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1 **Contamination of marine fauna by chlordecone in Guadeloupe: evidence of a seaward**
2 **decreasing gradient**

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14

15 **Abstract**

16 Chlordecone is an organochlorine pesticide, used in the Lesser Antilles from 1972 to 1993 to
17 fight against a banana weevil. That molecule is very persistent in the natural environment and
18 ends up in the sea with runoff waters. The objective of the present study is to evaluate the
19 level of contamination in several trophic groups of marine animals according to their distance
20 from the source of pollution. Samples of suspended matter, macroalgae, herbivorous fishes,
21 detritivorous crustaceans, zooplanktivorous fishes, first and second order of carnivorous fishes
22 and piscivorous fishes have been collected in two sites, located downstream the contaminated
23 sites (Goyave and Petit-Bourg), in three marine habitats (coastal mangroves, seagrass beds
24 located 1.5 km from the shoreline and coral reefs at 3 km offshore). Animals collected in
25 mangroves were the most contaminated (mean concentrations: 193 $\mu\text{g.kg}^{-1}$ in Goyave and 213
26 $\mu\text{g.kg}^{-1}$ in Petit-Bourg). Samples from seagrass beds presented intermediate concentrations of
27 chlordecone (85 $\mu\text{g.kg}^{-1}$ in Goyave and 107 $\mu\text{g.kg}^{-1}$ in Petit-Bourg). Finally, samples from
28 coral reefs were the less contaminated (71 $\mu\text{g.kg}^{-1}$ in Goyave and 74 $\mu\text{g.kg}^{-1}$ in Petit-Bourg).
29 Reef samples, collected 3 km offshore, were two to three times less contaminated than those
30 collected in mangroves.

31

32 **Key words:** chlordecone, trophic food-web, inshore-offshore gradient, marine fauna,

33 Guadeloupe, coral reefs, mangrove, seagrass beds

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40 **Introduction**

41 Chlordecone is an organochlorine pesticide, used from 1972 to 1993 in the Lesser Antilles, to
42 fight against the banana weevil. The manufacturing of this chemical (commercialized as
43 Kepone® and then as Curlone®) was first done in Virginia and stopped in 1975, when
44 workers from the site of production began to show severe and diverse pathologies associated
45 to their exposure. Due to the sewage system of the factory, the local environment and wildlife
46 was also impacted (Epstein 1978; Huff and Gerstner 1978). In the French West Indies, the use
47 of this chemical however continued until 1993. As a consequence, approximately 6 200
48 hectares are moderately to heavily polluted by chlordecone in Guadeloupe (Cabidoche and
49 Lesueur Jannoyer 2011), which represents about 25% of the land surface used for agriculture.
50 Chlordecone is a very persistent molecule in the environment with a half-life estimated to 600
51 years (Cabidoche et al. 2009).

52 In Guadeloupe, banana plants grow in the southern part of Basse-Terre (one of the two islands
53 of Guadeloupe), which is mountainous and, as a consequence of tropical humid weather,
54 characterized by intense rainfall events. Organochlorine molecules are hydrophobic and
55 adsorbed onto organic matter of the soil. With the erosion of soil particles, desorption
56 phenomena, slow solubilization and infiltration processes, these compounds reach runoff and
57 ground waters that end up directly into the sea (Cattan et al. 2006; Coat et al. 2006; Cabidoche
58 et al. 2009).

59 Since 2003, several samplings surveys have been conducted in Guadeloupe to evaluate the
60 level of contamination by chlordecone of some species of fishes, crustaceans and mollusks
61 (Bouchon and Lemoine 2003, 2007; Bertrand et al. 2013; Dromard et al. 2016a,b). In 2008,
62 the French food and safety authorities lowered the maximal residue limit (MRL) for
63 chlordecone, authorized for human consumption and commercialization of sea products, from
64 200 to 20 $\mu\text{g.kg}^{-1}$ of wet weight and regulated the fishing activities around the island. The

65 most contaminated marine areas, located downstream of the banana plantations, are now
66 totally closed to fishing activities. The boundary areas are classified as areas of fishing
67 restrictions in which it is not possible to fish a list of targeted species. These rules have been
68 established to protect the health of the local population, especially because seafood represents
69 a large part of the Caribbean food trade.

