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Thomas Baudry, Eric Gismondi, Jean-Pierre Goût, Alexandre Arqué, Juliette Smith-Ravin, et al.. The invasive crayfish Cherax quadricarinatus facing chlordecone in Martinique: Bioaccumulation and depuration study. Chemosphere, 2022, 286, Part 3, pp.131926. 10.1016/j.chemosphere.2021.131926 . hal-04063266

HAL Id: hal-04063266 https://hal.science/hal-04063266

Submitted on 21 Jun2023

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The invasive crayfish *Cherax quadricarinatus* facing chlordecone in Martinique: bioaccumulation and depuration study

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1 Abstract

2 The redclaw crayfish, Cherax quadricarinatus, was introduced to Martinique Island 3 for aquaculture purposes at the beginning of the 21st century, in an attempt to revitalize the 4 freshwater crustacean aquaculture sector. Mainly due to its high economical value, it was intentionally released in the wild and was caught and sold by fishermen. Martinican rivers are 5 polluted by chlordecone, considered as one of the worst Persistant Organic Pollutants (POP). 6 Despite its dangerousness, it was used until 1993 in the French West Indies against a banana 7 8 pest and was always found in the ecosystems. This study aimed to investigate the level of contamination in the muscle of crayfish caught in the wild, as well as the potential of 9 bioconcentration and depuration in the C. quadricarinatus muscle. This study could allow us 10 11 to quantify the risk for consumers but also, to evaluate a depuration process to reduce the risk 12 related to its consumption. Using both *in-vitro* and *in-situ* experiments, results highlighted the 13 importance of the chlordecone concentration in the water and the time of exposure to the 14 pollutant. The bioconcentration seems to be very quick and continuous in crayfish muscle, as 15 chlordecone can be detectable as early as 6 hours of exposure, whatever the concentration 16 tested. Finally, it appears that, even after 20 days of depuration in chlordecone-free water, chlordecone concentrations remained higher to the residual maximum limit (i.e. 20 ng/g wet 17 weight), concluding that the decontamination of the muscle seems not very efficient, and the 18 19 risk for the Martinican people could be serious.

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- 21
- 22 <u>Keywords:</u> Aquatic ecotoxicology, Decapoda, French West Indies, Invasive species, POP
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26 1. Introduction

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Worldwide aquaculture development is known to be a pathway for the introduction of nonnative species to new countries (FAO, 2016). It can lead to the transfer of organisms into new ecosystems facilitated by humans especially when sold alive and at a high price. It can result in serious aquatic invasions threatening native aquatic ecosystems (Filipová et al., 2013).

32 The Australian redclaw crayfish *Cherax quadricarinatus*, is a good example as it was 33 translocated to many territories in tropical and sub-tropical areas (references in Baudry et al., 34 2020) for aquaculture, due to its high reproductive capacity and rapid growth (Jones, 1990; 35 Patoka et al., 2014; Azofeifa-Solano et al., 2017). In Martinique, a tense ecological situation led to the closure of many aquaculture facilities raising the shrimps Macrobrachium 36 37 rosenbergii. In fact, most of the soils where this species was produced became contaminated with chlordecone (CLD). Only one farm was able to survive by exporting its activity to a 38 39 smaller site, making the production of *M. rosenbergii* unprofitable and forcing the farmer to 40 find an alternative (Baudry et al., 2020). Cherax quadricarinatus was finally introduced to the 41 island in the early 2000s, from Cuba, in order to reinvigorate this sector (Baudry et al., 2020). Following intentional releases, crayfish were found in the natural environment and, thanks to 42 43 their high dispersal capacity and its high tolerance of environmental quality, were able to 44 colonize a large part of the island's river systems (Baudry et al., 2021).

45

In the international toxicological context, several studies have shown that invasive species have a higher absorption potential than native ones, particularly in the presence of abiotic stressors (Fedorenkova et al., 2013; Pedro et al., 2017). For example, Pedro et al. (2017) show a higher concentration of POPs in non-native fish species capelin and sandlance (*Mallotus villotus* and *Ammodytes spp.*) than in the native Arctic cod (*Boreogadus saida*). Weir and Salice (2012) show the higher resistance of the snail species *Melanoides tuberculatus* to malathion compared to the native *Biomphalaria glabrata*. In bryozoans, large drops in biomass, often with no recovery, are observed in native species exposed to heavy metals, whereas in non-native species, an increase in biomass is sometimes even observed (Crooks et al., 2011).

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French West Indies soils and rivers are contaminated by chlordecone (CLD) 57 58 (C₁₀Cl₁₀O; CAS number 143-50-0) (Coat et al., 2006; Lafontaine et al., 2017), considered to be the worst Persistent Organic Pollutant (POP) for these areas. It was widely used to 59 eliminate the banana weevil (Cosmopolites sordidus), considered the most important banana 60 crop pest (Serge, 1994; Cabidoche and Lesueur-Jannoyer, 2012). The hazards of this pollutant 61 are known and led to a refusal to register it by the Toxics Commission and the ban of its use 62 63 in 1977 by the United States (Cannon et al., 1978). Indeed, chlordecone is well known to be an endocrine disruptor and could also be responsible for other diseases such as prostate 64 65 cancer, for example (Multigner et al., 2007, 2010). In France, CLD was only prohibited in 66 1990 and the French West Indies obtained a 3-year derogation, until 1993, waiting to find an alternative to this pesticide (Cabidoche et al., 2009; Cabidoche and Lesueur-Jannoyer, 2012). 67

