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Spatial variability of metallic and organic contamination of anguilliform fish in New Caledonia

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ABSTRACT: New Caledonia is one of the main hot spots of biodiversity on the planet. Large amounts of contaminants are discharged into the lagoon as a result of increasing anthropogenic activities such as intense mining, urbanization and industrialization. Concentrations of 14 trace elements and 26 persistent organic pollutants (POPs: PCBs and pesticides) were measured in the muscles of two anguilliform fish species, over a coast to barrier reef gradient in two lagoon areas differently exposed to anthropic disturbances. This study emphasizes the high trace element contamination status of anguilliform fish and also highlights slight but perceptible organic pollution. The contamination extends throughout the lagoon, from coast to barrier reef, even in areas remote from emission points. High levels of trace elements, especially those linked to mining activities (*i.e.* Co, Cr, Fe, Mn and Ni), were detected in coastal sites. Furthermore the large dispersion of most POPs throughout the entire lagoon poses the question of their potential toxicity on marine organisms from numerous habitats. Our results underline the need for long term monitoring of various contaminants over large spatial and time scales.

Keywords: trace elements; PCBs; pesticides; POPs; carnivorous fish; coral reefs; SW Pacific Ocean.

1. Introduction

Coral reefs are threatened by a wide range of natural and anthropic disturbances, including chemical pollution (Richmond, 1993). Three main contaminant classes are particularly involved: polychlorinated biphenyls (PCBs), pesticides (both classes are part of persistent organic pollutants or POPs), and metallic trace elements. These ubiquitous contaminants can be toxic, even at low concentrations; they are resistant to degradation, transported over long distances and can bioaccumulate in marine organisms (*e.g.* Phillips 1995; Robertson and Hansen 2001). The half-life of contaminants in the marine environment is estimated to be at least a decade for the most persistent PCBs (Sinkkonen and Paasivirta 2000; Robertson and Hansen 2001), several years to a decade for trace elements such as Hg (Lodenius 1991), and several months to years for pesticides (Hellawell 1988). After their release into the marine environment, some contaminants can be strongly accumulated by organisms of different trophic levels (Van Ael *et al.* 2012; Dummee *et al.* 2012).

Southwest Pacific coral reefs are generally in good health. However, for a few decades, toxic wastes released by human activities threaten some of them (Richmond 1993). New Caledonia's lagoon, the largest in the world, is no exception. This hot spot of biodiversity is subjected to increasing contamination pressure resulting from industrialisation and urbanisation. New Caledonia is the third producer of nickel ore in the world (Dalvi *et al.* 2004). Since the end of the 19th century, many open-cast mines and three metallurgical factories have been opened. Mining activities and natural soil erosion due to rainfall (Ambatsian *et al.* 1997) generate massive sediment deposits and discharge of associated trace elements (Co, Cr, Fe, Mn and Ni,) into the lagoon. Furthermore, with the expansion of industrial factories and urban development, the lack of efficient treatment of wastewater and the use of pesticides for agriculture, the contamination by POPs is also an important issue. Nearly 250 pesticides are officially authorized in New Caledonia (DAVAR, 2001). Twenty-six of these are banned but in Europe are still extensively used in NC.

Several studies focused on trace metal contaminants in coastal waters, in particular around Nouméa (the main urban region) (Fernandez *et al.* 2006; Metian *et al.* 2008a, b; Hédouin *et al.* 2008, 2009, 2011). Bioaccumulation of mining (Co, Cr, Fe, Mn and Ni) and urban trace elements (Ag, As, Cd, Cu, Hg, Pb, Se, V and Zn) have been investigated in various taxa (crustaceans, molluscs, ascidians) (Bustamante *et al.* 2000; Hédouin *et al.* 2006, 2007, 2009, 2010, 2011; Pernice *et al.* 2009; Metian *et al.* 2008a, 2010). One study recently

revealed high contamination levels by trace elements in fish from the south-western lagoon, extending from the coast to the barrier reef (Bonnet *et al.* 2014). However, there is still little information available about trace element contamination of fish across the whole lagoon in New Caledonia (Chouvelon *et al.* 2009, Fernandez and Breau 2011; Metian *et al.* 2013). Furthermore, to our knowledge there is no information available to date on PCB and pesticide concentrations in marine organisms in New Caledonia.

Analyses of spatial patterns of contamination allow the identification of their environmental sources. They also lead to a better understanding of the physical, chemical and biological processes involved in contaminant accumulation (Robertson and Hansen 2001; Johnson *et al.* 2005). However, with the exception of the coastal region (especially in Nouméa), contamination by trace elements is insufficiently documented and its impact on marine ecosystems remains unclear. Considering the immense surface of the south-western lagoon of New Caledonia, it is necessary to extend investigations to a large spatial scale and to other contaminants such as POPs.

Marine top predators, mainly long-living species, bioaccumulate high levels of metallic elements (*e.g.* Cd and Hg) or organochlorine pollutants in their tissues (*e.g.* Adams and McMichael 1999; Wafo *et al.* 2012). Fish are particularly useful to assess contamination status and distribution of pollutants; especially if they are site-attached or even territorial, living in benthic habitats and have a high trophic level (carnivorous) (*e.g.* Kojadinovic *et al.* 2007; Dierking *et al.* 2009). The sedentary nature of fish is a crucial aspect in spatial variability studies of contamination. Anguilliform fish (morays, congers and snake eels), which are ubiquitous organisms highly represented in tropical marine waters, fulfil the conditions of good bioindicators in contamination monitoring (Bonnet *et al.* 2014). These sedentary benthic predators, which mainly live in coral reef matrices of shallow waters, are good candidates for studying the impact and spatial variability of contamination in marine environments. Surprisingly, to date they have received little interest, which could be due to their cryptic lifestyle and the difficulty to collect them (Ineich *et al.* 2007).

In this general context, the main purposes of this study were 1) to determine the level of contamination of two anguilliform fish species by trace elements and POPs, 2) to study the spatial variability of these contaminants along two coast-barrier reef gradients which are differently exposed to human activities. As the influence of ecological and biological factors on the concentration of contaminants is important, three biological parameters (body size,

trophic position, and lipid content in tissue) and their link to the bioaccumulation processes were also investigated.

2. Materials and methods

2.1 Fish sampling and study areas

Two anguilliform species were studied, *Gymnothorax chilospilus* (Muraenidae) and *Conger* sp. (Congridae). Capture of specimens was carried out using sea kraits (*Laticauda* spp.) because the prey of these predators is almost exclusively anguilliform fish. This method has been successfully used in previous studies (e.g. Reed *et al.* 2002; Ineich *et al.* 2007; Brischoux *et al.* 2011). The prey items were obtained by a gentle massage of the sea krait abdomen. Because the eels consumed are non-spiny, the snake readily regurgitates their prey (Brischoux and Bonnet 2009) without any effect on their survival (Fauvel *et al.* 2012). The spatial accuracy of sea snake sampling and their use as sentinels was described in previous studies (Brischoux *et al.* 2007a, 2009; Brischoux and Bonnet 2008; Bonnet 2012). Sea kraits probe the surrounding seafloors within a radius of 10-15km and anguilliform fish are extremely sedentary. Considering the large spatial scale of the current study, this accuracy was sufficient to examine possible local influences along a coast-barrier reef gradient.

The study was realized in the South Western lagoon of New Caledonia. Both fish species were caught in two main areas: Grand Nouméa (GN) and Grand Lagon Sud (GS) (Fig. 1). Grand Nouméa, which is close to the main city of Nouméa, is subject to a variety of anthropic pollution such as industrial activities, farming industries and wastewater. In particular a nickel factory has functioned since 1880 (SLN, Société Le Nickel, Fig. 1). Grand Lagon Sud is less influenced by industrial and urban pollution. Nevertheless, since 1950 mining activity has developed near the Bay of Prony (Goro-Nickel, Vale Inco, Fig. 1), increasing the erosion of lateritic soil in this region. For each area, three stations located on a coast-barrier reef gradient inside the lagoon were sampled: Kuendu beach (coast; CO1), Signal islet (intermediate-reef; IR1) and Amédée islet (barrier-reef; BR1) inside GN and Ouen island (coast; CO2), Mato islet (intermediate-reef; IR2) and N'da islet (barrier-reef; BR2) inside GS (Fig. 1). Sampling was carried out from January to April 2011 and from August to

September 2011, corresponding respectively to the hot and wet *versus* cool and dry seasons in New Caledonia.

Each fish was identified, measured and weighed prior to dissection (Table 1). As some fish collected were partially digested, their total length (TL) was estimated applying allometric equations using either the snout vent length, or the tail length (Brischoux *et al.* 2007b). For each individual of both species, a piece of white muscle was sampled on the non-digested part and immediately frozen at -30°C for subsequent analyses. Muscle tissues were freeze-dried and ground to powder with a porcelain mortar and pestle.

2.2 Trace element analyses

The total Hg concentration in the powder obtained from the tissues was determined by analysing the Hg, with an Advanced Mercury Analyzer (ALTEC AMA 254), directly on aliquots ranging from 5 to 50 mg of dry sample weighed to the nearest 0.01 mg (Bustamante *et al.* 2006). From 150 to 300 mg of each sample were digested using a 3:1 v:v nitric-hydrochloric acid mixture with 65% ultrapure HNO₃ and ultrapure 37% HCl. The acidic digestion was performed overnight under ambient temperature and then heated in a microwave for 30 min, increasing the temperature up to 105 °C, and 15 min at 105 °C (1200 W). After the mineralization process, each sample was diluted to 30 or 50 ml with milli-Q quality water, according to the volume of acid added to the mineralization (3 and 4.5 ml, respectively).

