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

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Abstract :

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

Graphical abstract

Highlights

► Evaluation of the effect of parasites on metal contamination in an engineer species ► Low influence of trematode parasites on the metal burden of their host ► Bopyrid parasites largely modulate the contamination burden of their host. ► Differential effects of the bopyrid on the host metal burden depending on season

Keywords : Mud shrimp, Parasites, Metal contamination, Seasonal fluctuation, Reproductive cycle

1. Introduction

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

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

In addition to parasitism, bioturbators are exposed to several other biotic and abiotic stressors which influence the intensity of their bioturbation activities (e.g., Diaz and Rosenberg, 1995; Duport et al., 2006; Ouellette et al., 2004). Among these stressors, pollutants are of major concern. Littoral environments in particular are endangered by anthropogenic inputs of contaminants, leading to high levels of contamination in localized areas (e.g., Pan and Wang, 2012; Tueros et al., 2009). Initially present in the water column, contaminants precipitate as solids or are adsorbed to suspended particles on the seafloor bottom (Förstner and Wittmann, 1981). Consequently, coastal environments constitute an important sink for a wide variety of contaminants which have a large range of detrimental effects on bioturbating organisms (e.g., Bat et al., 1998; Chapman and Fink, 1984; Moreira et al., 2006).

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

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

2. Materials and methods

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

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

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

The mud shrimp *U.* cf. *pusilla* is also parasitized by the digenean trematode *Maritrema* sp.. This parasite infects the gills, the visceral mass and the abdominal muscle of its host (Dairain et al., 2017). The trematode occurs as metacercariae in the mud shrimp, meaning the mud shrimp is the second intermediate host of the parasite. The effects of this parasite on *U.* cf. *pusilla* have never been documented. Bopyrid and trematode parasites are negatively associated within their host. This pattern is likely due to the bopyrid parasite interfering with trematode infection *via* alteration of the fitness of the host and associated filtration activity (Dairain et al., 2017; Pascal, 2017).

2.2. Study sites and sampling strategy

Located on the French Atlantic coast, Arcachon Bay (44°42' N, 1°11' W) is a 180-km^2 macrotidal lagoon opening to the ocean through two channels (Fig. 1). The tidal cycle is semi-diurnal with an amplitude of 0.8–4.6 m. Mean sea surface temperature changes from 6 °C in winter to 22.5 °C in summer. In 2007, 39 % of the 110-km^2 intertidal area was covered by the dwarf eelgrass *Zostera noltei* (Plus et al., 2010).

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

2.3. Dissection

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

2.4. Metal determination in biological samples

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

2.5. Metal determination in sediment samples

Sediments samples were freeze-dried and ca. 30 mg DW was added to the polypropylene tubes. Samples were digested with 1.5 mL HCl (12 M, Merck), 0.75 mL HNO_3 (14 M, Merck) and 2 mL HF (22 M, Merck) at 110 °C for 2 h (DigiPREP, SCP Sciences). After complete evaporation, the residues were dissolved in 0.25 mL HNO_3 (14M, Merck) and 5 mL of ultrapure water (Milli-Q), heated to 65 °C for 15 min and brought to 10 mL in volumetric flasks using ultrapure water (Milli-Q) once cooled. Metal concentrations were determined using ICP-MS (XSeries, Thermo Scientific), except Fe and Mn which were determined using ICP-OES (700 Series ICP-OES, Agilent). Certified reference materials (RM 8704 and BCR-277R, NIST) were used in order to check the quality of the analytical method. Detection limits and recovery rates are given in Appendix A.

2.6. Expression dynamics of the *vtg* gene

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

2.6.1. Total RNA extraction and reverse transcription

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

2.6.2. Real-time PCR

A partial sequence of the gene *vtg* was amplified using real-time PCR using forward and reverse primers previously described by Dairain et al. (2018a) . The amplification of cDNA was monitored using the DNA intercaling dye SybrGreen I. Real-time PCR reactions were carried out in a total volume of 20 µL consisting of 10 µL GoTaq® qPCR Master Mix (2X; Promega), 2 µL of a mix of the primer pairs at a final concentration of 2 µM for each primer, 5 µL of a ten-time diluted cDNA mixture and 3 µL of nuclease-free water. Real-time PCR were performed in an Mx3000P (Stratagene). Cycling conditions were as follows: 1 cycle of initial activation at 95 °C for 10 min; followed by 45 cycles at 95 °C for 30 s, 60 °C for 30s and 72 °C for 30 s. Reaction specificity was determined from the dissociation curve of the PCR product. This dissociation curve was obtained by following the SybrGreen I fluorescence level during a gradual heating of the PCR products from 65 to 95 °C. The relative quantification of *vtg* expression was normalized according to the elongation factor 1-alpha (*ef1-α*) gene expression. This reference gene had a constant level of expression during all seasons, between the two sites, in bopyrid-infested and uninfested mud shrimp, and between males and females. The $2^{-\Delta Ct}$ method (Livak and Schmittgen, 2001) was used to generate relative mRNA expression.

