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1 **Seasonal influence of parasitism on contamination patterns of the**
2 **mud shrimp *Upogebia cf. pusilla* in an area of low pollution**

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12 **Abstract**

13 Very few studies have characterized the concentrations of pollutants in bioturbating species.
14 These species are considered as ecosystem engineers and characterizing stressors, such as
15 contaminants, that impact them could lead to a better understanding of the functioning of ecosystems.
16 In addition to contaminants, bioturbators are affected by a wide range of stressors, which can influence
17 their physiological status and their ability to accumulate pollutants. Among these stressors, parasitism
18 is of particular concern due to the ubiquity of parasites in natural environments and their influence on
19 the fitness of their host. This study aims to assess the relationship between parasitism and metal
20 accumulation in the bioturbating mud shrimp *Upogebia cf. pusilla*. A one-year seasonal survey was
21 conducted in Arcachon Bay, France, with the aims of (1) characterizing the levels of metals in the mud
22 shrimp and (2) evaluating the influence of two macroparasites (a bopyrid isopod and a trematode) on
23 the variation of the metal content in mud shrimp. The bopyrid parasite castrates its female host and a
24 particular attention has therefore been paid to the reproductive cycle of female mud shrimp by
25 quantifying the expression of the *vitellogenin* gene that encodes the major yolk protein in female
26 crustaceans. The levels of contaminants in mud shrimp appeared low compared to those reported in
27 other crustaceans in areas of higher pollution. Even at these low contamination levels, we observed a
28 significant impact by the bopyrid parasite that depends on season: bopyrid-infested organisms are
29 generally more contaminated than their uninfested conspecifics except in summer when the opposite
30 trend was observed. We suggest that the bopyrid indirectly interferes with the metal accumulation
31 process by altering the reproductive capabilities of the mud shrimp. On the opposite, very low
32 influence of the trematode parasite on the metal content of the host was found.

33 **Key words:** mud shrimp; parasites; metal contamination; seasonal fluctuation; reproductive cycle

34 **1. Introduction**

35 Despite their relative small size and discretion, parasites are widespread in natural
36 environments, representing as much as 40 % of the known animal species (Dobson et al., 2008). These
37 abundant organisms play a key role in the fitness of their hosts, modifying their physiological status
38 (McLaughlin and Faisal, 1998; Stier et al., 2015), fecundity (Lauckner, 1980; Mautner et al., 2007),
39 behaviour (Pascal, 2017; Thomas and Poulin, 1998) and survival (de Montaudouin et al., 2003; Jensen
40 and Mouritsen, 1992). In estuarine systems, parasites contribute substantially to biomass and
41 production within the ecosystem (Kuris et al., 2008; Thieltges et al., 2008). Parasitism can also shape
42 the community structure of free-living organisms (Minchella and Scott, 1991; Mouritsen and Poulin,
43 2002; Poulin, 1999). For instance, by infesting ecosystem engineer species (Jones et al., 1994),
44 parasites can interfere with functional traits of their host involved in their engineering functions. In
45 this way, parasites themselves can be considered ecosystem engineers (Dairain et al., 2019; Thomas et
46 al., 1999).

47 In marine benthic ecosystems, bioturbation is a typical example of ecosystem engineering
48 (e.g., Levinton, 1995; Mermillod-Blondin and Rosenberg, 2006; Meysman et al., 2006). The process
49 of bioturbation is described as any modification of the sediment matrix and interstitial water fluxes due
50 to the activities of organisms that mainly reside in or on the substratum (Kristensen et al., 2012). The
51 influence of bioturbators as ecosystem engineers depends on the magnitude of their bioturbation and,
52 inherently, on their well-being. Parasites could impair the physiological status and/or modify the
53 behaviour of bioturbators, and, by cascade effects, interfere with the roles of hosts in ecosystem
54 functioning (Thomas et al., 1999).

55 In addition to parasitism, bioturbators are exposed to several other biotic and abiotic stressors
56 which influence the intensity of their bioturbation activities (e.g., Diaz and Rosenberg, 1995; Duport et
57 al., 2006; Ouellette et al., 2004). Among these stressors, pollutants are of major concern. Littoral
58 environments in particular are endangered by anthropogenic inputs of contaminants, leading to high
59 levels of contamination in localized areas (e.g., Pan and Wang, 2012; Tueros et al., 2009). Initially
60 present in the water column, contaminants precipitate as solids or are adsorbed to suspended particles

61 on the seafloor bottom (Förstner and Wittmann, 1981). Consequently, coastal environments constitute
62 an important sink for a wide variety of contaminants which have a large range of detrimental effects
63 on bioturbating organisms (e.g., Bat et al., 1998; Chapman and Fink, 1984; Moreira et al., 2006).

64 Over the last two decades, an increasing number of studies has investigated the link between
65 parasitism and pollution in aquatic organisms. Both stressors likely act together with additive,
66 synergistic or antagonistic effects on the health of organisms (Marcogliese and Pietrock, 2011; Sures,
67 2008). Parasites can interfere with pollutant accumulation processes, increasing or decreasing the
68 levels of contaminants in organisms (Baudrimont and de Montaudouin, 2007; Dairain et al., 2018b).
69 Parasites can also modulate the detoxification responses of their host to pollutants (Gismondi et al.,
70 2012; Sures and Radszuweit, 2007). Finally, parasites accumulate contaminants (Bergey et al., 2002;
71 Siddall and Sures, 1998) with potential toxic effects on parasites themselves. For instance, the free-
72 living stages of several parasites show reduced infectivity or increased mortality rates when exposed to
73 pollutants (Morley et al., 2003; Pietrock and Marcogliese, 2003). The negative effects of contaminants
74 on parasites may benefit parasitized organisms if they are more harmful to the parasites than to the
75 hosts.

76 Most of these studies are experiment-based, and few environmental surveys have been
77 conducted with the aim of characterizing any correlation between contaminant levels and parasitism
78 (Cross et al., 2003; de Montaudouin et al., 2010; Kim et al., 2008, 1998). Furthermore, almost none of
79 these studies focused on bioturbating species. Here, we evaluate the influence of parasitism on
80 environmental levels of metals in the bioturbating mud shrimp *Upogebia cf. pusilla*. A one-year
81 seasonal survey was initiated in fall 2016 in Arcachon Bay (France) at two sites with contrasting
82 trematode parasite pressure (Dairain et al., 2017), in order to assess the influence of parasitism and
83 season, as well as their interactive effects, on the accumulation of metals in mud shrimp. Potential
84 seasonal effects are likely related to changes in the physiology of organisms, especially with regards to
85 gametogenesis (e.g., Boyden and Phillips, 1981; Fattorini et al., 2008; Páez-Osuna et al., 1995).
86 Therefore, particular attention has been paid to the reproductive status of organisms, that was
87 evaluated by quantifying the expression of the vitellogenin (*vlg*) gene.

88 **2. Materials and methods**

89 **2.1. Biology of *Upogebia cf. pusilla* and its parasites**

90 The gebiidean mud shrimp *Upogebia cf. pusilla* is a gonochoric crustacean decapod with a life
91 span estimated at > 5 years (Dworschak, 1988). It occurs in intertidal and upper sublittoral zones along
92 the Northeast Atlantic and Mediterranean coasts (de Saint Laurent and Le Loeuff, 1979; Dworschak,
93 1983). In Arcachon Bay (France), *U. cf. pusilla* preferentially inhabits intertidal seagrass meadows
94 where densities can reach 39 ind. m⁻². It lives in a deep (up to 49 cm) and complex burrow connected
95 to the sediment-water interface by several distant openings (Pascal, 2017). The fossorial life style of
96 these organisms is associated with a large sediment reworking activity as well as important
97 bioirrigation (Pascal, 2017). Therefore, *U. cf. pusilla* plays a key role on the physical structure of
98 sediments and greatly influences oxygen and nutrient fluxes at the sediment-water interface (Pascal et
99 al., 2016b; Pascal, 2017). Only one individual inhabits a burrow, except during the breeding period
100 when two adult mud shrimp have been found to occupy the same burrow (personal observation). In
101 Arcachon Bay, females can reproduce after reaching a total length (TL) of approximately 31 mm, and
102 are ovigerous in May–August.

103 The crustacean bopyrid *Gyge branchialis* is a parasite of *U. cf. pusilla* (Bonnier, 1900;
104 Tucker, 1930) which lives in one (or very rarely both) of the two gill chambers of its host (Pascal et
105 al., 2016a). The bopyrid settles early in the mud shrimp's life, grows with its host and finally occupies
106 most of the space in the gill chamber (Pascal et al., 2016a; Tucker, 1930). This ectoparasite disrupts
107 the mud shrimp's reproduction: females are castrated (at least never ovigerous), males develop female
108 secondary sexual characteristics and their gonads are atrophied (Pascal et al., 2016a; Tucker, 1930).
109 The parasite also impairs the levels of activity of its host (Pascal, 2017).

110 The mud shrimp *U. cf. pusilla* is also parasitized by the digenean trematode *Maritrema* sp..
111 This parasite infects the gills, the visceral mass and the abdominal muscle of its host (Dairain et al.,
112 2017). The trematode occurs as metacercariae in the mud shrimp, meaning the mud shrimp is the
113 second intermediate host of the parasite. The effects of this parasite on *U. cf. pusilla* have never been
114 documented. Bopyrid and trematode parasites are negatively associated within their host. This pattern

115 is likely due to the bopyrid parasite interfering with trematode infection *via* alteration of the fitness of
116 the host and associated filtration activity (Dairain et al., 2017; Pascal, 2017).

117 **2.2. Study sites and sampling strategy**

118 Located on the French Atlantic coast, Arcachon Bay (44°42' N, 1°11' W) is a 180-km²
119 macrotidal lagoon opening to the ocean through two channels (Fig. 1). The tidal cycle is semi-diurnal
120 with an amplitude of 0.8–4.6 m. Mean sea surface temperature changes from 6 °C in winter to 22.5 °C
121 in summer. In 2007, 39 % of the 110-km² intertidal area was covered by the dwarf eelgrass *Zostera*
122 *noletii* (Plus et al., 2010).

123 We initiated a one year seasonal sampling survey beginning fall 2016 at two intertidal sites
124 (“La Réousse” and “Le Teychan”) in Arcachon Bay (Fig. 1, Table 1). Both sites harbour population of
125 *U. cf. pusilla* with a significant proportion infested with the bopyrid isopod *G. branchialis* (Pascal et
126 al., 2016a). These two sites were selected based on differences in trematode infection (*Maritrema* sp.).
127 Trematodes are largely prevalent in mud shrimp at both sites, but organisms inhabiting La Réousse
128 show 6-fold higher levels of infection (mean number of parasites per host) with metacercariae than
129 specimens at Le Teychan (Dairain et al., 2017). For each site and at each season, 20 bopyrid-infested
130 and 20 bopyrid-uninfested individuals (half males and half females) were sampled using a bait piston
131 pump. The occurrence of the bopyrid parasite can easily be determined in the field as it causes a large
132 swelling of one of the two gill chambers of its host. The sex of the mud shrimp was determined as
133 follows: females have a gonopore on each coxae of the 3rd pereopod and have a first pair of pleopods
134 while males show neither of these two characteristics. Once collected, each specimen was isolated in a
135 plastic bag and kept on ice before immediate laboratory processing (see below). In parallel, sediment
136 samples were collected for quantification of metals (Ag, As, Cd, Cu, Fe, Mn, Ni, and Zn), except
137 during fall 2016. For this purpose, three sediment cores were taken at each site with 30-cm long
138 Plexiglas tubes. The three samples were combined and considered representative of the sampling site.
139 Surface sediments (ca. 0–2 cm depth) were differentiated from deeper sediments (ca. 18–20 cm depth).
140 Samples were placed in plastic bags and frozen (- 20 °C) once back at the laboratory.

141 **2.3. Dissection**

142 Each *U. cf. pusilla* was measured from the tip of the rostrum to the extremity of the telson
143 (Total Length, TL) using digital callipers. The measured size was rounded down to the nearest mm.
144 The gill infection caused by the trematode parasite is a proxy for the whole body infection (Dairain et
145 al., 2017). The abundance of trematodes was therefore determined by exclusively counting parasites
146 found in the gills of mud shrimp. The gills were extracted, squeezed between two transparent glass
147 plates and the metacercariae were counted under a stereomicroscope (Nikon, SMZ1500). The term
148 'infestation' was used for the ectoparasite (bopyrid) and the term 'infection' was used for the
149 endoparasite (trematode). Finally, the hepatopancreas of the mud shrimp was removed for metal and
150 genetic analyses (see below). Since the mass tissue was small, the hepatopancreases of two mud
151 shrimp of the same site, sex and TL were combined. Thus, metal and genetic analyses on five
152 replicates for male and female bopyrid-infested and bopyrid-uninfested mud shrimp ($N = 20$) were
153 performed for each season and each site. For the genetic analysis, a portion of the pooled
154 hepatopancreases was placed in an RNA-later buffer and were kept at $-20\text{ }^{\circ}\text{C}$ until RNA extraction
155 was performed. The remaining tissue was used for metals quantification. Thus, the survey consisted of
156 160 samples: 2 sites (La Réousse and Le Teychan) x 4 seasons x 2 parasitic status (bopyrid-uninfested
157 vs. bopyrid-infested) x 2 sexes (males and females) x 5 replicates.

158 **2.4. Metal determination in biological samples**

159 The biological samples were dried (at least 48 h at $45\text{ }^{\circ}\text{C}$), weighted and placed in
160 polypropylene tubes. They were then digested with nitric acid (1 to 3 mL depending on samples dry
161 weight DW, HNO_3 65 %; Carlo Erba Reagents) at $100\text{ }^{\circ}\text{C}$ for 3 h (HotBlock; Environmental Express).
162 Ultrapure water (Milli-Q) was added after cooling to dilute samples (5 to 15 mL depending on the
163 volume of nitric acid used to digest the tissue). Metal content in the hepatopancreas of mud shrimp
164 was measured by ICP-OES (700 Series ICP-OES, Agilent). In each analytical batch method blanks
165 and certified reference materials (DOLT-5, dogfish liver, and TORT-3, lobster hepatopancreas;
166 NRCC-CNRC) were included and treated and analysed in the same way as the biological samples.
167 Detection limits and recovery rates are given in Appendix A. Metals concentrations in the biological
168 samples were expressed in $\mu\text{g g}^{-1}$ DW.

