

## Status Level of Total Mercury (T-Hg) in Barracuda (*Sphyraena putnamae*) from the Gulf of Thailand

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### Abstract

Mercury a major contributor to global environmental pollution and a source of human health issues and could have been transferred to the environment *via* bioaccumulation in food chain and trophic levels in aquatic environments. Barracuda (*Sphyraena putnamae*) of different sizes were caught from various locations from the Gulf of Thailand (GoT) and determined for total mercury (T-Hg) using a direct thermal decomposition and gold amalgamation trapped mercury analyzer and evaluated for the accumulative human health risk for consumption. The range of T-Hg in edible muscle was from 9.58 to 314 µg/kg wet weight with an average of  $59.1 \pm 66.2$  µg/kg wet weight, respectively. T-Hg in Barracuda caught from the middle and the lower GoT was found with a higher concentration than the upper GoT, which might be influenced by internal anthropogenic activities in the GoT and external sources from the land *via* river runoff. Fish length, weight, feeding habit and habitat were those influencing factors that significantly related to T-Hg accumulation in Barracuda. Risk assessment revealed that most of Barracuda had the estimated daily intake (EDI) values (0.03 µg/kg bodyweight per day) lower than provisional tolerable daily intake ((PTDI); 0.23 µg/kg bodyweight per day) and hazard quotient (HQ) was lower than 1, indicating no potential risk for adults associated with consumption the muscle of Barracuda muscle from the GoT. The maximum allowable daily consumption (MSDC) for Barracuda was 0.54 kg per day.

**Keywords:** Anthropogenic effects, Animals, Bioaccumulation, Food chain, Humans

### Introduction

The GoT receive a discharge of contaminants from naturally occurring source in the environment and human activities such as untreated industrial water, local waste, oil and gas mining, land emission, cement and coal-power plants [1,2]. Among pollutants that had been exploited in the GoT, mercury is a concern because it is a highly toxic and non-essential substance to marine organisms and human health [3]. Mercury from both natural and anthropogenic sources may be deposited in the estuary, transported, and accumulated in the marine food web [4]. In this context, the Barracuda (*Sphyraena putnamae*) is a species of fish that usually found in estuarine and coastal water in a tropical and sub-tropical environment. They are known as pelagic, carnivorous, fast growth and swimming, long-lived species that need more supply energy to support their high metabolic activity. Thus, their consumption and predation rates were often very high allowing more pollutants including mercury accumulation in their organs and tissues [5,6].

Fish consumption is considered an important source of nutrients for the human body, particularly protein, amino acids, omega-3, calcium, iron and zinc [7]. Then, Barracuda one of the protein sources for people in Thailand have been widely used in the food industry in Thailand such as surimi production. According to Lertwittayanon *et al.* [8], investigation that Barracuda fish was chosen for surimi production because of its high yield and white colour. As one of the largest surimi-exporting countries to Asia, Europe and the USA, the production of surimi in Thailand increased from 119,000 metric tons in 2002 to about 143,000 metric tons in 2005 [9].

Not only nutrient have been found in fish, but fish is also known for the accumulation of mercury especially predatory fish [10]. Exposure to mercury in fish has been linked to risk for human health and

affected the endothelial, cardiovascular and central nervous systems [11]. To assure safety for fish consumers, United States Environmental Protection Agency [12], had been established guidelines for fish consumption evaluating by levels of mercury, consumption amount, and exposure duration in several approaches known as the estimated daily intake, HQ and maximum safe human consumption rates.

A study on mercury concentration in different biota and locations has been reported in Thailand [1,13-16], but only for a few samples and locations reported for Barracuda [17]. The purposes of this study are to; 1) Determine T-Hg concentration in Barracuda (*Sphyraena putnamae*) caught from a different location in the GoT; 2) Evaluate the relationship between T-Hg and fish size and 3) Assess the human risk of T-Hg exposure through Barracuda consumption.

