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Mercury and Selenium Levels in Swordfish (*Xiphias gladius*) Fished in the Exclusive Economic Zone of the Republic of Seychelles

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***Abstract*—**Total mercury (Hg), selenium (Se) and Hg-Se ratios were analyzed in the white muscle, liver and gonads of swordfish, in order to compare concentration between the different tissues and sex, and also the effect of size (fork length). The results show significant difference between tissue types, with the liver having the highest concentration of both Hg and Se. Positive significant correlations between moles of Hg and Se were obtained in the liver and white muscle, but no relationship was obtained in the gonads. No difference in the concentration of Hg and Se was obtained between the sexes in the tissue types, except for Hg in the gonads, which were found to be higher in males. Significant negative relationships were obtained when the Hg-Se ratio was plotted against fork length in all three tissue types.

***Keywords*—**Bioaccumulation, large pelagic fish, mercury, selenium, Western Indian Ocean.

I. Introduction

MERCURY (Hg) is a toxic heavy metal found in the environment and has both natural sources such as volcanoes and forest fires, as well as anthropogenic sources such as mining and power plant discharge [1]. Hg in the marine environment bioaccumulates and biomagnifies as it goes up the trophic levels, so that large, long-live top predators such as tuna and billfishes, usually have high concentrations [2]. The toxic form of Hg in the marine environment is methylmercury (MeHg), which can form up to 90% of total Hg in fish tissue [3]. Selenium (Se) is an element which is naturally occurring in rocks and soil [4]. Se is considered an essential element as trace amounts are necessary for the human body in the production of selenoproteins [5]; although, excess Se is also toxic. Selenoproteins have been noted to have antioxidant and anti-inflammatory properties [6] and are also very important in the production of thyroid hormones [4]. Swordfish (*Xiphias gladius*) are large migratory top predators [7], and are the main target species of the Seychelles local semi-industrial long line fisheries sector. High levels of Hg have been reported in swordfish worldwide. As with other large pelagic fish, concerns are often raised due to the levels of Hg reported in swordfish flesh above the maximum sanitary limit of 1 $\mu g.g^{-1}$ fresh weight, which is the level that has been advised as safe for frequent human consumption [8], [9]. Many studies [10]-[12] have observed a Hg-Se interaction in marine top predators often at a ratio of 1:1. This has led to the theory that Se may have an effect in reducing the toxicity of Hg [13], even if the exact mechanism is unknown. Many factors such as age, size, sex and location can also influence the concentrations of Hg and Se in swordfish, and consequently the Se:Hg ratio. In such a context, the aim of our study was to determine Hg contamination of the swordfish population caught in Seychelles waters together with the levels of Se. The distribution of both elements in white muscle, liver and gonads was examined, and for each tissue type, we studied the effect of fish size (fork length) and sex on Hg and Se accumulation. Finally, Hg levels and Hg:Se ratio are discussed from a health point of view.

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II. Methods

A total of 74 swordfish was caught and processed on board a longliner in the Seychelles EEZ from November 2013-December 2014. Briefly, each fish was identified and measured (i.e. fork length), and the fishing date and location were recorded. The liver, gonads and a piece of dorsal white muscle (approximately 100g) were collected, identified with the swordfish ID and stored frozen on board until the end of the vessel trip (typically two weeks).

Total Hg (hereafter Hg) analyses were carried out using a solid sample atomic absorption spectrometer AMA-254 (Advanced Mercury Analyser-254 from Altec). Around 1-3 mg of sample was directly introduced into the machine. The samples were dried for 10s, and then were combust at 750°C for 150 sec which leads to the release of Hg. For the remaining 45s, the Hg vapors produced are carried by an oxygen flow to a gold amalgamator, and trapped on its surface. Thereafter, the collected Hg is released from the amalgamator by a short heat-up to 800°C, and carried in a pulse through a spectrophotometer, where it is detected by atomic absorption. Each sample was analyzed in duplicates. The analytical quality of the Hg measurements by the AMA-254 was controlled by certified reference material TORT- 2 (Lobster hepatopancreas) from the National Research Council of Canada (NRCC by running it every 10 analyses) and blanks at the beginning and at the end of the analytical cycle.

