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Research Article

Evaluating Benefits and Risks of Polyunsaturated Fatty Acids and Methyl Mercury from Fish and Seafood Consumption in Peninsular Malaysia

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The risks and benefits associated with methyl mercury (meHg) and polyunsaturated fatty acid (PUFA) from seafood consumption were assessed in adults and adolescents from Peninsular Malaysia. Seafood samples were collected for meHg analysis while the consumption survey was conducted among adults and pupils ≥10 years old. Long-chain omega-3 fatty acids (LC ω-3 PUFA; DHA and EPA) data were obtained from locally published articles. The estimated weekly intake (EWI), provisional tolerable weekly intakes (PTWIs), hazard quotient (HQ), and maximum safe weekly consumption (MSWC) were calculated for each seafood studied. The average range of LC ω-3 PUFA concentration was between 11.7 and 2,210.5 mg/100 g, where 27% of samples contained >500 mg/100 g and were predominant in pelagic fish and mollusks. MeHg concentrations in seafood samples ranged from 0.0426 to 0.4576 mg/kg of wet weight (WW) and showed significant variations between all species at a median concentration of 0.0621 ± 0.0573 mg/kg WW. Total seafood consumed by the adolescents was 84.7 ± 103.7 g/day, with significant marginal differences compared to the adult population at 90.5 ± 100 g/day. Long-tail tuna, yellow-stripe shad, slender shad, and long-tail shad contributed to a higher LC ω-3 PUFA intake than other species. These fish also contributed to a low HQ value level, lowering the risk of health effects. Mangrove red snapper has a low LC ω-3 PUFA content, but the HQ value was the highest of all, and it is advised to consume less frequently. Double the intake of cephalopod and a threefold increase in crustacean consumption would still minimize the meHg risk and may increase the intake of LC ω-3 PUFA.

1. Introduction

Malaysians eat a lot of fish and rank fifth globally or second in Asia in yearly per capita fish intake [1]. They consumed fish at least once daily in the amounts of one and a half medium fish per day [2]. A recent study showed that they consumed 168 g/day of fish, with Malay ethnicities consuming fish significantly more than other ethnicities, the Chinese, Indians, and others [3]. Fish is recognized to have high-quality protein, making up 17% of all animal protein, 7% of which is consumed globally [4]. Eating fish and seafood has several health advantages: good energy sources, proteins, and essential vitamins and minerals [5, 6]. Fish also contains beneficial fatty acid composition, such as the long-chain n-3 polyunsaturated fatty acids ($n-3$ PUFA), eicosapentaenoic acid (EPA; C20: $5n-3$), and docosahexaenoic acid (DHA; C22: $6n-3$) [4, 6–8]. Protecting many ailments, including coronary heart disease, inflammation, the hypotriglyceridemic impact, allergies, hypertension, arthritis, autoimmune disorders, and cancer, is one of the well-established positive health effects of $n-3$ PUFAs. According to reports, atherosclerosis and thrombosis may also be avoided by eating a diet rich in marine n-3 fatty acids. Studies with newborns indicate that DHA is essential for the normal functional development of the retina and brain, specifically in premature infants [7–10].

Marine organisms can also be a potential source of human exposure to pollutants, for example, meHg, and the extent to human meHg exposure depends on the species of fish, their frequency, and the amount of consumption [11–13]. MeHg is a potent toxin that primarily affects the brain and other parts of the central nervous system [14]. It is highly mobile in the human body, and because it may traverse the placental and blood-brain barriers, it can harm unborn children and cause damage to developing children. It is known that high transmission of meHg to the developing fetus and child causes severe developmental impairment, brain damage with mental retardation, cerebral palsy, blindness, etc. [14–16]. The journey of meHg into the human body is explained through the formation of water-soluble methyl mercury complexes in body tissues, and the main route of its elimination from the body is through feces, which is as much as 90% of total excretion [14]. Adults with meHg poisoning typically have paraesthesia or numbness in the hands and feet, coordination problems, concentric constriction of the visual field, auditory complaints, ischemic stroke, dementia, depression, and gastrointestinal dysfunction [14, 17].

This study aims to evaluate the health benefits and risks analysis of fish and seafood consumed by the Malaysian population. It observed the intake of EPA, DHA (benefits), and meHg pollutants (risk) through consuming various edible fish and seafood captured by Malaysian fishery industries. This study notifies levels of meHg concentration in fish and seafood obtained from fish landing ports and wholesale wet markets across Peninsular Malaysia. It also examined consumption patterns of fish and seafood among adolescents and adults. A potential alarm for human health hazards was evaluated by calculating the EWI and HQ, and finally, the MSWC was identified for each fish and seafood species studied. The intake of LC ω-3 PUFA per day by these 2 population groups was also calculated.

2. Methodology

2.1. Fish and Seafood Consumption Survey. Detail on procedures for fish and consumption survey among adults in Peninsular Malaysia was reported by Ahmad et al. [3], while those for adolescents were reported later in the year 2019 [18]. Both groups' demographic backgrounds were presented in published articles accordingly [3, 11, 18]. The Ministry of Health Malaysia funded the study, and the Medical Research and Ethics Committee from the same ministry approved the ethical issues. Informed consent was obtained from the subjects earlier, a household-based, cross-sectional study was conducted, and data were collected through face-to-face interviews using predesigned questionnaires. The Department of Statistics, Malaysia, consulted the sampling frame and household addresses.

To begin the survey, a total of 2,996 individuals were selected who met the criteria of living in families with at least 2 adults and adolescents aged 10 to 17 years old. A total of 2,675 adults (89.2%) completed the survey, whereas only 484 adolescents (54.4%) did. The study tool was a prior validated questionnaire that consisted of two parts. The first part was a self-administered questionnaire consisting of a section of socio-demographic information, patterns, and frequency of fish consumption, knowledge, perception, and practices towards fish consumption. The second part was a three-day record of 24-hour dietary diary forms. Research assistants assisted with the survey and were equipped with tools such as dish pictures, fish, and standard household measures. The questionnaire was given at 9.00 am or until late at night if necessary. Parents were requested to assist their children in answering questionnaires and filling out the dietary forms [3, 11, 18]. The portion weight of food was referred to in the local food atlas by Suzana et al. [19, 20] and the composition of Malaysian foods by Tee et al. [21]. Five different nonlisted food sources were obtained, and mean weights were calculated. The polls were taken on weekdays and weekends.

2.2. Fish and Seafood Collection and Preparation for Total Mercury (tHg) Analysis. Details on fish collection and contaminant analysis methods are reported by Ahmad and coworkers (2015[ab]). Sampling was conducted from June to December 2009; samples were obtained from 6 principal Fisheries Development Authority of Malaysia (LKIM) fish landing complexes and 5 wholesale markets across Peninsular Malaysia. Samples were collected from three fishing boats landed at the complexes and three randomly selected business units at the wholesale market. The distribution of respondents with fish and seafood sampling survey locations is presented in Figure 1. One kg of sample was purchased for each species selected, with finally a total of 394 seafood collected. The total length and weight of samples were measured in millimeters and grams, respectively. Seafood samples were packed, labeled, and put into an icebox before being transported to the laboratory and stored at −21°C. Samples were thawed at room temperature before processing, filleted, chopped, and homogenized. Finally, samples were dried at 65°C to constant dry weight and ground using mortar.

