Different transfer pathways of an organochlorine pesticide across marine tropical food webs assessed with stable isotope analysis

Charlotte R. Dromard1,*, Yolande Bouchon-Navaro1, Sébastien Cordonnier1‡, Mathilde Guéné1‡, Mireille Harmelin-Vivien2‡, Claude Bouchon1

1 UMR BOREA, CNRS 7208 –MNHN–UPMC–UCBN–IRD 207 –UA, Laboratoire d’Excellence « CORAIL », Université des Antilles, Pointe-à-Pitre, Guadeloupe, 2 CNRS/IRD UM 110, Institut Méditerranéen d’Océanologie (MIO), Aix-Marseille Université, Marseille, France

* These authors contributed equally to this work.
‡ These authors also contributed equally to this work.
* charlotte.dromard@univ-antilles.fr

Abstract

Chlordecone is a persistent organochlorine pesticide used in the banana fields of the French West Indies from 1972 to 1993. Three marine habitats (mangroves, seagrass beds and coral reefs) of two study sites located downstream contaminated rivers were chosen to evaluate the level of contamination of marine food webs. On each habitat, the food chain collected included suspended organic matter, primary producers (macroalgae, algal turf, seagrass), zooplankton, symbiotic organisms (corals, sea anemones), primary consumers (herbivores, suspension feeders, biofilm feeders), omnivores and detritivores (lobsters, fish), secondary consumers (carnivores 1: invertebrate feeders, planktivores) and tertiary consumers (carnivores 2: invertebrate and fish feeders, piscivores). Log-linear regressions of the concentrations of chlordecone versus nitrogen isotopic ratios (δ15N) were used to assess the bioaccumulation of chlordecone along trophic food webs. At each site, bioconcentration and bioamplification take part on the transfer of chlordecone in marine organisms. In mangroves (i.e. close to the source of pollution), lower trophic magnification factors (TMF) indicated that bioconcentration prevailed over bioamplification phenomenon. The opposite phenomenon appeared on coral reefs in which bioconcentration processes were less important and bioamplification pathway became dominant. Far from the source of pollution, molecules of chlordecone seemed to be transferred to organisms mostly via trophic interactions rather than water contact.

Introduction

Persistent organic pollutants (POPs) are organic substances, persistent, bioaccumulative and possess toxic characteristics likely to cause adverse human health or environmental effects.
Many thousands of organic trace pollutants, such as organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) or polycyclic aromatic hydrocarbons (PAHs) are generated by agricultural and industrial activities and are, in part, released into the environment [1,2]. Coastal marine areas are generally the ultimate depositories of these anthropogenic compounds that reach the sea by riverine and atmospheric pathways [3].

In the French West Indies, an organochlorine insecticide called “chlordecone” (commercialized as Kepone, then as Curlone) was applied to banana fields from 1973 to 1993 to control root borers [4]. This hydrophobic molecule undergoes no significant biotic or abiotic degradation. Consequently, chlordecone is still present in soils where it was applied and the chlordecone pollution of soils in the French West Indies has been estimated to last for decades or centuries [5]. In Guadeloupe, approximately 6 200 hectares are moderately to heavily polluted by chlordecone [6], which represents about 25% of the land surface used for agriculture. Banana plants grow in the southern part of Basse-Terre (one of the two islands of Guadeloupe), which is mountainous and, as a consequence of tropical humid weather, characterized by intense rainfall events. Organochlorine molecules, adsorbed onto organic matter of the soil, end up in the ocean due to runoff and infiltration [5,7,8].

Toxicological studies have demonstrated that chlordecone is a reproductive and developmental toxicant, neurotoxic and carcinogenic for rodents and is an endocrine-disrupting chemical [9,10]. Several surveys have confirmed that French West Indies human population continues to be exposed to this chemical through consumption of contaminated foodstuffs and a correlation between chlordecone exposure and risk of prostate cancer has been evidenced for human [11,12].

Since 2003, several samplings surveys have been conducted in Guadeloupe to evaluate the level of contamination by chlordecone of some species of fish, crustaceans and mollusks, principally species with a high economical interest in order to regulate the fishing activities around the island [13–18]. In 2008, the European Commission of Food Safety set the maximal residue limit (MRL) to 20 μg.kg⁻¹ wet weight for the consumption and commercialization of sea products. Research surveys indicated that the most contaminated marine areas are located downstream to banana plantations [16]. These areas are now totally closed to fishing activities and the boundary areas are classified as areas of fishing restrictions in which it is not possible to fish a list of targeted species.

While spatial variations in the level of contamination of marine areas have been identified [15,16], no study has been conducted to understand the contamination dynamics, including the potential ways of transfer and bioaccumulation of the molecule in the food chain.

Persistent hydrophobic chemicals, such as chlordecone, may accumulate in marine organisms through different mechanisms: via the direct uptake from surrounding contaminated waters by gills or teguments (bioconcentration) and via the consumption of contaminated food (bioamplification or biomagnification) [19]. Bioconcentration is defined as the uptake of a chemical by an organism directly from the abiotic environment, resulting in a concentration of chemical in the organism higher than in the environment [20]. This uptake of chemicals from water probably follows a passive diffusion mechanism analogous to that of oxygen uptake until reaching an equilibrium partitioning between water and biota level [19,21]. Recently, a negative relationship between the level of chlordecone in the lionfish, Pterois volitans, and the distance of individuals from the source of pollution has been demonstrated [17]. These results indicated a potential bioconcentration of chlordecone in marine fishes. Bioamplification (or biomagnification) is a contamination by dietary exposure along the food chain. The transfer of chemicals, such as POPs, results in elevated concentrations of the chemical with increasing trophic level, due to their resistance to chemical and metabolic degradations [22].
Biomagnification mechanisms in marine food webs can be evaluated with stable isotope analyses (SIA). Generally, quantitative comparisons are performed using nitrogen stable isotope ratios ($\delta^{15}N$) as a proxy for the trophic position of species, because $\delta^{15}N$ value increases with increasing trophic level [23]. The use of stable isotopes to assess trophic position has numerous advantages over traditional methods such as analysis of gut contents as it averages dietary assimilation over a longer period of time [24]. The regression slope of the concentration of pollutants versus $\delta^{15}N$ signatures of fish has been used to model and predict bioamplification processes [25–27]. At the same time, the $y$-intercept of the regression line gives an estimation of the level of contamination in the environment, i.e. the level of the background concentration [25].

