

# First evidence of linked physiological and demographic effects of chlordecone in the freshwater shrimp *Macrobrachium faustinum* (Decapoda, Palaemonidae) in the Pérou River in Guadeloupe (FWI)

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## 1. Introduction

The organochlorine insecticide chlordecone was first produced in the United States in the early 1950s (IARC, 1979). It was introduced commercially in 1958 (Bus and Leber, 2001). Following the now world-famous “Kepone Episode” in Virginia, US (Raloff, 1975; Guzelian, 1982), its use was banned in 1975 in the United States. From 1972 to 1993, chlordecone was intensively used as Kepone<sup>®</sup> and Curlone<sup>®</sup> against the banana root borer in the French West Indies. The consecutive adverse effects were only discovered in the 2000's, with a massive contamination of drinking water resources, mainly originating from rivers (up to x103 the chlordecone legal limit (Bonan and Prime, 2001). Chlordecone is resistant to degradation in the environment, and it is adsorbed for hundreds of years in soils of Caribbean islands where it was used. Driven by water cycle, it steadily gets transferred to aquatic ecosystems. Recent studies showed that all compartments of aquatic ecosystems are affected, both in Guadeloupe (Coat *et al.* 2011) and Martinique Islands. Chlordecone has a high potential for bioaccumulation in freshwater fish and shrimps, but its physiological and demographic impacts on aquatic species have been poorly investigated. The present study aims at providing data on these impacts in the tropical freshwater shrimp *Macrobrachium faustinum*, a very common species and opportunistic feeder, exposed to chlordecone in the Pérou River located southeast of the Basse-Terre island of Guadeloupe (FWI).

## 2. Materials and methods

### 2.1. Study site

This study was performed from May 2009 to February 2010 in the Pérou River (Southeast of the Basse-Terre island of Guadeloupe, FWI). Upstream and downstream locations were defined, which correspond to contrasted conditions of water contamination: spatially, since upstream and downstream locations were at the beginning and at the end of the cultivated area of the Pérou watershed, respectively, and temporarily since, at a given location, chlordecone concentration may vary with time. In these two sites, crustacean microhabitats were strictly similar and human pressures very limited, with no fishing activities on this species.

### 2.2. Shrimp and water sampling

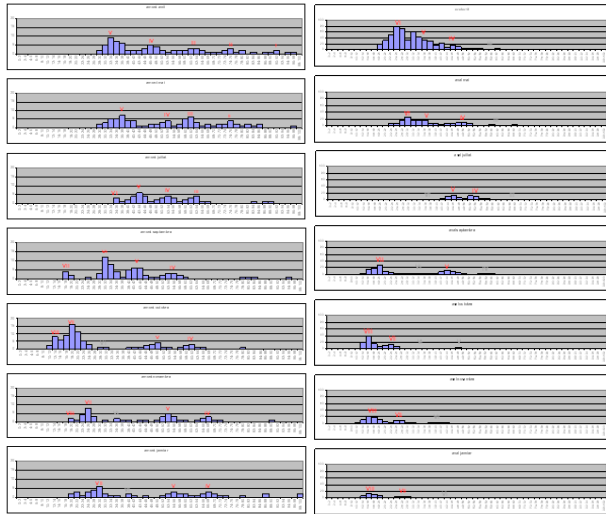
*Macrobrachium faustinum* was sampled by electrofishing, at 7 dates, in 100 m river reaches located upstream and downstream. All adults and juveniles collected in the river were measured to the nearest mm (total length) for demographic analysis, and a minimum of 15 adults were immediately frozen in nitrogen pellets and stored at -80°C. Water samples were taken at each sampling date in both locations and stored at 4°C until analysis.

