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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 with 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. Differences in levels between fish from the Mozambique Channel and Reunion Island probably reflect differences of diets rather than differences of elemental availability in both environments.

Capsule : Metal bioaccumulation was quantified in four species of pelagic fish.

Key words: Metals, Fish, Contamination, Detoxification, Guidelines.

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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, and provides an incentive to study 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 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., 2007).

Large fish such as Swordfish (*Xiphias gladius*), Yellowfin Tunas (*Tunnus albacares*), Skipjacks (*Katsuwonus pelamis*) and Common Dolfinfish (*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 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, kidney and 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 were investigated. Furthermore, species' elemental impregnations were compared within study zones, as well as between them. Because the levels of certain elements were dependent of 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. 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

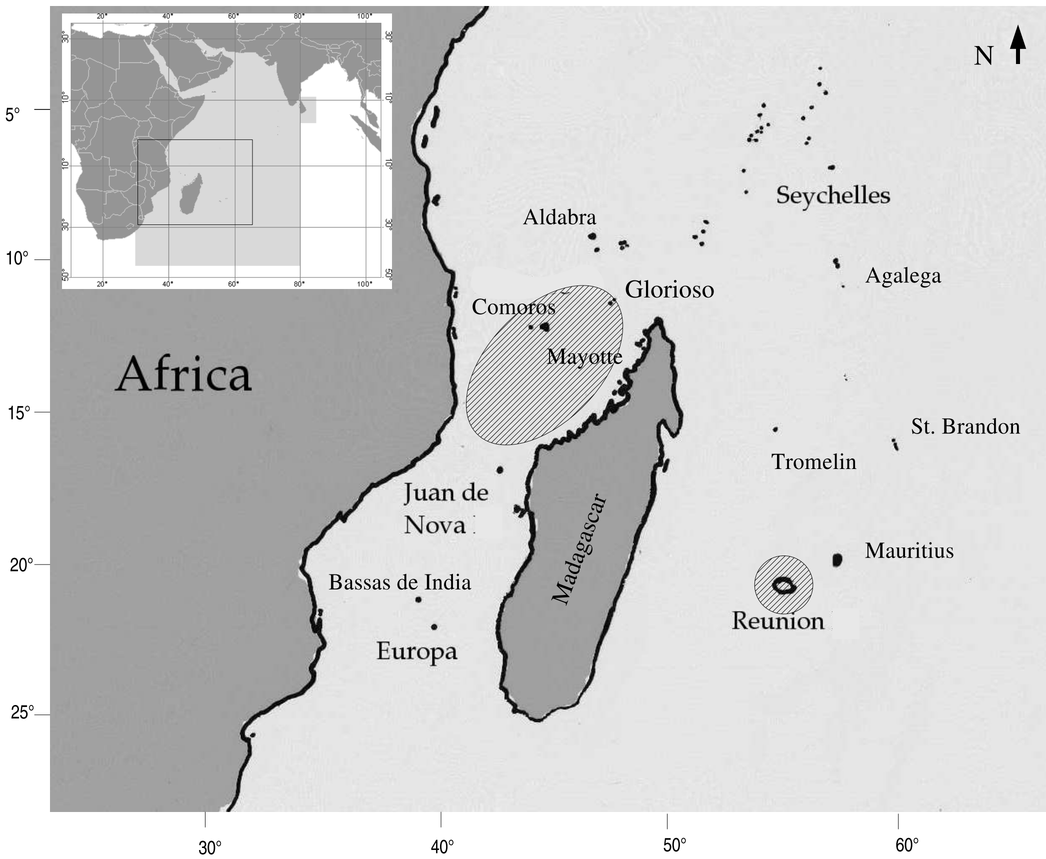

Fig. 1. Map of the study area with sampling zones materialized by shaded circles.

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 2 512 km^2 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 (Figure 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 Dolfinfish) were chosen for this study with respect to their bioaccumulaion capacities and their importance in human consumption.

2.2 Fish sampling and preparation

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

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

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.

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

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.

Table 2
Relationships between weight and length for 4 tropical pelagic fish: data from studies in the Indian Ocean. These relationships are presented as exponential regressions on the following model: W=a.L^b where W is the fish weight and L the length.

Species	n	a	b	r ²	References
Swordfish	430	$1.75.10^{-6}$	3.34	0.96	Poisson & Taquet 2001
Yellowfin Tuna (Females)	194	54.10^{-6}	2.72	0.97	Tantivala 2000
Yellowfin Tuna (Males)	174	41.10^{-6}	2.79	0.97	Tantivala 2000
Skipjack Tuna	22	$6.65.10^{-6}$	3.28	0.98	This study
Common Dolphinfish	51	$5.94.10^{-6}$	3.04	0.93	This study, Bourjea pers. comm.

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°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°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 to 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 $ml.l^{-1}$) and chlorhydric (50 $ml.l^{-1}$) acids for a minimum of 24 h, rinsed 3 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-3) 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 3
Detection limits (DL), quantification limits (QL) and recovery rates.

	DL (µg.g^{-1})	QL (µg.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

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 analyses were 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 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 were 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 homocedaticity by plotting fitted values *vs.* residuals (Venables and Ripley, 2002; Faraway, 2005). Furthermore, the potential influence of the reproductive status (GSI) and body condition (HSI) on elemental burdens were 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 Figures 2 to 4.

The level sequence of the tested elements were, 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 $\backsimeq$ Se $>$ Hg $\backsimeq$ Mn $>$ Pb and Fe $>$ Zn $>$ Se $\backsimeq$ Cd $\backsimeq$ Cu $>$ Hg $\backsimeq$ Mn $>$ Pb respectively (where $\backsimeq$ 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 $\backsimeq$ Hg $\backsimeq$ Cd $\backsimeq$ Mn $>$ Pb). Furthermore, the sequence of non-essential metal is noteworthy since, in all species-location subgroups but Mozambique Channel Common Dolfinfish, 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 3 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.

