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ENVIRONMENTAL POLLUTION

Environmental Pollution 146 (2007) 548-566

Bioaccumulation of trace elements in pelagic fish from the Western Indian Ocean

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Received 7 April 2006; received in revised form 17 July 2006; accepted 28 July 2006

Metal bioaccumulation was quantified in four species of pelagic fish.

Abstract

Trace elements were analyzed in fish of commercial interest to determine their importance in marine systems of the Western Indian Ocean and their bioaccumulation patterns. The results are equivalent or lower than levels reported in ichthyofauna worldwide. Certain values of muscular Cd, Hg, Pb and Zn were, however, above thresholds for human consumption. Levels varied among tissues, species and fish length, but were seldom influenced by the nutritional condition of the fish, its gender and its reproductive status. Correlations between hepatic Hg and Se levels in Swordfish ($r^2 = 0.747$) and Yellowfin Tunas ($r^2 = 0.226$), and among metallothionein linking metals imply the existence of detoxification processes in these species. Level differences between fish from the Mozambique Channel and Reunion Island reflect differences of diets rather than differences of elemental availability in both environments.

Keywords: Metals; Fish; Contamination; Detoxification; Guidelines

1. Introduction

Metals and metalloids, whether of natural or anthropogenic origin, are present in all ecosystems throughout the world. The occurrence of unnaturally high levels of metals in regions distant from anthropogenic activities, such as the Arctic and the Antarctic, is of concern, as it is an incentive to the study of metals in food webs of other isolated areas. Tropical waters are less monitored than marine environments from temperate and polar regions, more particularly southern tropical oceans which are often considered as less contaminated than the

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northern ones. Accordingly, the tropical zone of the Indian Ocean has, up to the present day, received little attention from researchers with reference to levels of trace elements in marine organisms (Kureishy et al., 1979; Matthews, 1983; Mwashote, 2003; Robinson and Shroff, 2004; Kojadinovic et al., in press).

Large fish such as Swordfish (*Xiphias gladius*), Yellowfin Tunas (*Thunnus albacares*), Skipjacks (*Katsuwonus pelamis*) and Common Dolphinfish (*Coryphaena hippurus*), which are at the top of marine food webs, are particularly exposed to high levels of trace elements through their food (Bryan, 1979). In addition, these pelagic organisms are high performance fish with very high metabolic rates, and consequently high food intake rates, a property that accentuates the exposure to trace elements. Because of their trophic position and bioaccumulation capacities, these fish will be used as bio-indicators

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of elemental levels to verify the pristine character of the tropical Western Indian Ocean waters.

Through the study of trace element levels in these four commercially important pelagic fish caught in the Mozambique Channel and in waters surrounding Reunion Island, this paper presents novel data on elemental impregnation of marine fish in the western part of the tropical Indian Ocean. Trace elements assimilated from food are transported in the blood, deposited in various tissues and excreted or stored. This paper aims to determine whether the global patterns of trace element accumulation and excretion are similar among species and between study sites. To address these points, three non-essential elements (cadmium (Cd), mercury (Hg) and lead (Pb)) and five essential elements (copper (Cu), iron (Fe), manganese (Mn), selenium (Se) and zinc (Zn)) were analyzed in the liver, the kidney and the muscle tissue.

The differences in elemental bioaccumulation among tissues were tested. In addition, the potential influence of factors such as physical condition, size, gender and reproductive status on elemental levels was investigated. Furthermore, species' elemental impregnations were compared within study zones, as well as between them. Because the levels of certain elements were dependent on the size of the fish, length-adjusted levels were used to compare their levels in each species found in Reunion Island waters and in the north of the Mozambique Channel. Finally, the relationships between elements were investigated through the study of the correlations existing among them. Furthermore, considering the nutritional value of fish and the large quantities consumed in this part of the world, our results are briefly discussed in the light of European guidelines for human consumption.

2. Materials and methods

2.1. Study site and species

The teleost species studied during this investigation were sampled in 2004 from two geographically distant sites in the Western Indian Ocean. The first site corresponds to waters surrounding Reunion Island, a 2512 km² French territory located 700 km east of Madagascar (21°7′S; 55°33′E). The second sampling zone was situated in the northern part of the Mozambique Channel, delimited by the following coordinates: 10°34′S and 17°07′S, and 41°11′E and 47°19′E (Fig. 1). These two sites correspond to two major fishing (purse seine and longline) areas of the Western Indian Ocean (Weenarain and Cayré, 1998).

Four pelagic marine fish (the Swordfish, the Yellowfin Tuna, the Skipjack and the Common Dolphinfish) were chosen for this study with respect to their bioaccumulation capacities and their importance in human consumption.

2.2. Fish sampling and preparation

A total of 72 fish were sampled in the northern part of the Mozambique Channel and 115 in waters surrounding Reunion Island. Sample information is given in Table 1. Each fish was measured, and weighed when possible. The LJFL (from the tip of the lower jaw to the fork of the caudal fin) was measured on Swordfish whereas the FL (from the tip of the snout to the fork of the caudal fin) was noted for the others species. Individuals were sexed during dissection by the examination of the gonads. The nutritional and reproductive states of each animal were assessed as they may affect the elemental levels. Consequently, gonads were weighed in order to calculate the gonadosomatic index (GSI) as it is generally considered a good measure of gonad maturation and spawning readiness. The GSI is based on the broad assumption that proportionally larger gonads indicate greater development (West, 1990). It was calculated as follows:

$$GSI = \frac{Gonad\ weight}{Body\ weight} \times 100$$

Furthermore, the liver was weighed and used to calculate a hepatosomatic index (HSI) which serves as an indicator of the body condition of the individual.

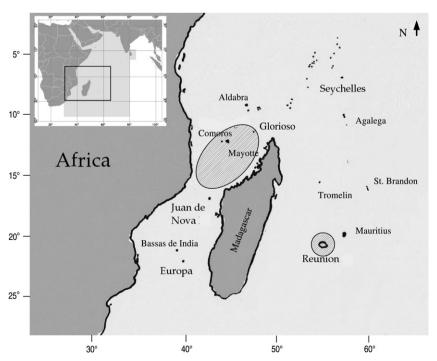


Fig. 1. Map of the study area with sampling zones materialized by shaded circles.

Table 1 Sample information on pelagic fish from the tropical Western Indian Ocean

Species	Moza	mbique Channel		Reunion Island					
	n	Mean length (cm) (min-max)	Weight (kg) Mean (min-max)	n	Mean length (cm) (min-max)	Weight (kg) Mean (min-max)			
Swordfish Xiphias gladius	42	122	21	14	127	22			
		(75-191)	(3-74)		(90-187)	(6-69)			
Yellowfin Tuna Thunnus albacares	24	107	21	21	104	24			
		(65-156)	(5-55)		(49-170)	(2-75)			
Skipjack Tuna Katsuwonus pelamis	0			38	68	9			
					(41-85)	(1-16)			
Common Dolphinfish Coryphaena hippurus	6	109	9	42	87	5			
		(100-115)	(7-11)		(61-112)	(1-10)			

$$HSI = \frac{Liver\ weight}{Body\ weight} \times 100$$

Due to the limited data on the body weight of the sampled fish, this parameter was extrapolated from the length using weight—length relationships presented in Table 2.

The liver, kidneys and digestive system were removed and placed in separate plastic bags. White muscle was sampled for analysis in the abdominal area above the vent of the fish. Samples were transported to the laboratory in coolers and frozen at $-20\,^{\circ}\text{C}$. To prepare for elemental determination livers, kidneys and muscles were partially thawed out, blended, dried and ground to a fine powder. Muscles were dried in an oven at 50 $^{\circ}\text{C}$ to constant mass for 72 h whereas liver and kidney samples were freeze dried.

2.3. Metal analysis

The analysis of Cd, Cu, Fe, Mn, Pb, Se and Zn calls for an extra step in the preparation protocol. Two aliquots of 0.2-0.4 g of each sample were digested with 3.5 ml of 15 N nitric acid at 60 °C for 48 h before being diluted to 10 ml with deionized water. To avoid metal contamination, all glass and plastic utensils were washed with detergent, plunged in a bath of mixed nitric (35 ml 1^{-1}) and chlorhydric (50 ml 1^{-1}) acids for a minimum of 24 h, rinsed three times in deionized (Milli-Q quality) water and dried in an oven at 50 °C before use.

Cadmium, Cu, Fe, Mn, Pb, Se and Zn were analyzed by Inductive Coupled Plasma Atomic Emission Spectrometry (ICP-AES Varian Vista Pro CCD). Total mercury (Hg) analysis was carried out with an Advanced Mercury Analyzer (ALTEC AMA 254) on aliquots ranging from 7 to 24 mg of dry sample weighed to the nearest 0.01 mg.

Accuracy and reproducibility of the preparation were tested by preparing replicates of lobster hepatopancreas (TORT-2), dogfish liver (DOLT-2) and dogfish muscle (DORM-2) reference standards (National Research Council, Canada) and blanks along with each set of samples. Detection limits and recovery rates are presented in Table 3. Results under the detection limit were considered as null values.

Table 2 Relationships between weight and length for four tropical pelagic fish: data from studies in the Indian Ocean

Species	n	a	b	r^2	References
Swordfish	430	1.75×10^{-6}	3.34	0.96	
V 11 C T (C 1)	104	54 10=6	0.70	0.07	Taquet (2001)
Yellowfin Tuna (females)		54×10^{-6}			Tantivala (2000)
Yellowfin Tuna (males)		41×10^{-6}			Tantivala (2000)
Skipjack Tuna		6.65×10^{-6}			This study
Common Dolphinfish	51	5.94×10^{-6}	3.04	0.93	This study,
					Bourjea, pers.
					comm.