70 Studying the evolution of pollution within an inshore-offshore gradient, with different habitats
71 and different species in the trophic food-web, is necessary to understand their dispersion
72 mechanism. Few studies have been conducted to evaluate the dispersion of pesticide in
73 marine environment with an inshore-offshore gradient (Rato et al. 2006; Briand et al. 2004;
74 Dromard et al. 2016b). Organochlorine pollution in marine food-webs has been studied in
75 mangroves (Paez-Osuna et al. 2002; Bayen et al. 2005), seagrass beds (Haynes et al. 2000;
76 Bouchon et al. 2016) and coral reefs (Glynn et al. 1995; Haynes and Johnson 2000) but few
77 works analyzed the dynamics of transfer of an organochlorine contamination in the continuum
78 “mangrove-seagrass beds-coral reefs” (Schaffelke et al. 2005). The degradation of these three
79 interlinked habitats has dramatic ecological and economical consequences (Wilkinson and
80 Salvat 2012).

81 In the present study, we examined the level of contamination in several trophic groups of
82 marine animals in relation with to their distance from the source of pollution. Concentrations
83 of chlordecone have been measured in three marine habitats: mangroves, seagrass beds and
84 coral reefs.

85

86 **Materials and methods**

87 Study sites

88 Two study sites (Goyave and Petit-Bourg) were chosen in the eastern coast of Basse-Terre in
89 Guadeloupe (Fig.1). These two sites are located in an area of fishing restriction due to their

90 position downstream the contaminated rivers and agricultural plots. These two sites include
91 three types of marine habitats: coastal mangroves, seagrass beds (located approximately at 1.5
92 km from the coast) and coral reefs (around 3 km offshore). Depths were comprised between 1
93 m in mangroves and 5 m in coral reefs ecosystems.

94

95 Sample collection and preparation

96 The sampling survey was carried out from January 2014 to February 2015. For this study, 205
97 samples were collected, 113 at Goyave and 92 at Petit-Bourg (Tables 1 and 2). Macroalgae,
98 fishes and crustaceans were collected by hand, spearfishing or using nets in seagrass beds and
99 mangroves. Fishes and crustaceans were clustered in trophic groups: detritivorous crustaceans
100 (Crust Det), herbivorous fishes (Fish HB), zooplanktivorous fishes (Fish PK), first order
101 carnivorous fishes (Fish CA1: invertebrate feeders), second order carnivorous fishes (Fish
102 CA2: invertebrates and fish feeders) and piscivorous fishes (Fish PV: fish feeders). Each
103 sample was rinsed, weighted to insure the minimal quantity required for chlordecone analysis
104 (10g wet weight) and frozen (-18°C) until analyses.

105 For sampling the suspended matter, seawater was collected in the three habitats of each site in
106 plastic drums. Water was then filtered at the laboratory on Whatman GF/F 47mm filters.

107

108 Chlordecone extraction and analysis

109 The laboratory Laboceca conducted the quantitative analyses of chlordecone. Molecules of
110 chlordecone were extracted from homogenized samples tissues with a solution of organic
111 solvents (hexane-acetone) and turned into chlordecone hydrate (hydrosoluble) in the presence
112 of soda. The aqueous phase was rinsed with hexane to eliminate fats. Chlordecone was then
113 reassembled in acid conditions, extracted with a solution of hexane and acetone.
114 Concentrations of chlordecone were quantified with liquid chromatography coupled to mass

115 spectrometry in tandem (UPLC-MS/MS). Chlordecone was extracted following the method
116 recommended by ANSES (“Agence nationale de sécurité sanitaire de l'alimentation, de
117 l'environnement et du travail”, French organization in charge of the sanitary security). The
118 lower limit of quantification with this method was $1 \mu\text{g.kg}^{-1}$ and the concentrations of
119 chlordecone were expressed in $\mu\text{g.kg}^{-1}$ (wet weight).

120

121 Statistical analysis

122 Shapiro-Wilk's tests attested of the non-normality of data distribution. Then, concentrations
123 of chlordecone were compared between types of habitat (mangrove, seagrass beds and coral
124 reefs) with Kruskal-Wallis tests. All statistical analyses were performed using the software
125 package R.

126

127 **Results**

128 Concentrations of chlordecone according to the habitats

129 Concentrations of chlordecone measured in this study varied from $1 \mu\text{g.kg}^{-1}$ (the limit of
130 quantification) to $1034 \mu\text{g.kg}^{-1}$. Concentrations of chlordecone were significantly different
131 between the three types of habitats at Goyave ($X^2=18.9$, $p<0.001$) and at Petit-Bourg ($X^2=5.5$,
132 $p<0.05$), and an inshore-offshore gradient of contamination was found (Fig.2). Samples
133 collected in mangroves were the most contaminated with a mean concentration of
134 chlordecone equal to $193 \mu\text{g.kg}^{-1}$ at Goyave and $213 \mu\text{g.kg}^{-1}$ at Petit-Bourg. Marine organisms
135 sampled in seagrass beds presented intermediate concentrations of chlordecone ($85 \mu\text{g.kg}^{-1}$ at
136 Goyave and $107 \mu\text{g.kg}^{-1}$ at Petit-Bourg). Finally, vegetal and animal samples from coral reefs
137 were the less contaminated ($71 \mu\text{g.kg}^{-1}$ at Goyave and $74 \mu\text{g.kg}^{-1}$ at Petit-Bourg).