Table 4
Hepatic, renal and muscular multielemental levels (Mean ± SD, $\mu g.g^{-1}$ $d.w.$) in fish from the Western Indian Ocean. 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; NS: non significative.

		MOZAMBIQUE CHANNEL							REUNION ISLAND								
		Swordfish n: L=42 ; M=41 ; K=14 Mean ± SD CV (%)		Yellowfin Tuna n: L=22 ; M=24 ; K=17 Mean ± SD CV (%)		Common Dolphinfish n: L=6 ; M=6 ; K=4 Mean ± SD CV (%)		Hypotheses tests results	Swordfish n: L=14 ; M=7 ; K=13 Mean ± SD CV (%)		Yellowfin Tuna n: L=21 ; M=17 ; K=13 Mean ± SD CV (%)		Skipjack Tuna n: L=38 ; M=37 ; K=27 Mean ± SD CV (%)		Common Dolphinfish n: L=39 ; M=42 ; K=38 Mean ± SD CV (%)		Hypotheses tests results
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	0.60 ± 0.45 75	a	0.23 ± 0.20 87	b	0.61 ± 0.37 60	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	22.2 ± 17.9 80	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 71	a	KW: 0.03
	Kidney	4.70 ± 2.15 46	a	2.56 ± 2.45 96	b	1.48 ± 0.94 63	b	KW: 0.01	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
	Muscle	22.4 ± 19.1 85	a	39.6 ± 16.8 43	b	12.6 ± 2.22 18	a	KW: < 0.001	18.9 ± 7.4 39	a	50.6 ± 34.0 67	b	70.2 ± 34.4 49	b	23.4 ± 19.0 81	a	KW: < 0.001
	Kidney	340 ± 121 35	a	2415 ± 825 34	b	6394 ± 3615 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 71	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 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 9	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 ± 0.33 54	b	1.00 ± 0.06 6	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	a	0.13 ± 0.12 91	a	0.21 ± 0.10 46	a	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	a	0.09 ± 0.14 148	a	0.14 ± 0.05 35	a	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	a	0.08 ± 0.09 125	a	0.15 ± 0.31 215	a	0.15 ± 0.19 131	a	KW: NS
Se	Liver	53.1 ± 0.82 101	a	90.8 ± 13.7 15	b	13.8 ± 7.07 51	c	KW: < 0.001	77.6 ± 54.2 70	a	83.5 ± 42.3 51	a	73.2 ± 25.1 34	a	13.5 ± 9.5 70	b	KW: < 0.001
	Muscle	2.45 ± 1.21 49	a	5.00 ± 1.78 36	b	1.85 ± 0.85 46	a	KW: < 0.001	4.00 ± 1.78 45	ab	6.26 ± 4.03 64	b	15.8 ± 12.2 78	c	3.17 ± 3.21 101	a	KW: < 0.001
	Kidney	49.4 ± 13.4 27	a	112 ± 23 21	b	24.6 ± 4.94 20	c	KW: < 0.001	98.6 ± 44.3 45	a	125 ± 58 47	a	40.0 ± 14.1 35	b	20.0 ± 3.2 16	c	KW: < 0.001
Zn	Liver	213 ± 73 35	a	439 ± 254 58	b	146 ± 35.4 24	c	KW: < 0.001	239 ± 45 19	ab	516 ± 680 132	a	208 ± 61 29	bc	135 ± 29 22	d	KW: < 0.001
	Muscle	41.7 ± 34.7 83	a	64.1 ± 47.3 74	a	44.7 ± 11.9 27	a	KW: NS	73.5 ± 49.8 68	a	160 ± 135 84	a	125 ± 94 75	a	65.8 ± 38.3 58	a	KW: NS
	Kidney	173 ± 33.6 19	a	23554 ± 2019 86	b	134 ± 41.71 31	a	KW: < 0.001	177 ± 16 9	ab	1467 ± 1851 126	b	114 ± 30 26	c	172 ± 72 42	a	KW: < 0.001

