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ABSTRACT

In Guadeloupe, many marine organisms are affected by an organochlorine pollution used in the past by the banana industry to fight against the banana weevil. In the present study, we evaluated the level of contamination of the invasive Indo-Pacific lionfish, *Pterois volitans*, all around the island. Concentrations of chlordecone varied from 3 to 144 $\mu g.kg^{-1}$ wet weight. The highest concentrations were recorded when samples were captured in the marine zones located downstream of the previous banana plantations. This contamination seemed to decrease rapidly with the distance from the coast. Mean concentration of chlordecone in *Pterois volitans* was higher than that of five others fish species collected in similar sites. Due to its position at the top of the trophic web, lionfish was affected by bioaccumulation of chlordecone and can be used as a sentinel species to assess and control the level of contamination of the marine environment by chlordecone.

Keywords: Lionfish, invasive species, contamination, chlordecone, sentinel species, Caribbean (Guadeloupe)

1. Introduction

Guadeloupe is an overseas French territory located in the Lesser Antilles. On the island, the production of bananas represents the one of the principal economical activity, with approximately 60 000 tones of commercial production of bananas in 2010. Banana plants grow in the southern Guadeloupe, which is characterized by the presence of a volcano and, as a consequence, by intense rainfall events. To maintain a good yield and fight against the banana weevil, an organochlorine pesticide called "chlordecone" was used in Lesser Antilles from 1972 to 1993. This chemical, commercialized as "Kepone", was first manufactured in Virginia from 1958. The manufacturing of kepone was stopped in 1975, when workers from the site of production began to show severe and diverse pathologies associated to their exposure. The local environment and wildlife was also impacted because of the sewage system of the factory (Epstein, 1978; Huff and Gerstner, 1978).
In contrast, in the French West Indies, the use of this chemical continued until 1993. Approximately 6 200 ha are moderately to heavily polluted by chlordecone (Cabidoche and Lesueur Jannoyer, 2011), which represents about 25% of the land surface used for agriculture in Guadeloupe. The risk of contamination of soils is the highest in the south of Basse-Terre (one of the two islands of Guadeloupe), where previous banana plantations were located. Chlordecone is a very persistent molecule in the environment with a half-life estimated to 600 years (Cabidoche et al., 2009). Organochlorine molecules are hydrophobic and adsorbed onto organic matter of the soil. With the erosion of soil particles, desorption phenomena, slow solubilization and infiltration processes, these compounds reach runoff and ground waters that end up directly into the sea (Cattan et al., 2006; Coat et al., 2006; Cabidoche et al., 2009). Since 2003, several samplings surveys have been conducted in Guadeloupe to evaluate the level of contamination by chlordecone of fishes, crustaceans and mollusks (Bouchon and Lemoine, 2003, 2007; Bertrand et al., 2013). In 2008, the French food and safety authorities lowered the maximal residue limit (MRL) authorized for human consumption and commercialization of sea products from 200 to 20 $\mu g.kg^{-1}$ of wet weight and regulated the fishing activities around the island. The most contaminated marine areas, located downstream of the banana plantations, are now totally closed to fishing activities. The boundary areas are classified as areas of fishing restrictions in which it is not possible to fish a list of targeted species. These rules have been established to protect the health of the local population, especially because seafood represents a large part of the Caribbean gastronomy.
In 2009, the occurrence of a new fish species, the invasive lionfish *Pterois volitans*, was recorded in Guadeloupe. This species, native of Indo-Pacific Ocean, first appeared in Florida in 1992 and then colonized most of the coasts of the Gulf of Mexico and the Caribbean Sea. The population of lionfish rapidly increased between 2004 and 2010, and their density reached around 400 individuals per hectares in Florida, i.e. five times higher than in their native environment (Fishelson, 1997; Green and Côté, 2009). This predator is now very abundant around Guadeloupe and represents a high threat for the local biodiversity (Albins and Hixon, 2008; Green et al., 2012; Côté et al., 2013). Several initiatives have been proposed to control and manage the lionfish invasion in the Caribbean (Morris and Whitfield, 2009; Akins, 2012). Human consumption of lionfish is a plausible option for creating harvest pressure, as lionfish meat has good organoleptic qualities. However, before supporting the

