Organochlorine pollution in tropical rivers (Guadeloupe): Role of ecological factors in food web bioaccumulation

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This paper determines the bioaccumulation and transfer processes of organochlorine pesticides within the stream food web in Guadeloupe (Caribbean).

ABSTRACT

Concentrations of organochlorine pesticides and stable isotope ratios of nitrogen and carbon were measured in a tropical freshwater ecosystem to evaluate the contamination level of biota and examine the bioaccumulation patterns of pollutants through the food web. Chemical analyses showed a general and heavy contamination of the entire food web. They revealed the strong accumulation of pollutants by juveniles of diadromous fishes and shrimps, as they re-enter the river. The role of ecological factors in the bioaccumulation of pesticides was evaluated. Whereas the most persistent pollutants (chlordecone and monohydro-chlordecone) were related to the organisms diet and habitat, bioaccumulation of β -HCH was only influenced by animal lipid content. The biomagnification potential of chlordecone through the food chain has been demonstrated. It highlighted the importance of trophic transfer in this compound bioaccumulation process. In contrast, bioconcentration by passive diffusion from water seemed to be the main exposure route of biota to β -HCH.

Keywords: Organochlorines; Bioaccumulation; Freshwater; Food web; Stable isotope

1. Introduction

Intensive agricultural or industrial use of persistent pollutants, such as organochlorine (OC) pesticides, has led to a widespread contamination of the environment. Since their introduction in the 1950s in Guadeloupe (Caribbean), OC pesticides have been extensively used on agricultural lands. Though they were banned from usage in the 1990s, these toxic and persistent molecules are still highly present in soils, especially in the most cultivated areas, in the south of Basse-Terre (Cabidoche et al., 2009). Concentrations reaching 10 mg kg⁻¹ of chlordecone (Kepone[®]), the most worrying OC residue in Guadeloupe, were measured in soils more than ten years after the last agricultural spreading (Cabidoche et al., 2004). Driven by water cycle, this terrestrial pollution progressively gets transferred to surrounding aquatic ecosystems. Two OC compounds, β- hexachlorocyclohexane and chlordecone (prohibited since 1972 and 1993, respectively) exhibited very high concentrations in freshwater fish and shrimp (Coat and Monti, 2006; Monti, 2005), far exceeding the French legal limit of 20 μg kg⁻¹ wet weight (determined by national ordinance in 2008). Monohydro-chlordecone, a chlordecone derivative produced by the replacement of a chlorine atom by hydrogen, has also been detected in biota (Monti and Lemoine, 2007). This contamination led to a regional ordinance (2005) which prohibits fishing, consumption and marketing of the organisms originating from most of the rivers in Guadeloupe. It also highlighted the urgent need for a precise evaluation of the bioaccumulation and transfer processes of OC pesticides along food webs, in order to guide future measures to preserve the fauna and protect the human population which largely consumed freshwater resources.

In the literature, two main processes leading to the accumulation of contaminants in aquatic organisms are evoked: direct partitioning from the abiotic environment (bioconcentration) and ingestion of contaminated dietary sources (trophic transfer) (Borga et al., 2004; Roche et al., 2009). If food is the dominant route of

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exposure and if the absorption rate exceeds the elimination rate, biomagnification will occur. By this process, the chemical concentration in an organism achieves a level that exceeds that in the organism's diet (Drouillard, 2008; Ramade, 1999). The determination of OC bioaccumulation and subsequent transfer within a food web first requires the identification of trophic interactions between species. The structure of the stream food web in Guadeloupe has been described during the dry (December to May) and the wet (June to November) seasons, using stable isotope analysis combined with literature dietary data (Coat et al., 2009; Lefrançois et al., 2010).

In this paper, concentrations of OC pesticides and stable isotope ratios of nitrogen and carbon as trophic tracer were measured in a freshwater ecosystem to evaluate the contamination level of biota and examine the bioaccumulation patterns of pollutants through the food web. The influence of ecological factors (body size, lipid content, diet, habitat and seasonality) in bioaccumulation was studied for each chemical. Habitat, which partly reflects chemical bioavailability, is considered as a potential explanatory factor (Borga et al., 2004). In mountainous tropical streams, the two main variables describing habitat variability are stream velocity and water depth (Monti and Gouézec, 2006). Because of the strong hydrodynamic differences that characterize seasonality in the tropics, the study was conducted during both the dry and wet seasons. The present survey is the first step in the elaboration of a model for estimating the contamination of aquatic species by OC pesticides in Guadeloupe.

2. Materials and methods

2.1. Study site

The study was performed in the lower reach of the Grande-Anse River, in the south of Basse-Terre, Guadeloupe (16°00'N and 61°30'W). This 8.7 km long river is bordered by an abundant ligneous vegetation. The sampled river bed (10 m wide and 2000 m long), extended from the river mouth to 50 m above sea level. It is characterized by small cascades, rapids, cobble-riffles, pools and large runs (according to the classification of Malavoi, 1989). This alternation of hydromorphological facies creates differences in stream velocity and constitutes a mosaic of habitats available for macrofauna. The study area is subjected to a humid tropical climate and received 2412 mm of rainfall in 2006 (Météo France).