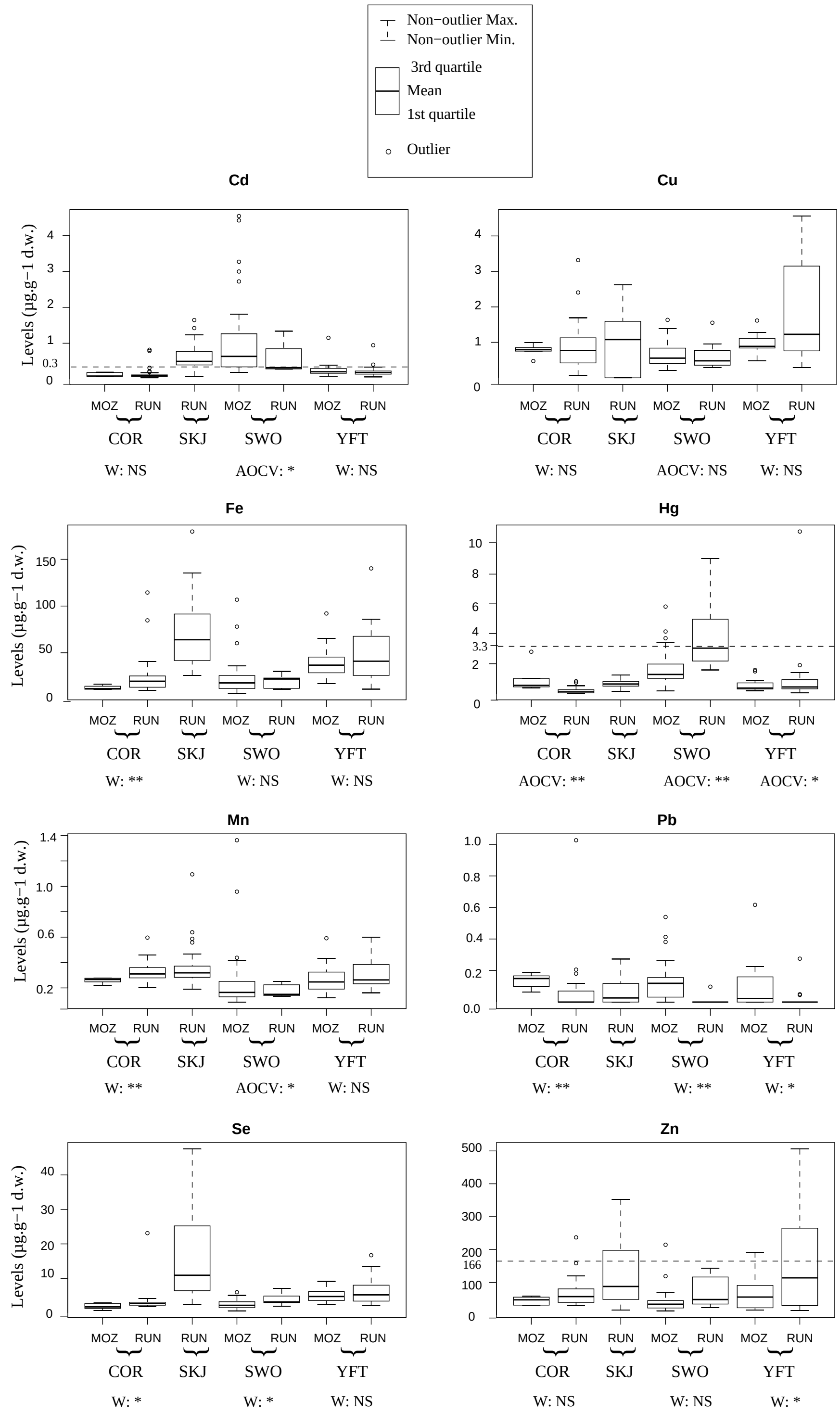

Fig. 2. Comparison of elemental levels ($\mu g.g^{-1}\ d.w.$) in muscle of fish caught in the Mozambique Channel (MOZ) and Reunion Island waters (RUN). For each species, the significances of the level differences between both study sites are indicated below the boxplots. Horizontal dotted lines mark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

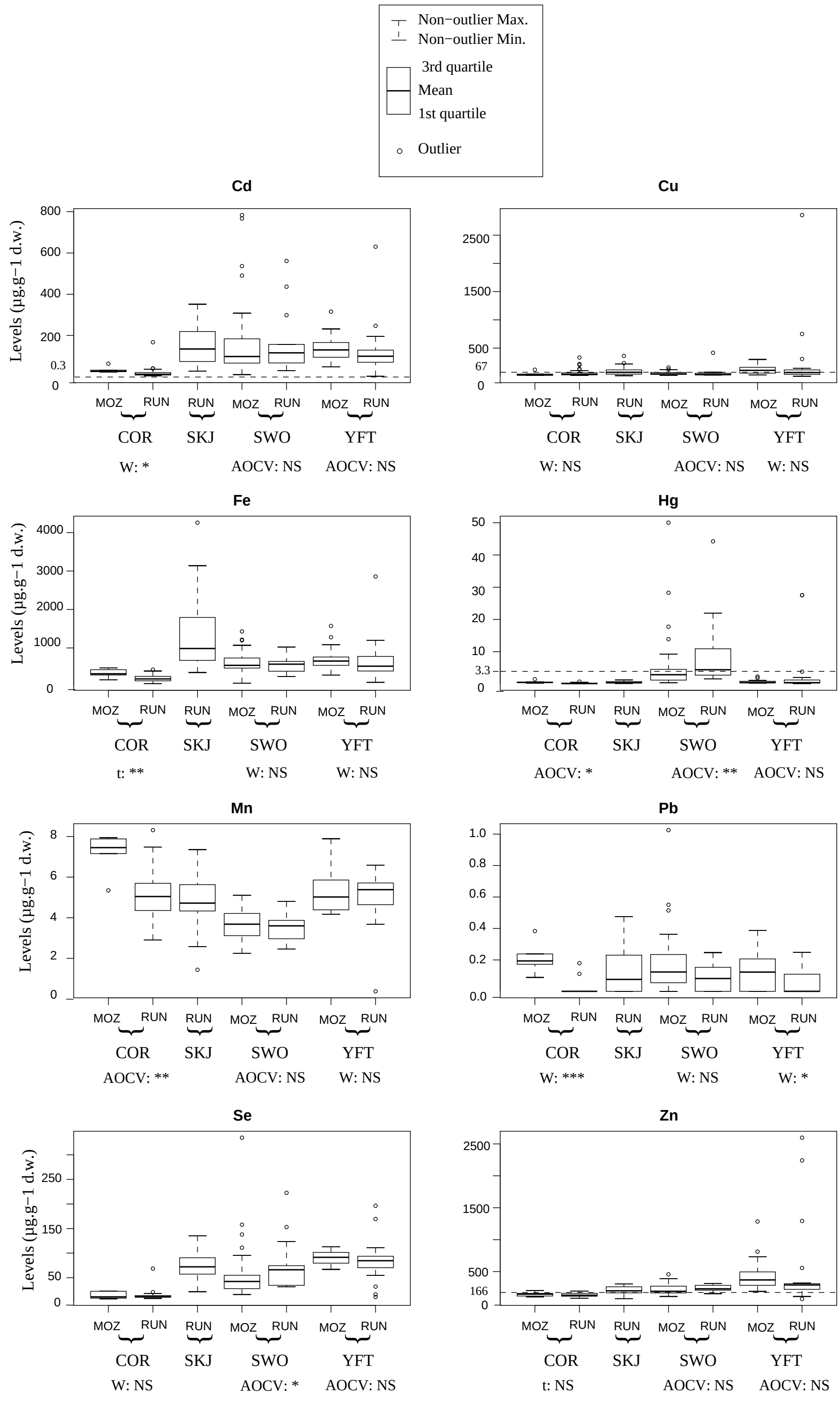

Fig. 3. Comparison of elemental levels ($\mu g.g^{-1}\ d.w.$) in liver of fish caught in the Mozambique Channel (MOZ) and Reunion Island waters (RUN). For each species, the significances of the level differences between both study sites are indicated below the boxplots. Horizontal dotted lines mark the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

