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Hg concentrations and related risk assessment in coral reef crustaceans, molluscs and fish from New Caledonia

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Abstract

There is a dramatic lack of data on Hg levels in marine organisms from tropical areas, and in particular from New Caledonia. For the first time, this study reports the total Hg concentrations in the tissues of several marine taxa from the New Caledonian lagoon. Seafood from both wild and farmed populations was considered. Hg concentrations varied over 3 orders of magnitudes according to factors including species, age (size/weight), trophic level, lifestyle and geographical origin. Taking into account the edible tissues, estimations of the amount of flesh that should be consumed by a 60-kg person to reach the Hg Provisional Tolerable Weekly Intake (PTWI) reveal acceptable risk for Human health in general. However, a risk was clearly identified in one site of the lagoon (i.e. Grande Rade) where high Hg concentrations were measured. These concentrations were higher than values reported in the current literature.

Capsule: This work reports the first assessment of Hg levels in edible organisms from the New Caledonian lagoon and the associated risk linked to their consumption by Human.

Keywords: Tropical environment; Metal; Marine products; Seafood; Risk assessment; PTWI

1. Introduction

Among heavy metals, Hg is of particular concern in marine environmental studies because it has both natural and anthropogenic sources, has no known biological function and is toxic to all living organisms (for a review, see Eisler, 1987). In the marine environment, Hg is naturally released in the water and then in the atmosphere through erosion of the sediments where it is present as sulphide salts (Salomon et al., 1987). However, about 30% of the total Hg emissions in the atmosphere are of anthropogenic origins. Moreover, these anthropogenic atmospheric emissions are ten times greater than direct inputs to water, contributing to a widespread contamination which is difficult to assess (Hylander, 2001). These emissions are mainly originating from industrial and domestic incineration (Cossa et al., 1990).

In the marine environment, Hg is mainly found under organic forms. Among organic Hg species, methyl-Hg is the most stable form in the marine environment; it is primarily produced by microorganisms in sediments. Methyl-Hg is also the most toxic form to organisms (WHO, 1990). Finally, Hg and methyl-Hg have the particularity to be bioaccumulated by marine organisms and to biomagnify along the food chain (Zizek et al., 2007). Therefore, the consumption of marine products represents a non negligible exposure pathway to Hg and, thereby, a risk for Human health (e.g. Buzina et al., 1989; Svensson et al., 1992). This is particularly the case for fish, since virtually 100% of the total Hg in fish muscles (edible tissues) are present as methyl-Hg (e.g. Bloom, 1992, Holsbeck et al. 1997), and is thus highly bioavailable for Human consumers and other predators.

In New Caledonia, coastal waters are subjected to large inputs of metals, mainly due to intense mining activities (especially Ni production), but also due to natural erosion of the soils associated with tropical rainfall, urban development and lack of efficient wastewater treatment (Ambastian et al., 1997). Surprisingly, few studies have been conducted to assess the marine contamination status of New Caledonia by metals (Labrosse et al., 2000).

Available studies are generally limited to Ni and its mining by-products and to a narrow range of species (Monniot et al., 1994; Bustamante et al., 2000; Hédouin et al., 2007, 2008; Metian et al., 2008a). To the best of our knowledge, data published on Hg in marine organisms from New Caledonia are limited to a single study on pelagic marine mammals (Bustamante et al., 2003), and so far none is available on non mammalian organisms from the lagoon.

The aim of the present study was therefore to provide baseline information on the Hg contamination status of the New Caledonia coastal marine environment. A wide range of species including Bivalves, Cephalopods and Teleosts from different locations were analysed for their Hg concentrations. Special emphasis was given to species that have already been recognised as valuable bioindicator species (i.e. the tumid venus *Gafrarium tumidum* and the radula scallop *Comptopallium radula*; Hédouin et al., 2007, 2008; Metian et al., 2008a), and to those that are major seafood for local consumption or exportation. The investigated organisms were collected either from the wild (i.e. scallops, clams, cockles, cephalopods and fish) or from mariculture farms (e.g. the Pacific blue shrimp *Litopenaeus stylirostris* and the Pacific cupped oyster *Crassostrea gigas*). Differences in Hg bioaccumulation among body compartments of a given species, within and among taxonomic groups, and among sites were tested. Size and weight were also taken into account as driving parameters. Finally, this paper provides a site-specific preliminary risk assessment for Hg consumption along with seafood.

2. Materials and methods

2.1. Sampling and sample preparation

Organisms belonging to 32 taxa (Table 1) were collected in March and October 2007 along the coast of New Caledonia (Fig. 1, Table 2), either by SCUBA diving (fish), by hand picking at low tide (bivalves), or bought on the central market of Noumea City (shrimp,

oysters, cephalopods and some fish). The sampling sites were selected because they were reported to have contrasting contamination status (Hédouin et al., 2008; Metian et al., 2008a; Warnau and Bustamante unpublished results): Grande Rade, Grande Rade-SLN, Koutio Bay, the edge of Noumea harbour, Sainte Marie Bay, Ouano Bay (which included 3 sub-sampling sites from the shore -S1- to open waters -S3), Maa Bay, Unia and Tomo (Fig. 1, Table 2). The geographical origin of the organisms bought on the Noumea central market was obtained from the selling fishermen: Bassin de La Foa, Dumbea Bay, southern and northern lagoon (Fig. 1, Table 2).

Grande Rade is subjected to anthropogenic inputs from the Ducos industrial zone, whereas Grande Rade-SLN is directly subjected to those of the metallurgic factory “Société Le Nickel” (SLN). Due to these anthropogenic inputs, Grande Rade sediments were shown to display high concentrations in several elements (Hédouin et al., 2008). Koutio Bay is characterised by the presence of an important rubbish dump and is influenced by inputs of domestic wastes from Noumea City. Sainte Marie Bay is located to the East of Noumea City and receives important sewage sludge and terrigenous inputs coming from the Coulée River. Dumbea Bay is also subjected to terrigenous inputs from the Dumbea River (Ambastian et al., 1997). In contrast, Unia (East coast), southern and northern lagoon, Maa Bay and Ouano Bay are preserved from important anthropogenic inputs. Finally, the Tomo site is located near the international airport of New Caledonia, between Ouano Bay and Noumea City, whereas the site of Bassin de La Foa is located near a shrimp farm on the West coast of New Caledonia.