138

139 Concentrations of chlordecone according to the trophic group

140 The level of contamination according to the habitat was studied for the different categories of
141 samples independently. At Goyave, a decreasing gradient of contamination was found for four
142 trophic groups: suspended matter ($X^2=6.0$, $p<0.05$), macroalgae ($X^2=8.9$, $p<0.001$),
143 detritivorous crustaceans ($X^2=6.7$, $p<0.05$), second order carnivorous fishes ($X^2=5.7$, $p<0.05$)
144 and planktivorous fishes ($X^2=4.7$, $p<0.05$, Fig.3). The concentrations of chlordecone were not
145 significantly different according to the habitat for the herbivorous fishes, first order
146 carnivorous fishes and piscivorous fishes. Herbivorous fishes presented low and similar
147 concentrations in the three habitats (42, 19 and 10 $\mu\text{g.kg}^{-1}$ in mangrove, seagrass bed and reef
148 respectively). First order carnivorous and piscivorous fishes were highly contaminated in
149 mangrove (295 and 338 $\mu\text{g.kg}^{-1}$ respectively) but showed similar levels of contamination in
150 seagrass bed and coral reef (114 and 112 $\mu\text{g.kg}^{-1}$ for CA1; 154 and 134 $\mu\text{g.kg}^{-1}$ for PV).

151 At Petit-Bourg, a decreasing gradient of contamination was found for the suspended matter
152 ($X^2=5.5$, $p<0.05$), macroalgae ($X^2=10.4$, $p<0.001$), first order carnivorous fishes ($X^2=9.7$,
153 $p<0.001$) and piscivorous fishes ($X^2=12.1$, $p<0.001$, Fig.4). No significant difference was
154 found for second order carnivorous fishes. For the latter, concentrations of chlordecone were
155 close between mangrove, seagrass bed and reef: 160, 203 and 160 $\mu\text{g.kg}^{-1}$ respectively.

156

157 **Discussion**

158 The concentrations of chlordecone measured in the present study indicate a high
159 contamination of marine organisms located in the coastal marine habitats in Guadeloupe. A
160 decreasing inshore-offshore gradient of contamination by chlordecone was found at both sites.
161 Samples collected in mangroves, located along the shore, were the most contaminated and
162 presented the highest concentrations of chlordecone measured in this study. Samples from
163 seagrass beds showed intermediate concentrations while samples from coral reefs were the

164 less contaminated. Concentrations of chlordecone in organisms were two to three times higher
165 in mangroves than in coral reefs.

166 In previous studies on chlordecone pollution in the James River (Virginia), mean
167 concentrations of chlordecone reached 4800 $\mu\text{g.kg}^{-1}$ in zooplankton and 1700 $\mu\text{g.kg}^{-1}$ for the
168 zooplanktivorous white perch, *Morone americana* (Nichols 1990; Luellen et al. 2006). In
169 Guadeloupe, the highest concentration measured was 1034 $\mu\text{g.kg}^{-1}$ for *Pomadasys*
170 *corvinaeformis*, which is a first order carnivorous fish (CA1), and the concentration of
171 chlordecone in zooplankton averaged 20 $\mu\text{g.kg}^{-1}$ at Petit Bourg and 6 $\mu\text{g.kg}^{-1}$ at Goyave. In
172 comparison, the level of contamination of marine organisms in Guadeloupe appears low.

173 However, in coral reefs, located approximately at 3 km offshore, concentrations of
174 chlordecone measured were still three times higher than the maximal residue limit authorized
175 for human consumption and commercialization of sea products (20 $\mu\text{g.kg}^{-1}$). These results
176 justify the interdiction of fishing on the continental shelf located on the eastern coast of
177 Basse-Terre due to high levels of contamination by chlordecone.

178 In Florida, Glynn et al. (1995) studied the dispersion of marine fauna contamination by
179 pesticides at three sites distributed on a 5 km distance from the coast and found no spatial
180 variation of the level of pollution between the sites. In other studies, the dispersion of
181 organochlorine pollutants was generally demonstrated over larger distances. Rato et al. (2006)
182 studied the dispersion of a pesticide over 25 km of the continental shelf in Portugal. In the
183 south of New Caledonia, a decreasing gradient of pollution was also found from the coast to
184 the reef barrier on a 45 km distance (Briand et al. 2014). In Guadeloupe, the width of the
185 continental shelf in front of Petit Bourg and Goyave is narrow (around 5 km) and exposed to
186 eastern winds and swell, which prevents the dispersion of the pollutants seaward. Indeed,
187 pollution is concentrated on this small area.