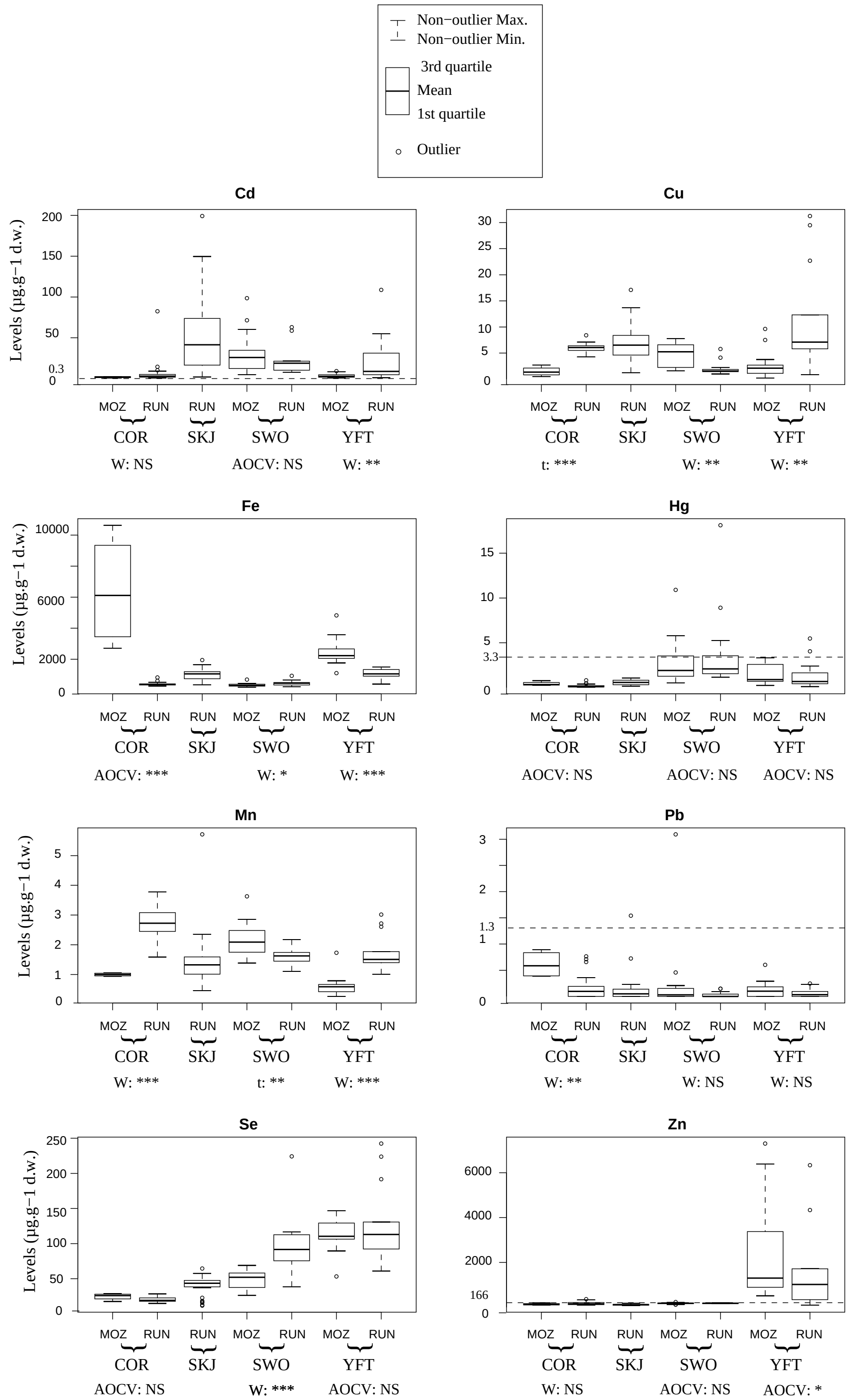

Fig. 4. Comparison of elemental levels ($\mu g.g^{-1}\ d.w.$) in kidney of fish caught in the Mozambique Channel (MOZ) and Reunion Island waters (RUN). For each species, the significances of the level differences between both study sites are indicated below the boxplots. Horizontal dotted lines mark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

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 ($p_{Friedman} = 0.15$), Swordfish ($p_{Friedman} = 0.184$; $p_{Friedman} = 0.05$) and Yellowfin Tunas ($p_{Friedman} = 0.683$; $p_{Friedman} = 0.076$) fished in the Mozambique Channel and Reunion waters respectively. In Common Dolfinfish, 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 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 Dolfinfish were oriented towards kidney (followed by liver and muscle), with the exception of Zn in Common Dolfinfish from the Mozambique Channel. Mercury levels in Mozambique Channel Common Dolfinfish were highest in muscle (followed by liver and kidney), while they were not significantly different in Common Dolfinfish from Reunion waters ($p_{Friedman} = 0.572$). 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).