All collected organisms were weighed (wet wt) and measured (total length for fish and shrimp, height and length of the shells for bivalves, and mantle length for cephalopods) upon return to the laboratory. Characteristics (origin, number of individuals, length and weight, sampling date) of each of the 32 species collected are given in Table 1. This table also indicates the trophic level (i.e. filter feeder, grazer/scavenger, predator of invertebrates,

predator of invertebrates and small fish, predator of small fish) and the water-column distribution (benthic, nectobenthic and neritic) for every species.

Organisms were dissected to collect specific body compartments. A piece of muscle (edible tissue) was taken from fish, shrimp and cephalopods. For fish, the liver was also collected. In cephalopods (*Sepioteuthis lessoniana*, *Sepia latimanus* and *Octopus cyanea*), the digestive gland and branchial hearts were collected whenever possible. In the case of Pectinidae (*Comptopallium radula* and *Mimachlamys gloriosa*), the edible tissues (muscle + gonads) were separated from the remaining soft tissues. For all other bivalves, the soft parts were considered as a whole. After dissection, the samples were weighed (wet wt) and immediately placed in individual plastic bags, frozen at -25°C , freeze-dried, and weighed again (dry wt). Freeze-dried tissues were then ground and stored in individual plastic vials until further metal analysis.

2.2. Total Hg analysis

Total Hg analyses were carried out with an Advanced Mercury Analyser (ALTEC AMA 254), on dried tissue aliquots ranging from 4 to 50 mg, weighed to the nearest 0.01 mg. For Hg determination, the metal was evaporated by progressive heating up to 800°C , then held under oxygen atmosphere for 3 minutes, and finally amalgamated on a gold net. Afterwards, the net was heated to liberate the collected Hg, which was measured by atomic absorption spectrophotometry. Mercury analyses were run according to a thorough quality control programme including the analysis of a NRC reference material (lobster hepatopancreas TORT-2; National Research Council, Canada). Standard aliquots were treated and analysed according to the same conditions as the samples. The results were in good agreement with the certified values, with an average recovery rate of 105%. Detection limit was 5 ng g^{-1} dry wt. All total Hg concentrations in tissues further reported are expressed in ng g^{-1} dry wt.

2.3. Data analysis

All data submitted to statistical tests were first checked for normality (Shapiro-Wilk test) and for homogeneity of variances (Bartlett test). When these conditions were satisfied, parametric tests were used in the subsequent analyses; otherwise, non-parametric analogues were used. Pearson or Spearman correlation coefficient test was used to analyse the correlations between size or weight and total Hg concentration in edible tissues in each species. Differences in Hg concentrations between tissues in species for which different body compartments were dissected were tested by Student *t*-test or Wilcoxon test for paired samples. To assess whether trophic levels (see Table 1) could influence Hg concentrations in fish (among the collected organisms, this taxa was the only one to display different diets; Table 1), analysis of covariance (ANCOVA) was performed, using length of individuals as covariable. Finally, species with a minimum of two individuals collected per different location were considered to test differences in Hg concentrations among sampling locations (Table 4), using a one-way analysis of variance (ANOVA) followed by the post-hoc Tukey test. In the case of species for which a correlation between size or weight and Hg concentration was previously revealed, ANCOVA was performed instead of ANOVA, using size or weight as covariable. One-way ANOVA and ANCOVA were performed on log-transformed Hg concentrations and length or weight values. When appropriate, the variability explained by each factor and their interaction was derived from the sum of squares (Warnau et al., 1996, 1998). The levels of significance for statistical analyses was always set at $\alpha = 0.05$.

2.4. Risk assessment for Human consumers

To prevent Human health impairments by Hg originating from dietary sources, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) allocated a Provisional Tolerable Weekly Intake (PTWI) for total Hg and methyl-Hg of 5000 and 1600 ng kg⁻¹ wk⁻¹, respectively (WHO, 2003). In this study, we calculated a “Maximum Safe Weekly Consumption” (MSWC), i.e. the weight of edible tissue (muscle for shrimp, cephalopods and fish, muscle + gonads or whole soft parts for bivalves) that should be consumed by a 60 kg adult to reach the PTWI. For this, mean Hg concentrations in ng g⁻¹ dry wt were first converted to wet wt, taking into account the dry wt/wet wt ratios measured in all samples. These ratios indicated a mean moisture content of 80% for bivalve flesh, shrimp and cephalopod muscle, and of 75% for fish muscle, which matched well those previously published for the same taxonomic groups (e.g. Trombini et al., 2003; Bustamante and Miramand, 2005). Then, the PTWI (ng kg⁻¹ wk⁻¹) multiplied by the consumer body weight (60 kg) was divided by the Hg concentration (ng g⁻¹ wet wt) in the considered seafood to obtain the MSWC (g wet wt). The latter value (g) was finally converted in kg (Tables 3 and 4). In the particular cases of the fish *Plectropomus leopardus* and the bivalves *Comptopallium radula*, *Gafrarium tumidum* and *Anadara scapha*, the MSWC was also calculated for each given site (Table 4). Finally, the MSWC was calculated for total-Hg and methyl-Hg, taking a 75% ratio of Me-Hg/T-Hg for shrimp muscle (Riisgard and Famme, 1986), a 80% ratio of Me-Hg/T-Hg for cephalopod muscle (Bustamante et al., 2006), and a 100% ratio for fish muscle (Bloom, 1992). MSWC for methyl-Hg was not calculated in bivalves, due the great variability of the ratio MeHg/T-Hg found in the literature (ranging from 20 to 95%), depending on species, age, season and location (Mohlenberg and Riigard, 1988; Kawaguchi et al., 1999; Otchere et al., 2003; Trombini et al., 2003).