188 In the present study, the gradient of contamination was analyzed for different trophic groups
189 of marine organisms. The majority of the studied trophic groups showed a decreasing gradient
190 of the contamination from the coast seaward. However, some trophic groups presented a
191 different pattern. In Goyave, the gradient of contamination was not observed for the first order
192 carnivorous fishes (CA1: invertebrates feeders) and piscivorous fishes (Table 1). These
193 species were highly contaminated in mangrove but showed similar levels of contamination in
194 seagrass bed and coral reef habitats. This could result from the mobility of these species
195 between seagrass beds and coral reefs (for example: *Sphyraena barracuda*). The absence of
196 gradient for some trophic groups can also be explained by the fact that a same trophic group
197 can be constituted by different species in the three habitats. Moreover, the lack of samples in
198 some habitat could lead to a bias in the comparison (for example there is a single fish species
199 of CA1 represented in the reef habitat). Still in Goyave, herbivorous fishes presented a low
200 and similar level of contamination in the three habitats (Table 1). That result could be
201 partially explained by their feeding patterns, as they consume macroalgae that were faintly
202 contaminated. At Petit Bourg, the gradient of contamination was not significant for the second
203 order carnivorous fishes (CA2: invertebrates and fish feeders, Table 2). The movements of
204 some of these species across the different habitats (for example: *Lutjanus apodus* and *L.*
205 *griseus*) could explain the similar level of contamination between the three habitats. These
206 movements can be carried out for dietary purposes or during post-settlement migrations
207 (Chapman and Kramer 2000; Cocheret de la Morinière et al. 2002).

208 To conclude, considering all the data combined, this study evidences a decreasing gradient of
209 the contamination by chlordecone from the coast to the coral reefs 3 km away from the source
210 of pollution. This spatial variation in chlordecone concentration suggests that uptake from the
211 water column is a significant source of contamination. Uptake through the trophic food web
212 *via* bioaccumulation is another potential source of contamination, but this hypothesis requires

213 further investigation. Future research will evaluate the relative contribution of uptake from the
214 water column vs. the food web.

215

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220

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288 the commons apply for the coral reefs, mangrove forests and seagrass beds. Mar Pollut
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- 291

292 **Figures captions**

293 **Fig. 1** Location of Guadeloupe in the Caribbean (a), location of the two study sites (b) and
294 location of the three habitats (M: mangroves, S: seagrass beds and R: reefs) in each study site

295

296 **Fig. 2** Mean concentrations of chlordecone (all species included) in $\mu\text{g.kg}^{-1}$ (\pm SE) measured
297 at Goyave and Petit-Bourg in mangroves, seagrass beds and coral reefs