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

	r^2	*p-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 Dolphinfish**		
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			

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 Dolfinfish 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 ($p_{t-test} = 0.029$, $p_{Wilcoxon} = 0.005$ and $p_{t-test} = 0.001$ respectively). Conversely, hepatic Cu levels were higher in females than in males ($p_{t-test} < 0.001$). In Common Dolfinfish, hepatic Fe, Mn and Zn levels were higher in females than in males ($p_{t-test} = 0.04$, 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%±0.13 and a HSI mean value of 1.06%±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 ($r^2 = 0.141,\ p = 0.024$). 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% ±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 ($HSI = 0.65\%\pm0.24$; $r^2 = 0.211,\ p = 0.011$). 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%±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%±0.31).

The Common Dolfinfish was the only species for which there was no relation between the HSI (1.15%±0.84) and elemental burdens. Nevertheless, the GSI (1.28%±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 Figures 2 to 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 Dolfinfish 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 Dolfinfish 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 Dolfinfish, 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 Dolfinfish from the Mozambique Channel. In the Yellowfin Tuna, Hg and Se burdens were similar between areas of origin. For Swordfish, Yellowfin Tunas and Common Dolfinfish, 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 Dolfinfish and Zn in Yellowfins).

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 Dolfinfish (Swordfish and Skipjacks showing intermediate values). Cadmium and Hg also shared common patterns: Swordfish being the most impregnated and Common Dolfinfish the least. It is however 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 Dolfinfish, 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 Figures 5 to 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 Dolfinfish. 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

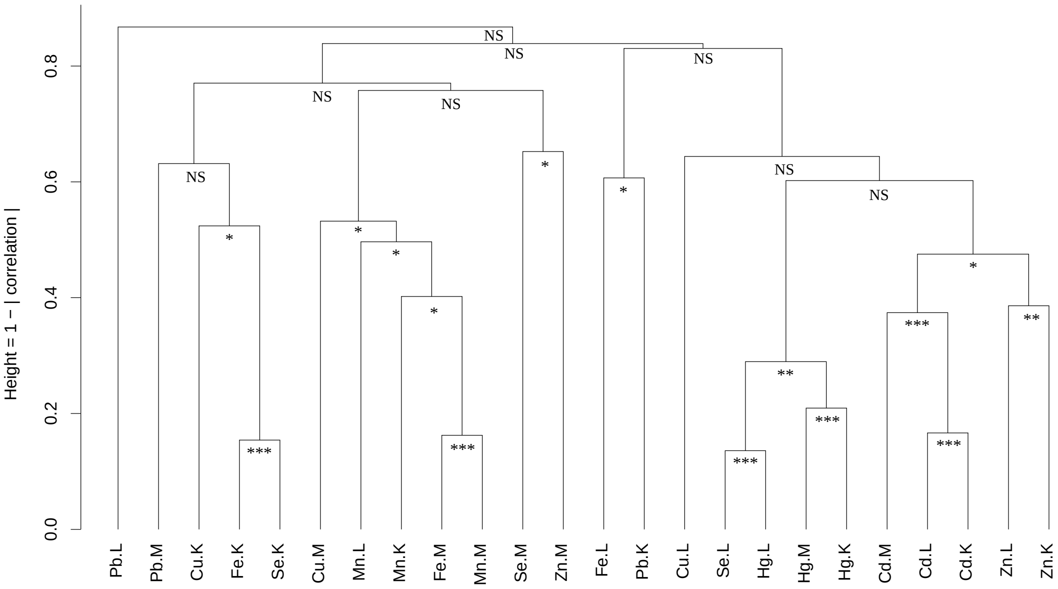

Fig. 5. Dendrogram illustrating linear correlations between trace elements in Swordfish. The minimum levels of significance of the linear correlation coefficients among groups of elemental levels are encoded in the dendrogram. NS: non significant. The letters ".L", ".K", ".M" and placed after the abbreviation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

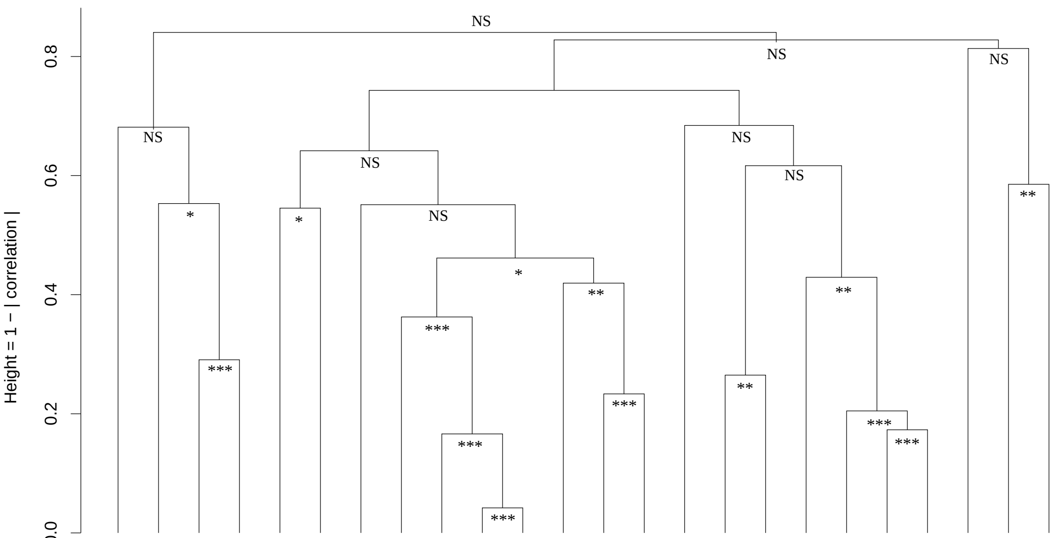

Fig. 6. Dendrogram illustrating linear correlations between trace elements in the Yellowfin Tuna. The minimum levels of significance of the linear correlation coefficients among groups of elemental levels are encoded in the dendrogram. NS: non significant. The letters ".L", ".K", ".M" and placed after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

