Chronic trace metals effects of mine tailings on estuarine assemblages revealed by environmental DNA

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Mine tailing disasters have occurred worldwide and contemporary release of tailings of large proportions raise concerns of the chronic impacts that trace metals associated with tailings may have on the aquatic biodiversity. Environmental metabarcoding (eDNA) offers an yet poorly explored opportunity for biological monitoring of impacted aquatic ecosystems from mine tailings and contaminated sediments. eDNA has been increasingly recognized to be an effective method to detect previously unrecognized small-sized Metazoan taxa, but their ecological responses to environmental pollution has not been assessed by metabarcoding. Here we evaluated chronic effects of trace metal contamination from sediment eDNA of the Rio Doce estuary, 1.7 years after the Samarco mine tailing disaster, which released over 40 million m^3 of iron tailings in the Rio Doce river basin. We identified 123 new sequence variants (eOTUs) of benthic taxa and an assemblage composition dominated by Nematoda, Crustacea and Platyhelminthes; typical of other estuarine ecosystems. We detected environmental filtering on the meiofaunal assemblages and multivariate analysis revealed strong influence of Fe contamination, supporting chronic impacts from mine tailing deposition in the estuary. This was in contrast to environmental filtering of meiofaunal assemblages of non-polluted estuaries. Here we suggest that the eDNA metabarcoding technique provides an opportunity to fill up biodiversity gaps in coastal marine ecology and may become a valid method for long term monitoring studies in mine tailing disasters and estuarine ecosystems with high trace metals content.

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Abstract

Mine tailing disasters have occurred worldwide and contemporary release of tailings of large proportions raise concerns of the chronic impacts that trace metals associated with tailings may have on the aquatic biodiversity. Environmental metabarcoding (eDNA) offers an yet poorly explored opportunity for biological monitoring of impacted aquatic ecosystems from mine tailings and contaminated sediments. eDNA has been increasingly recognized to be an effective method to detect previously unrecognized small-sized Metazoan taxa, but their ecological responses to environmental pollution has not been assessed by metabarcoding. Here we evaluated chronic effects of trace metal contamination from sediment eDNA of the Rio Doce estuary, 1.7 years after the Samarco mine tailing disaster, which released over 40 million m^3 of iron tailings in the Rio Doce river basin. We identified 123 new sequence variants (eOTUs) of benthic taxa and an assemblage composition dominated by Nematoda, Crustacea and Platyhelminthes; typical of other estuarine ecosystems. We detected environmental filtering on the meiofaunal assemblages and multivariate analysis revealed strong influence of Fe contamination, supporting chronic impacts from mine tailing deposition in the estuary. This was in contrast to environmental filtering of meiofaunal assemblages of non-polluted estuaries. Here we suggest that the eDNA metabarcoding technique provides an opportunity to fill up biodiversity gaps in coastal marine ecology and may become a valid method for long term monitoring studies in mine tailing disasters and estuarine ecosystems with high trace metals content.

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Introduction

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44   Environmental assessment studies rely on accurate detection of biodiversity of an extremely

45   diverse and small-sized benthic fauna. For decades, morphological methods are the basis to

46   impact assessment (IA) protocols at the cost of neglecting an enormous number of meiofaunal

47   species that could not be accurately identified (Bhadury et al., 2006; Fonseca et al., 2010). There

48   have been considerable advances in recent years by applying DNA-sequence based techniques,

49   ⁻⁻ commonly referred as metabarcoding, to identify and quantify meiofaunal biodiversity

50   (Creer et al., 2010; Bik et al. 2012; Brannock et al., 2014). These modern approaches offer fast

51   assessments of marine Metazoan meiofaunal assemblages and are particularly useful for the

52   identification of new species in areas with poorly reported biodiversity, which may be of special

53   interest in IA studies.

54   Estuarine coastal ecosystems offer an opportunistic case to evaluate biodiversity-environmental

55   relationships through environmental DNA (eDNA) since many estuaries are widely impacted by

56   pollutants with deleterious effects to benthic assemblages (Lotze et al., 2006; Chariton et al.,

57   2015; Hadlich et al., 2018). The Samarco mine tailing disaster that occurred in Brazil on

58   November 2015, released near 43 million m3 of tailings in the Rio Doce river basin, which were

59   transported for over 600 km until reaching the estuary and the Atlantic Ocean (Carmo et al.,

60   2017; Magris et al., 2019). The tailings severely impacted the Rio Doce riverine and estuarine

61   ecosystems causing rapid sediment accumulation, burial and death of benthic organisms, and

62   rapidly (1-2 days) increased sediment heavy metal accumulation by orders of magnitude from

63   pre-impact conditions (Gomes et al., 2017). Although the released tailings had trace metal

64   concentrations that were within the Brazilian legislation (Segura et al., 2016); the iron tailings

65   deposited in the estuarine soils were heavily associated with trace metals which are potentially

66   bioavailable given the redox conditions of estuarine soils (Queiroz et al., 2018).

67   Trace metal accumulation in coastal ecosystems are reported to be highly associated with

68   changes in benthic assemblages and to increase human health risks due to potential

69   bioaccumulation in food webs (Venturini et al., 2002; Muniz et al., 2004; Rainbow, 2007;

70   Hauser-Davis, 2015). As a result, impact assessment studies that followed the Samarco disaster

71   were also based on traditional morphological biodiversity assessments (Gomes et al., 2017). The

72   potential chronic pollution effects in the Rio Doce estuary will likely demand long term

73   monitoring programs for this ecosystem. To that end, technical and taxonomic expertise will be

74   of key importance to monitor the estuarine biodiversity, but these efforts are typically limited to

75   the macrofaunal and megafaunal benthic taxa. Therefore, monitoring this environmental disaster

76   by increasing its biodiversity assessment to a broader range of cryptic and meiobenthic taxa may

77   bring valuable information on the extension of impacts.