While bioconcentration processes received not much attention in the literature, bioamplification of POPs, which include organochlorine pesticides, have been widely studied along food webs of the arctic region [28,29], cold seas [30–32], freshwater systems [26,33–36] or temperate marine ecosystems [37–40]. On the contrary, few studies dealt with bioaccumulation processes of POPs on entire trophic food webs in marine tropical ecosystems. In these areas, the level of contamination of organochlorine pollutions has been measured in mangrove food chains [41–45], in vegetal and sediments in seagrass beds [44,46,47] and in macrophytes, invertebrates and fish in coral reefs [44,48–50]. In parallel, few works investigated the contamination of trophic food webs within the continuum “mangrove-seagrass bed-coral reef” [44].

The principal objective of the present study was to evaluate the part of bioconcentration and bioamplification mechanisms in the transfer of chlordecone in marine coastal food webs of Guadeloupe along three interlinked habitats: mangroves, seagrass beds and coral reefs. For that, regression models of the concentrations of chlordecone versus nitrogen isotopic ratios were performed on each habitat of two study sites. These models were conducted on the entire food chain from suspended organic matter to piscivorous predators.

**Material and methods**

**Study sites**

Two study sites (Goyave and Petit-Bourg) were chosen on the eastern coast of Basse-Terre in Guadeloupe. They are located in an area of fishing restrictions due to their position downstream contaminated rivers and banana fields. At each site, three types of marine habitats were investigated: coastal mangroves, seagrass beds (located approximately at 400 m from the coast) and coral reefs (between 500 m and 3 km offshore), representing a seaward gradient of decreasing land influence. Sampling depth was comprised between 1 m in mangroves and 5 m in coral reefs ecosystems.

**Sample collection and preparation**

The sampling survey was carried out from January 2014 to February 2015. For this study, 387 samples were collected, 193 at Goyave and 194 at Petit-Bourg (Table 1). The sampling protocol (location of the captures, method of capture and list of the collected species) has been approved and covered by a field permit delivered by the Direction de la Mer of Guadeloupe. The full list of collected species is given in Table S1. Macroalgae, fishes and crustaceans were collected by hand, spearfishing or using nets in seagrass beds and mangroves. Whenever possible, three replicates were made for each species. To sample the suspended organic matter (SOM), 40 L of seawater were collected in the three habitats at each site in acid-cleaned plastic drums. In order to have three replicates of SOM per habitat at each site, 10 L of seawater were filtered on each Whatman GF/F 47 mm filter. Samples were grouped into eight trophic categories: SOM, primary producers (macroalgae, algal turf, seagrass), zooplankton, symbiotic organisms (corals,
sea anemones), primary consumers (herbivores, suspension feeders, biofilm feeders), omni-
vores and detritivores (lobsters, fish), secondary consumers (carnivores 1: invertebrates feed-
ers, planktivores) and tertiary consumers (carnivores 2: invertebrates and fish feeders,
piscivores). Each individual was rinsed, measured (total length, TL, in cm) and weighed (wet
weight, ww, in g). For each organism, 50 g of flesh for animal (white muscle) and vegetative tis-
sue were collected and frozen (-18°C) until analyses. Due to the small amount collected, 0.03 g
ww of SOM were used for the analyses. Chlordecone concentrations and isotopic ratios were
measured on the same sample. Same type (flesh or tissue) and amount of sample was collected
at the two sites.

### Chlordecone extraction and analysis

Chlordecone was extracted from homogenized sampled tissues with a solution of organic sol-
vents (hexane-acetone) and turned into chlordecone hydrate (hydrophilic) in the presence of
sodium hydroxide. The aqueous phase was rinsed with hexane to eliminate fats. Chlordecone
was then reassembled in acid conditions, extracted with a solution of hexane and acetone.
Concentration of chlordecone was quantified with liquid chromatography coupled to mass
spectrometry in tandem (UPLC-MS/MS). Chlordecone concentrations were expressed in μg.
kg⁻¹ (ww) and the lower limit of quantification (LOQ) with this method was 1 μg.kg⁻¹ (ww)
with measurement precision of 0.1 μg.kg⁻¹ when data were superior to the LOQ. In this study,
chlordecone concentration was not lipid-corrected as the maximal residue limit indicated by
European regulations is expressed in wet weight.

### Stable isotope analyses

All samples were cut into small pieces, oven dried at 50°C to a constant weight and ground
into a homogenous fine powder. For each sample, 1 mg of dry weight (dw) was used for analy-
sis. Nitrogen isotope ratios were determined by a continuous flow mass spectrometer (Thermo
Fisher™, delta V Advantage). Nitrogen isotope ratios were expressed in standard delta notation
(δ¹⁵N) in ‰ according to the following formula: δ¹⁵N = [(Rsample/Rstandard− 1)] x 1000, where
R is the ratio ¹⁵N/¹⁴N of samples or international standard (atmospheric air). The analytical
measurement precision was <0.1‰ (replicate measurements of internal laboratory
standards).

---

### Table 1. Number of individuals and species (into brackets) collected in each habitat.

| Sites       | Goyave          |                                      | Petit-Bourg          |                                      |
|-------------|-----------------|--------------------------------------|----------------------|--------------------------------------|
| Habitats    | Mangrove        | Seagrass | Reef   | Mangrove | Seagrass | Reef   | Mangrove | Seagrass | Reef   |
| SOM         | 3               | 3        | 3      | 3        | 3        | 3      | 3        | 3        | 3      |
| Zooplankton | 3               | 3        | 3      | 3        | 3        | 3      | 3        | 3        | 3      |
| Primary producers | 6 (2) | 15 (5)   | 12 (4) | 6 (2)    | 15 (5)   | 11 (4) |
| Symbiotic organisms | 3 (1) | 8 (3)    | 3 (1)  | 22 (9)   | 12 (6)   |
| Primary consumers | 6 (2) | 16 (6)   | 19 (7) | 3 (1)    | 22 (9)   | 9 (3)  |
| Detritivores—omnivores | 9 (5) | 4 (2)    | 9 (3)  | 14 (6)   | 3 (1)    | 14 (5) |
| Secondary consumers | 23 (11) | 14 (7)  | 9 (4)  | 18 (8)   | 13 (6)   | 15 (5) |
| Tertiary consumers | 12 (10) | 4 (2)    | 12 (5) | 17 (8)   | 7 (3)    | 12 (5) |
| Total       | 59 (30)         | 59 (23)  | 75 (26)| 61 (25)  | 63 (24)  | 70 (25)|