Dolfinfish. (ii) The following significant correlation between elements were

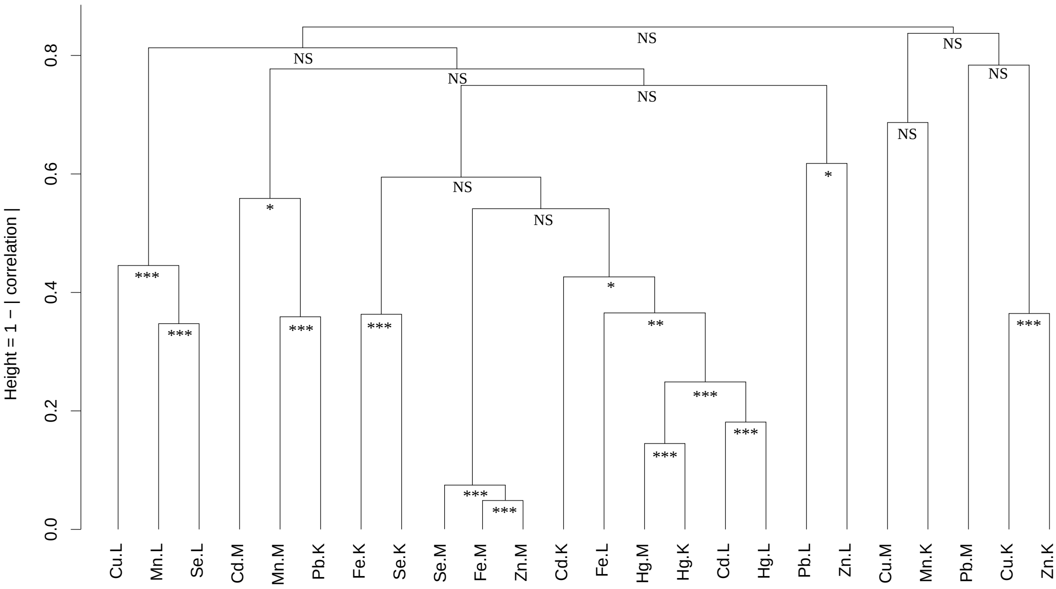

Fig. 7. Dendrogram illustrating linear correlations between trace elements in the Skipjack Tuna. The minimum levels of significance of the linear correlation coefficients among groups of elemental levels are encoded in the dendrogram. NS: non significant. The letters ".L", ".K", ".M" and placed after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

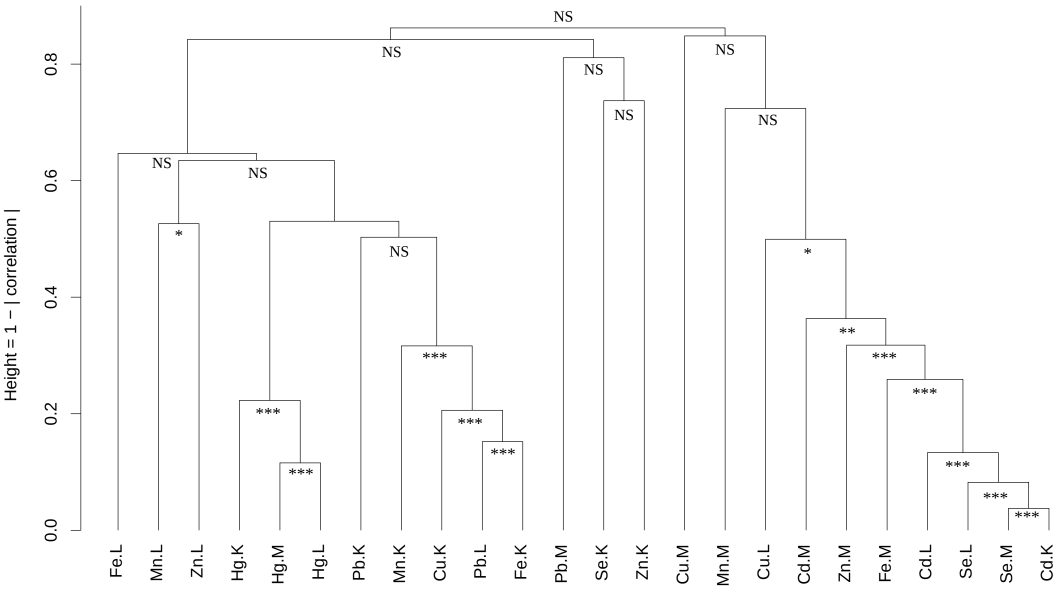

Fig. 8. Dendrogram illustrating linear correlations between trace elements in the Common Dolphinfish. The minimum levels of significance of the linear correlation coefficients among groups of elemental levels are encoded in the dendrogram. NS: non significant. The letters ".L", ".K", ".M" and placed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

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 Dolfinfish. Moreover, the correlation between Hg and Se in liver increased with the Hg impregnation among species (Figures 5-8): Common Dolfinfish ($r^2 = 0.007$; $p > 0.05$), 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 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., 2005; 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; Furness, 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 Dolfinfish.

Hg in fish tissues usually follows the liver>muscle order in terms of accumulation levels (Mackay et al., 1975; Storelli et al., 2005). Yet, dominating Hg levels in muscle, as it was noted for Common Dolfinfish 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 Dolfinfish 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 Dolfinfish in the northern Mozambique Channel.