2.3. tHg Concentrations Determination in Fish and Seafood. The sample was mixed with concentrated nitric acid and hydrogen peroxide in a polytetrafluoroethylene digestion vessel, sealed, and placed into the microwave digester (Multiwave 3000, Anton Paar) rotor before digestion. Total mercury was analyzed by the cold vapor atomic absorption spectrometry technique (Flow Injection Mercury System (FIMS-400), Perkin Elmer) following the method by Mohd Fairulnizal et al. [23]. LOD was identified from mercury concentration tallying 3 times the SD of 10 reagent blanks, 0.72 μg/L. The average recovery of reference standards (NIST SRM® 1946, Lake Superior Fish Tissue) was 91%, and RSD was <5%. Total mercury concentration levels were converted into wet basis values using the formulation: dry weight concentration = wet weight concentration × ((100−moisture content)/100). Moisture content was referred to by Tee et al. [21] and Nurnadia et al. [24]; and details on the analysis were published elsewhere [25, 26].

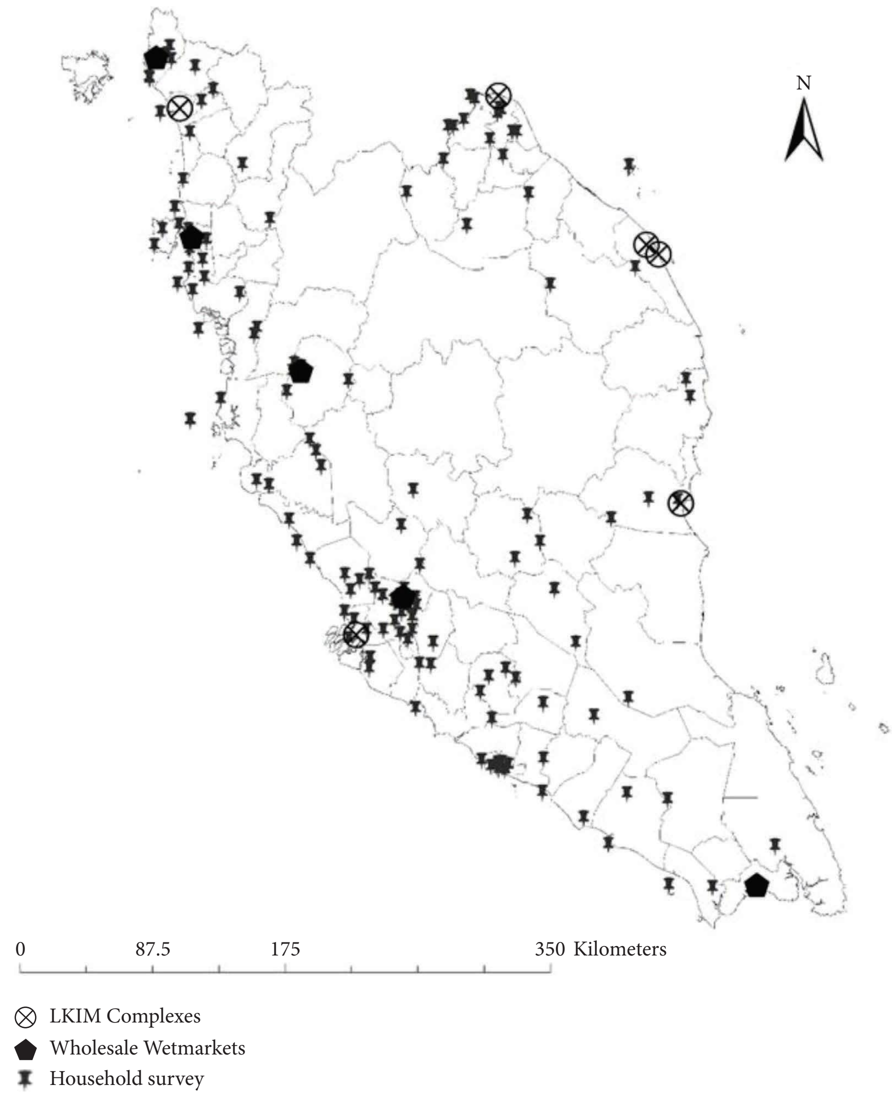


Figure 1: Map of fish sampling locations (LKIM complexes and selected wholesale wet markets) and locations for consumption and seafood household survey throughout Peninsular Malaysia (the distribution of locations was mapped using ArcGIS Desktop [22]).

2.4. DHA and EPA Data in Fish and Seafood. The DHA and EPA concentrations were sourced from Nurnadia et al. [27] and Wan Rosli et al. [28], where fish and seafood were captured from the east and west coasts of Peninsular Malaysia. The re-calculated average levels for both the DHA and EPA in fish and seafood were ordered into mg/100g samples, as shown in Table 1. The population intake of DHA and EPA was calculated and compared to the global authority target recommendation per day for healthy adults (250 mg DHA and EPA per day) and reduce coronary heart disease (CHD) risk in healthy adults (minimum of 500 mg DHA and EPA per day) [29].

2.5. Methyl Mercury (meHg) Data in Fish and Seafood. The calculation for meHg per species was using the percentages of tHg at 93%, 81%, and 50% for fish, cephalopods, and crustaceans, respectively [30] (Table 2). The FAO/WHO (2006) and the Malaysian Food Regulation [31] (Food Act 1983) compared results to guideline levels.

2.6. Health Risk Assessment of Mercury from Fish and Seafood Consumption

2.6.1. Estimated Weekly Intake (EWI) and Maximum Safe Weekly Consumption (MSWC). The EWI of total Hg and/or meHg was calculated using the following equation:

$$\mathrm{EWI} = \frac{\text{conc of meHg (mg/kgWW)} \times \text{weekly consumption (WC) (g)}}{\text{body weight (BW) (kg)}}. \quad (1)$$

Table 1: Total mercury (tHg) and methylmercury (meHg) levels in fish and seafood from Peninsular Malaysia.

Species no.	Groups/family/species	Common name	n	tHg ± IQR (mg/kg) DW	MC (%)	tHg (mg/kg) WW	*#meHg (mg/kg) WW
◆*Pelagic fish*							
1	*Selaroides leptolepis*	Yellow-stripe scad	13	0.252 ± 0.125	79.5	0.0635	0.0591
2	*Decapterus russelli*	Slender scad	10	0.195 ± 0.108	74.7	0.0458	0.0426
3	*Megalaspis cordyla*	Torpedo scad	20	0.319 ± 0.198	74.8	0.0750	0.0697
4	*Parastromateus niger*	Black pomfret	15	0.242 ± 0.121	76.5	0.0569	0.0529
5	*Rastrelliger kanagurta*	Indian mackerel	13	0.180 ± 0.066	73.1	0.0522	0.0485
6	*Scomberomorus guttatus*	Indo-Pacific king mackerel	12	0.262 ± 0.355	75.9	0.0650	0.0604
7	*Thunnus tonggol*	Long-tail tuna	8	0.358 ± 0.173	71.0	0.3580	0.3329
8	*Euthymus affinis*	Kawakawa	3	0.289	75.2	0.0699	0.0650
						Median	**0.0597**
◆*Demersal fish*							
9	*Lutjanus argentimaculatus*	Mangrove red snapper	3	0.856	75.8	0.1875	0.1743
10	*Lutjanus russellii*	John's snapper	4	0.884 ± 1.789	80.2	0.1848	0.1718
11	*Lates calcarifer*	Giant-sea perch	11	0.537 ± 0.436	78.1	0.1240	0.1154
12	*Gymnura poecilura*	Long-tail butterfly ray	25	0.492 ± 1.251	79.1	0.4920	0.4576
13	*Nemipterus japonicus*	Japanese threadfin bream	11	0.464 ± 0.724	76.9	0.1063	0.0988
						Median	**0.1718**
Freshwater fish							
14	*Clarias batrachus*	Walking catfish	9	0.334 ± 0.325	77.1	0.0621	0.0578
Cephalopods							
15	*Sepia officinalis*	Common cuttlefish	8	0.280 ± 0.101	81.4	0.0959	0.0777
Crustaceans							
16	*Metapenaeus affinis*	Rainbow shrimp	4	0.280 ± 0.501	80.5	0.2800	0.1400