2.7. Statistical analyses

2.7.1. Univariate analyses

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

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

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

2.7.2. Multivariate analyses

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

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

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

3. Results

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

3.1. Trematode infection and influence of the bopyrid parasite

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

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

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

3.2. Concentrations of metals in the sediment

Due to lack of replication, the significance of differences of sediment metal concentrations relative to the season or the depth in the sediment column could not be tested. Thus, changes in metal contents in the sediment were assessed qualitatively (Table 3). At La Réousse, there were few differences in metal concentrations in the sediment according to season and depth in the sediment column, except for Ag and Mn (Table 3). For these two metals, concentrations were markedly higher in the upper part of the sediment column than deeper in the sediment column (Table 3). At Le Teychan, the concentrations of several metals were higher in spring than in winter and summer (Ag, Cd, Cu and Zn). There were also some differences in metal concentrations in the sediment according to depth in the sediment column but without consistent pattern (Table 3). Annual average concentrations of metals in sediments at both sites were calculated and showed that sediments at Le Teychan were 1.6 to 4.5 times more contaminated than at La Réousse (Table 3).

3.3. Metal content in mud shrimp

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

3.3.1.Multivariate analyses

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

At Le Teychan, there were significant differences in metal content between mud shrimp according to the season and, to a lesser extent, according to bopyrid occurrence and sex of the organism, with a significant interaction between these three factors (Table 5). PERMANOVA pairwise tests showed a marked effect of season on metal content in bopyrid-uninfested females and males (Table 7). Conversely, there was no significant influence of season on metal content in bopyrid-infested males. With regards to bopyrid-infested females, a significant difference in metal content was only observed in organisms sampled in spring and summer compared with specimens sampled in fall (Table 7, Appendix D). The metal contents were different between bopyrid-infested and uninfested females that were sampled in summer, while there was no difference between bopyrid-infested and uninfested males. Finally, metal content was significantly different between bopyrid-uninfested males and females sampled in fall and summer, while no significant differences between bopyrid-infested males and females were found in the other seasons. The first axis of the PCO explained 36.7 % of the inertia and the second axis 22.6 % (Fig. 3B). The concentrations of Cd, Cu, Fe, Mn and Zn were negatively correlated with Axis 1. None of the two axes of the PCO allowed for a distinct separation of mud shrimp according to their sex, parasitic status or season. Nonetheless, it seemed that bopyrid-uninfested mud shrimp were more positively associated with the first axis than uninfested organisms, independently of their sex, except in summer when bopyrid-infested females were negatively associated with this axis (Fig. 3B, Appendix D).

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

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

3.4. Expression of the *vtg* gene

A significant influence of the sex of mud shrimp, bopyrid occurrence and season on the relative expression of the *vtg* gene was noticed in organisms sampled at La Réousse and Le Teychan, with a significant interaction between the three factors (Table 8, Fig. 4). In bopyrid-uninfested organisms, the level of expression of *vtg* was higher in females than in males at both sites. In females, a significant increase of the expression of *vtg* was noticed in summer (females were ovigerous at that time) compared with fall and winter (Fig. 4). In females, the bopyrid parasite was associated with a significant downregulation of the expression level of *vtg* and the influence of the season was low (Fig. 4). Overall, there was no influence of the bopyrid parasite on the expression of *vtg* at any of the seasons in males. The only exception was bopyrid-infested males sampled in summer at La Réousse, for which the expression of *vtg* was significantly enhanced, but with a high inter-individual variability (Fig. 4).

4. Discussion

4.1. Trematode infection and influence of the bopyrid isopod

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

4.2. Concentrations of metals in sediments

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

Sediments from Le Teychan displayed higher metal concentrations than those from La Réousse. Le Teychan is closer to the main harbour of Arcachon Bay and also to the Leyre River, which is the main input of freshwater into the lagoon (Manaud et al., 1997). Although industrial and urban wastes have not been dumped into the lagoon since the 1970s', inputs of metals from freshwater sources are possible because the Leyre River drains from a watershed dominated by urban and agriculture areas. Particle size of sediments also affects the distribution of metals, with coarse sediments harbouring reduced concentrations of metals compared with finer grain size sediments (Horowitz, 1985). Sediments at La Réousse are coarser than at Le Teychan, which could contribute to their lower concentrations of metals.