The only organotropism of Pb was noted in Common Dolfinfish where levels were highest in kidney. It is difficult to 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., 2005). 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 Dolfinfish, 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 which levels 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 Cu^{2+}, Zn^{2+} and Ca^{2+}) (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 Figures 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

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

	n	*r²*	*p-value*
Swordfish			
Hepatic Cd – Cu	56	0.35	< 0.001
Hepatic Cd – Hg	56	0.46	< 0.001
Hepatic Cd – Zn	56	0.55	< 0.001
Renal Cd – Hg	27	0.28	0.005
Renal Cd – Zn	27	0.29	0.003
Yellowfin Tuna			
Hepatic Cd – Cu	43	0.72	< 0.001
Hepatic Cd – Hg	43	0.32	< 0.001
Hepatic Cd – Zn	43	0.23	0.001
Renal Cd – Cu	30	0.21	0.012
Muscular Cd – Hg	38	0.69	< 0.001

	n	*r²*	*p-value*
Skipjack Tuna			
Hepatic Cd – Hg	38	0.67	< 0.001
Hepatic Cd – Zn	38	0.28	0.001
Renal Cd – Cu	27	0.23	0.011
Renal Cd – Hg	27	0.66	< 0.001
Renal Cd – Zn	27	0.19	0.023
Common Dolphinfish			
Hepatic Cd – Cu	45	0.30	< 0.001
Hepatic Cd – Hg	45	0.14	< 0.001
Muscular Cd – Zn	48	0.20	0.001

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 Dolfinfish ($r^2 = 0.007$; $p > 0.05$), 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 provides an argument for the existence of Se mediated Hg detoxification in both species. Indeed, it is possible that the presence of large quantities of Se in the liver conceal Se:Hg granules. Further investigation is needed to confirm the presence of tiemmanite 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 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., 2005). However, these 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 long-term bioaccumulation. Furthermore, Swordfish feed on larger preys than Yellowfin Tunas and Common Dolfinfish, 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 transfering 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 Dolfinfish feed almost exclusively on epipelagic preys (Quéro and Vayne, 1997; Opic et al., 1994; Potier et al., 2004). Yellowfin Tunas, for their part, had 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 Dolfinfish. This probably results from the Dolfinfish's small size, short lifespan (4 years) and very high growth rate (Massuti and Morales-Nin, 1997; NOAA fisheries, 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 Dolfinfish sampled in the Mozambique Channel than in Reunion waters can be explained by a larger amount of cephalopods in their diet (50% and 38% resp. in the Mozambique Channel vs 31% and 2% resp. in Reunion, determined by stomac 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 Dolfinfish 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 & 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.

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 $\mu g.g^{-1}$ *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 (Figure 2). This last result on Reunion Swordfish should however be taken very cautiously and not generalized owing to the very small sample size (7 fish). In Swordfish liver, 40% of Hg values were above the permissible level in Mozambique Channel

Table 7
Trace element levels (Mean ± 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 Is.	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 et al. 2001
	Atlantic Ocean	192	F/M						0.62 ± 0.35					Mendez *et al.* 2001
	Atlantic Ocean	88	F						0.93 ± 0.07					Monteiro & Lopes 1990
	Atlantic Ocean	48	M						1.30 ± 0.17					Monteiro & Lopes 1990
Black Marlin	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
Makaira indica														
Blue Marlin	Taiwan	-	-			0.04	0.32		3.09		0.43		10.45	Han *et al.* 1998
Makaira mazara														
Yellowfin Tuna	Mozambique Channel	24	F/M	21	107	0.06 ± 0.05	0.24 ± 0.06	9.78 ± 4.15	0.13 ± 0.09	0.07 ± 0.03	0.02 ± 0.03	1.24 ± 0.44	15.8 ± 11.7	This study
Thunnus albacares	Reunion Is.	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					Matthiews 1983
	Taiwan	-	-			0.02	0.26		2.93		0.06		5.23	Han *et al.* 1998
	Atlantic Ocean	56	-		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 & Gochfeld 2005
Bluefin Tuna	Arabian Sea	17	-	0.01 (?)		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 & Ashraf 1988
Thunnus thynnus	Black Sea					0.14 ± 0.03	3.69 ± 3.21	39.0 ± 12.0	0.62 ± 0.30	<0.05	<0.15	1.29 ± 0.32	6.28 ± 1.56	Topcuoğlu *et al.* 1990
	Mediterranean Sea	73	F/M	3.6	58	0.02 ± 0.01			0.20 ± 0.07		0.10 ± 0.03			Storelli *et al.* 2005a
	Mediterranean Sea	14	F/M	50-190	162-235	0.03 ± 0.07	1.15 ± 1.25		3.03 ± 0.55	0.09 ± 0.09	0.02 ± 0.06		30.3 ± 16.8	Licata *et al.* 2005
	Atlantic Ocean	10	F/M		98				0.04 ± 0.02					Garcia Sellanes *et al.* 2002
Northern Bluefin Tuna	Indian Ocean	1	F						0.12 ± 0.01					Kureishy *et al.* 1979
Kishinoella tonggol														
Longtail Tuna	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 & Ashraf 1988
Thunnus tonggol														
Skipjack Tuna	Reunion Is.	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 pelamis	Seychelles	5	-	5					0.34 ± 0.11					Matthiews 1983
	Indian Ocean	1	M						0.16					Kureishy *et al.* 1979
Bonito Tuna	Seychelles	9	-						0.05 ± 0.02			0.41 ± 0.07		Robinson & Shroff 2004
Euthynnus affinis														
Common Dolphinfish	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	0.33 ± 0.32	7.96 ± 8.42	This study
Coryphanea hippurus	Reunion Is.	42	F/M	5	87	0.03 ± 0.04	0.22 ± 0.15	5.83 ± 4.73	0.01 ± 0.05	0.08 ± 0.02	0.01 ± 0.04	0.79 ± 0.80	16.4 ± 9.5	This study
	Indian Ocean	4	F						0.07 ± 0.004					Kureishy *et al.* 1979
	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 ± SD or range, $\mu g.g^{-1}$ $w.w.$) in the liver (a) and kidney (b) of large pelagic fish.