The analysis of Ag, As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Se, V and Zn required an extra step in the preparation protocol. These elements were analysed by Inductively Coupled Plasma Atomic Emission Spectrometry (Varian Vista-Pro ICP-OES) and Mass Spectrometry (ICP-MS II Series Thermo Fisher Scientific). Reference tissues - dogfish liver DOLT-4 (NRCC), lobster hepatopancreas TORT-2 (NRCC) - were treated and analysed in the same way as the samples. Results were in line with the certified values, and the standard deviations were low, proving good repeatability of the method. The results for standard reference materials displayed recovery of the elements ranging from 73% to 116%. For each set of analyses, blanks were included in each analytical batch. The detection limits ($\mu\text{g}\cdot\text{g}^{-1}$ dw) were 0.005 (Hg), 0.015 (Cd), 0.017 (Ag), 0.02 (Cr, Co, Pb), 0.03 (Ni), 0.08 (Mn), 0.1 (Cu, Se), 0.2 (As), 0.3 (V) and 3.3 (Fe and Zn). All trace element concentrations are given on a dry weight basis ($\mu\text{g}\cdot\text{g}^{-1}$ dw).

2.3. POP analyses

2.3.1 Choice of PCB congeners and pesticides

The concentrations of 15 individual congeners (IUPAC Nos. 18, 20, 28, 31, 44, 52, 101, 105, 118, 138, 149, 153, 170, 180, 194; Ballschmiter and Zell 1980) were determined in each sample. This list contains the seven target congeners (PCBs 28, 52, 101, 118, 138, 153, 180) proposed by the International Council for the Exploration of the Sea (ICES) as indicators of PCB contamination (Valoppi *et al.* 2000).

The samples and blank were analyzed for the following pesticides: Aldrin, Diazinon, Dieldrin, Endosulfan I, Endosulfan II, Endrin, Heptachlor, Heptachlor-epoxide A, Heptachlor-epoxide B, Lindane and, pp'-DDD, pp'-DDE, pp'-DDT.

2.3.2 Sample extraction and quantification

Compounds were extracted and quantified following the procedures described by Dierking *et al.* (2009). About 1g of lyophilized sample was extracted and concentrated to 2 ml. About 100 μ L of this extract was reserved to determine the content of lipid (Lp, %) of each sample (see next paragraph). The remaining fraction was purified with concentrated sulphuric acid, followed by additional purification by liquid chromatography on a silica-alumina column (Murphy 1972; Wells *et al.* 1985). Four fractions were eluted: fraction Ia (Aldrin, pp'-DDT, Heptachlor (50%)), fraction Ib (Heptachlor (50%), pp'-DDT), fraction II (Endosulfan I, Endosulfan II, Lindane, pp'-DDD) and fraction III (Dieldrin, Endrin, Heptachlor-epox A and B).

PCBs and pesticides were analysed by gas chromatography (GC) coupled to mass spectrometry (MS). Calibration was done using fifteen individual standard solutions for PCBs and a standard mixture containing all pesticides was used for pesticides. All organic pollutant concentrations are given on a dry weight basis ($\text{ng}\cdot\text{g}^{-1}$ dw). Detection limits were $0.01 \text{ ng}\cdot\text{g}^{-1}$ for PBC congeners, $0.1 \text{ ng}\cdot\text{g}^{-1}$ for DDT and its metabolites, $0.01 \text{ ng}\cdot\text{g}^{-1}$ for Heptachlor-epoxide A and B, $0.1 \text{ ng}\cdot\text{g}^{-1}$ for Lindane, Diazinon, Heptachlor, Aldrin, Endosulfan II, Endrin and $0.2 \text{ ng}\cdot\text{g}^{-1}$ for Endosulfan I and Dieldrin.

2.4 $\delta^{15}\text{N}$ and lipid quantification

The nitrogen stable-isotope ratio ($\delta^{15}\text{N}$) was used to give an approximate trophic position. Analyses were carried out on dorsal white muscle, as this tissue gives the most reliable values (Pinnegar and Polunin 1999). Then 1 ± 0.1 mg of powdered freeze-dried samples was weighed out and sealed in tin capsules for analyses. $^{15}\text{N}/^{14}\text{N}$ ratios were determined by continuous-flow isotope-ratio mass spectrometry with a Thermo Scientific Delta V Advantage mass spectrometer coupled to a Thermo Scientific Flash EA1112 elemental analyser (Chouvelon *et al.* 2011). Results are expressed as isotope ratios $\delta^{15}\text{N}$ (‰) relative to international standard (atmospheric N_2 for nitrogen), according to the formula:

$$\delta^{15}\text{N} = [(R_{\text{sample}} / R_{\text{standard}}) - 1] * 10^3$$

where $R = ^{15}\text{N}/^{14}\text{N}$ (Peterson and Fry 1987). Replicate measurements of internal laboratory standards (acetanilide) indicated a precision of approximately 0.2‰ for $\delta^{15}\text{N}$ values.

To evaluate the lipid content (Lp, %) in each fish, 100 μL of the evaporated extraction residue was introduced in an initially tarred (tablet) container; then the whole was deposited into a desiccator. After drying to constant mass, the lipid content is determined by gravimetry.

2.5 Statistical analysis

Several indices were used to characterize the POP contamination. Our PCB profiles revealed that PCBs detected in the environment mainly came from capacitors (pyralene: DP3, DP4 or Arochlor1230, 1242). Consequently it was decided to express the global footprint of PCB contamination ($PCBTot$) using the estimation proposed by Annema *et al.* (1995): $PCBTot = \Sigma (28 + 52 + 101 + 138 + 153 + 180) * 5$. $\Sigma ICES$ (sum of concentrations of the 7 ICES congeners), and the proportional contribution of each PCB class (tri- to octa-chlorinated) was also calculated using all of the PCBs analysed. The total DDT concentration, ΣDDT , was calculated as the sum of pp'-DDD, pp'-DDE and pp'-DDT. An indication of the timing of PCB inputs was estimated through the ratio of the two congeners CB153/CB138 (Monod *et al.* 1995; Wafo *et al.* 2005: a high ratio (> 1) indicates continuous inputs or persistence of PCBs in the environment, whereas a low ratio (< 1) reflects a decrease or

sporadicity of PCB inputs or non-persistence. Finally, the ratio of the total concentration of pesticides to total concentration of PCBs analysed ($\Sigma Pest/\Sigma PCB$) was used as an approximate indication of the relative importance of agricultural versus industrial sources of organic pollutants in the environment (de Mora *et al.* 2004); a ratio higher than 1 indicates prevalence of agricultural sources, whereas a ratio less than 1 indicates prevalence of industrial sources.

Data were log transformed to satisfy conditions of normality (Shapiro-Wilk test) and homogeneity of variance (Bartlett test). However for some compounds (Aldrin, Diazinon, Dieldrin, Endrin, pp'-DDD and pp'-DDT), transformation was not sufficient. When conditions were satisfied, parametric tests were used. In other cases non-parametric analogues were applied.

Principal component analysis (PCA) was used to obtain an overview of the contribution of each contaminant according to sites. Trace elements and POP concentrations were analysed in separate PCAs and as no clear patterns were revealed from POP analysis, only trace element results were presented. Spatial variability of contaminant concentrations in each species was also tested using 2-way ANOVA and Tukey's *post hoc* multiple comparisons. For all contaminants, Pearson or Spearman correlations were used to explore the relationship between both contaminant concentration and fish size (TL) and contaminant concentration and trophic level ($\delta^{15}N$). For organic pollutants the relationship between contaminant concentration and the percentage of lipids in muscle (Lp) was also examined. When a correlation existed, ANCOVA, instead of ANOVA was applied, using TL, $\delta^{15}N$ or Lp as a covariate. R project (R version 2.13.2) was the statistical program used for all analyses. The levels of significance for statistical analyses was always set at $\alpha = 0.05$.

3. Results

3.1 Accumulation levels in anguilliform fish

All trace elements measured were detected in anguilliform fish, except Ag and V which were below the detection limit. Mean concentrations and ranges of detected values of contaminants (trace elements and POPs) in anguilliform fish muscle are presented in Table 2. Several elements showed a high inter-individual variability (*i.e.* Cd, Cr, Ni, Pb; coefficient of variation $CV > 100\%$). However it was most often the same individuals that accumulated high

concentrations of several trace elements linked to their origin (e.g. a *G. chilospilus* specimen from the coastal site of Grand Nouméa; 35.7 $\mu\text{g}\cdot\text{g}^{-1}$ dw of Cr and 17.4 $\mu\text{g}\cdot\text{g}^{-1}$ dw of Ni).

Overall, few differences in mean contaminant concentrations were found between species. However, significantly higher concentrations of As, Hg and Se were measured in *Conger* sp., whereas *G. chilospilus* exhibited higher concentrations of Cd and Zn ($p_{\text{ANOVA}} < 0.001$). PCBs had been similarly accumulated by both species, albeit weak interspecific differences were detected for pesticides: *Conger* sp. presented significantly higher concentrations of Heptachlor, Heptachlor-epoxy B ($p_{\text{ANOVA}} < 0.01$), while slightly higher concentrations of Endosulfan I and Aldrin were measured in *G. chilospilus* ($p_{\text{ANOVA}} < 0.05$).

3.2. Spatial patterns in contaminants

3.2.1. Trace elements

A significant spatial variability of several trace elements, in particular those linked with mining activity (Co, Cr, Fe, Mn and Ni), was detected along the coast-barrier reef gradient (Fig. 2 and Table 3). The PCAs in *G. chilospilus* indicated that the coastal sites were characterized by Co, Mn and Ni elements, in contrast to the barrier reef sites which were characterised by As and Cd concentrations (the plot of the first two PCA dimensions explained respectively 51.1% and 59.0% of data variability in *G. chilospilus* and *Conger* sp., Fig. 2). In *Conger* sp., the difference between sites was even stronger, with most trace elements (As, Co, Cr, Fe, Ni, Se, and Zn) characterising coastal sites. These general trends were confirmed by the significantly higher concentrations of mine elements at coastal sites for both species (p_{ANOVA} or $p_{\text{ANCOVA}} < 0.05$, Table 3).

The same spatial pattern (coast vs. barrier reef) was revealed in the two studied areas (GN and GS, Table 3 and S1). The coastal site of Grand Nouméa showed significantly higher concentrations of Ni in *G. chilospilus* ($p_{\text{ANOVA}} = 0.02$) and Cr and Fe in *Conger* sp. ($p_{\text{ANOVA}} < 0.05$, Table 3 and S1), with the same trends followed by several other trace elements (Cr, Fe in *G. chilospilus*, As, Co, Cu, Ni, Se, and Zn in *Conger* sp., results of PCA not shown). However in Grand Lagon Sud the spatial gradient was less pronounced. The coastal site was characterized by higher Co and Mn concentrations in both species (results of PCA not shown), but spatial analyses only suggested significantly lower concentrations of As and Cd in *G. chilospilus* ($p_{\text{ANCOVA}} = 0.001$, Tables 3 and S1).