68

Like most pesticides, CLD can be bioaccumulated along trophic chains (Bahner et al., 1977; Hansen et al., 1977). This is rather worrying given that aquatic ecosystems are well known to be the final receptacle of these toxic molecules through soil leaching and/or erosion processes (Snegaroff, 1977; Coat et al., 2006; Cabidoche and Lesueur-Jannoyer, 2012). Because of its very high persistence in soils, CLD is still present today in soils and rivers of Martinique (Merlin et al., 2016) and also in biota. Indeed, several studies highlighted a positive correlation between CLD concentrations in the water and CLD concentrations

measured in organisms (Hansen et al., 1977) with potentially very high bioconcentration 76 factors (Bahner et al., 1977). This highlights the dangerousness of the situation in Martinique 77 given that C. quadricarinatus, having a preference for slow-moving waters, often located 78 79 downstream and therefore receiving a large dose of pollutants, is highly likely to accumulate substantial doses of CLD. Highly appreciated by local fishermen, C. quadricarinatus is fished 80 in the wild and resold at high prices on the markets or to restaurants, but this could raise a 81 major human health problem in the population, given the high doses of chlordecone found in 82 83 the water at some sites, especially in the North of Martinique (Figure 1). CLD concentrations in Martinican rivers can be very variable (Figure 1). The figure 1 exposed the variability in 84 85 terms of CLD concentration in the water. This variability can be explained, for example, by different CLD amount used in the past by each banana plantation owner, known to be largely 86 87 located in the North. This observation could also by due to the type of soil which plays a role 88 in the CLD retention and depending on the location of the different plantations, from north to 89 south, the soil composition is not the same. Indeed, Cabidoche et al. (2009) modeled the 90 persistence of chlordecone in different type of soil and highlighted that CLD is more 91 persistent in andosol than nitisol. Finally, CLD concentrations measured in rivers are also dependent to the rainfall, responsible for soil leaching, and the North of the island is known to 92 93 be more exposed to the rainfalls. As the problem is destined to persist over time, given that 94 CLD has a very long persistence in the environment, the French health authorities have set a 95 Maximum Residual Limit (MRL), above which products are considered consumable, which is at 20 ng.g⁻¹ wet weight. 96

97

98 Therefore, the aims of this study were to (i) investigate the presence of CLD in the 99 abdominal muscle (part consumed by humans) of *C. quadricarinatus* collected from three 100 Martinican rivers according to a contamination gradient (biomonitoring), then (ii) determine the kinetics of bioconcentration of CLD in *C. quadricarinatus* muscle under controlled
laboratory conditions to understand the bioconcentration mechanism and finally, (iii)
investigate a potential decontamination of the crayfish before its consumption, to assess its
risk as a food product.



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Figure 1: Map showing the presence of *Cherax quadricarinatus* in Martinique and the stations
 where the chlordecone concentration in water is known. Colored points correspond to these
 stations, darker points indicate more polluted stations. Invaded watersheds by *Cherax quadricarinatus* are highlighted by red dotted lines, from data in Baudry et al. (2021).

110

111 2. <u>Material and method</u>

112 2.1. Is CLD bioaccumulated in wild crayfish?

113 Three sites, known to be contaminated by chlordecone, and a control site 114 (uncontaminated) were selected to investigate the potential of *C. quadricarinatus* to 115 accumulate CLD.

The four sites were selected according to a CLD-gradient of contamination, representing 116 the main CLD concentrations measured in Martinique rivers where crayfish populations were 117 118 observed (Baudry et al., 2021). These four sites were: a non-contaminated site, corresponding to the hatchery (CLD concentration in the water < limit of detection (LOD)), a low 119 contaminated site "Manzo" (below quantification limit 0.03 µg.L⁻¹), an intermediate station 120 on the Coulisses stream (annual mean $0.45 \ \mu g.L^{-1}$) and a highly contaminated station, on the 121 Lezarde river (annual mean 2.48 μ g.L⁻¹). At each sampling site, five male crayfish were 122 123 collected, between 10 and 14 cm (i.e. adult stage), weighed and measured. Only males were analyzed to avoid possible bias in analysis due to the reproductive cycle in females 124 (Lafontaine et al., 2017). After sampling, abdominal muscle was dissected in laboratory, 125 weighed and stored at -20°C until analysis. 126

127

- 128 2.2.What are the CLD bioconcentration and depuration kinetics in crayfish in laboratory129 conditions?
- 130 2.2.1. <u>Kinetics of bioconcentration</u>

A total of 180 crayfish males were provided by a hatchery in Carbet (Martinique), a CLDfree area, according to results of 'Office de l'Eau de Martinique' (ODE). They were all between 10 and 14 cm body length and between 15 and 59 grams (the most common size range in the Martinique rivers, *Personal observation*) and were transferred to the laboratory of IFREMER, Le Robert, Martinique. As for *in-situ* experiments, only males were used, to avoid bias due to reproduction cycle in females.

137 Thirty 20L aquaria were filled with CLD-free water. Crayfishes were acclimated, for138 seven days before experiments, under constant oxygenation aeration and a natural light/dark

photoperiod. Water temperature and pH were maintained at $27^{\circ}C \pm 0.11$ and 7.72 ± 0.28 corresponding to optimal values for *C. quadricarinatus* development (de Moor, 2002; Tropea et al., 2010). Crayfish were fed daily with pieces of carrot.

Exposure was carried out using two chlordecone conditions: $0.2 \ \mu g.L^{-1}$, $2 \ \mu g.L^{-1}$, chosen 142 for their environmental relevance in rivers of Martinique, and a control (containing 0.01%) 143 acetone, corresponding to the use of prepared stock solutions of CLD in acetone). 144 145 Intermediate solutions were prepared with two successive dilutions from CLD (GC purity area \geq 98%; Sigma-Aldrich, USA): first, a 1 g.L⁻¹ CLD stock solution was prepared with 146 147 100% acetone and then, a 10 mg.L⁻¹ CLD solution was prepared with water (therefore containing 1% acetone), used in exposure media preparation. The real CLD concentrations of 148 149 exposure were measured at the Laboratoire Territorial d'Analyse (LTA) of Martinique (Table 150 1).