## Materials and methods

### Sampling

A total of 49 Barracuda (*Sphyraena putnamae*) samples were caught using a bottom trawl from 15 different sampling stations under the collaborative research survey on marine fisheries resources and marine environment in the GoT from 17 August to 18 October 2018 on board M.V. SEAFDEC-2 (**Figure 1**). Each fish was randomly chosen, double-wrapped, placed into double zip-lock plastic and kept in the freezer ( $-20\text{ }^{\circ}\text{C}$ ) until transported back to the laboratory and analysed [18]. Barracuda was selected in this study as it is a popular species for consumption and is widely distributed throughout the GoT [19].

Before analysis, each sample was thawed ( $\pm 3\text{ h}$ ), and cleaned using distilled water, then identified, species by identification book [20]. Barracuda feeding habits, habitat, and trophic levels were extracted from <https://www.fishbase.org> [21]. Later, morphometric data of each sample e.g. length and weight were recorded. The muscles from the dorsal fin to the caudal fin were separated using a stainless-steel knife, later homogenized, placed in a plastic zip-lock bag and labelled with a code number. All samples were stored in a refrigerator at  $-20\text{ }^{\circ}\text{C}$  until further analysis.

### Reagent and glassware

All preparation and digestion steps used Milli-Q water from the Millipore Milli-Q lab water system ( $> 18.2\text{ M}\Omega\text{-cm}$ ). Polyethylene bottles and glassware were pre-cleaned before use by soaking in 10 %  $\text{HNO}_3$  (v/v) overnight ( $\pm 24\text{ h}$ ), washed and dried in a horizontal laminar airflow cabinet. The T-Hg working standards prepared by diluting a standard solution (1,014 mg-Hg /L from Kanto Chemical, Japan) with 0.01 % (w/v) L-cysteine at 0.2 % (v/v)  $\text{HNO}_3$  to concentrations of 0.2, 0.4, 0.6, 0.8, 1, 1.2, 1.4 and 1.6 ng/L, respectively. This set of standards was prepared daily and has given the calibration curve of the T-Hg solution in the linear range ( $r = 0.999$ ) during analysis.

### Mercury analysis

T-Hg concentrations in Barracuda muscle were analysed by mercury analyzer using thermal decomposition, amalgamation, and atomic absorption technique equivalent to U.S. Environmental Protection Agency (USEPA) Method 7,473 [22]. Weighed  $\pm 100\text{ mg}$  of homogenized fresh tissue and introduced it into the analyzer. The sample was dried for the 60 s at  $150\text{ }^{\circ}\text{C}$  and decomposed to elemental mercury ( $\text{Hg}^0$ ) by multi-steps thermal decomposition (from 200 to  $850\text{ }^{\circ}\text{C}$ ).  $\text{Hg}^0$  was trapped and later heated in a gold amalgamator. Liberated  $\text{Hg}^0$  was carried by oxygen flow ( $> 90\text{ }\%$  purity) and measured by a detector at the wavelength (253.7 nm). T-Hg concentrations are expressed as  $\mu\text{g}/\text{kg}$  on wet weight.

### Quality assurance/quality control (QA/QC)

Procedural blanks prepared along with the standards were analysed to ensure no contamination during the analysis process. The method detection limit (MDL) and limit of quantitation (LOQ) were calculated from 3 and 10 times the standard deviation from the 10 measurements of the procedural blank. MDL and LOQ values for T-Hg were 0.002 and 0.006  $\mu\text{g}/\text{kg}$ , respectively. The method of T-Hg analysis was verified with the analysis of certified reference materials (CRMs), namely BCR-422, DORM-4 and TORT-3. Percentage of recovery reported at a range from 86.9 to 94.2. In addition, about 10 % of all samples were analysed with replication and the percentage of relative standard deviation [23] reported for T-Hg in fish was  $< 8.47\text{ }\%$ .