### 2.3. Chlordecone analysis in shrimps and water

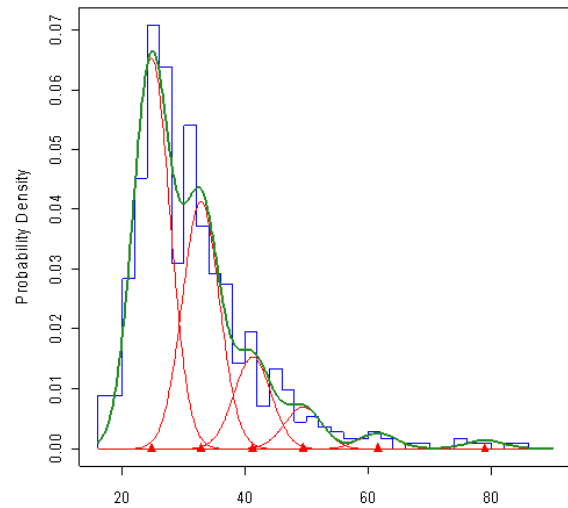
Chlordecone concentration was quantified in the abdomen of *M. faustinum*. Cuticle and muscle were isolated, weighted and lyophilized for 16 hrs using a Benchtop 3L Lyophilisator. Chlordecone and lipids were extracted with n-hexane/dichlorométhane (90 :10 ; v:v) using an Accelerated Solvent Extraction (ASE) method. A surrogate, PCB 112, was added to quantify the recovery efficiency of each extraction. After weighting the lipids, the extracts were diluted with 3 ml n-hexane and subjected to clean-up with sulphuric acid in order to remove organic matter (lipids, lipoproteins, glucides). Finally, the samples were conditioned after evaporation to dryness under nitrogen flow in n-hexane with PCB 209 as internal standard. The purified extracts were analysed by high-resolution gas chromatography using a Thermo Quest Trace 2000 gas chromatograph equipped with a Ni<sup>63</sup> ECD detector. Chemicals were separated on a 30 m x 0.25 mm (0.25 µm film thickness). An integrator calculated chemical peak areas, and peaks were identified according to their retention time. Quantification was performed using the internal standard method. The linear calibration curve which concentrations ranged from 1 to 375 pg.µl<sup>-1</sup> was established with a certified calibration mixture.

## 2.4 Demographic analyses

Size distribution histograms (size-class interval 0-2 mm) were plotted through time (Fig.1). Due to strong overlap between age groups, cohorts were modelled and extracted (package mixdist, R environment, Peter Mc Donald) (Fig. 2). This previous steps allows the precise computation of growth parameters, and a von Bertalanffy growth function (VBGF) was adjusted to the data (software FISAT II, FAO). This step allow to obtain very useful information pertaining to growth (asymptotic length  $L_{\infty}$ , growth coefficient  $K$ ), mortality ( $Z$ ) and physiological condition ( $\phi'$ ) of the individuals living in the river reaches.



**Fig. 1.** Size distribution histograms (size-class interval 0-2 mm) for *Macrobrachium fastinum* sampled upstream (left panel) and downstream (right panel) of the Pérou River in Guadeloupe.



**Fig. 2.** Example of cohort extraction from a plurimodal size distribution. Blue: original size data; red: reconstituted size groups; green: merged size groups

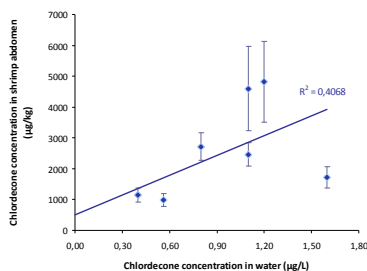
## 2.5. Biochemical measurements

Biochemical markers of chlordecone effects in *M. fastinum* were chosen according to the knowledge on its mode of action in vertebrates (Desaiah & Koch, 1975; Desaiah *et al.*, 1977; End *et al.*, 1979; Couch *et al.*, 1977; Guzelian, 1982; Chetty *et al.*, 1983; Belfiore *et al.*, 2007; Multigner *et al.*, 2010).  $\text{Na}^+/\text{K}^+$ -ATPase activity was measured in gills according to Zaugg (1982). Glutathione S-transferase (GST) activity and vitellogenin (Vtg) concentration were determined in hepatopancreas. GST assay was conducted as described in Habig *et al.* (1974). A specific western blot – slot blot method was developed to quantify Vtg expression. Finally, quantification of 20-hydroxyecdysone in the abdomen muscle was performed using an EIA specifically developed for *M. fastinum*.