These relationships are presented as exponential regressions on the following model: $W = aL^b$ where W is the fish weight and L the length.

Elemental levels are expressed in $\mu g \, g^{-1}$ of dry weight (d.w.). The mean moisture content in each species went from 67 to 73% in liver, from 65 to 77% in kidney and from 67 to 82% in muscle. They were used for the conversion of our results to wet weight basis (w.w.) for comparison with other studies.

2.4. Data analysis

Statistical analysis was performed using the GNU R statistical system (R Development Core Team, 2005). All statistical samples submitted to tests were first checked for normality and homogeneity of the variances by means of Shapiro—Wilk and Bartlett tests, respectively. In the case of non-departure from normality, parametric tests were used in the subsequent analysis, otherwise, non-parametric analogues were used.

The significance of differences of trace element levels among tissues was tested by one way repeated measures analysis of variance (ANOVA) or Friedman (F) tests and followed by multiple pairwise comparison t-tests (t) or Wilcoxon (W) tests for paired samples using Bonferroni's p-value correction. The influence of species on elemental levels was tested by means of Kruskal-Wallis (KW) tests followed by Wilcoxon tests for independent samples using Bonferroni's p-value correction. The influence of sex and sampling location on trace element levels was tested by means of t-tests or Wilcoxon tests. Trace element content in fish tissues may be influenced by the size of the individual (e.g. Jaffar and Ashraf, 1988; Monteiro and Lopes, 1990; Bloom, 1992; Mormede and Davies, 2001; Canli and Atli, 2003; Agusa et al., 2005). The difference in the average length of fish is thus a bias when comparing subsamples using comparison tests such as those described above. In order to truly measure the effect of the sampling location, tested subgroups should contain fish of equal mean size. An analysis of covariance (ANCOVA) was thus performed for each species, in order to adjust the elemental levels in both sampling sites (Mozambique Channel and Reunion Island) using fish length as a covariate. The ANCOVAs were realized on log metal values and log lengths. Residuals were checked for normality by means of Shapiro tests, and for homoscedaticity by plotting fitted values vs. residuals (Venables and Ripley, 2002; Faraway, 2005). Furthermore, the potential influence of the reproductive status (GSI)

Table 3
Detection limits (DL), quantification limits (QL) and recovery rates

	,			•							
	DL ($\mu g g^{-1}$)	QL ($\mu g g^{-1}$)	Recovery rates (%)								
			DOLT-3	DORM-2	TORT-2						
Cd	0.0007	0.0024	90 ± 12	Not measured	86 ± 17						
Cu	0.0211	0.0706	72 ± 15	Not measured	76 ± 19						
Fe	0.1435	0.4781	84 ± 12	43 ± 3	72 ± 13						
Hg	0.0007	0.0023	Not measured	Not measured	101 ± 3						
Mn	0.0021	0.0072	_	45 ± 1	77 ± 13						
Pb	0.0096	0.0322	108 ± 24	141 ± 83	133 ± 41						
Se	0.0295	0.0986	85 ± 11	115 ± 35	91 ± 14						
Zn	0.1075	0.3582	98 ± 16	106 ± 14	86 ± 18						

and body condition (HSI) on elemental burdens was investigated, for each species (data from both areas combined), by means of Pearson's linear correlation coefficient. This method was also applied to detect dependencies among elemental levels. The resulting correlation matrices were represented by dendrograms built using the average linkage as aggregation criterion.

Levels of significance of the null hypotheses associated with these tests will be divided into classes of p-values represented by the following codes: NS \geq 0.05; *<0.05; **<0.01; ***<0.001. SD will stand for standard deviation and CV for coefficient of variation.

3. Results

3.1. Trace element levels

Levels of Cd, Cu, Fe, Hg, Mn, Pb, Se and Zn in liver, muscle and kidney of Swordfish, Yellowfin Tunas, Skipjack Tunas and Common Dolphinfish caught in the north of the Mozambique Channel and in Reunion Island waters are presented in Table 4, and their dispersion illustrated in Figs. 2–4.

The level sequence of the tested elements was, in most cases, equal in both areas, and very similar among species (Table 4). Trends were alike in liver and kidney, as they generally adopted the following sequence: Fe > $Zn > Cd > Cu \simeq Se >$ $Hg \simeq Mn > Pb$ and $Fe > Zn > Se \simeq Cd \simeq Cu > Hg \simeq Mn > Pb$, respectively (which indicates that the two adjacent elements exchange places in the sequence according to the species and the location). In muscle, Zn levels were always higher than Fe levels contrarily to what was noted above $(Zn > Fe > Se > Cu \simeq Hg \simeq Cd \simeq Mn > Pb)$. Furthermore, the sequence of non-essential metal is noteworthy since, in all species-location subgroups except Mozambique Channel Common Dolphinfish, it was as follows: Cd > Hg > Pb in liver and kidney, and Hg > Cd > Pb in muscle. Lead levels were low in all subgroups, for the three tissues, with a certain number of values under the detection limit and 50% under the quantification limit (QL) of the ICP-AES (Table 3). The reader should keep in mind that, consequently, the precision of these values is not maximal. Concerning the other elements, all levels were above the QL except for 12 values of Cu in Skipjack muscle. Contrarily, hepatic Cu levels were exceptionally high in Yellowfin Tunas sampled in Reunion waters.

3.2. Factors influencing trace element levels

3.2.1. Tissue related differences

The two major vectors of trace element assimilation in fish are water, through the gills, and more importantly food (Dallinger et al., 1987; Reinfelder et al., 1998; Smith et al., 2002). Metals and selenium circulate through the body via blood and are accumulated in various tissues. Statistical tests applied on all the sampled individuals showed that the accumulation of elements varied between liver, kidney and muscle. Lead was the exception since Pb levels did not differ significantly between tissues for Skipjacks ($p_{\rm Friedman}=0.15$), Swordfish ($p_{\rm Friedman}=0.184$; $p_{\rm Friedman}=0.05$) and Yellowfin Tunas ($p_{\rm Friedman}=0.683$; $p_{\rm Friedman}=0.076$) fished in the Mozambique Channel and Reunion waters, respectively. In Common Dolphinfish, levels of Pb were, however, higher in kidney

than in the other two tissues (p < 0.015; Table 4). The general accumulation pattern of Cd, Cu, Mn showed that liver (followed by kidney, then muscle) was a privileged tissue in all the four species. In Swordfish and Skipjacks, liver was also the target tissue for Fe, Hg, Se (except in Swordfish from Reunion) and Zn. Conversely, the allocations of Fe, Se and Zn in Yellowfin Tunas and Common Dolphinfish were oriented towards kidney (followed by liver and muscle), with the exception of Zn in Common Dolphinfish from the Mozambique Channel, Mercury levels in Mozambique Channel Common Dolphinfish were highest in muscle (followed by liver and kidney), while they were not significantly different in Common Dolphinfish from Reunion waters ($p_{\text{Friedman}} = 0.572$). Mercury accumulation in Yellowfin Tunas was also different between sites. In Yellowfin Tunas from the Mozambique Channel Hg accumulated mostly in kidney (followed by liver and muscle), while in individuals caught in Reunion waters Hg was mainly found in liver (followed by kidney, then muscle).

3.2.2. Influence of size

Hepatic, renal and muscular Hg levels were indeed correlated with the length of the fish in the four species (considering results of both sites combined). Cadmium levels in Skipjacks and Swordfish were also positively correlated to fish length in all three tissues. Selenium levels were influenced by size in all Skipjack tissues and in either liver or kidney of the other species. On the contrary, the low Pb levels did not show any correlation with fish length. Although essential elements are regulated by the organism, some were nevertheless correlated to fish length (Table 5).

3.2.3. Influence of sex, reproduction status and body condition

In the previous sections, males and females were pooled since, in most cases, no influence of sex was revealed within the species-location subgroups which were tested (all but Mozambique Common Dolphinfish and Reunion Yellowfin Tunas). Certain exceptions were, however, noted. In Skipjacks, muscular Fe and Se, and hepatic Zn were significantly higher in males than in females ($p_{t\text{-test}} = 0.029$, $p_{\text{Wilcoxon}} = 0.005$ and $p_{t\text{-test}} = 0.001$, respectively). Conversely, hepatic Cu levels were higher in females than in males ($p_{t\text{-test}} < 0.001$). In Common Dolphinfish, hepatic Fe, Mn and Zn levels were higher in females than in males ($p_{t\text{-test}} = 0.04$, 0.047 and 0.019, respectively). These results must be regarded cautiously since the effect of inter-sex fish length differences was not tested because of a lack of data.