3. Results

3.1. Concentrations and tissue distribution

Total Hg concentrations in edible tissues varied greatly within and among species groups (i.e. fish, crustaceans, mollusc cephalopods, mollusc bivalves). They ranged from 26 to 2063 ng g⁻¹ dry wt in fish, from 128 to 297 ng g⁻¹ dry wt in crustaceans (i.e. *Litopenaeus stylirostris*), from 40 to 218 ng g⁻¹ dry wt in cephalopods, and from 73 to 2531 ng g⁻¹ dry wt in bivalves (see Table 3). Even within a single species, the variability was sometimes quite important, e.g. for *Mimachlamys gloriosa*, Hg concentrations ranging from 244 to 890 ng g⁻¹ dry wt. As it could be expected the variability was even greater when animals of a single species were sampled in different sites. Indeed, Hg concentrations ranged from 173 to 2063 ng g⁻¹ dry wt in *Plectropomus leopardus*, from 73 to 1015 ng g⁻¹ dry wt in *Anadara scapha*, and from 104 to 2531 ng g⁻¹ dry wt in *Gafrarium tumidum*. In a single sampling location, Hg concentrations varied greatly among species of the same taxonomic group. For example in Grande Rade, average Hg concentrations were significantly different (Kruskal-Wallis test, $p < 0.01$) among the three bivalves *C. radula*, *A. scapha* and *G. tumidum*: 234 ± 24 , 723 ± 414 and 1633 ± 565 ng g⁻¹ dry wt, respectively (Table 4). Interestingly, this pattern *C. radula* < *A. scapha* < *G. tumidum* was also observed in Koutio and Maa bays, even though the differences among species were significant only in Maa Bay (Kruskal-Wallis test, $p < 0.01$).

In Ouano Bay (S3), average Hg concentrations in the fish muscles were significantly different (Kruskal-Wallis test, $p < 0.01$) among *Kyphosus vaigiensis*, *Priacanthus hamrur* and *Plectropomus leopardus*, with 55 ± 22 , 188 ± 58 and 856 ± 330 ng g⁻¹ dry wt, respectively (Tables 3 and 4).

With very few exceptions, total Hg concentrations in fish were always higher in the liver (detoxification organ) than in the muscle (edible tissue) (Fig. 2). Moreover, this difference

was statistically significant for most of the species (t or Wilcoxon tests for paired samples, $p < 0.05$). In contrast, in cephalopods, there was no significant difference in Hg concentrations between muscles and detoxification tissues, i.e. the digestive gland for the squid *Sepioteuthis lessoniana* (t -test for paired samples, $p > 0.05$) and the branchial hearts for the octopus *Octopus cyanea* (Wilcoxon tests for paired samples, $p > 0.05$) (Fig. 3). However, in this latter species, the concentrations recorded in the digestive gland of one specimen was far higher than those of the branchial hearts and muscle (Fig. 3). In *Sepia latimanus*, Hg concentrations followed the decreasing order: branchial hearts > digestive gland > muscle (Fig. 3). Finally, for the Pectinidae (i.e. *Comptopallium radula* and *Mimachlamys gloriosa*), Hg concentrations were significantly lower (Wilcoxon test, $p < 0.05$) in the edible tissues (muscle + gonads) than in the remaining tissues (Fig. 4).

3.2. Influence of size, weight and trophic level

Correlations between Hg concentrations in edible tissues and individual size or weight were established for each species, considering all sites together. Few Spearman correlations were significant and Hg levels only varied positively with size for 2 out of the 32 species collected: in the muscles of the fish *P. leopardus* ($r = 0.773$, $p < 0.001$) and in the muscle + gonad of the pectinid *C. radula* ($r = 0.422$, $p = 0.040$). Interestingly, Hg concentrations in whole soft parts of *C. radula* were also correlated significantly with size ($r = 0.472$, $p = 0.020$). Positive correlations with the individual weight were significant for 3 out of the 32 species: in the muscles of *P. leopardus* ($r = 0.782$, $p < 0.001$), and in the whole soft tissues of the bivalves *G. tumidum* and *Periglypta chemnitzii* ($r = 0.239$, $p = 0.024$ and $r = 0.663$, $p = 0.037$, respectively).

Differences in Hg concentrations among trophic levels in fish are shown in Fig. 5. The ANCOVA revealed that trophic level, length and their interaction were all factors affecting significantly Hg concentrations ($p < 0.005$). Trophic level was the most important factor

explaining ca. 60% of Hg concentration variability, followed by length (15%) and their interaction (6%). The remaining 19% of the variability were due to other factors (residual term). It was not possible to perform ANCOVA with the sites or species as covariable, as was done for trophic levels, because the residuals did not satisfy normality and/or homoscedasticity pre-requisites.

3.3. Differences among sampling sites

Table 4 displays the comparison among Hg concentrations measured in different sites of New Caledonia for four species, i.e. the fish *P. leopardus* and the bivalves *A. scapha*, *C. radula* and *G. tumidum*. As it was previously found for the first 3 species that a significant positive correlation occurred between Hg concentrations and size or weight, the appropriate factor was used as covariable in the ANCOVA performed to test the differences among sites.

For all the four species, Hg concentrations in the edible tissues displayed significant differences among sampling sites ($p_{\text{ANCOVA or ANOVA}}$ always <0.001). Post-hoc Tukey test also showed that for all species, the highest Hg concentrations were always measured in Grande Rade. With respect to *A. scapha* and *G. tumidum*, relatively low Hg values were found for individuals collected in Ouano Bay sites (ranging from 73 to 492 ng g⁻¹ dry wt). In contrast, the fish collected in the latter bay displayed quite high Hg concentration (from 590 to 1338 ng g⁻¹ dry wt in muscles). Koutio, Maa and Sainte Marie Bays were generally characterized by intermediate values for the three bivalves, and by the lowest values for the fish (Table 4). Regarding the clam *G. tumidum*, Hg concentrations were the lowest in Unia, whereas they were relatively high in Tomo (154 ± 38 and 497 ± 124 ng g⁻¹ dry wt respectively). Nevertheless, values in Tomo were significantly lower than those measured in Grande Rade (Table 4). At a smaller geographic scale, possible difference between Grande Rade and Grande Rade-SLN and among the sub-stations in Ouano Bay (S1, S2, S3) could be

investigated for 2 out of the 4 species: *A. scapha* and *G. tumidum*. For *G. tumidum*, Hg concentrations were significantly higher ($p < 0.001$) in Grande Rade than in Grande Rade-SLN, whereas no significant difference was detected for *A. scapha*. In Ouano Bay, no significant difference could be detected among the three sampling sites (Table 4).