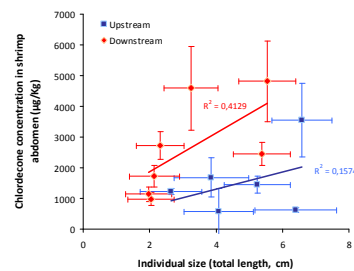
## 3. Results and discussion

### 3.1. Chlordecone concentration in water and shrimps

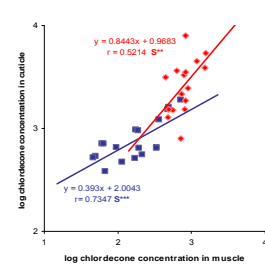
A linear correlation between water concentration in chlordecone and the amount of compound accumulated in the abdominal tissue of *M. fastinum* has been observed (Fig. 3). Water and abdomen tissue concentrations were higher downstream, as compared to upstream (Fig. 4), with concentration factors by *M. fastinum* of 2600 and 1000, respectively. Finally, per mass unit, the accumulation factor of chlordecone was 4-fold higher in the cuticle than in the muscle (Fig. 5).



**Fig. 3.** Relation between chlordecone concentration in the abdomen of *M. fastinum* and in water downstream of the Pérou River in Guadeloupe.



**Fig. 4.** Relation between chlordecone concentration in the abdomen of *M. fastinum* and individual size (total body length).



**Fig. 5.** Relation between chlordecone concentration in the cuticle and muscle of *M. fastinum* abdomen. Data have been plotted using a log scale.

### 3.2. Effects on field *M. faustinum* populations

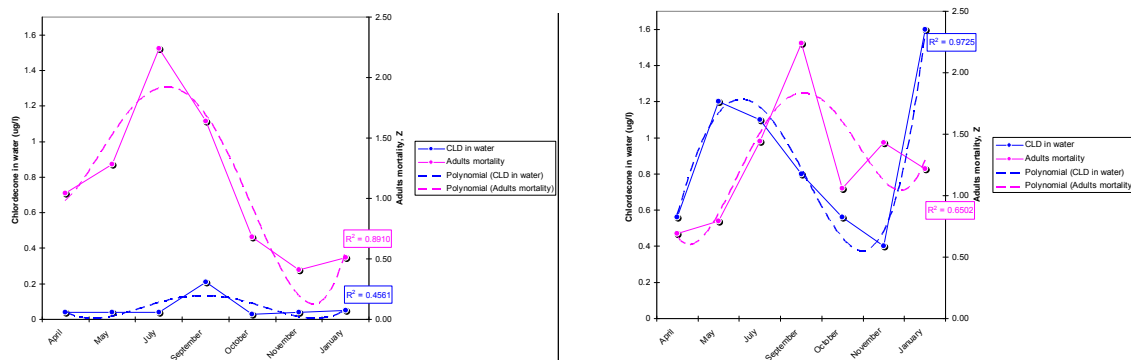
All samples of length frequency data were analyzed to estimate population growth parameters (Table 1). Whatever the season, the mean asymptotic length ( $L_{\infty}$ ) was greater upstream than downstream (mean length 78.8 mm versus 60.6 mm), showing the poor ability for this crustacean species to reach important sizes in the contaminated site. The growth coefficient (K, which is the period of time needed to double the stock) fluctuated with time, but its mean value was  $0.55 \text{ yr}^{-1}$  upstream, whereas it reached  $0.72 \text{ yr}^{-1}$  for the population living downstream, indicating a slower growth of those individuals.