In the light of the results of correlations between elemental levels, and the GSI and the HSI, the influence of both factors appeared to be species dependent. A GSI value of $0.25 \pm 0.13\%$ and an HSI mean value of $1.06 \pm 0.35\%$ were calculated for all Swordfish combined. The reproduction status was positively correlated only to hepatic Cu $(r^2 = 0.42, p = 0.031)$. Negative correlations were observed between HSI and renal $(r^2 = 0.304, p = 0.004)$, muscular $(r^2 = 0.24, p = 0.009)$ and hepatic $(r^2 = 0.134, p = 0.028)$ Hg, and hepatic Cd $(r^2 = 0.164, p = 0.014)$ and Se $(r^2 = 0.141, p = 0.014)$

Table 4
Hepatic, renal and muscular multielemental levels (mean \pm SD, μ g g⁻¹ d.w.) in fish from the Western Indian Ocean

Reunion Island

		Mozambique Ch	anne	el					Reunion Island									
		n: L = 42;		= 42; $n: L = 22;$ $n: L = 6; M = 6;$			Hypotheses tests results	n: L = 14; M = 7; K = 13		Yellowfin Tuna n: L = 21; M = 17; K = 13		Skipjack Tuna n: L = 38; M = 37; K = 27	,	Common Dolphinfish n: L = 39; M = 42; K = 38		Hypotheses tests results		
		$Mean \pm SD$	Mean \pm SD Mean \pm SD Mean \pm SD		·-	$\text{Mean} \pm \text{SD}$				Mean ± SD		Mean \pm SD						
		CV (%)		CV (%)		CV (%)		-	CV (%)		CV (%)		CV (%)		CV (%)			
Cd	Liver	163 ± 178 109	a	138 ± 60 44	a	32.3 ± 15.3 47	b	KW: 0.001	169 ± 156 92	a	126 ± 130 103	a	153 ± 95 62	a	18.7 ± 26.4 142	b	KW: <0.001	
	Muscle	1.04 ± 1.09 105	a	0.25 ± 0.21 82	b	0.12 ± 0.06 49	c	KW: <0.001		a	0.23 ± 0.20 87	b	0.61 ± 0.37	a	0.13 ± 0.16 119	c	KW: <0.001	
	Kidney	31.8 ± 27.2 85	a	3.39 ± 2.49 74	b	1.79 ± 0.76 42	b	KW: <0.001		a	24.1 ± 31.2 129	a	55.7 ± 50.8 91	a	5.91 ± 13.09 221	b	KW: <0.001	
Cu	Liver	54.7 ± 31.5 58	a	121 ± 74 61	b	40.2 ± 38.5 96	a	KW: <0.001	65.4 ± 102.6 157	ab	240 ± 620 259	ac	93.6 ± 75.3 81	bc	60.7 ± 64.3 106	a	KW: 0.01	
	Muscle	0.64 ± 0.32 50	a	0.97 ± 0.23 24	b	0.78 ± 0.17 22	ab	KW: <0.001	0.65 ± 0.46 70	ac	1.99 ± 1.47 74	bc	1.02 ± 0.89 88	ab	0.88 ± 0.62	a	KW: 0.03	
	Kidney	4.70 ± 2.15 46	a	2.56 ± 2.45 96	b	1.48 ± 0.94 63	b	KW: 0.001	2.09 ± 1.35 64	a	11.2 ± 10.2 91	b	6.97 ± 3.54 51	b	5.98 ± 0.83 14	b	KW: <0.001	
Fe	Liver	617 ± 278 45	a	734 ± 290 39	a	341 ± 110 32	b	KW: 0.002	558 ± 197 35	a	690 ± 571 83	a	1296 ± 897 69	b	211 ± 91 43	c	KW: <0.001	
		22.4 ± 19.1 85	a	39.6 ± 16.8 43		12.6 ± 2.22 18		KW: <0.001	39	a	50.6 ± 34.0 67		70.2 ± 34.4 49		23.4 ± 19.0 81	a	KW: <0.001	
	Kidney	340 ± 121 35	a	2415 ± 825 34	b	6394 ± 3614 57	c	KW: <0.001	469 ± 185 39	a	1068 ± 372 35	b	1033 ± 371 36	b	392 ± 98 25	a	KW: <0.001	
Hg	Liver	5.33 ± 8.76 164	a	0.65 ± 0.52 81	b	0.61 ± 0.43	b	KW: <0.001	9.44 ± 11.81 125	a	3.27 ± 8.11 248	b	0.51 ± 0.28 55	b	0.20 ± 0.16 82	c	KW: <0.001	
	Muscle	1.61 ± 1.11 69	a	0.56 ± 0.38 67	b	0.98 ± 0.92 94	ab	KW: <0.001	3.97 ± 2.67	a	1.15 ± 2.30 200	b	0.67 ± 0.26 39	b	0.21 ± 0.19 91	c	KW: <0.001	
	Kidney	2.93 ± 2.71 95	a	1.57 ± 1.23 78	ab	0.43 ± 0.23 55	b	KW: 0.02	4.03 ± 4.74 118	a	1.48 ± 1.76 119	ab	0.57 ± 0.29 50	b	0.17 ± 0.15 90	c	KW: <0.001	
Mn	Liver	3.73 ± 0.72 19	a	5.16 ± 0.96 19	b	7.20 ± 0.96 13	c	KW: <0.001	3.51 ± 0.67 19	a	5.05 ± 1.31 26	b	4.84 ± 1.19 25	b	5.17 ± 1.21 23	b	KW: <0.001	
	Muscle	0.24 ± 0.23 98	a	0.27 ± 0.11 41	b	0.26 ± 0.02	ab	KW: 0.004	0.18 ± 0.05 28	a	0.30 ± 0.12 40	b	0.36 ± 0.16 44	b	0.33 ± 0.07 23	b	KW: <0.001	
	Kidney	2.17 ± 0.59 27	a	$0.61 \pm 0.33b$ 54		1.00 ± 0.06	c	KW: <0.001	1.62 ± 0.27 17	a	1.73 ± 0.64 37	a	1.50 ± 0.96 64	a	2.75 ± 0.51 18	b	KW: <0.001	
Pb	Liver	0.18 ± 0.19 105		0.13 ± 0.12 91		0.21 ± 0.10 46		KW: NS	0.09 ± 0.08 95	a	0.05 ± 0.08 149	a	0.12 ± 0.14 111	a	0.01 ± 0.03 448	b	KW: <0.001	
	Muscle	0.12 ± 0.12 97		0.09 ± 0.14 148		0.14 ± 0.05 35		KW: NS	0.01 ± 0.04 265	ab	0.02 ± 0.07 309	b	0.07 ± 0.08 124	a	0.06 ± 0.16 292	ab	KW: 0.01	
	Kidney	0.29 ± 0.59 277	ab	0.13 ± 0.15 116	b	0.61 ± 0.26 42	a	KW: 0.014	0.04 ± 0.06 153		0.08 ± 0.09 125		0.15 ± 0.31 215		0.15 ± 0.19 131		KW: NS	

b KW: <0.001	a KW: <0.001	c KW: <0.001	d KW: <0.001	KW: NS	a KW: <0.001
a 13.5 ± 9.5 70	c 3.17 ± 3.21	b 20.0 ± 3.2 16	bc 135 ± 29	65.8 ± 38.3 58	c 172 ± 72 42
a 73.2 ± 25.1 34	b 15.8 ± 12.2 78	a 40.0 ± 14.1 35	a 208 ± 61	125 ± 94	$ \begin{array}{ccc} 5 & 114 \pm 30 \\ 26 & 26 \end{array} $
a 83.5 ± 42.3 51	ab 6.26 ± 4.03	a 125 ± 58 47	ab 516 ± 680	160 ± 135 84	ab 1467 ± 1851 126
c KW: <0.001 77.6 \pm 54.2	a KW: $<0.001 4.00 \pm 1.78$	c KW: <0.001 98.6 ± 44.3	c KW: $<0.001 239 \pm 45$	KW: NS 73.5 ± 49.8	a KW: <0.001 177 \pm 16
b 13.8 ± 7.07 51	b 1.85 ± 0.85 46	b 24.6 ± 4.94 20	b 146 ± 3	44.7 ± 11.9	b 134 ± 41.7 31
a 90.8 ± 13.7 15	a 5.00 ± 1.78	a 112 ± 23 21	a 439 ± 254	64.1 ± 47.3	a 23554 ± 2019 86
Se Liver 53.1 ± 0.82 101	Muscle 2.45 ± 1.21	Kidney 49.4 ± 13.4 27	Zn Liver 213 ± 73	Muscle 41.7 ± 34.7	Kidney 173 ± 33.6 19

Inter-specific comparison test results for each study site are given in the last columns. The significances of the differences among species are indicated by letters. L: Liver; M: Muscle; K: Kidney; KW: Kruskal Wallis test; NS: non significant. p = 0.024). Positive correlations were noted for renal and muscular Mn ($r^2 = 0.188$, p = 0.034; $r^2 = 0.139$, p = 0.042, respectively).

In Yellowfin Tunas, the absence of dependencies between elemental levels and GSI $(0.28 \pm 0.21\%)$ attests that there was no influence of the reproduction status on metal levels. Renal Pb was positively correlated to the fish's body condition (HSI = $0.65 \pm 0.24\%$; $r^2 = 0.211$, p = 0.011). Negative correlations were noted between HSI and Zn in liver ($r^2 = 0.185$, p = 0.007) and muscle ($r^2 = 0.126$, p = 0.031), and Hg in muscle ($r^2 = 0.147$, p = 0.021).

The GSI in Skipjacks $(2.03 \pm 0.90\%)$ was correlated to the largest number of elements: hepatic $(r^2 = 0.16, p = 0.021)$ and muscular $(r^2 = (-) 0.146, p = 0.034)$ Mn, hepatic Se $(r^2 = 0.159, p = 0.021)$, renal Fe $(r^2 = 0.156, p = 0.046)$ and hepatic Cd $(r^2 = 0.149, p = 0.026)$. Furthermore, hepatic $(r^2 = 0.292, p = 0.001)$ and muscular $(r^2 = 0.18, p = 0.022)$ Cd, and muscular Fe $(r^2 = 0.137, p = 0.048)$ were negatively correlated to the HSI $(0.80 \pm 0.31\%)$.