2.2. Sample collection and preparation

Sampling aimed at collecting all the potential trophic links in the study river. Surface sediments (5 cm depth) were collected in depositional areas with a grab sampler and wet sieved (6 mm pore size metal sieve) to remove coarse sands and organic debris. Biofilm was scraped from the surfaces of submerged rocks with a knife. Filamentous green algae were collected in the river bed. Leaf and fruit detritus were collected from depositional areas where they accumulate naturally. Drifting paniculate matter (composed of terrestrial plant fragments and other paniculate organic matter) was collected with a 30 µm meshed-net set up in the water column. Molluscs were picked up by hand and aquatic insects were collected using a Surber sampler. Fishes and shrimps were captured using a backpack electrofishing device (DEKA 3000 Gerätebau, Marsberg, Germany). The planktonic compartment, negligible in such torrential streams, was sampled out of the study reach. Plankton samples were collected in the river mouth and in coastal waters by towing a 30 µm meshed-net through the water column. The sampled material was rinsed with clear water and frozen at -30 °C before being prepared for analyses. Samples were pooled to create a representative composite sample. When sufficiently numerous, individuals were classified into different cohorts in order to compare the level of contamination of both adults and juveniles.

2.3. Isotopic analysis

Measurements of carbon and nitrogen isotopic ratios were performed on samples with a mass spectrometer (Optima, GV Instrument, UK) coupled to a C-N-S elemental analyser (Carlo Erba, Italy) for combustion and automated analysis. Isotopic ratios were presented as δ values (‰) relative to the vPDB (Vienna PeeDee Belemnite) standard and to atmospheric N_2 for carbon and nitrogen, respectively. $\delta^{13}C$ versus $\delta^{15}N$ values were plotted to determine the trophic linkages between producers and consumers. For each species, the potential diet items (identified from isotopic signatures and literature) were tested with a multiple-source mixed model (Isosource version 1.3, a Microsoft Visual Basic program used to determine the contribution of each food source to consumer diets (Phillips and Gregg, 2003)). Complete details concerning isotopic analysis are in Coat et al., 2009. Here, we re-use isotopic composition as proxy for carbon food web sources ($\delta^{13}C$) and consumer trophic level ($\delta^{15}N$), in order to explain OC biocontamination.

2.4. Pesticides extraction and analysis

Chlordecone ($C_{10}Cl_{10}O_0$), monohydro-chlordecone ($C_{10}HOCl_9$) and β -hexa-chloroyclohexane ($C_6H_6Cl_6$) were quantified in the sampled material. Animals were analysed in their entirety to evaluate their exact pollutant load. Processing of samples (extraction, purification and quantification of OC) was carried out at the Laboratoire Départemental d'Analyse de la Drôme (Valence, France), accredited by the French Accreditation Committee (COFRAC). The chemical analyses differed for animal and plant material. Lipid fraction, containing OC pesticides, was extracted from homogenised animal tissues in 1:1 acetone-dichloromethane using an Accelerated Solvent Extractor 200 apparatus (Dionex, Voisin le bretonneux, France). The solvent was removed by evaporation while purification was achieved by acid hydrolysis. Concentrations of OC were measured by gaschromatography with electron capture detector (GC/ECD: Varion, Les Ulis, France) and confirmed by gas chromatography coupled to mass spectrometry (GC/MS: Varion, Les Ulis, France). Pure chlordecone and β-HCH were used as reference compounds. They were introduced every ten samples in the analysis process. Five points determined the concentrations range and linearity was situated between 3 and 100 μg 1¹. The calibration curve allowed to determine the pesticide concentration, according to the area of the peak we obtained. PCB 101 and HBB-TPP (hexabromobenzene triphenylphosphate) with known concentrations were used as injection and extraction tracers, respectively. For plant material, pesticides were first extracted from gross samples by acetone, and from the filtered extract by acetone-dichloromethane partitioning. Purification and quantification of OC then followed the same method as described above. Results are reported as wet weight values. Detection and quantification limits depended on the sensibility of the instrument and on the chemical preparation (the mass extracted from animal or plant material and the volume from which this extract was concentrated). Since chemical analyses differed for animal and plant material, detection and quantification limits were also different: the highest values were then chosen as references in the study (3 and 10 µg kg 1 w.w. for detection and quantification values, respectively). Concentrations less than method detection limit were set to zero. Concentrations between detection and quantification limits were assigned the median value of 6.5 µg kg ¹ w.w. for calculations.

2.5. Statistical analysis

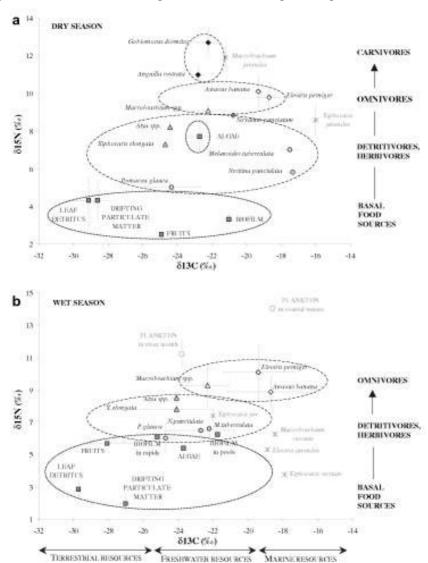
Analysis of contamination data as function of different factors was done with the R statistical language (R Development Core Team, 2007). Forward selection ('packfor' library, Dray et al., 2007) was first used to identify the variables (ecological factors) that statistically explained the contamination of biota (OC concentrations), in both seasons (significance level α = 0.1). Redundancy analysis (RDA, Legendre and Legendre, 1998) was then performed with the selected explanatory variables to determine the patterns of contamination for each studied molecule. The significance of the analysis was tested by permutation tests and the results were presented in an ordination diagram to visualize the main structure of the multivariate data.

