anguilla* [32, 33] were aligned with the assembled genome of *A.*  
 146 *marmorata* (GCA\_901111315.1) using the Blast program. Sequences with high homologies were then  
 147 used to design specific primers for *A. marmorata* by means of the primer3plus software (Table 1;  
 148 [34] ). Total mRNA extraction was performed from liver, kidneys, gills, brain, spleen and muscles  
 149 samples using a commercially available NucleoZOL (RNA isolation, nucleozol, Marcherey-nagel®,  
 150 USA), according to the manufacturer's recommendations. Reverse transcription was done by M-MLV  
 151 (Moloney Murine Leukemia Virus) Reverse Transcriptase kit (Promega). Finally, PCR reactions were  
 152 performed using a LC480 (Roche), 95°C for 2 min, followed by 45 cycles of 95°C for 15 sec and 60°C  
 153 for 1 min. Each 15 µl reaction contained 7.5 µl GoTaq qPCR Master mix (Promega), 5 µl template and  
 154 the specific primer pairs at a final concentration of 200 nM each. The reaction specificity was  
 155 determined for each reaction from the dissociation curve of the PCR product. The ribosomal protein  
 156 L7 (*rpl7*) and β-actin (*β-act*) genes were used as reference genes. Relative quantification of each  
 157 gene expression was normalized according to the reference genes and generated using the 2<sup>-ΔΔCt</sup>  
 158 method described by [35]. Amplification efficiencies for all primer sets were calculated; all values

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

161 **Table 2:** Specific primer pairs used for RT-qPCR

| Gene name  | Gene code                       | Function                              | Forward primer              | Reverse primer             | Efficiency (mean $\pm$ SEM) |
|--|---------------------------------|---------------------------------------|-----------------------------|----------------------------|-----------------------------|
| <i>ribosomal protein l7</i>                      | <i>rpl7</i>                     | Ribosomal components (reference gene) | 5'-GCATCTGGGCAATTACCATT-3'  | 5'-CAAGGAACTGGCTACCAAGC-3' | 98.2 $\pm$ 8 %              |
| <i><math>\beta</math>-actin</i>                  | <i><math>\beta</math>-actin</i> | Cell structure (reference gene)       | 5'-CTCTATCGTCCACCGCAAAT-3'  | 5'-CACCTTCACCGTTTCCAGT-3'  | 93.4 $\pm$ 5 %              |
| <i>clathrin b</i>                                | <i>cltb</i>                     | Internalization                       | 5'-GCAGAAGTCCCATAGGTCCA-3'  | 5'-AACACCGAAGCGTAATGTC-3'  | 91.2 $\pm$ 8 %              |
| <i>superoxide dismutase zinc/copper</i>          | <i>sod<sub>zn/cu</sub></i>      | Oxidative stress (copper/zinc)        | 5'-TCAAGGACAGAATGCTCACG-3'  | 5'-TGCTTTGGGACACCTTAC-3'   | 90.8 $\pm$ 5 %              |