The Common Dolphinfish was the only species for which there was no relation between the HSI (1.15 \pm 0.84%) and elemental burdens. Nevertheless, the GSI (1.28 \pm 1.21%) was positively correlated to renal ($r^2 = 0.224$, p = 0.005) and muscular ($r^2 = 0.169$, p = 0.017) Hg and hepatic Mn ($r^2 = 0.206$, p = 0.013).

3.2.4. Geographical differences

The comparison of the two study areas in term of fish elemental impregnation is presented in Figs. 2—4. The significance of the level difference, which is indicated for each species below the boxplots, corresponds to the result of a *t*-test or a Wilcoxon test when the given element was not correlated to the length of the fish considered. Otherwise (Table 5), ANCOVAs were applied in order to eliminate the bias of having different fish sizes between sampling areas. In this case, the result which is given corresponds to the level of significance of the null hypotheses associated to the regression slope of the elemental level against fish length.

Cadmium levels in the kidney of Reunion Yellowfin Tunas were above those found in the Mozambique Channel. In contrast, Cd levels in Swordfish muscle and Common Dolphinfish liver were highest in the Mozambique Channel. For Cu, muscular and hepatic levels were not different from one zone to the other, contrarily to renal levels which were higher in Reunion for Common Dolphinfish and Yellowfin Tunas and in the Mozambique Channel for Swordfish. Similar muscular and hepatic Fe burdens were found in Swordfish and Yellowfin Tunas from both areas. However, in Common Dolphinfish, Fe levels were significantly different in all tissues. Dolphinfish Mn and Pb levels were also significantly different between areas in all tissues, with higher Pb burdens in Mozambique Channel fish. The results obtained for the other species were variable. Mercury and selenium followed the same trends in the three tissues. Whether significantly or not higher levels were found in Swordfish from Reunion waters and in Common Dolphinfish from the Mozambique Channel. In the Yellowfin Tuna, Hg and Se burdens were similar between areas

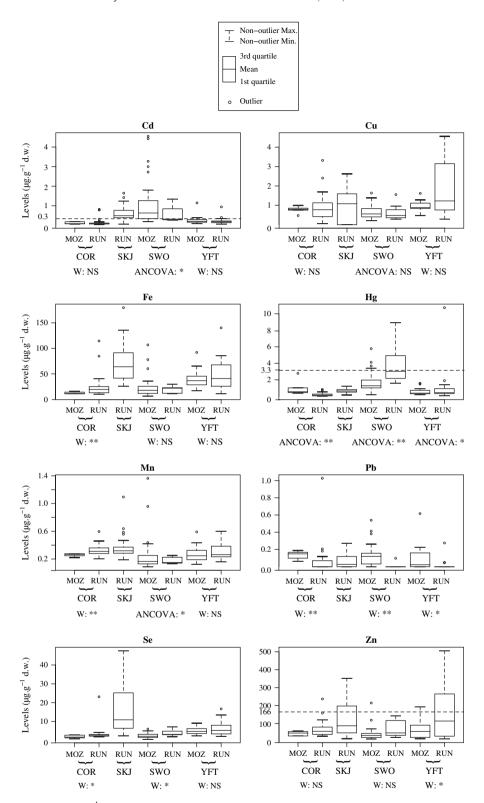


Fig. 2. Comparison of elemental levels ($\mu g g^{-1} d.w.$) in muscle of fish caught in the Mozambique Channel (MOZ) and Reunion Island waters (RUN). For each species, the significances of the level differences between both study sites are indicated below the boxplots. Horizontal dotted lines mark the European guidance levels for human consumption. COR: Common Dolphinfish; SKJ: Skipjack Tunas; SWO: Swordfish; YFT: Yellowfin Tunas. W: Wilcoxon test; ANCOVA: Analysis of covariance; NS: non significant.

of origin. For Swordfish, Yellowfin Tunas and Common Dolphinfish, Zn levels were not significantly different between areas regardless of the tissue, except for Yellowfin Tuna kidney and muscle. It is interesting to note that within a species the level of an element was, in some cases, higher in Mozambique Channel fish for one tissue and higher in Reunion fish for another tissue (e.g. Fe and Mn in Dolphinfish and Zn in Yellowfins).

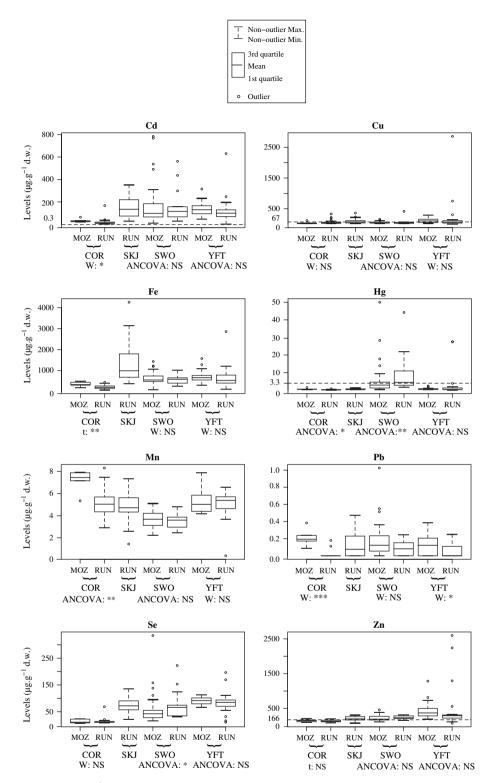


Fig. 3. Comparison of elemental levels ($\mu g g^{-1} d.w.$) in liver of fish caught in the Mozambique Channel (MOZ) and Reunion Island waters (RUN). For each species, the significances of the level differences between both study sites are indicated below the boxplots. Horizontal dotted lines mark the European guidance levels for human consumption. COR: Common Dolphinfish; SKJ: Skipjack Tunas; SWO: Swordfish; YFT: Yellowfin Tunas. W: Wilcoxon test; t: t-test; ANCOVA: Analysis of covariance; NS: non significant.

3.2.5. Inter-specific differences

Detailed results on inter-specific differences are presented in Table 4. Generally the ranking of the four species with respect to their elemental burdens was equivalent in both study zones. Several general trends can be highlighted, as for Se, and Cu, Fe and Zn in liver, which levels were highest in Yellowfin Tunas and lowest in Common Dolphinfish (Swordfish and Skipjacks showing intermediate values). Cadmium and Hg also shared common patterns: Swordfish being the most impregnated and Common Dolphinfish the least. It is however

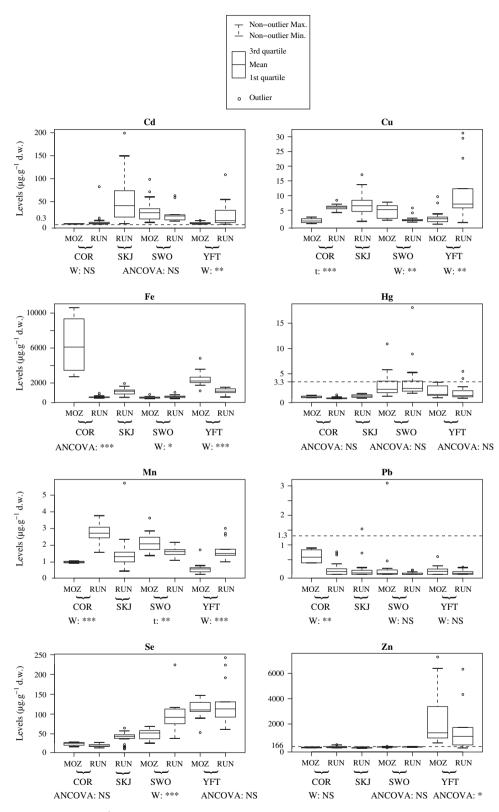


Fig. 4. Comparison of elemental levels ($\mu g g^{-1} d.w.$) in kidney of fish caught in the Mozambique Channel (MOZ) and Reunion Island waters (RUN). For each species, the significances of the level differences between both study sites are indicated below the boxplots. Horizontal dotted lines mark the European guidance levels for human consumption. COR: Common Dolphinfish; SKJ: Skipjack Tunas; SWO: Swordfish; YFT: Yellowfin Tunas. *W*: Wilcoxon test; *t*: *t*-test; ANCOVA: Analysis of covariance; NS: non significant.

Table 5 Correlations between elemental levels and fish length, expressed by determination coefficients (r^2) and p-values

	· / 1				
	r^2	<i>p</i> -value		r^2	p-value
Swordfish			Skipjack Tuna		
Hepatic Cd	0.35	< 0.001	Hepatic Cd	0.58	< 0.001
Muscular Cd	0.20	< 0.001	Muscular Cd	0.24	0.01
Renal Cd	0.53	< 0.001	Renal Cd	0.46	< 0.001
Hepatic Cu	0.14	0.01	Hepatic Fe	0.29	< 0.001
Muscular Cu	(-) 0.20	< 0.001	Muscular Fe	0.43	< 0.001
Hepatic Hg	0.49	< 0.001	Hepatic Hg	0.49	< 0.001
Muscular Hg	0.34	< 0.001	Muscular Hg	0.60	< 0.001
Renal Hg	0.51	< 0.001	Renal Hg	0.69	< 0.001
Hepatic Mn	(-) 0.14	0.01	Renal Mn	0.13	0.09
Muscular Mn	(-) 0.18	< 0.001	Hepatic Se	0.13	0.05
Hepatic Se	0.29	< 0.001	Muscular Se	0.34	< 0.001
Hepatic Zn	0.09	0.03	Renal Se	0.61	< 0.001
Renal Zn	0.19	0.03			
Yellowfin Tuna			Common Dolph	ninfish	
Hepatic Fe	0.31	< 0.001	Renal Fe	0.10	0.05
Hepatic Hg	0.11	0.03	Hepatic Hg	0.26	< 0.001
Muscular Hg	0.30	< 0.001	Muscular Hg	0.16	0.01
Renal Hg	0.61	< 0.001	Renal Hg	0.37	< 0.001
Hepatic Se	0.33	< 0.001	Hepatic Mn	0.16	0.01
Renal Se	0.26	< 0.001	Renal Se	0.14	0.02
Hepatic Zn	0.45	< 0.001			
Renal Zn	0.33	< 0.001			

Negative correlations are marked by (-).

noteworthy that, in spite of its small size and short lifespan, Skipjacks had Cd levels equivalent to those found in the Swordfish. Hardly any differences existed among species in terms of Pb burdens. Furthermore, the Common Dolphinfish, which is the smallest species in terms of weight, had the highest levels of renal Fe and Mn (in all tissues for fish caught around Reunion and in liver for Mozambique Channel fish).