78   In this study, we used an eDNA metabarcoding approach to evaluate the benthic biodiversity in

79   the Rio Doce estuary 1.7 years after the initial impacts of the Samarco disaster. We hypothesized

80   that spatial patterns of chronic metal contamination in the estuary would be significantly

81   associated with patterns of meiofaunal environmental taxonomic units (eOTUs), evidencing the

82   potential use of this technique for long term impact assessment of the estuary. We targeted

83   benthic meiofaunal eukaryote organisms by amplifying and sequencing the V9 hypervariable

84   region of the 18S ribosomal gene from purified eDNA. In addition, sediment variables (particle

85   size, organic carbon content) and trace metals concentrations were used to test for spatial

86   changes in benthic assemblages in response to contamination levels in the estuary.

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Materials & Methods

Study site

The Rio Doce estuary (19°38′ to 19°45′S, 39°45′ to 39°55′W; Figure 1), is located on the Eastern

Marine Ecoregion of Brazil that has two well-defined seasons, dry winter (April to September)

and wet summer (October to March), with an average monthly rainfall of 145 mm and

temperatures of 24 to 26 °C (Bernardino et al., 2018; Bissoli and Bernardino, 2018). The Rio

Doce estuary has been altered by historical human occupation, but ecosystem health of the

estuary was poorly known before the Samarco disaster that occurred in November 2015

(Bernardino et al., 2016; Gomes et al., 2017). The initial impacts of the Samarco disaster in the

estuary were reported by Gomes et al. (2017), and a standard monitoring of benthic assemblages

and contamination levels were established in 2017 with a disaster-response program funded by

Brazilian government agencies (Fapes, Capes and CNPq). The first monitoring campaign

occurred in August 2017 (SISBIO sampling license N 24700-1), approximately 1.7 years after

the initial impacts were observed in the estuary, when we aimed to quantify the potential chronic

effects of trace metal pollution that were first observed in November 2015 (Gomes et al., 2017).

Sample collection and DNA isolation

Environmental DNA was obtained from two biological replicates of estuarine undisturbed

surface (0-5 cm) sediments samples at 22 sites on the Rio Doce estuary in August 2017 (Figure

1). The top 5 cm (~300g wet weight) sediment was sampled with DNA-free sterile material and

immediately frozen in liquid nitrogen. In the laboratory, all glassware was cleaned and

autoclaved between samples to avoid cross contamination. Sediment samples were elutriated in

DNA-free material to concentrate benthic metazoans and eDNA was extracted following

113   protocols of Brannock and Halanych (2015)), stored at -20 °C and sent to the Genomic Services

114   Laboratory at Hudson Alpha Institute for Biotechnology (Huntsville, Alabama) for

115   metabarcoding sequencing. Briefly, the total DNA from 200 g (ww) of frozen sediments were

116   extracted from each replicate separately with a Mobio PowerSoil(R) kit according to

117   manufacturer's protocol with a 2 min bead-beating step. DNA integrity was evaluated using

118   electrophoresis on 1% agarose gels and DNA purity was assessed with a NanoDrop

119   spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). Accurate DNA

120   quantification was obtained using a Qubit® 3.0 Fluorometer (Life Technologies-Invitrogen,

121   Carlsbad, CA, USA). Only 20 stations had enough bulk DNA after extraction and 7 samples out

122   of the expected 40 replicates did not yield high quantities of purified eDNA. In total 33 sediment

123   eDNA samples from the Rio Doce estuary were then submitted to amplicon library preparation

124   and Illumina sequencing (Table 1).

125   Sediment samples were obtained for trace metals, grain size and total organic matter analysis and

126   frozen (-20oC). Grain size was analyzed by sieving and pipetting techniques (Suguio, 1973).

127   Total Organic Matter (TOM) content was quantified gravimetrically as the weight loss after

128   combustion (500 °C for 3 h). In each station, metal contamination was evaluated from two

129   independent replicate samples. For the total trace metal contents, ~ 1g of dry sediment samples

130   were digested by an acid mixture (HCl + HNO3 + HF; USEPA, 1996) in a microwave digestion

131   system. Following digestion, concentrations of trace metals (Al, Ba, Cr, As, Fe, Zn, Mn, Pb, Cd,

132   Co) in all samples were determined using an inductively coupled plasma optical emission

133   spectroscopy (ICP-OES; Thermo Scientific - iCAP 6200).

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135   Illumina sequencing and bioinformatic pipelines

136   eDNA samples were sent to the Genomic Services Laboratory at Hudson Alpha Institute for

137   Biotechnology (Huntsville, Alabama) for amplicon sequencing. The Eukaryotic-specific V9

138   hypervariable region of 18S SSU rRNA gene was amplified using primers Illumina_Euk_1391f

139   forward primer [GTACACACCGCCCGTC] and Illumina_EukBr reverse primer

140   [TGATCCTTCTGCAGGTTCACCTAC] (Caporaso et al., 2010). Library size distribution was

141   accessed using a 2100 Bioanalyzer (Agilent, Santa Clara, CA, USA). Amplicons were sequenced

142   on MiSeq (Illumina, San Diego, CA, USA) using the Reagent Kit v3 (300bp PE).

143   Demultiplexed raw single-end reads for each sample were processed and analyzed using the

144   2018.8 distribution of the QIIME2 software suite to estimate the observed taxa across replicates

145   (Bolyen et al., 2018). Fastq files were first imported as QIIME2 artifacts with the appropriate

146   import plugin. Single-end reads were then denoised via DADA2 (Callahan et al., 2016) with the

147   dada2 denoise-single plugin, where the --p-trunc parameter was set to 270 to remove low-quality

148   bases and the --p-trim was set to 20 to remove primer sequence. The taxonomic composition of

149   the amplicon sequence variants, generated after running the dada2 plugin, were assigned using

150   the machine learning Python library scikit-learn (Pedregosa et al., 2011). A pre-trained Naïve

151   Bayes classifier, trained on Silva 132 database (Quast et al., 2013) clustered at 99% similarity,

152   was downloaded from QIIME2 website [https://docs.qiime2.org/]. The feature-classifier plugin

153   was used to generate de classification results, and the taxonomic profiles of each sample were

154   visualized using the taxa barplot plugin.