### Statistical analysis

The descriptive statistics (range, mean and standard deviation) were conducted by Microsoft office excel (2010) and SPSS Statistical software (Ver. 22.0) for Windows. Because the data was not in a normal distribution while testing with the Shapiro-Wilk test, the non-parametric (Kruskal-Wallis) test was

performed to test the significant study. The relationship between T-Hg levels and length and weight was carried out with the Spearman correlation ( $r$ ) test. All statistic test was ran at a  $p < 0.05$  confidence level.

### Spatial distribution of T-Hg

The spatial distribution of T-Hg in Barracuda (*S. putnamae*) in each different survey area in the GoT was mapped using ArcGIS software (version 10.2). The T-Hg distribution in Barracuda can be used to reflect the GoT environmental conditions.

### Risk assessment for consumer health

To evaluate health risks associated with mercury exposure from consumption of Barracuda. The following risk assessment; estimated daily intake (EDI), HQ and the maximum safe daily consumption (MSDC) were estimated using equations from the USEPA [12].

$$\text{Estimated daily intake (EDI)} = \frac{C_m \times IR \times EF \times ED}{BW \times AT} \quad (1)$$

where;  $C_m$  is methylmercury concentrations ( $\mu\text{g}/\text{kg}$ ), which MeHg was determined using the T-Hg/MeHg ratio (93 %) proposed by Annual *et al.* [24], and Windom and Cranmer [25], IR is the ingestion rate of fish consumption in Thailand (0.086 kg/serving) according to Needhan and Funge-Smith [26]. EF is the frequency of methylmercury exposure (365 servings/year). ED is the duration of exposure, calculated as the lifetime mean between men and women at 72.05 years [27], BW; average body weight of adults (56 kg) in Thailand [28], and AT; mean time of exposure to non-carcinogenic substances (365 days/year  $\times$  72.05 years).

$$HQ = \frac{EDI}{RfD} \quad (2)$$

HQ is defined as the concentration ratio of an element to its maximum allowance concentration to the human body or the reference dose (RfD). In mercury, RfD value is 0.1  $\mu\text{g}/\text{kg}/\text{day}$  body weight [12]. When HQ value  $< 1$  indicates a level with no adverse effect on human health [12].

$$MSDC = \frac{PTDI \times BW}{C_m} \quad (3)$$

The maximum allowable daily consumption (MSDC) is determined by the average body weight of adults in Thailand (56 kg) and methylmercury concentrations ( $C_m$ ). The PTDI of T-Hg; the maximum allowance of intake value, at 0.23  $\mu\text{g}/\text{kg}/\text{day}$  [29].