3.3. Relationships between trace element levels

Accumulation behaviors of trace elements in the organism result in part from their interaction. The correlation matrices

linking trace element levels in the various tissues are represented, for each species, by the dendrograms of Figs. 5–8.

Two types of linear correlations were obvious: (i) correlations of levels of one element in various tissues and, (ii) correlations between different elements in the same tissue. (i) Cadmium and Hg stood out by their strong tissue-to-tissue correlations in Swordfish, Skipjacks and Common Dolphinfish. Mn was also correlated in the liver, kidney and muscle of Swordfish. Selenium was correlated in at least two tissues in the Yellowfin Tunas and the Common Dolphinfish. (ii) The following significant correlation between elements was common to the four species: Fe, Se and Zn in muscle, and Cu, Cd and Se in liver (limited to Cu and Se in Skipjacks). Relationships among elements were more species-specific in kidneys with evident correlations among Cu, Fe and Se in Swordfish; Fe, Mn and Zn in Yellowfin Tunas; Fe and Se, and Cu and Zn in Skipjacks; and Cu, Fe and Mn in Common Dolphinfish. Moreover, the correlation between Hg and Se in liver increased with the Hg impregnation among species (Figs. 5–8): Common Dolphinfish $(r^2 = 0.007; p > 0.05)$, Skipjacks ($r^2 = 0.063$; p > 0.05), Yellowfin Tunas ($r^2 =$ 0.226; p = 0.001) and Swordfish ($r^2 = 0.747$; p < 0.001).

4. Discussion

Element levels varied among tissues and species, and in many cases, with fish length. Individual patterns of elemental bioaccumulation will thus be discussed according to the tissue, the fish's physiology and detoxification processes. Furthermore, the differences in levels observed between fish of the Mozambique Channel and Reunion Island will be interpreted and levels compared with other oceans and health guidelines.

4.1. Individual patterns of elemental bioaccumulation

4.1.1. Elemental distribution among tissues

The general accumulation pattern of Cd, Cu, Mn (liver > kidney > muscle) confirmed what has previously been

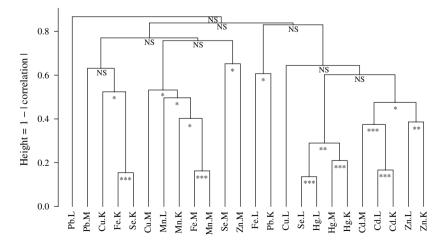


Fig. 5. Dendrogram illustrating linear correlations between trace elements in the Swordfish. The minimum levels of significance of the linear correlation coefficients among groups of elemental levels are encoded in the dendrogram. NS: non significant. The letters ".L", ".K", ".M" and placed after the abbreviation of an element indicate the tissues (respectively, liver, kidney and muscle) in which the element is considered.

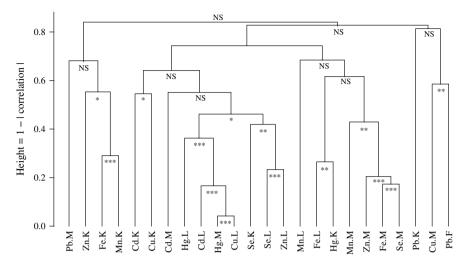


Fig. 6. Dendrogram illustrating linear correlations between trace elements in the Yellowfin Tuna. The minimum levels of significance of the linear correlation coefficients among groups of elemental levels are encoded in the dendrogram. NS: non significant. The letters ".L", ".K", ".M" and placed after the abbreviation of an element indicate the tissues (respectively, liver, kidney and muscle) in which the element is considered.

observed by most authors (e.g. Mackay et al., 1975; Jaffar and Ashraf, 1988; Canli and Atli, 2003; Bustamante et al., 2003; Storelli et al., 2005a; Licata et al., 2005). Interestingly the accumulation pattern of Cd in fish differs from that of other vertebrates such as birds, mammals and turtles in which kidney is the target tissue for Cd (e.g. Furness and Rainbow, 1990; Furness et al., 1993; Thompson et al., 1996; Dietz et al., 1996; Storelli et al., 2005b).

Literature on the distribution of Fe, Se and Zn shows that there exists a certain variation in accumulation patterns among tissues according to the species (Mackay et al., 1975; Canli and Atli, 2003; Deheyn et al., 2005). These observations corroborate our results since the main target tissue of these elements was different in Swordfish and Skipjacks vs. Yellowfin Tunas and Common Dolphinfish.

Hg in fish tissues usually follows the liver > muscle order in terms of accumulation levels (Mackay et al., 1975; Storelli

et al., 2005a). Yet, dominating Hg levels in muscle, as it was noted for Common Dolphinfish of the Mozambique Channel, have also been observed in Bluefin Tunas fished in the Messina straits (Licata et al., 2005) and in benthic fish caught around the Kerguelen Islands (Bustamante et al., 2003). Moreover, elemental turnover rates are, as a general rule, higher in liver and kidney than in muscle. Hence, elemental levels in the muscle reflect the elemental intake on a longer time frame than the other tissues. Since Common Dolphinfish are susceptible to cover large distances, it can be suggested that their muscle impregnation is the result of the accumulation of trace elements from preys living in other environments than where the fish were caught and which contained higher Hg levels than those consumed by Common Dolphinfish in the Northern Mozambique Channel.

The only organotropism of Pb was noted in Common Dolphinfish where levels were highest in kidney. It is difficult to

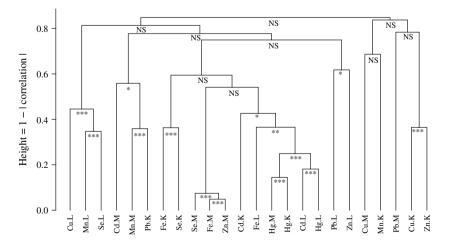


Fig. 7. Dendrogram illustrating linear correlations between trace elements in the Skipjack Tuna. The minimum levels of significance of the linear correlation coefficients among groups of elemental levels are encoded in the dendrogram. NS: non significant. The letters ".L", ".K", ".M" and placed after the abbreviation of an element indicate the tissues (respectively, liver, kidney and muscle) in which the element is considered.

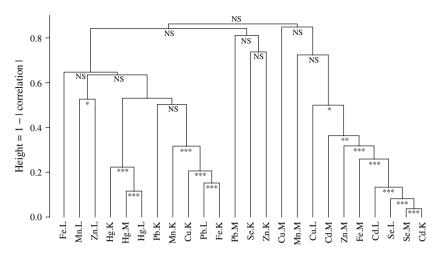


Fig. 8. Dendrogram illustrating linear correlations between trace elements in the Common Dolphinfish. The minimum levels of significance of the linear correlation coefficients among groups of elemental levels are encoded in the dendrogram. NS: non significant. The letters ".L", ".K", ".M" and placed after the abbreviation of an element indicate the tissues (respectively, liver, kidney and muscle) in which the element is considered.

compare this result to others since renal concentrations are rarely investigated in fish. Most studies in fish include liver and muscle and conclude that Pb levels are higher in the former than in the latter (Mackay et al., 1975; Mormede and Davies, 2001; Canli and Atli, 2003; Storelli et al., 2005a). The absence of organotropism in the other species may be attributed to the low Pb levels found in these fish.

4.1.2. Physiology dependent element accumulation

Metabolic activity in young individuals is usually higher than in older fish (Canli and Atli, 2003). Since the contaminant uptake rate is positively linked to the metabolic rate in marine animals, it can be supposed that metal accumulation would be highest in young fish. Furthermore a dilution effect of growth on elemental levels may also explain the negative correlation observed in this study with muscular Cu and Mn in Swordfish. The variability of the relationships observed for the other essential elements (positive or no correlation) may simply be attributed to their contrasted elemental regulation in relation with the environmental conditions, and by consequent physiological needs, at a given period. As for Cd, Hg and Se, their constant accumulation over the fish's lifetime and the absence (or limited) excretion of these elements results in their positive correlation with fish length. Added to the large range of sizes of the fish in each subgroup (Table 1), the bioaccumulation of Cd, Hg and Se with size is most probably the cause of the large individual variations in levels observed within species-location subgroups (high CVs in Table 4). Furthermore, in Yellowfin Tunas and Common Dolphinfish, for which Cd burden was not correlated with fish length, it is possible that both phenomena described above counteracted leading to a stabilization of Cd levels. This hypothesis could also be made for Pb, the levels of which do not correlate with the age/size of animals (Mackay et al., 1975; Dietz et al., 1996).