Total Hg in median ± IQR; DW: dry weight; IQR: interquartile range; MC: moisture content; MC content was based on the works by Tee et al. [21] and Nurnadia et al. [24]. WW: wet weight; conversion of DW mercury concentrations in fish samples to WW was by means formula: DW = WW × (100/100 MC). #Details on total mercury concentrations in seafood are referred to Ahmad et al. [25, 26]; *calculation of meHg concentrations was based on the mean percentage of methyl mercury to total mercury at 93% for fish, 81% for cephalopods, and 50% for crustaceans [11, 30]. #Comparison of meHg levels for different fish/seafood species: $\chi^{2MW} = 71.385$, p value ≤ 0.001, $N = 172$ and median meHg for all seafood = 0.0621 ± 0.0573 mg/kg WW. ◆Comparison of meHg levels for different groups of pelagic and demersal: $\chi^{2MW} = 1145.000$, p value ≤ 0.001, $N = 150$ (pelagic; $n = 96$; demersal, $n = 54$). Median meHg for pelagic fish = 0.0513 ± 0.0392 mg/kg WW and median meHg for demersal fish = 0.0988 ± 0.1332 mg/kg WW.

Table 2: Concentration of DHA and EPA from selected fish samples (mg/100 g fish samples) collected from the east and west coast of Peninsular Malaysia.

No.	Species	Common name	DHA (mg/100 g)	EPA (mg/100 g)	*DHA + EPA (mg/100 g)
Pelagic fish					
1	*Selaroides leptolepis*	Yellow-stripe scad	870.28	231.45	1,101.73
2	*Decapterus russelli*	Slender scad	1,162.50	353.80	1,516.30
3	*Megalaspis cordyla*	Torpedo scad	827.60	182.25	1,009.85
4	*Parastromateus niger*	Black pomfret	642.95	159.10	802.05
5	*Trachinotus blochii*	Snub nose pompano	122.60	176.70	299.30
6	*Pampus argenteus*	Silver pomfret	492.70	207.60	700.30
7	*Rastrelliger kanagurta*	Indian mackerel	348.95	115.50	464.45
8	*Scomberomorus guttatus*	Indo-Pacific king mackerel	624.95	182.55	807.50
9	*Thunnus tonggol*	Long-tail tuna	760.20	246.30	1,006.50
10	*Euthymus affinis*	Kawakawa	886.40	228.30	1114.70
		Median	701.58	195.08	907.00
Demersal fish					
11	*Lutjanus argentimaculatus*	Mangrove red snapper	209.90	24.10	234.00
12	*Lutjanus johnii*	Golden snapper	18.60	7.30	25.90
13	*Lates calcarifer*	Giant-sea perch	594.85	346.65	941.50
14	*Gymnura poecilura*	Long-tailed butterfly rays	9.00	2.70	11.70
15	*Nemipterus japonicus*	Japanese threadfin bream	723.75	292.10	1,015.85
16	*Polynemus indicus*	Indian threadfin	82.20	23.70	105.90
17	*Eleutheronema tetradactylum*	Four-finger threadfin	53.10	96.20	149.30
18	*Epinephelus fasciatus*	Red-banded grouper	924.90	216.10	1,141.00
19	*Chirocentrus dorab*	Wolf herring	54.30	23.90	78.20
20	*Plotosus canius*	Gray eel-catfish	88.80	145.80	234.60
21	*Cynoglossus arel*	Large-scale tongue sole	113.40	8.30	121.70
22	*Clupea fimbriata*	Fringe scale sardine	225.40	211.50	436.90
23	*Hilsa macrura*	Long-tail shad	168.70	2,041.80	2,210.50
		Median	113.40	96.20	234.00
Freshwater fish					
24	*Clarias batrachus*	Walking catfish	ND	14.50	14.50
Cephalopod/crustacean/bivalve					
25	*Sepia officinalis*	Common cuttlefish	472.85	159.05	631.90
26	*Metapenaeus affinis*	Greasy-back pink prawn	391.25	284.25	675.50
27	*Liocarcinus vernalis*	Shallow-water crab	749.70	517.10	1,266.80
28	*Arca granosa*	Blood cockle	156.80	298.00	454.80
		Median	432.05	291.13	653.70

DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid. *Calculated by Nurnadia et al. [27] and Wan Rosli et al. [28].

The average body weights of 60 and 45 kg were used for adults and adolescents, respectively [3, 18]. The PTWI used for inorganic meHg is 1.6 μg/kg body weight/week [32].

The calculation for estimating the MSWC at PTWI 1.6 μg/kg body weight/week is as follows:

$$\mathrm{PTWI} = \frac{\text{conc of meHg (mg/kgWW)} \times \mathrm{MSWC}\,(g)}{\mathrm{BW\,(kg)}},$$

$$\mathrm{MSWC}\,(g) = \frac{1.6\,\mu g/\text{kg body weight/week} \times \mathrm{BW\,(kg)}}{\text{conc of meHg (mg/kgWW)}}. \quad (2)$$

2.6.2. Hazard Quotient (HQ). Risk assessment is a tool to estimate the probability of health effects due to exposure to a hazard; this study is the exposure through the consumption of fish. USEPA developed the oral reference doses (RfDs) for Hg at 1×10^{-4} (mg/kg/day) (risk information system (IRIS) [33]. The HQ meHg was calculated based on the following equation [11, 34]:

$$\mathrm{HQ} = \frac{\mathrm{EF} \times \mathrm{ED} \times \mathrm{FIR} \times \mathrm{C} \times 10^{-3}}{\mathrm{RfD} \times \mathrm{BW} \times \mathrm{AT}}, \quad (3)$$

where EF = exposure frequency at 350 days/year; ED = duration of human exposure for children and adults at 6 and 30 years, respectively; FIR = seafood ingestion rate (total intake per day in gram); C = meHg concentration in the seafood (mg/kg wet weight); RfD = oral reference dose (IRIS, USEPA); BW = average body weight of population group; and AT = average time of human exposure to noncarcinogen (AT = ED × 365 days).

Target hazard is a ratio of the determined dose of a contaminant to the oral reference dose considered detrimental.

When HQ ≥ 1, there is potential for noncarcinogenic health risks from the intake of meHg through the consumption of seafood by the studied population.

2.7. Statistical Analysis. Statistical analysis in this study was conducted using IBM SPSS Statistics 26. The distribution of locations covered during the study was mapped using ArcGIS Desktop [22]. The analysis began with data checking and filtering where tHg data were cleaned and verified for inconsistencies. The final dataset used was households with complete information, and those with missing values were filtered out from the analysis. These included merging incomplete addresses into the same locality, district, and state. Nonparametric statistics such as median and interquartile range (IQR) were applied to the data due to nonnormal distribution. Mann–Whitney U and Kruskal–Wallis tests also were analyzed to compare the difference between groups of fish and seafood. A 5% significance level was used for all tests in the study.