155

156   Statistical analysis

157   Only Metazoan variant calls were selected for ecological analysis. Comparisons of community

158   composition were based on replicate averages of eOTU reads from benthic taxonomic groups.

159   Benthic taxa were grouped for taxonomic comparisons into main taxa including Gastrotricha,

160   Platyhelminthes, Nematoda, Annelida, Crustacea, Mollusca and Cnidaria. Other invertebrate taxa

161   including Gnathostomulida, Micrognathozoa, Tardigrada, Rotifera and Bryozoa were grouped

162   into "Other invertebrates". Unassigned or other taxa (e.g. Insecta) were represented as "Other

163   Metazoa". Taxonomic (eOTUs) accumulation curves (Chao1) were compared across datasets by

164   using: i. full eOTU matrices (Table S1), ii. dominant eOTUs with over 0.1% of total Metazoan

165   reads (Table S2); and iii) the baseline benthic morphological diversity from the Rio Doce estuary

166   (Gomes et al., 2017). Chao 1 curves were based on presence-absence eOTU matrices integrated

167   between replicates from each station and were estimated in Primer-e V6 (Clarke and Gorley,

168   2006).

169   The spatial consistency of metal contamination with benthic assemblage composition was tested

170   with a Canonical Analysis of Principal coordinates (CAP; Anderson and Willis, 2003)

171   complemented with multidimensional scaling (Anderson, 2001; McArdle & Anderson, 2001;

172   Oksanen et al., 2018). Before the CAP analysis was run, the existence of highly correlated

173   variables (trace metals) was assessed and trace metals with significant correlation with Fe

174   contents were removed. The resulting multivariate analysis was only run with sediment contents

175   of Fe, As and Pb, given their non-significant auto-correlations (Table S3). In addition, these trace

176   metals (Fe and Pb) markedly increased (5 to 20-fold) in concentration with the impact (Gomes et

177   al., 2017) and were often above the recommended limits within the Brazilian legislation (Guerra

178   et al., 2017; Gabriel et al., in review). Given that the concentration of other trace metals were

179   highly correlated with Fe, Fe contents likely represent the overall effect of mine tailings

180   deposited in the estuary (Queiroz et al., 2018).

181   The CAP was run based on presence or absence matrices with full Metazoan eOTUs and with the

182   reduced assemblage composed of dominant reads (>0.1% of reads; Table S2). The CAP eOTU

183   matrices were then compared with environmental (trace metal concentrations, sediment OM, %

184   sand and salinity) spatial patterns based on an Euclidean distances matrix to determine vectors

185   that contributed to differences among samples (Mazzuco et al., 2019). Graphical and analytical

186   processing were performed in R project (R Core Team, 2016) with the packages: 'stats' and

187   'vegan' (Oksanen et al., 2018).

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189
190   **Results**
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192   The Rio Doce estuary exhibited low salinities at the time of sampling (0.1 to 3.7). Sediments

193   were dominated by sand particles (>62% sand), with the exception of site 2 which showed less

194   sand-sized particles (12%; Table 1). Sediment total organic matter (TOM) varied from 1.5 to

195   16.8 %, with the highest organic content at stations 3, 13 and 22 (16.8, 13.8 and 10.2 %;

196   respectively). Several estuarine areas had TOM in a similar range of 2 to 6.2 % (Table 1). The

197   concentration of trace metals in the estuarine sediments also varied markedly along the studied

198   area. The concentrations of Fe, As and Pb in sediment samples were also spatially heterogeneous

199   (Table 1). Fe concentrations ranged from 18,814 to 54,982 mg.kg-1 and were highly correlated

200   with several other trace metals including Al, Cd, Cr, Co, Cu, Mn and Zn (Table S3).

201   We obtained a total of 9,836,039 sequence reads, of which 6,840,886 were of high quality. The

202   number of sequence reads per station ranged from 35,915 (St 16) to 359,718 (St 4), with an

203   average of 207,285 total sequence variants per station. Stations that had only one replicate

204   sequenced had a lower (e.g. stations 14 and 16) or a similar number of reads (e.g. stations 19 and

205   20) of sites that had two replicates sequenced. On average, 55.4% of reads corresponded to

206   aquatic or marine Metazoan taxa (Table S1). The eOTU richness per station ranged over three-

207   fold from 16 to 54 eOTUs (Table 1). Assemblages were dominated by Nematoda (34 eOTUs),

208   Platyhelminthes (19), Crustacea (18), Gastrotricha and Annelida (12 eOTUs each; Table S1;

209   Figure 2). Most sites had over 80% of sequence variant reads represented by two to three

210   meiofaunal taxa, including the dominant Gastrotricha, Nematoda and Crustacea. The number of

211   unassigned Metazoan taxa was large (> 50%) at stations 16 and 17; whereas it remained less than

212   20% in most sites.

213   The eDNA species accumulation curves did not reach an asymptote with addition samples

214   suggesting an yet incomplete biodiversity characterization of the estuary (Figure 3). Several

215   eOTUs (N=88) were represented by less than 0.1% of sequence variant reads. When we removed

216   the eOTUs that had less than 0.1% of sequence reads, the species accumulation stabilized at 32

217   eOTUs with 5 to 7 samples, with no additional gain of taxa. The species accumulation asymptote

218   with dominant eOTUs was reached in about half the number of samples necessary in

219   morphology-based studies (12 to 14 samples; Figure 3).