In most cases, elemental levels measured in male and female fish were not different in spite of physiological specificities of each gender, such as higher growth rates in female Swordfish. This observation was substantiated by the relatively small impact that the physiological changes implicated in reproduction had on trace element levels in these marine fish. The correlations between GSI and elemental levels, mostly essential elements, were different among the four species. The accumulation of essential elements during a period such as reproduction seems natural to account for the increased activity of the metabolism. The decrease of Mn levels with GSI in Skipjacks muscle indicates that this element is probably mobilized from muscle during the period of gonad development to be used elsewhere.

The decrease of some trace element levels with the increase of the HSI, or body condition, in Swordfish, Yellowfin Tunas and Skipjacks is probably due to a differential increase of the liver and the body weight during fish growth.

4.1.3. Detoxification processes

The basis of metal toxicity is the inhibition of enzymatic systems of cells, resulting from substitution of other metal ions (mainly Cu²⁺, Zn²⁺ and Ca²⁺) (Jackson, 1998). One of the most common detoxification strategies observed in marine vertebrates is the binding of metals to metallothioneins (Mason and Jenkins, 1995). Metallothioneins (MTs) provide protection against toxic effects of certain metals, Cd in particular, by sequestrating and reducing the amount of free metal ions and acting as a "rescue" function for structures impaired by inappropriate metal-binding (Hamilton and Mehrle, 1986; Vallee, 1995; Roesijadi, 1996). Since their discovery, in 1957, the existence of MTs was firmly established in a large number of animals including fish, in which they occur in liver, kidney, gill and muscle (Hogstrand and Haux, 1991; Hamza-Chaffai et al., 1995; Olsson et al., 1996; De Boeck et al., 2003; Scudiero et al., 2005). Besides Cd, MTs also have high affinities to Ag, Cu, Hg and Zn. These ions may be simultaneously linked to this protein. Hence, although MTs were not investigated in the present study, the correlations between Cd, Cu, Hg and Zn may provide clues to whether MTs play

a detoxifying role in the studied fish. The comparatively very low Cd levels in the muscle of all fish (Table 4) suppose effective processes of Cd sequestration in liver and kidney. Moreover positive correlations were noted between levels of Cd and levels of Cu, Hg and Zn in liver and kidney in all four species (Table 6 and Figs. 5–8). It is thus possible that renal and hepatic MTs could act as a sink for toxic Cd and Hg or for the excess Cu and Zn in these species. This hypothesis should, of course, be substantiated by MTs analysis.

Another prevalent detoxification process relies on the insolubilization of metals as mineral concretions. A typical example is the formation of non-toxic insoluble tiemannite granules after the mobilization and demethylization of Hg by Se in the liver of many marine mammals, as well as certain birds and fish (Koeman et al., 1973; Mackay et al., 1975; Martoja and Berry, 1980; Nigro et al., 2002; Decataldo et al., 2004; Ikemoto et al., 2004). Among the four species considered here, the correlation between Hg and Se in liver increased with the Hg impregnation of the given species: Common Dolphinfish $(r^2 = 0.007; p > 0.05)$, Skipjacks $(r^2 = 0.063; p > 0.05)$, Yellowfin Tunas $(r^2 = 0.226; p = 0.001)$ and Swordfish $(r^2 = 0.747; p < 0.001)$. Although the mean molar ratios of Se:Hg in liver were different from 1:1 in Swordfish (57:1) and in Yellowfin Tunas (483:1), the significant correlations between these two elements provide an argument for the existence of Se mediated Hg detoxification in both species. Indeed, it is possible that the large quantities of Se in the liver conceal the presence of Se:Hg granules. Further investigation is needed to confirm the presence of tiemannite granules in large fish such as Swordfish.

4.2. Trace element levels in fish of the Western Indian Ocean

The pelagic fish considered in this investigation are ideal bioaccumulators as they are characterized by metabolic rates approximately three folds higher than the most active fish species (Korsmeyer and Dewar, 2001), as well as digestion and growth rates two to five times higher than those of other species of equal body size (Storelli et al., 2005a). However, these

Table 6 Correlations between Cd and Cu, Hg and Zn levels, expressed by determination coefficients (r^2) and p-values

	n	r^2	<i>p</i> -value		n	r^2	<i>p</i> -value
Swordfish				Skipjack Tuna			
Hepatic Cd-Cu	56	0.35	< 0.001	Hepatic Cd-Hg	38	0.67	< 0.001
Hepatic Cd-Hg	56	0.46	< 0.001	Hepatic Cd-Zn	38	0.28	0.001
Hepatic Cd-Zn	56	0.55	< 0.001	Renal Cd-Cu	27	0.23	0.011
Renal Cd-Hg	27	0.28	0.005	Renal Cd-Hg	27	0.66	< 0.001
Renal Cd-Zn	27	0.29	0.003	Renal Cd-Zn	27	0.19	0.023
Yellowfin Tuna				Common Dolphini	fish		
Hepatic Cd-Cu	43	0.72	< 0.001	Hepatic Cd-Cu	45	0.30	< 0.001
Hepatic Cd-Hg	43	0.32	< 0.001	Hepatic Cd-Hg	45	0.14	< 0.001
Hepatic Cd-Zn	43	0.23	0.001	Muscular Cd-Zn	48	0.20	0.001
Renal Cd-Cu	30	0.21	0.012				
Muscular Cd-Hg	38	0.69	< 0.001				

four fish cover a large ranges of sizes, habitats (in terms of depth), and differ somewhat in terms of their dietary preferences. These are potential factors explaining the differences in their trace element burdens. Swordfish, for example, were the most impregnated in Hg and Cd. These fish stand out by their very large size and their long lifespan that allow longterm bioaccumulation. Furthermore, Swordfish feed on larger preys than Yellowfin Tunas and Common Dolphinfish, especially concerning cephalopods. Moreover, the high levels of Cd may be related to the high proportion of cephalopods in the diet of the sampled Swordfish, in comparison to the other three species which mostly preyed on fish (Potier, Unpubl. data). Indeed, cephalopods have been reported as a vector of the transfer of cadmium to top marine predators (Bustamante et al., 1998). Swordfish feed in epipelagic and mesopelagic waters. Mesopelagic waters, that are poorly oxygenated, house the process of Hg methylation, transformation of Hg from an inorganic to an organic form more easily absorbed by living organisms. Mesopelagic animals are thus more susceptible of having high Hg burdens and transferring them to organisms of higher trophic levels (Monteiro et al., 1996; Thompson et al., 1998). Among the four species, Swordfish is the most susceptible of integrating Hg through such a pathway since Yellowfin Tunas, Skipjacks and Common Dolphinfish feed almost exclusively on epipelagic preys (Quéro and Vayne, 1997; Opic et al., 1994; Potier et al., 2004). Yellowfin Tunas, for their part, had the highest levels of most essential elements. This latter species is phyllogenetically close to the Skipjack (Collette et al., 2001) which presented similar trace element values in many cases. Furthermore, with the exception of Mn, elemental levels were lowest in the Common Dolphinfish. This probably results from the Dolphinfish's small size, short lifespan (4 years) and very high growth rate (Massuti and Morales-Nin, 1997; NOAA fisheries, October 2005).

Non-essential metals do not present any function for the fish's metabolism and are by consequent not regulated by the metabolism. The amount of Cd, Hg and Pb in fish organisms can thus serve as an indication of environmental levels of these metals. For example, although not always in significant proportions, Pb mean levels were highest in tissues of all species caught in the Mozambique Channel. Hence, it could be assumed that Pb levels are higher in prey species inhabiting the north of the Mozambique Channel than in those found in waters surrounding Reunion Island. However, biological and ecological characteristics of each teleost species vary from one area to the other and may also affect metal levels. This was most likely the case for levels of Cd and Hg which predominance area was not always the same for the three species. These differences may be explained by shifts in the species' dietary prey composition and/or trophic position between areas, rather than differences in environmental levels. More specifically, the higher Cd levels in Swordfish and Common Dolphinfish sampled in the Mozambique Channel than in Reunion waters can be explained by a larger amount of cephalopods in their diet (50 and 38%, respectively, in the Mozambique Channel vs. 31 and 2%, respectively, in Reunion, determined by stomach content analysis, Potier, Unpubl. data). These conclusions are in accordance

with the results obtained for Hg in Swordfish. Mercury levels were highest in Reunion waters where Swordfish feed mainly on fish (68% with respect to 44% in the Mozambique Channel) which are regarded as Hg bioaccumulating prey (Arcos et al., 2002). A larger dataset of Common Dolphinfish from the Mozambique Channel (>6 fish) is needed to confirm that these fish are more impregnated in Cd, Hg and Pb than those collected in Reunion waters, irrespectively of their size. In Yellowfin Tunas, the higher Cd levels in Reunion were not in accordance with their piscivorous diet (99% fish in Reunion and 86% in the Mozambique Channel, Potier, Unpubl. data) as most fish species generally contain relatively low Cd levels (Bustamante et al., 2003). In the light of these results, it seems likely that the diet of Yellowfin Tunas from Reunion waters changes during the year and includes a significant proportion of cephalopods, which increases their exposure to Cd (Bustamante et al., 1998); changes in diet which may be linked to changes in habitat.

4.3. Comparison with other oceans and guidelines

Despite the human fishing pressure on Swordfish, tunas and, to a lesser extent, Dolphinfish from the Indian Ocean (Pianet, 1998), there is a great lack of knowledge on trace element levels in these fish. A compilation of data on trace element values from various studies (Tables 7 and 8) allows to compare the present results to those of phylogenetically related, or similar size species from the Indian Ocean and other locations in the world.

