Evaluation of Digestion Procedures for the determination of Total Mercury in Fish tissue and Optimization of a simple one suitable for a Low Technology Environment.

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INTRODUCTION

- Hg is of concern in the world among the heavy metals because of its toxicity, persistence on the earth and bio magnification in aquatic animals.

- Major human exposure to Hg has been through fish consumption.

- This has led many countries and organisations to set limits for mercury level in fish to protect the population.

INTRODUCTION

- Therefore, there is need for regular monitoring of its level in fish and other aquatic animals used as food.

- However, there are limitations to use of some of the available methods for determination of mercury.

- There is therefore continuous need to seek to improve on the available methods and develop methods suitable for use especially in low technology environments.

The Specific Objectives

1. Compare nine acid digestion procedures.
2. Optimize and validate the most accurate procedure.
3. Determine Hg levels in various fish species consumed using the optimized and validated procedure.
4. Determine whether mean Hg levels in the fish consumed in Lagos are within the permissible limit set by FAO/WHO.
5. Assess whether an average fish consumer resident in Lagos is liable to harmful effects of Hg.

Justification

- Most of the Hg pollution end up in water bodies.
- The pollutants are picked up by aquatic biota and magnify along the food chain.
- Fish is the major source of the most poisonous organic Hg species to humans.
- Measurement of THg in the axial muscle of fish provides a valid estimate of MHg concentration.

Justification (continued)

- Decomposition of organic matrices to release mercury in measurable form is critical in the determination of mercury.

- Some of the available analytical procedures have limitations for use in low technology laboratories (apparatus, digestion periods and high cost of equipment).

- Consequently, there is paucity of research studies on Hg levels in Africa due to unavailability of appropriate resources or required technology

Table 1. Modifications of the Selected Digestion Procedures

Procedure	Conditions in Literature	Reference	Modifications made
1	2 g of sample, heating on steam bath, no temperature stated.	Boscoe & Steve (2013)	0.5 g of sample, heating in Pyrex tube on hot plate, at 160 ^{o}C for 2 hr.
2	Mass of sample, volume of reagents, and digestion temperature not stated.	Pineau *et al* (1990)	0.5 g of sample, 5 ml HNO_3, Digestion at 160 ^{o}C for 2 hr, oxidise with 10 ml of 1% $KMnO_4$, and add 5% $HONH_2$.HCl.
3	0.126 g of fish tissue, digestion temp was 180 ^{o}C, oxidation was by $KMnO_4$-$KHSO_4$ mixture and reduction of excess by $HONH_2.HSO_4$.	Baker *et al* (2004).	0.5 g of sample, Digestion temp was 160 ^{o}C, 1 % $KMnO_4$ was used for oxidation and 5 % $HONH_2$.HCl was used to remove excess $KMnO_4$.
4	0.2 g of sample digested with HNO_3-H_2SO_4 mixture (1:4 v/v) at 60 ^{o}C until solvent evaporated.	Wagemann *et al* (1997).	0.5 g of sample was digested with 5 ml of 1:4 of the acid mixture at 160 ^{o}C for 3 hr.
5	As in procedure 4.	Wagemann *et al* (1997).	0.5 g of sample was digested with 5 ml of 4:1 of the acid mixture at 160 ^{o}C for 3 hr.
6	0.5 – 0.8 g, digestion with 5 ml of H_2SO_4 in water bath at 70 ^{o}C for 1hr, add 50 ml of 6 % $KMnO_4$, heating continued at 55 ^{o}C for further 2 hr. excess $KMnO_4$ was removed with $HONH_2$.HCl.	Rizea *et al* (2007).	0.5 g of sample, digestion on hot plate at 160 ^{o}C for 1 hr, 10 ml of 1 % $KMnO_4$, + further heating at 140 ^{o}C for 2 hr, removal of excess $KMnO_4$ with 5 % $HONH_2$.HCl.
7	NIMD Method	Voegborlo & Adimado (2010b)	Digestion with acids but without H_2SO_4 at 160 ^{o}C for 3 hr.
8	NIMD Method	Voegborlo & Adimado (2010b)	No modification.
9	NIMD Method	Voegborlo & Adimado (2010b)	Introduction of 10 ml of 1 % $KMnO_4$, after digestion and cooling, no further heating and removal of excess $KMnO_4$ with 5 % $HONH_2$.HCl after standing for 10 min.

Table 2: Summary of Digestion Procedures

Proced	HCl (ml)	HNO_3 (ml)	$HClO_4$ (ml)	H_2SO_4 (ml)	1 % $KMnO_4$ (ml)	D. Temp. (± 5 °C)	D. Period (hr)
1	-	5	-	-	-	160	2.0
		2	-	-	-	160	1.0
2	-	5	-	-	-	160	2.0
	-	-	-	-	10	160	0.5
3	-	2	-	3	-	160	5.0
	-	-	-	-	15	-	-
4	-	1	-	4	-	160	3.0
5	-	4	-	1	-	160	3.0
6	-	-	-	5	-	160	1.0
	-	-	-	-	10	140	2.0
7	-	4	1	-	-	160	3.0
8	1	1	1	5	-	200	0.5
9	1	1	1	5	-	200	0.5
	-	-	-	-	10	-	-

RESULTS AND DISCUSSION

- All the procedures showed good precision with standard deviations ranging from 0.005 to 0.230 µg/g.

- The mean THg levels ranged from 0.021 to 0.369 µg/g.

- Five of the procedures (**2**, **4**, **5**, **8** and **9**) were found suitable for determination of THg in fish muscle.

- The results of repeated digestion (**Fig. 1**) showed no significant difference when subjected to ANOVA.

- CRM was used to test for the method accuracy of the procedures.

- Linsinger's Test was used to determine method performance.