3. Results

3.1. DHA and EPA in Fish and Seafood. Average concentrations of DHA and EPA from selected fish samples (mg/100 g) collected from the east and west coasts of Peninsular Malaysia are shown in Table 1. Overall, the median concentration of DHA and EPA content in pelagic fish is the highest (907.00 mg/100 g) compared to the other groups: demersal fish (234.00 mg/100 g) and shellfish (653.70 mg/100 g). The ratio between DHA and EPA was also the highest in these fish (3.6) compared to the demersal fish (1.2) and shellfish (1.5). Pelagic fish with high content of both DHA and EPA are yellowstripe scad (*Selaroides leptolepis*) (1,101.73 mg/100 g), slender scad (*Decapterus russelli*) (1,516.30 mg/100 g), torpedo scad (*Megalaspis cordyla*) (1,009.85 mg/100 g), long-tail tuna (*Thunnus tonggol*) (1,006.50 mg/100 g), and kawakawa (*Euthynnus affinis)* (1, 114.7 mg/kg) while, for demersal fish, both DHA and EPA were at high concentrations in the Japanese threadfin bream (*Nemipterus japonicus*) (1,015.85 mg/100 g), red-banded grouper (*Epinephelus fasciatus*) (1,141.00 mg/100 g), and long-tail shad (*Hilsa macrura*) (2,210.50 mg/100 g). Shallow-water crab (*Liocarcinus vernalis*) (1,266.80 mg/100 g) also showed a high concentration of these PUFA compared to other shellfish. Among different fish and seafood species, DHA was the highest in slender scads (*Decapterus russelli*) (1,162.50 mg/100 g), while nine of the other species showed DHA levels of more than 500 mg/100 g samples. The EPA was highest in long-tail shad (*Hilsa macrura*) (2,041.80 mg/100 g) and was the only species that could be considered an excellent source of EPA compared to other species studied.

When long-chain PUFA in samples were positioned into 3 categories, as reported by Weaver et al. [35] (Figure 2), results revealed that 27% of fish and seafood studied were in category 1, with half of the samples containing PUFA less than 150 g/100 g edible portion (category 3) (Figure 2(a)). PUFA was found to be predominant in the pelagic fish and mollusks (Figure 2(b)), and among demersal fish studied, only 31% were categorized in category 1, with nearly half of the demersal species studied containing less than 150 mg/100g PUFA.

3.2. Fish and Seafood meHg Concentration Level. Table 2 presents the tHg and meHg concentrations in fish and seafood in median and IQR. Sixteen fish and seafood species were identified, and the meHg levels ranged from 0.0426 to 0.4576 mg/kg of wet weight (WW). A species of cephalopods, crustaceans, freshwater fish, five demersal fish, and eight pelagic fish were included as they represented the group of fish and seafood. The meHg levels significantly varied in fish and seafood groups ($\chi^2_{KW} = 71.385$; $p \leq 0.001$). The median concentration for meHg was at 0.0621 ± 0.0573 mg/kg WW. All demersal fish showed meHg levels at ≥0.1 mg/kg WW but not long-tail tuna (*Thunnus tonggol*) (0.3329 mg/kg WW) of pelagic fish, which showed higher levels of this metal. Similar results were shown for the crustacean and rainbow shrimp (*Metapenaeus affinis*) (0.1400 mg/kg WW).

However, for cephalopods (*Sepia officinalis*) and freshwater fish (*Clarias batrachus*), the concentration levels were very much lower. Comparison of meHg levels for different groups of pelagic and demersal fish showed significant differences at $\chi^2_{MW} = 1145.000$ and $p \leq 0.001$. Median concentrations of meHg for pelagic fish were significantly lower (0.0597 ± 0.0392 mg/kg WW) compared to the demersal fish (0.1718 ± 0.1332 mg/kg WW) at $p \leq 0.001$. Moreover, looking at median concentrations of meHg in fish and seafood groups in descending orders, demersal fish (0.1718 mg/kg WW) > crustaceans (0.1400 mg/kg WW) > cephalopods (0.0777 mg/kg WW) > pelagic fish (0.0597 mg/kg WW) > freshwater fish (0.0578 mg/kg WW). Results of the meHg levels in fish and seafood groups from this study do not exceed both the national and international guidelines.

3.3. Fish and PUFA Intake (g/day) by Adults and Adolescents in Peninsular Malaysia. A summary of fish and PUFA (DHA and EPA) daily intake by adults and adolescents in Peninsular Malaysia is shown in Tables 3 and 4. The fish consumed was expressed in grams daily, while PUFA intake was expressed in milligrams per day (mg/day). The median intake of fish among adult populations was 24.91 g/day, ranging between 11.11 and 66.39 g/day for pelagic fish, while for demersal fish, the average intake was at a higher rate (41.28 g/day) that ranges between 26.67 and 72.67 g/day.

Freshwater fish and other seafood intake fall within the range of these two fish groups (Table 3). In addition, fish and seafood intake patterns were similar for adolescents, but the amount of intake for all categories of fish and seafood was higher than that of adults (Table 4). The adolescent populations also consumed more freshwater fish and cephalopods than the adult population. Total fish consumed by adolescents were 84.7 ± 103.7 g/day which is at the margin of statistical significance ($p = 0.069$) compared to the adult population (90.5 ± 100 g/day) with the presence of one extreme seafood intake of outliers (Figure 3(a)). Reanalyzed fish consumption data without the outlier resulted in data close to statistical significance at $p = 0.058$ (Figure 3(b)). The subsequent analysis was based on the data, which included the outlier of a girl aged 17 years old who consumed a bowl of prawn tom-yam during dinner.

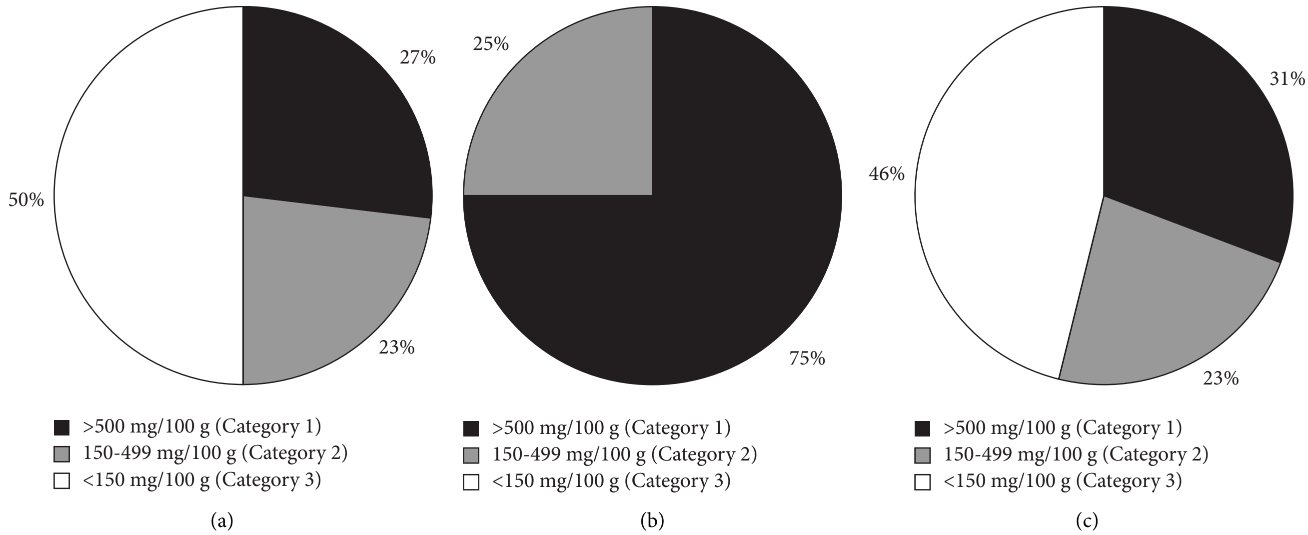


Figure 2: PUFA (DHA + EPA) content in fish and seafood samples based on 3 categories by Weaver et al. [35]. (a) All seafood samples. (b) Pelagic fish and Mollusca. (c) Demersal fish.