Few guidelines for effects of metals in fish have been established for the protection of the fish. Although they lack uniformity, guideline values are, however, available for fish consumers, both human and wildlife. The European Union has set regulations for Cd, Hg and Pb in muscle (legislations 466/2001 and 221/2002). Legal thresholds are inexistent for essential elements in Europe. Guidance values in muscle are, however, recommended in some countries, such as 20 $\mu g \, g^{-1} \, w.w.$ for Cu and 50 $\mu g \, g^{-1} \, w.w.$ for Zn (MAFF, 1995). None of the muscle samples reached the limits set for Cu. For Zn, although all mean values were inferior to 50 $\mu g \, g^{-1} \, w.w.$, some of the most Zn-enriched Yellowfin Tuna and Skipjack muscle samples were superior to the guidance level.

4.3.1. *Mercury*

A great deal of publications on marine fish report only Hg levels because this metal is of particular concern for human health (e.g. Carrington and Bolger, 2002; Marcotrigiano and Storelli, 2003; Burger and Gochfeld, 2004, 2005; Burger et al., 2005; Rasmussen et al., 2006). Inorganic mercury occurring naturally or from pollution is converted to methyl-mercury (MeHg) by microorganisms and is biomagnified up the food chain. Consequently, a large percentage of Hg is present as toxic MeHg in the edible portions of fish consumed by man (Cappon and Smith, 1981; Bloom, 1992; Wagemann et al., 1997). The most widely established guideline value for Hg levels in marine predatory fish is 1 μ g g⁻¹ w.w. (IPCS, 1987; EPA, 1994). In the Mozambique Channel and Reunion Island, 3 and 43% of the

Swordfish, respectively, had muscular Hg levels exceeding the authorized limits (Fig. 2). This last result on Reunion Swordfish should, however, be taken very cautiously and not generalized owing to the very small sample size (seven fish). In Swordfish liver, 40% of Hg values were above the permissible level in Mozambique Channel samples, and 64% in Reunion samples (Fig. 3). In kidney, the percentage of exceeding values was 29% in the Mozambique Channel samples, and 31% in Reunion samples (Fig. 4). For Yellowfin Tunas caught in Reunion Island waters the Hg guideline was reached in 5, 14 and 18% of muscle. liver and kidney samples, respectively. Overall, the Hg levels detected during this study were nevertheless congruent with, or lower than, Hg level found in the literature. For example, Hg levels in Yellowfin Tunas from Taiwan waters were 23 and 10 times superior to levels in Yellowfin Tunas from the Mozambique Channel and Reunion waters, respectively. Furthermore, Hg values in muscle of Common Dolphinfish from Reunion waters were five times lower than those measured in Common Dolphinfish of equal mean length fished in the Atlantic. The comparison of Hg values in marlins with those found in the present species is also noteworthy as the former are so much superior to the latter (Tables 7 and 8). In this section, hepatic and renal levels are compared to thresholds established for muscle. However, there are no health standards for liver and kidney because these organs are seldom, if ever, consumed or used for oil production, therefore concentrations exceeding the guideline values are unlikely to be a health hazard.

4.3.2. Cadmium

The accumulation of Cd is proportionally less oriented towards the muscle than Hg. Cadmium is, however, highly toxic (e.g. Järup, 2003). The threshold concentration of this metal in fish muscle destine to human consumption set by the European Commission is $0.1 \,\mu g \, g^{-1} \, w.w.$, thus 10 times lower than that set for Hg. Muscular levels of Cd exceeded the legal limit in 43, 18, 81 and 5% of cases for Swordfish, Yellowfin Tunas, Skipjacks and Common Dolphinfish caught in Reunion waters, respectively. In fish from the Mozambique Channel, percentages were as follows: 76, 0 and 0 in Swordfish, Yellowfin Tunas and Common Dolphinfish, respectively. Hepatic and renal values of Cd were higher than authorized limits in all fish. However, the reader should bear in mind that these standards have been established for muscle with a margin of safety. It is difficult to compare the Cd levels of Swordfish from this study to those from the Mediterranean (Tables 7 and 8) because of the propensity of Cd to accumulate with length in this species and the large size difference between Swordfish sampled in both basins (the mean length being approximately twice shorter in the Mediterranean sample). For Yellowfin Tunas, in which Cd was not correlated to length, muscular Cd levels are in line with that of other Tunas, although slightly higher than Cd values in Yellowfin Tunas from Taiwan waters and lower than those in Bluefin Tuna from the Black Sea.

4.3.3. Lead

Fish caught in the Northern Mozambique Channel and around Reunion Island presented lower Pb levels than

Table 7 Trace element levels (mean \pm SD, $\mu g \ g^{-1}$ w.w.) in the muscle of large pelagic fish

Species	Origin	n	Sex	Weight (kg)	Length (cm)	Cd	Cu	Fe	Hg	Mn	Pb	Se	Zn	References
Swordfish	Mozambique Channel	41	F/M	21	122	0.25 ± 0.26	0.15 ± 0.08	5.33 ± 4.55	0.38 ± 0.26	0.06 ± 0.05	0.03 ± 0.03	0.58 ± 0.29	9.92 ± 8.26	This study
Xiphias gladius	Reunion Island	7	F/M	30	136	0.19 ± 0.14	0.20 ± 0.14	5.90 ± 2.31	1.24 ± 0.83	0.06 ± 0.02	0.03 ± 0.01	1.25 ± 0.56	22.9 ± 15.5	This study
	Mediterranean Sea	58	F/M	1.9	66	0.01 ± 2.00			0.07 ± 0.04		0.05 ± 0.01			Storelli et al. (2005a)
	Mediterranean Sea	162	_	47					0.49 ± 0.26					Storelli and Marcotrigiano (2001)
	Atlantic Ocean	192	F/M						0.62 ± 0.35					Mendez et al. (2001)
	Atlantic Ocean	88	F						0.93 ± 0.07					Monteiro and Lopes (1990)
	Atlantic Ocean	48	M						1.30 ± 0.17					Monteiro and Lopes (1990)
Black Marlin Makaira indica	Pacific Ocean	42	F/M			0.90 ± 0.01	0.40 ± 0.03		7.30 ± 0.60		0.60 ± 0.05	2.20 ± 0.15	8.60 ± 0.29	Mackay et al. (1975)
Blue Marlin Makaira mazara	Taiwan	_	_			0.04	0.32		3.09		0.43		10.45	Han et al. (1998)
Yellowfin Tuna	Mozambique Channel		F/M		107			9.78 ± 4.15	0.13 ± 0.09			1.24 ± 0.44		•
Thunnus albacares	Reunion Island	17	F/M	25	88	0.06 ± 0.05	0.52 ± 0.39	13.3 ± 8.9	0.30 ± 0.60	0.08 ± 0.03	0.01 ± 0.02	1.65 ± 1.06	42.1 ± 35.5	This study
	Pacific Ocean	105	_						0.21 ± 0.11					Kraepiel et al. (2003)
	Seychelles	5	_	17					0.23 ± 1.10					Matthews (1983)
	Taiwan	_	_			0.02	0.26		2.93		0.06		5.23	Han et al. (1998)
	Atlantic Ocean		_		85				0.25 ± 0.12					Adams (2004)
	Unidentified	50	_			0.03 ± 0.01			0.65 ± 0.10	0.15 ± 0.01	0.04 ± 0.01	0.75 ± 0.10		Burger and Gochfeld (2005)
Bluefin Tuna Thunnus thynnus	Arabian Sea	17	_			0.02 ± 0.01	0.21 ± 0.01	2.18 ± 0.41	0.08 ± 0.01	0.08 ± 0.03	0.08 ± 0.01		1.27 ± 0.47	Jaffar and Ashraf (1988)
	Black Sea						3.69 ± 3.21	39.0 ± 12.0	0.62 ± 0.30	< 0.05	< 0.15	1.29 ± 0.32	6.28 ± 1.56	Topcuoglu et al. (1990)
	Mediterranean Sea		F/M		58	0.02 ± 0.01			0.20 ± 0.07		0.10 ± 0.03			Storelli et al. (2005a)
	Mediterranean Sea Atlantic Ocean		F/M F/M	50-190	162–235 98	0.03 ± 0.07	1.15 ± 1.25		3.03 ± 0.55 0.04 ± 0.02	0.09 ± 0.09	0.02 ± 0.06		30.3 ± 16.8	Licata et al. (2005) Garcia Sellanes et al. (2002)
Northern Bluefin Tuna Kishinoella tonggol	Indian Ocean	1	F						0.12 ± 0.01					Kureishy et al. (1979)
Longtail Tuna Thunnus tonggol	Arabian Sea	18	_			0.03 ± 0.01	0.16 ± 0.04	0.43 ± 0.08	0.03 ± 0.01	0.05 ± 0.02	0.09 ± 0.02		3.49 ± 0.60	Jaffar and Ashraf (1988)
Skipjack Tuna	Reunion Island	37	F/M	9	68	0.18 ± 0.11	0.29 ± 0.26	20.1 ± 9.9	0.19 ± 0.66	0.10 ± 0.05	0.02 ± 0.02	4.53 ± 3.5	35.9 ± 27.0	This study
Katsuwonus	Seychelles	5	_	5					0.34 ± 0.11					Matthews (1983)
pelamis	Indian Ocean	1	M						0.16					Kureishy et al. (1979)
Bonito Tuna Euthynnus affinis	Seychelles	9	_						0.05 ± 0.02			0.41 ± 0.07		Robinson and Shroff (2004)
Common	Mozambique Channel	6	F/M	9	109	0.02 ± 0.04	0.14 ± 0.04	2.24 ± 2.99	0.17 ± 0.16	0.05 ± 0.02	0.02 ± 0.02	$\boldsymbol{0.33 \pm 0.32}$	7.96 ± 8.42	This study
Dolphinfish	Reunion Island	42	F/M	5	87	0.03 ± 0.04	0.22 ± 0.15	$\boldsymbol{5.83 \pm 4.73}$	0.01 ± 0.05	0.08 ± 0.02	0.01 ± 0.04	0.79 ± 0.80	16.4 ± 9.5	This study
Coryphaena	Indian Ocean	4	F						0.07 ± 0.004					Kureishy et al. (1979)
hippurus	Indian Ocean	1	F						0.14 ± 0.01					Kureishy et al. (1979)
	Indian Ocean	1	F						0.12 ± 0.01					Kureishy et al. (1979)
	Atlantic Ocean	20	F/M		86				0.05 ± 0.02					Garcia Sellanes et al. (2002)