169 **2.5. Metal determination in sediment samples**

170 Sediments samples were freeze-dried and ca. 30 mg DW was added to the polypropylene
171 tubes. Samples were digested with 1.5 mL HCl (12 M, Merck), 0.75 mL HNO₃ (14 M, Merck) and 2
172 mL HF (22 M, Merck) at 110 °C for 2 h (DigiPREP, SCP Sciences). After complete evaporation, the
173 residues were dissolved in 0.25 mL HNO₃ (14M, Merck) and 5 mL of ultrapure water (Milli-Q),
174 heated to 65 °C for 15 min and brought to 10 mL in volumetric flasks using ultrapure water (Milli-Q)
175 once cooled. Metal concentrations were determined using ICP-MS (XSeries, Thermo Scientific),
176 except Fe and Mn which were determined using ICP-OES (700 Series ICP-OES, Agilent). Certified
177 reference materials (RM 8704 and BCR-277R, NIST) were used in order to check the quality of the
178 analytical method. Detection limits and recovery rates are given in Appendix A.

179 **2.6. Expression dynamics of the *vtg* gene**

180 Potential variations of metal content in mud shrimp were compared with variations in the
181 expression of the vitellogenin gene (*vtg*). This gene encodes for the vitellogenin protein, a precursor of
182 the major yolk protein in female crustaceans (i.e. the vitellin). The expression dynamics of *vtg* depends
183 on the sex of crustaceans, fluctuates with the reproductive cycle (and therefore the season) (Thongda
184 et al., 2015; Wilder et al., 2010) and may be modulated by bopyrid parasites because of their
185 significant effects on the reproductive function of their host (Pascal et al., 2016a; Tucker, 1930).
186 Bopyrid-infested females are castrated (Tucker, 1930) and it may be that the expression of *vtg* is
187 downregulated in these organisms compared with bopyrid-uninfested females, especially during the
188 reproductive period (spring and summer). We expected that the level of expression of *vtg* in bopyrid-
189 uninfested males is lower than in their female counterparts. It may also be that the expression of *vtg* is
190 enhanced in bopyrid-infested males compared with bopyrid-uninfested organisms since bopyrid
191 parasites feminize their male hosts (Pascal et al., 2016a; Tucker, 1930). Finally, the expression of *vtg*
192 could be highly impaired by metal contamination since some contaminants act as endocrine disruptors
193 (Hannas et al., 2011; Park et al., 2014; Rodríguez et al., 2007).

194 *2.6.1. Total RNA extraction and reverse transcription*

195 Total RNAs were extracted from the hepatopancreas of the mud shrimp using the “SV Total
196 RNA Isolation System” kit (Promega; Fitchburg, WI, USA) according to the manufacturer-
197 recommended protocol. The quality of RNA extractions as well as RNA concentrations were assessed
198 by spectrophotometry (Spectro Multivolume Epoch; BioTek). Immediately following total RNA
199 extractions, reverse transcriptions of RNA (RT) were performed. First-strand cDNA were synthesized
200 using the “GoScript Reverse Transcription System” kit (Promega) according to the manufacturer’s
201 instructions. RT reactions were performed from 1 µg of total RNA. The cDNA mixture was stored at -
202 20 °C pending real-time PCR reactions.

203 2.6.2. Real-time PCR

204 A partial sequence of the gene *vtg* was amplified using real-time PCR using forward and
205 reverse primers previously described by Dairain et al. (2018a) . The amplification of cDNA was
206 monitored using the DNA intercalating dye SybrGreen I. Real-time PCR reactions were carried out in a
207 total volume of 20 µL consisting of 10 µL GoTaq® qPCR Master Mix (2X; Promega), 2 µL of a mix of
208 the primer pairs at a final concentration of 2 µM for each primer, 5 µL of a ten-time diluted cDNA
209 mixture and 3 µL of nuclease-free water. Real-time PCR were performed in an Mx3000P (Stratagene).
210 Cycling conditions were as follows: 1 cycle of initial activation at 95 °C for 10 min; followed by 45
211 cycles at 95 °C for 30 s, 60 °C for 30s and 72 °C for 30 s. Reaction specificity was determined from
212 the dissociation curve of the PCR product. This dissociation curve was obtained by following the
213 SybrGreen I fluorescence level during a gradual heating of the PCR products from 65 to 95 °C. The
214 relative quantification of *vtg* expression was normalized according to the elongation factor 1-alpha
215 (*efl-α*) gene expression. This reference gene had a constant level of expression during all seasons,
216 between the two sites, in bopyrid-infested and uninfested mud shrimp, and between males and
217 females. The $2^{-\Delta Ct}$ method (Livak and Schmittgen, 2001) was used to generate relative mRNA
218 expression.

219 2.7. Statistical analyses

220 2.7.1. Univariate analyses

221 The abundance of trematodes was defined as the mean number of metacercariae per sampled
222 organism, taking into account both trematode-infected and uninfected mud shrimp (Bush et al., 1997).
223 Differences in the abundance of trematodes in mud shrimp between the two sampling sites was
224 assessed using a univariate PERmutational Multivariate ANalyses Of VAriances (PERMANOVA)
225 (Anderson, 2001; McArdle and Anderson, 2001) without data transformation. Euclidean distance was
226 used and the design consisted of one single “site” factor (2 levels).

227 At each site, differences in the abundance of trematodes in mud shrimp and in the relative
228 expression of *vtg* in the hepatopancreas of organisms were assessed according to season, bopyrid
229 occurrence and the sex of mud shrimp by carrying out PERMANOVAs on the basis of Euclidean
230 distances on untransformed data. The design consisted of three crossed factors, namely “Season”
231 (fixed, 4 levels), “Bopyrid occurrence” (fixed, 2 levels) and “Sex” (fixed, 2 levels). Pairwise tests were
232 also performed to highlight differences among factor modalities.

233 To get an overview of metal concentrations in mud shrimp at each site and depending on
234 season and bopyrid occurrence, the average concentration of each metal was calculated. Additionally,
235 the metal uptake in mud shrimp at each site depending on season and bopyrid occurrence was assessed
236 using bioconcentration factors. Bioconcentration factors were calculated by dividing the concentration
237 of each metal present in the hepatopancreas of organism by the concentration of that element found in
238 the sediment of the sampling site ($BCF = C_{\text{organism}}/C_{\text{sediment}}$). The median of the concentration of metal
239 in the sediment between concentrations in the sediment surface (0–2 cm) and deeper in the sediment
240 column (18–20 cm) was used. We tested for differences in metal concentrations in mud shrimp and
241 bioconcentration factors depending on season and bopyrid occurrence using Wilcoxon test.

242 2.7.2. *Multivariate analyses*

243 The differences between multivariate metal content patterns in the hepatopancreas of mud
244 shrimp amongst seasons, bopyrid occurrence and sex were explored by carrying out PERMANOVAs
245 on normalized data on the basis of Euclidean distances for each site. The design consisted of three
246 crossed factors, namely “Season” (fixed, 4 levels), “Bopyrid occurrence” (fixed, 2 levels) and “Sex”
247 (fixed, 2 levels). Pairwise tests were also performed to highlight differences among factor modalities.

248 A principal coordinates analysis (PCO) based on Euclidean distances was used to visualize the results.
249 Finally, the potential relationship between the multivariate metal content pattern in the hepatopancreas
250 of bopyrid-uninfested mud shrimp and the abundance of trematode parasites in organisms (predictor
251 variable) was tested for using a multivariate regression analysis (DISTLM) with distance-based
252 redundancy analysis (dbRDA) (Anderson et al., 2008).

253 Results are reported as the mean \pm SE (standard error) of N replicate measurements. We used
254 the PRIMER® v6 package with the PERMANOVA+ add-on software to perform PERMANOVAs,
255 DISTLMs and associated tests (Anderson et al., 2008). Differences were considered significant for $p <$
256 0.05.

257 **3. Results**

258 Female and male mud shrimp collected at La Réousse and Le Teychan were similar in size,
259 with $TL = 45.8 \pm 0.4$ mm for female mud shrimp and $TL = 45.6 \pm 0.4$ mm for male organisms.

260 **3.1. Trematode infection and influence of the bopyrid parasite**

261 The number of trematodes in the gills of *Upogebia cf. pusilla* ranged from 0–305 and 0–13
262 metacercariae per mud shrimp at La Réousse and Le Teychan respectively. On average, mud shrimp
263 from La Réousse harboured 35 times more metacercariae in their gills than organisms from Le
264 Teychan (1-way PERMANOVA, Pseudo-F = 102.7, $P(\text{perm}) = 0.0001$) (Fig. 2).

265 At La Réousse, bopyrid-infested organisms harboured fewer trematode parasites in the gills
266 than their bopyrid-uninfested conspecifics (Fig. 2, Table 2). Conversely, there was no significant
267 influence of the season nor of the sex of mud shrimp on the number of metacercariae in mud shrimp,
268 while there was a significant interaction between the season, the bopyrid parasite and the sex of mud
269 shrimp on the abundance of trematodes in organisms sampled at La Réousse (Fig. 2, Table 2).

270 At Le Teychan, bopyrid-infested specimens harboured significantly fewer trematodes in the
271 gills than their bopyrid-uninfested conspecifics (Fig. 2, Table 2). Mud shrimp sampled in fall
272 harboured a higher abundance of trematodes in the gills than organisms sampled over the other
273 seasons (Fig. 2, Table 2). There were no significant influence of the sex of mud shrimp and no
274 significant interaction between the season, the bopyrid parasite and the sex of mud shrimp on the
275 abundance of trematodes in organisms sampled at Le Teychan (Table 2).

276 **3.2. Concentrations of metals in the sediment**

277 Due to lack of replication, the significance of differences of sediment metal concentrations
278 relative to the season or the depth in the sediment column could not be tested. Thus, changes in metal
279 contents in the sediment were assessed qualitatively (Table 3). At La Réousse, there were few
280 differences in metal concentrations in the sediment according to season and depth in the sediment
281 column, except for Ag and Mn (Table 3). For these two metals, concentrations were markedly higher
282 in the upper part of the sediment column than deeper in the sediment column (Table 3). At Le
283 Teychan, the concentrations of several metals were higher in spring than in winter and summer (Ag,

284 Cd, Cu and Zn). There were also some differences in metal concentrations in the sediment according
285 to depth in the sediment column but without consistent pattern (Table 3). Annual average
286 concentrations of metals in sediments at both sites were calculated and showed that sediments at Le
287 Teychan were 1.6 to 4.5 times more contaminated than at La Réousse (Table 3).

288 **3.3. Metal content in mud shrimp**

289 Overall, the concentrations of metals were low in the hepatopancreas of mud shrimp sampled
290 at La Réousse and Le Teychan (Table 4). Nonetheless, there was a significant bioconcentration of
291 several metals (Ag, As, Cd, Mn and Ni) in the hepatopancreas of mud shrimp at both sites (Appendix
292 B). At La Réousse, there was an overall higher bioconcentration of Ag, As, Cu, Mn, Ni and Zn in
293 bopyrid-infested organisms than in uninfested mud shrimp, with rare inverse cases (As, Mn and Ni in
294 summer). Bopyrid infestation had few effects on Cd and Fe bioconcentration in mud shrimp at La
295 Réousse. The effect of bopyrid infestation on metal bioconcentration in mud shrimp was negligible at
296 Le Teychan (Table 4).

297 *3.3.1. Multivariate analyses*

298 At La Réousse, significant differences in metal content between mud shrimp were found and
299 depended mainly on the presence of the bopyrid parasite and, to a lesser extent, on the season, with a
300 significant interaction between both factors (Table 5). PERMANOVA pair-wise tests showed that
301 metal content in mud shrimp differed according to the season in bopyrid-infested and bopyrid-
302 uninfested organisms except during spring (Table 6, Appendix C). Fig. 3A shows the PCO ordination
303 graph based on results obtained with PERMANOVA. The first axis explained 51.7 % of the inertia
304 and the second axis 14.5 %. The concentration of As, Cu, Ni and Zn were negatively correlated with
305 Axis 1. The first axis discriminated mud shrimp according to the bopyrid parasite occurrence and the
306 season. Bopyrid-infested mud shrimp were negatively associated with the first axis of the PCO, except
307 during summer when the opposite trend is noticed (Fig.3A, Appendix C).

308 At Le Teychan, there were significant differences in metal content between mud shrimp
309 according to the season and, to a lesser extent, according to bopyrid occurrence and sex of the
310 organism, with a significant interaction between these three factors (Table 5). PERMANOVA pair-

311 wise tests showed a marked effect of season on metal content in bopyrid-uninfested females and males
312 (Table 7). Conversely, there was no significant influence of season on metal content in bopyrid-
313 infested males. With regards to bopyrid-infested females, a significant difference in metal content was
314 only observed in organisms sampled in spring and summer compared with specimens sampled in fall
315 (Table 7, Appendix D). The metal contents were different between bopyrid-infested and uninfested
316 females that were sampled in summer, while there was no difference between bopyrid-infested and
317 uninfested males. Finally, metal content was significantly different between bopyrid-uninfested males
318 and females sampled in fall and summer, while no significant differences between bopyrid-infested
319 males and females were found in the other seasons. The first axis of the PCO explained 36.7 % of the
320 inertia and the second axis 22.6 % (Fig. 3B). The concentrations of Cd, Cu, Fe, Mn and Zn were
321 negatively correlated with Axis 1. None of the two axes of the PCO allowed for a distinct separation of
322 mud shrimp according to their sex, parasitic status or season. Nonetheless, it seemed that bopyrid-
323 uninfested mud shrimp were more positively associated with the first axis than uninfested organisms,
324 independently of their sex, except in summer when bopyrid-infested females were negatively
325 associated with this axis (Fig. 3B, Appendix D).

326 3.3.2. Influence of the trematode parasite *Maritrema* sp.

327 Only 6.6 % of the total variation in multivariate metal content patterns in bopyrid-uninfested
328 mud shrimp sampled at La Réousse could be significantly explained by the abundance of the
329 trematode *Maritrema* sp. (DISTLM, $p = 0.050$). Conversely, there was no significant relationship
330 between the abundance of the trematode *Maritrema* sp. and the total variation in multivariate metal
331 content patterns in bopyrid-uninfested mud shrimp sampled at Le Teychan (DISTLM, $p = 0.91$).