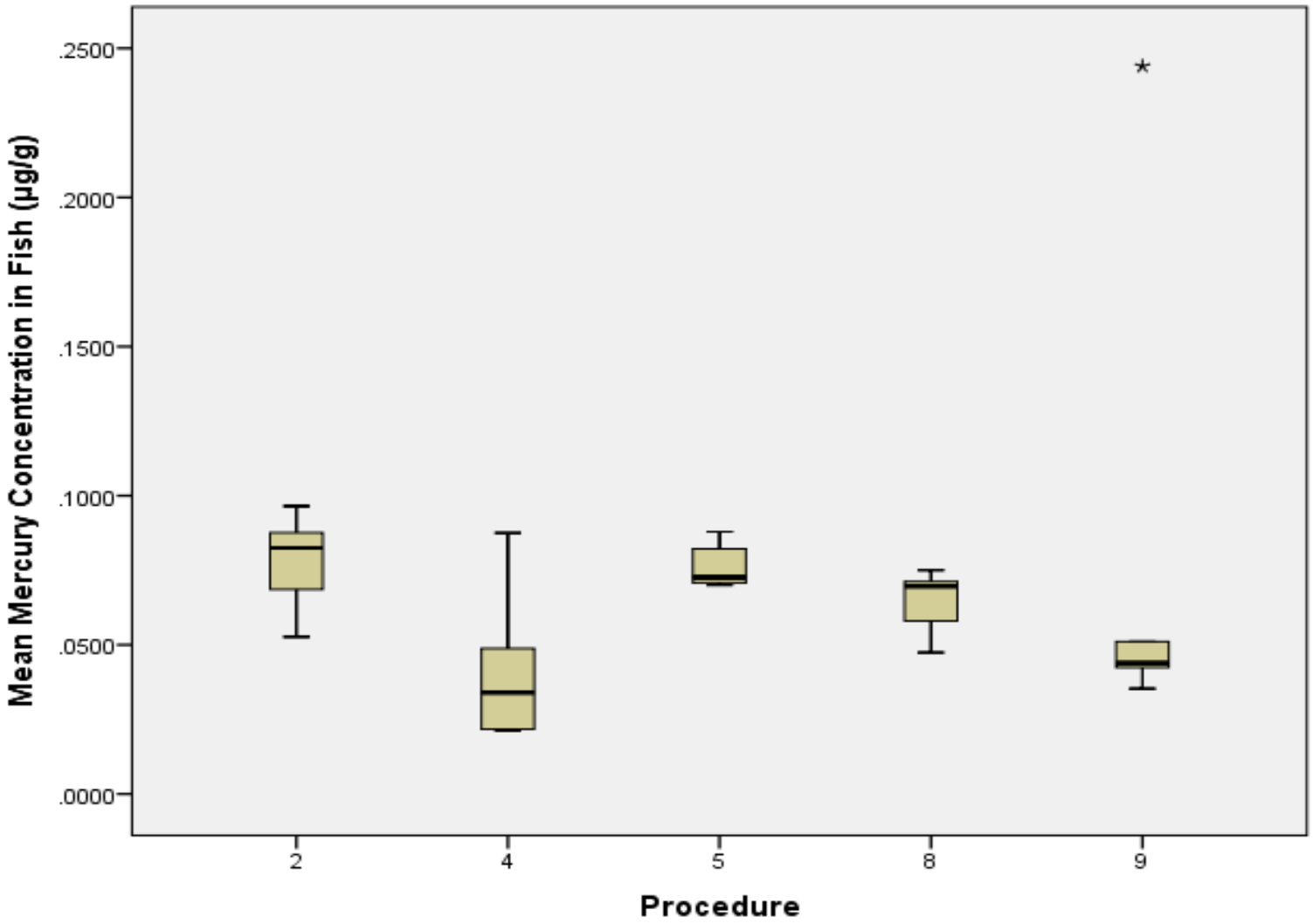

Fig 1 Mean THg levels in a fish sample by different Digestion Procedures.

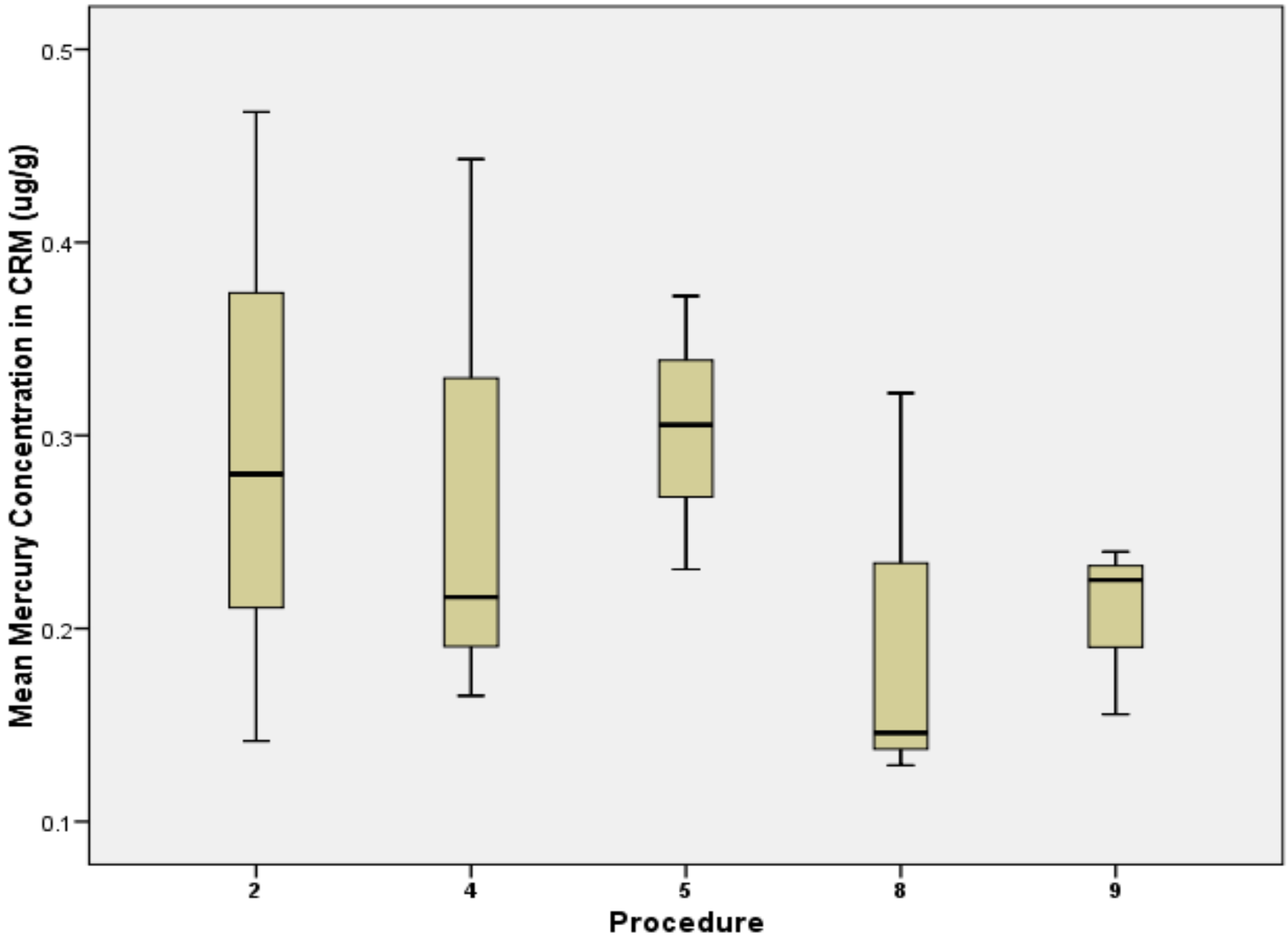

Fig. 2 Mean THg concentration in CRM using the Procedures.

Table 3 Linsinger's Test for Method Performance of the selected procedures.

Proced	Mean measured conc, Cm (µg/g)	Abs. diffce from certified value, Δm (µg/g)	Standard Deviation (µg/g)	Expanded Uncertainty U_Δ	$\Delta m - U_\Delta$	Inference
2	0.296	0.074	0.164	0.189	-0.115	NSD
4	0.275	0.053	0.148	0.171	-0.115	NSD
5	0.303	0.081	0.071	0.080	+0.001	SD
8	0.202	0.023	0.020	0.124	-0.095	NSD
9 .	0.207	0.015	0.045	0.052	-0.037	NSD

- The order of performance of the procedure is: **9** > **8** > **4** > **2** > **5**.

- The performance of procedures **8** and **9** could be explained by the efficiency of perchloric acid as a good oxidising agent and use of HCl which improved decomposition efficiency.

- Procedure **9** was optimized by varying temperature and digestion period.

- The optimum conditions for the digestion procedure were investigated by varying the digestion temperature and period.

- Generally, Mean THg obtained increased with increase in temperature and period of digestion.

- The maximum mean THg concentration of 0.492 ± 0.131 µg/g was achieved at 220 ^{o}C and 50 minutes of digestion (**Fig. 3**).