Table 8 Trace element levels (mean \pm SD or range, $\mu g g^{-1}$ w.w.) in the liver (a) and kidney (b) of large pelagic fish

Species	Origin	n	Sex	Weight (Kg)	Length (cm)	Cd	Cu	Fe	Hg	Mn	Pb	Se	Zn	References
Liver														
Swordfish Xiphias	Mozambique Channel		F/M		122	46.9 ± 51.3	15.7 ± 9.1	178 ± 80		1.07 ± 0.21			61.3 ± 21.0	·
gladius	Reunion Island Mediterranean Sea	192			127	46.3 ± 42.7 $1-28$	17.9 ± 28.1	153 ± 54	1-22		0 - 1.6	21.3 ± 14.8	65.5 ± 12.3	This study Fossi et al. (2004)
Black Marlin Makaira indica	Mediterranean Sea Pacific Ocean		F/M F/M	1.9	66	$0.16 \pm .07$ 9.2 ± 2.1	4.6 ± 0.8		0.19 ± 0.09 10.4 ± 2.0		0.09 0.70 ± 0.03	0.01 5.40 ± 0.85	47.5 ± 9.8	Storelli et al. (2005a) Mackay et al. (1975)
Yellowfin Tuna Thunnus	Mozambique Channel	22	F/M	22	107	39.5 ± 17.2	34.6 ± 21.1	210 ± 83	0.19 ± 0.15	1.48 ± 0.27	0.04 ± 0.03	26.0 ± 3.9	125 ± 73	This study
albacares	Reunion Island	21	F/M	24	104	40.4 ± 41.7	77 ± 199	221 ± 183	1.05 ± 2.60	1.62 ± 0.42	0.02 ± 0.03	26.8 ± 13.6	166 ± 218	This study
Bluefin Tuna Thunnus	Arabian Sea	17	_			0.05 ± 0.01	0.86 ± 0.22	17.1 ± 4.0	0.32 ± 0.11	0.25 ± 0.07	0.17 ± 0.05		7.65 ± 2.15	Jaffar and Ashraf (1988)
thynnus	Mediterranean Sea		F/M		58.5	1.5 ± 0.8			0.39 ± 0.10		0.21 ± 0.11			Storelli et al. (2005a)
	Mediterranean Sea			50-190	162-235	1.19 ± 0.46	18.4 ± 21.0			0.84 ± 0.20				Licata et al. (2005)
Longtail Tuna Thunnus tonggol	Arabian Sea	18	_			0.41 ± 0.12	1.42 ± 0.26	14.6 ± 2.8	0.81 ± 0.03	2.32 ± 0.41	0.28 ± 0.06		39.7 ± 6.1	Jaffar and Ashraf (1988)
Skipjack Tuna Katsuwonus pelamis	Reunion Island	38	F/M	9	68	50.9 ± 31.6	31.2 ± 25.1	432 ± 299	0.17 ± 0.09	1.61 ± 0.40	0.04 ± 0.05	24.4 ± 8.4	69.3 ± 20.3	This study
Common Dolphinfish Coryphaena hippurus	Mozambique Channel	6	F/M	9	109	8.59 ± 16.09	10.7 ± 19.7	90.7 ± 77.1	0.16 ± 0.14	1.92 ± 0.26	0.06 ± 0.03	3.67 ± 3.64	38.8 ± 67.6	This study
	Reunion Island	39	F/M	5	87	5.48 ± 7.74	17.8 ± 18.8	61.8 ± 26.7	0.06 ± 0.05	1.51 ± 0.35	0.03 ± 0.01	3.96 ± 2.78	39.6 ± 8.5	This study
Species	Origin	n	Se	ex Cd		Cu	Fe	Hg	Mn	Pb)	Se	Zn	References
Kidney														
Swordfish Xiphias gladius	Mozambique Channel	14			1 ± 6.34	1.10 ± 0.50	$79.2 \pm 28.$				07 ± 0.14	11.5 ± 3.1	40.3 ± 7.8	·
	Reunion Island	13			22 ± 4.21	0.49 ± 0.32	110 ± 43	$0.95 \pm$			01 ± 0.01	23.2 ± 10.4	41.6 ± 3.8	•
Bluefin Tuna Thunnus thynnus	Arabian Sea	17	_	0.4	42 ± 0.13	0.80 ± 0.21	7.68 ± 3.0	0.19 ±	0.04 0.19	± 0.05 0.4	41 ± 0.09		9.17 ± 2.1	2 Jaffar and Ashrai (1988)
Longtail Tuna Thunnus tonggol	Arabian Sea	18	_	1.2	29 ± 0.35	1.02 ± 2.28	4.42 ± 1.2	1 0.08 ±	0.02 2.28	± 0.42 0.3	25 ± 0.07		30.6 ± 5.2	Jaffar and Ashrai (1988)
Yellowfin Tuna Thunnus	Mozambique Channel	17	F/	M 0.9	07 ± 0.71	0.73 ± 0.70	693 ± 237	7 0.45 ±	0.35 0.18	± 0.09 0.0	04 ± 0.04	32.1 ± 6.6	6760 ± 579	This study
albacares	Reunion Island	13	F/	M 6.3	31 ± 8.17	2.93 ± 2.67	280 ± 97	$0.39 \pm$	0.46 0.45	± 0.17 0.0	02 ± 0.02	32.7 ± 15.2	384 ± 485	This study
Skipjack Tuna Katsuwonus pelamis	Reunion Island	27	F/	M 19.	.4 ± 17.7	2.43 ± 1.24	361 ± 129	9 0.20 ±	0.10 0.52	± 0.34 0.0	05 ± 0.11	14.0 ± 4.9	$39.8 \pm 10.$	5 This study
Common Dolphinfish Coryphaena hippurus	Mozambique Channel	4	F/	M 0.4	45 ± 0.62	0.37 ± 0.61	1598 ± 200	6 0.11 ±	0.31 0.25	± 0.08 0.	15 ± 0.04	6.15 ± 5.88	33.5 ± 505	5 This study
_	Reunion Island	38	F/	M 1.4	1 ± 3.12	1.42 ± 0.20	$93.3 \pm 23.$	$3 0.04 \pm$	0.04 0.65	± 0.12 0.0	04 ± 0.05	4.76 ± 0.76	40.9 ± 17 .	1 This study

equivalent species fished in the Mediterranean or in Taiwan waters. For comparison sake, the present Pb values in muscle are similar to those found in small sculpins and halibut from Greenland (Dietz et al., 1996). Lead found in wild fish can, however, reach very high levels such as in the Streamlined spinefoot, Siganus oramin, from Pearl River Estuary, in South China, in which Pb values were 33 times higher than the maximum level recorded during this study (30.7 vs. $0.93 \,\mu g \, g^{-1} \, w.w.$) (Ip et al., 2005). Volcanic activity is one of the largest natural input of Pb into the atmosphere (Ramade, 1992). In the light of the low Pb levels in Reunion fish, it appears that the volcanic activity in Reunion Island has no measurable affect on the surrounding marine ecosystem Pb levels. Furthermore, the isolation of the Western Indian Ocean with respect to large anthropogenic activity centers, added to the short half-life of Pb in water (25 days), and the absence of biomagnification proprieties for this metal, have apparently preserved the fish living in this region from anthropogenic Pb (mostly originating from anti-knock additive in petroleum, waste incineration, metallurgy, paints, etc). The guideline value adopted the European Commission for Pb in marine fish muscle 0.4 µg g⁻¹ w.w. Levels in fish sampled during this study were all under this limit except one Swordfish caught in the Mozambique Channel and one Skipjack.

This study of pelagic fish shows that the tropical waters of the Western Indian Ocean have low levels of non-essential metals. Monitoring studies should, however, be set up as some fish contain values of muscular Cd, Hg and Zn superior to thresholds for human consumption, particularly Swordfish caught in waters surrounding Reunion Island.

Acknowledgments

This research was supported by the *Conseil Général de La Réunion*. J.K. also benefited from support of the *Conseil Régional de La Réunion* and the *European Social Fund* through a PhD grant. The authors would like to thank J. Bourjea and M. Taquet (Ifremer — La Réunion), as well as Maeva Pêche au Gros, Réunion Fishing Club, L. & P. Berthier, and other anonymous Reunionese fishermen for their assistance in the sampling effort. We would also like thank E. Robert, M. Rouquette, P. Grondin, N. Ghanem (University of La Réunion) and C. Churlaud, (*Centre Commun d'Analyse* —La Rochelle), for their help in the sample preparation and analysis. We also acknowledge the referees for their helpful comments on the manuscript.

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