4.3. Concentrations of metals in mud shrimp

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

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

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

4.4. Metals in mud shrimp: seasonal fluctuations

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

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

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

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

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

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

During this field survey, a substantial interactive effect between the bopyrid parasite and the season on the variation of metal content in the mud shrimp *U.* cf. *pusilla* was also observed. Metal accumulation was generally higher in bopyrid-infested organisms, except in summer when this trend was reversed. This pattern was not influenced by the sex of mud shrimp at La Réousse, while it was only noticed in females at Le Teychan. The lower metal contamination in bopyrid-infested organisms in summer could be a side-effect of the negative impact of the parasite on its host. The intensity of the activities of mud shrimp is seasonal: mud shrimp are highly active during the summer period, spending a considerable amount of time transporting sediments and ventilating their burrow. In winter, the intensity of their activities is highly reduced (Pascal, 2017). The bopyrid parasite *G. branchialis* globally reduces the activity levels of its host but its deleterious role is more pronounced in summer (Pascal, 2017). The variations in the activity levels of mud shrimp could also be associated to changes in the energetic demand of organisms. The mud shrimp *U.* cf. *pusilla* is a suspension feeder, but it can also directly feed on sediments (Dworschak, 1987). Depending on the bioavailability of metals in sediments, the marked discrepancy in the metal content between bopyrid-infested and uninfested mud shrimp in summer may be due to a higher accumulation of contaminants in uninfested mud shrimp as a consequence of modifications of the feeding rate, compared with infested individuals. However, this mechanism is probably not the main driver of the differences in metal contamination between bopyrid-infested and uninfested organisms in summer because the potential increase in the feeding rate of mud shrimp would likely be associated with a weight gain, which would lead to an enhanced "dilution effect". Instead, we suggest that the seasonal effect of the bopyrid *G. branchialis* on the bioaccumulation pattern of its host is an indirect consequence of the influence of the parasite on the reproductive processes of *U.* cf. *pusilla.* Bopyrid-infested females are not ovigerous (Pascal et al., 2016a) and their ovaries are reduced, sometimes disappearing completely (Tucker, 1930). We also observed that *G. branchialis* largely down-regulates the expression levels of *vtg* in females during the reproductive period, cementing at the molecular scale the negative influence of this parasite on the reproduction functions of its female host. By doing so, *G. branchialis* interferes with the "spawning dilution effect" noticed in summer in bopyrid-uninfested females, especially at Le Teychan (see above). Regarding males, the bopyrid parasite induces their feminization, i.e. males exhibit altered secondary sexual characters (Pascal et al., 2016a; Tucker, 1930). However, the influence of this parasite on the reproductive functions of its male host appears widely variable. For instance, Tucker (1930) reported that the structure of testes and their functioning were not impaired by the presence of the bopyrid parasite in some males, while in other individuals testes were not detectable. The presence of an important number of egg-cells in the testes of several bopyrid-infested males has also been documented (Tucker, 1930). In this study, the *vtg* gene was not up-regulated in bopyrid-infested males, except in summer at La Réousse, but the levels of expression remained well below those reported for females at the same time. Overall, it suggests that the bopyrid did not stimulate oogenesis in infested males. Even if we assume that the bopyrid parasite diminishes the spermatogenesis in its mud shrimp host, the "spawning dilution effect" would be less intense in male mud shrimp than in females because changes in the weight and in the lipid content of the hepatopancreas of crustaceans over the reproductive cycle are more pronounced in females than in males (del R. Gonzalez-Baro and Pollero, 1988; Pillay and Nair, 1973). This contributes to explain the interactive effect of the season, bopyrid occurrence and sex of mud shrimp on the variations in metal content in organisms sampled at Le Teychan. The fact that the interactive effect of the season and of the bopyrid occurrence did not depend on the sex of mud shrimp at La Réousse highlights that others factors control metal contamination. Since both sites are deeply different, the reasons behind this pattern remain unclear.

5. Conclusion

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

Compliance with ethical standards

Declarations of interest: none.

Animal rights: All applicable international, national and/or institutional guidelines for the care and use of animals were followed.

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Dissolved arsenic in the upper Paraguay River basin and Pantanal wetlands

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Abstract

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

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

Dissolved arsenic in the upper Paraguay River basin and Pantanal wetlands

1 Introduction

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

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

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

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

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

2 Regional setting

The Upper Paraguay River Basin: being around 2.8×10^6 km^2, the Upper Plata River drainage system is the second largest basin in South America after the Amazonian basin. Its upstream section consists of two basins of similar size, namely the Parana and Paraguay basins. The major difference between these tributaries is the presence of the vast Pantanal floodplain located in the Paraguay headwaters (Fig. 1). The Pantanal (about 0.2×10^6 km^2) is a biodiversity hotspot classified as UNESCO Natural World Heritage site, and a priority region for environmental conservation (Olson and Dinerstein, 2002). Unlike the upper Parana basin, whose river chemistry is clearly impacted by the Brazilian megacities, the upper Paraguay basin is still relatively preserved, although some alterations in the water chemical profile have already been detected in downstream areas with extensive cropping (Rezende Filho et al., 2015).