Table 1 Species habitat affinities (with current velocity (V) and water depth (D) preferences) and trophic guild (TG) for each species observed in the study stream.

Group	Habitat affinities	V	D	TG
Species		(cm s ⁻¹)	(cm)	
Mollusc				
Neritina punctulata (Lamarck)	Emergent rocks, river margins	5	8	Herb.
Melanoides tuberculata (Müller)	Emergent rocks or sand, river margins	12	21	Detr.
Pomacea glauca (Linné)	Bottom, buried in terrestrial litter	10	50	Detr.
Shrimp				
Atya innocous (Herbst)	Cascades, rapids, riffles	50*	35*	Detr.
Atya scabra (Leach)	Cascades, rapids, riffles	63*	26*	Detr.
Xiphocaris elongata (Guérin-Méneville)	Pools close to the river banks	14*	38*	Detr.
Macrobrachium faustinum (De Saussure)	Calm biotopes under blockages	13*	22*	Omn
Macrobrachium crenulatum (Holthuis)	Rapids, riffles and deep runs	28*	65*	Omn.
Macrobrachium acanthurus (Wiegmann)	Calm biotopes close to the sea	6	34	Omn.
Macrobrachium heterchirus (Wiegmann)	Rapids, riffles	66*	36*	Omn.
Macrobrachium carcinus (Linnaeus)	Deep features under falls or blocks	50*	43*	Omn.
Fish				
Sicydium punctatum (Perugia)	On rocks in current, rapids, riffles	39*	34*	Herb.
Awaous banana (Valenciennes)	On sand or gravel, in current	40	39	Omn
Eleotris perniger (Cope)	On substrate or litter, in runs or margins	53*	33*	Omn.
Anguilla rostrata (Lesueur)	Calm biotopes, within rock crevices	6	48	Carn.
Gobiomorus dormitor (Lacépède)	Deep pools, margins of rivers	nd	nd	Carn.

Note: species current velocity and water depth preferences with an asterisk come from literature (Monti and Gouézec, 2006), others have been measured for this study. nd: no data.

Fig. 1. Stable isotope "map" of the stream food web during the dry (a) and wet (b) seasons, with $\delta^{13}C$ (mean \pm sd) representing the links between sources (squares) and consumers (circles for molluscs, triangles for shrimps, and diamonds for fish), and $\delta^{15}N$ indicating the trophic levels of the organisms. The carbon sources ranging from terrestrial to marine origin are indicated, along with trophic levels.



3. Results

3.1. Stream food web components

Drifting particulate matter, leaf detritus, fruits, biofilm and filamentous green algae were the dominant basal food sources collected in the freshwater food web in both seasons. Insect biomass was negligible in the study site. Plankton was absent from the stream but was collected during the rainy season in the river mouth and coastal waters, where larvae of the diadromous fish and shrimp species grow before re-entering freshwater system. A total of 16 consumers were identified, including 3 molluses, 8 shrimp and 5 fish species (Table 1). Cohorts were identified within two shrimp and one fish populations which presented, in addition to adult individuals, post-larvae (newly recruits) and/or juveniles. Species composition slightly differed between seasons. Fish had a lower species richness and the juvenile community of fishes and shrimps was much larger during the wet season.

3.2. Isotopic signatures

Producers and consumers isotopic signatures are reported in Fig. 1, which describes the food web structure examined during the dry (part a) and the wet (part b) seasons (data redraw from Coat et al., 2009 and Lefrançois et al., 2010).

 δ^{13} C signatures of basal food sources ranged from 13 C-depleted values for terrestrial resources (plant detritus, drifting particulate matter) to 13 C-enriched values for freshwater ones (biofilm, algae). The carbon signature of plankton sampled in river mouth (-23.8‰) gathered the δ^{13} C values of freshwater resources, while plankton from coastal waters had a much 13 C-enriched signature (-18.6‰), indicative of the marine ecosystem. Animals δ^{13} C varied from 13 C-depleted values for species living in the upper part of the study reach (e.g.: *Pomacea glauca*, *Atya* spp.) to 13 C-enriched values for fish and shrimp juveniles, collected near the river mouth.

Mean δ^{15} N values detected during both seasons ranged from 2‰ (basal food sources) to 12.7‰ (adult consumers). δ^{15} N signature of marine plankton (14.1‰) was enriched compared to all the δ^{15} N values determined in the freshwater food web.

Details regarding trophic relationships and diets were given in previous papers (Coat et al., 2009; Lefrançois et al., 2010).

Table 2 Average residue levels ($\mu g \ kg^{-1} \ w.w.$) of chlordecone (chlord.), monohydro-chlordecone (mh-chl.) and β -HCH in producers and consumers (adults: A, juveniles: J and post-larvae: PL) of Grande-Anse River, with their lipid content (% lipids) measured during the dry and wet seasons ('p' is the number of pooled samples and 'n' the total number of individuals analysed for pesticides).