332 3.4. Expression of the *vtg* gene

333 A significant influence of the sex of mud shrimp, bopyrid occurrence and season on the
334 relative expression of the *vtg* gene was noticed in organisms sampled at La Réousse and Le Teychan,
335 with a significant interaction between the three factors (Table 8, Fig. 4). In bopyrid-uninfested
336 organisms, the level of expression of *vtg* was higher in females than in males at both sites. In females,
337 a significant increase of the expression of *vtg* was noticed in summer (females were ovigerous at that

338 time) compared with fall and winter (Fig. 4). In females, the bopyrid parasite was associated with a
339 significant downregulation of the expression level of *vtg* and the influence of the season was low (Fig.
340 4). Overall, there was no influence of the bopyrid parasite on the expression of *vtg* at any of the
341 seasons in males. The only exception was bopyrid-infested males sampled in summer at La Réousse,
342 for which the expression of *vtg* was significantly enhanced, but with a high inter-individual variability
343 (Fig. 4).

344 **4. Discussion**

345 **4.1. Trematode infection and influence of the bopyrid isopod**

346 The two sampling sites were selected based on previous results showing that mud shrimp from
347 La Réousse are heavily infected with the trematode parasite *Maritrema* sp. compared with organisms
348 from Le Teychan (Dairain et al., 2017). A similar pattern was observed in this study. We also reported
349 that the abundance of trematodes in mud shrimp was influenced by the bopyrid parasite at both sites.
350 Bopyrid-infested organisms harbour fewer trematodes than bopyrid-uninfested specimens. These
351 results are in accordance with a previous study suggesting that the bopyrid parasite interferes with the
352 trematode infection process, probably by reducing the ventilation activity of its mud shrimp host
353 (Dairain et al., 2017).

354 **4.2. Concentrations of metals in sediments**

355 Metals in sediments displayed relatively low concentrations at both sites, with values
356 significantly inferior to the effects range-low thresholds determined by Long et al. (1995). The only
357 exception was As, for which the concentration in sediments from Le Teychan was 2.2 times the effects
358 range-low threshold (Long et al., 1995). The same tendency was documented by de Montaudouin et al.
359 (2016) in 2010–2012. This small As-enrichment of sediments is difficult to explain since Arcachon
360 Bay is not an industrialized area. However, the increase in human population densities in this area as
361 well as the intense anthropogenic pressure (agriculture, tourism and oyster farming) have been
362 associated with an increase in the release of pollutants into the lagoon over the last several years
363 (Tapie and Budzinski, 2018) and an increase in the levels of various contaminants in benthic
364 organisms (Oger-Jeanneret et al., 2016).

365 Sediments from Le Teychan displayed higher metal concentrations than those from La
366 Réousse. Le Teychan is closer to the main harbour of Arcachon Bay and also to the Leyre River,
367 which is the main input of freshwater into the lagoon (Manaud et al., 1997). Although industrial and
368 urban wastes have not been dumped into the lagoon since the 1970s', inputs of metals from freshwater
369 sources are possible because the Leyre River drains from a watershed dominated by urban and
370 agriculture areas. Particle size of sediments also affects the distribution of metals, with coarse

371 sediments harbouring reduced concentrations of metals compared with finer grain size sediments
372 (Horowitz, 1985). Sediments at La Réousse are coarser than at Le Teychan, which could contribute to
373 their lower concentrations of metals.

374 **4.3. Concentrations of metals in mud shrimp**

375 Concentrations of metals in the hepatopancreas of mud shrimp *Upogebia cf. pusilla* sampled at
376 two sites in Arcachon Bay, France, remained generally low over this one-year seasonal survey
377 compared to metal levels exhibited by other crustacean species sampled in areas of higher metal
378 pollution. For instance, goose barnacles *Pollicipes pollicipes* sampled along the northwest coast of
379 Portugal showed concentrations of Cd and Zn up to 10-fold higher than those reported in the mud
380 shrimp in Arcachon Bay (Reis et al., 2013). Similarly, shrimp *Palaemon elegans* in the commercial
381 harbour in Santa Cruz de Tenerife (Canary Islands) and its fishery dock dependency showed
382 concentrations of Ni 5-fold higher than those noticed over this survey in *U. cf. pusilla* (Lozano et al.,
383 2010). One of the most extreme example concerns the crustacean *Balanus balanoides* sampled in the
384 Huelva estuary (Spain), considered as one of the most polluted European estuary. This crustacean
385 species showed concentrations of metals 3 to 1000-fold higher, depending on the metal, than those
386 showed by the mud shrimp in Arcachon Bay (Morillo et al., 2005).

387 The concentrations of metals in mud shrimp were similar to levels documented over previous
388 field surveys conducted in Arcachon and focusing on the Manila clam *Ruditapes philippinarum* (de
389 Montaudouin et al., 2016), the common cockle *Cerastoderma edule* (de Montaudouin et al., 2010) or
390 the blue mussel *Mytilus* sp. (Devier et al., 2005). Nonetheless, there are some discrepancies between
391 these bivalves and the mud shrimp. For instance, the concentrations of Cu in mud shrimp are up to ca.
392 100-fold superior to those documented for the bivalves. Copper is an essential element but can be toxic
393 to organisms at relatively high concentration. As an essential element, Cu acts as a protein cofactor for
394 several enzymes or can be a key element in proteins without any enzymatic function, such as
395 hemocyanins which are the copper-dependent oxygen-carrying proteins of crustaceans. Thus, it is not
396 surprising to notice high levels of Cu in mud shrimp. However, hemocyanins have also been
397 evidenced in bivalves (Morse et al., 1986). While it is not clear if all bivalves have hemocyanins, it

398 might be suggested that the large difference of contamination with Cu between mud shrimp and
399 bivalves is related to diverse processes of Cu detoxification between the two clades.

400 Bioconcentration factors were calculated to evaluate the efficiency of metal accumulation in the
401 hepatopancreas of *U. cf. pusilla*. Some metals bioconcentrate in the hepatopancreas of mud shrimp
402 (Ag, As, Cd, Mn or Ni), while the bioconcentration factor of other remain extremely low (Cu, Fe and
403 Zn). This discrepancy may be due to differences of bioavailability between the different metals and/or
404 to different processes of detoxification. Indeed, the hepatopancreas is an important organ for metal
405 sequestration (Marsden and Rainbow, 2004) that also plays a key role in detoxification processes in
406 crustaceans (Ahearn et al., 2004). Even though mud shrimp bioconcentrate several metals, the
407 bioconcentration factors remain low compared to those reported in the crustacean *B. balanoides*
408 sampled in the Huelva estuary (Morillo et al., 2005). The only exception is As which is 4-fold more
409 bioconcentrated by the mud shrimp in Arcachon Bay than by *B. balanoides* in the Huelva estuary.

410 **4.4. Metals in mud shrimp: seasonal fluctuations**

411 We reported a significant effect of the season on the pattern of metal content in the mud
412 shrimp *Upogebia cf. pusilla*. In particular, the metal concentrations in organisms sampled in summer
413 largely differ from individuals sampled in other seasons. Such variations of metal content over the
414 seasonal cycle are common and have been reported for several invertebrates. For instance,
415 concentrations of Cu and Fe significantly fluctuated in periwinkles *Littorina littorea* from Northern
416 Ireland over a one-year survey, with a peak in concentration in spring for both metals (Cross et al.,
417 2003). In contrast, concentrations of metals in the blue mussel *Mytilus* sp. increased over the autumn-
418 winter period in Arcachon Bay (Devier et al., 2005). It has been suggested that seasonal variations in
419 the physiological processes of organisms, especially with regards to growth and reproduction, drive
420 the fluctuations in concentration of contaminants in these invertebrates (Boyden and Phillips, 1981;
421 Cain and Luoma, 1990, 1986; Fattorini et al., 2008; Páez-Osuna et al., 1995). For instance, changes in
422 metal concentrations in the bivalves *Limecola* (= *Macoma*) *balthica* were inversely correlated with
423 modifications in mass of the soft tissue of organisms (Cain and Luoma, 1986). In addition to growth,
424 the sexual cycle of organisms can govern changes in tissue weight. In oysters *Crassostrea gigas*, the

425 important development of gonadic tissues led to a “dilution” of metals in organisms. After spawning,
426 the body weight of organisms decreased while the concentration of contaminants increased (Boyden
427 and Phillips, 1981).

428 The cycling pattern of metal concentrations in oyster depending on their reproductive state has
429 been evidenced by determining concentrations of metals in the total body (Boyden and Phillips, 1981).
430 Here, only the accumulation of metals in the hepatopancreas of mud shrimp was quantified.
431 Nonetheless, great changes in the biochemical composition and in the weight of the hepatopancreas of
432 other crustacean species during the reproductive cycle were previously observed. During the breeding
433 period, there is a decrease in the weight and in the lipid content of the hepatopancreas, while the
434 opposite occurs in the gonads, especially in females (del R. Gonzalez-Baro and Pollero, 1988; Haefner
435 and Spaargaren, 1993; Pillay and Nair, 1973). This suggests that lipids could be transferred from the
436 hepatopancreas to gonads during the breeding period. In the hepatopancreas of crustaceans, metals are
437 complexed with metal-binding proteins and enclosed in lysosomes but generally not associated with
438 hydrophobic components such as lipids (Ahearn et al., 2004). Consequently, the decrease in the weight
439 of the hepatopancreas associated with the transfer of lipids to the gonadic tissue could induce an
440 increase of the concentration of contaminants in the hepatopancreas. In Arcachon Bay, gametogenesis
441 in *U. cf. pusilla* occurs in spring and reproduction in summer, with females ovigerous from June to
442 September. Thus, we suggest that the higher metal burden observed in the hepatopancreas in
443 organisms in summer is related to a decrease of the weight of organisms governed by their
444 reproductive cycle similarly to what was observed in molluscs such as oysters. Finally, it should not be
445 excluded that seasonal fluctuations in metal content in mud shrimp may be related to changes in
446 climatic conditions influencing the inputs of metals in the environment as well as their bioavailability
447 in sediments. In particular, the bioavailability of contaminants to marine organisms depends on their
448 speciation, which is determined by the physicochemical characteristics of the water and the sediment
449 column (pH, salinity, etc) (Förstner and Wittmann, 1981; Mason, 2013). For instance, Fowler and
450 Oregoni (1976) observed that the highest levels of contamination in mussel *M. galloprovincialis*
451 coincided with a period of important precipitations and runoffs. They suggested that this would

452 increase the quantity of suspended matter and thus the concentrations of metals in soluble and
453 particulate forms.

454 **4.5. Influence of parasitism on metal contamination over a seasonal cycle**

455 The main aim of this study was to investigate the relationship between the parasitic pressure
456 and the levels of metals in the mud shrimp *U. cf. pusilla* over a one year survey, thus taking into
457 account the reproductive cycle of the host. In Arcachon Bay, mud shrimp host two parasite species:
458 the bopyrid isopod *Gyge branchialis* and the trematode *Maritrema* sp.. Both trematode and bopyrid
459 parasites have a substantial influence on contaminant accumulation in their host (Bergey et al., 2002;
460 Paul-Pont et al., 2010b, 2010a; Williamson et al., 2009). For instance, the bopyrid parasites
461 *Probopyrus pandalicola* and *Gyge branchialis* reduce the accumulation of inorganic contaminants in
462 their hosts, the grass shrimp *Palaemonetes pugio* and the mud shrimp *U. cf. pusilla* (Bergey et al.,
463 2002; Dairain et al., 2018b). Trematodes also modulate the sensibility of their hosts to pollutants (e.g.,
464 Baudrimont and de Montaudouin, 2007; Paul-Pont et al., 2010b). While most of these studies deal
465 with experimental work, few have attempted to investigate the influence of parasites on the process of
466 contaminant bioaccumulation in a realistic ecological context (de Montaudouin et al., 2016, 2010; Kim
467 et al., 1998; Powell et al., 1999). These studies evidenced various relationships between parasites and
468 their host's contaminant burden, depending on the type of pollutants (organic contaminants vs.
469 metals), the type of parasites (micro vs. macroparasites) and the location of the study.

470 Our study reported a very low relationship between the metal burden in the mud shrimp *U. cf.*
471 *pusilla* and the abundance of the trematode *Maritrema* sp. metacercariae in organisms sampled at La
472 Réousse whereas no significant relationship was noticed in mud shrimp at Le Teychan. Literature
473 evaluating the influence of trematodes on the contamination burden of their host is scarce. Studies that
474 did investigate host-parasite associations have generally involved a molluscan host and a trematode
475 parasite, with the mollusc acting as the first intermediate host (Cross et al., 2003; Evans et al., 2001;
476 Kim et al., 2008, 1998), i.e. a stage reputed to be much more detrimental for the host than the
477 metacercarial stage (Lauckner, 1983, 1980). Hence comparisons of our results with these studies are
478 irrelevant.

479 At both sites, we reported a significant influence of the bopyrid parasite *G. branchialis* on the
480 metal content of its mud shrimp host, with bopyrid-infested organisms being generally more
481 contaminated than their uninfested conspecifics. This discrepancy could be due to the parasite *G.*
482 *branchialis* interfering with the “metal dilution effect” which postulates that, in low polluted areas,
483 organisms grow faster than they accumulate contaminants. As a result there is no overall increase in
484 contaminant concentrations in the organism even though the animal continues to accumulate them
485 (Rainbow et al., 1990). The effects of bopyrid parasites on the growth dynamics of their host are still
486 unclear (O’Brien and Van Wyk, 1985). Nonetheless, bopyrid-infested mud shrimp *U. cf. pusilla* are
487 lighter than their uninfested conspecifics, when standardized for TL (Pascal et al., 2016a). Thus, it is
488 possible that *G. branchialis* reduces the metal dilution effect in its host *U. cf. pusilla* by lowering its
489 weight.