Table 1: Comparison of nominal values of the different treatments run during the experiments with the mean measured values, performed by the Laboratoire Territorial d'Analyses de Martinique. One water sampling per aquarium, in 3 different aquaria (3 replicates) per condition was done.

Treatment	Nominal value (µg.L ⁻¹)	Mean value ($\mu g.L^{\cdot 1}$) ± Standard deviation		
Control	0	< 0.01*		
C1	0.2	0.23 ± 0.03		
C2	2	2.75 ± 0.21		
1 day-depuration	-	0.02 ± 0.003		
3 day-depuration	-	0.06 ± 0.02		

155 *0.01 μ g.L⁻¹ representing the Limit of Detection of chlordecone.

156 Ten aquaria per condition were used (six individuals per aquarium). As for the157 acclimation period, crayfishes were fed daily with pieces of carrot, small enough (around

0.125 cm³) to be eaten quickly and avoid a possible adsorption of pollutant. According to
Lafontaine et al. (2017), to keep a constant CLD concentration in water during all the
experiments, it is necessary to perform a 7-day period to saturate the aquariums' walls before
exposure, and renew the exposure medium every 96h.

To analyze the CLD concentration in *C. quadricarinatus*, five individuals per condition
were randomly sampled in aquariums after six exposure times: 6h, 1day, 2d, 6d, 12d and 20d.
The abdominal muscle was dissected, weighed and conserved at -20°C until analysis.

165 2.2.2. <u>Kinetics of depuration</u>

166 At the end of the bioconcentration experiment (i.e. 20 days), the remaining individuals (from 0.2 and 2 µg.L⁻¹ exposure) were rinsed thoroughly with CLD-free water (to avoid 167 168 possible traces of CLD adsorbed on the cuticle) and transferred to different aquaria filled with 169 CLD-free water, to analyze a possible decontamination. Experimental conditions in terms of 170 feeding, photoperiod and physico-chemical values were the same as for the bioconcentration 171 kinetics experiment. Moreover, to avoid a possible recontamination of individuals, water was 172 renewed every 3 days. Five crayfishes per condition were collected after 1, 2, 8 and 20 days of depuration. The abdominal muscle was dissected, weighed and stored at -20°C until CLD 173 analysis. Only for aquaria where crayfish initially exposed to 2 μ g.L⁻¹ of CLD were placed, 174 175 the CLD concentration was measured at day 1 and day 3 to quantify a possible CLD release in the depuration water (Table 1). 176

177 2.3.<u>Chlordecone concentration analysis</u>

178 CLD concentration in *C. quadricarinatus* muscles was analyzed according to the protocol
179 detailed in Lafontaine et al. (2017). Briefly, muscles were freeze-dried and an average of 250
180 mg of lyophilized samples were used to CLD extraction, which was performed in Accelerated

Solvent Extraction (ASE) with a mixture of n-hexane:dichloromethane (90:10; v:v; Biosolve-Chimie, France). After fat fraction extraction, samples were evaporated, resuspended in 2 mL hexane, and purified with a volume of 2 mL 98% sulfuric acid, used to remove organic matter. Then, a 3-min centrifugation at 3000 rpm allowed recovery of the organic layer, which was evaporated under gentle nitrogen flow. Finally, the extract was resuspended with 50 μ L n-hexane and 50 μ L of a solution PCB 209 (100 pg/ μ L in n-hexane) as an injection internal injection standard.

A procedural blank and a Quality Control were analysed alongside the samples. The procedural blank consisted of ASE extraction without biological matrix, while the Quality Control consisted of ASE extraction with biological matrix (i.e. *Cherax quadricarinatus* muscle, CLD-free) spiked with CLD solution to obtain a final concentration of 2.5 ng.g⁻¹ wet weight.

193 CLD concentrations were analyzed in GC-ECD (Thermo Scientific, USA) using the 194 parameters described in Lafontaine et al. (2017). The limit of detection (LOD) was fixed at 195 0.02 ng/g wet weight (i.e. three times the background noise of chromatogram) and the limit of 196 quantification was 0.06 ng/g wet weight. All CLD concentrations were expressed as ng.g⁻¹ 197 wet weight.

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199 2.4. <u>CLD uptake and depuration rate constants</u>

The toxicokinetic model was done according to the OECD 305 Fish Bioconcentration Guidelines (OECD, Test No. 305), with some adaptations because our experimental design does not allow consideration of the fecal and gill elimination constants. Thus, to estimate the uptake rate constant (k_1) and the depuration rate constant (k_2) , we assumed that CLD bioconcentration followed a first order kinetics. With this hypothesis, a linear model wasobtained, and the depuration rate constant (k₂) correspond to the slope of the equation (1):

206
$$ln(CLD_{Cq}) = -k_2 \cdot t + c$$

with $ln(CLD_{Cq})$ the natural logarithm of the CLD amount in *C. quadricarinatus* muscle (ng), k₂ the depuration rate constant and c the intercept, corresponding to the natural logarithm of the CLD concentration in *C. quadricarinatus* muscle (ng.g⁻¹) at the end of the accumulation phase (i.e. start of the depuration phase). As the experiment time was relatively short (40 days in total), we considered no growth variation, and therefore the depuration rate constant was not corrected for organism growth. The uptake rate constant (k₁) can be estimated from k₂ with this equation (2):

214
$$C_{Cq} = C_W \cdot \frac{k_1}{k_2} \cdot (1 - e^{-k_2 \cdot t})$$

with C_{Cq} the concentration of CLD in the organism (ng.g⁻¹), Cw the concentration of CLD in the exposure medium (µg.L⁻¹) and t the time in days.