Se was analyzed on a Varian Vista-Pro ICP-AES (for Inductively Coupled Plasma Atomic Emission Spectroscopy)

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and a Thermofisher Scientific XSeries 2 ICP-MS (Inductively Coupled Plasma Mass Spectrometry) [14]. Briefly, around 250 mg of dry homogenized samples were digested with a mixture of nitric acid (67–70% HNO_3) and Hydrochloric acid 34–37% HCl) (3:1, v/v). Acid digestion of the samples was carried out overnight at room temperature and then in a Milestone microwave oven (30 min with constantly increasing temperature up to 120 °C, then 15 min at this maximal temperature). Each sample was made up to 50 ml with Milli-Q water. Two certified reference materials (DOLT-4 (dogfish liver) and TORT-2 (lobster hepatopancreas) from the NRCC) and one blank were included in each analytical batch.

Hg and Se results are stated in $\mu g.g^{-1}$ wet weight (WW). All data were tested for normality using Shapiro-Wilk, if the data was not normally distributed it was log transformed. Analysis of variance (ANOVA) was used to test for the difference of Hg and Se between tissues and between sexes. Pearson's test of correlation was used to test the relationship between length and Se-Hg ratio and the molar concentration of Hg against the molar concentration of Se in each tissue.

III. Results and Discussion

A. Distribution of Hg and Se in Swordfish Tissues

A total of 71 white muscles, 73 liver and 65 gonads were analyzed for Hg and Se. The highest concentrations of Hg and Se were found in the liver (Table I). The levels of Hg in the white muscle (0.63±0.32 $\mu g.g^{-1}$ WW) were slightly higher than in the gonads (0.39± 0.27 $\mu g.g^{-1}$ WW), while an opposite pattern was observed for Se being more accumulated in the gonads (3.25 ±1.10 $\mu g.g^{-1}$ WW) compared to the white muscle (0.56 ±0.24 $\mu g.g^{-1}$ WW). The differences in the concentrations of Hg and Se between the three tissue types are significant (Hg: F-value = 61.83, p- value <0.001; Se: F-value = 968.9 p-value <0.001) and the higher concentration of Hg and Se observed in the liver of Seychelles swordfish is consistent with what was observed by Branco *et al.* [15]. The mean level of Hg obtained in the white muscle is far below the recommended safe intake of 1 $\mu g.g^{-1}$ WW, and only 13% of studied swordfish had Hg concentration above this limit in their flesh. The highest concentration of Hg obtained in swordfish white muscle was 1.80 $\mu g.g^{-1}$ WW. The concentration of Hg in white muscle is consistent with other studies done on swordfish from other oceanic basins [16]-[18]. The levels of Se where in excess of those Hg (i.e., Se:Hg > 1; Table I), which is noted by Kaneko and Ralston [11] to be the observation obtained in marine fish. According to these results, all swordfish from Seychelles waters are safe for consumption [11].

TABLE I
Concentrations (Mean±Standard Deviation and Min-Max) of Hg and Se (mg.g^{-1} WW) in White Muscle, Gonads and Liver of Swordfish from Seychelles Waters

Tissue	Hg		Se		Se:Hg
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD
White muscle	0.63 ± 0.32	0.16 - 1.80	0.56 ± 0.24	0.20 - 1.37	2.74 ± 1.91
Gonads	0.39 ± 0.27	0.03 - 1.32	3.25 ± 1.10	1.88 - 6.02	33.02 ± 29.65
Liver	1.63 ± 1.29	0.15 - 7.01	9.40 ± 4.14	3.70 - 23.48	24.24 ± 28.55