Table 3: Intake of PUFA, meHg, and health risk assessment data from consumption of fish/seafood by adult populations in Peninsular Malaysia.

No.	Groups/family/species	Common name	meHg (mg/kg WW)	Fish intake (g/day)	PUFA intake (g/day)	EWI (μgkg^{-1})	EWI/PTWI (%)	MSWC (kg/week)	HQ
Pelagic fish									
1	*Selaroides leptolepis*	Yellowstripe scad	0.0480	66.39	879.20	0.3721	23.3	2.00	0.5098
2	*Decapterus russelli*	Slender scad	0.0459	27.00	409.40	0.1445	9.0	2.09	0.1980
3	*Megalaspis cordyla*	Torpedo scad	0.0748	37.52	443.73	0.3273	20.5	1.28	0.4483
4	*Parastromateus niger*	Black pomfret	0.0529	11.11	102.70	0.0686	4.3	1.82	0.0939
5	*Rastrelliger kanagurta*	Indian mackerel	0.0450	36.67	202.79	0.1926	12.0	2.13	0.2639
6	*Scomberomorus guttatus*	Indo-Pacific king mackerel	0.0587	20.00	195.19	0.1370	8.6	1.63	0.1877
7	*Thunnus tonggol*	Long-tail tuna	0.0966	22.83	229.78	0.2572	16.1	0.99	0.3523
8	*Euthymus affinis*	Kawakawa	0.0667	22.83	254.49	0.1775	11.1	1.44	0.2432
		Median	0.0600	24.91	242.14	0.1851	11.6	0.88	0.2536
Demersal fish									
9	*Lutjanus argentimaculatus*	Mangrove red snapper	0.1927	72.67	170.05	1.6333	102.1	0.50	2.2374
10	*Lutjanus russellii*	John's snapper	0.1628	33.33	8.63	0.6330	39.6	0.59	0.8671
11	*Lates calcarifer*	Giant-sea perch	0.1094	26.67	851.04	0.3403	21.3	0.88	0.4662
12	*Gymnura poecilura*	Long-tail butterfly ray	0.0956	41.28	4.83	0.4606	28.8	1.00	0.6309
13	*Nemipterus japonicus*	Japanese threadfin bream	0.0997	53.97	636.09	0.6276	39.2	0.96	0.8598
		Median	0.1176	41.28	170.05	0.6276	39.2	0.88	0.8598
Freshwater fish									
14	*Clarias batrachus*	Walking catfish	0.0711	35.00	98.79	0.2905	18.2	1.35	0.3979
Cephalopods									
15	*Sepia officinalis*	Common cuttlefish	0.0422	44.17	512.53	0.2174	13.6	2.28	0.2978
Crustaceans									
16	*Metapenaeus affinis*	Rainbow shrimp	0.0273	19.63	183.62	0.0625	3.9	3.52	0.0856

Adult age ≥18 years old; body weight (BW) for adult = 60 kg; WW: wet weight; EWI: estimated weekly intake (μg/kg BW/week); provisional tolerable weekly intake (PTWI) for MeHg = 1.6 μg/kg BW/week [32]; MSWC: maximum safe weekly consumption (kg); HQ: hazard quotient.

Higher intake of PUFA was contributed from the consumption of yellow-stripe scad (*Selaroides leptolepis*), giant-sea perch (*Lates calcarifer*), Japanese threadfin bream (*Nemipterus japonicus*), and common cuttlefish (*Sepia officinalis*) for both population groups (Tables 3 and 4). In addition, for the adult population, the consumption of slender scad (*Decapterus russelli*) (409.4 mg/day) and torpedo scad (*Megalaspis cordyla*) (443.73 mg/day) also contributed to a high amount of PUFA. The intake of PUFA through consumption of black pomfret *(Parastromateus niger)* and Indo-Pacific king mackerel *(Scomberomorus guttatus)* by adolescents was nearly two times or more when

Table 4: Intake of PUFA, meHg, and health risk assessment data from consumption of fish/seafood by adolescents in Peninsular Malaysia.