| <i>superoxide dismutase manganese</i>            | <i>sod<sub>mn</sub></i>         | Oxidative stress (manganese)          | 5'-CGCCACATATGTCAACAACC-3'  | 5'-TAGGGGACAGTTTGTCCAG-3'  | 106.2 $\pm$ 8 %             |
| <i>metallothioneine</i>                          | <i>mt</i>                       | Metal homeostasis detoxification      | 5'-CGAGCTGCTGTCAAGTGAAGA-3' | 5'-GCTCTGCATGGATGACAAAA-3' | 88.7 $\pm$ 4 %              |
| <i>ribosomal rna 12s</i>                         | <i>12S</i>                      | Mitochondrial metabolism              | 5'-CTCACCATCCCTGCCTAAA-3'   | 5'-CTCAGAGCCGGTTTCAAAG-3'  | 91.7 $\pm$ 1 %              |
| <i>Cytochrome c oxidase subunit 1</i>            | <i>cox1</i>                     | Mitochondrial metabolism              | 5'-CCAGATGCATACACCCTGTG-3'  | 5'-CGAATGTGTGGTATGGTGA-3'  | 91.3 $\pm$ 3 %              |
| <i>atp synthase membrane subunit 6-8</i>         | <i>atp6-8</i>                   | Mitochondrial metabolism              | 5'-TGCAGTCCCAATATGACTCG-3'  | 5'-ATGGCCTGCTGTAGTTTG-3'   | 90 $\pm$ 4 %                |
| <i>acetyl-coa carboxylase</i>                    | <i>acc</i>                      | Lipid metabolism                      | 5'-GAGCAGTACAAGCCCGACAT-3'  | 5'-AGCATGGTCACCAGGATGT-3'  | 97.4 $\pm$ 8 %              |
| <i>triglyceride lipase</i>                       | <i>tgl</i>                      | Lipid metabolism                      | 5'-GACGCTGTTGTTCCAGATGA-3'  | 5'-GGGAACACGTACGAAGCCTA-3' | 95.8 $\pm$ 10 %             |
| <i>glyceraldehyde-3-phosphate dehydrogenase</i>  | <i>gapdh</i>                    | Lipid metabolism                      | 5'-TCTTTTGGGGGATCAAAGT-3'   | 5'-TGGCCTCAAAGGAGTAGAGC-3' | 99.3 $\pm$ 9 %              |
| <i>vitellogenin</i>                              | <i>vtg</i>                      | precursor of egg yolk                 | 5'-CAGCTGCTTCTCAGCACAAG-3'  | 5'-AGCCTGAAGAACTGGCAGAA-3' | 91.7 $\pm$ 2 %              |
| <i>bcl-2-associated x protein</i>                | <i>bax</i>                      | Cell cycle arrest/apoptosis           | 5'-GCGACCCTAACCCACAAGAAG-3' | 5'-GTCAAGCTCGTCTCCGATTT-3' | 91.2 $\pm$ 11 %             |
| <i>tumor protein 53</i>                          | <i>p53</i>                      | Cell cycle arrest/apoptosis           | 5'-GAGCCCAAGAAAGGAAAAG-3'   | 5'-GACATGGAAGTACCCCAAC-3'  | 98.9 $\pm$ 9 %              |
| <i>growth arrest and dna damage-inducible 45</i> | <i>gadd45</i>                   | DNA repair                            | 5'-CCGTTGTTGTCCACTGTGTC-3'  | 5'-GATGAGGAAGACGAGGACGA-3' | 98.4 $\pm$ 12 %             |
| <i>nlr family card domain containing 3</i>       | <i>nlr3</i>                     | Immune system                         | 5'-CCTCCAGAGTCCGTGTTAGC-3'  | 5'-TGCCTTTCTGTCTGGTATG-3'  | 98.7 $\pm$ 13 %             |