220   The multivariate patterns of dominant meiofaunal (S= 32) eOTU composition were significantly

221   related to Fe contents in sediments (F= 2.89, p=0.018, Figure 4; Table 2). The CAP axes 1 and 2

222   explained 44% and 21% of multivariate variability; respectively (Table 2). Fe contents in

223   sediments was associated to the multivariate distribution of meiofaunal eOTUs including the

224   Nematoda Mesodorylaimus nigritulus and Epitobrilus stefanskii, Harpacticoid copepods, the

225   Platyhelminthes Cirrifera dumosa and Bothrioplana sinensis, and Ostracods (Chrissia

226   dongqianhuensis). Monhysteridae and Desmodorida spp. nematode worms were negatively

227   correlated to Fe concentrations (CAP1 score= -0.25 to -0.18). Pb and As contamination were not

228   correlated to Fe concentrations in sediments and were not significantly associated with the

229   meiofaunal multivariate composition (Table 2).

230
231
232   **Discussion**

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234   Our study demonstrates that environmental DNA can be an effective method to indicate chronic

235   contamination effects on benthic assemblages of the Rio Doce estuary, supporting our

236   hypothesis. This first eDNA survey in the Rio Doce estuary also revealed a previously

237   unrecognized benthic biodiversity, even with significant impacts by trace metal levels 1.7 years

238   after the initial impacts. Although there is no baseline eDNA assessment from the Rio Doce

239   estuary, the impacted sediments potentially supported over 32 dominant meiofaunal taxa

240   (eOTUs), with a spatial distribution significantly related to Fe contamination.

241   The Rio Doce eDNA composition was similar to other estuarine and marine sediments assessed

242   by metabarcoding methods (Fonseca et al., 2010; Faria et al., 2018). Nematoda, Gastrotricha and

243   Crustaceans were highly dominant in the estuary with local changes in relative abundance across

244   sites sampled. The marked spatial variability in assemblage composition within the estuary

245   indicates that benthic assemblages were spatially structured; which is a similar pattern commonly

246   observed in morphology-based assessments. Environmental filtering in benthic assemblages may

247   result from a combination of sediment and water variables, with grain size, salinity and food

248   availability being critical to species turnover and replacement in estuarine benthos (Menegotto et

249   al., 2019). Although our study design does not allow for an hierarchical spatial analysis of

250   variables that determined the observed environmental filtering, the detection of spatial variance

251   in assemblages from eDNA samples suggests that the biodiversity assessment is likely

252   representing living benthic organisms instead of predominantly ancient or allochthone DNA.

253   There is now strong evidence supporting that eDNA techniques can detect complex spatial

254   variability in estuarine and coastal marine ecosystems (Chariton et al., 2015; Faria et al., 2018);

255   and our data additionally supports its use to biodiversity assessment in a heavily impacted

256   estuary.

257   Most eOTUs represented new occurrences for the estuary, but yet with several unassigned taxa,

258   stressing the complementarity value of molecular and morphological approaches to ecological

259   and impact assessment studies (Leasi et al., 2018). We recovered a total of 123 environmental

260   OTUs (eOTUs) in the Rio Doce estuarine sediments, increasing by over 20-fold the previous

261   richness of benthic taxa based on morphological identifications (Gomes et al., 2017). The species

262   accumulation curves did not reach an asymptote with addition of eDNA samples, and most

263   eOTUs (N=88) were represented by less than 0.1% of sequence variant reads, suggesting an yet

264   incomplete biodiversity assessment of the Rio Doce estuary even with high levels of trace

265   metals. However, estuaries are highly connected to continental and marine ecosystems and it is

266   unlikely that species accumulation curves would reach an asymptote with a single biodiversity

267   assessment (Chariton et al., 2015; Nascimento et al., 2018). The rapid increase and stabilization

268   of the number of dominant meiofaunal OTUs with the addition of new samples suggests a

269   reasonable beta-diversity assessment of the Rio Doce estuary with the effort taken. Sites that had

270   only one sequenced replicate due to low DNA stocks attained similar or lower OTUs richness if

271   compared to other stations, but the sediment volumes used in this study (> 200g) were well over

272   the necessary to avoid technical bias in the detection of Metazoan diversity (Brannock and

273   Halanych, 2015; Nascimento et al., 2018).

274   This single eDNA survey was efficient in assembling benthic meiofaunal assemblages in the Rio

275   Doce estuary. The species accumulation curves indicate that half of the sampling effort would be

276   necessary to characterize the dominant meiofaunal groups in the estuary if compared to the

277   species accumulation rate of previous morphological assessments. eDNA metabarcoding can be

278   more efficient at characterizing marine taxa (Lobo et al., 2017), and our data supports its use on

279   long term studies where taxonomic and technical limitations cannot be controlled (Bista et al.,

280   2017). The lack of controlled methods may be a crucial problem to the biodiversity monitoring

281   that followed the Samarco disaster on the Rio Doce, given the extremely large scale and diversity

282   of impacted ecosystems. It is estimated that over 2,000 ha of terrestrial, limnetic and estuarine

283   ecosystems along the Rio Doce basin were directly impacted by the disaster (Carmo et al., 2017);

284   with additional potential effects on nearby coastal zones (Magris et al., 2019). Given the large

285   area, the diversity and natural complexity of ecosystems to be monitored, it is likely that the

286   number of biological samples needed to reach reasonable statistical power to detect biodiversity

287   impacts would be prohibitive (Fairweather, 1991). Therefore, the massive amount of data

288   obtained in metabarcoding techniques could have a profound contribution to environmental

289   monitoring in this scenario, which would also increase dramatically the discovery of cryptic

290   species on a range of aquatic and terrestrial ecosystems.