[Insert Fig. 1 here]

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

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

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

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

Nhecolândia sub-region: Nhecolândia lies in the floodplain on the southern half of the Taquari fan (Fig. 1). It comprises an area of approximately 24,000 km^2, delimited in the north by the Taquari River, in the south by the Negro River, in the west by a portion of the Paraguay River, and in the east by the Maracajú Plateau, which corresponds to the southeastern edge of the Pantanal wetland. Aside from these rivers, the region has a relatively closed drainage with little connection to the major river system, and the water usually flows below the surface within sandy soils and along drainage fields called "vazantes". The peculiarity of this region is the presence of about 15,000 lakes, including about 500 saline-alkaline ones (Furian et al., 2013). While freshwater lakes supply the regional water-table during almost all seasons (Freitas et al., 2019), saline alkaline lakes are disconnected by low permeability soil horizons, and supply the aquifer only fleetingly during strong rainy events (Barbiero et al., 2008; Furian et al., 2013). Previous studies have shown that the alkaline lakes may be classified within 3 different types, depending on their biogeochemical functioning (Andreote et al., 2018, 2014; Barbiero et al., 2018; Martins, 2012; Vaz et al., 2015), i.e. green, black and crystalline water lakes. Their electrical conductivity ranges usually from 1500 to 15,000 $\mu S\ cm^{-1}$, with exceptional values recorded up to 80,000 $\mu S\ cm^{-1}$ at the end of the dry season. In parallel, the pH oscillates from 8.9 to 10.7. High pH and EC result from cumulative evaporation over years of water supplied near the surface from the vazantes and/or freshwater lakes towards the saline lakes (Barbiero et al., 2008; Furian et al., 2013). Soils around alkaline lakes have a standard organization of which a simplified model is shown in Figure 2 (adapted from Barbiero et al., 2016). It mainly consists of 5 contrasting horizons. Close to the lake, a grey-brown topsoil loamy sand horizon (1) is observed usually with numerous calcareous precipitations. The occurrence of this horizon is limited to the oscillation zone of the lakeshore between the wet and dry seasons. Below, there is a light brown sandy material (2) with less than 1% clay. Within horizon 2, high water pH conditions favor large dissolved organic carbon contents, which precipitate into blackish volumes at the base of this material and defines horizon (3). Subjacent to this, there is a massive (single grain), greyish loamy sand material (horizon 4) with about 15% clay. The top of this horizon (4) is wavy. Further below lies a loamy sand, olive to light olive-grey colored horizon (5), with 15–20% clay, massive structure (coherent and cemented) and locally extremely firm consistency.

[Insert Fig. 2 here]

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

3 Materials and Methods

3.1 Sampling and Database

3.1.1 Regional UPRB study: For the study at the UPRB scale, we used 4 datasets. Dataset 1, 2 and 3 consists of 56 river samples each, collected in December 2012, March 2013 and May 2013, respectively, *i.e.* at the beginning, middle and end of the wet season. The collection took place on the uplands, at the south-eastern and southern border of the Pantanal from the cities of Coxím to Porto Murtinho (Fig. 1). Datasets 4 was collected from November 2010 to January 2011 along the main drainage axis of the floodplain, that is, the Paraguay (21 samples) and Cuiaba (nine samples) Rivers, and a few kilometers upstream the confluence with their major tributaries (88 samples). All samples were collected at approximately 0.3-m depth in the middle of the river section. The sampling procedure as well as the major ion chemistry were detailed in Rezende Filho et al., (2015, Supplemental Material S1 and S2). All samples for trace element determination were filtered (0.45µm cellulose acetate) in the field and acidified with ultrapure HNO_3.

3.1.2 Local study at Nhecolândia : Water, sediment and soil collection was carried out in two sites of Nhecolândia (São Roque and Centenário farms) and compared with previous results obtained at Nhumirím farm in 2 alkaline lakes and surrounding piezometers (Barbiero et al., 2007). These three sites, located in the central and southern part of Nhecolândia, cover complementary geographical positions intersecting the regional drainage oriented east northeast – west southwest (Fig. 2). At São Roque farm, water samples were collected in extreme dry (September and October 2017) and wet conditions (August and September 2018) in 6 saline lakes (referred to as SR06 with black water, SR01, SR04, SR05, SR08 and SR09 with green water, and SR07 with crystalline water) and three freshwater lakes (BSR03, BSR04, BSR05). Lake sediments were collected in 2017. At the Centenário farm, sampling was carried out in 3 saline lakes (referred as CN01 with green water, CN02 with black water and CN03 with crystalline water), 1 vazante and 1 fresh water lake. Samples were collected during the dry season (September 2015 and 2016, and October 2017) and at the beginning (November 2015) and the end of the wet season (June 2016 and August 2018). Lake sediments (0-20 cm) were sampled in 2016. In addition, water samples were also taken in the perched shallow water-table of the soil systems surrounding the lakes at Centenário site. For this, fifteen water-table samplers (lysimeters) consisting of pierced polyethylene containers (Maître, 1991) were installed in the water-table through auger holes (7cm in diam.). They are further referred to as (G01S, G02S and G01 to G13). The 120-ml containers were wrapped with a synthetic tissue to prevent clogging by soil particles. Two capillary tubes (1 mm inner diam.), inserted into the container, reach the soil surface. The first one ends at the upper part of the container just below the cap and is used for injecting N_2 gas. The second one, down to the lower part of the container, is used to collect the groundwater sample by depression. After installing the samplers, the holes were filled with the initial material preserving the order of the different layers up to the soil surface. The sampling device prevents contact between the water-table and the atmosphere and thus preserves the redox conditions of the water-table within the sampler. The samples were collected from the lysimeters with a hand-held vacuum pump by gentle pumping while injecting N_2 flow at a maximum pressure of 0.05 atm, in order to avoid turbulences and to prevent drastic changes in redox conditions in the sampler. The samples first reached a closed Erlenmeyer previously filled with N_2 to avoid rapid oxidation. The first drops were driven toward the sensitive part of the potential Pt-probe (ref HI3620D) and the lowest value (usually after ~5 seconds) was noted. A value of +203 mV was added to the measured potential for its conversion into redox potential Eh, assuming that the temperature was almost constant close to 30 °C. Then the samples were stored into 120 ml acid washed HDPE container filled up and closed without air bubbles. All samples were preserved in a cold and dark place until filtration. Temperature (T), electrical conductivity (EC) and pH were determined in the field in aliquots. Soil horizons in contact with the lysimeters were also collected for arsenic contents determination.