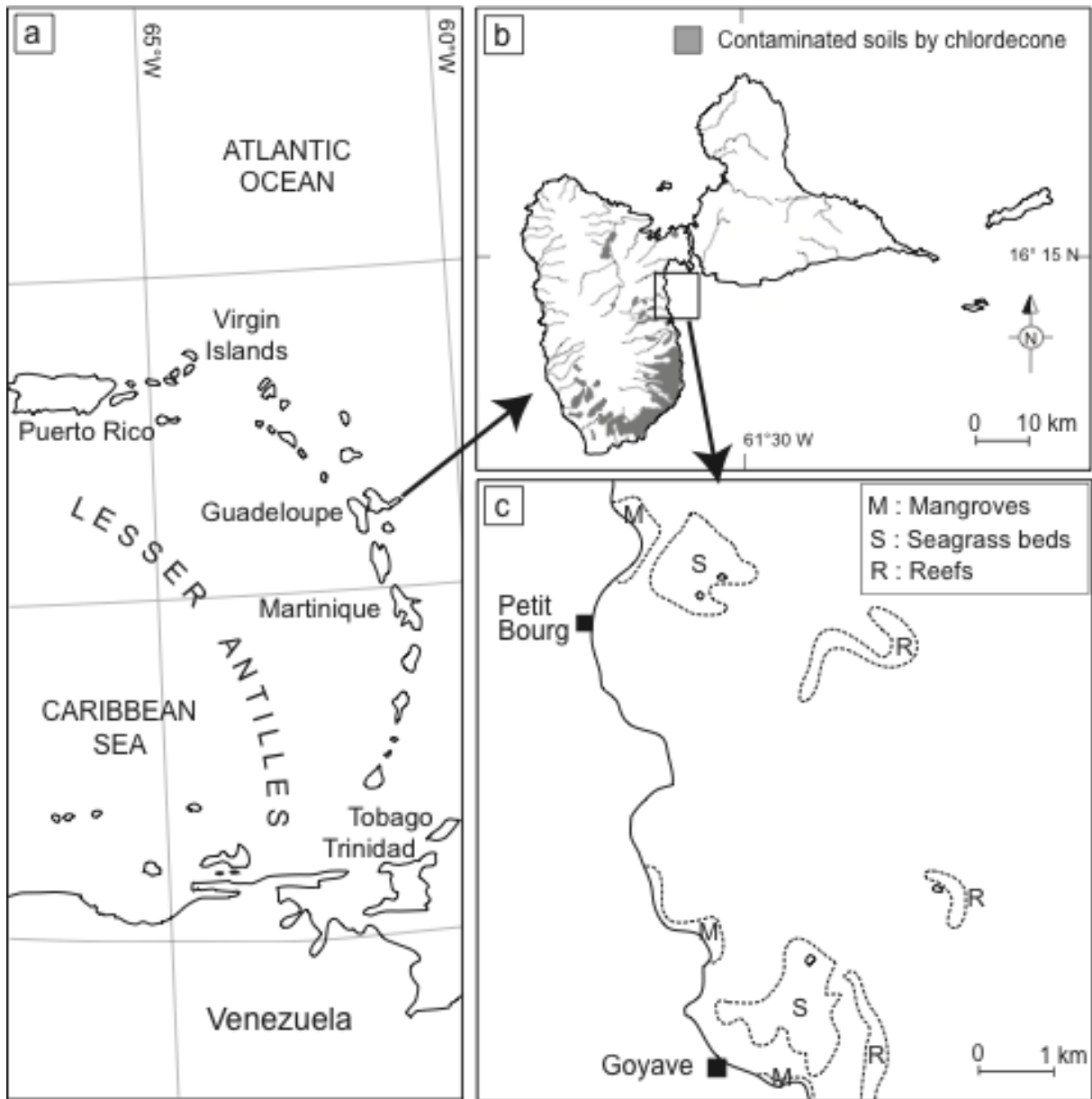
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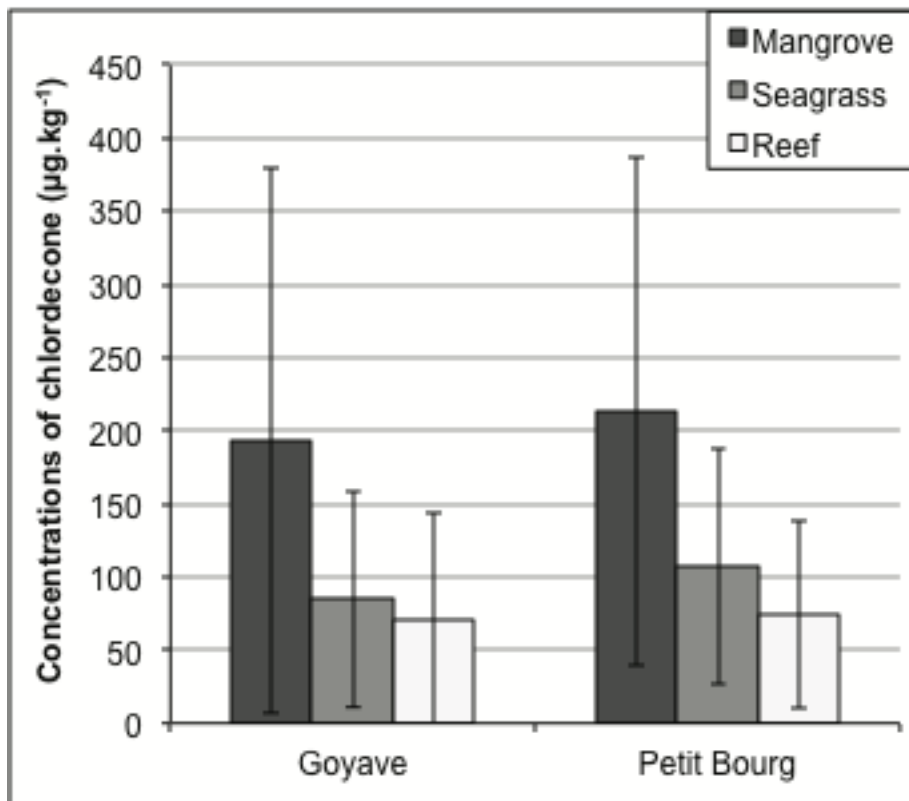
299 **Fig. 3** Mean concentrations of chlordecone by trophic groups (in $\mu\text{g.kg}^{-1} \pm$ SE) measured at
300 Goyave in mangroves, seagrass beds and coral reefs. SM: suspended matter, Crust Det:
301 detritivorous crustaceans, Fish HB: herbivorous fishes, Fish PK: planktivorous fishes, Fish
302 CA1: carnivorous fishes 1, Fish CA2: carnivorous fishes 2 and Fish PV: piscivorous fishes