291   Multivariate analysis revealed that Fe contents are partially structuring spatial patterns of

292   dominant benthic meiofaunal assemblages in the Rio Doce estuary 1.7 years after the disaster.

293   The sediment Fe contents were significant predictors of changes in dominant meiofaunal eOTUs

294   including nematodes, copepods, ostracods and flatworms. These groups corresponded to over 2/3

295   of meiofaunal OTUs in the estuary and revealed that trace metal contents are driving spatial

296   patterns of the Rio Doce estuarine biodiversity. Our data suggest that benthic assemblages were

297   highly sensitive to chronic metal contamination in polluted estuaries, and partially explains a

298   lower effect of sediment grain size and organic matter on local meiofauna (e.g. Faria et al., 2018;

299   Menegotto et al., 2019). This could indicate that the Rio Doce estuarine assemblages were

300   strongly impacted after the disaster through the exclusion of intolerant species, although we lack

301   baseline eDNA to fully support that hypothesis. However, the extremely high Fe contents allied

302   to covariance of several potentially toxic trace metals that are adhered to iron oxides present in

303   the tailings strongly suggest that the tailings have led to major changes in the estuarine benthic

304   biodiversity since the initial impact (Queiroz et al., 2018).

305   The statistical lack of As and Pb effects on the multivariate distribution and composition of

306   meiofaunal assemblages have important implications for future environmental monitoring in the

307   estuary. One plausible cause is that not all elements that are accumulated in the sediments are

308   bioavailable and have toxicity to the estuarine biota. However, given the amplitude of trace

309   metals accumulated in the Rio Doce sediments it is very likely that a combination of these

310   contaminants lead to changes in the estuarine benthos. The sediment concentrations of Pb in

311   August 2017 were over 20 times higher than baseline values (Gomes et al., 2017); and several

312   other trace metals also increased with time since the impact (Gabriel et al., in review). The iron

313   oxides from tailings deposited in the estuary have a strong capacity of metal retention (Cornell

314   and Schwertmann 2003; Yin et al. 2016); and they are likely to be released due dissimilatory iron

315   reduction under estuarine conditions (Bonneville et al. 2009; Queiroz et al., 2018; Xia et al.

316   2019). As a result, the observed relationship of meiofaunal assemblages with Fe contents suggest

317   that the tailings have toxicity to benthic organisms even though some contaminants may not

318   achieve alarming concentrations. The effects of trace metal contents on the Rio Doce benthic

319   assemblages resemble impacts in other areas that are highly polluted with trace metals, but these

320   effects could be confounded with the constant environmental changes that typically occur in

321   these ecosystems (Krull et al., 2014; Martins et al., 2015). Our approach of selecting dominant

322 meiofaunal OTUs to multivariate analysis led to positive detection of Fe contents effects. This

323 approach was justifiable given that we detected 88 eOTUs with less than 0.1% of sequence

324 variant reads, which could be potentially associated with allochthone DNA from connected river

325 or ocean ecosystems and would not be under influence of local contaminants. The use of

326 indicator taxa or functional groups to eDNA biodiversity assessment studies is becoming practice

327 in ecological studies (e.g. Bista et al., 2017) and our approach offers an important

328 methodological approach for detection of trace metals effects in aquatic biota that need to be

329 further investigated in other case studies.

330

331 **Conclusions**

332 In conclusion, our results support our hypothesis of impacts of tailings on benthic estuarine

333 assemblages. Our study is also in agreement with previous assertions that ecological inferences

334 from eDNA analysis may increase the performance of biodiversity assessments in marine

335 ecosystems by capturing a range of cryptic taxa, thus greatly improving current short and long-

336 term impact assessment studies. The use of eDNA to the Samarco mine tailing disaster would

337 benefit monitoring assessments with standard techniques and dramatically increase our

338 knowledge of the biodiversity of cryptic aquatic species. The continued sampling and monitoring

339 would also increase the precision of the eDNA assessments, especially if allied to detailed

340 morphological work.