3.4. Risk assessment for Human consumers

The “Maximum Safe Weekly Consumption” (MSWC, in kg wet wt) of edible flesh was estimated taking into account the “Provisional Tolerable Weekly Intake” (PTWI) recommended by JECFA (WHO, 2003) for total Hg and methyl-Hg. MSWC values are given on a species basis in Table 3 (for the species collected in more than one site, the MSWC is thus an average value taking into account the different sampling sites), and on a site-specific basis in Table 4 (for the four species that were collected in sufficient quantity in several sites). The amount of shrimp muscle (*L. stylirostris*) from Bassin de La Foa (i.e. a shrimp farm) which should be eaten by a 60-kg person to reach the PTWI for total Hg ($5000 \text{ ng kg}^{-1} \text{ wk}^{-1}$) would be ca. 7.5 kg. For the oyster *Crassostrea gigas* from Dumbea Bay, this amount would be about 13.5 kg of oyster flesh. Regarding cephalopods from the southern and northern lagoon, the MSWC for total Hg ranged from 10 kg of edible flesh (muscle) for the squid *Sepioteuthis lessoniana* to 36.7 kg per week for the cuttlefish *Sepia latimanus* (Table 3). With respect to fish consumption, the MSWC for total Hg was between ca. 1 kg of flesh for some carnivorous species from Grande Rade (*Plectorhinchus chaetodonoides*, *P. flavomaculatus*) to more than 32 kg per week for some herbivorous species coming from Ouano Bay (*Naso unicornis*, *Scarus microrhinos*, *S. rivulatus*, *S. schlegeli*) (Tables 3 and 4). In Grande Rade, a 60 kg person should eat 6.4 kg, 2.1 kg and 920 g wet wt per week of flesh of *C. radula*, *A. scapha* and *G. tumidum*, respectively, to reach the PTWI in total Hg (Table 4). In the same site, the MSWC for total Hg was 830 g of edible flesh of the *P. leopardus* fish. In all cases,

MSWCs for methyl-Hg were far lower than those computed for total Hg, especially in fish (Tables 3 and 4). For example, a 60 kg person should eat about 260 g wet wt of flesh of *P. leopardus* from Grande Rade to reach the PTWI in methyl-Hg (vs. the aforementioned 830 g for total Hg).

4. Discussion

The whole dataset of the present study provides substantial baseline information of major importance for future investigations on Hg contamination status in New Caledonia as well as for the evaluation of the risk related to local seafood consumption. Indeed, despite the well-known toxicity of Hg to marine organisms and its potential effects on Human health, this metal has been poorly investigated in this tropical zone, with only one published study reporting Hg measurements in tissues of two marine mammals (Bustamante et al., 2003). So far, there are no published data on Hg levels in invertebrate or vertebrate species used as food by local populations, whether they are fished from the wild or cultured in artificial (shrimp) or natural (oysters) environments.

In species for which several tissues were examined, the lowest concentrations were almost always recorded in the muscles (see Figs 2 to 4). In cephalopods, the digestive gland and the branchial hearts are actually known to be major sites for concentrating metals, among which Hg, compared to muscles (e.g. Miramand and Bentley, 1992; Bustamante et al., 2000, 2006). The digestive gland is generally supposed to play a major role in the storage and detoxification of many essential and non essential elements such as Ag, Cd, Cu or Zn (Miramand and Bentley, 1992; Bustamante et al., 2000, 2002, 2006). Concerning the Pectinidae family, the digestive gland and the kidneys have been identified for their storage and detoxification function for many elements (e.g. Bustamante and Miramand, 2004, 2005; Metian et al., 2008a), especially in the case of Hg (Metian et al., 2008b). This explains the higher Hg values that we measured in the “remaining soft tissues” body compartment of *C.*

radula and *M. gloriosa* (Fig. 4) as this compartment comprises all tissues and organs other than the adductor muscle and the gonad (i.e. it includes both the kidneys and the digestive gland). Finally, in fish, hepatic levels of Hg are generally reported to be higher than the muscular ones (e.g. Storelli et al., 2005; Kojadinovic et al., 2007), probably due to the demethylation process that has been suggested to occur in fish liver, as indicated by the occurrence of almost only inorganic Hg in this organ (Oliveira Ribeiro et al., 1996; Maury-Bachet et al., 2006). In our study, liver was similarly found to be the major organ for Hg storage in fish. Pertaining to edible tissues, Hg concentrations varied greatly in some bivalves collected from different sites in the New Caledonia lagoon. Hg levels ranged from 73 to 1015 and from 104 to 2531 ng g⁻¹ dry wt in the whole soft parts of *A. scapha* and *G. tumidum*, respectively. In fish, *P. leopardus*, which was also collected in several sites, displayed muscular Hg concentrations ranging from 173 to 2063 ng g⁻¹ dry wt. On the other hand, in some species of fish (i.e. *Scarus sp.*, *Lutjanus monostigma*, *Kyphosus vaigiensis*), Hg concentrations were very low (<60 ng g⁻¹ dry wt) for most of the individuals collected (Table 3). Similarly, Hg concentrations measured in the cultured species did not exceed 105 and 297 ng g⁻¹ dry wt for the oyster *C. gigas* and the shrimp *L. stylirostris*, respectively.

Comparison with other tropical areas (i.e. NE coast of Australia, Thailand, and Gulf of Oman) indicates that the total Hg concentrations reported in similar organisms were generally below the mean values that we obtained in this study. Indeed, in bivalves from Thailand, total Hg in the clam *Paphia undulate*, the blood cockle *Anadara granosa* and the oyster *Crassostrea commercialis* never exceeded 100 ng g⁻¹ dry wt (Phillips and Muttarasin, 1985) whereas in the Gulf of Oman, Hg concentrations were below 315 ng g⁻¹ dry wt for the clam *Circentia callipyga*, the spiny oyster *Spondylus sp.* and the oyster *Saccostrea cucullata* (de Mora et al., 2004). Regarding fish, relatively low concentrations were reported for species from the NE coast of Australia with Hg levels ranging from 120 to 760 ng g⁻¹ dry wt in muscle of *Acanthopagrus berda*, *Cymbacephalus nematophthalmus*, *Lutjanus carponotatus*,

L. sanguineus and *Plectorhynchus flavimaculatus* (Denton and Breck, 1981). However, de Mora et al. (2004) found relatively high values in fish from the Gulf of Oman, i.e. ranging from 343 to 522 and from 498 to 2350 ng g⁻¹ dry wt in *Lethrinus nebulosus* and *Epinephelus coicoides*, respectively. Also, Denton and Breck (1981) reported values from 250 to 1250 ng Hg g⁻¹ dry wt in muscles of cephalopods (*Loligo sp.* and *Sepia sp.*), and a Hg concentration of ca. 1000 ng g⁻¹ dry wt for the tumid venus *Gafrarium tumidum*. Moreover, in chronically polluted, non tropical areas (i.e. Western Limfjord, Denmark and Lavaca Bay, Texas, USA), Hg concentrations ranging from 414 to 2517 ng g⁻¹ dry wt were reported in the cockle *Cardium sp.* and from 430 and 10 100 ng g⁻¹ dry wt in several other species of bivalves (Mohlenberg and Riigard, 1988; Locarnini and Presley, 1996). In our study, some values recorded in the edible *A. scapha* and *G. tumidum* from Grande Rade were thus on the same order of magnitude than those reported in well known Hg-contaminated areas. Hence, along with our data on Hg levels in fish muscles, the former comparisons indicate that New Caledonia may suffer Hg contamination to some extent and that additional studies would be necessary for further assessing the contamination status in Hg of the New Caledonia lagoon. Nonetheless, the assessment of the risk for Human consuming marine products from this lagoon appeared of particular concern, particularly for the Grande Rade area.