490 During this field survey, a substantial interactive effect between the bopyrid parasite and the
491 season on the variation of metal content in the mud shrimp *U. cf. pusilla* was also observed. Metal
492 accumulation was generally higher in bopyrid-infested organisms, except in summer when this trend
493 was reversed. This pattern was not influenced by the sex of mud shrimp at La Réousse, while it was
494 only noticed in females at Le Teychan. The lower metal contamination in bopyrid-infested organisms
495 in summer could be a side-effect of the negative impact of the parasite on its host. The intensity of the
496 activities of mud shrimp is seasonal: mud shrimp are highly active during the summer period,
497 spending a considerable amount of time transporting sediments and ventilating their burrow. In winter,
498 the intensity of their activities is highly reduced (Pascal, 2017). The bopyrid parasite *G. branchialis*
499 globally reduces the activity levels of its host but its deleterious role is more pronounced in summer
500 (Pascal, 2017). The variations in the activity levels of mud shrimp could also be associated to changes
501 in the energetic demand of organisms. The mud shrimp *U. cf. pusilla* is a suspension feeder, but it can
502 also directly feed on sediments (Dworschak, 1987). Depending on the bioavailability of metals in
503 sediments, the marked discrepancy in the metal content between bopyrid-infested and uninfested mud
504 shrimp in summer may be due to a higher accumulation of contaminants in uninfested mud shrimp as a
505 consequence of modifications of the feeding rate, compared with infested individuals. However, this

506 mechanism is probably not the main driver of the differences in metal contamination between bopyrid-
507 infested and uninfested organisms in summer because the potential increase in the feeding rate of mud
508 shrimp would likely be associated with a weight gain, which would lead to an enhanced “dilution
509 effect”. Instead, we suggest that the seasonal effect of the bopyrid *G. branchialis* on the
510 bioaccumulation pattern of its host is an indirect consequence of the influence of the parasite on the
511 reproductive processes of *U. cf. pusilla*. Bopyrid-infested females are not ovigerous (Pascal et al.,
512 2016a) and their ovaries are reduced, sometimes disappearing completely (Tucker, 1930). We also
513 observed that *G. branchialis* largely down-regulates the expression levels of *vgt* in females during the
514 reproductive period, cementing at the molecular scale the negative influence of this parasite on the
515 reproduction functions of its female host. By doing so, *G. branchialis* interferes with the “spawning
516 dilution effect” noticed in summer in bopyrid-uninfested females, especially at Le Teychan (see
517 above). Regarding males, the bopyrid parasite induces their feminization, i.e. males exhibit altered
518 secondary sexual characters (Pascal et al., 2016a; Tucker, 1930). However, the influence of this
519 parasite on the reproductive functions of its male host appears widely variable. For instance, Tucker
520 (1930) reported that the structure of testes and their functioning were not impaired by the presence of
521 the bopyrid parasite in some males, while in other individuals testes were not detectable. The presence
522 of an important number of egg-cells in the testes of several bopyrid-infested males has also been
523 documented (Tucker, 1930). In this study, the *vgt* gene was not up-regulated in bopyrid-infested
524 males, except in summer at La Réousse, but the levels of expression remained well below those
525 reported for females at the same time. Overall, it suggests that the bopyrid did not stimulate oogenesis
526 in infested males. Even if we assume that the bopyrid parasite diminishes the spermatogenesis in its
527 mud shrimp host, the “spawning dilution effect” would be less intense in male mud shrimp than in
528 females because changes in the weight and in the lipid content of the hepatopancreas of crustaceans
529 over the reproductive cycle are more pronounced in females than in males (del R. Gonzalez-Baro and
530 Pollero, 1988; Pillay and Nair, 1973). This contributes to explain the interactive effect of the season,
531 bopyrid occurrence and sex of mud shrimp on the variations in metal content in organisms sampled at
532 Le Teychan. The fact that the interactive effect of the season and of the bopyrid occurrence did not

533 depend on the sex of mud shrimp at La Réousse highlights that others factors control metal
534 contamination. Since both sites are deeply different, the reasons behind this pattern remain unclear.

535 **5. Conclusion**

536 The aim of this study was to characterize interactions between parasites and contaminants in
537 an ecosystem engineer species, the mud shrimp *Upogebia cf. pusilla*. The field survey conducted in
538 Arcachon Bay, France, showed that mud shrimp harbour low levels of contaminants compared to
539 levels reported in crustaceans occurring in areas of higher pollution (Lozano et al., 2010; Morillo et
540 al., 2005; Reis et al., 2013). Despite these low levels of metal contamination in mud shrimp, we
541 reported significant changes in the metal burden throughout the seasonal sampling, with organisms
542 showing higher metal contamination in summer compared with the rest of the year, corresponding to
543 the period of maximum bioturbation activity (Pascal, 2017). These seasonal fluctuations could be
544 related to changes in physiological processes of organisms, especially with regards to reproduction.
545 The parasitic status of organisms appears also to greatly influence the contamination burden in *U. cf.*
546 *pusilla*. As previously noticed by Kim et al. (1998), the influence of parasites on the metal content in
547 mud shrimp is, however, species-specific. The bopyrid parasite *Gyge branchialis* greatly interferes
548 with the metal accumulation process of its host whereas the effect of the trematode parasite *Maritrema*
549 sp. is less obvious. In addition, we reported an interactive effect between the bopyrid parasite and the
550 season on the levels of metals in the mud shrimp, especially in females; bopyrid-infested organisms
551 being generally more contaminated than their uninfested conspecifics except in summer when the
552 opposite trend was observed. Considering that parasites can influence the levels of metals in mud
553 shrimp exposed to such a low contamination pressure, we strongly recommend parasites be taken into
554 account during eco-toxicological studies targeting mud shrimp species.

555 **Compliance with ethical standards**

556 **Declarations of interest:** none.

557 **Animal rights:** All applicable international, national and/or institutional guidelines for the care and
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859

1 **Dissolved arsenic in the upper Paraguay River basin and Pantanal wetlands**

2

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14

15 **Abstract**

16 Although high levels of dissolved arsenic were detected in surface and ground waters of Nhecolândia, a sub-
17 region of the vast Pantanal wetlands in Brazil, the possible sources have not been clearly identified and the
18 potential release from the wetland to the draining rivers has not been investigated. In this study we measured
19 the dissolved As content in all the rivers and small streams that supply the southern Pantanal region, as well
20 as in the two main rivers draining the wetland, i.e., the Cuiaba and Paraguay rivers and tributaries. In
21 addition, Arsenic in surface waters, perched water-table, soils and sediments from 3 experimental sites
22 located in the heart of Nhecolândia were compared. On the one hand, the results show the absence of As
23 contamination in rivers that supply the Pantanal floodplain, as well as a lack of significant release from the
24 floodplain to the main drains. The As contents in the rivers are less than 2 µg L⁻¹, with variations that depend
25 on the lithology and on the geomorphology at the collection point (uplands or floodplain). On the other hand,
26 they confirm the regional extension of As contamination in Nhecolândia's alkaline waters with some values
27 above 3 mg L⁻¹. Arsenic is mainly in the arsenate form, and increases with the evaporation process estimated
28 from sodium ion concentrations. The pH of soil solution and surface water increases rapidly during evapo-
29 concentration up to values above 9 or 10, preventing adsorption processes on oxides and clay minerals and

30 promoting the retention of dissolved arsenic in solution. Solutions from organic soil horizons show higher As
31 contents in relation to Na, attributed to the formation of ternary complex As-(Fe/Al)-OM. In this alkaline pH
32 range, despite high levels of dissolved As, soil horizons and lake sediments in contact with these waters
33 show As values that correspond to uncontaminated environments.

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37 **Dissolved arsenic in the upper Paraguay River basin and Pantanal wetlands**

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40 **1 Introduction**

41 Arsenic (As) is known to be a serious health risk, a toxic and carcinogenic chemical element. In addition to
42 naturally occurring arsenic, its presence also depends on anthropogenic, mining, industrial or agricultural
43 activities. It is present in the environment under four oxidation states (- 3, 0, + 3, and + 5) and different
44 organic and inorganic species. The amounts and relative proportion of oxidation states and chemical species
45 of As in water are the result of a complex reactivity including oxidation / reduction, complexation,
46 adsorption/desorption, precipitation and biological transformations (Bhattacharya et al., 2006; Hasegawa et
47 al., 2010; Redman et al., 2002; Sharma and Sohn, 2009; Welch and Lico, 1998). Average arsenic
48 concentrations of surface water are around 0.1 – 2 $\mu\text{g L}^{-1}$ in river and lake waters (Gaillardet et al., 2014;
49 Rahman and Hasegawa, 2012), although concentrations may be higher (up to 12 mg L^{-1}) in areas containing
50 natural As sources (WHO, 2018). Guidelines are usually set at the limit of 10 $\mu\text{g L}^{-1}$ for drinking water
51 (Brazil, 2011; EPA, 1991; WHO, 2008) and for the protection of aquatic life in freshwaters (CCME, 2001;
52 CONAMA, 2011; EPA, 1991).

53

54 High arsenic contents in surface and groundwater in Latin America have only recently been reported
55 (Bundschuh et al., 2012a). In addition to pollution and contamination related to human activities (mainly
56 mining), high levels of naturally occurring arsenic in water have been detected in Mexico (Armienta and
57 Segovia, 2008; Castro de Esparza, 2010), Nicaragua (Mcclintock et al., 2012), Uruguay (Guérèquiz et al.,
58 2009), Argentina (Bundschuh et al., 2004; Nicolli et al., 2012), Chile (Arriaza et al., 2010). In most of these
59 cases, high contents result from weathering products in the Andean volcanic chain and geothermal surface
60 manifestations (López et al., 2012). Although high arsenic levels have been reported in the Pantanal of
61 Nhecolândia in Brazil, with values approaching 3 mg L^{-1} (Barbiero et al., 2007), this area has not been
62 mentioned in recent research on arsenic occurrence (Bundschuh et al., 2012b).

63 The Upper Paraguay River Basin (UPRB) can be divided into the plateaus (or uplands) and the enormous
64 Pantanal floodplain, considered the world's largest wetland (Por, 1995). The floodplain is drained by the
65 Paraguay River on its western side and is supplied by about 90 rivers or small watercourses arising from the

66 Brazilian craton that consists of a variety of rocks, i.e. potential sources of arsenic. On the one hand, very
67 few data are available on the chemistry of the rivers that supply the Pantanal, although recent studies have
68 shown that extensive agricultural activities on the highlands are affecting the major ion composition of some
69 rivers down to the floodplain (Rezende Filho et al., 2015, 2012). On the other hand, despite the presence of
70 high arsenic content in the shallow perched water-table and the surface water of Nhecolândia, the most
71 alkaline region of the Pantanal, no study has been directed towards a possible release of arsenic from the
72 wetland towards the main draining rivers. The potential release mainly depends on both, the fate of As
73 during the reduction and trapping mechanisms that favor As stabilization in the wetland (Guénet et al.,
74 2017), and the behavior of arsenic during the re-oxidation process occurring at the wetland-river interface
75 (Pédrot et al., 2015).

76 In this framework, the objective of this study is double: first, to identify, among the rivers on the uplands,
77 the possible sources for the high arsenic contents observed in the floodplain and to verify if the floodplain is
78 releasing arsenic to the nearby river network, according to the hydrological connectivity between the wetland
79 and the main draining rivers; second, to verify whether arsenic occurrence detected in an alkaline soil system
80 of Nhecolândia is related to local or regional processes and to identify the factors responsible for these high
81 arsenic contents.

82

83 **2 Regional setting**

84 *The Upper Paraguay River Basin:* being around 2.8×10^6 km², the Upper Plata River drainage system is the
85 second largest basin in South America after the Amazonian basin. Its upstream section consists of two basins
86 of similar size, namely the Parana and Paraguay basins. The major difference between these tributaries is the
87 presence of the vast Pantanal floodplain located in the Paraguay headwaters (Fig. 1). The Pantanal (about 0.2
88 $\times 10^6$ km²) is a biodiversity hotspot classified as UNESCO Natural World Heritage site, and a priority region
89 for environmental conservation (Olson and Dinerstein, 2002). Unlike the upper Parana basin, whose river
90 chemistry is clearly impacted by the Brazilian megacities, the upper Paraguay basin is still relatively
91 preserved, although some alterations in the water chemical profile have already been detected in downstream
92 areas with extensive cropping (Rezende Filho et al., 2015).

93

94

[Insert Fig. 1 here]

95

96 Figure 1: Map of the Upper Paraguay basin and Pantanal wetland: geological framework and river water
97 sampling and location of the Nhecolândia sub-region and of the 3 studied sites Nhumirim, São Roque and
98 Centenário farms

99

100 The population density in the UPRB is quite low on the uplands (<4 people km⁻² in Mato Grosso state and <7
101 people km⁻² in South Mato Grosso state, (IBGE, n.d.), and it is low in the Pantanal with less than 0.5
102 inhabitant km⁻². In addition to extensive livestock ranching, two kinds of crop systems are cultivated on the
103 uplands: sugar cane and a simple system of rotation of cotton, soybeans and corn. The Pantanal is essentially
104 privately owned and the main land use activity is livestock reproduction and extensive ranching.

105 The climate is classified as tropical humid with short dry season (July to October), i.e. “Aw” type in Köppen
106 classification. Climate patterns are controlled by the seasonal migration of the Intertropical Convergence
107 Zone (ITCZ). The mean annual temperature is about 25°C, from 21°C to 32°C during dry winters and wet
108 summers, respectively. Mean precipitation is about 1100 mm, whereas evapotranspiration is about 1400 mm,
109 resulting in an annual hydrological deficit of about 300 mm. The flood pulse in the floodplain occurs from
110 November in the northern part, to March in the southern part of the Pantanal (Junk and Nunes de Cunha,
111 2005).

112 Figure 1 shows the UPRB geological context, with calcareous formations located in the north (Serra das
113 Araras) and in the south (Serra da Bodoquena), basalts of the Serra Geral formation mainly in the upper part
114 of the Aquidauana and Miranda watersheds, sandstone formations on the eastern part of the basin, and some
115 crystalline rocks interspersed in the eastern, north-western and the narrow southern part of the wetland. The
116 floodplain, covered by quaternary sediments, consists of several sub-regions with their own specificities
117 regarding the date and duration of the flooding (Por, 1995), the transport, deposition, and mineralogy of the
118 sediments (Bergier, 2013) and the water chemical composition (Rezende Filho et al., 2012). It is made up of
119 several alluvial fans (Assine et al., 2015), including one formed by the Taquari River, referred to as one of
120 the largest alluvial fans of the world (Buehler et al., 2011).