217 Finally, the bioconcentration factor (BCF) can be estimated using the equation (3):

$$BCF = \frac{k_1}{k_2}$$

Finally, the depuration rate constant (k₂) was used to determine the time of depuration phase
necessary to reach (or be) under the MRL (i.e. 20 ng.g⁻¹) in the muscle of *C. quadricarinatus*.
For that, estimation was performed with the equation (4):

$$C_{Cq} = C_{Cq}0 \cdot e^{-k2 \cdot t}$$

where C_{Cq} equals 20 ng.g⁻¹ and $C_{Cq}0$ corresponding to the mean CLD concentration in the muscle tissue at the end of the bioconcentration phase (i.e. 15.91 ± 7.44 ng.g⁻¹ for the C1 treatment and 91.19 ± 20.73 ng.g⁻¹ for the C2 treatment). Similarly, half-life of CLD in the muscle tissue was calculated, for each condition using the equation (5):

227
$$T_{1/2} = ln(2)/k_2$$

228 with $T_{1/2}$ corresponding to the half-life of CLD and k_2 the depuration rate constant.

From the equation 4 and using k_2 of each exposure conditions, theoretical values of CLD concentration at each sampling time of depuration can be calculated. For that, C_{Cq0} correspond to the mean CLD concentration measured in the crayfish muscle at the end of the accumulation phase (91.19 ng.g⁻¹ for 2 µg/L exposure and 15.91 ng.g⁻¹ for 0.2 µg/L exposure), k_2 to the depuration rate constant for each condition (0.007 for 0.2 µg/L exposure and 0.03 for 2 µg/L) and t to each sampling time, during the depuration experiment. Thus, these theoretical values can be compared with the measured ones.

236 2.5.<u>Statistical analysis</u>

237 Statistical analyses were all performed using RStudio v1.1.463 (Core Team R Development, 2019). Normality and homogeneity of variance were tested with Shapiro and 238 239 Bartlett tests (p > 0.05). The effect of the exposure time and CLD concentrations of exposure 240 on the concentration measured in C. quadricarinatus muscle were analyzed with a two-way 241 ANOVA, with a significant probability less than 0.05. Kruskal-Wallis test was performed on 242 k₁ values, for each treatment, between each exposure time, to visualize a possible significative 243 influence of the exposure time on the uptake rate constant. The same tests were used on the CLD concentration, to highlight the presence of different statistical groups between each 244 245 treatment, at each exposure time. After that, each group were compared using a posthoc Kruskal test (Dunn's-test). A Generalized Linear Model (GLM) was used to investigate the
correlation between the CLD concentration in the water (*in-situ*) and the CLD concentration
in the crayfish tissues but also the influence of biotic parameters (body length and total
weight) on the CLD bioconcentration in muscle. Student t-test and an Analyze of Variance
were utilized to test respectively the difference of BCF between in lab-conditions and *in-situ*conditions.

- 252
- 253 **3.** <u>Results</u>

254 *3.1._CLD accumulation in crayfish wild population*

A significant positive correlation was observed between the CLD concentration in water and the CLD concentration accumulated in *C. quadricarinatus* muscle of wild populations (\mathbb{R}^2 = 0.64; p-value < 0.001) (Figure 2).

In the hatchery, where no CLD concentration was measured (i.e. below the LOD), the 258 CLD concentration in the C. quadricarinatus muscle reached 0.13 ± 0.12 ng.g⁻¹ while, at the 259 most CLD-polluted station (i.e. Lezarde, with 2.48 μ g.L⁻¹ of CLD concentration all year 260 round), the CLD concentration in the organism muscles reached $74.9 \pm 51.01 \text{ ng.g}^{-1}$ (with a 261 bioconcentration factor of 30.21 ± 20.58). At the intermediate stations, Manzo (below 262 quantification limit 0.03 μ g.L⁻¹) and Coulisses (mean 0.45 μ g.L⁻¹ all year round), the CLD 263 concentrations measured in C. quadricarinatus muscles were 0.65 ± 0.62 ng.g⁻¹ (21.52 ± 264 20.71 of bioconcentration factor) and 9.82 \pm 11.65 ng.g⁻¹ (22 \pm 26.12 of bioconcentration 265 266 factor), respectively (Table 2; Figure 2). No significant difference was observed between the BCF estimated for each of the three contaminated stations (F = 0.4; p-value = 0.54). 267

268



269 CLD concentration in water (µg.L⁻¹)
 270 Figure 2: Changes in CLD concentration (ng.g⁻¹) in the *Cherax quadricarinatus* muscle
 271 (captured in the wild) depending on CLD concentration measured in the river water (µg.L⁻¹).
 273

Table 2: Bioconcentration factor (L kg⁻¹) of chlordecone for *in lab* experiments and for *in situ* conditions, calculated at the end of the 20 days-experiment for the lab conditions (i.e. 0.23 μ g.L⁻¹ and 2.75 μ g.L⁻¹) and from individuals (five per site) caught in the four reference stations in the wild (i.e. Long-term exposure experiment: Hatchery, Manzo, Coulisses and Lezarde). Letters in the statistical analysis represent the significant difference between each condition (Student t-test for *in-lab* experiments: t = 2.59, p-value = 0.04; and ANOVA for *in situ* experiments: F = 0.4 ; p-value = 0.54).

	In lab		In situ			
			Hatchery	Manzo	Coulisses	Lezarde
Measured CLD concentration	0.23 µg.L ⁻¹	2.75 μg.L ⁻¹	< LOD	< 0.03 µg.L ⁻¹	0.4 5 μg.L ⁻¹	2.4 8 µg.L ⁻¹
BCF	$560.3 \pm 464.3^{\text{A}}$	$67.4 \pm 29.9^{\text{B}}$	-	$21.52 \pm 20.71^{\text{A}}$	$22 \pm 26.12^{\text{A}}$	30.21 ±
						20.58 ^A

281

282 *3.2. Short term exposure (lab experiment)*

283 *3.2.1. <u>Kinetics of bioconcentration</u>*

The real CLD concentrations, measured in the exposure medium, were close to the nominal values: $0.23 \pm 0.03 \ \mu g.L^{-1}$ against $0.2 \ \mu g.L^{-1}$ and $2.75 \pm 0.21 \ \mu g.L^{-1}$ against $2 \ \mu g.L^{-1}$ (Table 1). As expected, no CLD was detected in the control condition.