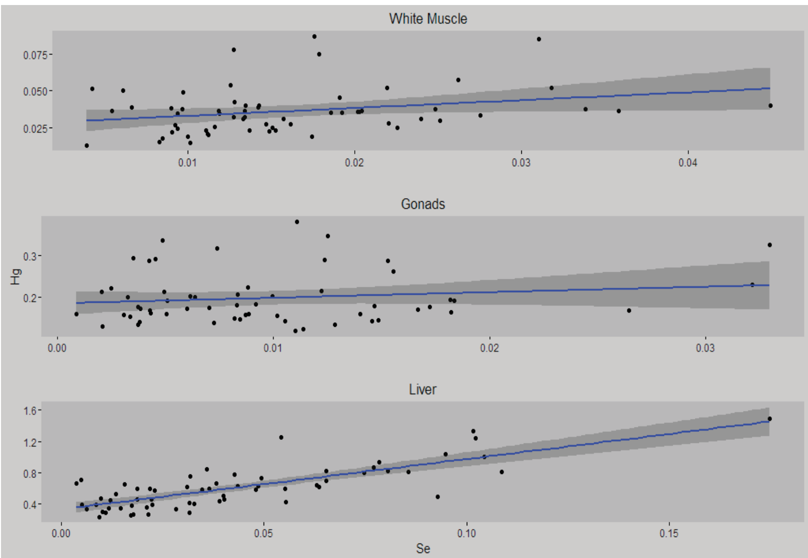

Fig. 1 Correlation between molar Se and molar Hg concentrations in the white muscle, liver and gonads of swordfish from Seychelles waters

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A significant positive relationship was obtained in the liver (R^2= 0.651, p-value<0.001) when the number of moles of Hg was plotted against the moles of Se. The relationship in white muscle (R^2=0.316 p-value<0.05) was weak while the one obtained in the gonads was not significant (R^2= 0.109, p-value =0.4145). A similar positive relationship was observed by [15] in their study on swordfish livers. This positive correlation could be due to the role of Se in reducing the toxicity of Hg.

The mean Hg level in white muscle of females was 0.62±0.38 µg.g^{-1} WW and 0.67±0.28 µg.g^{-1} WW in males. Muscular Se was 0.47±0.14 µg.mg^{-1} WW in females and 0.66± 0.30 µg.g^{-1} WW in males. No difference was found in Hg levels between the sexes (p-value= 0.326), while for Se, the concentrations in males were significantly higher than in females (p-value<0.01). The Hg-Se ratio in the white muscle was found to be 2.43±1.36 in females and 3.03±2.29 in males.

In the gonads, the mean Hg level in females was 0.32±0.23 µg.g^{-1} WW and in males 0.46±0.23 µg.g^{-1} WW. Hg levels were found to be significantly higher in male gonads than in females (p-value<0.05). No sex effect was found in the levels of Se in the gonads (p-value = 0.098), with a mean of 2.89±0.67 µg.g^{-1} WW obtained in females and 3.38±1.18 µg.g^{-1} WW obtained in males. Hg-Se ratio was 38.80±36.45 in females and 27.24±19.79 in males.

No difference was found in the mean levels of Hg (p-value= 0.129) and Se (p-value=0.372) in the liver of both male (mean Hg= 1.98±1.47 µg.g^{-1} WW, mean Se= 9.89±3.98 µg.g^{-1} WW) and female (mean Hg= 1.41±1.16 µg.g^{-1} WW, mean Se= 9.23±4.75 µg.g^{-1} WW) swordfish. The Se-Hg ratio was 23.65±15.12 in females and 24.79±37.27.