Group	Cohort	Cohort Nb, OC contamination and lipids during the dry					Nb, OC contamination and lipids during the wet				
		season				season					
Species	A, J, PL	p (n)	chlord.	mh-chl.	β-НСН	% lipids	p (n)	chlord.	mh-chl.	β-НСН	% lipids
Sediment	-	1	401	Presence	Presence	-	3	91	bdl	bdl	-
Drifting particulate matter	-	1	4733	37	126	-	2	1250	bdl	75	-
Leaf detritus	-	3	1661	37	110	-	3	350	7	27	-
Fruits	-	-	-	-	-	-	1	18	1	3	-
Biofilm	-	3	980	9	19	-	2	348	12.5	9	-
Filamentous green algae	-	3	2406	13	Presence	-	3	1056	9	8	-
River mouth plankton	-	-	-	-	-	-	1	5100	bdl	40	-
Marine plankton	-	-	-	-	-	-	1	3500	bdl	50	-
Mollusc											
Nentina punctulata	-	2 (822)	1109	17	58	1.6	3 (667)	3271	38	26	1.9
Melanoides tuberculata	-	1 (1043)	1008	9	15	4.8	1 (92)	3570	bdl	130	7.8
Pomacea glauca	-	2 (31)	1379	19	38	1	3 (54)	2014	37	41	2
Shrimp											
Atya innocous	A	7 (955)	1541	13	98	2.9	9 (1079)	1023	6.5	122	3.4
Atya scabra	A	1 (88)	1604	7	43	2.2	1 (70)	177	bdl	92	4.5
Xiphocaris elongata	A	3 (668)	2637	26	33	2.7	3 (510)	1241	19	68	3.4
_	J	1 (1000)	3987	106	39	1.4	3 (1080)	2493	58	55	2.6
	PL	-	-	-	-	-	1 (700)	114	14	7	3
Macrobrachium faustinum	A	3 (1249)	5338	84	44	2.1	3 (499)	2096	bdl	81	3.6
Macrobrachium crenulatum	A	1 (55)	2079	13	70	2.5	1 (30)	5124	28	120	6.1
Macrobrachium acanthurus	A	1 (39)	4486	54	23	1.5	1 (13)	2325	25	15	3.3
Macrobrachium heterochirus	A	1 (35)	2599	17	41	2.5	1 (45)	4810	52	46	4.9
Macrobrachium carcinus	A	1(1)	1841	21	16	0.7	1(1)	1005	bdl	20	2
Macrobrachium spp.	J	1 (1000)	14624	35	9	0.9	-	-	-	-	-
	PL	-	-	-	-	-	3 (3000)	36	bdl	10	1.5
Fish											
Sicydium punctatum	A	1 (16)	2122	57	64	2	-	-	-	-	-
Awaous banana	A	1 (15)	1350	23	62	2	2 (12)	12366	188	138	3
Eleotris perniger	A	1 (33)	1878	35	36	3	4 (52)	5801	75	34	1.5
	J	-	-	-	-	-	1 (150)	6700	bdl	50	62.3
Anguilla rostrata	A	2(2)	5863	68	986	22.3	-	-	-	-	-

bdl: concentrations below detection limit (<3 µg kg⁻¹ w.w.).

Presence: concentrations between detection and quantification limits (between 3 and 10 µg kg⁻¹ w.w.).

^{&#}x27;-': no data

3.3. Biocontamination

Mean OC concentrations measured in all food web compartments during the two seasons are reported in Table 2.

Chlordecone was detected in 100% of the samples analysed. During the dry season, average concentrations ranged from 401 µg kg⁻¹ wet weight in sediments to 14624 µg kg⁻¹ w.w. in shrimp juveniles (*Macrobrachium*) spp. measuring 15-20 mm). Drifting particulate matter showed a particularly high concentration (4733 μg kg⁻¹ w.w.), which exceeded the contamination levels of most of the animals. Among adult consumers, molluscs species had the lowest chlordecone concentrations (1008 to 1379 µg kg⁻¹ w.w.) whereas the highest level was reached by the carnivorous fish Anguilla rostrata (5863 µg kg⁻¹ w.w.). During the wet season, average concentrations ranged from 18 µg kg⁻¹ w.w. in fruits to 12,366 µg kg⁻¹ w.w. in the omnivorous fish Awaous banana. The lack of fishes from the carnivorous guild in the second campaign prevents the comparison between upper contamination levels. Yet, omnivorous fish species showed higher chlordecone concentrations during the wet season (varying from 5801 to 12,366 μg kg⁻¹ w.w.). Drifting particulate matter concentrated less chlordecone during the wet season (1250 µg kg⁻¹ w.w.), but still represented the most contaminated freshwater basal food source. Plankton showed very high concentrations, reaching 5100 µg kg⁻¹ w.w. in river mouth and 3500 µg kg⁻¹ w.w. in coastal waters. Important differences were observed between the contamination levels of shrimp cohorts: concentrations measured in *Macrobrachium* spp. varied from 36 µg kg⁻¹ w.w. for post-larvae (10-15 mm) to 14,624 µg kg⁻¹ w.w. for juveniles. This trend between young cohorts was confirmed by the values detected in Xiphocaris elongata, ranging from 114 μg kg⁻¹ w.w. in post-larvae (10 mm) to 2493 μg kg⁻¹ w.w. in juveniles (20-25 mm). The contamination level of adults was situated between these two results (1241 μg kg⁻¹ w.w.). Juveniles of *Eleotris perniger* also exhibited a higher contamination level (6700 µg kg⁻¹ w.w.) than this measured in adult fishes (5801 µg kg⁻¹ w.w.). In brief, all the chlordecone concentrations detected in animals widely exceeded the consumption limit of 20 µg kg⁻¹ w.w., until 730 times.