fishing and the commercialization of lionfish in Guadeloupe, a study was necessary to evaluate the risks of its consumption linked with its potential contamination by chlordecone. Following this study, we suggest that lionfish, which are particularly abundant and sedentary, could be used as sentinel species to evaluate the level of contamination by pesticides around the island and to begin long-term monitoring of the contamination. The concept of "sentinel species" is important in the environmental health sciences because sentinel species can provide integrated and relevant information on the types, the amounts and the bioavailability fraction of pollutant in an ecosystem by retaining the pollutant in their tissues (Beeby, 2001; Basu et al., 2007). Fish are often used as sentinel species to assess the level of contamination of a region by organochlorine pollutants or metals (Albalat et al., 2002; Gibbons et al., 2009; Cresson et al., 2014). But few studies have exploited the abundance of invasive marine species to use them as sentinel species (Linde-Arias et al., 2008). Invasive species, like lionfish, could represent excellent models due to their abundance, their widespread distribution, their sedentary behavior and their high trophic level (Beeby, 2001; Basu et al., 2007).

In the present study, we studied chlordecone contamination of the invasive Indo-Pacific lionfish *Pterois volitans* in Guadeloupe. The objectives of this study were: 1) to evaluate the level of contamination of lionfish around the island, 2) to study the bioaccumulation of pesticide in this species and 3) to define if lionfish could be used as sentinel species.

2. Material and methods

2.1. Samplings

The present study was carried out in Guadeloupe (16°15'N; 61°34'W), Lesser Antilles (Fig.1). Samplings were conducted during two surveys: in 2013 with the help of professional fishers and in 2014. During both surveys, 97 lionfish were captured with spear guns or fish pots. Fish were collected within ten marine areas (from G01 to G10), previously marked out for the implementation of the European DCE ("Directive Cadre de l'Eau"). These marine areas have been described as homogeneous water masses according to various criteria as the morphology of the coasts, hydrology or hydrodynamic conditions (Fig.1). Each fish sample was characterized by its geographical location (GPS coordinates). Wet weight (in g) and total length (in cm) of each individual collected in 2014 were recorded before dissections. Fish samples were filleted with the skin, were then wrapped in aluminum foil and kept frozen until analyses.

2.2. Measures of the concentrations of chlordecone

The laboratory LABOCEA conducted the quantitative analyses of chlordecone. Chlordecone was extracted from homogenized samples tissues with a solution of organic solvents (hexane-acetone) and turned into chlordecone hydrate (hydrosoluble) in the presence of soda. 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. Concentrations of chlordecone were quantified with liquid chromatography coupled to mass spectrometry in tandem (UPLC-MS/MS). The lower quantification limit with this method was 3 $\mu g.kg^{-1}$ of wet weight. Chlordecone was extracted following the method recommended by ANSES (Laboratory for Studies and Research on hygiene and quality of foods; Anon., 2011). Concentrations of chlordecone in animal tissues were expressed in $\mu g.kg^{-1}$ (wet weight).

2.3. Statistical analyses

Data were tested for normality with the Shapiro-Wilk's test. Concentrations of chlordecone measured in lionfish were compared between the locations of their capture, i.e. between the marine areas in which lionfish were collected, with a one-way analysis of variance. ANOVAs were also performed to compare the concentrations of chlordecone measured in lionfish with the concentrations measured in five other fish species, in contaminated and non-contaminated areas. Spearman's rank correlations were used to test 1) the correlation between the concentrations of chlordecone in fish and the distance from the coast of their capture and 2) the correlation between the concentrations of chlordecone measured in fish and their size.