3.2 Analytical Methods and data treatment

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

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

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

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

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

For samples collected in 2018 on the SR farm, speciation analysis was also performed through HPLC-HG-AFS (Millennium Excalibur 10.055, PS Analytical). The separation of only four As species, As(III), DMA, MMA and As(V) was carried out with an anion exchange column (Hamilton PRP-X100 (10 µm, 250 mm x 4.1 mm). A chromatographic method was adapted from PSAnalytical Application Note APP 160, using Na_2HPO_4 and NaH_2PO_4 20 mmol L^{-1} (Sigma-Aldrich reagents with purity ≥ 99%) as a mobile phase at pH 6.2 in isocratic mode. The chromatographic run was 13 minutes with an injection volume of 200 µL. LOD and LOQ (µg L^{-1}) for each species were 0.46 and 1.38 for As(III), 0.31and 0.93 for DMA, 1.31 and 3.92 for MMA, and 1.12 and 3.35 for As(V). Some As organic species do not produce a hydride. Therefore, for each sample, a qualitative analysis was performed to confirm the absence of these species. The method was adapted from Ma et al., (2014), including a UV digestion step. The chromatographic separation used the same anion exchange column and a mobile phases, (A): 4 mmol L^{-1} $NaHCO_3$ and (B): 4/40 mmol L^{-1} $NaHCO_3/NaNO_3$ solutions at pH 9.5. The chromatographic run time was 15 minutes with the following gradient elution program: 100 % A, 3 min.; 50 % A and 50% B 4 min.; 100 % B 5 min. and 100 % A 3 min.

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

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

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

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

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

4 Results

4.1 As concentration in the rivers from the UPRB

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

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

[Insert Fig. 3 here]

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

Regarding the Cuiaba and Paraguay rivers (dataset 4), which are the two main draining rivers of the floodplain, there was a trend of increasing As levels towards downstream (Fig. 4). Arsenic values in the Cuiaba River (Fig. 4a) gradually increased from 0.25 µg L^{-1} just downstream the city of Cuiaba to 0.65 µg L^{-1} at its confluence with the Paraguay River. The first increase to a value of about 0.35 µg L^{-1} occurred after the mixing with the waters of “Baia do Agapito” (1.02 µg L^{-1}) and the confluence with River Urutubinha (0.37 to 0.49 µg L^{-1}). The second increase (0.40 µg L^{-1}) occurred after the contribution of the Muquem River (0.64 µg L^{-1}). The confluence with São Lourenço River (0.73 and 1.04 µg L^{-1}) and its secondary channels (0.93 to 1.8 µg L^{-1}) caused an increase in As content up to 0.52 µg L^{-1}, then a final contribution of the Piquiri River (0.76 µg L^{-1}) stabilized the value at about 0.65 µg L^{-1} down to the Cuiaba-Paraguay confluence. Throughout the upper stretch of the Paraguay (from Caceres to “Baia do Tamengo” close to the city of Corumba) (Fig. 4b), As water contents were rather stable ranging from 0.21 to 0.32 µg L^{-1}. This stability can be attributed to two characteristics: on the one hand the high Paraguay River flow compared to that of its tributaries (from field estimate, no quantitative data are available), and on the other hand the moderate As levels in the tributaries that ranged from 0.17 to 0.56 µg L^{-1} (0.35 ± 0.12 µg L^{-1}), with the only exceptions of two tributaries (“Boca inferior da Baia Branca” and “Boca do Tuiuiu”) in which the As concentrations were 0.70 and 0.80 µg L^{-1}, respectively. Downriver from Corumba city, As concentration in the Paraguay water kept increasing gradually, first up to 0.43 µg L^{-1} after the confluence with Taquari (0.48 to 0.58 µg L^{-1}) and Negro Rivers (0.91 µg L^{-1}), then 0.50, 0.60, and 0.65 µg L^{-1} after receiving the water from the Abobral (0.53 µg L^{-1}), Miranda (0.41 to 0.62 µg L^{-1}), and Nabileque (1.88 µg L^{-1}) Rivers, respectively.