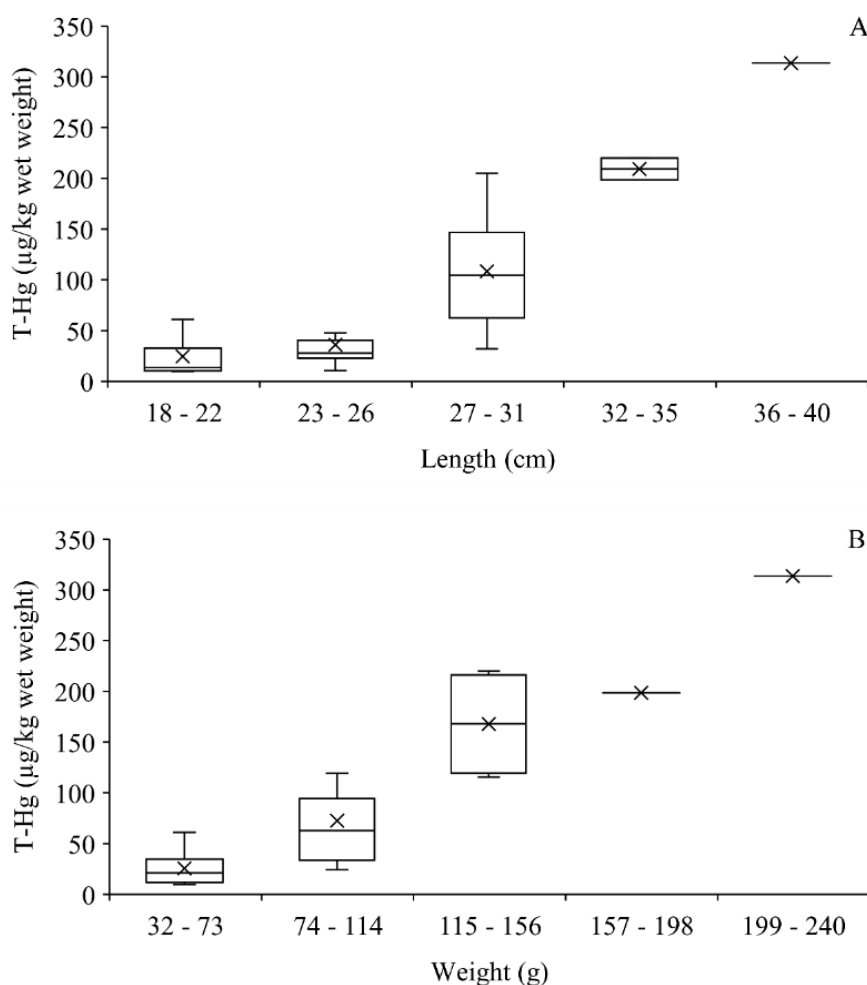
## Results and discussion

### Total mercury concentration and its bioaccumulation in Barracuda in the GoT

T-Hg concentration range of Barracuda in this study was from 9.58 to 314  $\mu\text{g}/\text{kg}$  wet weight with an average of  $59.1 \pm 66.2$   $\mu\text{g}/\text{kg}$  wet weight, respectively. In dividually, T-Hg levels in each Barracuda muscle could be differentiated due to it detoxification and excretion processes [30]. Mercury enters the fish body through gills and food, laterally absorbed and accumulated in tissues *via* blood circulation. Mercury, then forms a complex compound such as Sulfhydryl groups [31], that is stable and can be stored in several organs, including fish muscles. Thus, the variation of T-Hg levels in their feeding habit and habitats in the GoT could be leaded to the diverse in T-Hg accumulation in Barracuda muscle than the variation in detoxification and excretion process. As carnivorous fish, they are found to live extending from coral reefs and mangrove ecosystems to offshore and feeding on fish, crustaceans, squid or cuttlefish, bivalves and octopuses [32,33]. Moreover, the average life span of Barracuda was reported as about 11 - 18 years [34]. Thus, the long life of Barracuda could allow more bioaccumulation of Hg from the environment. Overall, T-Hg concentration in Barracuda was less than 1,000  $\mu\text{g}/\text{kg}$  wet weight; the maximum T-Hg allowance in predatory fish in guidelines of the European Commission Regulation (EC) No 1881/2006 [35], and the Ministry of Public Health of Thailand [36].

T-Hg concentration in fish was reported to vary with fish size and caught location [5,14,16,17,37-44]. This study, the significant positive correlations were found between length and weight ( $r = 0.96$ ,  $p < 0.05$ ), T-Hg and length ( $r = 0.84$ ,  $p < 0.05$ ), and T-Hg and weight ( $r = 0.89$ ,  $p < 0.05$ ). The bigger size (199 - 240 g) with longer length (36 - 40 cm) fish contained 12 times higher T-Hg concentration than the smaller fish (32 - 73 g, 18 - 22 cm) (**Figure 1**). In other words, the larger size of the Barracuda or adults have much

more time to accumulate mercury in their body. This general finding indicated that T-Hg accumulation increased along with Barracuda growth and was consistent with other studies in different areas [24].