3. Results

3.1. Contamination of lionfish according to the location of their capture

Concentrations of chlordecone measured in lionfish during this study were comprised between the limit of detection (<3 $\mu g.kg^{-1}$) and 144 $\mu g.kg^{-1}$. The mean concentration of chlordecone varies according to the location of the fish capture (ANOVA, $F_{(7,89)} = 12.4$, $p < 0.0001$). The fish that were the most contaminated by chlordecone were collected in zone G01, with a mean concentration equal to 61.7 ± 17.6 $\mu g.kg^{-1}$ w.w. (Table 1). Fish showed an intermediate level of contamination in zones G02 (29.3 ± 10.5 $\mu g.kg^{-1}$) and G03 (17.0 ± 4.9 $\mu g.kg^{-1}$) while the other sampling areas were characterized by a mean fish contamination less than 10 $\mu g.kg^{-1}$. No sampling was done in zones G07 and G08, the most remote areas from the contaminated terrestrial areas (Fig.1).

3.2. Contamination of lionfish according to their distance from the coast

In zone G01, the most contaminated areas, 13 samples were used to study the correlation between concentrations of chlordecone and distance from the coast of the capture (Fig.2). Among the 13 samples, five fish were collected around an islet located at 1.2 km from the coast; six were found around a wreck at 3.8 km from the coast and two others fish were samples at 4.1 and 5.7 km from the coastline. Fish size was comprised between 20 and 25 cm for all these specimens.

Fish collected closer from the coast presented the highest concentrations of chlordecone (Fig.2) and a significant correlation was found between these two variables (Spearman's rank correlation, $p < 0.001$). Beyond the distance of 4 km from the coast, concentrations of chlordecone measured were less than 15 $\mu g.kg^{-1}$ (Fig.2).

3.3. Contamination of lionfish according to the fish size

Correlations between total lengths of fish and concentrations of chlordecone were studied in three sites located in the most contaminated marine zone (G01), to avoid variations due to the location of the capture (Fig.3). Total lengths of fish were comprised between 19.5 cm and 33.2 cm. In the three sites, a significant positive correlation was found between the concentrations of chlordecone measured in fish and their size (Spearman's rank correlation, $p < 0.01$ for the three sites). Thus, the biggest individuals were the most contaminated by chlordecone in each of these three sites (Fig.3).

3.4. Comparisons with the level of contamination of other fish species

Concentrations of chlordecone measured in lionfish were compared to the concentrations of five others fish species reported in the literature (Dromard et al., 2016): white grunt, *Haemulon plumierii*, spotted goatfish, *Pseudopeneus maculatus*, redtail parrotfish, *Sparisoma chrysopterum*, ocean surgeonfish, *Acanthurus bahianus* and yellowtail snapper, *Ocyurus chrysurus* (Table 2). These values come from a chlordecone database combining all concentrations of chlordecone measured in the marine fauna since 2003. Values come from several campaigns conducted by different governmental institutions: DAAF (Department of Food, Agriculture and Forests), DEAL (Department of Environment, Land settlement and Housing), UA (Université des Antilles) and IFREMER (French Institute of Study and Exploitation of the Sea).
In the contaminated areas (i.e. in zones G01 and G02), all fish species showed a mean concentration of chlordecone higher than 20 $\mu g.kg^{-1}$, except for the surgeonfish *Acanthurus bahianus*. Among the six fish species, lionfish showed the highest concentrations of chlordecone (ANOVA, $F_{(5,102)} = 1.5$, $p < 0.01$).
In the non-contaminated areas, mean concentrations measured for the six fish species were less than 7 $\mu g.kg^{-1}$ (Table 2). Mean concentrations were significantly different according to the fish species (ANOVA, $F_{(5,277)} = 2.7$, $p < 0.05$). Lionfish was not the most contaminated species in these areas, however the level of contamination was low for all species.