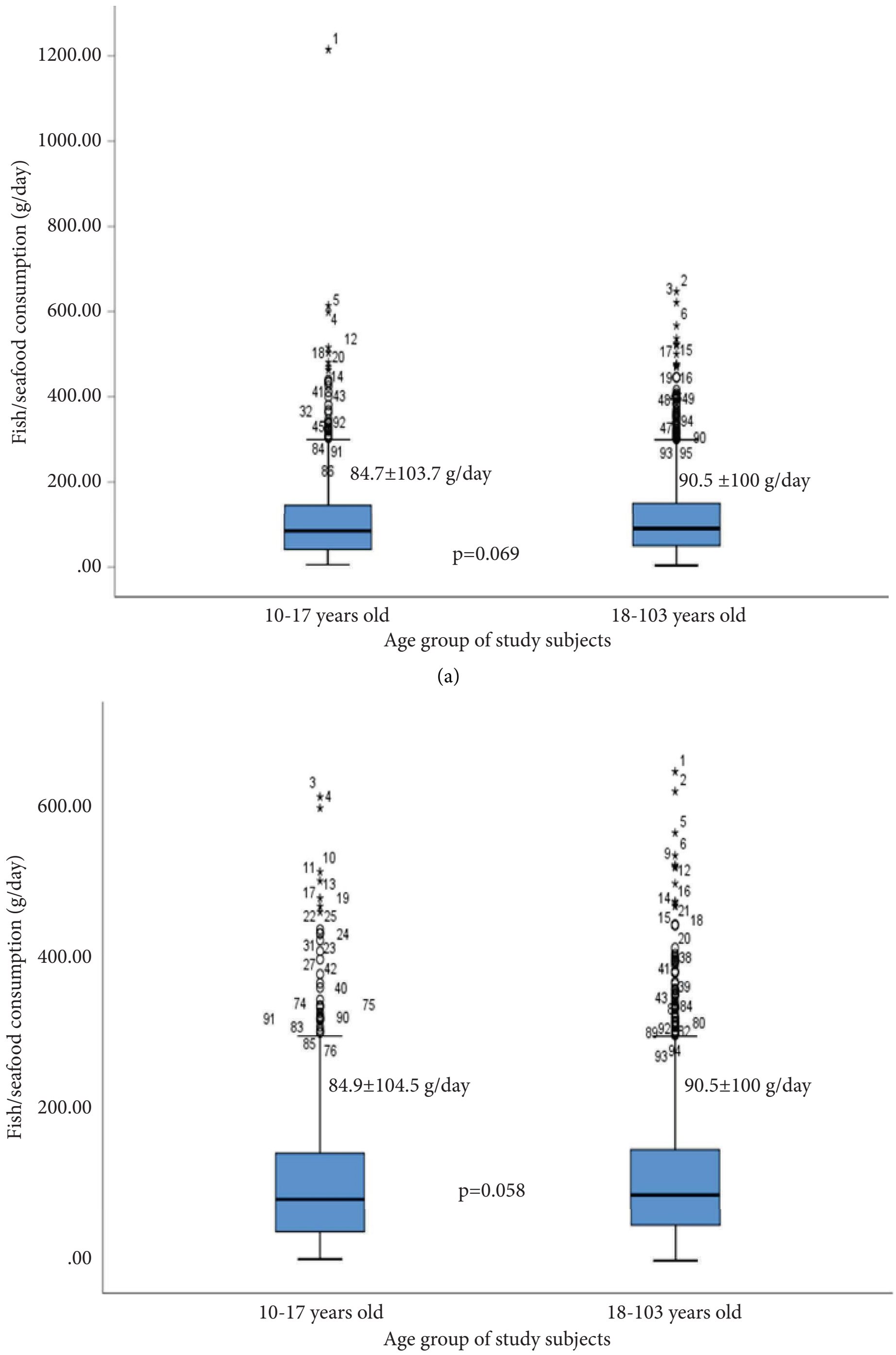
No.	Groups/family/species	Common name	meHg (mg/kg WW)	Fish intake (g/day)	PUFA intake (g/day)	EWI (μgkg^{-1})	EWI/PTWI (%)	MSCW (kg/week)	HQ
Pelagic fish									
1	*Selaroides leptolepis*	Yellow-stripe scad	0.0480	53.64	710.35	0.4009	25.1	1.50	0.5491
2	*Decapterus russelli*	Slender scad	0.0459	22.67	343.75	0.1618	10.1	1.57	0.2216
3	*Megalaspis cordyla*	Torpedo scad	0.0748	37.52	443.73	0.4363	27.3	0.96	0.5977
4	*Parastromateus niger*	Black pomfret	0.0529	39.07	361.16	0.3214	20.1	1.36	0.4403
5	*Rastrelliger kanagurta*	Indian mackerel	0.0450	24.33	134.54	0.1704	10.7	1.60	0.2335
6	*Scomberomorus guttatus*	Indo-Pacific king mackerel	0.0587	35.53	346.76	0.3246	20.3	1.23	0.4446
7	*Thunnus tonggol*	Long-tail tuna	0.0966	31.30	315.03	0.4701	29.4	0.75	0.6440
8	*Euthymus affinis*	Kawakawa	0.0667	31.30	348.90	0.3245	20.3	1.08	0.4446
		Median	0.0600	33.42	347.83	0.3245	20.3	1.29	0.4446
Demersal fish									
9	*Lutjanus argentimaculatus*	Mangrove red snapper	0.1927	101.69	237.95	3.0474	190.5	0.37	4.1746
10	*Lutjanus russellii*	John's snapper	0.1628	39.46	10.22	0.9992	62.4	0.44	1.3687
11	*Lates calcarifer*	Giant-sea perch	0.1094	25.33	808.28	0.4309	26.9	0.66	0.5903
12	*Gymnura poecilura*	Long-tail butterfly ray	0.0956	54.37	6.36	0.8088	50.5	0.75	1.1079
13	*Nemipterus japonicus*	Japanese threadfin bream	0.0997	43.83	516.58	0.6796	42.5	0.72	0.9310
		Median	0.1176	43.83	273.95	0.8088	50.55	0.66	1.1079
Freshwater fish									
14	*Clarias batrachus*	Walking catfish	0.0711	40.56	114.48	0.4488	28.0	1.01	0.6148
Cephalopods									
15	*Sepia officinalis*	Common cuttlefish	0.0422	39.24	455.32	0.2575	16.1	1.71	0.3527
Crustaceans									
16	*Metapenaeus affinis*	Rainbow shrimp	0.0273	21.00	196.43	0.0892	5.6	2.64	0.1222

Adolescents age 10–17 years old; BW for adolescents = 45 kg; WW: wet weight. EWI: estimated weekly intake (μg/kg BW/week); provisional tolerable weekly intake (PTWI) for MeHg = 1.6 μg/kg BW/week [32]. MSWC: maximum safe weekly consumption (kg); HQ: hazard quotient.

compared to the consumption by the adult population. The highest intake of PUFA for both population groups was from the cephalopods (455.32 mg/day and 512.53 mg/day, respectively), followed by the pelagic fish (347.83 mg/day) for the adolescent population. For both groups, the adults and adolescents, the pelagic (242.14 mg/day; 347.83 mg/day) fish contributed higher amounts of PUFA for daily consumption compared to the demersal (170.05 mg/day; 273.95 mg/day). The last source of PUFA for both population groups was from the consumption of John's snapper (*Lutjanus russellii*), long-tail butterfly ray (*Gymnura poecilura*), and walking catfish (*Clarius bathachus*). Overall, additional calculations showed that although adolescents consume more fish and shellfish (40.05 g/day) than adults (35.66 g/day), the final intake of PUFA for both groups was comparable at 334.37 mg/day and 323.93 mg/day, respectively.

3.4. Health Risk Assessment for Adults and Adolescents. Tables 3 and 4 show the health risk assessment (EWI and HQ) for adults and adolescents in Peninsular Malaysia, resulting from the consumption of fish and seafood data. The level of EWI was calculated in micrograms per unit of body weight per week (μg/kg BW/week). These EWI estimated values showed results below 1.6 μg/kg BW/week, the acceptable or tolerable levels recommended by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) for all different fish and seafood species. However, for adult populations, the results exceeded the EWI recommended levels for consuming Mangrove red snapper (*Lutjanus argentimaculatus*) (1.6333 μg/kg BW/week). The adolescents showed EWI values more than adults, with even double the values at 3.0474 μg/kg BW/week. The EWI values for both population groups corresponded to 102.1% and 190.5% of the PTWI value from the consumption of this fish, respectively. Despite this, the EWI values for demersal fish other than this species showed lower than the recommended value for both population groups. Compared to the values from other fish and seafood consumption, these values were higher by two to five times in both population groups.

A summary of the HQ values for meHg from the consumption of fish and seafood by different population in Peninsular Malaysia was also studied (Tables 3 and 4). The HQ is a risk index that compares the quantity of meHg



(a)

(b)

Figure 3: Boxplot of fish and seafood consumption data (g/day) for adolescents and adults of Peninsular Malaysia before and (a) after deleting one outlier from adolescents (b).

consumed to a specified reference dosage. For adults, the consumption risk is from mangrove red snapper (*Lutjanus argentimaculatus*); unlikely for adolescents, the risk was from the exposure through the consumption of all demersal fish studied, except the giant-sea perch (*Lates calcarifer*) (HQ = 0.5903). Despite this, the HQ values for other fish and seafood groups were below one, assuming that daily exposure is not likely to cause adverse health effects during the lifetime of adults and adolescents in Malaysia. In addition, lower HQ values were shown from the consumption of the other seafood groups, and the lowest values were from the consumption of crustaceans for both population groups.

3.5. Maximum Safe Weekly Consumption (MSWC) of Fish and Seafood among Adults and Adolescents. MSWC values in kilograms are reported for each fish and seafood category, as shown in Tables 3 and 4. The proportion of demersal fish from Peninsular Malaysia that all population groups should consume to fulfil the PTWI for meHg would be less than 1 kg/week (range 500 to 1 kg/week) for all population groups and much less for adolescents. For all forms of fish, the adolescents were permitted to consume up to 1 kilogram per week (range: 370 g to 750 g/week), with a more significant amount for seafood (range: 1.71 to 2.64 kg). Conversely, adults were permitted to consume more marine and freshwater fish each week (790 g to 1.67 kg).