**Figure 1** Relationship between T-Hg and; length (A) and weight (B) of *S. putnamae* from the GoT.

#### Comparative study of T-Hg in Barracuda

T-Hg in Barracuda (*S. putnamae*) in the GoT were compared across species and locations (Table 2). In this study, concentrations of T-Hg in Barracuda muscle were generally lower than another study [5,16,17,37-40,42-44]. Still, the average T-Hg in Barracuda in this study was higher than other observed Barracuda species in Thailand [14], and subtropical Chi-ku Lagoon, SW Taiwan [41]. Differences species, sampling locations, as well as mercury sources such as agriculture and mining could play an important role in T-Hg accumulation. In the GoT, the source of mercury content usually came from naturally occurring and anthropogenic activities such as untreated industrial water waste, agriculture, oil refinery, oil and gas mining and habitat degradation [1,16,45,46]. However, another factor that could be influenced T-Hg accumulation in this study is fish size. The differences in fish size (Table 1) among studies are likely to account for some of the observed T-Hg consistencies. A study from Suriname, South America [37], indicated that 7 times higher T-Hg levels than this study may be attributed to differences in body size, as organisms in this study are slightly smaller (range from 18.1 to 36.2 cm) than those reported (between 36.9 and 57.0 cm). Similarly, for various species and relatively smaller body sizes, T-Hg levels in Barracuda from the Gulf of Florida, Laguna Indian River, USA [5], Cambodia [16] and Gulf Persia [38], respectively were higher than 2 times as much as in this study.

**Table 2** (T-Hg in wet weight) levels in the edible muscle of Barracuda (*S. putnamae*) from GoT in comparison to other locations.

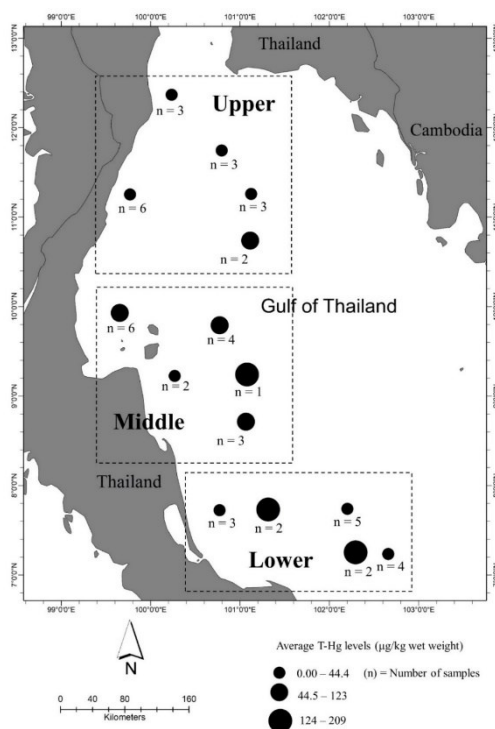
Species	Locations (year)	n	Length (cm)	Weight (g)	T-Hg ( $\mu\text{g}/\text{kg}$ )	References
<i>S. obtusata</i>	Chon Buri Coastal, Thailand (1976)	3	19.0 - 83.0	43 - 2,600	$32.3 \pm 27.9$	[14]
<i>S. guachancho</i>	Suriname, South Amerika (2001)	13	36.9 - 57.0	380 - 1,150	$390 \pm 240$	[39]
<i>S. putnamae</i>	Laguna Chi-ku, southwestern Taiwan (2002)	8	27.6 - 52.2	-	< 25	[41]
<i>S. barracuda</i>	Gulf of Florida & Laguna Indian River, USA (2003)	81	11.9 - 110	-	160 - 870	[5]
<i>S. obtusata</i>	Cambodia (2007)	6	44.0 - 49.7	55 - 600	$177 - 347^*$	[16]
<i>S. jello</i>	Gulf of Persia (2010)	12	64 - 71	819 - 927	$199 \pm 97$	[40]
<i>S. chrysotaenia</i>	Lebanon markets (2011)	4	-	-	160 - 225	[42]
<i>S. chrysotaenia</i>	Gulf of Oman (2013)	28	-	-	$120 \pm 160$	[43]
<i>S. qenie</i>	Samoa Island (2015)	3	-	3,570 - 9,050	105 - 741	[37]
<i>S. jello</i>	Kao Bay, Indonesia (2017)	3	25.5**	29.1**	150**	[44]
<i>S. jello</i>	East Afrika (2020)	9	-	-	$360 \pm 310^{**}$	[38]
<i>S. putnamae</i>	Bangkok markets (2022)	2	-	-	280 - 290	[17]
<i>S. putnamae</i>	Gulf of Thailand (2022)	52	18.1 - 36.2	32.0 - 326	$59.1 \pm 66.2$	This study