SOM: suspended organic matter. The full list of species is given in S1 Table.

https://doi.org/10.1371/journal.pone.0191335.t001
Trophic magnification factor (TMF)

The trophic transfer of chlordecone through the food chain is based on the relationship between nitrogen isotope ratios ($\delta^{15}\text{N}$ in ‰) and chlordecone concentrations ([CHD] in μg kg$^{-1}$). The trophic transfer of chlordecone was estimated following the formula [32]:

$$\log_{10}[\text{CHD}] = a \delta^{15}\text{N} + b,$$

where $b$ is the y-intercept (constant dependent on the background concentration) and $a$ is the slope of the regression $\log_{10}[\text{CHD}]$ function to $\delta^{15}\text{N}$ (indicating the biomagnification power of the contaminant).

The trophic magnification factor (TMF), also called “food web magnification factor” (FWMF), is calculated from the slope using the following formula: TMF = $e^a$.

We replace trophic level (TL) (used in [32]) by $\delta^{15}\text{N}$ values as a more accurate estimation of the organism position in the food chain. Contaminant with TMF greater than 1 is considered to biomagnify in the food chain while, TMF values comprised between 0 and 1 indicates that the contaminant is not biomagnified in the food web. A TMF value inferior to zero indicates that the contaminant is excreted by the organisms in the food chain [25,45].

Statistical analysis

Normality of data was verified with Shapiro-Wilk tests. Concentrations of chlordecone were compared between habitats (mangrove, seagrass beds and reef) with one-way analyses of variance (ANOVA), followed by multiple comparison tests, performed with post hoc Tukey’s honestly significant difference (HSD) test. Simple linear regression analyses were used to investigate the relationship between the logarithm of chlordecone concentrations ($\log_{10}[\text{CHD}]$) and the trophic level of species ($\delta^{15}\text{N}$). Comparisons of the regression slopes at each site were performed with Student’s t-tests.

Differences of chlordecone concentrations between Goyave and Petit-Bourg were tested with one-way analyses of variance on ranks (Kruskal-Wallis tests) on species present at both sites in the same habitat. To avoid an influence of fish size on between-site comparisons, differences of the mean total length of fish between sites were previously tested with Kruskal-Wallis tests, as the influence of fish size on their level of contamination has already been demonstrated [17,39].

Results

Levels of contamination by chlordecone according to habitat

In Goyave, the concentrations of chlordecone varied from 1.1 μg kg$^{-1}$ for *Halimeda incrassata* to 1034 μg kg$^{-1}$ for an individual of *Pomadasys corvinaeformis*. At this site, concentrations of chlordecone were significantly different according to habitat (ANOVA, $F_{(2,190)} = 26.9$, $p < 0.0001$). The highest mean concentration of chlordecone (± standard error) was found in mangrove (182.4 ± 22.6 μg kg$^{-1}$). This mean value was significantly higher from those measured in seagrass beds and coral reef (Tukey’s HSD, $p < 0.0001$). Mean concentration of chlordecone in seagrass bed (54.1 ± 8.9 μg kg$^{-1}$) and coral reef (61.1 ± 8.4 μg kg$^{-1}$) did not differ significantly (Tukey’s HSD, $p = 0.94$). In mangrove, only 6 samples (10% of mangrove samples) presented a concentration lower to the MRL (= 20 μg kg$^{-1}$). The number of samples compliant with the MRL was 28 in seagrass beds (47%) and 37 in coral reefs (45%).

In Petit-Bourg, concentrations of chlordecone varied from 1.1 μg kg$^{-1}$ for *Megalops atlanticus* to 3012 μg kg$^{-1}$ for an individual *Callinectes* sp. Similarly to Goyave, the mean concentration of chlordecone measured in mangrove was the highest (251.0 ± 52.7 μg kg$^{-1}$) and was significantly different from those of the two other habitats (ANOVA, $F_{(2,191)} = 13.4$, $p < 0.0001$). No significant difference was found between the concentrations measured in
seagrass \((71.5 \pm 10.6 \, \mu g.kg^{-1})\) and coral reef communities \((48.5 \pm 6.0 \, \mu g.kg^{-1})\) (Tukey’s HSD, \(p < 0.85\)). The number of samples with a concentration of chlordecone lower to the MRL was 8 in mangrove (13% of mangrove samples), 23 in seagrass beds (36%) and 32 in coral reefs (45%). The level of contamination in each habitat was also reflected by the intercept \((b)\) of simple linear regressions \(\log_{10}[\text{CHD}]\) versus \(\delta^{15}\text{N}\) of organisms (Fig 1). The values of the constant \(b\) differed significantly with habitat, showing a consistent pattern. At the two sites, the intercept was the highest in mangroves \((b = 1.01\) in Goyave and \(b = 1.61\) in Petit-Bourg), intermediate in seagrass beds \((b = 0.19\) in Goyave and \(b = 0.55\) in Petit-Bourg), and the lowest in coral reefs \((b = -0.12\) in Goyave and \(b = 0.01\) in Petit-Bourg).

**Bioamplification of chlordecone along marine food webs**

A significant relationship was found between log-transformed concentrations of chlordecone \((\log_{10}[\text{CHD}])\) versus \(\delta^{15}\text{N}\) signatures of organisms in each habitat in Goyave (mangrove: \(r^2 = 0.21\), \(p < 0.01\), seagrass bed: \(r^2 = 0.70\), \(p < 0.0001\), coral reef: \(r^2 = 0.64\), \(p < 0.0001\)) and in Petit-Bourg (mangrove: \(r^2 = 0.09\), \(p < 0.01\), seagrass bed: \(r^2 = 0.42\), \(p < 0.0001\), coral reef: \(r^2 = 0.63\), \(p < 0.0001\)) (Fig 1A).