From a global health standpoint, Hg concentrations measured in the investigated organisms of this study did not reveal excessive risk for Humans consuming seafood in New Caledonia. Indeed, even in the most contaminated site (Grande Rade), the amount of bivalve flesh to be eaten on a weekly basis by a 60 kg person for a given species to reach the PTWI for total Hg was relatively elevated: 920 g, 2.1 kg and 6.4 kg wet wt for *G. tumidum*, *A. scapha* and *C. radula*, respectively (Table 4). Also, regarding the fish *P. leopardus* in the same location, a 60 kg person should eat more than 830 g of fish muscle to exceed the PTWI in total Hg. Hence, except in local Human groups where sea products constitute the main food source, the former quantities of fish or bivalves are not very likely to be reached over a one-

week period, as far as total Hg is considered. However, if we assume that ca. 100% of total Hg in fish muscle is occurring as methyl-Hg as it is typically the case in this group (e.g. Bloom, 1992; Holsbeek et al., 1997), the PTWI in methyl-Hg would be reached with the consumption of approximately 260 g wet wt of edible flesh of *P. leopardus* from Grande Rade. Such a low quantity of flesh can be easily consumed during a single meal. Indeed, in New Caledonia, fish consumption is particularly high, varying between 23 to 50 kg/inhabitant/year, i.e. between 0.5 to 1 kg per week (Labrosse et al., 2006). Moreover, the “Maximum Safe Weekly Consumption” (MSWC) for methyl-Hg assessed for the other fish species collected in Grande Rade (i.e. *Plectorhinchus chaetodonoides*, *P. flavomaculatus* and *Bodianus perditio*) were similarly quite low and always below 400 g wet wt of flesh per week for a 60-kg consumer (Table 3). Although these low MSWC values are of concern, they were only found in the single area of Grande Rade.

As for other metals, Hg concentrations in marine organisms may vary with biological and environmental factors such as age (size/weight), trophic level, lifestyle and geographical origin (e.g. Monteiro et al., 1992; Otchere et al., 2003; Burger et al., 2007; Zizek et al., 2007). Size has been reported as a factor to which Hg concentrations are well correlated in fish (Monteiro and Lopes, 1990; Mathieson and McLusky, 1995; Adams, 2004; Kojadinovic et al., 2006), due to the continuous bioaccumulation of Hg in muscular tissues all along their life (Braune, 1987; Burger and Gochfeld, 2007). However, among the fish species examined in the present study, only *P. leopardus* displayed a positive correlation with length and weight. The lack of positive correlation for the other fish investigated may be due to the small number of individuals within those species, or, alternatively to the fact that the sampled individuals were of about the same age (Braune, 1987; Mathieson and McLusky, 1995).

In cephalopods, it is also generally admitted that Hg concentrations are positively correlated with size (Rossi et al., 1993; Bustamante et al., 2006; Pierce et al., 2008). However,

no correlation was found either for *Sepioteuthis lessoniana* or for *Octopus cyanea*, probably because of the too narrow size range of the individuals examined.

Hg concentrations were found to differ among species, and notably among species belonging to the same taxonomic group. Interestingly, for a given sampling location (Grande Rade, Koutio Bay, Maa Bay), the three species of bivalves (*C. radula*, *A. scapha* and *G. tumidum*) did not bioaccumulate Hg to the same extent. The increasing order of concentrations (*C. radula* < *A. scapha* < *G. tumidum*) always occurred in each site, the clam and the cockle concentrating Hg much more than the scallop. These three species were of very different sizes: average length was 77 ± 14 mm for *C. radula*, 58 ± 9 mm for *A. scapha* and 38 ± 5 mm for *G. tumidum*. It is well-established that metal uptake efficiency can decrease with organism size increase (e.g. Swaileh and Adelung, 1995; Warnau et al., 1995; Hédouin et al., 2006). This trend is generally explained by the decreasing surface/volume ratio of an organism with increasing body size, the decreasing metabolic activity in older individuals, and/or by the dilution of the metal in larger organisms (e.g. Braune, 1987; Swaileh and Adelung, 1995; Warnau et al., 1995). Moreover, different species of bivalves may have different diet and filtration rates. Although further studies are needed to better explain the differences in Hg bioaccumulation among marine bivalves from New Caledonia, it is effectively well-known that scallops have a quite elevated filtration rate compared to other bivalves (Meyhöfer, 1985). As they displayed lower values of Hg than other bivalves in this study, it could be suggested that diet composition could be a major factor in Hg bioaccumulation in bivalves. This is in accordance with the few evidences available in the related literature (e.g. Wang and Wong, 2003; Metian et al., 2008b). This apparently also holds true in fish, for which the trophic level clearly appeared to affect Hg concentrations in the investigated species (see Fig. 5). Indeed, grazers/scavengers displayed the lowest values of Hg in their muscular tissues, whereas predators of small fish displayed the highest ones.

These observations are in good agreement with the Hg biomagnification process occurring in marine food webs (e.g. Burger et al., 2007; Zizek et al., 2007).

Species, size or weight and trophic level as factors of variation for Hg concentrations were also well illustrated by the case of the three fish species collected in Ouano Bay S3. Indeed, they displayed significant differences in their mean muscle values of total Hg, according to the following order: *Kyphosus vaigiensis* < *Priacanthus hamrur* < *Plectropomus leopardus*. Actually, *K. vaigiensis* and *P. hamrur* are similar in size, but the former is a grazer whereas the second is a predator of invertebrates and small fish; as for *P. leopardus*, it is much heavier and is a predator of small fish, and displayed thus, as could be expected, the highest Hg concentrations among the three species.