Note: \*Values of T-Hg reported in dry weight [16]; converted to wet weight using a 77 % moisture content [47]. \*\*The average value of the calculation.

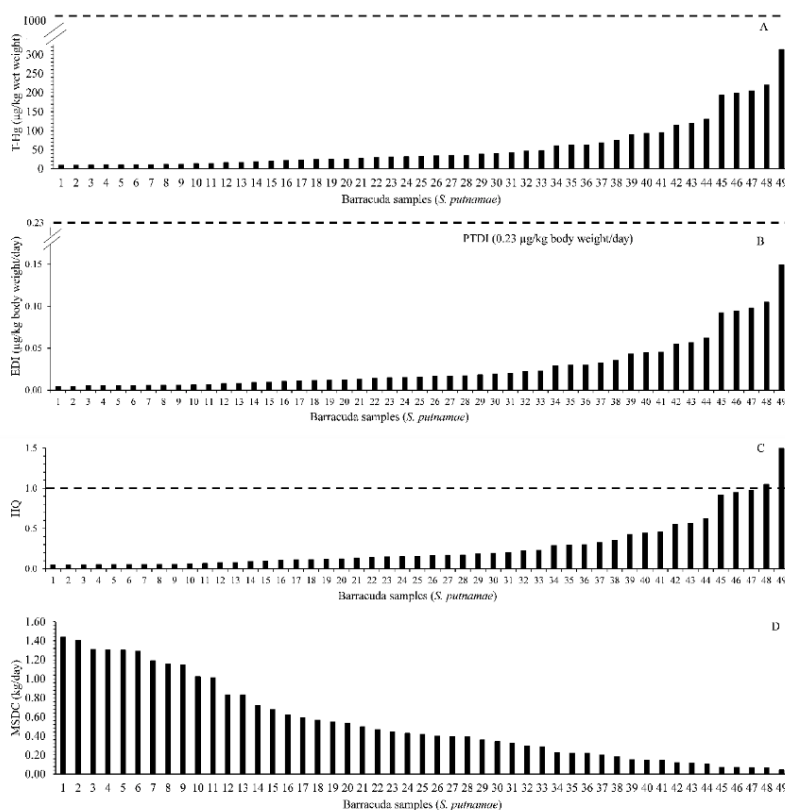
### T-Hg distribution of Barracuda caught in the Gulf of Thailand

The distribution pattern of T-Hg in Barracuda in GoT was analysed based on location in the upper, the middle and the lower GoT. The highest T-Hg was found in the middle GoT ( $74.9 \pm 56.2 \mu\text{g}/\text{kg}$ ), followed by the lower GoT ( $74.3 \pm 88.8 \mu\text{g}/\text{kg}$ ) and the upper GoT ( $29.9 \pm 37.7 \mu\text{g}/\text{kg}$ ) GoT. The Kruskal-Wallis test showed a significant difference (Chi-Square = 18.8,  $p < 0.05$ ) in T-Hg concentration by location. Between the middle and the lower GoT, the Mann Whitney test observed no significant different ( $p > 0.05$ ) of T-Hg contents in these two location.