The coastal site of Grand Nouméa was characterized by significantly higher levels of several trace elements compared to Grand Lagon Sud (*e.g.* As, Cd and Ni in *G. chilospilus*, and Cr, Fe in *Conger sp.*; $p_{\text{ANCOVA}} < 0.05$, Tables 3 and S1).

3.2.2 Organic pollutants

The PCAs did not reveal any clear spatial pattern for POPs in either species (results not shown). Analyses suggested a low spatial variability along the coast-barrier reef gradient (Table 4).

The PCB contamination in *G. chilospilus* did not vary significantly between sites (see $PCBTot$ index; $p_{\text{ANOVA}} > 0.05$), in spite of a slight increase from the coast to the barrier reef. However in *Conger sp.* the intermediate sites were characterized by high concentrations ($p_{\text{ANCOVA}} < 0.05$, Table 4). The $CB153/CB138$ ratio, which was generally below 1, showed few spatial variations and only *G. chilospilus* from coastal sites showed a significantly higher ratio ($p_{\text{ANCOVA}} < 0.05$, Tables 4 and S2). The PCB chlorination classes differed slightly along the coast-barrier reef gradient and between studied areas, with a low variability detected in each case ($< 10\%$, p_{ANCOVA} or $p_{\text{ANOVA}} < 0.05$, Table 4). However the results highlighted a remarkable increase in the proportion of the most toxic chlorinated classes in coastal sites (*i.e.* 8Cl in GN for *G. chilospilus* and 7Cl in GS for *Conger sp.*, $p_{\text{ANOVA}} < 0.05$).

The results for pesticides indicated few spatial variations (Tables 4 and S2). The coastal sites highlighted significantly higher concentrations of some pesticides in *G. chilospilus* (Aldrin and Heptachlor, p_{ANCOVA} or $p_{\text{KW}} < 0.05$), whereas the intermediate reef sites revealed significantly higher rates of several other pesticides in *Conger sp.* (*e.g.* Diazinon, Heptachlor epoxide A and pp'-DDT; p_{ANCOVA} or $p_{\text{KW}} < 0.05$).

Finally, spatial analyses of POP contamination according to sources of pollution (agriculture *vs.* industrial) reinforce previous results (see $\Sigma Pest/\Sigma PCBs$; Table 4). Along the gradient, *G. chilospilus* from the coastal sites were significantly different from other sites, with quite homogeneous sources of contamination (ratio ≥ 1). In comparison industrial sources dominated in other sites (ratio $\cong 0.50$, $p_{\text{ANOVA}} < 0.05$, Table 4). A strong influence of industrial pollution in intermediate sites (ratio $\cong 0.50$, $p_{\text{ANCOVA}} < 0.05$) was also highlighted in *Conger sp.*

3.3 Influence of size, trophic position and lipid content on contamination level

Several trace elements (Co, Cr, Ni, Pb, Se, and Zn in *G. chilospilus*, Cd and Mn in *Conger* sp.) were not correlated with either the size or the trophic position of fish ($p_{\text{Pearson}} > 0.05$), but some trace elements were significantly correlated with fish size (Table 5). Only As and Hg highlighted a positive relationship with the trophic position of fish ($\delta^{15}\text{N}$) in *Conger* sp., whereas no correlation was found for *G. chilospilus*.

Organic pollutant concentrations were linked to size, trophic position or lipid content, depending on the contaminant. Most of them were mainly significantly correlated with the lipid contents in fish (Table 5). For example, PCBs were only correlated with lipids in both species. Pesticides were also correlated with Lp, and to a lesser extent with $\delta^{15}\text{N}$ and TL (Table 5).

4. Discussion

This study confirms, reinforces and extends the high trace element contamination status of anguilliform fish in New Caledonia (Bonnet *et al.* 2014). It also constitutes the first substantial baseline on organic pollutant contamination of fish in New Caledonia. Importantly, our results show that large scale contamination reaches remote parts of the lagoon, more than 30km offshore (SW lagoon), and thus well beyond the urbanized Nouméa region.

4.1. Contaminant levels and spatial variability

4.1.1. Trace element patterns

Anguilliform fish concentrate both significant levels of trace elements associated with mining exploitation (Cr, Fe, Mn, and Ni for both species), and also some elements linked to urban activities (*e.g.* Cd, Cu and Zn, especially in *G. chilospilus*) (Table 2). This elevated typical mining signature detected in sedentary benthic species contrasts with other studies which highlight low contaminant levels in some more mobile fish in both the lagoons of New Caledonia and other tropical regions (Eisler 2010; Bonnet *et al.* 2014; Table 6).

Although coastal sites in New Caledonia are highly contaminated by trace elements, directly or indirectly linked to mining activity (Co, Cr, Fe, Mn and Ni), the contamination has

spread throughout the entire lagoon as far as the remote barrier reef of the extremely vast South-Western lagoon. This is probably due to dispersal by local currents (Fichez *et al.* 2008). However, it should be noted that concentrations decrease significantly from the coast to the barrier reef.

Unsurprisingly, fish from the coastal site in Grand Nouméa present important concentrations in trace elements, especially Cr, Fe and Ni (Table 3). This urbanized and industrialized region is subjected to clear impact from the metallurgic industry (Hédouin *et al.* 2009, 2011; Metian *et al.* 2008, 2013). In contrast, the Grand Lagon Sud is less impacted by trace element inputs. Even if coastal sites in this area seem to be less threatened, trends still reveal important concentrations of Co, Mn and Ni (Table 3). This strong contamination level likely results from severe soil erosion, due to deforestation and mining exploitation in this region over several decades. Given the numerous mines in New Caledonia and the intensive development of mining activities, assessing to what extent other areas of the New Caledonian lagoon have been affected is timely.

We expected a strong impact of urban pollution at least in Grand Nouméa sites, given the intense activity in the coastal industrial area of Ducos. However, while spatial patterns are obvious for trace elements associated with mining activities, those associated with urban pollution (As, Cd, Cu, Pb and Zn) are less clear. Whereas Se and Zn are mostly concentrated in coastal sites for *Conger* sp., Cu and Pb are homogeneously distributed along the coast-barrier reef gradient. Furthermore, the cases of As and Cd are more complex with sometimes higher concentrations (*e.g.* As in *Conger* sp.) and sometimes lower concentrations in coastal sites (*e.g.* As and Cd in *G. chilospilus*). Finally elements issued from urban pollution concern the entire lagoon, maybe due to their wide dispersal by local currents (Fichez *et al.* 2008). Another possible explanation is that in the past some islets were used as rubbish tips for decades (*e.g.* Amédée islet in Nouméa region, Bonnet *et al.* 2014). Further information on these issues is thus needed.

4.1.2. Organic pollutant patterns

Contamination by POPs detected in anguilliform fish was rather low. However, considering the high toxicity of some POPs, even at very low doses, this contamination should be considered as important. Levels of PCB concentrations recorded in New Caledonia are generally lower than in fish from other tropical or temperate regions (*e.g.* *ΣICES* index in

New Caledonia: $14.0 \pm 12.2 \text{ ng.g}^{-1} \text{ dw}$ (*G. chilospilus*) and $16.5 \pm 14.3 \text{ ng.g}^{-1} \text{ dw}$ (*Conger* sp.); in Wallis Central Pacific: $31.3 \pm 9.6 \text{ ng.g}^{-1} \text{ dw}$ (*Cephalopholis argus*) and $49.7 \pm 45.3 \text{ ng.g}^{-1} \text{ dw}$ (*Epinephelus merra*) (Letourneur *et al.*, unpublished data); in the Mediterranean Sea: $100.6 \text{ ng.g}^{-1} \text{ dw}$ (*Solea solea*) (Dierking *et al.* 2009)). Exposure profiles of PCBs showed a contribution of chlorination classes in both species, with the prevalence of moderately chlorinated classes (5Cl and 6Cl) and the scarcity of highly chlorinated classes (7Cl and 8Cl). These results emphasize a recent or even current use of PCBs in New Caledonia, otherwise only the more persistent classes (highly chlorinated) should be detected. Further studies are needed to confirm this hypothesis.

In New Caledonian fish, pesticide concentrations are equivalent or lower than in other regions (*e.g.* Lindane in New Caledonia: $1.3 \pm 0.6 \text{ ng.g}^{-1} \text{ dw}$ (*G. chilospilus*) and 1.7 ± 1.6 (*Conger* sp.); in Wallis Central Pacific: $3.5 \pm 1.03 \text{ ng.g}^{-1} \text{ dw}$ (*C. argus*) and $3.6 \pm 2.07 \text{ ng.g}^{-1} \text{ dw}$ (*E. merra*) (Letourneur *et al.*, unpublished data); in French Polynesia: $73.3 \pm 34.5 \text{ ng.g}^{-1} \text{ dw}$ (*Epinephelus hexagonatus*) (Salvat *et al.* 2012); in the Mediterranean Sea: $35.4 \text{ ng.g}^{-1} \text{ dw}$ (*Solea solea*) (Dierking *et al.* 2009)). Despite the quantities and diversity of pesticides imported into New Caledonia, these low levels detected reflect their relatively moderate use, which probably comes from local and non-professional activities rather than from the agricultural industry. In addition, Nouméa is located far from the most important agricultural areas. This type of non-professional use could also explain a non-negligible detection of pesticides such as DDT and its metabolites (*i.e.* ΣDDT : $1.55 \pm 1.50 \text{ ng.g}^{-1} \text{ dw}$ in *G. chilospilus* and 1.57 ± 1.48 in *Conger* sp.), which are supposed to be prohibited by international regulations and not homologated in New Caledonia (DAVAR, 2001).