[Insert Fig. 4 here]

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

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

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

[Insert Fig. 5 here]

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

4.2 Arsenic concentrations in the Nhecolândia region.

4.2.1 Concentration ranges in waters

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

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

[Insert Table 1 here]

By contrast, dissolved arsenic contents in alkaline lakes were much higher, ranging from 28.8 to 2,916 μg L^{-1} (Table 1), and fluctuate depending of the season and year of collection. This effect is particularly clear when comparing the concentrations in samples collected from the same lakes at the CN site in October 2017 (atypical dry season with very low water level and high concentrations) and in August 2018 (atypical dry season with high water level and low concentrations). The lowest concentrations were always found in the crystalline water lakes (CN03 and SR07), whereas the highest ones were observed in the green and black water lakes. The As concentrations reported for the two lakes of the Nhumirím Farm, collected during a particularly dry episode, were of the same order of magnitude (Table 1). In water-table, samples collected from lysimeters at the CN site, arsenic concentrations ranged from 0.8 to 3581.5 µg L^{-1} (Table 2). Low values were observed in deep samples collected on higher grounds (G03, G05 and G12), whereas the highest concentrations were found in samples collected within organic horizons (G01S, G02S and G13) around the alkaline lakes. In comparison, dissolved As in groundwater around the lakes on the Nhumirím farm (collected from piezometers) ranged from 0.14 to 266 µg L^{-1}.

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

[Insert Table 2 here]

4.2.2 As and major elements

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

[Insert Fig. 6 here]

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

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

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

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

[Insert Fig. 7 here]

Figure 7: Arsenic Pourbaix diagram showing the As speciation expected for the watertable samples collected in surface water (S) and soil horizons (Hig, Cly or Org) at Centenário farm (see Table 1 for the corresponding total As concentrations). Both, pH and Eh were measured in the field, under N_2 flux.

[Insert Fig. 8 here]

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

4.2.4 As in lake sediments and soil horizons

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

5 Discussion

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

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

On the other hand, the results are much more contrasting in the Nhecolândia floodplain. At all three studied sites, elevated As levels are noted, indicating that its occurrence is a consequence of processes that operate on a regional scale. In addition, the similar behavior of arsenic with respect to sodium confirms that the same processes are at work in these three sites. Huge As variations are observed over short distances, i.e. a few hundred meters that separate the vazantes and freshwater lakes from the alkaline lakes, as well as the few tens of meters that separate the higher grounds at the top of the beaches from the border of the alkaline lakes. The results of the speciation carried out at Centenário site show that As mainly occurs as arsenate (Fig. 8). The As contents variability must be related to the hydrological and hydrochemical functioning of this system of lakes. The vazantes are the water supplying areas (Furian et al., 2013). In the short term, they mainly receive water from the seasonal rains, but over the long term, they are also supplied by the overflows of the Taquari River. Therefore, this river arising from sandstone area imposes its chemical characteristics, in particular the low arsenic contents (Table 1) and a positive calcite residual alkalinity ($RA_{calcite}$) (Oliveira Junior et al., 2019; Rezende Filho et al., 2012). During the wet season, while freshwater lakes are generally supplied by overflow during the flood pulse, alkaline saline lakes receive a reduced amount of freshwater through sub-surface flows from the vazantes. During the dry season, waters concentrate under the effect of evaporation, not only in the alkaline lakes but also in the perched water-table and of surrounding beaches. In the geochemical context of positive $RA_{calcite}$ (Barbiero et al., 2002), alkalinity increases with increasing evapo-concentration while calcium levels remain very low due to calcite precipitation. The solution pH increases, mainly controlled by carbonate species. Close to alkaline lakeshores, the solutions are more concentrated due to higher evaporation by wicking in the sandy material (Barbiero et al., 2016). Magnesium silicates precipitate (in horizon 1, see Fig. 2), controlling dissolved Mg at a low level, while alkalinity keep increasing (Furquim et al., 2008). Finally, the mineralization of dissolved organic matter a few tens of meters from the lake shore releases iron and aluminum allowing the synthesis of Fe-micas (in horizons 4 and 5, Barbiero et al., 2016; Furquim et al., 2010). This succession of saline precipitations (calcite, Mg-silicate, Fe-micas) is standard in alkaline environments (Barbiero et al., 2004).