Concentrations of monohydro-chlordecone, detected in 78% of the samples, were about 100 times lower than those of chlordecone. These two contaminants followed proximate distributions, involving the same samples for maximal values: highest monohydro-chlordecone concentrations were measured in shrimp juveniles during the dry season (356 μ g kg⁻¹ w.w. for *Macrobrachium* spp.) and in adult fishes during the wet season (225 μ g kg⁻¹ w.w. for *Awaous banana*). Positive correlations were observed between contamination levels of chlordecone and monohydro-chlordecone in biological samples, in both the dry (r = 0.911, p < 0.05) and wet (r = 0.880, p < 0.05) seasons.

β-HCH was detected in 97% of the samples, and showed concentrations about 50 times lower than those of chlordecone. During the dry season, average concentrations ranged from a minimal value included between 3 and 10 μg kg⁻¹ w.w. in sediments and algae, to 986 μg kg⁻¹ w.w. in *Anguilla rostrata*. Drifting particulate matter was distinguished by its high contamination (126 μg kg⁻¹ w.w.). β-HCH levels in consumers varied widely depending on species. Lowest concentrations were found in juveniles of *Macrobrachium* spp. (9 μg kg⁻¹ w.w.), which conversely represented the most contaminated group by chlordecone. Adult shrimps showed higher β-HCH concentrations than juveniles. During the wet season, sediments constituted the single uncontaminated compartment. Again, drifting particulate matter was the most contaminated basal food source (75 μg kg⁻¹ w.w.), followed by the plankton sampled in coastal waters (50 μg kg⁻¹ w.w.) and in river mouth (40 μg kg⁻¹ w.w.). Levels detected in consumers varied from 7 μg kg⁻¹ w.w. in post-larvae of *Macrobrachium* spp. to 130 μg kg⁻¹ w.w. in *Melanoides tuberculata*.

3.4. Relationships among contaminant concentrations and ecological factors

Statistical analyses were carried out to investigate possible relationships between the bioaccumulation of pesticides as the response variables (the three OC contaminants in Table 2) and the following explanatory variables: trophic level (δ^{15} N, Fig. 1b), carbon source (δ^{13} C, Fig. 1b), lipid content (Table 2), current velocity and water depth (as species preferenda, Table 1). Testing two sets of data (i.e. pesticide concentrations during the dry and wet seasons) allowed to understand the effect of seasonality. As juveniles (coming from seawater) were not good indicators of freshwater systems, they were excluded from the analysis. Age was examined separately, considering the differences between the contamination levels in adults, juveniles and post-larvae.

Forward selection was applied to the response data to identify variables that statistically explained the distribution of OC in biota. The results differed between seasons: three variables were selected for the dry season (trophic level, lipid content and current velocity) and two for the wet season (carbon source and lipid content). Water depth did not significantly affect pollutant load in biota. Redundancy analyses (RDA) were conducted for the two seasons separately, using the explanatory variables that were selected in each case. Correlation triplots

(Fig. 2a and b) illustrate the OC contamination patterns, with the response and explanatory variables displaying the relationships between contaminant concentrations and the trophic (δ^{15} N, δ^{13} C), biological (lipid content) and environmental (current velocity) explanatory factors.

For the dry season (global R^2 for the RDA = 0.67, global adjusted R^2 = 0.61, permutational p-value = 0.001 after 999 random permutations), the first two canonical axis explained 68% of the variation associated to the contamination data. The variables 'chlordecone' and 'monohydro-chlordecone' provided similar responses: they were both positively correlated with species trophic level (δ^{15} N) and negatively with current velocity. Thus, the analysis separated species with high trophic levels and high chlordecone and monohydro-chlordecone concentrations from species with habitat in rapid running waters and low contamination levels. A bivariate diagram between chlordecone concentrations and species trophic levels (Fig. 3) confirmed the biomagnification of this pollutant (a significant positive relationship was observed for the entire population with r = 0.488, p < 0.05) but revealed that a better correlation coefficient was obtained when only species living in calm habitats were considered (r = 0.922, p < 0.05). It also showed that species from the same genus (e.g. Macrobrachium) within a trophic level exhibited contamination levels twice more important when living in calm habitats (Macrobrachium faustinum and M. Contamination faustinum and Contaminati

The variable 'β-HCH' was positively correlated with organisms lipid content. Species with high β-HCH contamination levels and high lipid contents were opposed to species with low β-HCH concentrations and low lipid contents. The correlation between these two variables was clear in log-log regression (r = 0.687, p < 0.05).

For the wet season (global $R^2 = 0.49$, global adjusted $R^2 = 0.42$, permutational *p*-value = 0.001 after 999 random permutations), the first two canonical axis explained 49% of the variation (Fig. 2b). The variables 'chlordecone' and 'monohydro-chlordecone' were again both positively correlated with a trophic factor: the organism carbon source (δ^{13} C). The analysis separated the species with high chlordecone and monohydro-chlordecone concentrations, which feed on ¹³C-enriched food sources, from the ones with low contamination levels, which feed on ¹³C-depleted food sources (Fig. 4).

The variable ' β -HCH' was still positively correlated with the organisms lipid content (r = 0.662, p < 0.05). This second analysis confirmed the independence of ' β -HCH' towards the other ecological factors tested in this study.

4. Discussion

4.1. Food web structure

 δ^{13} C values of carbon sources showed a linear progression along the *X*-axis, with a 13 C-enrichment from terrestrial to freshwater and then marine resources. This longitudinal distribution of basal food sources along the river continuum was reflected among primary consumers, which stood near the sources they exploit: species feeding on plant-derived material (e.g. *Pomacea glauca, Xiphocaris elongata*) were 13 C-depleted in comparison with species feeding on algae-derived material (e.g. *Neritina tuberculata, Melanoides tuberculata*). Fish and shrimp juveniles that depend on zooplankton during their early life stages in coastal waters (Choudhury, 1971a,b; Cutolo de Araujo and Valenti, 2007) showed the most enriched δ^{13} C values. Secondary consumers had intermediate δ^{13} C values that related the integration of various carbon sources via the assimilation of consumers.