Trophic magnification factors (TMF) were calculated based on the slope of each model of linear regression. In Goyave, TMF was higher than 1 in mangrove (TMF\(_{\text{mangrove}} = 1.16\)), seagrass bed (TMF\(_{\text{seagrass}} = 1.25\)) and coral reef (TMF\(_{\text{reef}} = 1.25\)). These values were also higher than 1 in the second site Petit-Bourg (TMF\(_{\text{mangrove}} = 1.07\), TMF\(_{\text{seagrass}} = 1.14\), TMF\(_{\text{reef}} = 1.21\)). At each site, increasing TMF values were found across a seaward spatial gradient from mangroves to coral reefs.

At Goyave, slopes of the linear regressions were significantly different between mangrove and seagrass beds (Student’s t-test, \(p < 0.05\)) and between mangrove and coral reef systems (Student’s t-test, \(p < 0.05\)), but were not different between seagrass beds and coral reef (Student’s t-test, \(p > 0.05\)).

At Petit-Bourg, slopes were not different between mangrove and seagrass beds (Student’s t-test, \(p > 0.05\)), but the slopes of these two systems differed significantly with those of coral reef (Student’s t-tests, \(p < 0.05\)).

**Difference of contamination between sites**

Differences of chlordecone concentrations between Goyave and Petit-Bourg were tested on a list of species present at both sites in the same habitat (Table 2). *Aulostomus maculatus*, *Hae- mulon carbonarium* and *Lutjanus apodus* were present in coral reefs at both sites, but were not considered for site comparisons because total of individuals differed significantly with site (Kruskal-Wallis, \(p < 0.05\) for each considered species). In mangrove, 57% (4/7) of the studied species showed a concentration of chlordecone higher at Petit-Bourg than at Goyave. In seagrass beds, 30% (3/10) of the studied species followed a similar tendency. Finally, higher concentrations of chlordecone were measured at Petit-Bourg for 20% (2/10) of samples collected in coral reefs. Two species, the sea anemone *Stichodactyla helianthus* and the West indian starsnail *Lithopoma tectum*, presented higher chlordecone concentrations at Goyave than Petit-Bourg (Table 2). The other species did not show any significant difference in their concentrations of chlordecone between Petit-Bourg and Goyave.

**Discussion**

The highest concentrations of chlordecone were measured in organisms collected in mangroves that are located on the coastline, close to river mouths. The level of chlordecone was
Transfer pathways of chlordecone in tropical marine food webs

A GOYAVE

Mangrove (●): Log[CHD] = 0.15 (δ^{15}N) + 1.01
r^2 = 0.21  p < 0.001

Seagrass (○): Log[CHD] = 0.22 (δ^{15}N) + 0.19
r^2 = 0.70  p < 0.0001

Reef (○): Log[CHD] = 0.25 (δ^{15}N) - 0.47
r^2 = 0.59  p < 0.0001

B PETIT-BOURG

Mangrove (●): Log[CHD] = 0.07 (δ^{15}N) + 1.61
r^2 = 0.09  p = 0.01

Seagrass (○): Log[CHD] = 0.13 (δ^{15}N) + 0.55
r^2 = 0.42  p < 0.0001

Reef (○): Log[CHD] = 0.21 (δ^{15}N) - 0.18
r^2 = 0.57  p < 0.0001
three to four times lower in the organisms living in seagrass beds and coral reefs, two habitats located between 400 m and 3 km from the coast respectively.

This decreasing gradient of chlordecone from the coast to the open sea was previously identified [18] when studying spatial variations of the level of chlordecone contamination in different trophic groups. These results indicated that marine organisms are contaminated by chlordecone by direct contact with seawater and highlighted the existence of a bioconcentration phenomenon in these areas. Through water contact, living organisms integrate organochlorine toxicant via gills and tegument epithelium, and their level of residue is related to the level of contamination the ambient water [1]. The measurement of chlordecone concentration

Table 2. Comparisons of concentrations of chlordecone between the two sites.

| Mangrove          | Petit-Bourg | Goyave | X²   | p values | Comparisons |
|-------------------|-------------|--------|------|----------|-------------|
| SOM               | 6           | 191.3 ± 38.5 | 60.0 ± 5.6 | 3.86   | p < 0.05              | PB > G          |
| Acanthophora spicifera | 6          | 11.3 ± 0.6 | 7.6 ± 0.6 | 3.97   | p < 0.05              | PB > G          |
| Anchoa lyolepis    | 6           | 323.7 ± 47.5 | 209.0 ± 101.9 | 2.33   | p = 0.13              | PB = G          |
| Callinectes sp.    | 6           | 1547.3 ± 1587.8 | 257.0 ± 52.1 | 2.33   | p = 0.13              | PB = G          |
| Crassostrea rhizophorae | 6        | 122.3 ± 3.8 | 74.7 ± 5.5 | 3.86   | p < 0.05              | PB > G          |
| Eucinostomus gula  | 6           | 202.3 ± 12.9 | 100.7 ± 14.6 | 3.87   | p < 0.05              | PB > G          |
| Harengula clupeola | 5           | 265.0 ± 137.2 | 113.0 ± 72.5 | 1.33   | p = 0.25              | PB = G          |

Seagrass beds

| SOM               | 6           | 31.7 ± 2.9 | 27.3 ± 9.5 | 0.44   | P = 0.5             | PB = G          |
| Caulerpa sertuloides | 6          | 16.6 ± 6.0 | 10.7 ± 2.0 | 1.19   | P = 0.4             | PB = G          |
| Cerithium vulgatum | 6           | 27.0 ± 1.0 | 22.7 ± 4.9 | 3.14   | p = 0.07            | PB = G          |
| Halophila stipulacea | 6         | 4.6 ± 0.9 | 4.1 ± 0.8 | 0.43   | P = 0.70            | PB = G          |
| Holothuria Mexicana | 6          | 3.9 ± 1.1 | 4.1 ± 2.1 | 0.20   | P = 0.66            | PB = G          |
| Neopetrosia carbonaria | 6        | 14.7 ± 1.5 | 8.8 ± 3.3 | 3.86   | p < 0.05            | PB > G          |
| Padina sp.         | 6           | 4.5 ± 0.3 | 1.8 ± 0.2 | 3.86   | p < 0.05            | PB > G          |
| Sparisoma radians  | 6           | 63.3 ± 37.2 | 19.0 ± 3.6 | 3.86   | p < 0.05            | PB > G          |
| Syringodium filiforme | 6         | 5.4 ± 0.8 | 6.9 ± 0.3 | 3.86   | p = 0.10            | PB = G          |
| Thalassia testudinum | 6          | 3.0 ± 0.6 | 2.7 ± 0.2 | 0.43   | P = 0.70            | PB = G          |