4. Discussion

This study was carried out to optimize the balance between the health benefits of omega-3 fatty acids and risks of meHg contamination from consuming freshwater fish and marine organisms by Malaysian adults and adolescents. We used the recommended intake of fish and fish oils worldwide [31] to compare the minimum intake of long-chain omega-3 PUFA (LC ω-3 PUFA) with targeted health benefits. The Joint FAO/WHO Expert Consultants [36] recommended a 250 g EPA and DHA daily to improve adults' health while the International Society for the Study of Fatty Acids and Lipids recommended a minimum intake of 500 mg of EPA and DHA daily to reduce CHD risk in healthy adults [37]. We expended an approach of estimating health risk assessment from intake of meHg among adults and adolescents who resided in Peninsular Malaysia using the PTWIs established by the WHO [32] and the HQ values by the USEPA [33]. The PTWI represents the amount of meHg that can be ingested over a lifetime without appreciative risks, while the HQ value is an integrated index that compares the ingested amount of meHg with the standard reference dose. The USEPA signifies the value of HQ < 1 as the exposure level is lower than the reference dose and assumes a daily exposure at this level is not likely to cause any adverse health effects during a lifetime in a human population.

Oily fish is the primary dietary source of LC ω-3 PUFA and the content of fish species and their environments are shown in Table 1. Results of the referred studies showed variabilities of PUFA content in seafood caught from both the east coast (South China Sea) and the west coast (the Straits of Malacca) of Peninsular Malaysia [28, 29]. Examples of a few pelagic species reported to contain high content of both PUFA are several Carangidae scads (the yellow-stripe scads, slender scads, and torpedo scads) and the long-tail tuna. Higher PUFA content was shown in the Japanese threadfin bream, red-banded grouper, and long-tail shad for demersal fish. Among species of shellfish studied, the shallow-water crab contained the highest concentration of these PUFA. Results also showed that DHA was the highest in slender scads, with another 9 species showing DHA levels of more than 500 mg/100 g samples (the yellow-stripe scad, torpedo scad, black pomfret, Indo-Pacific king mackerel, long-tail tuna, kawakawa, giant-sea perch, Japanese threadfin bream, and red-banded grouper). The long-tail shad was the only species that could be considered as an excellent source of EPA compared to other species studied.

LC ω-3 PUFA content in fish has been linked to their dietary niche, variety of dietary sources, and seasonal variations in food availability. Pelagic fish often have the largest concentration of these fatty acids, especially the DHA, because they inhabit cold water [38]. Researchers used an ecological factor that divided fish species into different habitats (pelagic, benthopelagic, and demersal), standard fish sizes as a proxy of trophic level, as well as the water temperature of their habitat (cold water, temperate, and warm water) to organize fish species by their EPA and DHA values [39]. Yet, these substitutions are not perfect. Significant differences in the fatty acids profile within species occurred due to other factors as well, for instance, fish species, genetics, harvesting seasons, reproductive cycle, and environmental characteristics [31, 38, 40–42]. Mahaffey [43] reported on a general coherent pattern of fatty acid composition for fish and shellfish species, but the relationship between ω-3 fatty acids and the fat content of all species is not a simple linear association. The study also provided an overview of the EPA and DHA contents in various fish and shellfish species from 1975 to 2000, represented on a fresh-weight basis. Results showed that the EPA concentration ranged from less than 0.01 g to 1.5 g/100 g, and the DHA concentrations ranged from less than 0.01 g/100 g to more than 2.00 g/100 g of species studied [43]. A study by Williams et al. [44] summarized fatty acids in fillets from freshwater fish sampled from the US water of all 5 Great Lakes and inland lakes and rivers in the Great Lakes region. The studies determined that fatty acid content in freshwater species varies across spatial, biological, physical, and chemical gradients. They highlighted all taxonomic families from Great Lakes species and in-land salmonid fillets containing ≥250 mg/227 g fillet (or ≥110 mg/100 g EPA + DHA). In contrast, Dellinger et al. [41] listed a few Great Lakes species (walleye, rainbow smelt, lake herring, yellow perch, whitefish, and lake trout) that contained a high concentration of these fatty acids at a range between 244.2 and 2,395.3 mg/100 g. Gribble et al. [39] summarized data on EPA and DHA across fish populations in their review. They discovered that order Clupeiformes (e.g., sardine, *Sardinops sagax*), followed by order Salmoniformes, has the highest concentrations of ω-3 PUFA. At the same time, lower quantities of these long-chain fatty acids were found in other order groups, including the Perciformes, Scorpaeniformes, and Gadiformes. Earlier studies by Weaver et al. [35] showed that the most widely farmed fish have relatively high amounts of ω-3 PUFA in trout and Atlantic salmon but not in tilapia and catfish. Still, there are differences in levels of this LC ω-3 PUFA in different species of tilapia, and research revealed that *O. niloticus* contained varieties of fatty acids and ω-3 PUFAs compared to other species: *L. niloticus*, *T. zilli*, and *R. argentea*. It is also helpful to value significant and relatively higher levels of EPA and DPA in *R. argentea* [42]. A study conducted among species captured from the Jamaican marine environment revealed the mean concentration of EPA and DHA at 123.1 ± 93.6 mg/100 g, and species with high content of these omega-3 fatty acids were codfish (*Gadus morhua*), pickled mackerel (*Scomberomorus regalis*), sea trout (*Macrodon ancylodon*), and winchman (*Pristipomoides aquilonaris*) (EPA and DHA range between 213 and 298 mg/100 g) [45]. A more significant amount of these long-chain ω-3 polyunsaturated fatty acids was found in some commercially significant New Zealand species, ranging from 2.01 to 20.5 mg/g [46], consistent with research from Malaysian waterbodies.

Elemental fatty acid composition, which is cumulative in fish, is influenced by the ingredients in their diet, mainly on microalgae [38, 39, 47–50] that actively synthesize and accumulate high amounts of LC ω-3 PUFA. These EPA and DHA occur widely in many unicellular species, especially marine ones. The microalgae form the bottom of a food web, with their long-chain ω-3 PUFA finally accumulating in the lipids of the fish that consume them [50]. Studies have shown that the Cryptophyceae, Dinophyceae, and flagellate algae were the highest sources of EPA and DHA for zooplankton, and another different species of *Bacillariophyceae* with a high EPA concentration was a better food source for Daphnia [40, 48, 51]. Additional examples of aquatic microorganisms that accumulate high amounts of EPA are the diatom, *Phaeodactylum tricornutum*, and the heterotrophic marine algae, *Crypthecodinium cohnii*, which were identified as species rich in DHA [48, 50]. Further, epilithic algae in freshwater ecosystems, especially the diatoms, are comparatively rich in EPA but low in DHA. There are also advantages for some freshwater fish; in the case of *Oncorhynchus mykiss* and *Salvelinus alpinus*, they can transform dietary alpha-linolenic acid (ALA) via stearidonic acid (SDA) and EPA to DHA [47]. Major lipid classes in algae are membrane components and storage lipids, but the proportion of polar membrane lipids may vary depending on algae species. The overall fatty acids composition and its patterns for individual lipid classes of algae depend on their growth condition, seasonal variations, and developmental stages [48].