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

[Insert Fig. 9 here]

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

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

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

6 Conclusion

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

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Tab. 1 Characteristics of the two sampling sites in Arcachon Bay, France. D_{50}: 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 *Gyge branchialis* (%)[2]	11.6	12.5
Prevalence of *Maritrema* sp. (%)[2]	93.4	58.9
Abundance of *Maritrema* sp. (metacercariae. ind^{-1}, mean ± SE)[2]	66.5 ± 7.7	10.9 ± 2.9

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

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

	La Réousse			**Le Teychan**		
	df	Pseudo-F	*P(perm)*	df	Pseudo-F	*P(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

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

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

Tab. 4 Average concentrations (mean ± SE; µg g^{-1} DW) of metals in the hepatopancreas of mud shrimp *Upogebia* cf. *pusilla* uninfested and infested with the bopyrid parasite *Gyge branchialis* over a one year seasonal survey conducted at two sampling sites (La Réousse and Le Teychan) in Arcachon 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**	***p-value***	**Bopyrid-uninfested**	**Bopyrid-infested**	***p-value***
Ag	Fall	2.0 ± 0.2	4.5 ± 0.5	**< 0.01**	3.5 ± 0.3	4.5 ± 0.4	0.14
	Winter	2.6 ± 0.1	4.6 ± 0.4	**< 0.01**	4.3 ± 0.2	5.1 ± 0.4	0.25
	Spring	3.0 ± 0.3	4.1 ± 0.3	**< 0.01**	3.3 ± 0.3	4.2 ± 0.4	0.09
	Summer	3.7 ± 0.4	3.0 ± 0.3	0.19	4.4 ± 0.4	4.4 ± 0.4	0.97
As	Fall	46.2 ± 2.2	65.0 ± 5.8	**< 0.01**	43.1 ± 4.1	40.5 ± 1.6	0.53
	Winter	42.3 ± 3.2	64.0 ± 3.9	**< 0.01**	50.3 ± 1.6	48.3 ± 1.7	0.44
	Spring	50.1 ± 5.4	60.1 ± 3.1	0.19	39.2 ± 3.0	46.4 ± 4.5	0.28
	Summer	63.1 ± 2.7	47.9 ± 4.1	**< 0.01**	41.0 ± 3.6	32.8 ± 2.4	0.11
Cd	Fall	0.32 ± 0.02	0.49 ± 0.04	**< 0.01**	0.43 ± 0.02	0.50 ± 0.05	0.35
	Winter	0.28 ± 0.03	0.33 ± 0.02	0.75	0.33 ± 0.03	0.46 ± 0.04	0.05
	Spring	0.48 ± 0.03	0.55 ± 0.03	0.11	0.47 ± 0.04	0.55 ± 0.05	0.25
	Summer	0.58 ± 0.06	0.42 ± 0.03	0.09	0.68 ± 0.05	0.55 ± 0.03	**< 0.05**
Cu	Fall	189.3 ± 31.3	652.0 ± 56.2	**< 0.01**	462.6 ± 63.1	761.6 ± 102.3	**< 0.05**
	Winter	264.0 ± 47.2	705.2 ± 86.6	**< 0.01**	592.2 ± 48.4	859.6 ± 64.8	**< 0.05**
	Spring	370.0 ± 63.3	499.1 ± 55.6	0.28	476.9 ± 47.9	688.5 ± 87.8	0.05
	Summer	530.9 ± 117.8	358.5 ± 69.1	0.11	874.8 ± 69.9	744.9 ± 46.9	0.19
Fe	Fall	187.7 ± 41.5	224.6 ± 35.0	0.44	209.8 ± 37.9	256.8 ± 38.5	0.39
	Winter	280.9 ± 34.6	316.8 ± 70.4	0.85	264.5 ± 66.1	259.7 ± 34.2	0.53
	Spring	192.0 ± 42.1	275.5 ± 104.8	1	212.3 ± 44.1	230.2 ± 39.1	0.58
	Summer	284.2 ± 40.9	178.0 ± 31.6	0.11	402.2 ± 73.5	239.6 ± 54.9	**< 0.05**
Mn	Fall	36.2 ± 2.0	47.5 ± 4.5	0.06	23.6 ± 0.9	27.7 ± 1.6	0.06
	Winter	25.9 ± 1.1	39.8 ± 2.7	**< 0.01**	23.7 ± 1.8	23.1 ± 1.0	0.97
	Spring	22.0 ± 1.2	29.7 ± 2.0	**< 0.01**	16.5 ± 1.5	18.7 ± 1.4	0.35
	Summer	34.4 ± 1.8	27.3 ± 1.7	**< 0.05**	34.1 ± 2.4	25.1 ± 1.4	**< 0.01**
Ni	Fall	1.9 ± 0.1	3.1 ± 0.2	**< 0.01**	1.5 ± 0.1	1.7 ± 0.1	0.17
	Winter	1.0 ± 0.1	1.4 ± 0.2	**< 0.05**	1.1 ± 0.2	1.0 ± 0.2	0.85
	Spring	1.6 ± 0.2	2.2 ± 0.2	**< 0.05**	1.1 ± 0.1	0.9 ± 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**

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

	La Réousse			**Le Teychan**		
	df	Pseudo-F	*P(perm)*	df	Pseudo-F	*P(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**