Coral reefs

| SOM               | 6           | 30.3 ± 2.1 | 20.7 ± 0.6 | 3.97   | p < 0.05            | PB > G          |
| Plankton          | 6           | 20.7 ± 2.1 | 6.3 ± 1.7 | 3.86   | p < 0.05            | PB > G          |
| Halimeda incrassata | 5           | 1.8 ± 1.1 | 1.3 ± 1.3 | 3.00   | P = 0.08            | PB = G          |
| Lithopoma tectum  | 6           | 13.0 ± 2.0 | 21.3 ± 1.5 | 3.86   | p < 0.05            | PB < G          |
| Panulirus argus    | 6           | 86.7 ± 18.5 | 86.7 ± 10.4 | 0.05   | P = 0.83            | PB = G          |
| Porites astreoides | 5           | 2.4 ± 0.5 | 1.6 ± 0.4 | 3.00   | p = 0.08            | PB = G          |
| Porites fuscata    | 6           | 1.9 ± 0.5 | 2.6 ± 0.4 | 3.14   | p = 0.07            | PB = G          |
| Pterois volitans   | 6           | 74.3 ± 11.7 | 87.7 ± 26.1 | 0.43   | P = 0.50            | PB = G          |
| Scarus taeniopterus| 6           | 11.2 ± 1.7 | 10.3 ± 3.2 | 0.43   | P = 0.51            | PB = G          |
| Stichodactyla helianthus | 6          | 11.5 ± 2.3 | 41.7 ± 6.0 | 3.86   | p < 0.05            | PB < G          |

Comparisons were done with Kruskal Wallis tests. [CHD]: concentrations of chlordecone (± SE in μg.kg⁻¹), SOM: suspended organic matter, PB: Petit-Bourg, G: Goyave, n is the number of samples.

https://doi.org/10.1371/journal.pone.0191335.t002
directly in seawater was not possible in the present study due to analytical issues. Therefore, it was not possible to calculate the concentration factor (relation between the contaminant concentration of organisms and those of the ambient water) in these food webs. However, the y-intercept of the regression lines (\(\log_{10}[\text{CHD}] \text{ vs } \delta^{15}\text{N}\)), the constant \(b\), gave a relative estimation of the ambient level of chlordecone in each habitat. At both sites, the constant \(b\) was maximal in mangroves, intermediate in seagrass beds and minimal in coral reefs, attesting to the existence of a decreasing gradient of contamination seawards from the coast. These results indicated that the mechanism of biocentration is prevailing in mangroves, inducing a contamination of the entire food chain, with less influence of the trophic level of the organisms. In mangroves, a large part of samples (53/59 samples) presented contamination values higher than the MRL authorized for consumption and commercialization of sea products in these areas. Bioconcentration seemed to be a less important driver of contamination by chlordecone in the two other habitats.

At each site, the logarithms of chlordecone concentrations were positively correlated to the trophic level of marine organisms estimated by their \(\delta^{15}\text{N}\). These observations indicated that the concentration of contaminant increased along the food chain, from lower to higher trophic levels, attesting of the existence of a bioamplification phenomenon in each habitat. This mode of transfer occurs through food consumption and the integration of pollutant via digestive pathways. Bioamplification of POPs is thus related to the feeding habits and trophic level of organisms \([26,51]\). However, it should be noted that the variability of chlordecone concentrations among individuals in one species and among species at each trophic level was much higher than the variability of their \(\delta^{15}\text{N}\) (see \(\text{S1 Table}\)). Such a high intra- and inter-specific variability of contaminant concentration is observed in all studies, whatever the type of contaminants \([39]\), as the level of contamination in one organism depends on many factors, such as the environmental conditions (temperature, salinity habitat, contaminant inputs,), and the biology (species, diet, trophic level) and physiology (metabolism, growth rate, sex, age) of the different organisms studied \([36,32]\) among many others). To fully apprehend the transfer of any contaminant in food webs it is necessary to analyze a sufficiently wide spectrum of species and individuals.

Bioamplification, increasing contaminant concentration with increasing trophic level, was demonstrated by the measurement of Trophic Magnification Factors (TMF), based on the slopes of the regression lines (\(\log_{10}[\text{CHD}] \text{ vs } \delta^{15}\text{N}\)). A contaminant presenting a TMF higher than 1 is considered to biomagnify in the food chain, while a TMF value comprised between 0 and 1 indicates that the contaminant is not biomagnified \([25,45]\). In the present study, all TMF values exceeded 1, indicating that the level of chlordecone bioamplify along the food webs. These TMF values ranged from 1.07 to 1.25, and reached similar thresholds than those calculated for POPs in entire food webs (from zooplankton to apex predators) in African lake (TMF\(_{\text{DDT}} = 1.16\), TMF\(_{\text{DDE}} = 1.25\), \([26]\)). On the contrary, the TMF values of the present study were lower than those calculated in South China mangroves (TMF\(_{\text{DDT}} = 2.61\), TMF\(_{\text{PCB}} = 2.76\), \([45]\)), in Artic food webs exposed to PCB and DDT (TMF = 2.41 and 2.20 respectively, \([37]\)), or exposed to Mirex pollution (TMF = 10.5, \([32]\)). However, the length of the food chain greatly influences the value of biomagnification factors. Studies conducted in the Artic or cold sea regions generally encompass a larger scale of trophic levels (from primary producers to marine mammals and sea birds) than in other ecosystems.