303

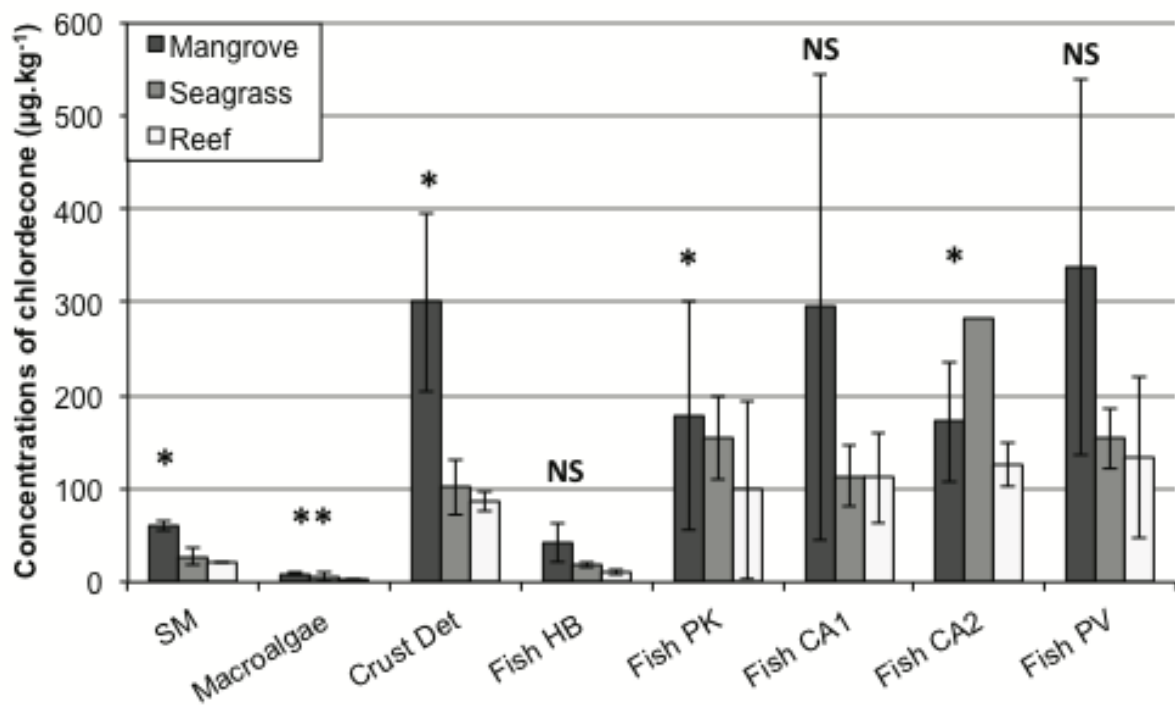
304 **Fig. 4** Mean concentrations of chlordecone by trophic groups (in $\mu\text{g.kg}^{-1} \pm$ SE) measured at
305 Petit-Bourg in mangroves, seagrass beds and coral reefs. SM: suspended matter, Fish CA1:
306 carnivorous fishes 1, Fish CA2: carnivorous fishes 2 and Fish PV: piscivorous fishes