162

## 163 2.4. Statistical analysis

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

167 Statistical analyses were performed using analysis of variance: one-way ANOVA followed by Tukey's  
168 post-hoc test for multiple comparisons, and t-test to compare each sites to reference site, Tivoli ( $*P <$   
169  $0.05$ ,  $**P < 0.01$  and  $***P < 0.001$ ) with GraphPad statistics software [36]. When assumptions  
170 necessary for using a parametric test were not met (normality, independence and homoscedasticity  
171 of the error term), we used non-parametric test.  $P$  values  $< 0.05$  were considered statistically  
172 significant. Correlations between measured parameters on livers were revealed by principal  
173 component analysis using Statistica 13.3 software.

### 174 **3. Results**

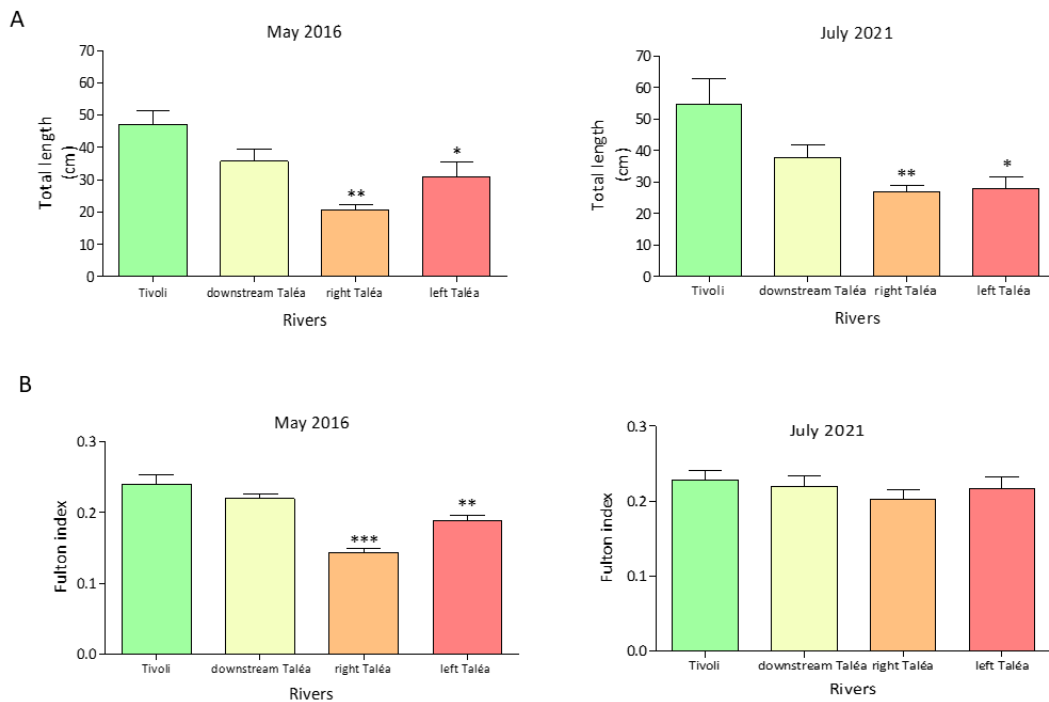
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#### 176 **3.1. Morphometric parameters**

177 The analyses of *A. marmorata* morphometric parameters showed that eels living in the rivers  
178 downstream the mines are significantly smaller ( $**P < 0.01$  and  $*P < 0.05$  respectively for RT and LT  
179 rivers) than eels from the reference site (Tiv), and this for the two periods (Fig.2A). Furthermore, eels  
180 taken from the same rivers had approximately the same size in 2016 and 2021, for example in LT  
181 they measured  $31.02 \pm 4.58$  cm in 2016 and  $27.75 \pm 3.1$  cm in 2021. The Fulton condition index did  
182 not vary among sites in July 2021, but in May 2016, eels living in Taléa rivers had a lower Fulton index  
183 as compared to those from Tivoli ( $**P < 0.01$ ). However, the hepato-somatic index varied in the same  
184 way for the two periods, with a significant lower value for eels living in Taléa rivers in 2016 ( $**P <$   
185  $0.01$ ) and 2021 ( $*P < 0.05$ ) compared to those collected in Tivoli (Fig. 3).

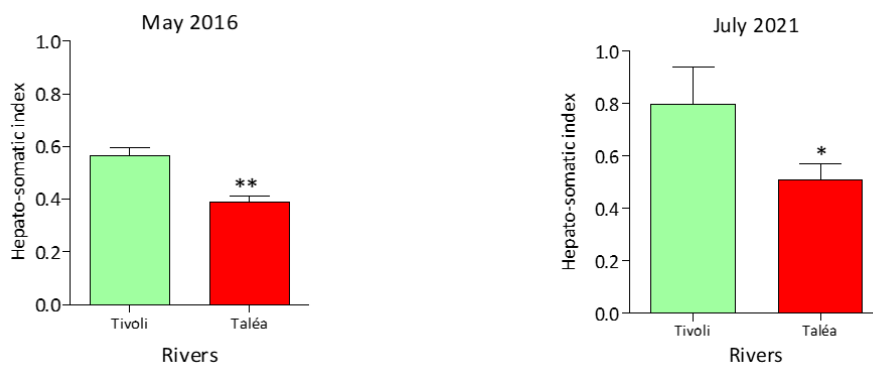
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187 **Figure 2:** A. Total length (cm, mean  $\pm$  SEM) of eels in May 2016 (n = 4-6) and July 2021 (n = 5). B. Fulton  
 188 condition index (mean  $\pm$  SEM) of eels in May 2016 (n = 4-6) and July 2021 (n = 5). Means designated with star  
 189 (\*) 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).