This study identified LC ω-3 PUFA was predominant in some pelagic fish and mollusk species. It also attempted to document the most consumed seafood species potential providers of these fatty acids to our population, categorized into groups I and 2 with EPA and DHA content ≥150 mg/100 g [35]. Total fish consumption between both population groups (adults = 84.7 ± 103.7 g/day; adolescents = 90.5 ± 100 g/day) was a marginally significant difference at $p = 0.058$, despite the amount of seafood consumed by the adolescents being higher for all seafood categories than the adults. In this dataset, consumption of four species of seafood, mainly the yellow-stripe scad, giant-sea perch, Japanese threadfin bream, and common cuttlefish had fulfilled the requirement established by the International Society for the Study of Fatty Acids and Lipids for a minimum intake of 500 mg EPA and DHA per day for reducing CHD risk in healthy adults [37]. While intake of another 3 species of slender scad, torpedo scad, and kawakawa is adequate per day, which contributed to ≥250 g of EPA and DHA, a daily adequate intake as proposed by The Joint FAO/WHO Expert Consultants [36] to gain healthiness for adults [31]. All listed pelagic fish species could contribute to the intake of ≥250 g of EPA and DHA for adolescents, except for an Indian mackerel. It may be accurate to suggest an intake of double amounts of species such as Indian mackerel, Indo-Pacific king mackerel, longtail tuna, and rainbow shrimp to Malaysian adults to meet the adequate daily intake of these PUFA.

Dietary intake levels and sources of ω-3 PUFA vary among countries. For Malaysians, other than fatty fish (e.g., Hoven's carp, seabass, tuna, and sardines), the basal RNI for omega-3 fatty acids of 0.3% energy may be achieved from the intake of local foods, namely, vegetables such as soybean/soybean products (tofu, tofu skin, and bean sprout), legumes, fortified foods (omega-3 eggs) and ready to drink omega-3 milk/soybean milk [52]. Similarly, in Japan, fish and shellfish, edible fats, and oils are the most essential sources of n-3 PUFAs, with the prior food group providing ranges between 1 and 2 g of $n-3$ PUFA/person/day. About 60% of this fat is from fresh fish, especially horse mackerels, sardines, and tuna, with an average seafood consumption rate of approximately 80 g per day among them [53]. Other findings from a population-based cohort study among the Japanese indicated an estimated daily fish intake of 111 g, 307 mg of EPA, or 123 mg of DPA, which could be related to a lower risk of depression [54]. For the US population, the most common species of fish and shellfish as sources of ω-3 fatty acids are salmon and shrimp, and researchers recognizing sources of ω-3 fatty acids present in foods in addition to fish are eggs and chicken [55]. Australian kids between the ages of 2 and 16 ate an average of 13 g of fish or shellfish daily, but only 50 to 60% of them got the recommended amount of LC ω-3 PUFA. Findings also showed that these kids ingested eight times as much meat as fish or seafood, which accounted for 33% of the LC ω-3 PUFA in these meals [56]. Malaysian adolescents appeared to have consumed roughly seven times more fish and seafood than indicated by this published data. Studies also revealed that estimations of the global dietary intake of DHA from 64% of 175 developed and developing nations were less than 200 mg/day [57]. In this study, we expanded the list of fish and seafood species that contain highly LC ω-3 PUFA to fully comply with the recommendations for improving health or lowering the risk of CHD in our populations.

A benefit-risk assessment was performed on LC ω-3 PUFA and meHg; this study found scenarios where consumption of some species of seafood, especially the pelagic fish, would confer the benefit of these fatty acids and meHg exposure would remain below the tolerable intake for the Malaysian population. Moreover, the findings demonstrated that adults and adolescents could consume more than one kilogram of pelagic, freshwater, and shellfish per week, but the risk index of HQ still assumes that daily exposure is not likely to cause negative health effects. The percent ratio of meHg exposure in adolescents towards the PTWI guideline value per week for pelagic fish is 20.3% compared to the demersal fish at 50.6%, which is higher when compared to the ratio for the adult population at 11.6% and 39.2%, respectively. The exposure by consumption of the crustacean is the lowest for both groups indicating protection of meHg accumulation by the most negligible permeability of the exoskeleton or several molting processes in the life cycle of crustaceans when new bioaccumulation starts in new exoskeleton formation [58]. In some cases, consumption of the demersals (*Lates calcarifer* and *Nemipterus japonicus*) not only contributed to a high intake of LC ω-3 PUFA but also increased the HQ to 1. However, these findings are contradicted by the consumption of cephalopods. Double the intake of cephalopod may increase the intake of LC ω-3 PUFA, with a lower risk compared to the guideline. Even a threefold increase in crustacean consumption would minimize the rise in meHg risk and help people achieve their daily LC ω-3 PUFA dietary requirements. Cressey et al. [46] used two approaches to compare the risk due to meHg and the benefits due to EPA and DHA from the consumption of six important commercial New Zealand fish species (the barracouta, black oreo, gemfish, ling, orange roughy, and smooth oreo). They calculated the dose-response equations for the impact of meHg and LC ω-3 PUFA on off-springs IQ and a hazard index from the dietary exposure estimation as a proportion of a health-based guidance value. The analysis indicates a different balance between risks and benefits for fish species studied and only one species; Ling showed the narrowest margin between the benefits and risks at 10 or more servings of this fish per week. Research on the nutritional benefits and mercury risk of commercial fish collected in the upper Laurentian Great Lakes concluded that six ounces of caught fish might benefit cardiovascular health and neonatal neurodevelopment [59]. Before this, Dellinger [60] reported that tribal members in the Upper Great Lakes region had moderately elevated mercury exposure, which is not high enough to call for widespread dietary restrictions. This was based on ten years of data collection and exposure assessment for the Ojibwe Health Study. The HQ values for all demersal fish species in this study were less than 1, indicating that the mercury toxicity risk is modest compared to the health advantages of LC ω-3 PUFA for Malaysian adolescents and adults. Similar outcomes were also observed in Jamaicans who ate typical deep slope and reef finfish species such as snappers, grunts, and parrotfish [45].

5. Conclusions

Quantitative assessments of the meHg risk must be matched with the benefits of PUFA to establish the significance of fish and shellfish as food sources. This study emphasized the species-specific variation in the LC ω-3 PUFA (EPA and DHA) concentration from the consumption of fish and seafood by adolescents and adults in Peninsular Malaysia in relation to meHg, which harms human health. This study revealed that meHg levels ranged from 0.0426 to 0.4576 mg/kg of wet weight in 16 fish and seafood species comprised of cephalopods, crustaceans, freshwater fish, and marine fish (the demersal and pelagic fish). meHg levels showed significant variations between all species at a median concentration of 0.0621 ± 0.0573 mg/kg WW. The content of DHA and EPA differed substantially by species, with PUFA found to be predominant in the pelagic fish and mollusks at the highest ratio in pelagic fish (3.6) compared to the demersal fish (1.2) and shellfish (1.5). Pelagic fish, including long-tail tuna, yellow-stripe shad, slender shad, and long-tail shad from the demersal fish, have been found to contribute to higher PUFA intake than other types of fish and seafood. These fish also contributed to a low HQ value level, lowering the risk of health effects. It is advised to consume mangrove red snapper less frequently since the PUFA content is relatively low, but the HQ value was the highest. Results from this study were used to justify a new project proposal entitled "Assessment of Heavy Metal Risks for the Optimization of Nutrient Benefits from Fish and Seafood in Malaysia" (NMRR ID: 22-01573-PGU 22-029). One of the objectives of this new study is to analyze fatty acid content in varieties of fish and seafood species distributed throughout Malaysia.

Data Availability

The datasets used during the current study are available from the corresponding author upon reasonable request.

Ethical Approval

The Medical Research and Ethics Committee, MOH Malaysia, reviewed and approved the proposal.

Consent

Informed consent forms were obtained from the subjects beforehand.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Ahmad NI substantively contributed to the conception of the work and data collection. Equally, contributions from both authors were made to the data analysis and interpretation, drafting and critical revision of the article, and final approval for publication.

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