Overall, POP concentrations are homogeneous throughout the lagoon, without any clear spatial pattern along the coast-barrier reef gradient, or between studied areas. Nevertheless, some high pesticide concentrations in *G. chilospilus* were observed in several coastal sites and in intermediate reefs, with high PCB and some high pesticide levels in *Conger* sp. (Tables 4 and S2). Storage organs (*i.e.* liver, kidney or digestive gland) usually accumulate higher concentrations than muscle (*e.g.* Bustamante *et al.* 2003; Chouvelon *et al.* 2009; Metian *et al.* 2013; Eisler 2010). Further research is required to test this notion in Anguilliforms and thus to better assess spatial contamination gradients, especially for POPs. The results underlined the complexity in identifying the agricultural and/or industrial origin of organic contamination for each site (see $\Sigma\text{Pest}/\Sigma\text{PCBs}$ ratio, Table 4). Trends seem to emphasize a slight prevalence of agricultural contamination sources at coastal sites, in

comparison with other sites dominated by industrial pollution. Furthermore, the Nouméa area generally seems to be more exposed to industrial contamination, whereas Grand Lagon Sud has revealed strong local agricultural pollution (except at intermediate reefs, Table S2). In Grand Nouméa, anguilliform fish have not been exposed to PCB inputs in recent times in view of their low *CB153/CB138* ratio (*i.e.* ratio < 0.8, ≈ 0.75 in *G. chilospilus* and ≈ 0.78 in *Conger* sp., Table S2). On the other hand, the PCB inputs in Grand Lagon Sud seem to be more recent mainly in coastal sites, as detected in both species (ratio > 1, 1.64 in *G. chilospilus* and 1.04 in *Conger* sp., Table S2). Industrial activities in this area, including the recent construction of a mining factory, could explain in part the continuing inputs of PCBs in this area.

4.2. Influence of biological parameters on contamination level

As mentioned in several studies, concentrations of contaminants can also vary according to ecological and biological factors such as fish size (or age), trophic position and feeding habits or lipid content (Monteiro *et al.* 1991; Geyer *et al.* 2000; Penedo de Pinho *et al.* 2002). These complex relationships can induce interspecific differences in bioaccumulation processes and so emphasize the relevance in detecting the contamination rate at a specific level.

Our results indicated the influence of the three factors tested (body size, trophic position and lipid content) on several contaminants in both species. The link between body size and bioaccumulation is complex, depending on both the contaminants and the fish species considered. In some cases, trace elements accumulated with increasing fish size (*e.g.* As, Cd and Hg in *G. chilospilus*, Table 5), which is consistent with continuous bioaccumulation in muscle during life span (Braune 1987; Burger and Gochfeld 2007; Bloom 1992). In some other cases, trace element concentration is negatively correlated with size (*e.g.* Cu and Fe in both species, or Co, Cr, Ni, Pb, Se and Zn in *Conger* sp.). This trend could be explained by a decrease in assimilation or by more efficient elimination processes with increasing fish size (Braune 1987, Swaileh and Adelung 1995, Warnau *et al.* 1995). Such a relationship can also suggest that an ontogenetic diet shift might occur, implying a variation in the exposure to contaminants through the food pathway, as found in other species (Chouvelon *et al.* 2011, Chouvelon *et al.* 2014). However, with the exception of Hg, and to a lesser extent Se, biomagnification of trace elements in trophic webs is still unclear and difficult to identify (Wang 2002). For anguilliform fish from New Caledonia, the trophic position influenced As

and Hg concentrations only in *Conger* sp. The POP results are in accordance with their hydrophobic nature, which allows their sequestration in fat tissues during the process of organochlorine accumulation (Robertson and Hansen 2001). Most of them were indeed positively linked to the lipid content of fish, in particular in *Conger* sp., where lipid variation ranged between 0.2 and 5.5% (Table 1).

Measures of $\delta^{15}\text{N}$ in fish confirm the high trophic level of anguilliform fish (Brischoux *et al.* 2011). When this is associated with other life history traits (size, age, longevity, philopatry, etc.), the detection of high trace element levels appears consistent. These benthic predators are also probably good candidates for monitoring organic pollution because of the strong relationship of POPs with lipid content (even if the flesh of these fish is not very fatty). The specific biological characteristics of each species may well explain interspecific differences in bioaccumulation of contaminants.

Moreover, contamination of anguilliform fish (high trophic level predators) suggests that trophic networks in general are contaminated (Monniot *et al.* 1994; Hédouin *et al.* 2006, 2007; Metian *et al.* 2008b). As a consequence, it is important to carry out contaminant analysis in prey, top predators (*e.g.* sea snakes, Bonnet 2012) and in the global trophic web. This will provide a more complete assessment of contamination of the environment and lead to better understanding of the mechanisms and processes involved (*e.g.* bioaccumulation, biomagnification, Bryan 1984; Rainbow 1993). A good understanding of both the organisms (*i.e.* biology, ecology and metabolic capacities) and the trophic network structures, plus environmental conditions, is necessary to understand the dynamics of bioaccumulation and biomagnification throughout the trophic webs (Harmelin-Vivien *et al.* 2009).

5. Conclusion

This study strongly confirms a clear accumulation of trace metal contaminants in the muscles of anguilliform fish and the obvious coastal contamination by trace elements linked to mining activities. It is also shown that contamination by POPs occurs in the whole lagoon. These results raise an alert about complex contaminations throughout the lagoon on a large spatial scale. Various contaminants may show different behaviour in abiotic and biotic environments. Due to their lipophilic nature, POPs have different targets compared to trace elements. This characteristic can explain in part their divergences in mobility in comparison to trace elements. Thus, the more widespread repartition of POPs within the lagoon is

probably due to their great capacity of dispersion, through atmospheric transport and current-driven dilution during tropical rain events. This highlights the importance of considering multiple sources of contamination. Moreover, the possibility of a cocktail effect of these various contaminants is not known and could increase the threat. Therefore, in the future, long term monitoring studies and experiments on toxicity are necessary.

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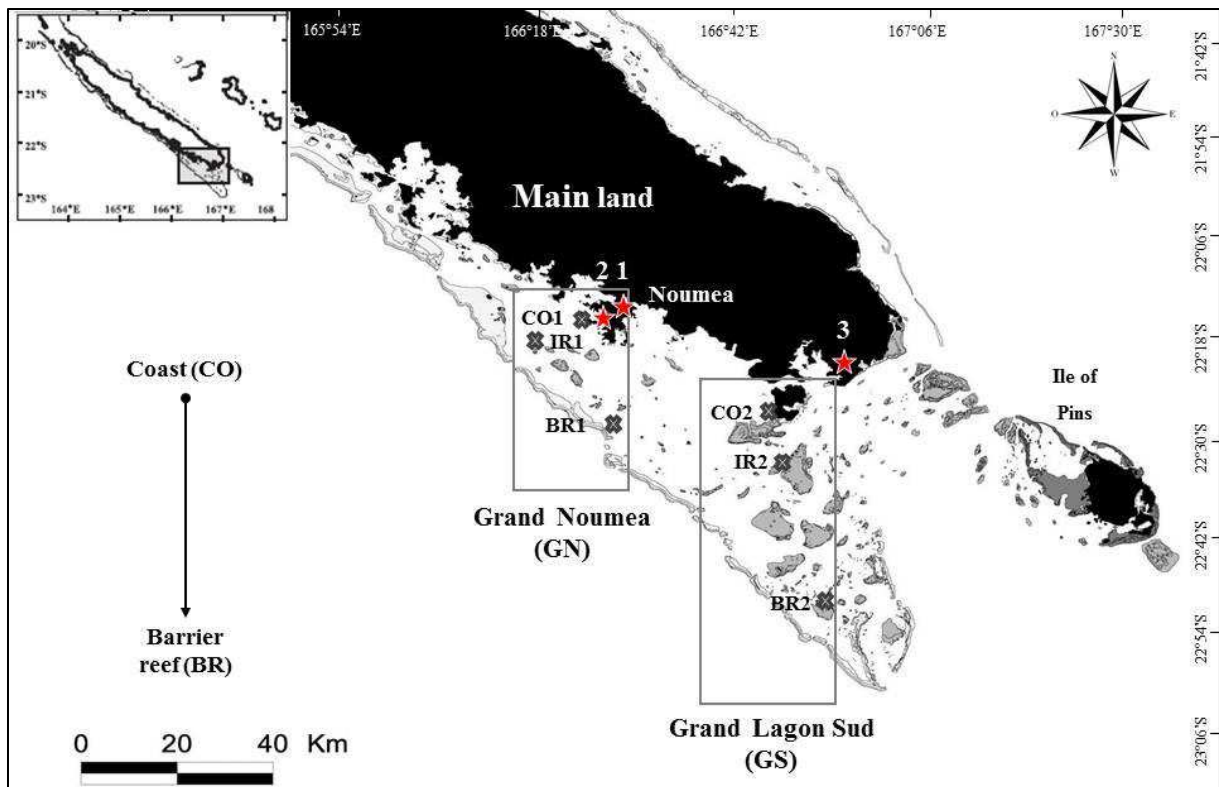


Figure 1. Location of the sampled sites distributed on two coast-barrier reef gradients in New Caledonia: Kuendu (CO1), Signal (IR1) and Amédée (BR1) in Grand Nouméa (GN) and Ouen (CO2), Grand Mato (IR2) and N'Da (BR2) in Grand Lagon Sud. CO = coastal sites; IR = intermediate reef sites; BR = barrier reef sites. Stars indicate areas of human activities, which are the industrial area of Ducos (1), the Nickel factory « SLN » (2) and the Nickel factory « Goro-Nikel » (3). The emerged land is indicated in black, grey areas represent coral reefs; the dark grey line represents the slope of the barrier reef.