121 *Nhecolândia sub-region*: Nhecolândia lies in the floodplain on the southern half of the Taquari fan (Fig. 1).
122 It comprises an area of approximately 24,000 km², delimited in the north by the Taquari River, in the south
123 by the Negro River, in the west by a portion of the Paraguay River, and in the east by the Maracajú Plateau,

124 which corresponds to the southeastern edge of the Pantanal wetland. Aside from these rivers, the region has a
125 relatively closed drainage with little connection to the major river system, and the water usually flows below
126 the surface within sandy soils and along drainage fields called “vazantes”. The peculiarity of this region is
127 the presence of about 15,000 lakes, including about 500 saline-alkaline ones (Furian et al., 2013). While
128 freshwater lakes supply the regional water-table during almost all seasons (Freitas et al., 2019), saline
129 alkaline lakes are disconnected by low permeability soil horizons, and supply the aquifer only fleetingly
130 during strong rainy events (Barbiero et al., 2008; Furian et al., 2013). Previous studies have shown that the
131 alkaline lakes may be classified within 3 different types, depending on their biogeochemical functioning
132 (Andreote et al., 2018, 2014; Barbiero et al., 2018; Martins, 2012; Vaz et al., 2015), i.e. green, black and
133 crystalline water lakes. Their electrical conductivity ranges usually from 1500 to 15,000 $\mu\text{S cm}^{-1}$, with
134 exceptional values recorded up to 80,000 $\mu\text{S cm}^{-1}$ at the end of the dry season. In parallel, the pH oscillates
135 from 8.9 to 10.7. High pH and EC result from cumulative evaporation over years of water supplied near the
136 surface from the vazantes and/or freshwater lakes towards the saline lakes (Barbiero et al., 2008; Furian et
137 al., 2013). Soils around alkaline lakes have a standard organization of which a simplified model is shown in
138 Figure 2 (adapted from Barbiero et al., 2016). It mainly consists of 5 contrasting horizons. Close to the lake,
139 a grey-brown topsoil loamy sand horizon (1) is observed usually with numerous calcareous precipitations.
140 The occurrence of this horizon is limited to the oscillation zone of the lakeshore between the wet and dry
141 seasons. Below, there is a light brown sandy material (2) with less than 1% clay. Within horizon 2, high
142 water pH conditions favor large dissolved organic carbon contents, which precipitate into blackish volumes
143 at the base of this material and defines horizon (3). Subjacent to this, there is a massive (single grain),
144 greyish loamy sand material (horizon 4) with about 15% clay. The top of this horizon (4) is wavy. Further
145 below lies a loamy sand, olive to light olive-grey colored horizon (5), with 15–20% clay, massive structure
146 (coherent and cemented) and locally extremely firm consistency.

147

148

[Insert Fig. 2 here]

149

150 Figure 2: Centenário study site showing a representative landscape, and a standard soil sequence around
151 saline alkaline lakes; see text for soil horizons description. ⊗ denotes the location of the lysimeters in the
152 perched water table.

153 **3 Materials and Methods**

154 **3.1 Sampling and Database**

155 **3.1.1 Regional UPRB study:** For the study at the UPRB scale, we used 4 datasets. Dataset 1, 2 and 3
156 consists of 56 river samples each, collected in December 2012, March 2013 and May 2013, respectively, *i.e.*
157 at the beginning, middle and end of the wet season. The collection took place on the uplands, at the south-
158 eastern and southern border of the Pantanal from the cities of Coxím to Porto Murtinho (Fig. 1). Datasets 4
159 was collected from November 2010 to January 2011 along the main drainage axis of the floodplain, that is,
160 the Paraguay (21 samples) and Cuiaba (nine samples) Rivers, and a few kilometers upstream the confluence
161 with their major tributaries (88 samples). All samples were collected at approximately 0.3-m depth in the
162 middle of the river section. The sampling procedure as well as the major ion chemistry were detailed in
163 Rezende Filho et al., (2015, Supplemental Material S1 and S2). All samples for trace element determination
164 were filtered (0.45 μ m cellulose acetate) in the field and acidified with ultrapure HNO₃.

165

166 **3.1.2 Local study at Nhecolândia :** Water, sediment and soil collection was carried out in two sites of
167 Nhecolândia (São Roque and Centenário farms) and compared with previous results obtained at Nhumirim
168 farm in 2 alkaline lakes and surrounding piezometers (Barbiero et al., 2007). These three sites, located in the
169 central and southern part of Nhecolândia, cover complementary geographical positions intersecting the
170 regional drainage oriented east northeast – west southwest (Fig. 2). At São Roque farm, water samples were
171 collected in extreme dry (September and October 2017) and wet conditions (August and September 2018) in
172 6 saline lakes (referred to as SR06 with black water, SR01, SR04, SR05, SR08 and SR09 with green water,
173 and SR07 with crystalline water) and three freshwater lakes (BSR03, BSR04, BSR05). Lake sediments were
174 collected in 2017. At the Centenário farm, sampling was carried out in 3 saline lakes (referred as CN01 with
175 green water, CN02 with black water and CN03 with crystalline water), 1 vazante and 1 fresh water lake.
176 Samples were collected during the dry season (September 2015 and 2016, and October 2017) and at the
177 beginning (November 2015) and the end of the wet season (June 2016 and August 2018). Lake sediments (0-
178 20 cm) were sampled in 2016. In addition, water samples were also taken in the perched shallow water-table
179 of the soil systems surrounding the lakes at Centenário site. For this, fifteen water-table samplers (lysimeters)
180 consisting of pierced polyethylene containers (Maître, 1991) were installed in the water-table through auger

181 holes (7cm in diam.). They are further referred to as (G01S, G02S and G01 to G13). The 120-ml containers
182 were wrapped with a synthetic tissue to prevent clogging by soil particles. Two capillary tubes (1 mm inner
183 diam.), inserted into the container, reach the soil surface. The first one ends at the upper part of the container
184 just below the cap and is used for injecting N₂ gas. The second one, down to the lower part of the container,
185 is used to collect the groundwater sample by depression. After installing the samplers, the holes were filled
186 with the initial material preserving the order of the different layers up to the soil surface. The sampling
187 device prevents contact between the water-table and the atmosphere and thus preserves the redox conditions
188 of the water-table within the sampler. The samples were collected from the lysimeters with a hand-held
189 vacuum pump by gentle pumping while injecting N₂ flow at a maximum pressure of 0.05 atm, in order to
190 avoid turbulences and to prevent drastic changes in redox conditions in the sampler. The samples first
191 reached a closed Erlenmeyer previously filled with N₂ to avoid rapid oxidation. The first drops were driven
192 toward the sensitive part of the potential Pt-probe (ref HI3620D) and the lowest value (usually after ~5
193 seconds) was noted. A value of +203 mV was added to the measured potential for its conversion into redox
194 potential Eh, assuming that the temperature was almost constant close to 30 °C. Then the samples were
195 stored into 120 ml acid washed HDPE container filled up and closed without air bubbles. All samples were
196 preserved in a cold and dark place until filtration. Temperature (T), electrical conductivity (EC) and pH were
197 determined in the field in aliquots. Soil horizons in contact with the lysimeters were also collected for arsenic
198 contents determination.

199

200 **3.2 Analytical Methods and data treatment**

201

202 In the laboratory, triplicates of each water sample were centrifuged (12,500 g for 30 min) and filtered
203 through a 0.22- μ m membrane (Milipore Millex-GV) before analyses. During centrifugation and filtration
204 very low amount of suspended material was obtained and therefore this fraction was not analyzed. Sediment
205 and soil samples were dried at room temperature and ground (< 100 mesh) with a ball mill (Minutem MLW
206 KM1).

207

208 **3.2.1 Total arsenic:** Due to the extension of the study period (2010 to 2018) determination of total As
209 concentration (AsTot) was performed in different laboratories and with different analytical methods.

210 Samples collected from 2010 to 2013 were analyzed by an inductively-coupled plasma mass spectrometer
211 (7500ce ICP-MS, Agilent Technology, USA) at the Géosciences Environnement Toulouse laboratory
212 (France). AsTot was determined together with other trace element concentrations. Indium (In) and Rhenium
213 (Re) were used as internal standards to correct instrumental drift. Accuracy (% of certified concentration)
214 and precision (relative standard deviation of three replicates) were assessed by analyzing the certified
215 reference material (CRM) NRC-NRCC SLRS-4 (Trace elements in natural river water) and reached 104%
216 and 4%. For samples collected from 2015 to 2016, AsTot was determined by ICP-MS (ELAN, Perkin
217 Elmer®) at the Institute of Chemistry from the University of Campinas (UNICAMP, Brazil). Accuracy and
218 precision assessed by analyzing the CRM NIST 1640a (*Trace elements in water*) reached 91% and 1.6%,
219 respectively. AsTot in water samples from 2017 to 2018 were also measured at UNICAMP but by hydride
220 generation atomic fluorescence spectrometer (HG-AFS) (Millennium Excalibur 10.055, PS Analytical).
221 Accuracy (105 %) and precision (4.5%) were also assessed by analyzing the CRM NIST 1640a. In all cases,
222 limits of detection (LOD) and quantification (LOQ) were calculated as $LOD = 3\sigma/S$ and $LOQ = 10\sigma/S$ where
223 σ is the standard deviation of blank replicates and S is the angular coefficient of the calibration curve. LOD
224 and LOQ were generally lower than 0.05 and 0.15 $\mu\text{g L}^{-1}$, respectively. All the samples were analyzed in
225 triplicate and relative standard deviation was typically lower than 5%.

226 Two decomposition methods were used to determine AsTot in soil and sediment samples. Both methods
227 used microwave radiation to enhance decomposition but different volumes of acids/oxidants and different
228 CRM for methods validation. For 2016 sediment samples, 250 mg of sediment sample were decomposed
229 with 10 mL sub-distilled HNO_3 , then analyses were performed by ICP-MS as described for 2015-2016 water
230 samples. LOD and LOQ were 0.02 and 0.08 $\mu\text{g L}^{-1}$, respectively. Accuracy (96%) and precision (4.3%) were
231 checked by analyzing certified marine sediment NRCC PACS-2. For soil and sediment collected in 2017 and
232 2018, 200-250 mg of sample were decomposed with 4 mL HNO_3 , 2 mL HF, 1 mL HCl and 0.5 mL H_2O_2 .
233 Boric acid was added post decomposition to avoid HF excess. Analyses were performed by Hydride
234 Generation Atomic Fluorescence Spectrometer (HG-AFS) (PSAnalytical 10.055 Millenium Excalibur
235 System). LOD and LOQ were always below 0.04 and 0.14 $\mu\text{g L}^{-1}$, respectively. Accuracy (96% and 103%)
236 and precision (6% and 2%) were checked by analyzing CRM NIST 2702 (Inorganics in Marine Sediment)
237 and CRM BCR 320 (River Sediment), respectively.

238

239

240 **3.2.2 As speciation analyses:** For water samples collected from 2015 to 2016 (Centenário farm) Speciation
241 analysis was performed by High Performance Liquid Chromatographer (HPLC) coupled to the ICP-MS
242 (ELAN, Perkin Elmer®). Five arsenic species were determined: AsB (Arsenobetaine), MMA
243 (Monomethylarsenate), DMA (Dimethylarsenate) and the ions As (III) (Arsenite, NaAsO_2) and As (V)
244 (Arsenate, Na_3AsO_4). Separation was carried out with an anion exchange column (Hamilton PRP-X100 (10
245 μm , 250 mm x 4.1 mm). The chromatographic method was adapted from Watts et al., (2008), with 4 and 60
246 mmol L^{-1} NH_4NO_3 solutions as mobile phases in a concentration gradient pumping program. The pH of both
247 solutions was adjusted at 8.7, the chromatographic run was 12.5 minutes, with an injection volume of 150
248 μL . Daily calibration curves were drawn in the 5-40 $\mu\text{g L}^{-1}$ linear range for all five species. All standards and
249 reagents used (Merck and Sigma-Aldrich) have high purity for trace metal analyses. LOD and LOQ ($\mu\text{g L}^{-1}$)
250 were 2.7 and 8.9 for AsB, 4.4 and 14.6 for As(III), 2.4 and 8.1 for DMA, 2.6 and 8.7 for MMA, and 2.8 and
251 9.0 for As(V).

252 For samples collected in 2018 on the SR farm, speciation analysis was also performed through HPLC-HG-
253 AFS (Millennium Excalibur 10.055, PS Analytical). The separation of only four As species, As(III), DMA,
254 MMA and As(V) was carried out with an anion exchange column (Hamilton PRP-X100 (10 μm , 250 mm x
255 4.1 mm). A chromatographic method was adapted from PSAnalytical Application Note APP 160, using
256 Na_2HPO_4 and NaH_2PO_4 20 mmol L^{-1} (Sigma-Aldrich reagents with purity $\geq 99\%$) as a mobile phase at pH
257 6.2 in isocratic mode. The chromatographic run was 13 minutes with an injection volume of 200 μL . LOD
258 and LOQ ($\mu\text{g L}^{-1}$) for each species were 0.46 and 1.38 for As(III), 0.31 and 0.93 for DMA, 1.31 and 3.92 for
259 MMA, and 1.12 and 3.35 for As(V). Some As organic species do not produce a hydride. Therefore, for each
260 sample, a qualitative analysis was performed to confirm the absence of these species. The method was
261 adapted from Ma et al., (2014), including a UV digestion step. The chromatographic separation used the
262 same anion exchange column and a mobile phases, (A): 4 mmol L^{-1} NaHCO_3 and (B): 4/40 mmol L^{-1}
263 $\text{NaHCO}_3/\text{NaNO}_3$ solutions at pH 9.5. The chromatographic run time was 15 minutes with the following
264 gradient elution program: 100 % A, 3 min.; 50 % A and 50% B 4 min.; 100 % B 5 min. and 100 % A 3 min.

265

266 **3.2.3 Other chemical analyses:** Alkalinity was determined by acid 10^{-1} or 10^{-2} mol L⁻¹ HCl titration, other
267 major elements (anions and cations) by ion chromatography, and DOC by combustion (TOC Analyser,
268 Shimadzu).