In the present study, TMF values were different according to the habitat, with minimal values in mangroves, intermediate values in seagrass beds (at Petit Bourg) and maximal values in coral reefs. The statistical comparisons of the regression slopes indicated that degrees of bioamplification were different according to the three habitats. At Goyave, the highest slopes were found in seagrass beds and coral reefs and these values differed significantly from the
slope calculated in mangrove. These results indicate that, at this site, bioamplification is maximal in seagrass beds and coral reefs food webs and minimal in mangrove systems. At Petit Bourg, the highest slope was found in coral reef ecosystem and differed from those calculated in mangrove and seagrass beds. At this site, bioamplification seemed to be maximal in coral reef, but lower and equivalent in seagrass beds and mangrove. In Singapore, higher POPs concentrations were found in predator species while organisms at lower trophic levels had in general, lower levels of POPs [43]. These results highlighted a bioamplification phenomenon in mangrove food webs in Singapore. In the present study, the difference of TMF values and the comparisons of the regression slopes between the three habitats showed that the bioamplification phenomenon is masked in mangroves by the predominance of bioconcentration. This tendency also appears in seagrass beds at Petit-Bourg, which can be explained by the difference of background concentrations between the two sites. At petit-Bourg, the level of contamination in the environment seems to be higher than at Goyave, leading to a higher influence of the source of pollution on the food web and a prevalence of bioconcentration phenomenon as far as seagrass beds.

Biomagnification is linked to the physical properties of contaminant such as the octanol-water partition coefficient (log $K_{OW}$). Concentrations of very hydrophobic substances (log $K_{OW} > 6.3$) increased with trophic positions, attesting that bioamplification occurred for these molecules [36]. For less hydrophobic substances of log $K_{OW} < 5.5$, biomagnification is not observed and the contamination of the food chain is the result of equilibrium partition of the chemical between water and biota level. Our colleagues [52] predicted that compounds with log $K_{OW} < 6$, such as chlordecone ($K_{OW} = 5.41$), attain equilibrium within 1 year. Some evidence for biomagnification of chemical with log $K_{OW}$ values between 5.5 and 6.3 has been demonstrated [36]. Moreover, the ability of Kepone to magnify, like in the present study, while the $K_{OW}$ of chlordecone is less than 5.5, has been evidenced before [53,54].

The level of contamination, respectively to each habitat, appears to be globally similar between the two sites (Goyave and Petit-Bourg). However, between 20 and 57% of the samples, depending on the habitat, showed higher concentrations of chlordecone in Petit-Bourg than in Goyave. The level of contamination of rivers cannot explain this result, because the river flowing at Goyave is more contaminated than these flowing at Petit-Bourg ("Rivière Moustique" and "Rivière la Rose") (Office de l’Eau Guadeloupe). However, the location of the two sites could give an explanation to this difference in the level of contamination. Petit-Bourg is located in the North of Goyave and is more enclosed in the bay of Petit Cul-de-Sac Marin, where the trade winds tend to accumulate seawater. This particularity could involve a trapping of the molecules of chlordecone in the north of the bay.

In summary, both phenomena (bioconcentration and bioamplification) are effective on the transfer of chlordecone in marine organisms in Guadeloupe, but bioconcentration prevails on bioamplification in mangroves at both sites, and in seagrass beds at Petit-Bourg. In coral reefs at both sites and seagrass beds in Goyave, the opposite tendency seems to appear. Far from the source of pollution, molecules of chlordecone were transferred more via trophic interactions than water contact.

Consequences of chlordecone exposure to marine organisms have not been widely studied while the knowledge on their damages on human health is well documented [11,12,54]. However, few studies were conducted to investigate the consequences of long-term exposure of marine food chain or the metabolic and physiologic responses of marine organisms facing with chlordecone exposure [55].
Supporting information

S1 Table. List of the species collected in each habitat at Goyave and Petit-Bourg. [CHD]: mean concentration of chlordecone (± SE in μg.kg⁻¹), δ¹⁵N: nitrogen isotope ratios (in ‰). n is the number of sample. T: Teleostei, E: Echidermata, P: Porifera; M: Mollusca, C: Crustacea, A: Annelida, Cn: Cnidaria.

Acknowledgments
The authors express theirs thanks to Dr. Pierre Richard and Gaël Guillou, Université de La Rochelle-CNRS UMR LIENSs, for performing stable isotope analyses, and to the Laboratory LABOCEA for performing the analyses of chlordecone.

Author Contributions
Conceptualization: Yolande Bouchon-Navaro, Claude Bouchon.
Data curation: Charlotte R. Dromard, Claude Bouchon.
Formal analysis: Charlotte R. Dromard, Yolande Bouchon-Navaro, Mireille Harmelin-Vivien, Claude Bouchon.
Funding acquisition: Claude Bouchon.
Investigation: Charlotte R. Dromard, Yolande Bouchon-Navaro, Sébastien Cordonnier, Mathilde Guéné, Claude Bouchon.
Methodology: Charlotte R. Dromard, Yolande Bouchon-Navaro, Claude Bouchon.
Project administration: Claude Bouchon.
Resources: Charlotte R. Dromard, Yolande Bouchon-Navaro, Sébastien Cordonnier, Claude Bouchon.
Supervision: Claude Bouchon.
Validation: Charlotte R. Dromard, Yolande Bouchon-Navaro, Mireille Harmelin-Vivien, Claude Bouchon.
Visualization: Charlotte R. Dromard, Yolande Bouchon-Navaro, Mireille Harmelin-Vivien, Claude Bouchon.
Writing – original draft: Charlotte R. Dromard, Mireille Harmelin-Vivien, Claude Bouchon.
Writing – review & editing: Charlotte R. Dromard, Yolande Bouchon-Navaro, Mireille Harmelin-Vivien, Claude Bouchon.

References
1. Loganathan BG, Kannan K. Global organochlorine contamination trends: an overview. Ambio. 1994;187–191.
2. Fernandez A, Singh A, Jaffé R. A literature review on trace metals and organic compounds of anthropogenic origin in the Wider Caribbean Region. Mar Pollut Bull. 2007; 54: 1681–1691. https://doi.org/10.1016/j.marpolbul.2007.08.007 PMID: 17904166
3. Simcock A, Halpern B, Kirubagaran R, Hossein MM, Polette M, Smith E, et al. Coastal, riverine and atmospheric inputs from land. In: Inniss L, Simcock A, coordinators. First Global Integrated Marine Assessment. United Nations; 2016. pp. 1–93.
4. Joly PB. La saga du chlordecone aux Antilles françaises. Reconstruction chronologique 1968–2008. Rapport AFSSER-INRA, Marne-la-Vallée. 2010. French
5. Cabidoche Y, Achard R, Cattan P, Clermont-Dauphin C, Massat F, Sansoulet J. Long-term pollution by chlordecone of tropical volcanic soils in the French West Indies: a simple leaching model accounts for current residue. Environ Pollut. 2009; 157: 1697–1705. [PMID: 19167793]

6. Cabidoche Y, Jannoyer-Lesueur M. Pollution durable des sols par la chlordecone aux Antilles: comment la gérer? Innovations agronomiques. 2011; 16: 117–133. French

7. Cattan P, Cabidoche YM, Lacas JG, Voltz M. Occurrence of runoff on high infiltrability under two banana cropping systems. Soil Tillage Res. 2006; 86: 38–51.