 δ^{15} N defined three main trophic guilds. The first one, composed of herbivorous and detritivorous species (molluscs, Atyidae and Xiphocarididae shrimps, *Sicydium punctatum*) showed the lowest δ^{15} N values. The second one included omnivorous species (*Macrobrachium* species, *Eleotris perniger* and *Awaous banana*). The carnivorous guild (*Anguilla rostrata* and *Gobiomorus dormitor*) was not represented during the wet season. The abundance of juveniles during the wet season corresponds to the intensification of juvenile recruitment that occurs during the increasing stream flows in tropical fresh waters (March et al., 1998; Tate, 1997). Juvenile community then provided an important food resource for freshwater species (Corujo-Flores, 1980; Cruz, 1987).

Fig. 2. Correlation triplots of the redundancy analyses of the OC contamination data (response variables represented by diamonds) during the dry (a) and wet (b) seasons with respect to trophic, biological and environmental factors (arrows, names in italics). Species codes: Pgl, Pomacea glauca; Npu, Neritina punctulata; Mtu, Melanoides tuberculata; Xel, Xiphocaris elongata; Ain (I,II or II), Atya innocous (adult cohort I, II or III); Asc, Atya scabra; Mfa (I or II), Macrobrachium faustinum (adult cohort I or II); Mcr, M. crenulatum; Mac, M. acanthurus; Mhe, M. heterochirus; Mca, M. carcinus; Epe (I or II), Eleotris perniger (adult cohort I or II); Aba, Awaous banana; Aro, Anguilla rostrata. R software: 'rdaTest' library from http://www.bio.umontreal.ca/legendre/.

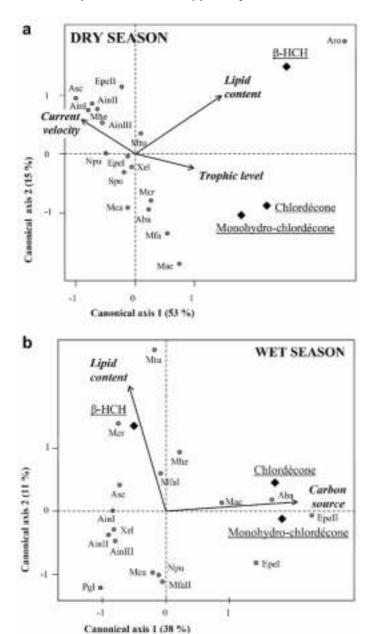


Fig. 3. Chlordecone concentrations versus trophic level measured in river samples during the dry season (the hatched regression line represents the statistically significant relationship in biota (all circles), the complete regression line only takes into account the species living in calm habitats (black circles), no relationship is observed for the species living in rapid running waters (grey circles)).

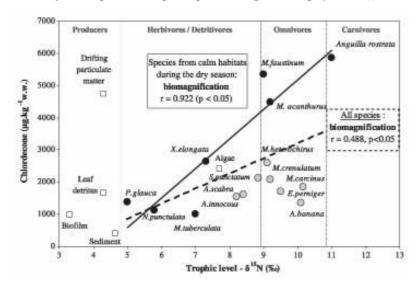
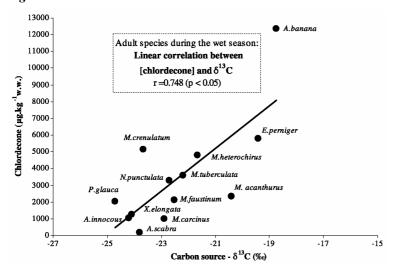


Fig. 4. Chlordecone concentrations versus $\delta^{13}C$ measured in biota during the wet season.



4.2. OC in the aquatic ecosystem

4.2.1. Chemical analyses revealed a strong and ubiquitous OC contamination of the aquatic ecosystem

All food web components were polluted. Predominance of chlordecone compared to β -HCH in contamination profiles resulted from (i) the anterior use of technical HCH (this pesticide had been forbidden twenty years before chlordecone), (ii) the lower quantities spread on plantations (β -HCH only represented 5-12% of the total isomers contained in technical HCH mixture (Xiao et al., 2004)) and (iii) the less persistent characteristics of β -HCH compared to chlordecone: the partitioning coefficient (K_{OC}) between the adsorbed part onto organic matter and the dissolved part in water is about ten times lower for β -HCH, and confers to this molecule higher mobility in soils and solubility in water.

The presence of monohydro-chlordecone in the environment suggested either an in situ dechlorination of chlordecone or the existence of background amounts attributed to uncompleted chlorination during the production process (Cabidoche et al., 2006).

The constant ratio observed between chlordecone and monohydro-chlordecone concentrations in most of the samples supported the second hypothesis: monohydro-chlordecone would be a residue of process. It accumulated in biota as its parent compound, in proportions that respected the initial concentrations ratio of these two molecules in the pesticide formulation.