Finally, there were clear differences in Hg concentrations among sites for a given species, the site factor explaining between 68 and 85% of the variability in the performed ANCOVA. In the three bivalve species *A. scapha*, *C. radula* and *G. tumidum*, and in the fish *P. leopardus*, individuals from Grande Rade always displayed the highest concentrations in Hg. Considering the concentrations of several metals in the sediment and in the clam *G. tumidum*, Grande Rade is regarded as a highly contaminated station (Hédouin et al., 2008). As this area is subjected to industrial inputs, it was not surprising to find high concentrations of Hg in the organisms living there. However, there was a spatial heterogeneity for bivalves from the Grande Rade area, with Hg concentrations in *G. tumidum* from Grande Rade-SLN that were surprisingly much lower than that from Grande Rade (individuals from both locations were of similar size). Very intense exposure to Ni and to Ni-ore co-occurring metals in Grande Rade-SLN lead to very high concentrations on these elements in the tissues of *G. tumidum* ($>10 \mu\text{g Cr g}^{-1}$, $>90 \mu\text{g Ni g}^{-1}$, $>300 \mu\text{g Mn g}^{-1}$; Hédouin et al., 2008) which could interfere with other elements such as Hg. However, the lack of similar trends in Hg concentrations for the other bivalve also collected in both sites (*A. scapha*) does not support this hypothesis. Further investigations are thus necessary to explain this peculiar observation in *G. tumidum*.

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- 634

Captions to figures

Fig. 1. Map of the sampling stations in the South of New Caledonia and in the vicinity of Noumea City. GR: Grande Rade; GR-SLN: Grande Rade-SLN.

Fig. 2. Relationship between total Hg concentrations (T-Hg; ng g^{-1} dry wt) in liver and muscle in fish species from New Caledonia.

Fig. 3. Total Hg concentrations (T-Hg; mean $\pm$ SD, ng g^{-1} dry wt) in the digestive gland, branchial hearts and muscles of three cephalopod species from New Caledonia.

Fig. 4. Total Hg concentrations (T-Hg; mean $\pm$ SD, ng g^{-1} dry wt) in the muscle + gonad and in the remaining tissues of two scallop species from New Caledonia.

* reconstructed data

Fig. 5. Comparison of total Hg concentrations (T-Hg, ng g^{-1} dry wt) in the muscles of fish from New Caledonia according to their trophic level. GS = grazer / scavenger ($n = 10$); PI = predator of invertebrates ($n = 5$); PISM = predator of invertebrates and small fish ($n = 23$); PSM = predator of small fish ($n = 24$).

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Table 1
Characteristics of the organisms collected and analysed

Family and Scientific Name	Common Name	<i>n</i>	Length (mm) Mean $\pm$ SD (Range)	Wet wt (g) Mean $\pm$ SD (Range)	Sampling date	Sampling site(s)	Trophic level	Life style
Crustaceans								
Penaeidae								
<i>Litopenaeus stylirostris</i>	Pacific blue shrimp	5	155 $\pm$ 4 (155 – 160)	21.5 $\pm$ 1.3 (20.2 – 23.3)	Mar 2007	Bassin de La Foa	Grazer / Scavenger	Benthic
Mollusc Bivalves								
Arcidae								
<i>Anadara scapha</i>	Antique ark	28	58.4 $\pm$ 8.8 (28 – 70)	60.8 $\pm$ 20.5 (9 – 101)	Mar 2007 Oct 2007	Grande Rade, Koutio, Ouano (S1, S2), Maa, Sainte Marie Grande Rade-SLN	Filter feeder	Benthic
Ostreidae								
<i>Crassostrea gigas</i>	Pacific cupped oyster	5	86.0 $\pm$ 4.7 (80 – 93)	62.0 $\pm$ 7.1 (54 – 73)	Mar 2007	Dumbea	Filter feeder	Benthic
Pectinidae								
<i>Comptopallium radula</i>	Radula scallop	24	77.3 $\pm$ 14.3 (44 – 99)	70.6 $\pm$ 28.4 (19.6 – 115)	Mar 2007 Oct 2007	Grande Rade, Koutio, Maa, Sainte Marie Noumea harbour	Filter feeder	Benthic
<i>Mimachlamys gloriosa</i>	Tropical scallop	10	72.6 $\pm$ 6.4 (63 – 80)	55.8 $\pm$ 14.9 (38 – 80)	Oct 2007	Noumea harbour	Filter feeder	Benthic
Veneridae								
<i>Gafrarium tumidum</i>	Tumid venus	89	37.6 $\pm$ 4.6 (29 – 45)	22.3 $\pm$ 6.4 (11.3 – 35.8)	Mar 2007 Oct 2007	Grande Rade, Grande Rade-SLN, Koutio, Ouano (S1, S2, S3), Maa Unia, Tomo	Filter feeder	Benthic
<i>Periglypta chemnitzii</i>	Chemnitz Venus	10	60.6 $\pm$ 2.8 (56 – 65)	111 $\pm$ 26.3 (73 – 172)	Oct 2007	Unia	Filter feeder	Benthic
Mollusc Cephalopods								
Loliginidae								
<i>Sepioteuthis lessoniana</i>	Big fin reef squid	9	215 $\pm$ 34 ^a	441 $\pm$ 185	Oct 2007	Southern Lagoon	Predator	Neritic
Octopodidae								
<i>Octopus cyanea</i>	Big blue octopus	7	113 $\pm$ 16.3 ^a (82 – 131) ^a	1521 $\pm$ 671 (700 – 2900)	Oct 2007	Northern Lagoon	Predator	Benthic
Sepiidae								
<i>Sepia latimanus</i>	Broad club cuttlefish	1	240 ^a	1300 ^a	Oct 2007	Southern Lagoon	Predator	Nectobenthic

^a Mantle length (not total length)

Table 1 (continued)