307

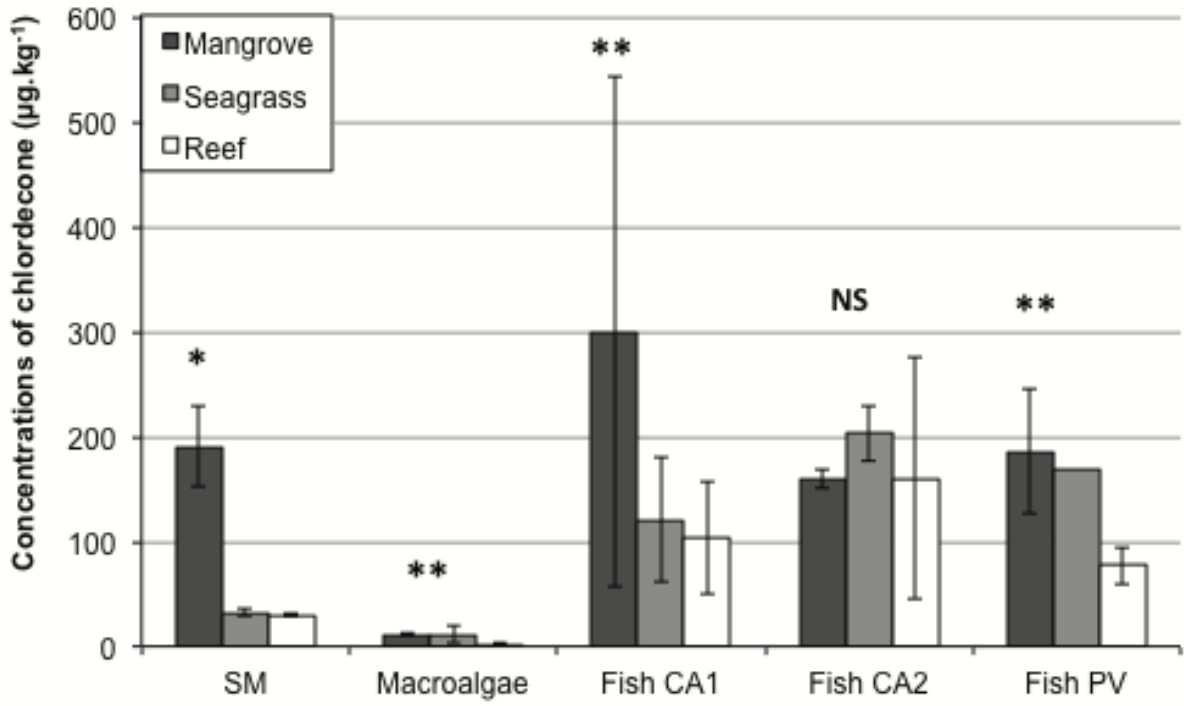




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314

315 Table 1 Mean concentrations of chlordécone in $\mu\text{g}\cdot\text{kg}^{-1}$ (\pm SE) of species and trophic groups collected
 316 at Goyave. n is the number of samples.

Samples	n	Mangrove	Seagrass bed	Coral reef
Phytoplankton-SM	9	60.0 \pm 5.6	27.3 \pm 9.5	20.7 \pm 0.6
Suspended matter	9	60.0 \pm 5.6	27.3 \pm 9.5	20.7 \pm 0.6
Macroalgae	21	8.6 \pm 1.3	6.2 \pm 5.1	1.8 \pm 0.7
<i>Acanthophora spicifera</i>	6	7.6 \pm 0.6		1.8 \pm 0.8
<i>Caulerpa sertularoides</i>	3		10.7 \pm 2.0	
<i>Enteromorpha flexuosa</i>	3	9.6 \pm 0.8		
<i>Galaxaura rugosa</i>	3			2.1 \pm 0.6
<i>Halimeda incrassata</i>	3			1.6 \pm 0.9
<i>Padina</i> sp	3		1.8 \pm 0.3	
Detritivorous crustaceans	10	300.3 \pm 96.4	102.0 \pm 29.7	86.7 \pm 10.4
<i>Callinectes</i>	3	257.0 \pm 52.1		
<i>Farfantepenaeus subtilis</i>	1	430.0		
<i>Panulirus argus</i>	6		102.0 \pm 29.7	86.7 \pm 10.4
Herbivorous fishes	9	42.0 \pm 20.3	19.0 \pm 3.6	10.3 \pm 3.2
<i>Scarus taeniopterus</i>	3			10.3 \pm 3.2
<i>Sparisoma radians</i>	6	42.0 \pm 20.3	19.0 \pm 3.6	
Planktivorous fishes	14	177.9 \pm 122.0	154.7 \pm 44.6	99.0 \pm 95.5
<i>Anchoa lyolepis</i>	3	209.0 \pm 101.9		
<i>Harengula clupeiola</i>	3	113.0 \pm 72.5		
<i>Hemiramphus balao</i>	4	228.5 \pm 228.4	129.0 \pm 4.2	
<i>Heteropriacanthus cruentatus</i>	2			44.0 \pm 8.5
<i>Myripristis jacobus</i>	2		206.0	209.0
Carnivorous fishes 1	24	295.4 \pm 249.8	113.8 \pm 32.6	112.3 \pm 48.9
<i>Eucinostomus gula</i>	3	100.7 \pm 14.6		
<i>Eucinostomus lefroyi</i>	3		91.3 \pm 11.0	
<i>Gerres cinereus</i>	1	207.0		
<i>Haemulon plumieri</i>	3			112.3 \pm 48.9
<i>Larimus breviceps</i>	1	522.0		
<i>Mulloidichthys martinicus</i>	1	204.0		
<i>Ocyurus chrysurus</i>	1		145.0	
<i>Polydactylus virginicus</i>	2	215.5 \pm 23.3		
<i>Pomadasys corvinaeformis</i>	3	524.3 \pm 458.9		
<i>Sphoeroides greeleyi</i>	5	254.3 \pm 196.6	132.0 \pm 43.8	
<i>Trachinotus falcatus</i>	1	429.0		
Carnivorous fishes 2	14	171.5 \pm 64.6	284.0	126.4 \pm 23.6
<i>Bairdiella ronchus</i>	3	110.0 \pm 24.8		