The CLD concentration bioaccumulated in C. quadricarinatus muscle was significantly 287 influenced by both time of exposure and CLD concentration of exposure (Table 3) but not by 288 body length of crayfish (R²: 0.007, p-value: 0.75) nor by crayfish weight (R²: -0.007, p-289 value: 0.84) (Figure 3). At the end of exposure (20 days), the mean CLD concentrations 290 measured were $15.91 \pm 7.44 \text{ ng.g}^{-1}$ for 0.2 µg.L⁻¹ condition and $91.19 \pm 20.73 \text{ ng.g}^{-1}$ for 2 291 292 μ g.L⁻¹ treatment (Figure 4). The CLD concentration in muscle of the crayfish control group reached 1.47 ± 1 ng.g⁻¹ (Figure 4). Results highlighted also that CLD concentrations 293 294 measured in muscle were over the LOD and LOQ from 6 hours of exposure, regardless the 295 exposure condition (Figure 4).





Body length (cm)
Figure 3: Influence of total weight (A) and total body length (B) of the crayfish on CLD concentration measured in the muscle of *Cherax quadricarinatus*, using a Generalized Linear Model (GLM). Neither of the two parameters seems to be significant (respectively for A: R²: -0.007, p-value: 0.84 and B: R²: 0.007, p-value: 0.75).





Figure 4: Accumulation and depuration phases of CLD in the *Cherax quadricarinatus* muscle for the two exposure conditions tested ($0.2 \ \mu g.L^{-1}$ chlordecone exposition condition in orange and $2 \ \mu g.L^{-1}$ in grey). Depuration was done by placing crayfish in CLD-free water during 20 days with a renewal of the water every 3 days. Letters represent the statistical differences between each condition at each time, following Kruskal-Wallis and posthoc tests.

- 308
- 309 Table 3: Two-way Analysis of Variance (ANOVA) investigating the effects of exposure time
- to CLD (Time) and the CLD concentration of exposure (Medium) on the CLD concentration
- 311 bioaccumulated in *Cherax quadricarinatus* muscle.

Source of variation	Sum of square	d.f.	F	<i>p</i> -Values
Time	20472	5	31.88	< 0.001
Medium	10319	1	80.36	< 0.001
Time x Medium	10550	5	16.43	< 0.001
Residuals	5650	44		

Values of k₁ (uptake rate constant) were calculated at each sampling time for both 313 exposure conditions (Figure 5). For the 0.2 μ g.L⁻¹ treatment, the maximal k₁ value (10.27 ± 314 2.93 L kg⁻¹ d⁻¹) was observed after 6h of CLD exposure, then it decreased to reach a plateau 315 around 3 L kg⁻¹ d⁻¹ until the end of the exposure (Figure 5). For the 2 μ g.L⁻¹ treatment, the 316 same trend was observed, except that the maximal k₁ value $(3.29 \pm 0.38 \text{ L kg}^{-1} \text{ d}^{-1})$ was 317 estimated for the 6th day of exposure (Figure 5). 318





319 320 Figure 5: Variation of the constant accumulation rate (k₁) for *Cherax quadricarinatus*, calculated from the constant elimination rate k_2 , over time, for the $0.2 \mu g.L^{-1}$ CLD exposition 321 condition (orange dot) and the $2 \mu g.L^{-1}$ chlordecone exposition condition (blue dot). Letters 322 represents the different statistical groups between each exposure time, for each condition. 323 324

BCF values, at the end of the absorption phase, showed that individuals exposed to the 325 lowest CLD concentration (i.e. $0.2 \ \mu g.L^{-1}$) had the highest bioconcentration factor: 560.3 ± 326 464.3 and 67.4 \pm 29.9 for the 0.2 µg.L⁻¹ and the 2 µg.L⁻¹ treatments, respectively (Table 2) 327 highlighting a significant difference between the two conditions (t = 2.59, p-value = 0.04). 328

329

331 Depuration rate constants (k_2) reached respectively 0.007 days⁻¹ (for 0.2 µg.L⁻¹ condition) 332 and 0.03 days⁻¹ (for 2 µg.L⁻¹ condition).

After 20 days of depuration, a very low decrease of CLD concentration in muscle was observed for crayfish exposed at $0.2 \ \mu g.L^{-1}$: the CLD concentration reached $10.8 \pm 6.26 \ ng.g^{-1}$ i, corresponding to a 32.12 % loss (CLD concentration at the end of bioconcentration period was $15.91 \pm 7.44 \ ng.g^{-1}$) (Figure 4). This maximum time of depuration tested seemed to reduce the CLD concentration in muscle, even though it was already below the MRL at the end of the exposure time.

For C. quadricarinatus exposed to 2 µg.L⁻¹, no decrease in CLD concentration was observed 339 during the six first days. Then, a slow decrease was measured and CLD concentration reached 340 59.18 ± 37.47 ng.g⁻¹ at the end of the decontamination period, corresponding to a 35.1 % loss 341 (CLD concentration at the end of the bioconcentration period was 91.19 ± 20.73 ng.g⁻¹) 342 343 (Figure 4). This CLD concentration at the end of the depuration phase was always higher than 344 the MRL, however, according to calculations from uptake and elimination constants, 50.57 days seems necessary to reach the MRL (20 ng.g⁻¹) in muscle of crayfish exposed for 20 days 345 346 at 2 μ g.L⁻¹.