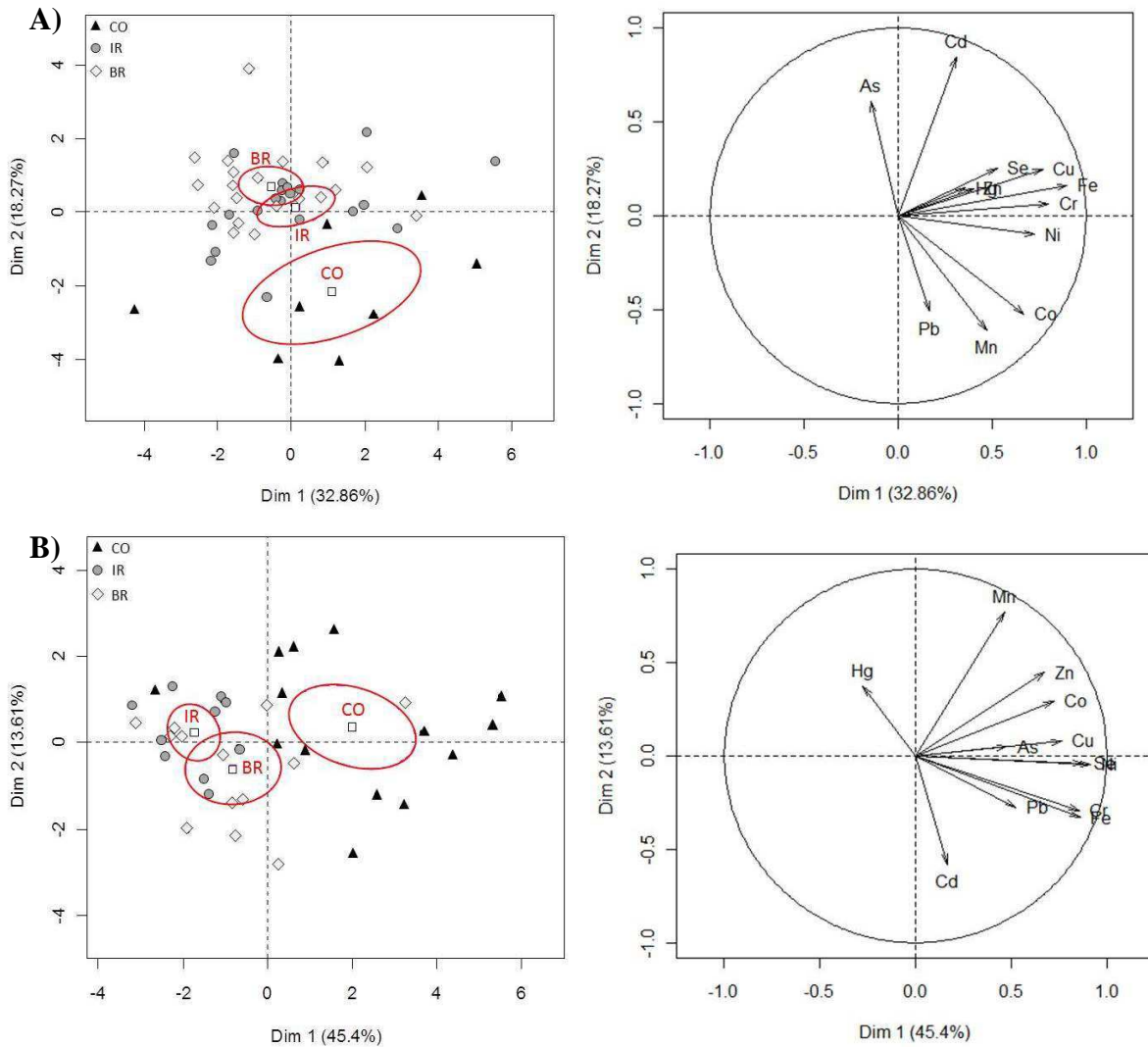


Figure 2. Plot of the principal component analysis (PCA) assessing spatial patterns of anguilliform fish contamination by trace elements on the coast-barrier reef gradient (left panel) and the contribution of the contaminants analyzed to site differentiation (right panel) in *G. chilospilus* (A) and *Conger sp.* (B). CO = coastal sites; IR = intermediate reef sites; BR = barrier reef sites. The most contributing variables in both species were Cr, Cu, Fe, Ni concentrations for the first axis and Cd and Mn concentrations for the second axis. The significant differences between sites are illustrated by confidence ellipses of centroids at 95%.

Table 1. Number (N) of fish analyzed from the coastal (CO), intermediate (IR) and barrier reef (BR) sites in Grand Nouméa (GN) and Grand Lagon Sud (GS). Mean (\pm SD) fish total length (TL, cm), trophic position (express with $\delta^{15}\text{N}$, ‰) and lipid content (Lp, %) are given, with minimal and maximal values (min-max) indicated in brackets. No variation of TL, $\delta^{15}\text{N}$ and Lp between sites was observed ($p_{\text{ANOVA}} > 0.05$).

Species	Site	N	N (GN)	N (GS)	TL (cm) (min-max)	$\delta^{15}\text{N}$ (‰) (min-max)	Lp (%) (min-max)
<i>Gymnothorax chilospilus</i>	CO	8	3	5	21.2 \pm 4.3 (14.7-28.4)	8.0 \pm 0.8 (7.1-9.3)	1.2 \pm 1.3 (0.2-3.6)
	IR	20	10	10	25.3 \pm 4.8 (14.0-37.2)	7.9 \pm 0.7 (6.7-9.5)	0.8 \pm 0.8 (0.2-3.3)
	BR	20	10	10	25.1 \pm 2.3 (21.4-30.8)	7.8 \pm 0.5 (7.1-8.8)	0.7 \pm 0.6 (0.2-1.8)
	Total	48	23	25	24.5 \pm 4.1 (21.7-31.2)	7.9 \pm 0.6 (6.7-9.5)	0.8 \pm 0.8 (0.2-3.6)
<i>Conger</i> sp.	CO	14	9	5	29.0 \pm 4.6 (23.2-38.3)	8.6 \pm 1.4 (6.6-10.5)	1.0 \pm 0.7 (0.2-1.9)
	IR	10	0	10	34.4 \pm 8.9 (20.3-48.7)	8.5 \pm 0.8 (7.7-9.9)	1.5 \pm 1.6 (0.3-5.5)
	BR	13	8	5	35.7 \pm 6.3 (27.7-51.7)	8.5 \pm 0.9 (7.1-10.3)	1.0 \pm 1.2 (0.3-3.6)
	Total	37	17	20	32.7 \pm 7.1 (20.3-51.7)	8.6 \pm 1.0 (6.6-10.5)	1.2 \pm 1.2 (0.2-5.5)

Table 2. Trace element and organic pollutant concentrations (mean \pm SD and range of values; $\mu\text{g}\cdot\text{g}^{-1}$ dw) measured in muscle of *G. chilospilus* and *Conger* sp., all sites confounded.

	<i>G. chilospilus</i>	<i>Conger</i> sp.	
Trace element	As	11 \pm 7.4 (0.9-31.9)	25.7 \pm 25.6 (3.0-117.9)
	Cd	0.41 \pm 0.47 (0.01-2.91)	0.04 \pm 0.04 (0.01-0.16)
	Co	0.11 \pm 0.08 (0.02-0.45)	0.13 \pm 0.15 (0.02-0.68)
	Cr	4.53 \pm 7.15 (0.86-35.71)	4.03 \pm 5.36 (0.45-23.60)
	Cu	1.6 \pm 0.7 (0.7-3.4)	1.9 \pm 0.9 (0.7-4.5)
	Fe	40.2 \pm 36.9 (12.2-196.8)	34.3 \pm 31.1 (7.9-134.4)
	Hg	0.049 \pm 0.028 (0.02-0.13)	0.117 \pm 0.112 (0.03-0.6)
	Mn	2.3 \pm 1.4 (0.6-7.2)	2.7 \pm 2.3 (0.4-10.1)
	Ni	1.95 \pm 3.19 (0.30-17.38)	1.44 \pm 1.77 (0.10-7.74)
	Pb	0.05 \pm 0.04 (0.01-0.2)	0.04 \pm 0.09 (12.2-196.8)
	Se	1.2 \pm 0.4 (0.7-2.6)	1.8 \pm 0.8 (0.9-4.6)
	Zn	56.5 \pm 16.4 (26.5-112.3)	45.4 \pm 23.8 (21.6-112.1)
Organic pollutant	PCBTot	59.1 \pm 52.7 (9.3 - 271.5)	63.2 \pm 54.8 (9.1 - 211.4)
	ΣICES	14.0 \pm 12.2 (2.0 - 64.3)	16.5 \pm 14.3 (2.1 - 60.9)
	Aldrin	0.1 \pm 0.06 (0.08 - 0.3)	0.1 \pm 0.05 (0.04 - 0.2)
	Diazinon	1.8 \pm 1.9 (<0.1 - 10.2)	2.4 \pm 3.3 (<0.1 - 15.3)
	Dieldrin	0.4 \pm 0.3 (<0.2 - 1.8)	0.5 \pm 0.4 (<0.2 - 2.2)
	Endosulfan I	1.3 \pm 1.3 (<0.2 - 6.1)	1.0 \pm 1.7 (0.09 - 9.7)
	Endosulfan II	2.1 \pm 1.7 (<0.1 - 7.3)	2.9 \pm 2.3 (<0.1 - 8.9)
	Endrin	0.3 \pm 0.8 (<0.1 - 4.5)	0.8 \pm 2.0 (0.07 - 9.9)
	Heptachlor	0.4 \pm 0.4 (<0.1 - 1.9)	0.5 \pm 0.4 (<0.1 - 1.9)
	Heptachlor-epox.A	1.5 \pm 1.2 (0.03 - 5.6)	1.8 \pm 1.9 (0.1 - 9.1)
	Heptachlor-epox.B	0.3 \pm 0.5 (<0.01 - 2.6)	0.5 \pm 0.6 (<0.01 - 2.7)
	Lindane	1.3 \pm 0.6 (0.3 - 3.1)	1.7 \pm 1.2 (0.2 - 5.1)
ΣDDT	1.6 \pm 1.5 (0.3 - 6.9)	1.6 \pm 1.5 (0.3 - 7.2)	

Table 3. Trace element concentrations (mean (SD); $\mu\text{g}\cdot\text{g}^{-1}$ dw) in *G. chilospilus* and *Conger* sp. along the coastal-barrier reef gradient. ANOVA (ANV) or ANCOVA (ACV) with Tukey's pairwise comparisons were used to analyze spatial patterns.