a) Liver

Species	Origin	n	Sex	Weight (Kg)	Length (cm)	Cd	Cu	Fe	Hg	Mn	Pb	Se	Zn	References
Swordfish	Mozambique Channel	42	F/M	21	122	46.9 ± 51.3	15.7 ± 9.1	178 ± 80	1.54 ± 2.52	1.07 ± 0.21	0.05 ± 0.05	15.3 ± 0.2	61.3 ± 21.0	This study
Xiphias gladius	Reunion Is.	14	F/M	22	127	46.3 ± 42.7	17.9 ± 28.1	153 ± 54	2.59 ± 3.24	0.96 ± 0.18	0.02 ± 0.02	21.3 ± 14.8	65.5 ± 12.3	This study
	Mediterranean Sea	192	F/M			1-28			1-22		0-1.6			Fossi et al. 2004
	Mediterranean Sea	58	F/M	1.9	66	0.16 ± 0.07			0.19 ± 0.09		0.09 0.01			Storelli *et al.* 2005a
Black Marlin	Pacific Ocean	42	F/M			9.2 ± 2.1	4.6 ± 0.8		10.4 ± 2.0		0.70 ± 0.03	5.40 ± 0.85	47.5 ± 9.8	Mackay et al. 1975
Makaira indica														
Yellowfin Tuna	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
Thunnus albacares	Reunion Is.	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	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 & Ashraf 1988
Thunnus thynnus	Mediterranean Sea	73	F/M	3.6	58.5	1.5 ± 0.8			0.39 ± 0.10		0.21 ± 0.11			Storelli *et al.* 2005a
	Mediterranean Sea	14	F/M	50-190	162-235	1.19 ± 0.46	18.4 ± 21.0		1.88 ± 0.54	0.84 ± 0.20	0.31 ± 0.35		4.47 ± 3.37	Licata *et al.* 2005
Longtail Tuna	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 & Ashraf 1988
Thunnus tonggol														
Skipjack Tuna	Reunion Is.	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
Katsuwonus pelamis														
Common Dolphinfish	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
Coryphanea hippurus	Reunion Is.	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

b) Kidney

Species	Origin	n	Sex	Cd	Cu	Fe	Hg	Mn	Pb	Se	Zn	References
Swordfish	Mozambique Channel	14	F/M	7.41 ± 6.34	1.10 ± 0.50	79.2 ± 28.2	0.68 ± 0.63	0.51 ± 0.14	0.07 ± 0.14	11.5 ± 3.1	40.3 ± 7.8	This study
Xiphias gladius	Reunion Is.	13	F/M	5.22 ± 4.21	0.49 ± 0.32	110 ± 43	0.95 ± 1.11	0.38 ± 0.06	0.01 ± 0.01	23.2 ± 10.4	41.6 ± 3.8	This study
Bluefin Tuna	Arabian Sea	17	-	0.42 ± 0.13	0.80 ± 0.21	7.68 ± 3.00	0.19 ± 0.04	0.19 ± 0.05	0.41 ± 0.09		9.17 ± 2.12	Jaffar & Ashraf 1988
Thunnus thynnus												
Longtail Tuna	Arabian Sea	18	-	1.29 ± 0.35	1.02 ± 2.28	4.42 ± 1.21	0.08 ± 0.02	2.28 ± 0.42	0.25 ± 0.07		30.6 ± 5.2	Jaffar & Ashraf 1988
Thunnus tonggol												
Yellowfin Tuna	Mozambique Channel	17	F/M	0.97 ± 0.71	0.73 ± 0.70	693 ± 237	0.45 ± 0.35	0.18 ± 0.09	0.04 ± 0.04	32.1 ± 6.6	6760 ± 579	This study
Thunnus albacares	Reunion Is.	13	F/M	6.31 ± 8.17	2.93 ± 2.67	280 ± 97	0.39 ± 0.46	0.45 ± 0.17	0.02 ± 0.02	32.7 ± 15.2	384 ± 485	This study
Skipjack Tuna	Reunion Is.	27	F/M	19.4 ± 17.7	2.43 ± 1.24	361 ± 129	0.20 ± 0.10	0.52 ± 0.34	0.05 ± 0.11	14.0 ± 4.9	39.8 ± 10.5	This study
Katsuwonus pelamis												
Common Dolphinfish	Mozambique Channel	4	F/M	0.45 ± 0.62	0.37 ± 0.61	1598 ± 206	0.11 ± 0.31	0.25 ± 0.08	0.15 ± 0.04	6.15 ± 5.88	33.5 ± 505	This study
Coryphanea hippurus	Reunion Is.	38	F/M	1.41 ± 3.12	1.42 ± 0.20	93.3 ± 23.3	0.04 ± 0.04	0.65 ± 0.12	0.04 ± 0.05	4.76 ± 0.76	40.9 ± 17.1	This study

samples, and 64% in Reunion samples (Figure 3). In kidney, the percentage of exceeding values was 29% in the Mozambique Channel samples, and 31% in Reunion samples (Figure 4). The Hg guideline was also reach in 5%, 14% and 18% of muscles, livers and kidneys of Yellowfin Tunas caught in Reunion Island waters. 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 Dolfinfish from Reunion waters were five times lower than those measured in Common Dolfinfish 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 & 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.

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 Dolfinfish 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 Dolfinfish 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 & 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.

Lead. Fish caught in the northern Mozambique Channel and around Reunion Island presented lower Pb levels than 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, wast incineration, metallurgy, paints, etc). The guideline value adopted the European Commission for Pb in marine fish muscle 0.4 $\mu g.g^{-1}$ *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.

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