The variation of T-Hg in each station (**Figure 2**) could be a reflect the presence of various mercury sources at the study site [1,15,45,48,49]. River discharge with wastewater from household in the river mouth was a primary mercury source for the upper GoT [50], while in the middle and the lower GoT, water circulation was different and influenced by South China Sea waters [51]. The high T-Hg in Barracuda in the middle of the GoT may be due to activities occurred in the GoT e.g. oil and gas exploration and production. In Sompongchaiyakul and Sanesith [52] study, T-Hg in seawater in the middle of the GoT ( $5.3 \pm 7.6 \text{ ng}/\text{L}$  as average) was relatively higher than measured in the Chao Phraya estuary; a part of the upper GoT ( $2.1 \pm 0.9 \text{ ng}/\text{L}$  as average) [53]. An increase of T-Hg in the surface sediment in the middle GoT from  $24.4 \pm 9.00$  (2003) to  $41.4 \pm 15.3 \mu\text{g}/\text{kg}$  (2013) also indicated a local effect from oil and gas exploration [45]. In addition, the supply of seawater and sediment from rivers in the southern part coupled with the deposition of Hg from the atmosphere and seawater from adjacent areas could play a role in the contribution and distribution of Hg in the middle and bottom of the GoT [48,51,54-57]. Latterly, mercury was transferred from seawater and sediments and was bioaccumulated in benthic organisms [58] and transferred to organisms in the higher trophic positions such as Barracuda species.



**Figure 2** Classification plot of T-Hg concentrations ( $\mu\text{g/kg}$  wet weight) in Barracuda (*S. putnamae*) caught during a research survey in the GoT in 2018.



**Figure 3** Individual plot of 49 Barracuda samples caught in the GoT displayed as (A) T-Hg concentration, (B) the estimated EDI, (C) HQ, and (D) MSDC in Barracuda; dash line showed the safety limit from the guideline value for predatory fish.

### Human health risk assessment

**Figure 3(A)** showed the concentration of T-Hg in all 49 Barracuda at the safe level for consumption (< 1,000 µg/kg wet weight). The estimated daily intake (EDI) ranged between 0.00 - 0.15 µg/kg body weight/day with an average of 0.03 µg/kg body weight/day. From **Figure 3(B)**, all EDI values were lower than the provisional tolerable daily intakes (PTDI); the threshold value recommended for fish at less than 0.23 µg/kg body weight/day [29]. A recent study by Ritonga *et al.* [17], reported an EDI value of 0.14 µg/kg/day in Barracuda *Sphyraena putnamae* from the Bangkok market in which the higher EDI was observed. This finding could be reassured that T-Hg concentration in the Barracuda from the GoT still be safe for consumption and the variation in T-Hg levels of fish could be linked to their size and location.

HQ values ranged between 0.05 and 1.49 with an average of 0.28. Almost all samples revealed HQ values < 1, indicating that there is no potential risk to adults consuming Barracuda muscle from the GoT. Even though there were only 2 samples (number 48 and 49; which accounted for 4 % of the total) where the HQ values exceeded the critical values (**Figure 3(C)**), a limited amount of Barracuda consumption was recommended to ensure non-exceeding MeHg exposure. The average of MSDC estimated for adults consumer in Thailand (56 kg) was 0.54 kg (ranged from 0.04 to 1.44 kg/day) of Barracuda per day (**Figure 3(D)**). From these results, it is not recommended to continuously consume Barracuda for a long time due to the bioaccumulation of mercury in the human body.

### Conclusions

T-Hg concentrations in Barracuda (*Sphyraena putnamae*) from the GoT were determined in various sampling locations. The accumulation of T-Hg in Barracuda was influenced by length, weight, diet and habitat. The highest T-Hg levels in Barracuda were found in fish caught in the middle GoT, followed by the lower and the upper GoT. Levels T-Hg in fish were influenced by local sources such as gas and oil exploration and external input from river runoff. T-Hg concentrations in Barracuda muscle were lower than the maximum allowance value by the European Union and Thailand's Ministry of Public Health. All EDI value in Barracuda was lower than the PTDI guideline. Most HQ value expressed the value lower than one. It is recommended that adults in Thailand should limit their MSDC of Barracuda to avoid health risks.

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