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343 **Acknowledgements**

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Figure 1

Map of the study site

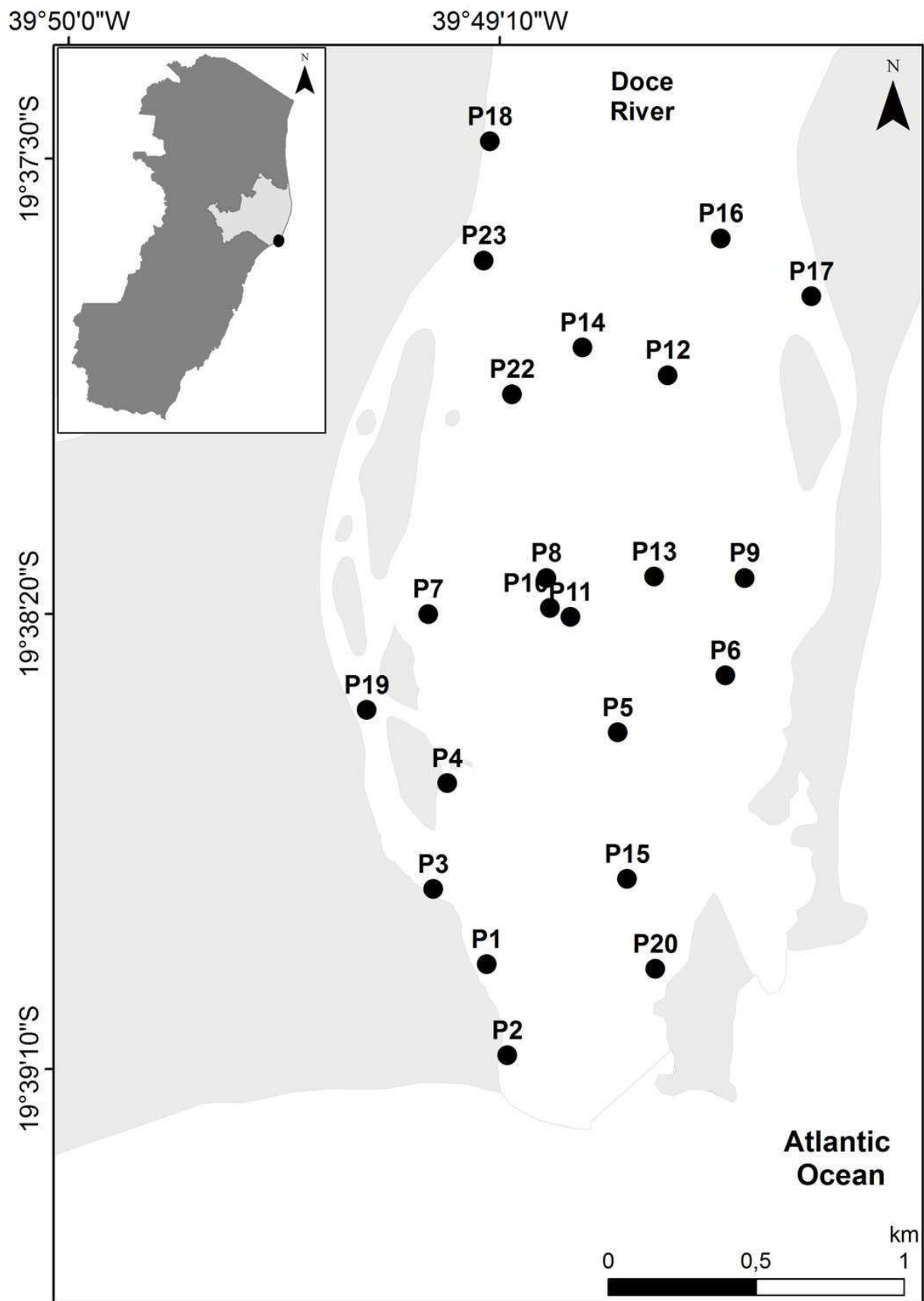Map of sediment sampling stations at the Rio Doce estuary, Brazil in August 2017

Figure 2

Benthic assemblage composition of the Rio Doce estuary

Benthic meiofaunal assemblage composition based on eDNA samples from the Rio Doce estuary in August 2017.
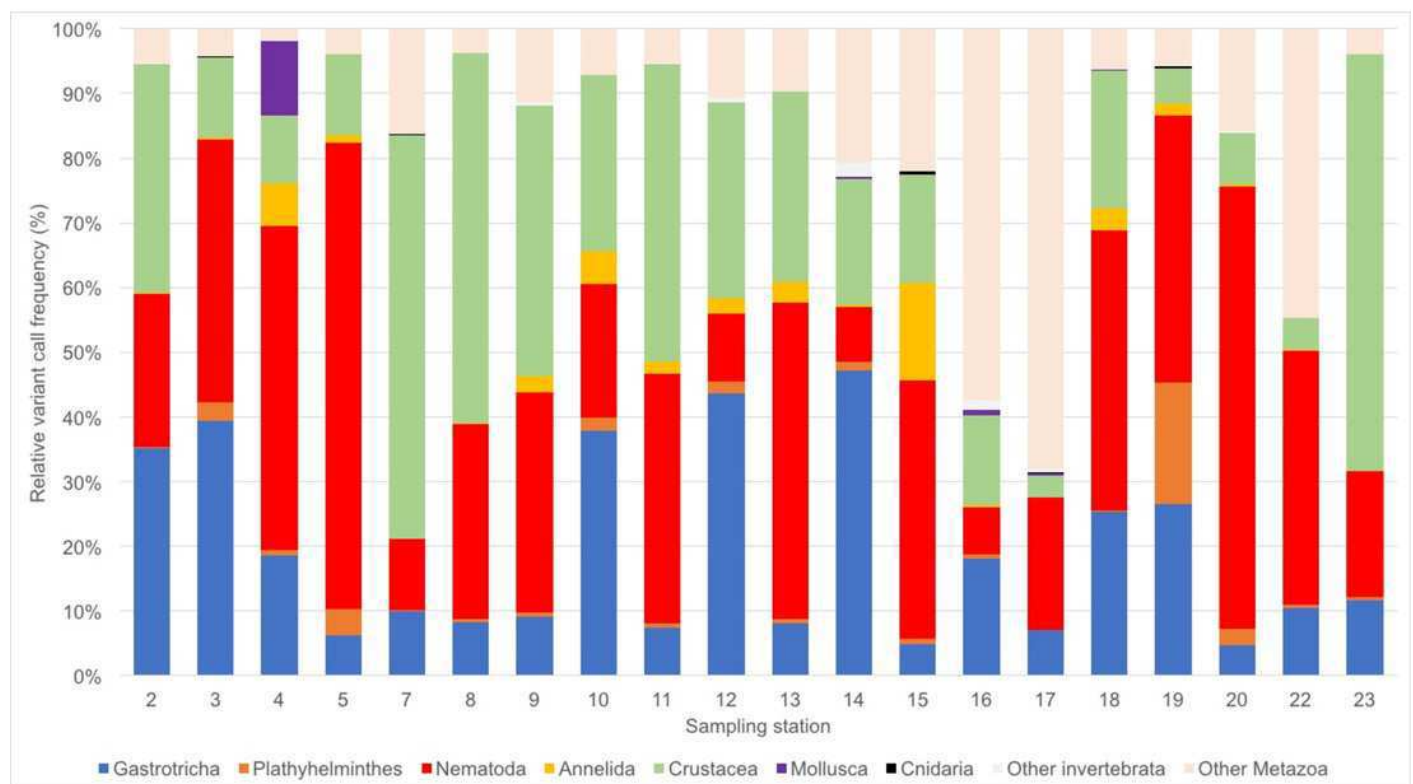
Figure 3