Discussion

The lionfish, *Pterois volitans*, is a new fish species in the marine ecosystems of the Gulf of Mexico and the Caribbean. In Guadeloupe, this fish was recorded for the first time in 2009 and can be found now in abundance all around the island. A study on the contamination of lionfish by chlordecone was necessary before supporting the fishing and the commercialization of this species on the local market. Even if the number of individuals studied was relatively low (less than a hundred), the present study brings relevant information on the level of contamination of lionfish by this pesticide and opens new perspectives of research.
Lionfish are principally contaminated in the marine areas located downstream of previous banana plantations, where the risk of contamination of soils is the highest that is in the southeast of Basse-Terre (Cabidoche and Lesueur Jannoyer, 2011). In this part of the island, runoff and ground waters coming from the lands lead to the contamination of the surrounding marine ecosystems. Thus, the highest concentrations of chlordecone measured in the present

study were found in lionfish collected in the southeast of Basse-Terre (zones G01 and G02). In 2009, regional authorities marked out an area of fishing ban, located along the coastline of zone G02. At the boundaries of this area, an area of fishing restrictions has been established, corresponding to zones G01 and G03. Due to its contamination, the lionfish *Petrois volitans* has been added to the list of species for which fishing is forbidden in the area of fishing restriction, because the maximal residue limit authorized for human consumption is 20 $\mu g.kg^{-1}$ w.w. The level of contamination of lionfish are relatively low in others marine areas (< 10 $\mu g.kg^{-1}$ w.w.) and consequently, fishing and commercialization of lionfish is allowed in these others parts of the island.

The level of contamination of lionfish seems to decrease rapidly with the distance from the coast. This observation could be explained by a dilution of the molecule in the marine environment especially because the southeast of Basse-Terre is exposed to wind and swell. However, the number of samples collected farther than 4 km from the coast was too low to define the maximal distance of influence of this organochlorine pollution on marine ecosystems.

In the most contaminated sites, concentrations of chlordecone increased with the total length of fish, showing increased bioaccumulation of the molecule with fish age. Compared to other fish species, collected in similar marine areas, *Pterois volitans* showed higher level of contamination. The five other fish species studied (white grunt, *Haemulon plumierii*, spotted goatfish, *Pseudopeneus maculatus*, redtail parrotfish, *Sparisoma chrysopterum*, ocean surgeonfish, *Acanthurus bahianus* and yellowtail snapper, *Ocyurus chrysurus)* are commonly preyed by lionfish (Morris and Akins, 2009). Indeed, lionfish are likely to strongly bioaccumulate pollutants like pesticides principally because of its top position in the coral reef food web. There is considerable evidence that the concentration of organochlorine pesticide residues increases through the food chain and that concentrations measured in predatory mammals or fish is higher than that found in their food (Portman and Bourne, 1975; Guo et al., 2008). Due to its sedentary behavior and its high capacity to accumulate chlordecone, lionfish could be used as a sentinel species to follow the level of contamination of marine fauna by chlordecone with long-term monitoring.

Furthermore, lionfish could be used to study the impact of chlordecone exposures on the fish physiology, such as mechanisms of development, growth or reproduction. As well as the impact on the fish physiology, environmental pollutants can also involve disruptions of fish behaviors associated with foraging, predator avoidance, reproduction or social hierarchies, for examples (Portman and Bourne, 1975; Little et al., 1990; Scott and Sloman, 2004; Weis and Weis 2004). To date, no study has been done yet on the impact of chlordecone on the biology and the behavior of marine fauna. Due to its abundance in the marine ecosystems of the Caribbean, the invasive lionfish seems to be an excellent model to test these topical concerns in ecotoxicology.