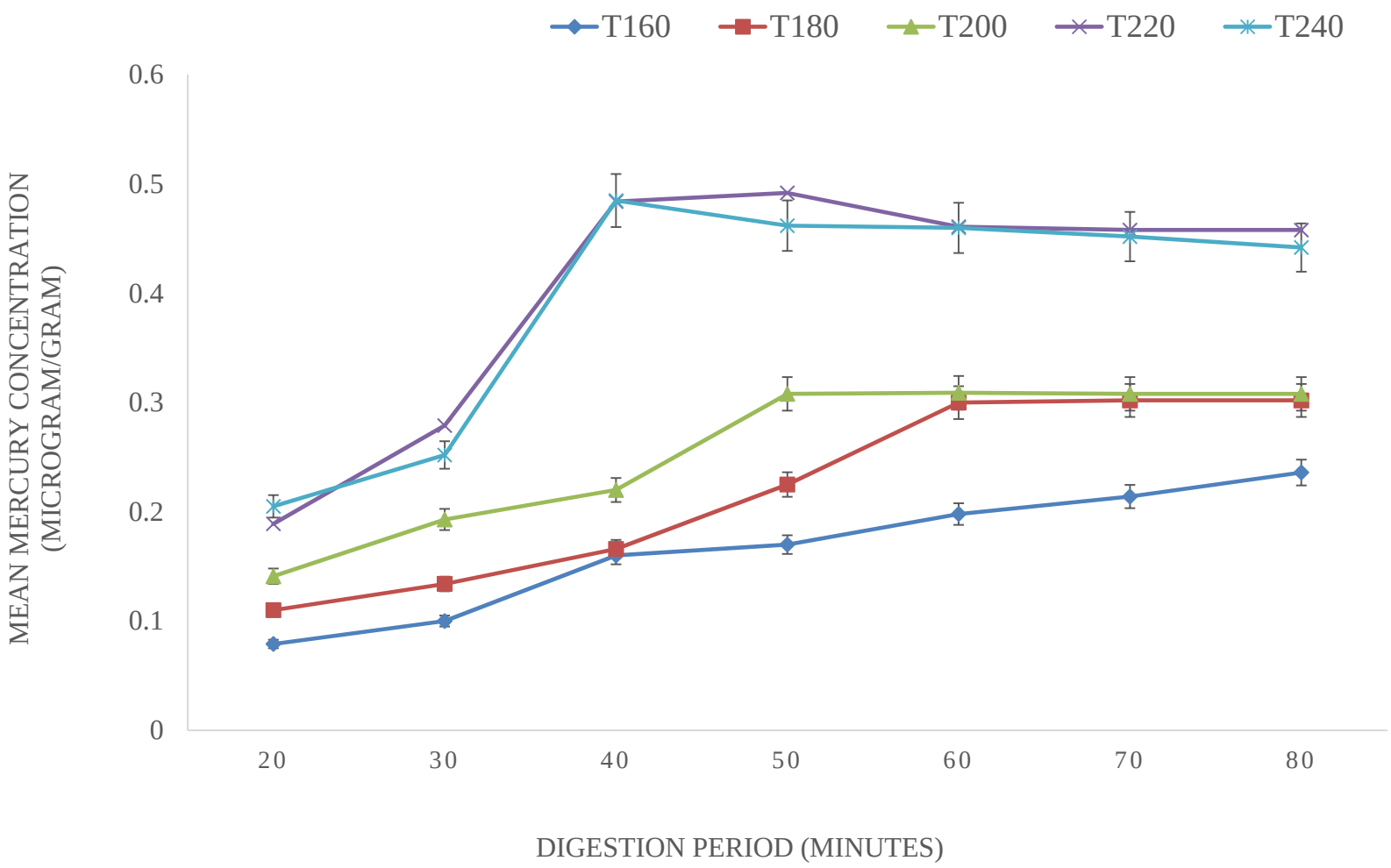

Fig. 3 Mean THg concentrations in the fish muscle at different temperatures against digestion period**.**

- The procedure was investigated for validity by subjecting 1.000 g of CRM fish homogenate IAEA-407 to optimum conditions of the digestion in five replicates.

- The measured THg concentration (0.219 ± 0.010 µg/g.) showed good agreement with the certified value (0.222 ± 0.006 µg/g).

- The mean percentage recovery was 98.56 ± 4.54 %.

- Linsinger’s Test showed no significant difference between the measured and certified THg concentrations for the CRM.

- Spiking studies carried out showed mean percentage recoveries of 96.67 ± 2.89, 106.60 ± 3.82 and 106.67 ± 2.54 % at the three levels of fortification respectively (**Figs. 4** and **5**).