Taxa accumulation curves from eDNA samples

Taxa accumulation curves (Chao1 index) based on full eOTU matrices (eDNA, blue dotted line), dominant eOTUS (>0.1% sequence reads; eDNA_dom black dotted line) and on morphology-based macrofaunal pre-impact assessments (author's data published on Gomes et al., 2017) in the Rio Doce estuary.
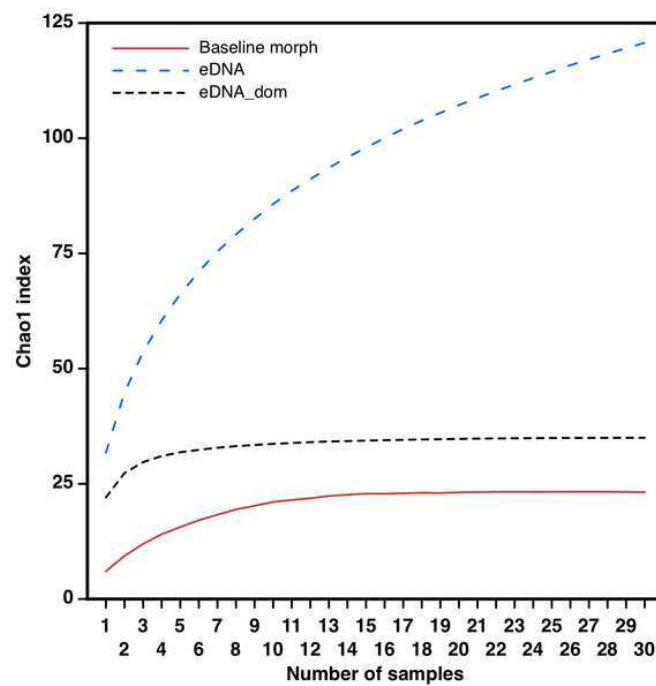
Figure 4

Multivariate analysis of assemblage composition and environmental filtering in the Rio Doce in August 2017

Canonical analyses of principal coordinates (CAP) ordination of samples according to multivariate distribution of dominant eOTUS (>0.1% total SV reads) in the Rio Doce estuary. The strength and direction of environmental effects (Spearman correlation values with p< 0.5 in red) on biological assemblages is represented by arrows of variable size. Environmental variables were based on Table 1 (Fe, Pb, As, Salinity, TOM and %Sand). Taxa scores indicate OTUs mostly correlated to site differences. Proportion of variance explained by axis 1 and 2 are in parenthesis. Symbol numbers indicate sampling station.
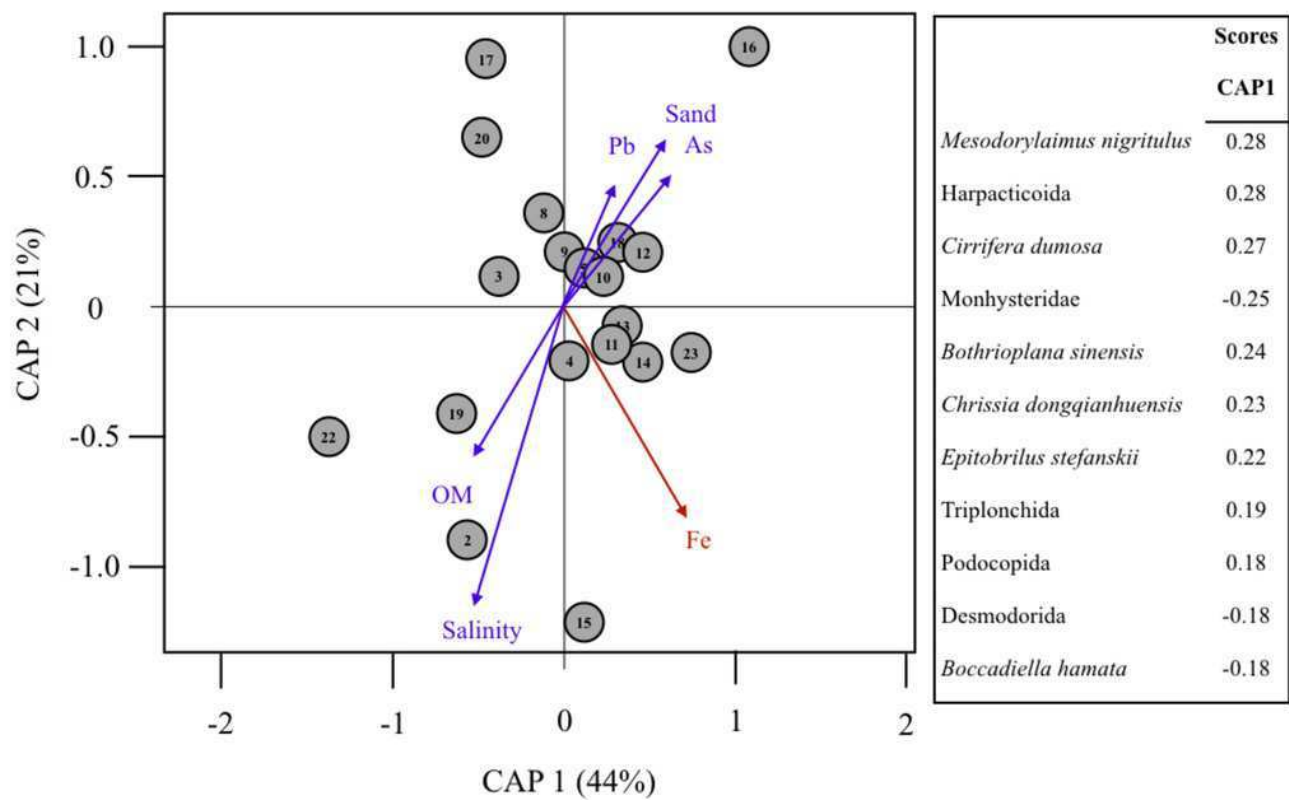
Table 1(on next page)

Sediment, eDNA and environmental variables in the Rio Doce estuary

Salinity, Sediment total organic matter (TOM, %), particle size (% sand), concentration of trace metals (Fe, As and Pb), and Number of sequence variant reads (reads SV) and richness per station. All data sampled in August 2017 or 1.7 years after the Samarco disaster. Trace metals averaged from N=2 replicates (SE). N. number of eDNA replicate samples sequenced per station. Reads SV. Total marine/aquatic meiofaunal sequence variants.