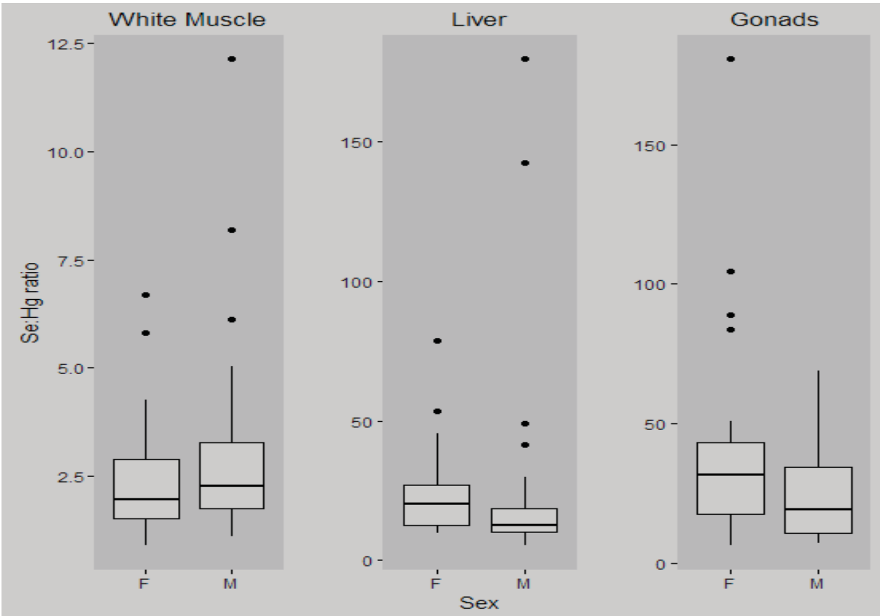

Fig. 2 Hg-Se molar ratio in the white muscle, liver and gonads of male (M) and female (F) swordfish from Seychelles waters

B. Effect of Swordfish Size

A strong positive relationship was obtained between concentrations of Hg in the liver and fish fork length (R^2 = 0.719, p-value <0.001), all the relationships obtained including in muscle (R^2 = 0.540, p-value <0.001) and gonads (R^2 = 0.476, p-value <0.001) were significant. The relationship between length and Se concentration in liver (R^2 = 0.333 p-value < 0.01), white muscle (R^2 = -0.158, p-value = 0.226) and gonads (R^2 = 0.003, p-value = 0.980) were not significant. Negative relationships have been obtained between length and Se-Hg ratio in all the three tissues: A strong relationship was obtained in the liver (R^2 = -0.715, p-value <0.001) and white muscle (R^2 = -0.622, p-value <0.001) and a moderate one was obtained in the gonads (R^2 =-0.459, p-value <0.001).

IV. Conclusion

The concentrations of both Hg and Se in Seychelles swordfish differed greatly between tissue types, which are consistent with previously reported results in swordfish from various areas. The liver was found to have the higher concentrations, while the muscle had higher Hg content than the gonads, but lower Se content. A strong positive correlation of Se molar concentrations and Hg molar concentrations was found in swordfish liver. Sex had no effect on the concentrations of both Hg and Se, except for Hg in the gonads, and fish length had an effect only on the concentration of Hg in all three tissue types and a negative effect on the Hg:Se ratio. Finally, all swordfish from Seychelles waters appeared safe for consumption regarding their Hg:Se molar ratio.

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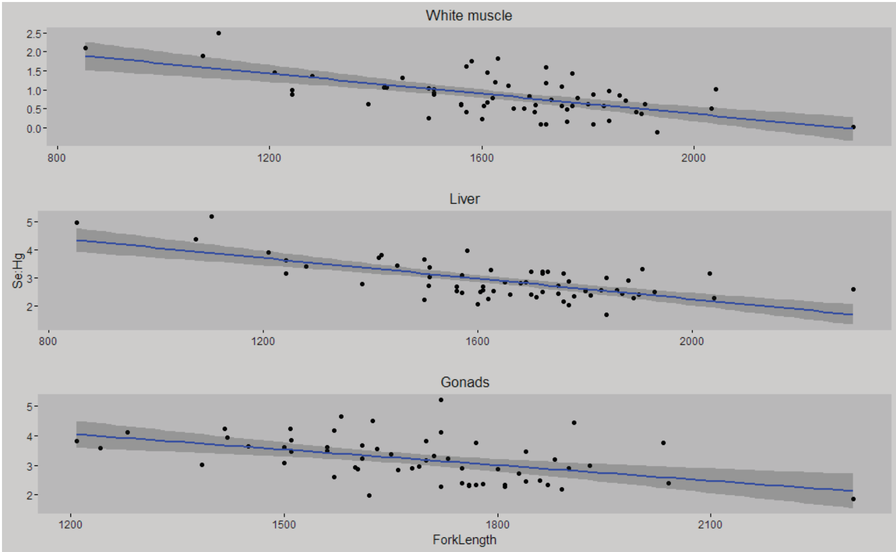

Fig. 3 Relationships between fish fork length and Hg-Se molar ratio in the white muscle, liver and gonads determined in swordfish from Seychelles waters

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