The half-life of CLD in the muscle tissue is 99 days for the 0.2 μ g.L⁻¹ condition and 23.1 days for the 2 μ g.L⁻¹ condition.

349

For the 0.2 μ g.L⁻¹ of CLD exposure condition, the mean measured values of CLD in the crayfish muscle are below the theoretical values at each depuration time, with an increase of the CLD concentration at Day 20 (6.8 ng.g⁻¹ at Day 1 and 10.1 ng.g⁻¹ at Day 20 for the measured values vs 15.8 ng.g⁻¹ at Day 1 and 13.8 ng.g⁻¹ at Day 20) (Table 4). For the 2 μ g.L⁻¹ of CLD exposure condition, the mean measured values raise until day 8 (82.9 ng.g⁻¹ at Day 1 to 103.9 ng.g⁻¹ at Day 8) and are above the theoretical values for the Day 2 and Day 8 of depuration. After that the mean CLD measured values decrease until Day 20 and reach 59.8 ng.g⁻¹, close to the theoretical value calculated (50.1 ng.g⁻¹) (Table 4).

358

359

360 Table 4: Comparison of theoretical values of CLD concentration in crayfish muscle at each361 sampling time during the depuration experiment, for each condition, based on calculations

362 with the equation 4 and the respective k_2 values, with the measured values.

	$0.2 \ \mu g.L^{-1}$ of CLD e	2 µg.L ⁻¹ of CLD exp)0	
	Theoretical values (ng.g ⁻¹)	Measured values (ng.g ⁻¹)	Theoretical values (ng.g ⁻¹)	M
Day 1	15.8	6.8	88.5	
Day 2	15.7	7.1	85.9	
Day 8	15	6.5	71.7	
Day 20	13.8	10.1	50.1	

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Finally, the CLD concentration measurements in water, after 1 and 3 days of depuration (for crayfish initially exposed to 2 μ g.L⁻¹ of CLD), revealed an increase of CLD in the water over time: $0.02 \pm 0.003 \mu$ g.L⁻¹ at Day 1 and $0.06 \pm 0.02 \mu$ g.L⁻¹ at Day 3 (Table 1).

367

368 **4.** <u>Discussion</u>

369 *4.1.CLD in wild crayfish populations*

The CLD concentration analysis in muscle of C. quadricarinatus wild population 370 highlighted a significant positive correlation between the CLD concentration in river water 371 372 and the CLD concentration bioaccumulated in organisms. This trend is consistent with several studies showing bioaccumulation of CLD in aquatic organisms like fish or crustaceans. Coat 373 et al. (2006) showed CLD concentrations ranging from 196 ng.g⁻¹ and 386 ng.g⁻¹ in the whole 374 375 body of *Oreochromis mossambicus* sampled in the Lezarde river in Martinique. Monti (2007) highlighted more impressive CLD bioaccumulation rates in aquatic organisms sampled in the 376 Grande Anse river (Guadeloupe, French West Indies), contaminated at 1.715 µg.L⁻¹ CLD: 377 from 1568 ng.g⁻¹ to 4002 ng.g⁻¹ for the crustaceans Atya innocuous and Xiphocaris elongata, 378 respectively, and up to 11733 ng.g⁻¹ for the freshwater fish *Eleotris perniger*. These higher 379 380 CLD bioconcentrations, as compared to the present study, seem to be due to the fact that CLD analysis was carried out in the whole-body organisms and not only using the muscle as in our 381 382 study. This assumption was confirmed by Lafontaine et al. (2017) who investigated the CLD distribution in the prawn *M. rosenbergii*. The authors observed that the CLD was mainly 383 384 distributed in the hepatopancreas (i.e. detoxification tissue) (between 50 and 65% of the total 385 CLD amount) and less in the muscle (between 15 and 20% of the total CLD amount).

386 *4.2. CLD behaviour under in-vitro conditions*

The laboratory approach was carried out to better evaluate the CLD toxicokinetic mechanism in the invasive crayfish *C. quadricarinatus*. Results highlighted that although CLD concentration in control conditions was below the LOD, a low concentration of CLD was measured in the muscle $(1.47 \pm 1 \text{ ng g}^{-1})$, thus confirming a much higher BCF calculated for a lower CLD concentration exposure. The same result was observed in the CLD analysis in wild populations, where *C. quadricarinatus* sampled in Manzo (CLD concentration < 0.03 µg/L) were contaminated by measurable CLD concentration. These results confirmed the fact 394 that the analysis of xenobiotic bioconcentration in biota could reinforce the biomonitoring of 395 aquatic ecosystem contaminations. Besides, a rapid CLD absorption in C. quadricarinatus 396 muscle happened from the first exposure time (6 hours), whatever the condition (i.e. 0.2 µg.L⁻ ¹ and 2 μ g.L⁻¹). This is consistent with the fact that the higher k₁ was estimated at the 397 398 beginning of the bioconcentration phase, as also observed in the prawn M. rosenbergii 399 (Lafontaine et al., 2017). Even if these results are in accordance with other studies, they must 400 be considered with cautious due to the fact that the steady-state, recommended for a BCF 401 calculation, was not reached in our study.