191



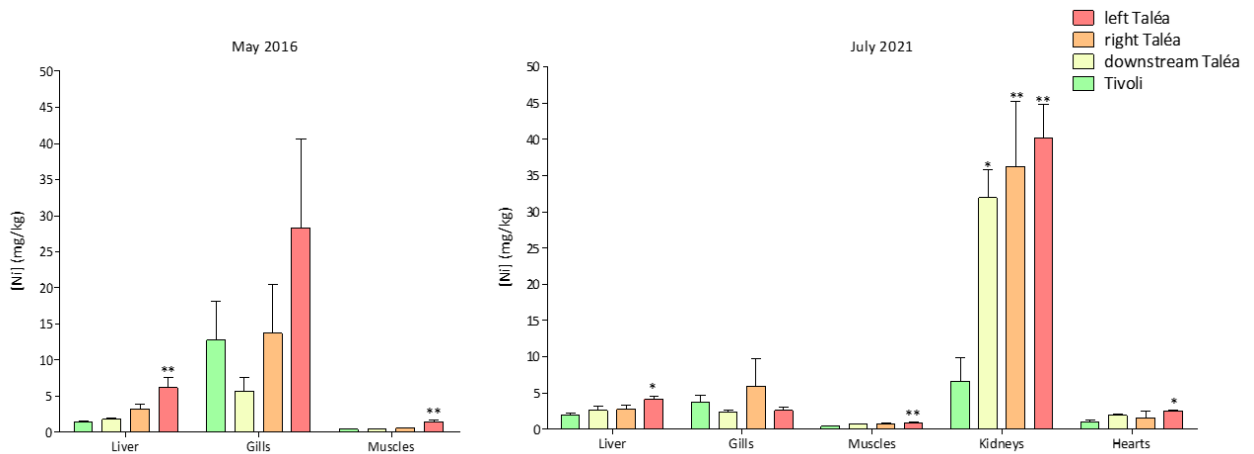
192

193 **Figure 3:** Hepato-somatic index (mean  $\pm$  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.

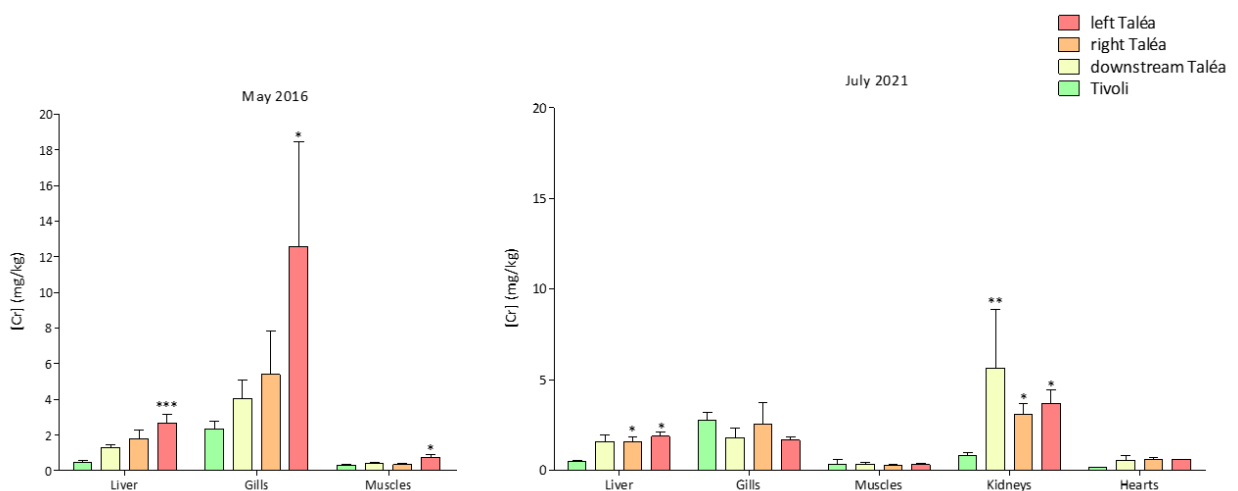
199 For Ni, the results showed hepatic concentrations significantly higher for eels living in Taléa river,  
 200 particularly in LT, than those living in Tivoli, either in 2016 or 2021 (Fig. 4). For muscle in 2016 and  
 201 2021, and for kidneys and hearts in 2021, the same patterns were observed, with highest  
 202 concentrations measured in eels from LT. The same tendencies were thus observed on both periods  
 203 except for gills, for which Ni concentrations were largely higher in 2016 than in 2021 (28.34 mg/kg  
 204 and only 2.58 mg/kg in LT respectively for 2016 and 2021).