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Figures

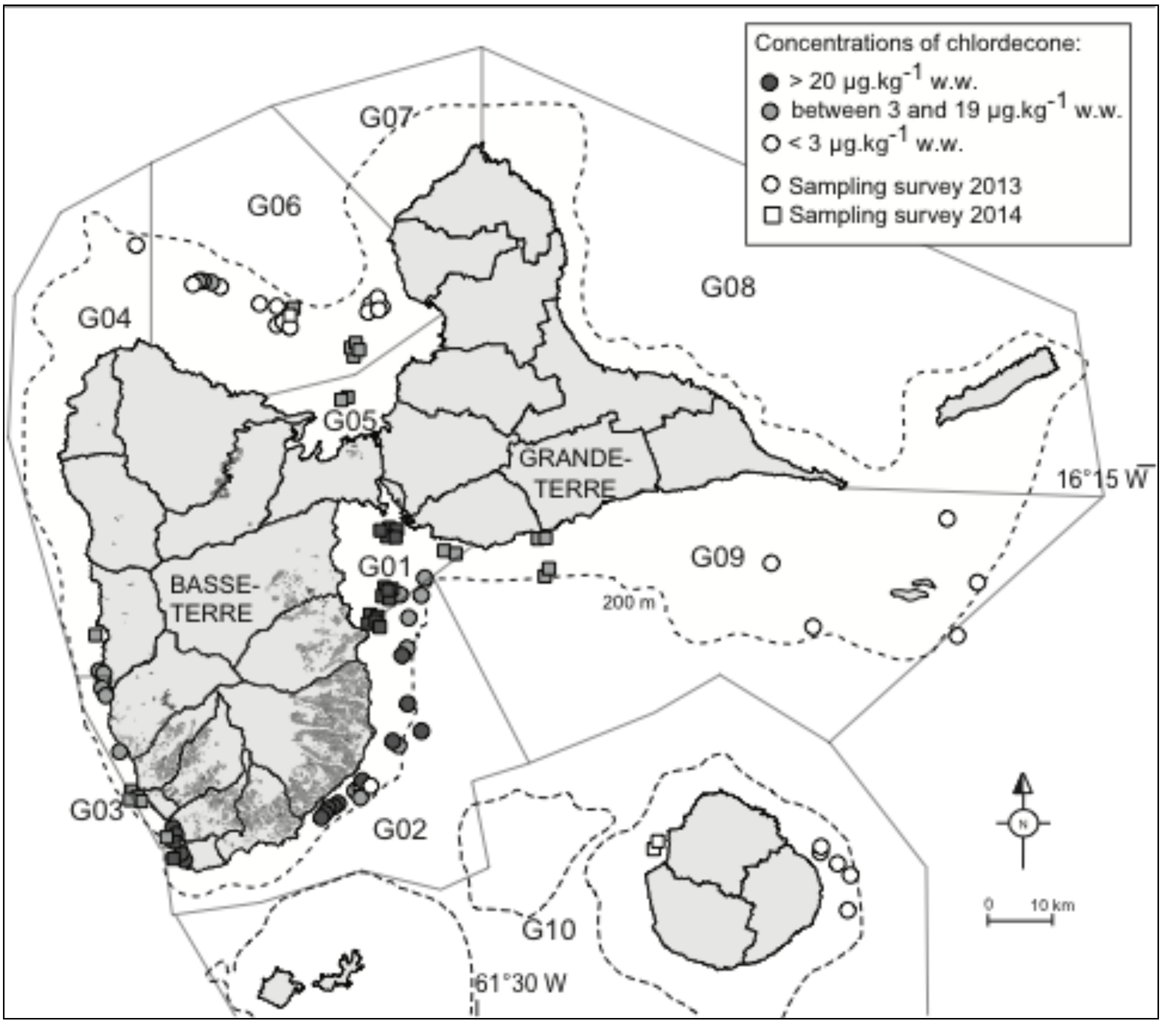

Fig.1 Location of sampling areas (G01 to G10) and concentrations of chlordecone (in $\mu g.kg^{-1}$ w.w.) measured in lionfish during the study. Circles: sampled in 2013; squares: sampled in 2014. Black symbols: concentrations of chlordecone higher than 20 $\mu g.kg^{-1}$; gray symbols: concentrations between 3 and 19 $\mu g.kg^{-1}$; white symbols: concentrations inferior to 3 $\mu g.kg^{-1}$. Terrestrial areas in gray indicate contaminated soils due to previous bananas plantations.