**Table 1.** Estimates of population growth parameters in *M. faustinum* sampled upstream and downstream of the Pérou River in Guadeloupe.

months	$L_{\infty}$		K		Growth performance	
	upstream	downstream	upstream	downstream	upstream	downstream
April	101.81	68.27	0.61	0.49	3.80	3.36
May	84.09	77.39	0.67	0.59	3.68	3.55
July	74.88	71.92	0.89	0.64	3.70	3.52
September	64.03	59.14	0.78	1.21	3.54	3.63
October	72.08	53.00	0.29	0.51	3.12	3.16
November	78.03	45.33	0.17	0.85	3.02	3.24
January	76.44	49.33	0.45	0.73	3.42	3.25
mean	78.77	60.63	0.55	0.72	3.47	3.39

The growth performance index  $\phi'$  varied with time, but showed cohesive periods of performance slowing down (dotted lines) in April-July 2009 and January 2010, corresponding to the dry season.

The mortality coefficients for adults (cohorts > 40 mm) showed no differences between upstream and downstream reaches (Fig. 6), and the high values of instantaneous mortalities occurring during the rainy season were attributed to natural fluctuations of *M. faustinum* populations, whatever the chlordecone concentration in water.



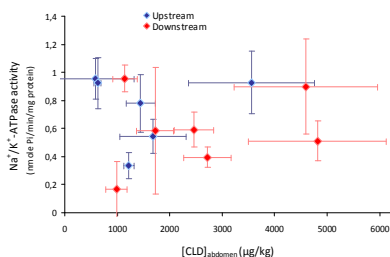
**Fig. 6.** Mortality coefficients for adults (cohorts > 40 mm) of *M. faustinum* upstream (left) and downstream (right) of the Pérou River.

### 3.3. Biochemical responses

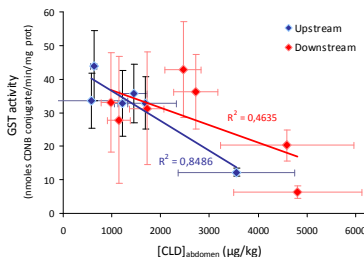
Chlordecone did not affect gill  $\text{Na}^+/\text{K}^+$ -ATPase activity in *M. faustinum* sampled upstream and downstream of the Pérou River (Fig. 7). This may result from a difference in the sensitivity of this enzyme between vertebrate and invertebrate species.

Glutathione S-transferase activity measured in the hepatopancreas of *M. faustinum* was inhibited by increasing amounts of chlordecone stored in the shrimps, with no difference between the individuals sampled upstream and downstream of the Pérou River (Fig. 8).

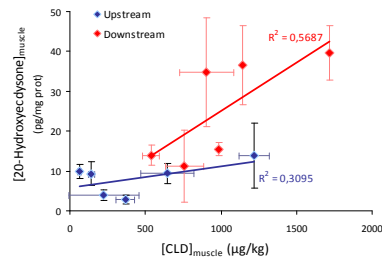
In *M. faustinum* sampled downstream, 20-hydroxyecdysone levels measured in the abdomen muscle were higher than those found in the individuals sampled upstream, and these levels of ecdysteroids were positively correlated to the concentration of chlordecone in muscle (Fig. 9). Furthermore, the results indicate an interesting link between the levels of circulating ecdysteroids and the levels of vitellogenin in the hepatopancreas of *M. faustinum* in agreement with the data of the literature which show the involvement of ecdysteroids in the maturation of gonads and synthesis of vitellogenin. Induction of vitellogenin was detected in all the males caught in the Pérou River. The presence of vtg in males could constitute a biomarker of chlordecone exposure in field populations.



**Fig. 7.** Gill  $\text{Na}^+/\text{K}^+$ -ATPase activity in *M. faustinum* sampled upstream and downstream of the Pérou River in Guadeloupe.



**Fig. 8.** Hepatopancreas glutathione S-transferase activity in *M. faustinum* sampled upstream and downstream of the Pérou River.



**Fig. 9.** Levels of 20-hydroxyecdysone measured in the abdomen muscle of *M. faustinum* sampled upstream and downstream of the Pérou River.

#### **4. Conclusions**

Slower growth of the individuals sampled downstream may result from high 20-hydroxyecdysone levels measured in those individuals. For the first time, it has been indicated that bioaccumulation of chlordane by a freshwater shrimp resulted in physiological alterations that might affect population dynamics.

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