269

270 **3.2.4 As relative mobility:** The abundance of arsenic in rivers depends both on its abundance in the
271 continental upper crust and its mobility during weathering and transport. The As mobility in the UPRB in
272 relation to Na was estimated using the dissolved As/Na ratio normalized to the As/Na ratio in the upper crust
273 as reference (Li, 2000).

$$274 \quad E_{As/Na} = (As/Na)_{\text{sample}} / (As/Na)_{\text{reference}} \quad (1)$$

275 The results were compared to relative As chemical mobility during weathering and transport processes from
276 the world compilation presented by Gaillardet et al., (2014).

277

278 **3.2.5 Statistical analysis:** For samples collected at Centenário site (70 samples), a covariance analysis
279 (ANCOVA) was conducted in order to test the effect of several parameters, and the location in the soil cover,
280 on dissolved arsenic. The analysis was performed using Xlstat software (AddInSoft) with a 95% reliability
281 threshold. In a first step, the analysis was carried out using quantitative variables representing the evapo-
282 concentration process (sodium and alkalinity (Furian et al., 2013)) and the effect of organic matter (DOC). In
283 a second step, qualitative variables reflecting 4 different origins of the collected sample were added, either
284 from surface (S) waters (lakes and vazantes), or from the lysimeters installed in the soil cover in the organic
285 horizons (Org), in the deep and more clay horizons (Cly) or in the sandy horizons of the higher grounds
286 (Hig) (Fig. 2).

287

288 **4 Results**

289 **4.1 As concentration in the rivers from the UPRB**

290 Descriptive statistics of water samples collected in the highlands (datasets 1, 2 and 3) are shown in Figure 3
291 and Supplementary Material S1, in which the sampling points were classified according to the lithology. The
292 lithology does not necessarily refer to the type of rock present at the sampling point, but to the type of rock
293 that has a dominant influence on the river geochemistry (Rezende Filho et al., 2015). The total dissolved
294 arsenic ranged from 0.05 to 1.69 $\mu\text{g L}^{-1}$, *i.e.* in a ratio of 34.

295 On the plateau, the contents were relatively similar for each river during the 3 field campaigns, which
296 suggests good stability of dissolved As values during the rainy season (Fig. 3a and b), and probably
297 throughout the year. It appears that dissolved As mainly depends on the lithology (Fig. 3c). The rivers
298 draining sandstone areas showed the lowest values, generally close to $0.26 \mu\text{g L}^{-1}$ (ranging from 0.05 to 0.54
299 $\mu\text{g L}^{-1}$), except for “Rio do Peixe” with a value close to $1 \mu\text{g L}^{-1}$ throughout the 3 campaigns (1.15, 0.86 and
300 $1.13 \mu\text{g L}^{-1}$, respectively). These contents increased slightly ($\sim 0.36 \mu\text{g L}^{-1}$) for rivers draining basaltic
301 formations in their upstream part, as it is the case for example for the rivers Taquarussu, Aquidauana,
302 Cachoeirão (Fig. 1). The streams flowing on the calcareous rocks of the Bodoquena region (*e.g.* rivers
303 Salobra, Betione, Chapena) have higher values, generally ranging between 0.32 and $0.86 \mu\text{g L}^{-1}$, with an
304 average concentration of $0.48 \mu\text{g L}^{-1}$, whereas in the 5 rivers draining crystalline rocks, the values were
305 between 0.26 and $0.69 \mu\text{g L}^{-1}$, with an average concentration of $0.50 \mu\text{g L}^{-1}$. The few rivers collected before
306 and after their entry into the Pantanal systematically showed an increase in the order of 20% to 60% of
307 dissolved As values in the floodplain (not shown). When these rivers separate into several channels in the
308 alluvial plain (Negro and Taboco rivers, for example), we observed that the secondary channels, with much
309 lower discharge, showed As contents approximately 20 to 30% higher than the main stream (not shown).
310 Finally, among all the rivers collected in datasets 1, 2 and 3, the Nabileque River, the only one with
311 headwaters in the alluvial plain and not in the surrounding uplands, showed the highest As contents (1.01,
312 1.69 and $1.37 \mu\text{g L}^{-1}$ during the 3 campaigns, respectively, Fig. 3c).

313

314

[Insert Fig. 3 here]

315

316 Figure 3: Total dissolved As contents (a and b), mean value and standard deviation (c) in rivers on the
317 highlands and alluvial plain (Nabileque River) during 3 campaigns in 2012-2013.

318

319 Regarding the Cuiaba and Paraguay rivers (dataset 4), which are the two main draining rivers of the
320 floodplain, there was a trend of increasing As levels towards downstream (Fig. 4). Arsenic values in the
321 Cuiaba River (Fig. 4a) gradually increased from $0.25 \mu\text{g L}^{-1}$ just downstream the city of Cuiaba to $0.65 \mu\text{g L}^{-1}$
322 ¹ at its confluence with the Paraguay River. The first increase to a value of about $0.35 \mu\text{g L}^{-1}$ occurred after

323 the mixing with the waters of “Baia do Agapito” ($1.02 \mu\text{g L}^{-1}$) and the confluence with River Urutubinha
324 (0.37 to $0.49 \mu\text{g L}^{-1}$). The second increase ($0.40 \mu\text{g L}^{-1}$) occurred after the contribution of the Muquem River
325 ($0.64 \mu\text{g L}^{-1}$). The confluence with São Lourenço River (0.73 and $1.04 \mu\text{g L}^{-1}$) and its secondary channels
326 (0.93 to $1.8 \mu\text{g L}^{-1}$) caused an increase in As content up to $0.52 \mu\text{g L}^{-1}$, then a final contribution of the Piquiri
327 River ($0.76 \mu\text{g L}^{-1}$) stabilized the value at about $0.65 \mu\text{g L}^{-1}$ down to the Cuiaba-Paraguay confluence.
328 Throughout the upper stretch of the Paraguay (from Cáceres to “Baia do Tamengo” close to the city of
329 Corumba) (Fig. 4b), As water contents were rather stable ranging from 0.21 to $0.32 \mu\text{g L}^{-1}$. This stability can
330 be attributed to two characteristics: on the one hand the high Paraguay River flow compared to that of its
331 tributaries (from field estimate, no quantitative data are available), and on the other hand the moderate As
332 levels in the tributaries that ranged from 0.17 to $0.56 \mu\text{g L}^{-1}$ ($0.35 \pm 0.12 \mu\text{g L}^{-1}$), with the only exceptions of
333 two tributaries (“Boca inferior da Baia Branca” and “Boca do Tuiuiu”) in which the As concentrations were
334 0.70 and $0.80 \mu\text{g L}^{-1}$, respectively. Downriver from Corumba city, As concentration in the Paraguay water
335 kept increasing gradually, first up to $0.43 \mu\text{g L}^{-1}$ after the confluence with Taquari (0.48 to $0.58 \mu\text{g L}^{-1}$) and
336 Negro Rivers ($0.91 \mu\text{g L}^{-1}$), then 0.50 , 0.60 , and $0.65 \mu\text{g L}^{-1}$ after receiving the water from the Abobral (0.53
337 $\mu\text{g L}^{-1}$), Miranda (0.41 to $0.62 \mu\text{g L}^{-1}$), and Nabileque ($1.88 \mu\text{g L}^{-1}$) Rivers, respectively.

338

339

[Insert Fig. 4 here]

340

341 Figure 4: Upstream-downstream As concentration throughout the (a) Cuiaba and tributaries, and (b)
342 Paraguay and tributaries. Note the gradual increase in As concentration throughout both, Cuiaba and
343 Paraguay rivers.

344

345 The histogram drawn in Figure 5 shows the distribution of the log-normal transformation for As mobility in
346 relation to Na ($E_{\text{As}/\text{Na}}$) in the UPRB. On the highlands (Fig. 5a), $\text{Log}(E_{\text{As}/\text{Na}})$ had a bimodal distribution with a
347 first mode focused on 0.2 ($E_{\text{As}/\text{Na}} \sim 2$) and most of these samples matched with rivers flowing from crystalline
348 bedrock in the southern part of the basin (e.g. rivers Naitaca, Terere, Branco) where dissolved Na is higher
349 than in the rest of the sampling (Rezende Filho et al., 2012). The second mode focused on the value 1.1

350 ($E_{As/Na} \sim 13$) and corresponded to the rivers coming from the sandstone formations mainly on the eastern part
351 of the basin. For these rivers with very low mineral charge, both the dissolved As and Na contents are close
352 to the limit of detection, which confers a high uncertainty on the calculation of $E_{As/Na}$ value. A bimodal
353 distribution was also observed for the samples collected in the floodplain (Fig. 5b, dataset 4) with a strong
354 mode centered on 0.6 ($E_{As/Na} \sim 4$), which corresponds to the general trend in As mobility, and a second one of
355 1.2 ($E_{As/Na} \sim 20$), which corresponded to some rivers at effluence of the wetland (São Lourenço and Piquiri
356 Rivers and secondary channels) just before their confluence with the Cuiaba River.

357

358 [Insert Fig. 5 here]

359

360 Figure 5: Frequency distribution of $\log(E_{As/Na})$ a) on the highlands and b) in the floodplain of the UPRB.

361

362

363 **4.2 Arsenic concentrations in the Nhecolândia region.**

364 **4.2.1 Concentration ranges in waters**

365 Arsenic concentration in vazante at the end of the dry period and in a freshwater lake at the end of the wet
366 season were 3.47 and $<0.04 \mu\text{g L}^{-1}$, respectively (Table 1).

367

368 **Table 1:** As concentrations in sediment (mg kg^{-1}) and water ($\mu\text{g L}^{-1}$) of green, black and crystalline alkaline
369 lakes and freshwater environments at Centenario (CN) and São Roque (SR) farms, compared with values
370 from Nhumirim (NH) farm (Barbiero et al., 2007).

371

372 [Insert Table 1 here]

373

374 By contrast, dissolved arsenic contents in alkaline lakes were much higher, ranging from 28.8 to $2,916 \mu\text{g L}^{-1}$
375 (Table 1), and fluctuate depending of the season and year of collection. This effect is particularly clear when
376 comparing the concentrations in samples collected from the same lakes at the CN site in October 2017
377 (atypical dry season with very low water level and high concentrations) and in August 2018 (atypical dry
378 season with high water level and low concentrations). The lowest concentrations were always found in the

379 crystalline water lakes (CN03 and SR07), whereas the highest ones were observed in the green and black
380 water lakes. The As concentrations reported for the two lakes of the Nhumirim Farm, collected during a
381 particularly dry episode, were of the same order of magnitude (Table 1). In water-table, samples collected
382 from lysimeters at the CN site, arsenic concentrations ranged from 0.8 to 3581.5 $\mu\text{g L}^{-1}$ (Table 2). Low
383 values were observed in deep samples collected on higher grounds (G03, G05 and G12), whereas the highest
384 concentrations were found in samples collected within organic horizons (G01S, G02S and G13) around the
385 alkaline lakes. In comparison, dissolved As in groundwater around the lakes on the Nhumirim farm
386 (collected from piezometers) ranged from 0.14 to 266 $\mu\text{g L}^{-1}$.

387

388 **Table 2:** As concentrations ($\mu\text{g L}^{-1}$) in shallow water-table samples collected from lysimeters at Centenário
389 farm around the three alkaline lakes (type of environment G, B and C) and As contents in the soil horizon
390 ($\mu\text{g g}^{-1}$) at the contact with the lysimeter.

391

392 [Insert Table 2 here]

393

394 4.2.2 As and major elements

395

396 The concentration diagrams based on Na contents (São Roque, Centenário and Nhumirim farms) are
397 presented in Figure 6. A similar increase in alkalinity was observed for the three sites, and the values were in
398 agreement with the Alk-Na relationship established by Furian et al. (2013) from a regional sampling (147
399 samples). Although the plots were scattered, dissolved arsenic concentrations increased in proportion to Na
400 at the Nhumirim and São Roque sites. At the Centenário site, a similar trend was observed for surface waters
401 (S), deep waters of the higher grounds (Hig), and waters collected in the clay (Cly) horizons. Nevertheless,
402 samples taken from organic horizons (Org) slightly departed from this trend, showing values about 5 to 10
403 times higher compared to Na.

404

405 [Insert Fig. 6 here]

406

407 Figure 6: Arsenic and carbonate alkalinity in concentration diagrams based on Na contents (Centenário, São
408 Roque and Nhumirím farms). The solid line denotes the regional Alk-Na relationship established by Furian
409 et al. (2013) from 147 samples of surface water.

410

411 The results of the ANCOVA are presented in Table 3. The results show a clear relationship between total
412 dissolved arsenic and alkalinity, sodium and DOC (Step 1). By including the origin of the water samples
413 according to the pedological system (step 2), it appears that the parameters DOC and the origin of an organic
414 horizon (Org) have significant influence on the total As contents.

415

416 **4.2.3 Arsenic speciation in alkaline lakes and perched shallow water-table around the alkaline lakes**

417 The samples collected from the lysimeters and surface waters on the CN site are plotted in the Pourbaix
418 diagram in Figure 7, showing that for the main part of the samples, As(V) may be expected. Only a few
419 samples from deep down and more clayey horizons could show a predominance of As(III). Analytical results
420 from water sampled at CN (Fig. 8) and SR sites (not shown) confirmed that As(V) was the main species
421 detected in the waters from both shallow perched water-table and alkaline lakes, accounting for more than
422 95% of the total As concentration. Although MMA and DMA appeared in some chromatograms, their
423 concentrations were below the limit of quantification, with the exception of SR04 and SR08 samples
424 collected in September 2018, for which DMA concentrations were 1.43 and 1.47 $\mu\text{g L}^{-1}$, respectively. As(III)
425 was not detected.

426

427 [Insert Fig. 7 here]

428

429 Figure 7: Arsenic Pourbaix diagram showing the As speciation expected for the watertable samples collected
430 in surface water (S) and soil horizons (Hig, Cly or Org) at Centenário farm (see Table 1 for the
431 corresponding total As concentrations). Both, pH and Eh were measured in the field, under N₂ flux.

432

433 [Insert Fig. 8 here]

434

435 Figure 8: Concentration of As(V) vs Total As in water samples from Centenário farm: S (surface water), Cly
436 (water from clay horizons), Org (water from organic horizons). In water samples from the higher grounds
437 (Hig), As(V) contents were below the limit of quantification.