1 *Table 1. Salinity, Sediment total organic matter (TOM, %), particle size (% sand), concentration*
2 *of trace metals (Fe, As and Pb), and Number of sequence variant reads (reads SV) and richness*
3 *per station. All data sampled in August 2017 or 1.7 years after the Samarco disaster. Trace metals*
4 *averaged from N=2 replicates (SE). N. number of eDNA replicate samples sequenced per station.*
5 *Reads SV. Total marine/aquatic meiofaunal sequence variants.*
6

Station (N)	Salinity	TOM (%)	%sand	Fe (mg.kg^{-1})	As (mg.kg^{-1})	Pb (mg.kg^{-1})	Reads SV	Total SV richness
2 (2)	1.0	6.2	12	42,343 (2,468)	2.3 (0.1)	56.9 (4.8)	256,072	32
3 (2)	0.6	16.8	72	41,808 (1,278)	10.1 (14)	77.8 (2.7)	265,363	33
4 (2)	0.3	2.1	90	33,681 (2,429)	4.5 (1.6)	173.3 (7.8)	359,718	40
5 (2)	0.3	2.2	95	28,710 (3,686)	1.6 (2.2)	115.4 (2.8)	293,669	51
7 (2)	0.4	2.1	64	36,142 (134)	0.1 (0.2)	74.5 (7.5)	101,127	23
8 (2)	0.2	1.5	96	21,419 (3,212)	0.1 (0)	134.8 (5.8)	238,735	39
9 (2)	1.0	1.9	91	28,155 (1,391)	28.8 (34.3)	111.4 (40.7)	254,335	44
10 (2)	0.2	3.5	89	27,184 (227)	0.1 (0)	83.1 (5.9)	226,548	35
11 (2)	0.2	5.2	70	43,116 (2,768)	0.1 (0)	67.3 (4.3)	272,299	34
12 (1)	0.1	2.4	84	39,029 (11,713)	13.3 (16.2)	174.4 (28)	132,722	54
13 (2)	0.2	13.8	91	54,983 (4,157)	3.9 (5.5)	117.3 (12.9)	236,707	53
14 (1)	1.6	2.4	86	27,920 (7,793)	0.1 (0)	30.3 (11.8)	71,648	40
15 (2)	3.7	6	85	34,532 (1,980)	16.7 (15.6)	78.2 (4.6)	320,192	41
16 (1)	0.3	3.9	90	31,539 (1,001)	0.0 (0)	33.1 (2.1)	35,915	44
17 (2)	0.1	1.7	90	21,191 (42)	2.1 (0.2)	192.9 (15.1)	54,355	50
18 (2)	0.2	3.2	88	37,781 (1,120)	11.2 (2.7)	160.8 (6.2)	222,481	46
19 (1)	0.4	2.3	93	36,244 (801)	3.7 (0.5)	118.0 (3.5)	103,987	31
20 (1)	1.9	1.9	62	18,814 (94)	0.1 (0)	14.2 (1.5)	139,712	29
22 (1)	1.3	10.2	91	24,501 (3,804)	0.1 (0)	16.0 (6.9)	69,713	16
23 (1)	0.3	2.5	89	44,506 (1,079)	4.9 (2.2)	99.1 (7.6)	133,700	26

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Table 2(on next page)

Results of the canonical analysis of principal coordinates

Results of the Canonical Analysis of Principal coordinates (CAP) testing the contribution of sediment (TOM%, sand content), water salinity and concentrations of trace metals in sediments (As, Fe, Pb) to the multivariate distribution of meiofaunal (eDNA) assemblages in the samples from Rio Doce estuary. Spearman correlation values for each sediment variable are described for in CAP axis 1-2. Note: proportion of variability explained by CAP axes are highlighted, F for statistic, significant results ($p < 0.05$) are in bold.

Table 2. Results of the Canonical Analysis of Principal coordinates (CAP) testing the contribution of sediment (TOM%, sand content), water salinity and concentrations of trace metals in sediments (As, Fe, Pb) to the multivariate distribution of meiofaunal (eDNA) assemblages in the samples from Rio Doce estuary. Spearman correlation values for each sediment variable are described for in CAP axis 1-2. Note: proportion of variability explained by CAP axes are highlighted, F for statistic, significant results ($p < 0.05$) are in bold.

	All eOTUS (N=123)				Dominant eOTUS (N=32)			
	axis1 0.33	axis2 0.29	F	*p*	axis1 0.44	axis2 0.21	F	*p*
Salinity	-0.63	0.09	1.24	0.223	-0.30	-0.71	1.60	1.77
OM	-0.55	-0.18	0.89	0.485	-0.33	-0.36	0.71	0.592
Sand	0.71	0.26	1.01	0.413	0.37	0.40	1.17	0.272
As	0.02	0.26	0.63	0.815	0.39	0.31	0.79	0.506
Fe	-0.29	0.46	1.45	0.135	0.43	-0.51	2.89	**0.018**
Pb	0.70	-0.08	1.11	0.303	0.19	0.28	1.59	0.160