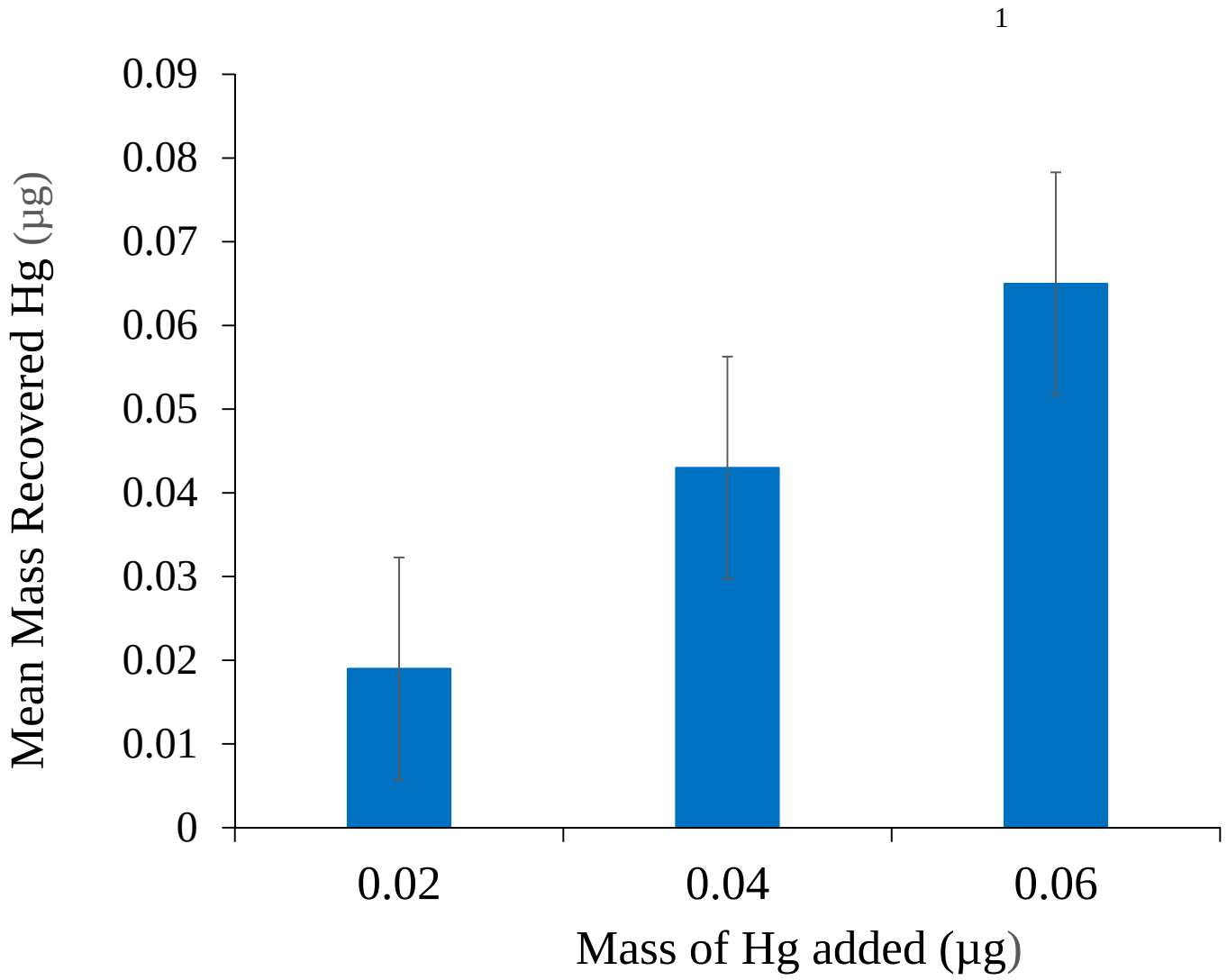

Fig. 4 Results of Spiking Studies I.