	Gradient			Stat test	p	Spatial patterns				
	CO	IR	BR			Gradient	GN	GS	GN vs. GS	
<i>G. chilospilus</i>	As	6.77 (10.27)	7.84 (5.75)	14.33 (5.85)	ACV ($\delta^{15}\text{N}$)	***	BR > CO/IR	BR1 > IR1	IR2/BR2 > CO2	CO1 > CO2
	Cd	0.16 (0.23)	0.44 (0.63)	0.43 (0.31)	ACV (TL)	***	IR/BR > CO		IR2/BR2 > CO2	CO1 > CO2
	Co	0.19 (0.12)	0.10 (0.05)	0.07 (0.05)	ANV	***	CO > IR > BR			
	Cr	6.59 (11.93)	3.57 (5.98)	3.97 (5.81)	ANV	NS				
	Cu	1.62 (0.76)	1.66 (0.78)	1.37 (0.40)	ACV (TL)	NS				
	Fe	46.5 (50.5)	38.2 (39.9)	33.8 (27.4)	ACV (TL)	NS				
	Hg	0.05 (0.02)	0.05 (0.03)	0.04 (0.03)	ACV (TL)	NS				
	Mn	3.77 (2.18)	1.84 (0.91)	1.76 (0.76)	ACV (TL)	***	CO > IR/BR			
	Ni	3.72 (5.65)	1.51 (2.33)	1.34 (2.32)	ANV	*	CO > BR	CO1 > IR1/BR1		CO1 > CO2
	Pb	0.06 (0.05)	0.04 (0.03)	0.04 (0.03)	ANV	NS				
	Se	0.98 (0.27)	1.20 (0.38)	1.07 (0.42)	ANV	NS				
	Zn	42.9 (9.9)	55.8 (17.3)	55.1 (17.1)	ANV	NS				
<i>Conger</i> sp.	As	37.96 (31.44)	20.57 (21.08)	16.45 (16.60)	ACV ($\delta^{15}\text{N}$)	*	CO > BR			
	Cd	0.04 (0.04)	0.03 (0.04)	0.06 (0.05)	ANV	ns				
	Co	0.23 (0.19)	0.10 (0.06)	0.05 (0.03)	ACV (TL)	***	CO/IR > BR			
	Cr	7.88 (7.09)	0.95 (0.31)	2.26 (1.68)	ACV (TL)	**	CO/IR > BR	CO1 > BR1		CO1 > CO2
	Cu	2.37 (1.05)	1.32 (0.43)	1.88 (0.79)	ACV (TL)	ns				
	Fe	57.4 (40.2)	15.3 (4.0)	24.2 (9.7)	ACV (TL)	**	CO > IR/BR	CO1 > BR1		CO1 > CO2
	Hg	0.11 (0.07)	0.07 (0.03)	0.15 (0.17)	ACV ($\delta^{15}\text{N}$)	**	CO/BR > IR			
	Mn	3.7 (2.30)	1.76 (0.95)	2.28 (2.66)	ANV	ns				
	Ni	2.78 (2.25)	0.35 (0.14)	0.83 (0.67)	ACV (TL)	***	CO > IR /BR			
	Pb	0.07 (0.05)	0.03 (0.01)	0.09 (0.14)	ACV (TL)	ns				
	Se	2.31 (1.06)	1.37 (0.21)	1.51 (0.43)	ACV (TL)	**	CO > BR			
	Zn	59.5 (26.2)	33.8 (6.3)	39.0 (23.10)	ACV (TL)	*	CO > IR/BR			

Note: GN: Grand Nouméa; GS: Grand Lagon Sud; CO: coastal sites; IR: intermediate reef sites; BR: barrier reef sites
 ns = not significant ($p > 0.05$); * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Table 4. PCBs and pesticide concentrations (mean (SD); ng.g⁻¹ dw, except % of classes) in *G. chilospilus* (A) and *Conger* sp. (B) along the coast-barrier reef gradient. ANOVA or ANCOVA with Tukey's pairwise comparisons were used to analyze spatial patterns

A)	Gradient			Stat test	p	Spatial patterns			
	CO	IR	BR			Gradient	GN	GS	GN vs. GS
<i>G. chilospilus</i>									
PCBs index (ng.g⁻¹ dw)									
<i>PCBTot</i>	26.96 (9.21)	68.64 (67.30)	60.62 (43.55)	ANV	ns				
<i>ΣICES</i>	6.02 (2.16)	17.00 (16.02)	13.74 (9.04)	ANV	ns				
<i>153/138</i>	1.17 (0.52)	0.76 (0.13)	0.80 (0.10)	ACV (Lp)	***	CO > IR/BR		CO2 > IR2/BR2	CO2 > CO1
PCB chlorination classes (% of CTotal PCBs)									
3 Cl	20.39 (7.67)	16.64 (5.30)	14.12 (3.22)	ANV	*	CO > BR		CO2/IR2 > BR2	CO2 > CO1
4 Cl	20.09 (9.93)	14.84 (7.94)	18.12 (5.31)	ACV (Lp)	ns				
5 Cl	25.65 (4.87)	34.32 (6.53)	30.48 (5.85)	ANV	*	IR > CO			
6 Cl	28.07 (6.89)	29.56 (8.73)	32.46 (7.44)	ANV	ns				
7 Cl	3.55 (1.15)	3.82 (1.99)	4.33 (2.72)	ACV (Lp)	ns				
8 Cl	2.25 (3.12)	0.82 (1.17)	0.50 (0.75)	ANV	**		CO1 > BR1		GN > GS
Pesticides (ng.g⁻¹ dw)									
Ald.	0.16 (0.11)	0.11 (0.03)	0.06 (0.04)	KW	**	CO > BR			
Dia.	3.91 (3.41)	1.47 (1.68)	1.35 (0.92)	KW	ns				
Diel.	0.41 (0.26)	0.36 (0.24)	0.37 (0.39)	KW	ns				
Endo.I	1.09 (1.06)	1.27 (1.04)	1.36 (1.66)	ACV (δ ¹⁵ N)	ns				
Endo.II	1.53 (0.86)	2.38 (2.16)	2.00 (1.54)	ANV	ns				
Endr.	0.16 (0.09)	0.28 (0.62)	0.34 (0.97)	KW	ns				
Hept.	0.83 (0.51)	0.43 (0.48)	0.20 (0.17)	ACV (Lp)	***	CO > IR/BR			
Hept.A	0.66 (0.22)	1.69 (1.49)	1.58 (1.09)	ANV	ns				
Hept.B	0.30 (0.23)	0.23 (0.33)	0.27 (0.60)	ANV	*			CO2 > IR2/BR2	IR1 > IR2
Lind.	1.32 (0.47)	1.50 (0.85)	1.22 (0.46)	ANV	ns				
DDE	0.23 (0.23)	0.34 (0.36)	0.50 (0.77)	ANV	ns				
DDD	0.29 (0.17)	0.24 (0.21)	0.41 (0.52)	KW	ns				
DDT	1.88 (1.58)	0.86 (1.19)	0.47 (0.63)	KW	ns				
<i>ΣDDT</i>	2.40 (1.70)	1.44 (1.58)	1.38 (1.36)	ANV	ns				
<i>ΣPest/ ΣPCB</i>	1.13 (0.30)	0.5 (0.12)	0.51 (0.12)	ANV	***	CO > IR/BR			

B)	Gradient			Stat test	p	Spatial patterns			
	CO	IR	BR			Gradient	GN	GS	GN vs. GS
PCBs (ng.g⁻¹ dw)									
<i>PCBTot</i>	67.66 (54.60)	99.47 (67.68)	31.91 (16.39)	ACV (Lp)	**	IR > BR			
<i>ΣICES</i>	17.17 (12.78)	26.32 (18.94)	8.58 (4.55)	ACV (Lp)	**	IR > BR			
<i>153/138</i>	0.82 (0.19)	0.88 (0.36)	1.0 (0.47)	ACV (Lp)	ns				
PCB chlorination classes (% of CTotal PCBs)									
3 Cl	15.75 (7.20)	19.94 (6.08)	16.09 (7.62)	ACV (Lp)	ns				
4 Cl	17.71 (6.99)	11.07 (4.59)	21.74 (8.23)	ACV (Lp)	*	IR < BR			
5 Cl	32.02 (8.23)	32.74 (7.86)	34.84 (5.25)	ACV (Lp)	ns				
6 Cl	26.5 (5.15)	32.37 (7.73)	22.28 (4.51)	ACV (Lp)	***	IR > CO > BR			
7 Cl	6.50 (5.97)	3.62 (1.38)	3.70 (2.12)	ANV	*			CO2 > BR2	
8 Cl	1.53 (1.38)	0.26 (0.24)	1.36 (1.03)	ACV (Lp)	ns				
Pesticides (ng.g⁻¹ dw)									
Conger sp.	Ald.	0.13 (0.06)	0.12 (0.04)	0.10 (0.03)	KW	ns			
	Dia.	2.02 (4.74)	3.99 (2.31)	1.54 (1.74)	KW	***	IR > CO		CO2 > CO1
	Diel.	0.77 (0.64)	0.25 (0.09)	0.36 (0.17)	KW	*	CO > IR		
	Endo.I	1.06 (0.89)	0.61 (0.73)	1.30 (2.69)	ACV (Lp)	ns			
	Endo.II	3.78 (2.46)	2.61 (2.60)	2.26 (1.83)	ACV (Lp)	ns			
	Endr.	0.24 (0.31)	1.69 (3.19)	0.56 (1.55)	KW	ns			
	Hept.	0.59 (0.40)	0.51 (0.55)	0.52 (0.31)	ACV (TL)	ns			
	Hept.A	2.37 (2.58)	2.47 (1.77)	0.88 (0.56)	ACV (Lp)	*	IR > BR		
	Hept.B	0.79 (0.80)	0.30 (0.48)	0.33 (0.19)	ACV (Lp)	ns			
	Lind.	2.56 (1.51)	1.25 (0.93)	1.23 (0.62)	ACV (Lp)	ns			
	DDE	0.44 (0.61)	0.37 (0.21)	0.36 (0.29)	ANV	ns			
	DDD	0.29 (0.30)	0.65 (0.22)	0.15 (0.09)	KW	***	IR > CO/BR		IR2 > BR2
	DDT	0.82 (1.07)	0.40 (0.61)	1.19 (1.90)	KW	ns			
	<i>ΣDDT</i>	1.56 (1.19)	1.42 (0.71)	1.70 (2.12)	ANV	ns			
	<i>ΣPest/ ΣPCB</i>	0.92 (1.29)	0.51 (0.21)	0.94 (1.02)	ACV (Lp)	**	CO/BR > IR		

Note: GN: Grand Nouméa; GS: Grand Lagon Sud; CO: coastal sites; IR: intermediate reef sites; BR: barrier reef sites
ns = not significant (p > 0.05); * p < 0.05, ** p < 0.01, *** p < 0.001 / (+) positive and (-) negative correlation. ^a only one value.