402 The kinetic results showed a significant positive influence of exposure time and CLD 403 concentration on the CLD bioaccumulated in the muscle. These findings are in accordance 404 with other studies, such as Lafontaine et al. (2017) who highlighted a quick accumulation of 405 CLD (measurable as early as 6 hours), in the prawn M. rosenbergii whole body, and a slow-406 down after 4 days of exposure: the uptake rate is lower after this time (and so, the 407 accumulation of pollutant) but the saturation (plateau phase) seems to be not reached 408 Lafontaine et al. (2017), as for C. quadricarinatus in our study. These results could be 409 explained by the fact that the muscle bioconcentrates CLD slower than other organs like the 410 hepatopancreas. Even if the plateau seems not be reached, we reached CLD concentration 411 close to those measured in the muscle of crayfish caught in the wild, exposed to CLD for 412 longer. This leads us to believe that this accumulation occurs throughout life without reaching 413 stabilization or that this plateau phase would have been reached with a longer exposure. 414 Relatively similar trends were found in terrestrial vertebrates: a great and quick assimilation 415 of CLD was observed in goat and in cows matrices (Lastel et al., 2016; Fournier et al., 2017 416 and references therein). Concerning the depuration of goat matrices studied by Lastel et al. (2016), contaminated with CLD at 1 mg.kg⁻¹ body weight, a steady-state was observed after 417 418 the accumulation phase and so, a delay before an efficient elimination of the CLD, fitting with our results. Interestingly, a relatively close half-life was found between our two experiments (20 days vs 23.1 days). Contrarily, for the muscle of beef, contaminated with CLD at 1 mg.kg⁻¹ body weight, the half-life of CLD reach 43 days. The accumulation and depuration were both linked to the physiological status and the metabolic capacities explaining why certain taxonomic groups accumulate more efficiently the CLD through their feeding habits and eliminate more or less efficiently the pollutants than other (via lactation or eggs laying for example) (Lastel et al., 2016 and references therein).

Interestingly, results obtained in the laboratory experiment highlighted a similar pattern to that of wild individuals, showing a dose-dependent accumulation of CLD (i.e. higher CLD concentration in muscles of crayfish sampled in rivers highly contaminated) : 15.91 ± 7.44 ng.g⁻¹ for 0.2 µg.L⁻¹ treatment against 9.82 ± 11.65 ng.g⁻¹ for Coulisses river (mean 0.45 µg.L⁻¹ all year) and 91.19 ± 20.73 ng.g⁻¹ for 2 µg.L⁻¹ treatment against 74.9 ± 51.01 ng.g⁻¹ for Lezarde river (2.48 µg.L⁻¹ of CLD concentration all year).

432 Higher levels of CLD contamination were expected in specimens caught in-situ. Indeed, 433 absorption via both water and food, contaminated at a lower level, is known to be more efficient (Bahner et al., 1977). This is accentuated in the case of our study, as like the majority 434 435 of invasive crayfish, C. quadricarinatus is omnivorous and opportunistic, capable of predating on macroinvertebrates, and therefore, placed quite high in the trophic chain 436 (Johnston et al., 2011; Jackson et al., 2017) and so, becoming more contaminated than other 437 438 species at a lower level. Hoekstra et al. (2003) highlighted that large mammals were more 439 contaminated than fish, themselves more contaminated than copepods. Although laboratory 440 experiments highlighting only CLD bioconcentration from water, the CLD concentration 441 measured in crayfish caught *in-situ* seems to be more realistic. This slight difference, less contaminated individuals in the wild, can be explained by the fact that the toxicokinetic 442

443 constants remains more difficult to estimate in the natural environment because it is necessary
444 to take into account many environmental factors (season, temperature, dissolved oxygen,
445 turbidity, predation, cocktail effect of different pollutants ...) (Rotchell and Ostrander, 2003).
446

Some studies highlighted the possibility for invertebrates to eliminate the CLD (more 447 or less quickly) from their own body, partly via their faeces, moulting (Bahner et al., 1977) or 448 449 other unknown mechanisms (Schimmel et al., 1977; Lafontaine et al., 2017). Here, we highlighted values of CLD concentration in the crayfish just below the MRL for individuals 450 451 exposed to 0.2 μ g.L⁻¹ for 20 days (15.91 ± 7.44 ng.g⁻¹ at the end of the 20 days-absorption phase against 20 ng.g⁻¹ for the MRL). But those exposed to higher concentrations were 452 453 contaminated above this value. Unfortunately, a very slow CLD decontamination was observed in the C. quadricarinatus muscle for the $2 \mu g.L^{-1}$ treatment, or even an absence of 454 decontamination for the 0.2 μ g.L⁻¹ treatment. Based on the depuration constant k₂ determined 455 in this work (0.03 days⁻¹), 50.57 days of depuration seem necessary to reach the MRL in 456 muscle of crayfish exposed for 20 days at $2 \mu g.L^{-1}$. This can be explained by the fact that the 457 muscle was not very efficient at eliminating the CLD. This time was not possible to be 458 estimated in organisms exposed to 0.2 µg.L⁻¹ because CLD concentration at the end of the 459 460 bioconcentration period was already below the MRL. Indeed, previous studies showing CLD 461 depuration, used the entire body, including the hepatopancreas, known to play an important 462 role in the accumulation, detoxification of pollutants in crustaceans (Lafontaine et al., 2017) 463 and their elimination (Sreeram and Menon, 2005; Zeng et al., 2010). The depuration observed 464 in M. rosenbergii by Lafontaine et al. (2017) may be mainly due to the detoxification of CLD 465 by the hepatopancreas. However, other detoxification system could explain this observation 466 such as the MXR (e.g. multi-xenobiotics resistance mechanism) pump system, protecting 467 aquatic species against pollutant molecules (Kurelec, 1992), as already investigated in other 468 aquatic invertebrates (i.e. bivalves and crustaceans) (Pain and Parant, 2007; Horion et al., 469 2015). This MXR system could explain the fact that CLD concentration decreased in the 470 organism and increased in the water over time during the depuration phase, until the renewal 471 at day 3. This release of CLD into the depuration water reinforces the idea that it is not 472 sufficient to simply place the organisms in clean water to allow them to be decontaminated 473 within a defined period, but that it is necessary to renew the medium at least every three days, to avoid possible recontamination of exposed individuals. Another explanation can be a 474 475 contamination due to traces of pollutant present on the crayfish cuticle after the accumulation 476 period but all the precautions were taken (i.e. thorough washing of crayfish before the transfer 477 in the CLD-free for the depuration period). However, this depuration system seems to be 478 ineffective at quickly reaching the MRL in crayfish muscle. In fact, it cannot be excluded that the presence of CLD in the depuration water could led to a recontamination of the organisms, 479 480 especially because our results highlight that crayfish didn't reach the steady-state of 481 bioconcentration, and we observed an increase of the CLD muscle concentration measured 482 even during the first time of depuration. This suggests that CLD released in water, even if at a 483 very low concentration, could be uptake again by organisms. This hypothesis is supported by the *in-situ* experiment showing a CLD bioconcentration in crayfishes sampled in the Manzo 484 river, having a similar CLD concentration as the depuration water (i.e. 0.03 ng.L⁻¹). This 485 486 could therefore also explain the low estimated k_2 after the depuration phase.