8. Coat S, Bocquéné G, Godard E. Contamination of some aquatic species with the organochlorine pesticide chlordecone in Martinique. Aquat Living Resour. 2006; 19: 181–187.

9. Epstein SS. Kepone-hazard evaluation. Sci Total Environ. 1978; 9: 1–62. [PMID: 74851]

10. Huff JE, Gerstner HB. Kepone: a literature summary. J Environ Pathol Toxicol. 1978; 1: 377–395. [PMID: 82598]

11. Multigner L, Kadhel P, Rouget F, Blanchet P, Cordier S. Chlordecone exposure and adverse effects in French West Indies populations. Environ Sci Pollut Res. 2016; 23: 3–8.

12. Multigner L, Ndong JR, Giusti A, Romana M, Delacroix-Mailiard H, Cordier S, et al. Chlordecone exposure and risk of prostate cancer. J Clin Oncol. 2010; 28: 3457–3462. [PMID: 2071253] [PMID: 20566993]

13. Bouchon C, Lemoine S. Niveau de contamination par les pesticides des chaînes trophiques des milieux marins côtiers de la Guadeloupe et recherche de biomarqueurs de génotoxicité. Pointe-à-Pitre: Rapport UAG-DIREN; 2003. French

14. Bouchon C, Lemoine S. Contamination par les pesticides des organismes marins de la baie du Grand Cul-de-Sac Marin (île de la Guadeloupe). Pointe-à-Pitre: Rapport UAG; 2007. French

15. Bertrand J, Guyader O, Reynal L. Caractérisation de la contamination de la faune halieutique par la chlordecone autour de la Guadeloupe. Résultats des campagnes de 2008 à 2011 (projet CarGual). Le Robert: Rapport Ifremer; 2013. French

16. Dromard CR, Bodiguel X, Lemoine S, Bouchon-Navarro Y, Reynal L, Thouard E, et al. Assessment of the contamination of marine fauna by chlordecone in Guadeloupe and Martinique (Lesser Antilles). Environ Sci Pollut Res. 2016; 23: 73–80.

17. Dromard CR, Bodiguel X, Lemoine S, Bouchon-Navarro Y, Cordonnier S, Bouchon C. The invasive lionfish, Pterois volitans, used as a sentinel species to assess the organochlorine pollution by chlordecone in Guadeloupe (Lesser Antilles). Mar Pollut Bull. 2016; 107: 102–106. [PMID: 27113021]

18. Dromard CR, Guénet M, Bouchon-Navarro Y, Lemoine S, Cordonnier S, Bouchon C. Contamination of marine fauna by chlordecone in Guadeloupe: evidence of a seaward decreasing gradient. Environ Sci Pollut Res. 2017; 23: 1–8.

19. Van der Oost R, Beyer J, Vermeulen NP. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environ Toxicol Pharmacol. 2003; 13: 57–149. [PMID: 21782649]

20. LeBlanc GA. Trophic-level differences in the bioconcentration of chemicals: implications in assessing environmental biomagnification. Environ Sci Technol. 1995; 29: 154–160. [PMID: 22200214]

21. Thomann RV, Connolly JP. Model of PCB in the Lake Michigan lake trout food chain. Environ Sci Technol. 1984; 18: 65–71. [PMID: 22292677]

22. Vallack HW, Bakker DJ, Brandt I, Broström-Lundén E, Brouwer A, Bull KR, et al. Controlling persistent organic pollutants—what next? Environ Toxicol Pharmacol. 1998; 6: 143–175. [PMID: 21781891]

23. Cabana G, Rasmussen JB. Modelling food chain structure and contaminant bioaccumulation using stable nitrogen isotopes. Nature. 1994; 372: 255–257.

24. De Niro ML, Epstein S. Influence of diet on the distribution of nitrogen isotopes in animals. Geochim Cosmochim Acta. 1981; 45: 341–351.

25. Broman D, Rolff C, Näf C, Zebrühr Y, Fry B, Hobbie J. Using ratios of stable nitrogen isotopes to estimate bioaccumulation and flux of polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) in two food chains from the northern Baltic. Environ Toxicol Chem. 1992; 11: 331–345.

26. Kidd KA, Bootsma HA, Hesslein RH, Muir DC, Hecky RE. Biomagnification of DDT through the benthic and pelagic food webs of Lake Malawi, East Africa: importance of trophic level and carbon source. Environ Sci Technol. 2001; 35: 14–20. [PMID: 11351999]