Table 5. Summary of correlations performed on trace elements and organic pollutants (PCBs and pesticides) concentrations *versus* total length (TL), trophic position ($\delta^{15}\text{N}$) and lipid content (Lp) in *G. chilospilus* and *Conger* sp.

<u><i>G. chilospilus</i></u>			
	<i>Trace elements</i>	<i>PCBs</i>	<i>Pesticides</i>
TL	<u>As*</u> , <u>Cd**</u> , Cu*, Fe*, <u>Hg*</u> , Mn*		Ald.**, Dia.**
$\delta^{15}\text{N}$			<u>Endo.*</u>
Lp		<u>CB153/138**</u> , <u>4 Cl**</u> , <u>8 Cl*</u>	<u>Hept.**</u>

<u><i>Conger</i> sp.</u>			
	<i>Trace elements</i>	<i>PCBs</i>	<i>Pesticides</i>
TL	Co**, Cr*, Cu**, Fe*, Ni*, Pb*, Se*, Zn*		Hept.**
$\delta^{15}\text{N}$	<u>As***</u> , <u>Hg***</u>		Ald.*
Lp		<u>CTotal PCBs**</u> , <u>SICES*</u> , <u>CB153/138*</u> , <u>3 Cl*</u> , <u>4 Cl***</u> , 5 Cl**, 6 Cl*, <u>8 Cl**</u>	<u>Dia.*</u> , <u>Diel.*</u> , <u>Endo.I**</u> , <u>Endo.II**</u> , <u>Endr.**</u> , <u>Hept.A*</u> , Hep.B*, <u>Lind.**</u> , DDT**

Note: 3 Cl to 8 Cl : classes of chlorination; Lind.: Lindane; Dia.: Diazinon; Hept.: Heptachlor; Ald.: Aldrin; Hept.B: Heptachlor-epox.B; Hept.A: Heptachlor-epox.A; Endo.I: Endosulfan I; Diel: Dieldrin; Endr.; Endrin; DDT : pp'-DDT.
Significativity: * p < 0.05, ** p < 0.01, *** p < 0.001.
Positive correlations are underlined.

Table 6. Comparison of several trace element concentrations (mean \pm SD and range of values; $\mu\text{g}\cdot\text{g}^{-1}$ dw) measured in muscle of *G. chilospilus*, *Conger* sp. and other lagoon fish species in New Caledonia.

Trace element	Concentration ($\mu\text{g}\cdot\text{g}^{-1}$ dw)	species	reference
As	11.0 \pm 7.4 (0.9-31.9)	<i>G. chilospilus</i>	This study
As	25.7 \pm 25.6 (3.0-117.9)	<i>Conger</i> sp.	This study
As	13.7 \pm 7.57 (<7.87-19.1)	<i>Lethrinus laticaudis</i>	Metian <i>et al.</i> (2013)
As	9.47 \pm 1.47 (<7.85-10.7)	<i>Priacanthus hamrur</i>	Metian <i>et al.</i> (2013)
As	< 6.01-7.77	<i>Cymbacephalus beauforti</i>	Metian <i>et al.</i> (2013)
As	1.2 - 52.5	mix	Fernandez and Breau (2011)
Cd	0.41 \pm 0.47 (0.01-2.91)	<i>G. chilospilus</i>	This study
Cd	0.04 \pm 0.04 (0.01-0.16)	<i>Conger</i> sp.	This study
Cd	<0.06	mix	Fernandez and Breau (2011)
Co	0.11 \pm 0.08 (0.02-0.45)	<i>G. chilospilus</i>	This study
Co	0.13 \pm 0.15 (0.02-0.68)	<i>Conger</i> sp.	This study
Co	< 0.03	<i>Lethrinus laticaudis</i>	Metian <i>et al.</i> (2013)
Co	< 0.03	<i>Priacanthus hamrur</i>	Metian <i>et al.</i> (2013)
Co	< 0.02	<i>Cymbacephalus beauforti</i>	Metian <i>et al.</i> (2013)
Co	<0.1 - 1.1	mix	Fernandez and Breau (2011)
Cr	4.53 \pm 7.15 (0.86-35.71)	<i>G. chilospilus</i>	This study
Cr	4.03 \pm 5.36 (0.45-23.60)	<i>Conger</i> sp.	This study
Cr	< 0.79	<i>Lethrinus laticaudis</i>	Metian <i>et al.</i> (2013)
Cr	1.39 (<0.70-1.39)	<i>Priacanthus hamrur</i>	Metian <i>et al.</i> (2013)
Cr	< 0.60	<i>Cymbacephalus beauforti</i>	Metian <i>et al.</i> (2013)
Cr	<0.1 - 5.7	mix	Fernandez and Breau (2011)
Cu	1.6 \pm 0.7 (0.7-3.4)	<i>G. chilospilus</i>	This study
Cu	1.9 \pm 0.9 (0.7-4.5)	<i>Conger</i> sp.	This study
Cu	< 0.79	<i>Lethrinus laticaudis</i>	Metian <i>et al.</i> (<i>in press</i>)
Cu	< 0.83	<i>Priacanthus hamrur</i>	Metian <i>et al.</i> (<i>in press</i>)
Cu	< 0.53-0.64	<i>Cymbacephalus beauforti</i>	Metian <i>et al.</i> (<i>in press</i>)
Cu	0.25 - 3.0	mix	Fernandez and Breau (2011)
Fe	40.2 \pm 36.9 (12.2-196.8)	<i>G. chilospilus</i>	This study
Fe	34.3 \pm 31.1 (7.9-134.4)	<i>Conger</i> sp.	This study
Fe	9.19 \pm 1.42 (7.56-10.1)	<i>Lethrinus laticaudis</i>	Metian <i>et al.</i> (2013)
Fe	10.4 \pm 3.56 (7.82-16.9)	<i>Priacanthus hamrur</i>	Metian <i>et al.</i> (2013)
Fe	7.01-7.60	<i>Cymbacephalus beauforti</i>	Metian <i>et al.</i> (2013)
Fe	4.0 - 211	mix	Fernandez and Breau (2011)
Mn	2.3 \pm 1.4 (0.6-7.2)	<i>G. chilospilus</i>	This study
Mn	2.7 \pm 2.3 (0.4-10.1)	<i>Conger</i> sp.	This study
Mn	< 0.79	<i>Lethrinus laticaudis</i>	Metian <i>et al.</i> (2013)
Mn	< 0.83	<i>Priacanthus hamrur</i>	Metian <i>et al.</i> (2013)
Mn	< 0.60	<i>Cymbacephalus beauforti</i>	Metian <i>et al.</i> (2013)
Mn	<0.03 - 3.3	mix	Fernandez and Breau (2011)
Ni	1.95 \pm 3.19 (0.30-17.38)	<i>G. chilospilus</i>	This study
Ni	1.44 \pm 1.77 (0.10-7.74)	<i>Conger</i> sp.	This study
Ni	<0.06 - 1.35	mix	Fernandez and Breau (2011)

Note: mix = 144 fish from 27 species and 10 families (Acanthuridae, Carangidae, Haemulidae, Labridae, Lethrinidae, Lutjanidae, Mugilidae, Scaridae, Serranidae and Siganidae).

Table S1. Trace element concentrations (mean (SD); $\mu\text{g}\cdot\text{g}^{-1}$ dw) in *G. chilospilus* and *Conger* sp. along the gradient in Grand Nouméa and Grand Lagon Sud.

	Grand Nouméa (GN)			Grand Lagon Sud (GS)		
	CO1	IR1	BR1	CO2	IR2	BR2
<i>G. chilospilus</i>						
As	16.27 (13.65)	8.57 (7.52)	16.75 (4.87)	2.42 (1.26)	7.90 (3.62)	13.34 (6.49)
Cd	0.36 (0.31)	0.36 (0.30)	0.36 (0.18)	0.06 (0.07)	0.56 (0.85)	0.54 (0.34)
Co	0.21 (0.10)	0.10 (0.05)	0.09 (0.06)	0.22 (0.14)	0.11 (0.06)	0.05 (0.03)
Cr	16.83 (16.87)	3.21 (2.04)	4.28 (3.30)	1.76 (0.74)	4.28 (8.41)	4.05 (7.77)
Cu	2.35 (0.85)	1.75 (0.89)	1.38 (0.30)	1.51 (0.57)	1.74 (0.69)	1.49 (0.50)
Fe	95.2 (64.4)	33.8 (19.0)	35.4 (15.7)	26.5 (13.0)	46.4 (54.0)	35.5 (36.6)
Hg	0.07 (0.03)	0.05 (0.02)	0.04 (0.02)	0.05 (0.04)	0.07 (0.04)	0.04 (0.02)
Mn	2.92 (0.89)	2.13 (1.18)	1.70 (0.43)	5.04 (2.41)	1.72 (0.51)	2.00 (0.98)
Ni	9.21 (7.09)	1.33 (0.79)	1.09 (0.81)	1.17 (0.76)	1.84 (3.27)	1.73 (3.23)
Pb	0.07 (0.03)	0.06 (0.07)	0.05 (0.04)	0.06 (0.03)	0.03 (0.03)	0.04 (0.03)
Se	1.17 (0.28)	1.21 (0.47)	1.00 (0.19)	1.07 (0.28)	1.31 (0.28)	1.25 (0.54)
Zn	50.2 (6.5)	59.9 (23.3)	52.7 (11.4)	47.0 (12.1)	57.3 (9.3)	63.0 (20.7)
	Grand Nouméa (GN)		Grand Lagon Sud (GS)			
	CO1	BR1	CO2	IR2	BR2	
<i>Conger</i> sp.						
As	42.51 (33.77)	20.68 (18.76)	29.77 (28.29)	20.57 (21.08)	9.67 (10.84)	
Cd	0.05 (0.04)	0.04 (0.04)	0.02 (0.01)	0.03 (0.04)	0.08 (0.07)	
Co	0.23 (0.20)	0.07 (0.04)	0.23 (0.21)	0.10 (0.06)	0.03 (0.01)	
Cr	11.69 (6.00)	3.13 (1.61)	1.02 (0.45)	0.95 (0.31)	0.86 (0.14)	
Cu	2.59 (1.21)	2.20 (0.81)	1.98 (0.61)	1.32 (0.43)	1.42 (0.55)	
Fe	78.7 (34.2)	25.7 (8.2)	19.1 (7.60)	15.3 (4.0)	21.6 (12.3)	
Hg	0.08 (0.04)	0.07 (0.03)	0.18 (0.09)	0.07 (0.03)	0.28 (0.22)	
Mn	3.06 (1.73)	2.61 (3.34)	4.86 (2.93)	1.76 (0.95)	1.74 (1.06)	
Ni	3.90 (2.05)	1.15 (0.67)	0.77 (0.25)	0.35 (0.14)	0.32 (0.20)	
Pb	0.08 (0.06)	0.12 (0.17)	0.06 (0.02)	0.03 (0.01)	0.03 (0.01)	
Se	2.77 (1.06)	1.65 (0.43)	1.50 (0.28)	1.37 (0.21)	1.28 (0.34)	
Zn	58.7 (27.6)	40.8 (29.6)	60.9 (26.5)	33.8 (6.3)	36.2 (6.9)	