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

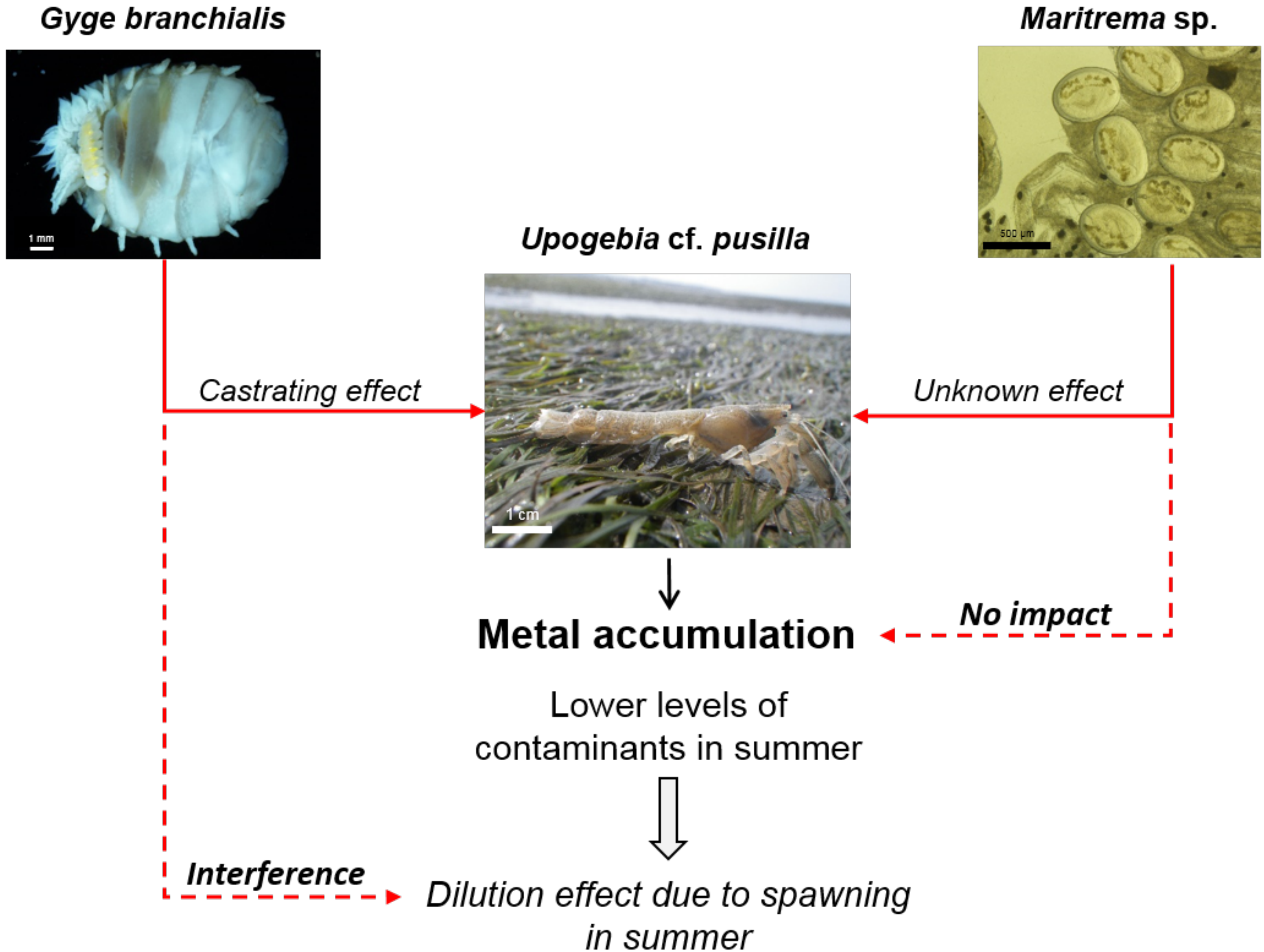
		Bopyrid-uninfested				**Bopyrid-infested**			
		Fall	**Winter**	**Spring**	**Summer**	**Fall**	**Winter**	**Spring**	**Summer**
Bopyrid-uninfested	**Fall**	/							
	Winter	**< 0.01**	/						
	Spring	**< 0.01**	**< 0.01**	/					
	Summer	**< 0.01**	**< 0.01**	**< 0.01**	/				
Bopyrid-infested	**Fall**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

Tab. 7 Results of pairwise comparisons evaluating dissimilarities in metal content (based on a normalised Euclidean resemblance matrix) between mud shrimp groups given by PERMANOVA analyses for organisms sampled at Le Teychan, Arcachon Bay (France) (cf. Table 5). P-values in bold 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	/

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

	La Réousse			**Le Teychan**		
	df	Pseudo-F	*P(perm)*	df	Pseudo-F	*P(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**



Gyge branchialis
Maritrema sp.
Upogebia cf. pusilla
1 mm
500 µm
1 cm
Castrating effect
Unknown effect
Metal accumulation
No impact
Lower levels of contaminants in summer
Interference
Dilution effect due to spawning in summer