Family and Scientific Name	Common Name	<i>n</i>	Length (mm) Mean $\pm$ SD (Range)	Wet wt (g) Mean $\pm$ SD (Range)	Sampling date	Sampling site(s)	Trophic level	Life style
Fish								
Acanthuridae								
<i>Naso unicornis</i>	Bluespine unicornfish	1	435	1600	Mar 2007	Ouano (S3)	Grazer / Scavenger	Neritic
Haemulidae								
<i>Plectorhinchus albovittatus</i>	Two-striped sweetlips	1	405	1300	Mar 2007	Ouano (S3)	Predator (invertebrates)	Neritic
<i>P. chaetodonoides</i>	Harlequin sweetlips	1	351	900	Mar 2007	Grande Rade	Predator (invertebrates and small fish)	Neritic
<i>P. flavomaculatus</i>	Lemon sweetlips	1	485	1600	Mar 2007	Grande Rade	Predator (crustaceans and small fish)	Neritic
<i>Diagramma pictum</i>	Painted sweetlips	1	451	1160	Oct 2007	Ouano (S3)	Predator (invertebrates and small fish)	Neritic
Kyphosidae								
<i>Kyphosus vaigiensis</i>	Brassy chub	5	317 $\pm$ 50 (260 – 370)	718 $\pm$ 330 (380 - 1100)	Mar 2007	Ouano (S3)	Grazer / Scavenger	Neritic
Labridae								
<i>Bodianus perditio</i>	Golden-spot hogfish	1	343	800	Mar 2007	Grande Rade	Predator (invertebrates)	Neritic
<i>Cheilinus chlorourus</i>	Floral wrasse	1	290	450	Mar 2007	Koutio	Predator (invertebrates)	Neritic
Lethrinidae								
<i>Lethrinus laticaudis</i>	Grass emperor	3	266 $\pm$ 11 (256 – 277)	376 $\pm$ 45 (331 – 420)	Mar 2007	Southern Lagoon	Predator (crustaceans and small fish)	Neritic
<i>Monotaxis grandoculis</i>	Humpnose big-eye bream	2	261 $\pm$ 7 (256 – 266)	420 $\pm$ 21 (405 – 434)	Mar 2007	Southern Lagoon	Predator (invertebrates)	Neritic
Lutjanidae								
<i>Lutjanus argentimaculatus</i>	Mangrove red snapper	5	449 $\pm$ 63 (348 – 510)	1345 $\pm$ 494 (625 – 1900)	Mar 2007	Grande Rade, Ouano (S3), Maa	Predator (crustaceans and small fish)	Neritic
<i>L. monostigma</i>	Onespot snapper	2	249 $\pm$ 37 (223 – 275)	475 $\pm$ 177 (350 – 600)	Mar 2007	Ouano (S3), Maa	Predator (crustaceans and small fish)	Neritic
Platycephalidae								
<i>Cymbacephalus beauforti</i>	Crocodile fish	2	510 $\pm$ 99 (440 – 580)	1425 $\pm$ 530 (1050 – 1800)	Oct 2007	Southern Lagoon	Predator (small fish)	Benthic
Priacanthidae								
<i>Priacanthus hamrur</i>	Moontail bullseye	7	307 $\pm$ 21 (285 – 340)	454 $\pm$ 96 (360 – 600)	Mar 2007	Ouano (S3)	Predator (invertebrates and small fish)	Neritic
Scaridae								
<i>Scarus ghobban</i>	Blue-barred parrotfish	1	247	245	Mar 2007	Koutio	Grazer / Scavenger	Neritic
<i>S. microrhinos</i>	Blunt-head parrotfish	1	508	3000	Mar 2007	Ouano (S3)	Grazer / Scavenger	Neritic
<i>S. rivulatus</i>	Rivulated parrotfish	1	355	900	Mar 2007	Ouano (S3)	Grazer / Scavenger	Neritic
<i>S. schlegeli</i>	Yellowband parrotfish	1	249	400	Mar 2007	Ouano (S3)	Grazer / Scavenger	Neritic
Serranidae								
<i>Plectropomus leopardus</i>	Leopard coralgroupier	21	413 $\pm$ 110 (265 – 615)	1294 $\pm$ 1051 (300 – 3800)	Mar 2007 Oct 2007	Grande Rade, Koutio, Ouano (S3), Maa, Sainte Marie Grande Rade, Ouano (S3), Southern Lagoon	Predator (small fish)	Neritic
<i>Epinephelus coeruleopunctatus</i>	Whitespotted grouper	1	470	1500	Oct 2007	Southern Lagoon	Predator (crustaceans and small fish)	Neritic
<i>E. maculatus</i>	Highfin grouper	1	340	480	Mar 2007	Ouano (S3)	Predator (invertebrates and small fish)	Neritic
Sparidae								
<i>Acanthopagrus berda</i>	Picnic seabream	1	278	600	Mar 2007	Ouano (S2)	Predator (invertebrates and small fish)	Neritic
		1	271	500	Oct 2007	Maa		

Table 2

Latitude and longitude coordinates of the sampling stations in the New Caledonia Lagoon

Sampling site	Geographical coordinates	
	Latitude	Longitude
Northern Lagoon	19°57 S	163°58 E
Bassin La Foa	21°45 S	165°43 E
Ouano Bay S1	21°51 S	165°50 E
Ouano Bay S2	21°49 S	165°46 E
Ouano Bay S3	21°52 S	165°49 E
Tomo	21°57 S	166°04 E
Maa Bay	22°12 S	166°19 E
Dumbea Bay	22°12 S	166°24 E
Koutio Bay	22°13 S	166°25 E
Grande Rade	22°16 S	166°25 E
Grande Rade-SLN	22°15 S	166° 26 E
Noumea harbour	22°17 S	166°25 E
Sainte Marie Bay	22°18 S	166°28 E
Unia	21°55 S	166°38 E
Southern Lagoon	22°25 S	166°53 E

Table 3

Total Hg concentrations (T-Hg; mean $\pm$ SD, ng g⁻¹ dry wt) in the edible tissues of the species collected, correlations with individual size and weight, and estimate of the “Maximum Safe Weekly Consumption” (MSWC) for total Hg (T-Hg) and methyl-Hg (Me-Hg) (kg of flesh wet wt)