27. Mancinelli G, Vizzini S. Assessing anthropogenic pressures on coastal marine ecosystems using stable CNS isotopes: State of the art, knowledge gaps, and community-scale perspectives. Estuar Coast Shelf Sci. 2015; 156: 195–204.
28. Atwell L, Hobson KA, Welch HE. Biomagnification and bioaccumulation of mercury in an arctic marine food web: insights from stable nitrogen isotope analysis. Can J Fish Aquat Sci. 1998; 55: 1114–1121.
29. Hoekstra P, O’hara T, Fisk A, Borgå K, Solomon KR, Muir D. Trophic transfer of persistent organochlorine contaminants (OCs) within an Arctic marine food web from the southern Beaufort–Chukchi Seas. Environ Pollut. 2003; 124: 509–522. PMID: 12758030
30. Strandberg B, Bandh C, Van Bavel B, Bergqvist P, Broman D, Nåf C, et al. Concentrations, biomagnification and spatial variation of organochlorine compounds in a pelagic food web in the northern part of the Baltic Sea. Sci Total Environ. 1998; 217: 143–154. PMID: 9695178
31. Borgå K, Gabrielsen G, Skaare J. Biomagnification of organochlorines along a Barents Sea food chain. Environ Pollut. 2001; 113: 187–198. PMID: 11383336
32. Fisk AT, Hobson KA, Norstrom RJ. Influence of chemical and biological factors on trophic transfer of persistent organic pollutants in the Northwater Polynya marine food web. Environ Sci Technol. 2001; 35: 732–738. PMID: 11349285
33. Evans MS, Noguchi GE, Rice CP. The biomagnification of polychlorinated biphenyls, toxaphene, and DDT compounds in a Lake Michigan offshore food web. Arch Environ Contam Toxicol. 1991; 20: 87–93. PMID: 1899993
34. Kyrkou RM, Servos MR, Whittle DM, Cabana G, Rasmussen JB. Using ratios of stable nitrogen and carbon isotopes to characterize the biomagnification of DDE, mirex, and PCB in a Lake Ontario pelagic food web. Can J Fish Aquat Sci. 1995; 52: 2660–2674.
35. Kucklick JR, Harvey HR, Ostrom PH, Ostrom NE, Baker JE. Organochlorine dynamics in the pelagic food web of Lake Baikal. Environ Toxicol Chem. 1996; 15: 1388–1400.
36. Russell RW, Gobas FA, Haffner GD. Role of chemical and ecological factors in trophic transfer of organic chemicals in aquatic food webs. Environ Toxicol Chem. 1999; 18: 1250–1257.
37. Jarman WM, Hobson KA, Sydeman WJ, Bacon CE, McLaren EB. Influence of trophic position and feeding location on contaminant levels in the Gulf of the Farallones food web revealed by stable isotope analysis. Environ Sci Technol. 1996; 30: 654–660.
38. Mizukawa K, Takada H, Takeuchi I, Ikemoto T, Omori K, Tsuchiya K. Bioconcentration and biomagnification of polybrominated diphenyl ethers (PBDEs) through lower-trophic-level coastal marine food web. Mar Pollut Bull. 2009; 58: 1217–1224. https://doi.org/10.1016/j.marpolbul.2009.03.008 PMID: 19376538
39. Harmelin-Vivien M, Bodiguel X, Charmasson S, Loizeau V, Mellon-Duval C, Tronczyński J, et al. Differential biomagnification of PCB, PBDE, Hg and Radiocesium in the food web of the European hake from the NW Mediterranean. Mar Pollut Bull. 2012; 64: 974–983. https://doi.org/10.1016/j.marpolbul.2012.02.014 PMID: 22404970
40. Ma X, Zhang H, Yao Z, Zhao X, Wang L, Wang Z, et al. Bioaccumulation and trophic transfer of polybrominated diphenyl ethers (PBDEs) in a marine food web from Liaodong Bay, North China. Mar Pollut Bull. 2013; 74: 110–115. https://doi.org/10.1016/j.marpolbul.2013.07.020 PMID: 23906472
41. Liang Y, Wong M, Shutes R, Revitt D. Ecological risk assessment of polychlorinated biphenyl contamination in the Mai Po Marshes Nature Reserve, Hong Kong. Water Res. 1999; 33: 1337–1346.
42. Páez-Osuna F, Ruiz-Fernández A, Botello A, Ponce-Vélez G, Osuna-López J, Frias-Espírcueta M, et al. Concentrations of selected trace metals (Cu, Pb, Zn), organochlorines (PCBs, HCB) and total PAHs in mangrove oysters from the Pacific Coast of Mexico: an overview. Mar Pollut Bull. 2002; 44: 1303–1308. PMID: 12523531
43. Bayen S, Wurl O, Karupiah S, Sivasothi N, Lee HK, Obbard JP. Persistent organic pollutants in mangrove food webs in Singapore. Chemosphere. 2005; 61: 303–313. https://doi.org/10.1016/j.chemosphere.2005.02.097 PMID: 16182847
44. Schaffelke B, Mellors J, Duke NC. Water quality in the Great Barrier Reef region: responses of mangrove, seagrass and macroalgal communities. Mar Pollut Bull. 2005; 51: 279–296. https://doi.org/10.1016/j.marpolbul.2004.10.025 PMID: 15757728
45. Sun Y, Zhang Z, Xu X, Hu Y, Luo X, Cai M, et al. Bioaccumulation and biomagnification of halogenated organic pollutants in mangrove biota from the Pearl River Estuary, South China. Mar Pollut Bull. 2015; 99: 150–156. https://doi.org/10.1016/j.marpolbul.2015.07.041 PMID: 26209127
46. Haynes D, Ralph P, Prange J, Dennison B. The impact of the herbicide diuron on photosynthesis in three species of tropical seagrass. Mar Pollut Bull. 2000; 41: 288–293.
47. Bouchon C, Lemoine S, Dromard C, Bouchon-Navaro Y. Level of contamination by metallic trace elements and organic molecules in the seagrass beds of Guadeloupe Island. Environ Sci Pollut Res. 2016; 23: 61–72.
48. Glynn P, Rumbold D, Snedaker S. Organochlorine pesticide residues in marine sediment and biota from the northern Florida reef tract. Mar Pollut Bull. 1995; 30: 397–402.
49. Haynes D, Johnson JE. Organochlorine, heavy metal and polyaromatic hydrocarbon pollutant concentrations in the Great Barrier Reef (Australia) environment: a review. Mar Pollut Bull. 2000; 41: 267–278.

50. Salvat B, Roche H, Ramade F. On the occurrence of a widespread contamination by herbicides of coral reef biota in French Polynesia. Environ Sci Pollut Res. 2016; 23: 49–60.

51. Dietz R, Riget F, Cleemann M, Aarkrog A, Johansen P, Hansen J. Comparison of contaminants from different trophic levels and ecosystems. Sci Total Environ. 2000; 245: 221–231. PMID: 10682369

52. Hawker DW, Connell DW. Prediction of bioconcentration factors under non-equilibrium conditions. Chemosphere. 1985; 14: 1835–1843.

53. Kay SH. Potential for biomagnification of contaminants within marine and freshwater food webs. Vicksburg: US Army Corps of Engineers; 1984.

54. Luellen DR, Vadas GG, Unger MA. Kepone in James River fish: 1976–2002. Sci Total Environ. 2006; 358: 286–297. https://doi.org/10.1016/j.scitotenv.2005.08.046 PMID: 16256173

55. Fisher DJ, Bender M, Roberts M. Effects of ingestion of Kepone-contaminated food by juvenile blue crabs (Callinectes sapidus Rathbun). Aquat Toxicol. 1983; 4: 219–234.