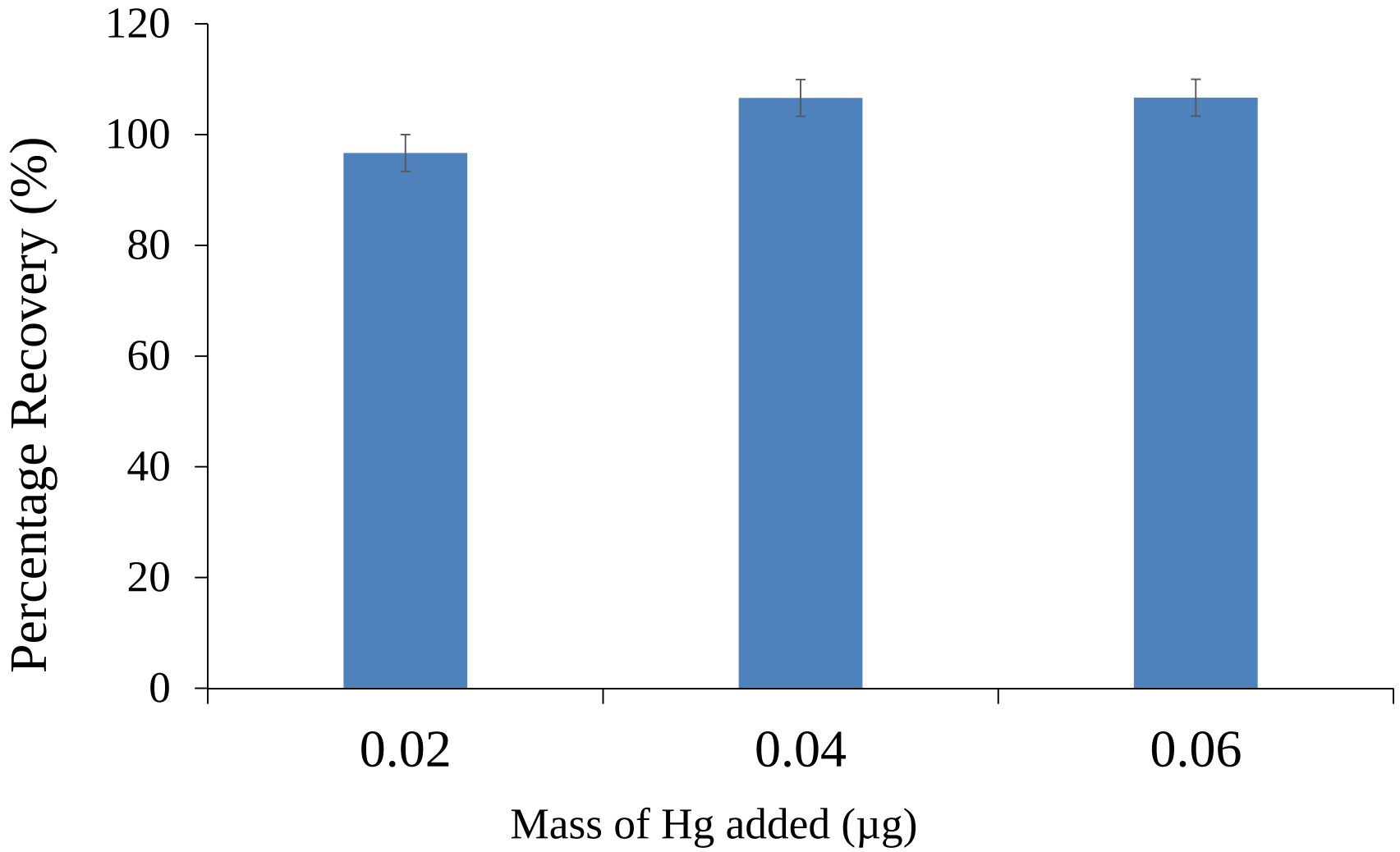

Fig. 5 Analytical Results of Spiking Studies II

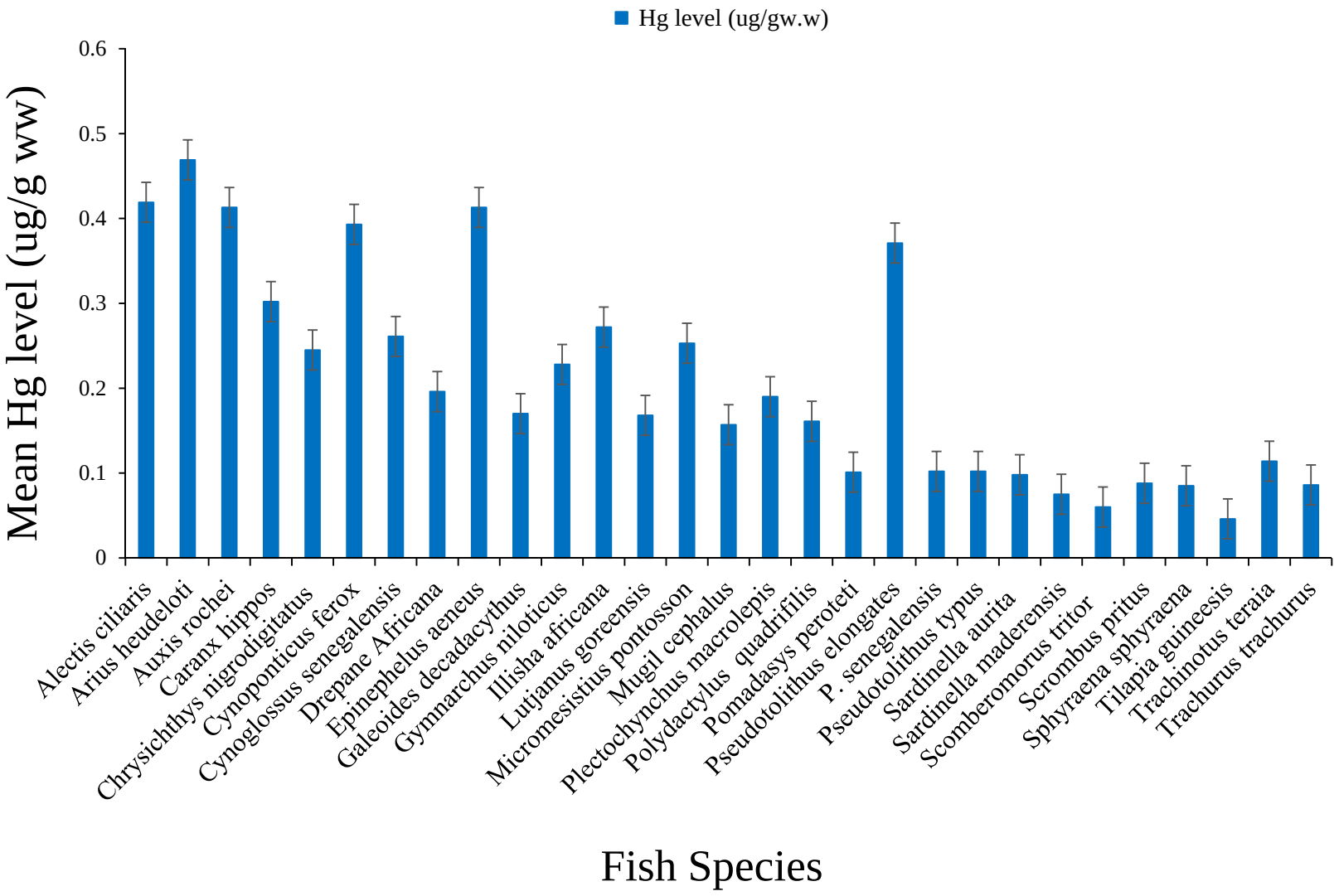

Fig. 6 Mean Hg Levels in Fish from Lagos Markets

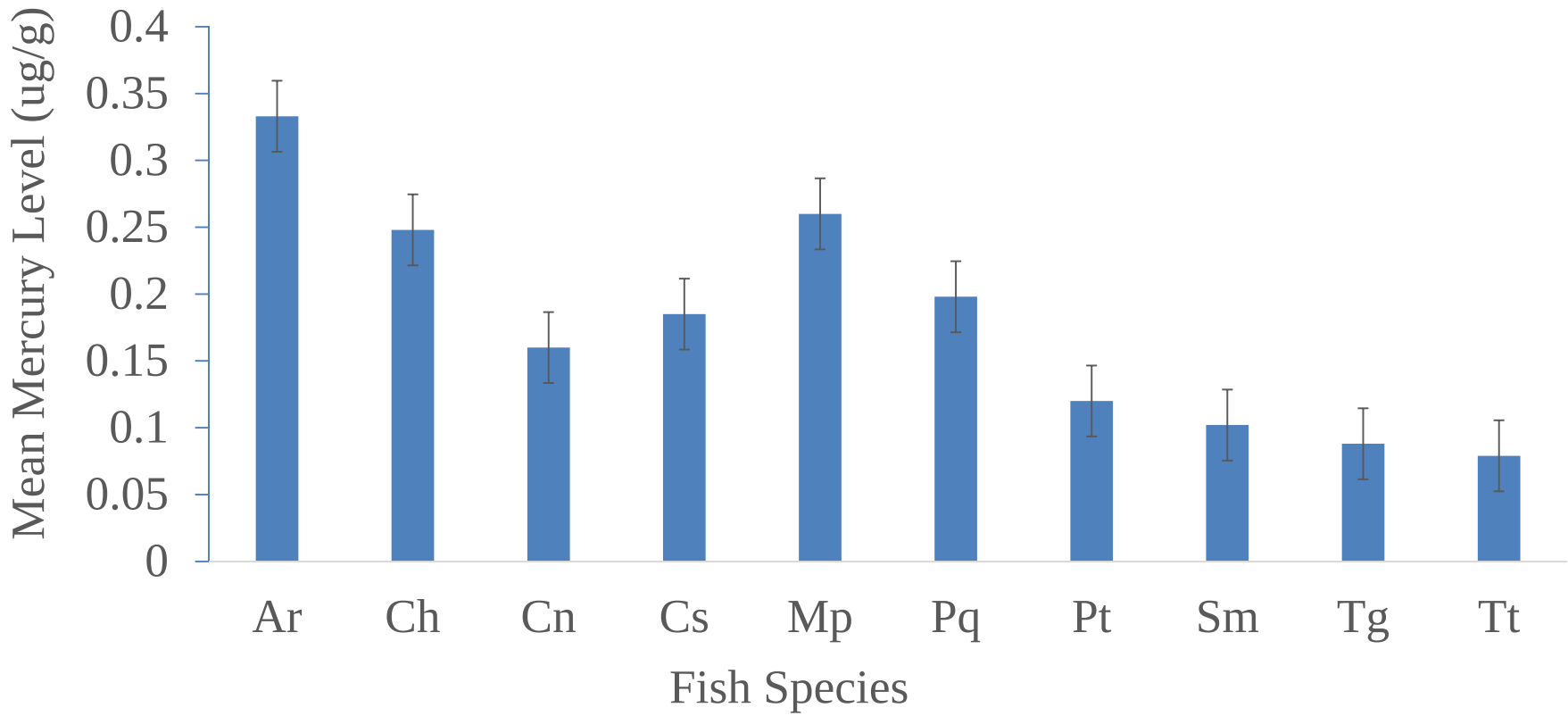

Fig.7 Mean THg Levels in Commonly Consumed Fish Species in Lagos

Ar = *Arius rochei*, **Ch** = *Caranx hippos*, **Cn** = *Chrysichthys nigrodigitatus*,

Cs = *Cynoglossus senegalensis*, **Mp** = *Micromesistius pontosson*,

Pq = *Polydactylus quadrifilis*, **Pt** = *Pseudotolithus typus*, **Sm** = *Sardinella manderensis*,

Tg = *Tilapia guineensis*, **Tt** = *Trachurus trachurus.*

Table 4. Weekly Mercury Intakes of Lagos Resident Fish Consumers.

Fish Species	Mean Hg Level (µg/g)	Weekly Hg Intake LFC (µg Hg/kg bw-wk)		Wkly Hg Intake MFC (µg Hg/kg bw-wk)		Wkly Hg Intake HFC (µg Hg/kg bw-wk)	
		WCBA	MA	WCBA	MA	WCBA	MA
A. rochei	0.333	0.46	0.42	1.14	1.06	1.84	1.71
		0.76	0.71	1.91	1.77	3.08	2.86
		1.00	1.00	2.69	2.50	4.34	4.03
C. hippos	0.248	0.34	0.32	0.85	0.79	1.40	1.30
		0.57	0.53	1.42	1.32	2.34	2.18
		0.80	0.74	2.00	1.86	3.29	3.06
C. nigrodigitatus	0.160	0.22	0.20	0.55	0.51	0.95	0.89
		0.37	0.34	0.92	0.85	1.60	1.48
		0.52	0.48	1.29	1.20	2.25	2.09
C. senegalensis	0.185	0.25	0.24	0.63	0.59	0.94	0.88
		0.42	0.39	1.06	0.98	1.58	1.46
		0.60	0.56	1.49	1.39	2.22	2.06
M. pontosson	0.260	0.36	0.33	0.89	0.83	1.45	1.34
		0.60	0.55	1.49	1.38	2.42	2.25
		0.84	0.78	2.10	1.95	3.41	3.17
P. quadrifilis	0.198	0.27	0.25	0.68	0.63	1.19	0.86
		0.45	0.42	1.13	1.05	1.99	1.85
		0.64	0.59	1.60	1.49	2.80	2.60