However, as our results doesn't really show a depuration, these theoretical calculations could be more accurate in the case of a longer depuration which could show a real decrease of CLD in the body. However, the muscle is not a detoxifying organ and so, we are not sur that a longer depuration time would result in a depuration of CLD from the muscle and the CLD concentration could remain the same in the long term.

493 *4.3. Risks for consumers*

494 This rapid and high CLD accumulation in combination with an inefficient CLD 495 decontamination of the muscle could pose a human health problem: even if fishing in the 496 Martinican rivers is prohibited, this recreational and ancestral practice remains very difficult 497 to control. Many fishermen put traps in the rivers every day (Personal observation) to catch 498 crustaceans for their personal consumption or to sell them on the markets. Moreover, this 499 practice is also very lucrative: C. quadricarinatus can be sold for up to 25€ / kg as it is 500 considered a luxury gourmet product. This species is now found and fished in high densities 501 in several martinican rivers (Baudry et al., 2021). The majority of rivers inhabit by C. 502 quadricarinatus are polluted by CLD beyond the potability thresholds set by the Regional 503 Health Agency $(0.1 \ \mu g.L^{-1})$ or even well above $0.2 \ \mu g.L^{-1}$, which is an exposure concentration resulting in the MRL being exceeded in crayfish muscle, according to our study (Figure 1). 504

505 Due to the transfer of CLD along the food chain, CLD can thus reach humans through the 506 consumption of contaminated products. Even if a recent study, based on the analysis of CLD 507 contamination before and after several cooking processes, highlighted a strike reduction of the 508 amount of CLD in beef meat (Martin et al., 2020), exposure to CLD is now chronic and it is 509 detectable in the blood of the majority of the Caribbean population (Multigner et al., 2007). 510 This could generate a health problem since it has been shown that CLD could be responsible 511 to the development of diseases, such as prostate cancer (Multigner et al., 2007). The 512 possibility of cooking products previously decontaminated by a simple depuration period, as 513 studied in this work, could have been an interesting alternative to the consumption of crayfish. 514 But the fact that the muscle of C. quadricarinatus is only slowly decontaminated in CLD-free water means that other methods must be tested to allow the safe consumption of these 515

products: a decontamination period longer than 20 days, in a tank, with potential losses of specimens due to cannibalism or inconvenience in an artificial closed environment (leading to mortality), is likely not to be followed by fishermen. At the moment, the most efficient way of stopping the spread of this poison is to inform the population on the dangers of this wild fishery. This social aspect is important as the problem is likely to persist for a very long time as modelling analyses have predicted that CLD in soils would persist for at least 300 years in Martinique (Cabidoche et al., 2009).

523

524 **5.** <u>Conclusion</u>

525 This study aimed to investigate the level of CLD contamination in *C. quadricarinatus* 526 muscle of wild populations and determine the CLD toxicokinetic (i.e. bioconcentration and 527 depuration) through *in-vitro* experiments in order to investigate the processes and to 528 communicate about an alternative to consume these fishery products. Here, we showed a clear positive correlation between the CLD concentration in water and the CLD concentration 529 530 measured in the muscle of crayfish caught in the wild. These results were in accordance with *in-vitro* experiments: a quick (as early as 6 hours of exposure) and efficient **bioconcentration** 531 over time, reaching approximatively 100 ng.g⁻¹ of CLD for crayfish exposed to 2 µg.L⁻¹ of 532 CLD and approximatively 20 ng.g⁻¹ (corresponding to the MRL) for cravfish exposed to 0.2 533 $\mu g.L^{\text{-}1}$ was observed. Worryingly, we were not able to decontaminate the muscle of 534 contaminated C. quadricarinatus: almost 3 weeks of decontamination did not reduce the 535 536 concentration of CLD below the MRL for crayfish exposed 2 μ g,L⁻¹ CLD. The crayfish resource is inexhaustible in the natural environment as this invasive species seems not to be 537 538 affected by this abiotic stressor, making the situation alarming: C. quadricarinatus is highly 539 prized in Martinique and chlordecone, highly present in a majority of Martinique watersheds,

is well known to cause significant health problems. Depuration experiments have shown that
the muscle seems to decontaminate too slowly to propose this as an alternative for the
consumption of products caught in the Martinique rivers.

543

544 Acknowledgement

We thank the Institut Français de Recherche pour l'Exploitation de la Mer (IFREMER) for welcoming us into their structure in order to carry out the experiments and the Laboratoire Territorial d'Analyses (LTA) de Martinique for having carried out the river water analyses. We also thank Catherine Adam, technician at the Laboratory of Animal Ecology and Ecotoxicology (LEAE) of the University of Liege, for the CLD analyses in the crayfish tissues.

551 We thank Julian Reynolds for reading the article and correcting the English.

This project was funded by the Direction de l'Environnement, de l'Aménagement et du
Logement de Martinique (DEAL), the Office de l'Eau de Martinique (ODE) and the
Collectivité Territoriale de Martinique (CTM).

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