<i>Gymnothorax funebris</i>	1	234.0		
<i>Lutjanus apodus</i>	4	180.0		123.7 ± 32.3
<i>Lutjanus griseus</i>	1		284.0	
<i>Lutjanus mahogani</i>	1	275.0		
<i>Lutjanus synagris</i>	3	146.0		130.5 ± 9.2
<i>Rypticus saponaceus</i>	1	207.0		
Piscivorous fishes	12	337.5 ± 201.5	154.0 ± 32.2	133.6 ± 87.1
<i>Aulostomus maculatus</i>	3			118.0 ± 39.1
<i>Caranx crysos</i>	3		154.0 ± 32.2	
<i>Caranx latus</i>	1	480.0		
<i>Pterois volitans</i>	3			87.7 ± 26.1
<i>Sphyraena barracuda</i>	1			318.0
<i>Sphyraena picudilla</i>	1	195.0		
All species pooled	113	193.0 ± 185.5	84.8 ± 74.0	71.4 ± 72.4

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320 Table 2 Mean concentrations of chlordécone in $\mu\text{g}\cdot\text{kg}^{-1}$ (\pm SE) of species and trophic groups collected
 321 at Petit-Bourg. n is the number of samples.

Samples	n	Mangrove	Seagrass bed	Coral reef
Phytoplankton-MS	9	191.3 \pm 38.5	31.7 \pm 2.9	30.3 \pm 2.1
Suspended matter	9	191.3 \pm 38.5	31.7 \pm 2.9	30.3 \pm 2.1
Macroalgae	15	11.3 \pm 0.6	10.6 \pm 7.6	2.3 \pm 1.1
<i>Acanthophora spicifera</i>	3	11.3 \pm 0.6		
<i>Caulerpa sertularoides</i>	3		16.6 \pm 6.0	
<i>Galaxaura rugosa</i>	3			1.4 \pm 0.7
<i>Halimeda incrassata</i>	3			3.2 \pm 0.5
<i>Padina</i> sp	3		4.5 \pm 0.3	
Carnivorous fishes 1	34	300.4 \pm 243.4	121.0 \pm 59.8	103.8 \pm 53.9
<i>Chaetodon capistratus</i>	2		196.0 \pm 18.4	
<i>Eucinostomus argenteus</i>	3		80.7 \pm 46.1	
<i>Eucinostomus gula</i>	6	202.3 \pm 12.9	75.7 \pm 30.0	
<i>Eugerres brasiliensis</i>	1	861.0		
<i>Gerres cinereus</i>	3	182.5 \pm 145.0	76.0	
<i>Haemulon carbonarium</i>	4			89.5 \pm 33.9
<i>Haemulon flavolineatum</i>	3			66.3 \pm 12.3
<i>Haemulon plumieri</i>				
<i>Halichoeres radiatus</i>	2			188.5 \pm 10.6
<i>Ocyurus chrysurus</i>	3		171.7 \pm 21.1	
<i>Pomadasys corvinaeformis</i>	1	121.0		
<i>Sphoeroides testudinum</i>	3	519.0 \pm 168.4		
<i>Trachinotus falcatus</i>	3	131.3 \pm 21.4		
Carnivorous fishes 2	11	159.5 \pm 9.2	203.3 \pm 26.0	160.3 \pm 114.9
<i>Lutjanus apodus</i>	6		196.3 \pm 28.0	160.3 \pm 114.9
<i>Lutjanus griseus</i>	4	153.0	210.3 \pm 27.4	
<i>Rypticus saponaceus</i>	1	166.0		
Piscivorous fishes	23	185.6 \pm 58.9	169.0	76.8 \pm 17.8
<i>Aulostomus maculatus</i>	3			83.0
<i>Carangoides bartholomaei</i>	3	173.0 \pm 10.4		
<i>Caranx crysos</i>	5	173.0 \pm 77.0		81.0 \pm 39.6
<i>Caranx latus</i>	4	180.3 \pm 81.3		
<i>Pterois volitans</i>	3			74.3 \pm 11.7
<i>Sphyraena barracuda</i>	3	278.0	169.0	57.0
<i>Sphyraena picudilla</i>				
<i>Tylosurus crocodilus</i>	2	188.0		
All species pooled	92	213.1 \pm 173.6	107.1 \pm 81.1	73.7 \pm 64.1