4.2.2. OC levels measured in Guadeloupe were among the highest values detected worldwide in freshwater ecosystems

Concentrations of β -HCH measured in aquatic species from China (Li et al., 2008), Vietnam (Nhan et al., 2001) or India (Kaur et al., 2008), countries that also widely used technical HCH, did not exceed 10 μ g kg⁻¹ w.w. while concentrations measured here averaged 51 μ g kg⁻¹ w.w. for molluscs and 219 μ g kg⁻¹ w.w. for fishes. Little is published about diffuse pollution of chlordecone due to an agricultural use. The few data available in the neighbouring island, Martinique, showed that chlordecone concentrations reached 386 μ g kg⁻¹ w.w. in freshwater fishes (Coat et al., 2006). Concentrations measured in Guadeloupe also exceeded the high levels monitored in the James River biota (Virginia, USA) (Nichols, 1990), consequently to industrial negligence in the chlordecone manufacturing. The particularly high OC concentrations measured in Guadeloupe denoted an important and current input of pesticide residues to the aquatic ecosystem, as a result of agricultural runoff and discharges in tropical storm waters.

4.2.3. Contamination patterns varied between the two main contaminants

The relatively low chlordecone concentrations in sediments were attributed to their low percentage of fine particles. While clay and silt have more adsorption capacities for OC (Sarkar et al., 2008), the coarse particles found in torrential stream sediments adsorb minor amounts of chemicals. Here, poor organic matter sediments did not constitute a reservoir of pesticides but enhanced water-biota exchanges. Inversely, high concentrations were detected in drifting particulate matter. This allochthonous compartment, mainly composed of organic matter from soils and plants, would adsorb contaminants and constitute the main source of OC to the river. The lowest concentration measured in drifting matter during the wet season would result from its dilution in a larger amount of detritus, transported by the more erosive storm waters occurring during that season. River mouth plankton was the most contaminated food source available in stream. Marine samples were little less polluted, probably because of chemical dilution in the open sea. The strong contamination of plankton mainly explained the elevated concentrations of chlordecone found in zooplanktotrophic shrimp juveniles. Among adult species, we noticed a global increase of contamination along the food web, with the maximum levels recorded in carnivorous species.

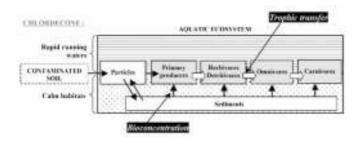
Seasonal fluctuations of chlordecone contamination showed a general decrease for sediments and producers during the wet season, because of the declining pollutant concentrations in greater river flows. No uniform seasonal trend was observed for animals, which revealed the improbability of a bioaccumulation that would only result of direct absorption, but either suggested a more complex bioaccumulation process for this chemical.

Results regarding monohydro-chlordecone indicated a proximate behaviour for the two parent compounds in aquatic ecosystem.

The low β -HCH concentrations detected in sediments confirmed the slight role they played in the storage of OC. The high β -HCH levels measured in drifting particulate matter confirmed the importance of allochthonous organic matter in the importation of contaminants to hydrosystems. Yet, the erratic distribution of β -HCH in aquatic species, which did not follow any uniform trend along the food web, contrasted with chlordecone distribution. It suggested that lipid content would explain a significant proportion of the variability in β -HCH bioconcentrations over and above that explained by trophic position.

Seasonal fluctuations of β -HCH concentrations showed a downward trend for sediments and producers during the wet season, which is attributed to the contaminant dilution in the major river flow, and an upward trend for animals, which seemed to follow the general increase of animals lipid content observed during the wet season (Table 2). This fat enrichment of species during the rainy season may reflect the lipid storage occurring prior to the reproduction period, which reaches a peak at the beginning of the wet season in Guadeloupean fresh waters (Gillet, 1983; Lim et al., 2002).

Fig. 5. Model of chlordecone transfer in the aquatic ecosystem, separated into calm and turbulent habitats (the transfer of chlordecone between abiotic, white, and biotic, grey, compartments is symbolised by black arrows for direct contamination (bioconcentration) or white arrows for contamination by food (trophic transfer)).



4.3. Interaction patterns between contaminants and biota

4.3.1. Chlordecone and monohydro-chlordecone

Habitat use, feeding ecology, seasonality and body size had been demonstrated to influence chlordecone concentrations in biota.

During the dry season, when low water allowed to distinguish habitats under low current velocity from rapid running waters, an excess of contamination was recorded for the species living in calm habitats. The deposition of contaminated particles that occurred in slow water flows increased the exposition level of animals to chlordecone, through their surrounding environment. This hypothesis agreed with other studies that reported increasing chlordecone biocontamination in turbid zones of the rivers (high suspended particulate concentrations, high sedimentation) (Luellen et al., 2006; Nichols, 1990). During the wet season, the recurrent floods standardized habitats (rapid running waters went through the whole study reach) and precluded the deposition of suspended matter, limiting the mass of pollutant available for biota.

The absence of significant effect of water depth on biocontamination may be due to the shallow water column in the study site (one meter depth): it was not high enough to create heterogeneous conditions within the water column.

The positive relationship observed during the dry season between chlordecone concentrations and organisms trophic level revealed (i) the importance of trophic transfer in this compound uptake and (ii) the biomagnification potential of chlordecone through the food chain. These results are in accordance with previous experimentations (Bahner et al., 1977; Fisher et al., 1986) that demonstrated the importance of dietary accumulation of chlordecone by aquatic species. Due to its important hydropho-bicity (log $K_{\rm OW}$ = 4.5 (Howard, 1991) to 5.4 (UNEP, 2006)), chlordecone tended to be associated to particulate matter, thus being prone to biomagnification. Besides, the differences observed between the concentrations measured in *Macrobrachium* species, which share the same trophic level but live in opposite habitats, suggested that organisms in calm habitats would, in addition to dietary uptake, accumulate chlordecone by bioconcentration (i.e., passive diffusion of contaminated water and suspended particulate matter through their gills). Fig. 5 illustrated the transfer pattern of chlordecone in the aquatic ecosystem, according to the results obtained during the dry season.