438

439

440 **4.2.4 As in lake sediments and soil horizons**

441 Arsenic contents in soil horizons at the contact with the lysimeters were low, ranging from values below the
442 limit of quantitation ($0.23 \mu\text{g g}^{-1}$) up to $7.4 \mu\text{g g}^{-1}$ (Mean = $2.2 \mu\text{g g}^{-1}$ and SD = $1.65 \mu\text{g g}^{-1}$). Values were
443 slightly higher in lake sediments, ranging from 1.7 to $8.2 \mu\text{g g}^{-1}$. The highest values were observed in
444 sediments from crystalline water alkaline lakes (CN01 and SR07) (Table 1).

445

446 **5 Discussion**

447 The uplands and floodplain data indicate standard levels ($0.1 - 1.7 \mu\text{g L}^{-1}$) of dissolved arsenic in UPRB
448 rivers, i.e. in the range of non-As-contaminated rivers. The waters draining the uplands show a dissolved As
449 variability depending on the type of rock that controls the chemistry of the major ions on each watershed
450 (Rezende Filho et al., 2015, 2012). These variations are in agreement with the As contents in rocks, reported
451 in the literature, with the following order: Sandstone ($0.5 - 1 \text{ mg kg}^{-1}$) <basalts and granites ($\sim 0.7 \text{ mg kg}^{-1}$)
452 <limestone ($1.0 - 1.5 \text{ mg kg}^{-1}$) (Matschullat, 2000). It confirms that dissolved As in rivers supplying the
453 floodplain is mainly controlled by the lithology of the uplands. Although a slightly higher arsenic relative
454 mobility was observed with respect to Na in UPRB (~ 5) (Fig. 5) than the world average (close to 2,
455 Gaillardet et al., 2014), these data clearly show that the waters that supply the floodplain are not
456 contaminated with As. A gradual upstream-downstream increase in dissolved As concentrations was
457 observed in the Cuiaba and Paraguay waters suggesting that arsenic concentrations may be explained by the
458 simple hydrological mixing with the tributaries that usually have higher As concentration and lower flow
459 than the main rivers (Fig. 4b). This is the opposite of what was reported for trace-elements in other hydro-
460 systems such as in the Mississippi basin (Shiller, 1997; Shim et al., 2016) or Amazon basin (Seyler and
461 Boaventura, 2003) where a decrease of trace-element concentration downstream was observed and attributed
462 to a dilution effect from the upstream source. Notwithstanding this trend of a slight increase in dissolved
463 arsenic, the values recorded in dataset 4 indicate a lack of high dissolved arsenic transfer from the floodplain

464 to the Cuiaba and Paraguay rivers. Some rivers at the exit of the wetland have arsenic levels significantly (p
465 <0.05) higher than the dataset mean value, as well as higher mobilities ($E_{As/Na} \sim 20$). This is particularly the
466 case of the São Lourenço and Piquiri rivers and their secondary channels just upstream of their confluence
467 with the Cuiabá River. A previous study conducted on the same dataset showed that the major ion chemistry
468 of these rivers is impacted by extensive agricultural activities on the uplands (Rezende Filho et al., 2015),
469 particularly by an increase in sulfate and ammonium contents together with a slight increase in alkalinity.
470 Such alteration of the chemical profile was detected at the entry of these rivers in the Pantanal. It has been
471 attributed to the fertilization practices together with field liming on the uplands, and it is detectable until the
472 confluence with the river Cuiabá. Therefore, slightly higher dissolved As levels at the confluence of these
473 rivers with the Cuiaba may also be a consequence of these activities. Unfortunately, we do not have the
474 dissolved As content of these waters as they enter the floodplain, which does not allow further discussion.
475 The highest As concentrations ($1.36 \pm 0.34 \mu\text{g L}^{-1}$) were found in the Nabileque river, the only one that has its
476 source in the alluvial plain. In this environment, during the high water levels, anaerobic conditions favor the
477 reductive dissolution of wetland-soil Fe-oxihydroxides and associated elements, such as arsenic and organic
478 matter (Guénet et al., 2017). Such a process could be responsible for the slightly higher As concentration
479 observed in the Nabileque river.

480 On the other hand, the results are much more contrasting in the Nhecolândia floodplain. At all three
481 studied sites, elevated As levels are noted, indicating that its occurrence is a consequence of processes that
482 operate on a regional scale. In addition, the similar behavior of arsenic with respect to sodium confirms that
483 the same processes are at work in these three sites. Huge As variations are observed over short distances, i.e.
484 a few hundred meters that separate the vazantes and freshwater lakes from the alkaline lakes, as well as the
485 few tens of meters that separate the higher grounds at the top of the beaches from the border of the alkaline
486 lakes. The results of the speciation carried out at Centenário site show that As mainly occurs as arsenate (Fig.
487 8). The As contents variability must be related to the hydrological and hydrochemical functioning of this
488 system of lakes. The vazantes are the water supplying areas (Furian et al., 2013). In the short term, they
489 mainly receive water from the seasonal rains, but over the long term, they are also supplied by the overflows
490 of the Taquari River. Therefore, this river arising from sandstone area imposes its chemical characteristics, in
491 particular the low arsenic contents (Table 1) and a positive calcite residual alkalinity (RA_{calcite}) (Oliveira
492 Junior et al., 2019; Rezende Filho et al., 2012). During the wet season, while freshwater lakes are generally

493 supplied by overflow during the flood pulse, alkaline saline lakes receive a reduced amount of freshwater
494 through sub-surface flows from the vazantes. During the dry season, waters concentrate under the effect of
495 evaporation, not only in the alkaline lakes but also in the perched water-table and of surrounding beaches. In
496 the geochemical context of positive RA_{calcite} (Barbiero et al., 2002), alkalinity increases with increasing
497 evapo-concentration while calcium levels remain very low due to calcite precipitation. The solution pH
498 increases, mainly controlled by carbonate species. Close to alkaline lakeshores, the solutions are more
499 concentrated due to higher evaporation by wicking in the sandy material (Barbiero et al., 2016). Magnesium
500 silicates precipitate (in horizon 1, see Fig. 2), controlling dissolved Mg at a low level, while alkalinity keep
501 increasing (Furquim et al., 2008). Finally, the mineralization of dissolved organic matter a few tens of meters
502 from the lake shore releases iron and aluminum allowing the synthesis of Fe-micas (in horizons 4 and 5,
503 Barbiero et al., 2016; Furquim et al., 2010). This succession of saline precipitations (calcite, Mg-silicate, Fe-
504 micas) is standard in alkaline environments (Barbiero et al., 2004).

505 Dissolved As is usually controlled by adsorption processes that can take place in three main different
506 adsorbents, namely metallic (Al, Fe and Mn) oxides and hydroxides, clay minerals and organic matter.
507 However, in this alkaline geochemical framework, three factors favor the maintenance of arsenic in solution.
508 First, from the beginning of the evapo-concentration, arsenic concentrates together with other dissolved
509 species such as carbonates and secondarily fluorides (Barbiero et al., 2008), and to a lesser extent chlorides
510 and sulfates. Competitive adsorption between As and those ions prevents As fixation onto any adsorbent
511 (Goldberg, 2002). Second, increasing dissolved As contents occur simultaneously with increasing pH (Fig.
512 9) and therefore a decrease in its adsorption affinities. Indeed, arsenate adsorption on oxides and clays is
513 highest at low pH and strongly decreases with increasing pH, namely, above pH 9 for Al oxide, pH 7 for Fe
514 oxide or hydroxide, illite and kaolinite (Cornu et al., 1999; Goldberg, 2002).

515

516

[Insert Fig. 9 here]

517

518 Figure 9. Dissolved As concentration vs pH in lysimeters, lakes and vazante at Centenário farm.

519

520 Third, high Fe and Al concentrations (Barbiero et al., 2016) likely favor the formation of ternary
521 complexes As-Fe/Al-humic acids. As can be seen in Table 2, the solutions sampled in organic horizons

522 (G01S, G02S, G13) have the highest As concentrations, suggesting that some of the As does not migrate in
523 free dissolved form, but likely complexes with aquatic humic substances (AHS). AHS represent one of the
524 main parts of the organic matter (Mariot et al., 2007) and act as complexing agents increasing As mobility
525 (Sharma et al., 2011; Warwick et al., 2005). As(V) is present in anionic forms (H_2AsO_4^- and HAsO_4^{2-} , Fig.
526 7), which results in repulsion forces between As and negatively charged AHS at high pH. However, the
527 presence of dissolved Fe and Al, as mentioned in Barbiero et al. (2016), leads to the formation of ternary
528 complexes (As-Fe/Al-AHS) (Oliveira et al., 2016). This behavior could be at the origin of the results of the
529 ANCOVA, emphasizing that the evaporation, but also the DOC content and the origin of the samples coming
530 from the organic horizons have a significant influence on dissolved As (Table 3) (Ghosh et al., 2015; Mariot
531 et al., 2007). In summary, the solid phase does not act as a factor controlling dissolved As, which appears to
532 be mainly regulated by the evapo-concentration process. Such a behavior of As in alkaline and/or
533 evaporative environment have already been mentioned by Bhattacharya et al. (2006) and Welch and Lico
534 (1998). Changes in the concentration of As first results from its conservative behavior during seasonal
535 evaporation and dilution as shown by the increase in proportion to Na (slope close to 1, Fig. 6). For water
536 samples arising from organic horizons and with high DOC contents, an additional fraction of arsenic is
537 maintained in the solution likely through the formation of ternary As-metals-AHS complexes. Then
538 dissolved As increase in a factor of 5 to 10 compared to sodium. This behavior of arsenic in this alkaline
539 environment is also demonstrated by the low levels of arsenic measured in soils. Despite high levels of As in
540 the solutions, soils in contact, including organic horizons, have low As levels (Table 2), in the range of non-
541 As-contaminated soils (Matschullat, 2000). The same is observed for lake sediments. By way of comparison
542 with the work of Caumette et al. (2011), although dissolved As contents in Canadian lakes were much lower
543 ($250 \pm 100 \mu\text{g L}^{-1}$) than in alkaline lakes of Nhecolândia (up to 3 mg L^{-1}), these authors reported As values in
544 sediments ranging from $34 \mu\text{g g}^{-1}$ in an uncontaminated freshwater lake to $698 \mu\text{g g}^{-1}$ in a highly
545 contaminated lake. Nevertheless, in these lakes, the pH ranging from 7.6 to 7.9 is more favorable to As
546 adsorption on particulate matter, as mentioned above. The values reported in Table 1 for alkaline lakes in the
547 Pantanal are much lower, in the range of uncontaminated soils and sediments. These low As concentrations
548 in soils and sediments confirm that an alkaline environment favors the maintenance of arsenic in solution and
549 that the solid phase acts as a non-reactive matrix. Arsenic accumulates in alkaline lake waters and
550 surrounding water-table from year to year as do sodium ions (Fig. 6).

551

552 **6 Conclusion**

553 The behavior of arsenic in alkaline environments is little documented and still poorly understood. A previous
554 study reported high levels of dissolved arsenic in the waters of the Pantanal, the largest wetland on the
555 planet, and more specifically in the vast sub region "Nhecolândia". On the one hand, our data collected at the
556 level of the UPRB show that the rivers that supply the alluvial plain of the Pantanal have low As contents.
557 All concentrations are below $2 \mu\text{g L}^{-1}$, that is to say in the range for non-arsenic-contaminated river waters.
558 The relative mobility of arsenic in relation to sodium is slightly higher than the global average, but remains
559 moderate. In addition to the absence of noticeable As source on the plateaus upstream of the alluvial plain,
560 the data show a lack of significant As release from the alluvial plain towards the main draining rivers,
561 namely the Cuiaba and Paraguay rivers. On the other hand, the study confirms the high dissolved As levels
562 in the alkaline waters of Nhecolândia. The relations between As and the major ions are similar in the 3 sites
563 studied, which confirms that As responds to the same control processes throughout the region. The chemical
564 speciation indicates that it mainly occurs in the form of As(V). In surface water, the proportions are
565 substantially the same in the 3 sites and increase with the sodium amount, itself resulting from long-term
566 cumulative evaporation over many years. In the soil solution, the As levels in the surface aquifer depend on
567 the type of saturated soil horizon, the organic horizons having As/Na ratio 5 to 10 times higher, compared to
568 the trend in the rest of the samples. Future studies should therefore focus on details of arsenic dynamics
569 within the alkaline lake and associated soil system.

570

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581

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1 **Tab. 1** Characteristics of the two sampling sites in Arcachon Bay, France. D₅₀: median grain size.

	La Réousse	Le Teychan
Position	44°41'17.1"N 1°12'5.5"W	44°40'18.9"N 1°6'26.6"W
Zonation	Intertidal	Intertidal
D ₅₀ (µm)	200.0	133.0
Gravel (%)	0.4	2.3
Sand (%)	95.7	70.7
Mud (%)	3.9	27.0
Density of mud shrimp (ind. m ⁻² , mean ± SD) ¹	6.8 ± 0.7	4.1 ± 1.7
Prevalence of <i>Gyge branchialis</i> (%) ²	11.6	12.5
Prevalence of <i>Maritrema</i> sp. (%) ²	93.4	58.9
Abundance of <i>Maritrema</i> sp. (metacercariae. ind ⁻¹ , mean ± SE) ²	66.5 ± 7.7	10.9 ± 2.9

2 ¹ Pascal, 2017; ² Dairain et al., 2017

3

4 **Tab. 2** Results of PERMANOVA analyses evaluating the influence of season, bopyrid *Gyge branchialis*
5 presence and sex of the mud shrimp *Upogebia cf. pusilla* on trematode abundances in the gills of mud
6 shrimp sampled at two sites in Arcachon Bay, France (La Réousse and Le Teychan). P-values in bold
7 indicate statistically significant effects.