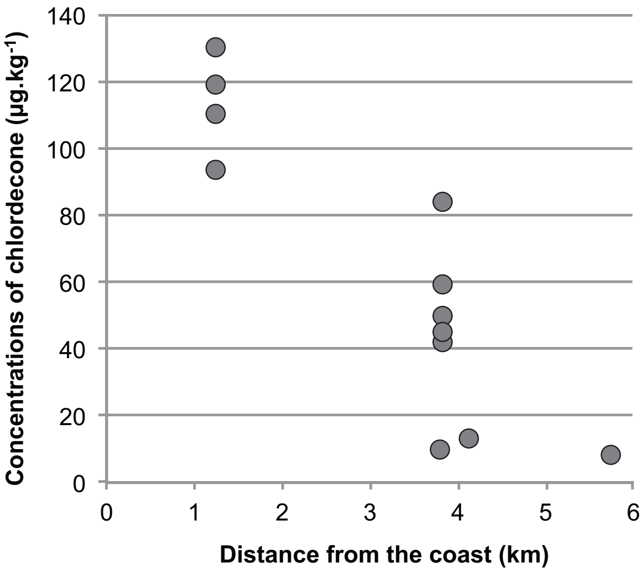

Fig.2 Concentrations of chlordecone (in µg.kg^{-1} w.w.) measured in fish according to the distance from the coast of their capture (in km).

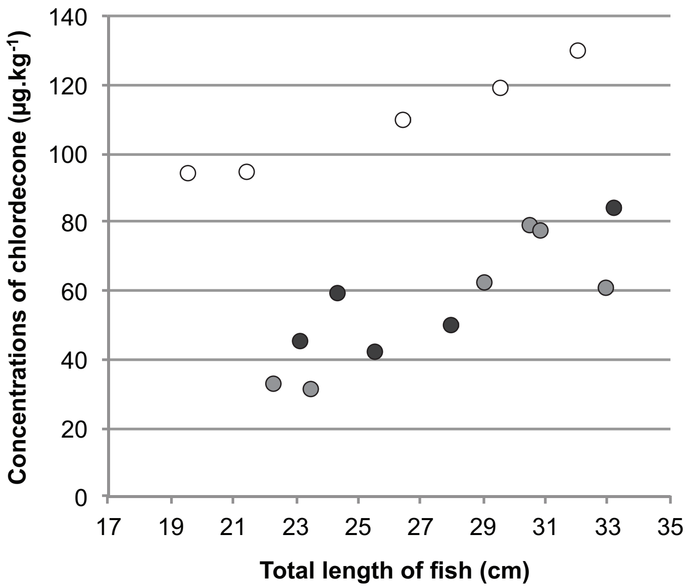

Fig.3 Concentrations of chlordecone (in µg.kg^{-1} w.w.) measured in fish according to their size (total length in cm). White, gray and black circles indicate that fish come from three different sites of the zone G01.

Tables

Table 1 Mean concentrations of chlordecone in $\mu g.kg^{-1}$ w.w. ([CHD] ± 95%CI) measured in lionfish according to the sampling areas in which they were collected (G01 to G10). The location of the sampling areas is shown in Figure 1. n is the number of samples analyzed.

Sampling areas	n	[CHD] ± CI
G01	24	61.7 ± 17.6
G02	18	29.3 ± 10.5
G03	10	17.0 ± 4.9
G04	6	7.8 ± 1.6
G05	2	6.5 ± 2.9
G06	21	3.4 ± 0.6
G07	0	-
G08	0	-
G09	9	5.2 ± 2.5
G10	7	2.1 ± 1.1

Table 2 Mean concentrations of chlordecone in $\mu g.kg^{-1}$ w.w. ([CHD] ± 95%CI) measured in lionfish and in five others fish species (Dromard et al., 2016), in contaminated areas (zones G01 and G02) and in non-contaminated areas (others zones). n is the number of samples analyzed for each fish species.

Fish species	n	Contaminated areas	Non-contaminated areas
Pterois volitans	97	47.8 ± 12.0	6.6 ± 1.7
Haemulon plumierii	60	31.4 ± 23.4	6.0 ± 2.9
Pseudopeneus maculatus	48	31.2 ±12.5	4.1 ± 3.9
Ocyurus chysurus	67	27.0 ±16.1	7.0 ± 5.0
Sparisoma chrusopterum	72	28.2 ± 32.0	1.8 ± 1.1
Acanthurus bahianus	46	16.4 ± 5.7	1.7 ± 1.0
Results of ANOVA		$p < 0.01$	$p < 0.05$