Note: CO: coastal sites; IR: intermediate reef sites; BR: barrier reef sites.

Table S2. Trace element concentrations (mean (SD); $\mu\text{g}\cdot\text{g}^{-1}$ dw) in *G. chilospilus* (A) and *Conger* sp. (B) along the gradient in Grand Nouméa and Grand Lagon Sud.

A)	Grand Nouméa (GN)			Grand Lagon Sud (GS)			
	CO1	IR1	BR1	CO2	IR2	BR2	
PCBs index ($\text{ng}\cdot\text{g}^{-1}$ dw)							
<i>PCBTot</i>	25.79 (8.98)	70.14 (78.71)	59.07 (56.06)	28.13 (11.28)	66.95 (57.15)	62.18 (29.27)	
ΣCES	5.80 (1.67)	17.59 (18.5)	13.65 (11.80)	6.25 (2.90)	16.33 (13.90)	13.83 (5.80)	
<i>153/138</i>	0.71 (0.10)	0.75 (0.15)	0.78 (0.08)	1.62 (0.20)	0.77 (0.13)	0.82 (0.11)	
PCB chlorination classes (% of <i>CTotal</i> PCBs)							
3 Cl	14.34 (4.50)	14.09 (4.40)	14.51 (4.11)	26.44 (4.10)	19.51 (4.90)	13.74 (2.16)	
4 Cl	24.48 (12.55)	16.06 (10.80)	18.52 (7.00)	15.71 (5.60)	13.47 (2.70)	17.71 (3.19)	
5 Cl	24.42 (5.30)	33.18 (7.50)	30.82 (4.90)	26.87 (5.20)	35.60 (5.40)	30.14 (6.92)	
6 Cl	28.72 (10.30)	30.73 (11.20)	30.32 (7.60)	27.42 (3.40)	28.24 (5.20)	34.59 (7.04)	
7 Cl	3.64 (1.58)	4.67 (2.30)	5.02 (3.50)	3.46 (0.89)	2.87 (0.88)	3.64 (1.44)	
8 Cl	4.40 (3.25)	1.27 (1.50)	0.81 (0.98)	0.11 (0.05)	0.31 (0.36)	0.18 (0.14)	
<i>G. chilospilus</i>	Pesticides ($\text{ng}\cdot\text{g}^{-1}$ dw)						
	Ald.	0.11 (0.03)	0.11 (0.03)	0.07 (0.04)	0.21 (0.15)	0.12 (0.04)	0.06 (0.04)
	Dia.	5.18 (4.63)	1.72 (2.30)	1.08 (1.00)	2.64 (1.70)	1.20 (0.67)	1.63 (0.78)
	Diel.	0.30 (0.17)	0.43 (0.28)	0.44 (0.50)	0.51 (0.33)	0.28 (0.16)	0.29 (0.23)
	Endo.I	0.83 (0.58)	0.95 (0.87)	1.17 (1.80)	1.35 (1.50)	1.64 (1.10)	1.56 (1.60)
	Endo.II	1.30 (0.09)	2.73 (2.10)	1.87 (1.60)	1.77 (1.30)	1.99 (2.30)	2.12 (1.60)
	Endr.	0.18 (0.13)	0.43 (0.85)	0.13 (0.06)	0.13 (0.05)	0.10 (0.01)	0.55 (1.40)
	Hept.	0.47 (0.17)	0.59 (0.57)	0.23 (0.17)	1.18 (0.49)	0.25 (0.28)	0.18 (0.18)
	Hept.A	0.59 (0.15)	1.66 (1.40)	1.46 (1.20)	0.73 (0.29)	1.73 (1.70)	1.71 (1.00)
	Hept.B	0.20 (0.16)	0.40 (0.39)	0.41 (0.78)	0.40 (0.27)	0.04 (0.03)	0.13 (0.32)
	Lind.	1.49 (0.51)	1.72 (0.91)	1.28 (0.58)	1.15 (0.46)	1.26 (0.77)	1.16 (0.32)
	DDE	0.35 (0.29)	0.45 (0.45)	0.68 (1.00)	0.11 (0.01)	0.22 (0.20)	0.33 (0.35)
	DDD	0.29 (0.18)	0.28 (0.29)	0.50 (0.72)	0.29 (0.21)	0.19 (0.05)	0.31 (0.19)
	DDT	3.10 (1.21)	0.94 (1.50)	0.52 (0.74)	0.65 (0.50)	0.77 (0.73)	0.42 (0.54)
	ΣDDT	3.74 (1.31)	1.67 (2.00)	1.71 (1.80)	1.05 (0.31)	1.18 (0.90)	1.06 (0.55)
	$\Sigma\text{Pest/}$						
	ΣPCB	1.27 (0.28)	0.53 (0.12)	0.52 (0.14)	0.99 (0.31)	0.47 (0.11)	0.51 (0.10)

B)	Grand Nouméa (GN)			Grand Lagon Sud (GS)			
	CO1	IR1	BR1	CO2	IR2	BR2	
<i>PCBs (ng.g⁻¹ dw)</i>							
<i>PCBTot</i>	75.59 (56.91)		40.16 (13.36)	31.95 (27.93)	99.47 (67.68)	15.43 (4.98)	
<i>SICES</i>	19.12 (13.14)		10.93 (3.53)	8.38 (7.86)	26.32 (18.94)	3.89 (1.70)	
<i>153/138</i>	0.77 (0.44)		0.78 (0.15)	1.04 (0.47)	0.88 (0.36)	1.43 (0.61)	
<i>PCB chlorination classes (% of CTotal PCBs)</i>							
3 Cl	15.28 (7.70)		11.24 (2.15)	17.86 (5.84)	19.94 (6.08)	25.78 (3.73)	
4 Cl	17.37 (3.83)		22.43 (4.85)	19.26 (19.14)	11.07 (4.59)	20.35 (13.76)	
5 Cl	33.38 (7.02)		36.39 (4.06)	25.86 (13.80)	32.74 (7.86)	31.72 (6.57)	
6 Cl	27.21 (2.42)		24.05 (2.66)	23.33 (13.93)	32.37 (7.73)	18.73 (5.74)	
7 Cl	5.04 (2.92)		4.24 (2.29)	13.04 (13.56)	3.62 (1.38)	2.63 (1.42)	
8 Cl	1.72 (1.43)		1.66 (1.07)	0.66 (0.87)	0.26 (0.24)	0.78 (0.74)	
<i>Conger sp.</i>	<i>Pesticides (ng.g⁻¹ dw)</i>						
	Ald.	0.13 (0.07)		0.09 (0.02)	0.10 ^a	0.12 (0.04)	0.12 (0.04)
	Dia.	0.10 (0.01)		0.69 (1.15)	10.64 (6.58)	3.99 (2.31)	3.26 (1.45)
	Diel.	0.88 (0.66)		0.41 (0.18)	0.29 (0.13)	0.25 (0.09)	0.24 (0.04)
	Endo.I	1.20 (0.93)		0.57 (0.59)	0.44 (0.33)	0.61 (0.73)	2.77 (4.64)
	Endo.II	4.24 (2.49)		2.18 (0.80)	1.71 (0.48)	2.61 (2.60)	2.44 (3.26)
	Endr.	0.10 (0.003)		0.10 (0.01)	0.86 (0.16)	1.69 (3.19)	1.47 (2.67)
	Hept.	0.59 (0.41)		0.47 (0.23)	0.55 (0.49)	0.51 (0.55)	0.60 (0.46)
	Hept.A	2.74 (2.72)		1.14 (0.49)	0.69 (0.66)	2.47 (1.77)	0.35 (0.21)
	Hept.B	0.89 (0.84)		0.43 (0.14)	0.30 (0.41)	0.30 (0.48)	0.12 (0.06)
	Lind.	2.82 (1.44)		1.53 (0.50)	1.37 (1.61)	1.25 (0.93)	0.62 (0.27)
	DDE	0.52 (0.65)		0.43 (0.31)	0.12 (0.02)	0.37 (0.21)	0.23 (0.24)
	DDD	0.33 (0.32)		0.17 (0.11)	0.10	0.65 (0.22)	0.10
	DDT	0.62 (0.71)		1.58 (2.27)	1.73 (2.30)	0.40 (0.61)	0.41 (0.27)
	Σ DDT	1.47 (1.03)		2.18 (2.49)	1.94 (2.32)	1.42 (0.71)	0.74 (0.39)
	<i>SPest/</i>						
	<i>SPCB</i>	0.50 (0.09)		0.52 (0.17)	2.82 (2.79)	0.51 (0.21)	1.78 (1.54)

Note: CO: coastal sites; IR: intermediate reef sites; BR: barrier reef sites. ^a only one value.