	La Réousse			Le Teychan		
	df	Pseudo-F	<i>P</i> (perm)	df	Pseudo-F	<i>P</i> (perm)
Season (1)	3	0.03	0.99	3	4.15	< 0.01
Bopyrid (2)	1	9.85	< 0.01	1	22.44	< 0.01
Sex (3)	2	0.06	0.80	2	0.54	0.47
(1) x (2)	3	0.23	0.88	3	1.67	0.18
(1) x (3)	3	2.02	0.11	3	2.33	0.07
(2) x (3)	1	0.12	0.74	1	2.16	0.14
(1) x (2) x (3)	3	2.65	< 0.05	3	1.88	0.14

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9

10 **Tab. 3** Concentrations of metals ($\mu\text{g g}^{-1}$ DW) in surface sediments (ca. 0–2 cm depth) and deeper in the sediment column (18–20 cm depth) over a
 11 one-year survey conducted in two sampling sites in Arcachon Bay, France (La Réousse and Le Teychan). Annual average concentrations (\pm standard
 12 error SE) at the two sampling sites are also given. Na.: no data available.

	La Réousse					Le Teychan				
	Fall	Winter	Spring	Summer	Mean \pm SE	Fall	Winter	Spring	Summer	Mean \pm SE
0-2 cm depth										
Ag	Na.	0.13	0.18	0.27	0.19 \pm 0.00	Na.	0.3	0.34	0.31	0.31 \pm 0.00
As	Na.	4.86	3.79	3.54	4.06 \pm 0.08	Na.	16.7	20.5	16	17.73 \pm 0.26
Cd	Na.	0.09	0.1	0.11	0.10 \pm 0.00	Na.	0.21	0.25	0.22	0.23 \pm 0.00
Cu	Na.	3.8	3.69	2.96	3.48 \pm 0.05	Na.	9.44	12.3	9.56	10.43 \pm 0.17
Fe	Na.	8216	7894	7087	7732 \pm 62	Na.	21640	27157	22120	23639 \pm 325
Mn	Na.	129	177	235	180 \pm 6	Na.	214	269	204	229 \pm 3
Ni	Na.	5.56	4.97	5.05	5.19 \pm 0.03	Na.	15.1	17.1	13.9	15.37 \pm 0.17
Zn	Na.	34.8	31.1	26.4	30.77 \pm 0.45	Na.	60.3	99	76.3	78.53 \pm 2.07
18-20 cm depth										
Ag	Na.	0.11	0.12	0.11	0.11 \pm 0.00	Na.	0.29	0.29	0.26	0.28 \pm 0.00
As	Na.	4.74	4.06	3.68	4.16 \pm 0.06	Na.	22.4	15.2	19.3	18.97 \pm 0.38
Cd	Na.	0.09	0.1	0.06	0.08 \pm 0.00	Na.	0.24	0.27	0.21	0.24 \pm 0.00
Cu	Na.	3.27	4.1	2.32	3.23 \pm 0.01	Na.	10.8	11.9	9.05	10.58 \pm 0.15
Fe	Na.	6354	6407	5067	5943 \pm 81	Na.	28161	24058	25771	25997 \pm 219
Mn	Na.	78	104	119	100 \pm 2	Na.	226	211	205	214 \pm 1.15
Ni	Na.	6.51	5.39	3.69	5.20 \pm 0.15	Na.	19.1	15	16.2	16.77 \pm 0.22
Zn	Na.	32.1	42.4	19.3	31.27 \pm 1.23	Na.	63.3	83.4	63.7	70.13 \pm 1.22

13

14

15 **Tab. 4** Average concentrations (mean \pm SE; $\mu\text{g g}^{-1}$ DW) of metals in the hepatopancreas of mud shrimp *Upogebia cf. pusilla* uninfested and infested
16 with the bopyrid parasite *Gyge branchialis* over a one year seasonal survey conducted at two sampling sites (La Réousse and Le Teychan) in Arcachon
17 Bay, France, and results of multiple comparisons (Wilcoxon tests). P-value in bold indicate statistically significant effect.

		La Réousse			Le Teychan		
		Bopyrid-uninfested	Bopyrid-infested	<i>p</i> -value	Bopyrid-uninfested	Bopyrid-infested	<i>p</i> -value
Ag	Fall	2.0 \pm 0.2	4.5 \pm 0.5	< 0.01	3.5 \pm 0.3	4.5 \pm 0.4	0.14
	Winter	2.6 \pm 0.1	4.6 \pm 0.4	< 0.01	4.3 \pm 0.2	5.1 \pm 0.4	0.25
	Spring	3.0 \pm 0.3	4.1 \pm 0.3	< 0.01	3.3 \pm 0.3	4.2 \pm 0.4	0.09
	Summer	3.7 \pm 0.4	3.0 \pm 0.3	0.19	4.4 \pm 0.4	4.4 \pm 0.4	0.97
As	Fall	46.2 \pm 2.2	65.0 \pm 5.8	< 0.01	43.1 \pm 4.1	40.5 \pm 1.6	0.53
	Winter	42.3 \pm 3.2	64.0 \pm 3.9	< 0.01	50.3 \pm 1.6	48.3 \pm 1.7	0.44
	Spring	50.1 \pm 5.4	60.1 \pm 3.1	0.19	39.2 \pm 3.0	46.4 \pm 4.5	0.28
	Summer	63.1 \pm 2.7	47.9 \pm 4.1	< 0.01	41.0 \pm 3.6	32.8 \pm 2.4	0.11
Cd	Fall	0.32 \pm 0.02	0.49 \pm 0.04	< 0.01	0.43 \pm 0.02	0.50 \pm 0.05	0.35
	Winter	0.28 \pm 0.03	0.33 \pm 0.02	0.75	0.33 \pm 0.03	0.46 \pm 0.04	0.05
	Spring	0.48 \pm 0.03	0.55 \pm 0.03	0.11	0.47 \pm 0.04	0.55 \pm 0.05	0.25
	Summer	0.58 \pm 0.06	0.42 \pm 0.03	0.09	0.68 \pm 0.05	0.55 \pm 0.03	< 0.05
Cu	Fall	189.3 \pm 31.3	652.0 \pm 56.2	< 0.01	462.6 \pm 63.1	761.6 \pm 102.3	< 0.05
	Winter	264.0 \pm 47.2	705.2 \pm 86.6	< 0.01	592.2 \pm 48.4	859.6 \pm 64.8	< 0.05
	Spring	370.0 \pm 63.3	499.1 \pm 55.6	0.28	476.9 \pm 47.9	688.5 \pm 87.8	0.05
	Summer	530.9 \pm 117.8	358.5 \pm 69.1	0.11	874.8 \pm 69.9	744.9 \pm 46.9	0.19
Fe	Fall	187.7 \pm 41.5	224.6 \pm 35.0	0.44	209.8 \pm 37.9	256.8 \pm 38.5	0.39
	Winter	280.9 \pm 34.6	316.8 \pm 70.4	0.85	264.5 \pm 66.1	259.7 \pm 34.2	0.53
	Spring	192.0 \pm 42.1	275.5 \pm 104.8	1	212.3 \pm 44.1	230.2 \pm 39.1	0.58
	Summer	284.2 \pm 40.9	178.0 \pm 31.6	0.11	402.2 \pm 73.5	239.6 \pm 54.9	< 0.05
Mn	Fall	36.2 \pm 2.0	47.5 \pm 4.5	0.06	23.6 \pm 0.9	27.7 \pm 1.6	0.06
	Winter	25.9 \pm 1.1	39.8 \pm 2.7	< 0.01	23.7 \pm 1.8	23.1 \pm 1.0	0.97
	Spring	22.0 \pm 1.2	29.7 \pm 2.0	< 0.01	16.5 \pm 1.5	18.7 \pm 1.4	0.35
	Summer	34.4 \pm 1.8	27.3 \pm 1.7	< 0.05	34.1 \pm 2.4	25.1 \pm 1.4	< 0.01
Ni	Fall	1.9 \pm 0.1	3.1 \pm 0.2	< 0.01	1.5 \pm 0.1	1.7 \pm 0.1	0.17
	Winter	1.0 \pm 0.1	1.4 \pm 0.2	< 0.05	1.1 \pm 0.2	1.0 \pm 0.2	0.85
	Spring	1.6 \pm 0.2	2.2 \pm 0.2	< 0.05	1.1 \pm 0.1	0.9 \pm 0.3	0.80

	Summer	3.0 ± 0.2	1.7 ± 0.1	< 0.01	1.7 ± 0.3	1.2 ± 0.1	
Zn	Fall	80.3 ± 3.0	97.4 ± 3.8	< 0.01	91.1 ± 4.6	87.2 ± 1.7	0.58
	Winter	68.5 ± 2.2	92.6 ± 3.6	< 0.01	80.5 ± 3.1	86.5 ± 3.2	0.25
	Spring	85.6 ± 4.6	94.9 ± 4.3	0.25	75.7 ± 2.6	83.5 ± 3.4	0.12
	Summer	108.3 ± 6.7	77.9 ± 3.0	< 0.01	103.5 ± 6.6	79.0 ± 3.3	< 0.01

18

19 **Tab. 5** Results of PERMANOVA analyses evaluating the influence of season, bopyrid *Gyge branchialis*
 20 presence and sex of the mud shrimp *Upogebia cf. pusilla* on metal content in the hepatopancreas of mud
 21 shrimp sampled at two sites in Arcachon Bay, France (La Réousse and Le Teychan). P-values in bold
 22 indicate statistically significant effects.

	La Réousse			Le Teychan		
	df	Pseudo-F	<i>P</i> (perm)	df	Pseudo-F	<i>P</i> (perm)
Season (1)	3	5.73	< 0.01	3	6.45	< 0.01
Bopyrid (2)	1	9.61	< 0.01	1	3.38	< 0.01
Sex (3)	2	1.67	0.14	2	2.34	< 0.05
(1) x (2)	3	9.00	< 0.01	3	3.57	< 0.01
(1) x (3)	3	0.60	0.87	3	1.71	0.052
(2) x (3)	1	1.39	0.22	1	2.65	< 0.05
(1) x (2) x (3)	3	1.00	0.44	3	1.88	< 0.05

23

24 **Tab. 6** Results of pairwise comparisons evaluating dissimilarities in metal content (based on a normalised Euclidean resemblance matrix) between
 25 mud shrimp groups given by PERMANOVA analyses for organisms sampled at La Réousse, Arcachon Bay (France) (cf. Table 5). P-values in bold
 26 indicate statistically significant effects. '-': Pairwise comparison not tested.

		Bopyrid-uninfested				Bopyrid-infested			
		Fall	Winter	Spring	Summer	Fall	Winter	Spring	Summer
Bopyrid-uninfested	Fall	/							
	Winter	< 0.01	/						
	Spring	< 0.01	< 0.01	/					
	Summer	< 0.01	< 0.01	< 0.01	/				
Bopyrid-infested	Fall	< 0.01	-	-	-	/			
	Winter	-	0.005	-	-	< 0.01	/		
	Spring	-	-	0.060	-	< 0.05	< 0.01	/	
	Summer	-	-	-	< 0.01	< 0.01	< 0.01	< 0.01	/

27

28

29 **Tab. 7** Results of pairwise comparisons evaluating dissimilarities in metal content (based on a normalised Euclidean resemblance matrix) between
 30 mud shrimp groups given by PERMANOVA analyses for organisms sampled at Le Teychan, Arcachon Bay (France) (cf. Table 5). P-values in bold
 31 indicate statistically significant effects. '-': Pairwise comparison not tested.

		FEMALES								MALES									
		Bopyrid-uninfested				Bopyrid-infested				Bopyrid-uninfested				Bopyrid-infested					
		Fall	Winter	Spring	Summer	Fall	Winter	Spring	Summer	Fall	Winter	Spring	Summer	Fall	Winter	Spring	Summer		
FEMALES	Bopyrid-uninfested	Fall	/																
		Winter	< 0.05	/															
		Spring	0.45	< 0.05	/														
		Summer	< 0.01	< 0.01	< 0.01	/													
	Bopyrid-infested	Fall	0.064	-	-	-	/												
		Winter	-	0.40	-	-	0.50	/											
		Spring	-	-	0.92	-	0.22	< 0.05	/										
		Summer	-	-	-	< 0.01	0.40	< 0.01	0.13	/									
MALES	Bopyrid-uninfested	Fall	< 0.05	-	-	-	-	-	-	/									
		Winter	-	0.055	-	-	-	-	-	-	< 0.01	/							
		Spring	-	-	0.62	-	-	-	-	-	< 0.01	< 0.05	/						
		Summer	-	-	-	< 0.01	-	-	-	-	< 0.05	< 0.01	< 0.01	/					
	Bopyrid-infested	Fall	-	-	-	-	0.97	-	-	-	0.19	-	-	-	/				
		Winter	-	-	-	-	-	0.64	-	-	-	0.056	-	-	0.062	/			
		Spring	-	-	-	-	-	-	0.056	-	-	-	0.064	-	0.067	0.48	/		
		Summer	-	-	-	-	-	-	-	0.71	-	-	-	0.48	0.68	0.20	0.18	/	

32

33 **Tab. 8** Results of PERMANOVA analyses evaluating the influence of season, bopyrid *Gyge branchialis*
 34 presence and sex of the mud shrimp *Upogebia cf. pusilla* on the relative expression of the gene *vtg* in the
 35 hepatopancreas of mud shrimp sampled at two sites in Arcachon Bay, France (La Réousse and Le Teychan).
 36 P-values in bold indicate statistically significant effects.

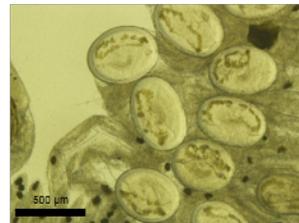
	La Réousse			Le Teychan		
	df	Pseudo-F	<i>P</i> (perm)	df	Pseudo-F	<i>P</i> (perm)
Season (1)	3	11.67	< 0.01	3	9.33	< 0.01
Bopyrid (2)	1	66.76	< 0.01	1	72.27	< 0.01
Sex (3)	2	80.57	< 0.01	2	79.82	< 0.01
(1) x (2)	3	6.97	< 0.01	3	6.23	< 0.01
(1) x (3)	3	9.45	< 0.01	3	7.87	< 0.01
(2) x (3)	1	72.75	< 0.01	1	67.20	< 0.01
(1) x (2) x (3)	3	9.47	< 0.01	3	5.21	< 0.01

37

Gyge branchialis



Maritrema sp.



Upogebia cf. *pusilla*



Castrating effect

Unknown effect

Metal accumulation

No impact

Lower levels of
contaminants in summer

Interference

Dilution effect due to spawning
in summer