205

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

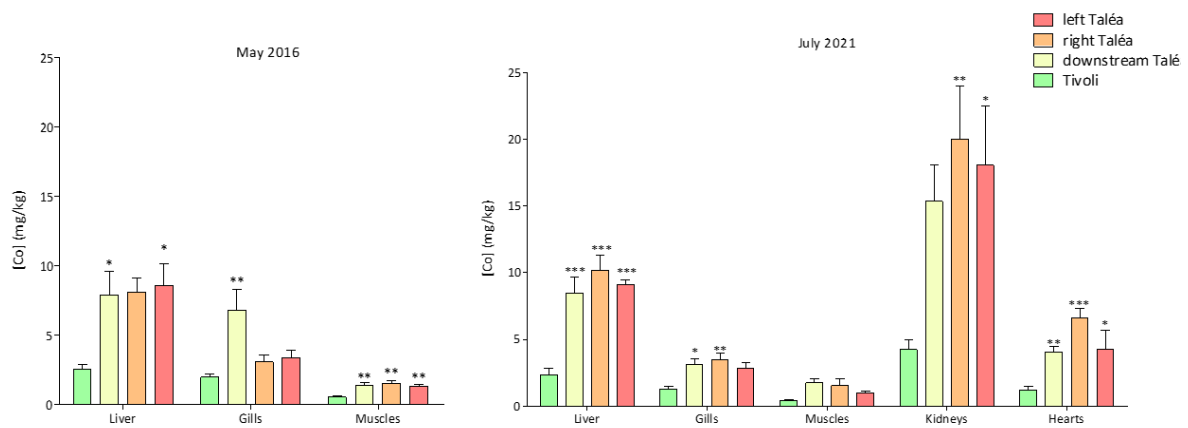
210 Eels collected from Taléa rivers accumulated also more Cr in their liver than eels living in Tivoli (p =  
 211 0.003 in 2016 and p = 0.024 in 2021) for the two periods of sampling (Fig. 5). Gills and muscle in 2016  
 212 bioaccumulated Cr to a greater extent in LT and kidneys in 2021 in all Taléa sites compared to Tiv.



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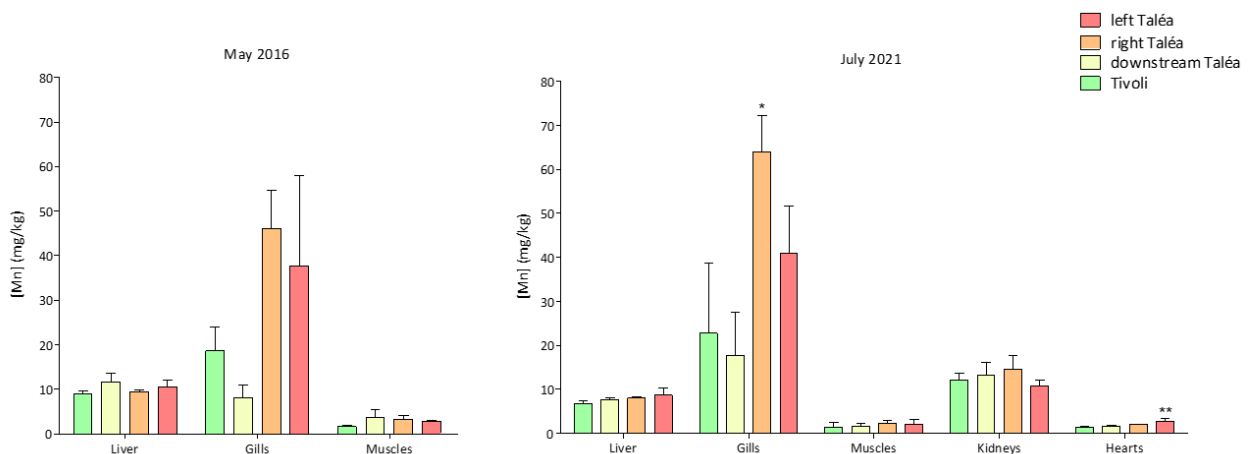
214 **Figure 5:** Cr concentrations (mg/kg) in liver, gills, muscle, kidneys and heart of eels sampled in Koniambo  
 215 Mount in New Caledonia (mean  $\pm$  SEM, n = 3-5 in 2016 and n = 4-6 in 2021). Means designated with star (\*) are  
 216 significantly different as compared to the reference Tivoli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