The group sampled during the wet season did not include carnivore fishes but gathered species from proximate trophic positions. This partly explained the lack of significant correlation between chlordecone concentrations and $\delta^{15}N$.

Yet, the positive relationship between biocontamination and $\delta^{13}C$ confirmed that chlordecone bioaccumulation pattern was governed by alimentation. It also indicated that ^{13}C -enriched food sources mostly contributed to biocontamination. As fish and shrimp juveniles (marked by a carbon-enriched isotopic signature) appeared highly contaminated, very abundant and widely consumed during the rainy season, they were considered as a main trophic source of contaminant to predator during the wet season.

Seasonality indirectly affected chlordecone concentrations in biota by changing the level of contaminant exposure, in terms of hydrodynamics and food availability.

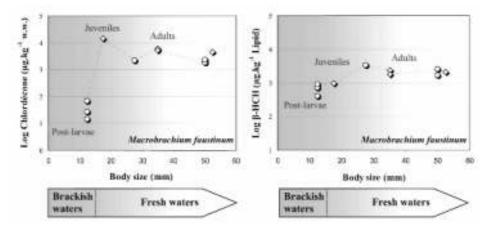
Lipids had no significant effect on chlordecone concentrations.

Body size influenced chlordecone concentrations in shrimps, through their changes in behaviour. Whereas post-larvae of *Macrobrachium* species were little exposed to pollutant (because of their non-feeding and planktonic behaviour in open sea), juveniles massively accumulated chlordecone when migrating back to the river mouth, where they eat plankton and live in calm habitats. The lower concentrations measured in adults may reflect either their changes in diet composition and habitat use or growth dilution, or both (Fig. 6).

Ecological properties of species were then considered as the main determinants of chlordecone accumulation process, in agreement with what is expected from highly hydophobic chemicals (De Laender et al., 2009).

Monohydro-chlordecone concentrations in biota were determined by these same parameters.

Fig. 6. OC contamination versus body size measured in Macrobrachium faustinum (diamonds, for the dry season, and circles, for the wet season, are representative of composite samples gathering 26 to 1000 individuals; colour gradation indicates the salinity gradient along which cohorts were sampled).



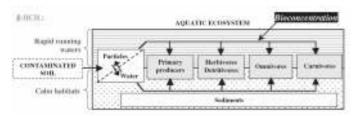
4.3.2. β-HCH

Whatever the season, lipid content was the only parameter that influenced β -HCH accumulation process. This conclusion coincided with other studies that reported a correlation between HCH isomers and organisms lipid content (Guo et al., 2008; Kidd et al., 1998). The lack of correlation between β -HCH and trophic factors precluded the hypothesis of a dietary accumulation and restrained the capacity of β -HCH to biomagnify. Bioconcentration by passive diffusion seemed to be the main exposure route of β -HCH in biota (Fig. 7).

Body size had no effect on biocontamination and lipid-normalized concentrations of β -HCH remained equivalent all along the shrimps life cycle (Fig. 6). These results coincided with the pollutant chemical properties, described by a moderate hydrophobicity (log $K_{\rm OW} = 3.78$) and a higher solubility (log $K_{\rm OC} = 3.57$) (Calvelo Pereira et al., 2008). Bioaccumulation for substances with log $K_{\rm OW} < 5$ suggests a minor role of species ecology (De Laender et al., 2009; Russell et al., 1999). Chemical partitioning between the external media (water, sediment, suspended particulate matter) and the organisms lipid fraction governs the uptake of these less hydrophobic compounds.

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Fig. 7. Model of β -HCH transfer in the aquatic ecosystem (arrows symbolised the direct contamination of abiotic, white, and biotic, grey, compartments by β -HCH; the contribution of sediments to β -HCH bioaccumulation is negligible and no difference is observed between the transfer patterns in calm and turbulent habitats).



5. Conclusion

In Guadeloupe, residues of OC pesticides were detected in very high concentrations in freshwater ecosystems; these concentrations widely exceeded the French consumption limit of 20 µg kg⁻¹ w.w. of chlordecone determined for fish and shrimp. Due to their hydrophobic nature, OC pesticides tend to be adsorbed onto terrestrial particulate matter, which became the main source of contaminants to surface water. The coarse composition of torrential stream sediment prevented this compartment to sequester pollutants and, inversely, made them highly available to river biota. Each food web component was contaminated, with differences according to the molecule, the species and the season analysed.

Whereas the most persistent pollutant (chlordecone) was demonstrated in this paper to be related to the organisms diet and habitat, bioaccumulation of β -HCH was only related to the animals lipid content. The biomagnification potential of chlordecone through the food chain highlights the importance of trophic transfer in the bioaccumulation pattern of that compound. A process of bioconcentration by passive diffusion from water and suspended particulate matter might add to dietary accumulation in calm habitats, where species showed higher chlordecone concentrations. Bioconcentration seems to be the main exposure route of β -HCH in biota.

As lixiviation appeared to be the main way to reduce pollution in soils (Cabidoche et al., 2009), the contamination of rivers is bound to last for decades or even centuries, and public education will play a central role in decreasing the risk for the population to be contaminated.

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