Species	Tissue	n	T-Hg concentration (ng g ⁻¹ dry wt)		MSCW (kg)	
			Mean ± SD	(Range)	T-Hg	Me-Hg
Crustaceans						
<i>Litopenaeus stylirostris</i>	muscle	5	201 ± 62	128 – 297	7.5	3.2
Cephalopods						
<i>Sepioteuthis lessoniana</i>	muscle	9	150 ± 36	113 – 218	10	4
<i>Octopus cyanea</i>	muscle	7	90 ± 23	69 – 139	16.7	6.7
<i>Sepia latimanus</i>	muscle	1	41	-	36.7	14.7
Bivalves						
<i>Comptopallium radula</i>	muscle + gonad	24	62 ± 20	30 – 108	24.3	-
<i>C. radula</i>	whole soft parts ^a	24	150 ± 53	84 – 261	10	-
<i>Mimachlamys gloriosa</i>	muscle + gonad	10	70 ± 12	55 – 91	21.5	-
<i>M. gloriosa</i>	whole soft parts ^a	10	107 ± 18	90 – 142	14	-
<i>Anadara scapha</i>	whole soft parts	28	297± 226	73 – 1015	5.1	-
<i>Crassostrea gigas</i>	whole soft parts	5	111 ± 15	92 – 105	13.5	-
<i>Gafrarium tumidum</i>	whole soft parts	89	418 ± 466	104 – 2531	3.6	-
<i>Periglypta chemnitzii</i>	whole soft parts	10	407 ± 222	244 – 890	3.7	-
Fish						
<i>Naso unicornis</i>	muscle	1	32	-	38	12.1
<i>Plectorhinchus albovittatus</i>	muscle	1	650	-	1.8	0.59
<i>P. chaetodonoides</i>	muscle	1	1200	-	1	0.32
<i>P. flavomaculatus</i>	muscle	1	1173	-	1	0.33
<i>Diagramma pictum</i>	muscle	1	329	-	3.6	1.2
<i>Kyphosus vaigiensis</i>	muscle	5	55 ± 22	36 – 92	22	7
<i>Bodianus perditio</i>	muscle	1	1020	-	1.2	0.38
<i>Cheilinus chlorourus</i>	muscle	1	362	-	3.3	1.1
<i>Lethrinus laticaudis</i>	muscle	3	165 ± 55	125 – 228	7.3	2.3
<i>Monotaxis grandoculis</i>	muscle	2	99 ± 18	87 – 112	12.1	3.9
<i>Lutjanus argentimaculatus</i>	muscle	5	430 ± 325	403 – 994	2.8	0.89
<i>L. monostigma</i>	muscle	2	51 ± 23	35 – 67	23.6	7.5
<i>Cymbacephalus beauforti</i>	muscle	2	1022 ± 62	979 – 1066	1.2	0.38
<i>Priacanthus hamrur</i>	muscle	7	188 ± 58	113 – 258	6.4	2
<i>Scarus ghobban</i>	muscle	1	53	-	22.7	7.3
<i>S. microrhinos</i>	muscle	1	30	-	39.5	12.6
<i>S. rivulatus</i>	muscle	1	26	-	46.1	14.8
<i>S. schlegeli</i>	muscle	1	37	-	32.2	10.3
<i>Plectropomus leopardus</i>	muscle	21	738 ± 576	173 – 2063	1.6	0.52
<i>Epinephelus coeruleopunctatus</i>	muscle	1	439	-	2.7	0.87
<i>E. maculatus</i>	muscle	1	389	-	3.1	0.99
<i>Acanthopagrus berda</i>	muscle	2	368 ± 168	249 – 486	3.2	1

^a reconstructed data

Table 4

Comparison of total Hg concentrations (T-Hg; mean $\pm$ SD, ng g⁻¹ dry wt) in the whole soft parts of the bivalves *Comptopallium radula*, *Anadara scapha* and *Gafrarium tumidum* and in the muscle of the fish *Plectropomus leopardus* collected from different sites in New Caledonia. MSWC is the estimate of the “Maximum Safe Weekly Consumption” for total Hg (T-Hg) and methyl-Hg (Me-Hg) (kg of flesh wet wt) from each site

Species	Site	n	T-Hg concentration (ng g ⁻¹ dry wt)		Group				MSCW (kg)	
			Mean ± SD	Range	1	2	3	4	T-Hg	Me-Hg
Bivalves (whole soft parts)										
<i>Anadara scapha</i>			One-way ANOVA and Tukey test ^a							
	Ouano Bay S2	5	103 ± 39	73 – 169					14.6	-
	Ouano Bay S1	3	146 ± 20	130 – 169					10.3	-
	Koutio Bay	5	230 ± 111	129 – 354					6.5	-
	Maa Bay	5	304 ± 165	164 – 588					4.9	-
	Sainte Marie Bay	5	337 ± 145	224 – 279					4.4	-
	Grande Rade-SLN	3	515 ± 290	184 – 724					2.9	-
	Grande Rade	2	723 ± 414	430 – 1015					2.1	-
<i>Comptopallium radula</i>			ANCOVA (cov.: length) and Tukey test ^a							
	Noumea harbour	3	99 ± 13	84 – 108					15.2	-
	Maa Bay	6	116 ± 19	93 – 144					12.9	-
	Sainte Marie Bay	7	125 ± 16	103 – 145					12.0	-
	Koutio Bay	4	199 ± 29	173 – 231					7.6	-
	Grande Rade	4	234 ± 24	203 – 261					6.4	-
<i>Gafrarium tumidum</i>			ANCOVA (cov.: weight) and Tukey test ^a							
	Unia	10	154 ± 38	111 – 224					9.7	-
	Ouano Bay S3	10	193 ± 45	146 – 281					7.8	-
	Ouano Bay S2	10	197 ± 68	106 – 295					7.6	-
	Grande Rade-SLN	10	253 ± 107	143 – 495					5.9	-
	Koutio Bay	10	299 ± 143	104 – 614					5.0	-
	Ouano Bay S1	10	310 ± 98	198 – 492					4.8	-
	Maa Bay	10	344 ± 194	113 – 741					4.4	-
	Tomo	10	497 ± 124	291 – 658					3.0	-
	Grande Rade	9	1633 ± 565	894 – 2531					0.92	-
Fish (muscle)										
<i>Plectropomus leopardus</i>			ANCOVA (cov.: length) and Tukey test ^a							
	Koutio Bay	4	336 ± 49	286 – 380					3.6	1.1
	Maa Bay	4	353 ± 82	245 – 445					3.4	1.1
	Sainte Marie Bay	3	629 ± 280	436 – 950					1.9	0.61
	Ouano Bay S3	4	856 ± 330	591 – 1338					1.4	0.45
	Grande Rade	5	1451 ± 697	449 – 2063					0.83	0.26

^a According to the results of ANOVA or ANCOVA followed by Tukey test, means which do not differ significantly within each species are joined by vertical bars (|)