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



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

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



231

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

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

### 239 **3.3. Gene transcription levels**

240 To assess the impact of Ni and other associated metals from mining activity in organs of eels, RT-  
241 qPCR analyses were carried out on six organs: liver, kidneys, gills, brain, spleen and muscle from eels  
242 sampled in 2021. Fifteen genes were analyzed to explore the internalization (*cltb*); the oxidative  
243 stress (*sod<sub>zn/cu</sub>* *sod<sub>mn</sub>*); the metal detoxification (*mt*); the mitochondrial metabolism (*12s*; *cox1* and  
244 *atp6-8*); the lipid metabolism (*acc*; *tgl* and *gapdh*); the reproductive function (*vtg*); the apoptosis  
245 (*p53* and *bax*); the DNA repair (*gadd45*) and the immune system (*nlr3*). The results showed that liver  
246 was the most impacted organ, as the transcription level of almost 12 genes on the 16 studied was  
247 differentially regulated in eels from one or several Taléa sites compared to Tivoli (up-regulation for  
248 oxidative stress and lipid metabolism and down-regulation for mitochondrial metabolism, DNA  
249 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      |   |
|                    |        | <i>sod<sub>cu</sub></i> | <i>sod<sub>mn</sub></i> | <i>cat</i> | <i>mt</i>            | <i>12S</i>               | <i>cox</i> | <i>atp6-8</i>  | <i>gapdh</i>     | <i>tgl</i>    | <i>AcCoo</i>   | <i>gadd45</i> | <i>p53</i>     | <i>bax</i>    | <i>cltb</i>  | <i>Vtg</i>     | <i>nlr3</i>   |   |
| 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   | /                | /             | /              | /             | /              | /             | /            | /              | /             | / |

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

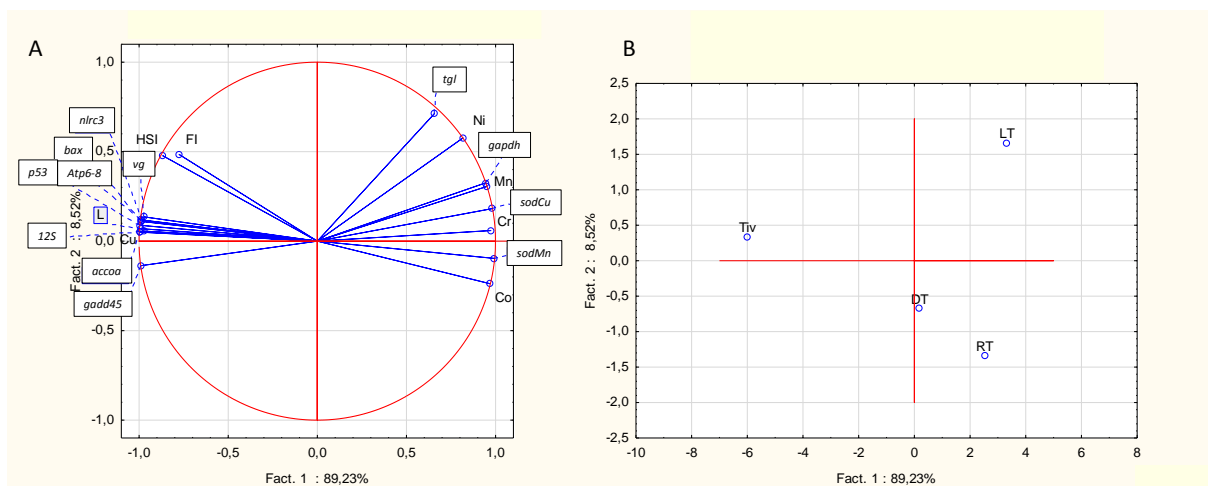
253 The gills were on the one hand up-regulated for genes involved in oxidative stress and metal  
254 detoxification and on the other hand down-regulated for genes involved in apoptosis.