Table 4 Continued

Fish Species	Mean Hg Level (µg/g)	Weekly Hg Intake LFC (µg Hg/kg bw-wk)		Weekly Hg Intake MFC (µg Hg/kg bw-wk)		Weekly Hg Intake HFC (µg Hg/kg bw-wk)	
		WCBA	MA	WCBA	MA	WCBA	MA
P. typus	0.120	0.16 0.27 0.39	0.15 0.26 0.36	0.41 0.69 0.97	0.38 0.64 0.97	0.64 1.06 1.50	0.59 0.99 1.39
S. maderensis	0.102	0.14 0.23 0.33	0.13 0.22 0.31	0.35 0.58 0.82	0.32 0.54 0.77	0.50 0.84 1.19	0.47 0.78 1.10
T. guineensis	0.088	0.12 0.20 0.28	0.11 0.19 0.26	0.30 0.50 0.71	0.28 0.47 0.66	0.22 0.38 0.53	0.21 0.35 0.49
T. trachurus	0.079	0.11 0.18 0.26	0.10 0.17 0.24	0.27 0.45 0.64	0.25 0.42 0.59	0.44 0.73 1.03	0.41 0.68 0.96
Mixed Consumption	0.177	0.24 0.41 0.57	0.22 0.38 0.53	0.61 1.01 1.43	0.56 0.94 1.33	0.93 1.56 2.20	0.86 1.45 2.04

- Two methods were used for assessment of Health Risks from mercury exposure: Use of data from Questionnaires and use of established and validated American Consumption Rate (ACR).

- In each of the methods two approaches were used: Estimation of Hazard Quotient (HQ) and Exposure Ratio (ER).

- In all the sub-groups of consumers (Questionnaires), consumption of large serving size per meal increased the risk of observable adverse health of mercury if consumption habits were maintained.