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

257 No difference in gene transcription level was observed in muscles.

### 258 3.4. Multivariate analysis on the liver

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



269

270

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

275 **4. Discussion**

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

287 **4.1. Morphometric parameters**

288 First of all, we noticed that eels living in rivers downstream the mines were smaller than eels living in  
289 the reference site, with a lower hepato-somatic index at both different periods, as it has been  
290 already observed in eels exposed to other metals such as Hg and Cd [22]. These results seemed to  
291 indicate that eels, in particular their liver function, are probably impacted by physico-chemical or  
292 nutrient conditions occurring in Taléa river compared to Tivoli, whatever the period and season.  
293 Interestingly, the Fulton index differed between the two periods. Indeed, no differences were  
294 observed in 2021, whereas, in 2016, eels living in Taléa presented a smaller Fulton index compared to  
295 Tivoli. In 2021, the sampling campaign was done after a long episode of rain which could explain at  
296 least in part the low dissolved metal concentration in the rivers. For example, the concentrations of  
297 dissolved Ni was 48 µg/L in 2016 compared to 8 µg/L few weeks before the fisheries in 2021. Indeed,  
298 in 2016 the cumulative rainfall in the 6 months preceding the fisheries (December 2015 to May 2016)  
299 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  
300 mm of rain per day) in 2021 (February 2021 to July 2021) (MeteoNC, 2016 ; MeteoNC, 2021). The  
301 higher dissolved metal levels in 2016, could further affect the food chain and eel nutrient quality, but  
302 also eels themselves. As the Fulton index reflects the physiological state of the fish [37], it is possible  
303 that in 2016, rivers under mining influence offered poor condition for growth and that eels inhabiting  
304 these sites were more impacted by metals, and therefore, had a lower condition index.

305 **4.2. Metals bioaccumulation**

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

312 The same trends were noticed in 2016 and 2021 but some variations especially for metal  
313 concentrations in livers and gills were nevertheless observed. In fact, for Ni and Cr, in 2016, eels  
314 appeared to be mainly contaminated by the direct route of exposure (*i.e.* dissolved metals in water)  
315 whereas in 2021 the main route of contamination of eels in Taléa seemed to be the trophic route.  
316 This was highlighted by higher metal concentrations in the gills compared to the livers for all sites in  
317 2016 in contrast to 2021 where Ni and Cr concentrations in gills were lower than in livers. Indeed,  
318 metals bioaccumulation in the gills is expected to be higher following waterborne exposure, while  
319 higher bioaccumulation levels in the liver is followed by absorption of metals from the gut [38]. It  
320 seems that few dissolved metals were assimilated by the gills in 2021, that could also be explained by  
321 the moment of the fisheries, which were realized after heavy rain episodes in 2021. As already  
322 evoked, these hydrologic conditions in 2021 would have probably diluted the metals in the  
323 freshwaters by high leaching of soils during several months before sampling and modified their  
324 chemical speciation, with a highest proportion of them adsorbed to particulate matter in 2021  
325 compared to 2016 [39, 40]. All these findings could suggest that the contamination of eels is  
326 dependent on the weather conditions and the fishing season. However, the livers are in all cases  
327 impacted by higher concentrations of metals measured in eels living under mining influence  
328 compared to Tivoli.

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



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

### 348 **4.3. Genes transcription**

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

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

377 Another important fact is that eels living in contaminated freshwaters seem to have an important  
378 inflammation with *nlr3* depletion in livers. On the contrary, an induction of *nlr3* observed in  
379 kidneys indicate a decrease of pro-inflammatory response leading to a decrease of response if eels  
380 are in contact with virus, parasites or bacteria with potentially serious consequences about their  
381 health condition [62, 63].

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

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

## 396 **5. Conclusion**

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

405

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410 **Statement of contributions**

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

413 Gunkel-Grillon Peggy: Methodology, Writing – review & editing

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416 Daffe Guillemine: Methodology, Writing – review & editing

417 Dassié Emilie: Methodology, Writing – review & editing

418 Pierron Fabien: Methodology, Writing – review & editing

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

421 Baudrimont Magalie: Project administration, Conceptualization, Funding acquisition, Methodology,  
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