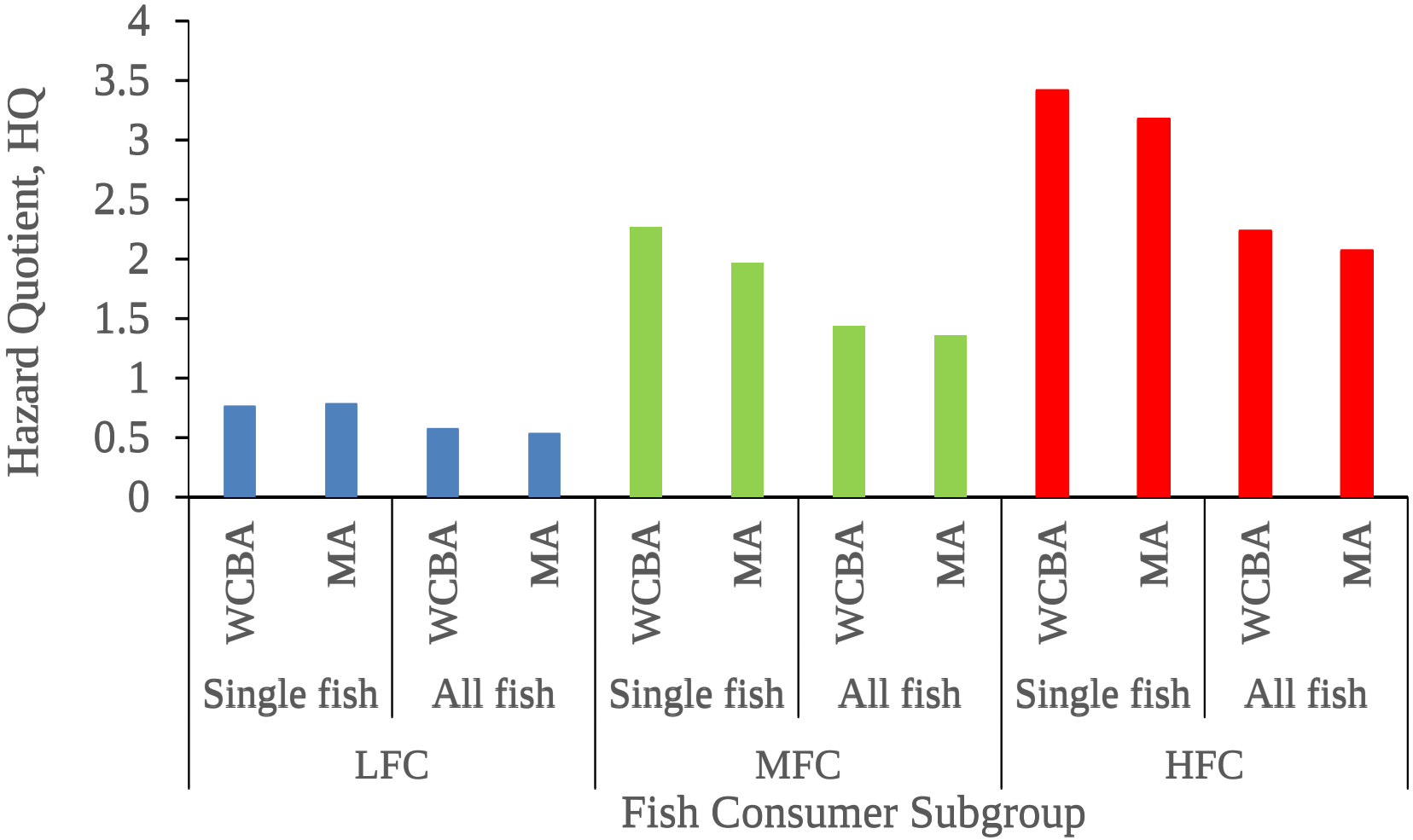

Fig. 8 Mean Hazard Quotients of Fish Consumer Sub-groups

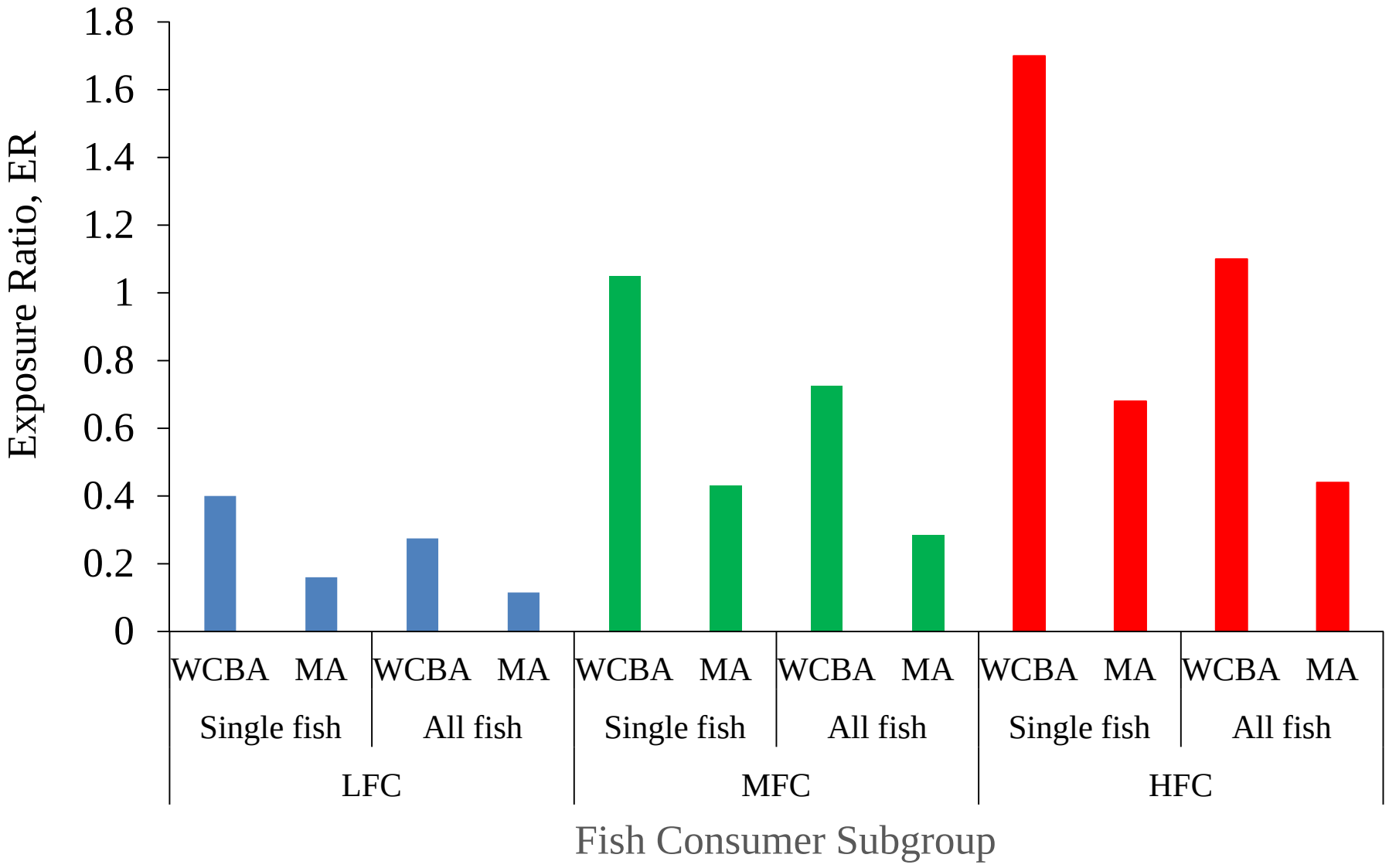

Fig.9 Mean Exposure Ratios of Various Fish Consumer Sub-groups

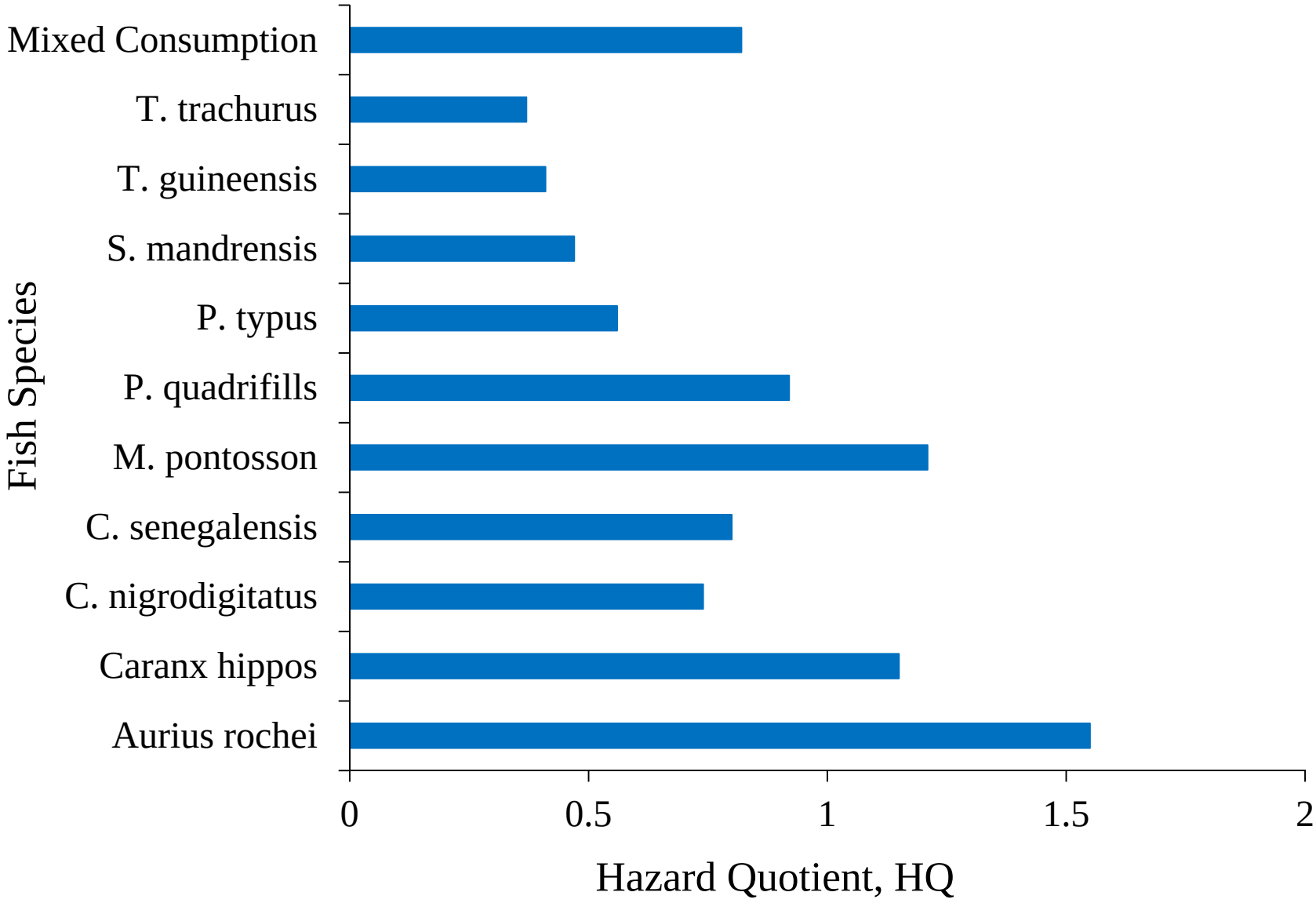

Fig. 10 The HQ values using the American Consumption Rate

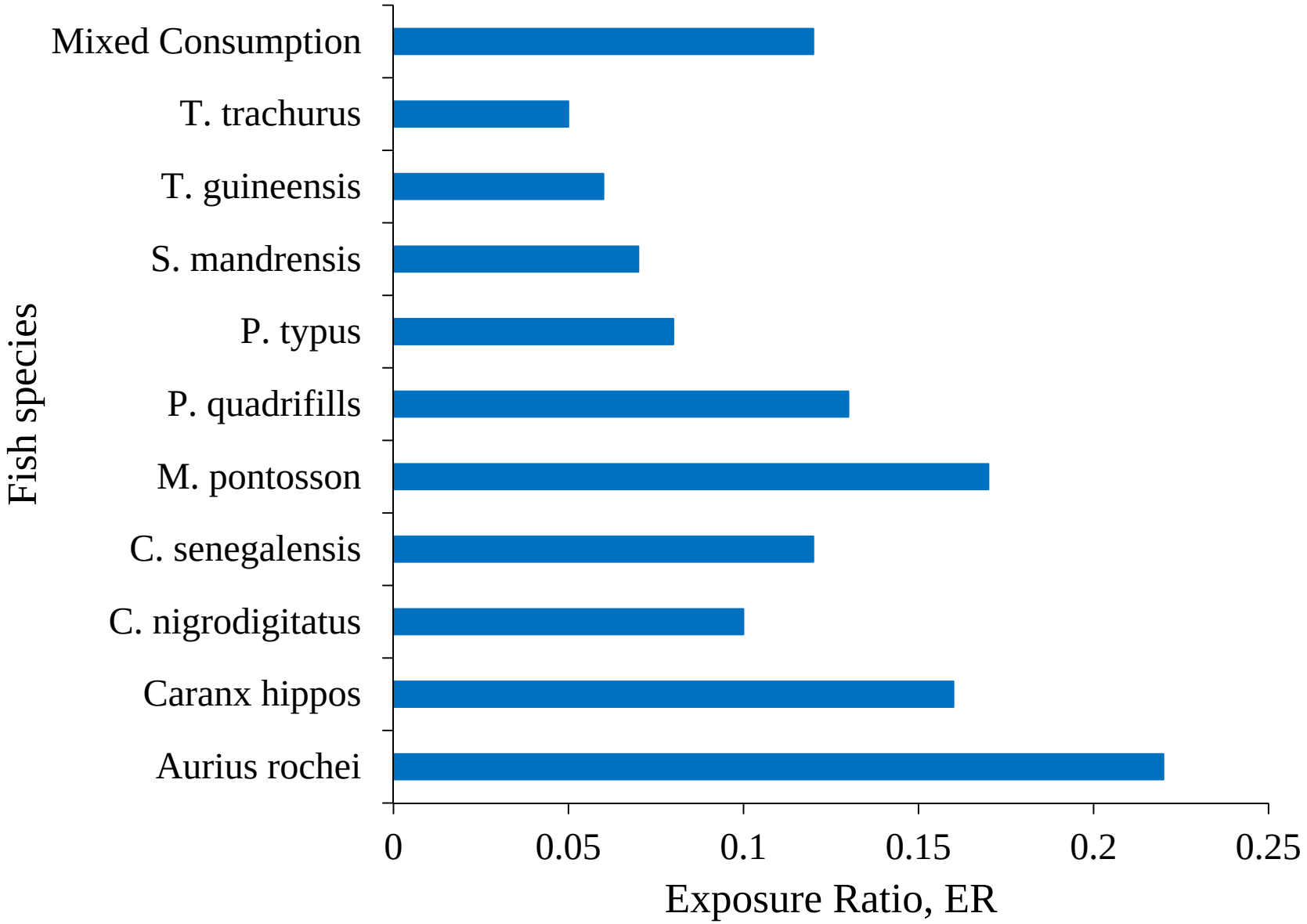

Fig.11 Estimates of Exposure Ratio using the American Consumption Rate

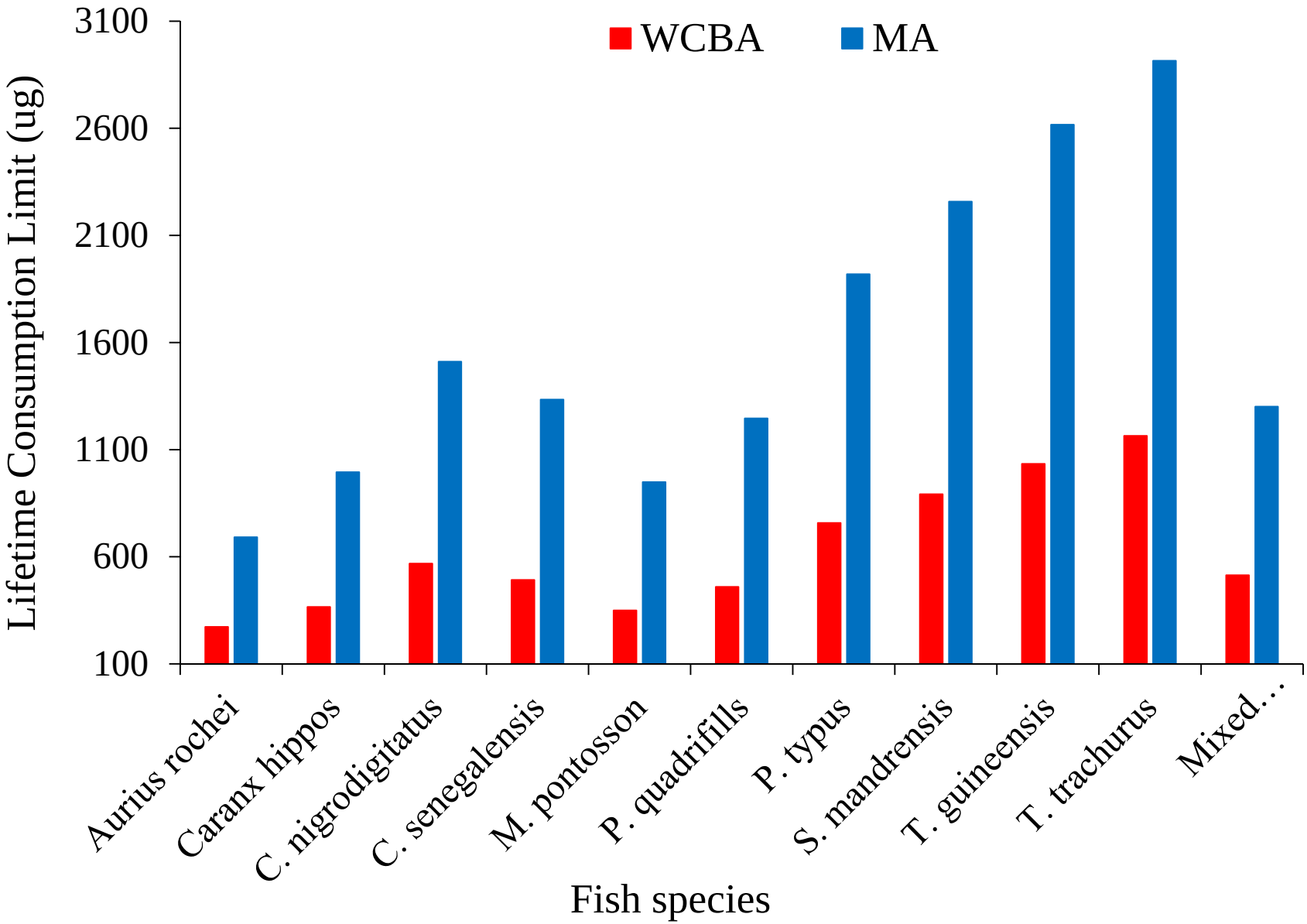

Fig.12 Lifetime Consumption Limits of Commonly Consumed Fish in Lagos

CONCLUSIONS

- There were no significant differences in the THg measured using the digestion procedures.
- Procedure **9** was found to be best in terms of accuracy and method performance.
- Procedure **9** was found to perform best at 220 ^{o}C and 50 minutes of digestion.
- The optimized procedure was validated using CRM, Spiking and Linsinger's Test of method performance.

- All the 205 samples covering twenty-nine species analysed using the optimized and validated procedure showed THg levels below the limit set by FAO/WHO for fish.

- Risk assessment (Hazard Quotient) of the fish consumption using American Consumption Rate of 228 g/wk ranged between 0.37 and 1.55.

- Among the commonly consumed fish species *A. rochei*, *C. hippos* and *M. pontosson* have HQ above 1.00; so their consumption could likely result in appreciable risk of observable adverse health effects from mercury over time.

THANK YOU