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RESEARCH ARTICLE

Nutrient Composition and Heavy Metal Contents of Freshwater Sardine, *Sardinella tawilis* (Herre, 1927), in Taal Lake, Philippines

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ABSTRACT

An assessment of the current nutrient composition and heavy metal contents of the only freshwater sardine, *Sardinella tawilis* (Herre, 1927), was conducted due to lake environment changes, recent episodes of volcanic eruptions, antiquated nutrient data, and food system influence. Freshwater sardines, commonly known as "tawilis", were acquired from four different sampling sites of Taal Lake, namely San Nicolas, Cuenca, Tanauan City, and Agoncillo, along with lake water samples. Proximate composition, fatty acid (FA) profile, and heavy metal concentrations (Hg, Pb, Cd) were analyzed. Mean proximate values for fish include moisture (69.15±3.01%), protein (17.48±0.72%), fat (8.98±1.84%), ash (3.32±0.24%), and carbohydrate (2.19±1.61%), which yields approximately 158±19 kcal/100g. There is no significant difference ($p>0.05$) in the proximate composition (%) among fish samples. Total saturated FA had the highest value (5.88±0.74 g/100g) among fatty acid groups, followed by monounsaturated FA (1.24±0.77 g/100g), then the polyunsaturated FA (0.71±0.46 g/100g), wherein palmitic acid is the most predominant FA. *Tawilis* is a "high protein" food since 100g of raw fish provides >20% of Filipino adults aged 19-59 daily protein requirement. Hg, Pb, and Cd contents of both fish and water samples were below the maximum allowable concentrations, thus, there is no heavy metal contamination. Parallel studies concerning variations in seasons, environmental conditions, cooking or processing methods, and analysis of other significant nutrient components could be conducted. The food composition of other native and endemic fishes may also be investigated, along with their potential contribution to food and nutrition security.

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1. INTRODUCTION

As an archipelagic country, the Philippines has abundant aquatic resources, making fisheries a major sector of the Philippine economy, agriculture, and food production. Fish, particularly small finfish, contributed significantly to healthy and diverse diets. It is an affordable source of high-quality protein, fatty acids, vitamins, and minerals, which can potentially alleviate nutrition problems, especially in low-income countries (Béné et al. 2016; FAO 2020). Fish and fish products are consumed throughout the country in many local and traditional dishes and were identified as the primary protein food source and top contributor of calcium in the Filipino diet (DOST-FNRI 2016).

Taal Lake is the third largest lake in the country and a part of the Taal Volcano environment. Most of the lake resources are utilized for open fisheries and aquaculture activities, providing livelihood and income for the people. Taal Lake also serves as a home for different native, migratory, and endemic aquatic species, including the only freshwater sardine in the world – the *tawilis*, *Sardinella tawilis* (Herre, 1927). *Tawilis* is the most commercially important and dominant fish species in Taal Lake's inland fisheries and is regarded as a local popular food, especially by the people in the nearby areas (Mutia et al. 2018a;

Muyot et al. 2021). However, its contribution to nutrition is not strongly investigated due to limited and outdated published sources regarding its food composition and nutritional value.

Food composition data is essential in nutrition assessment, dietetics, health, and epidemiological research, as with other related disciplines such as food science, agriculture, and trade, among others The nutrient composition of foods may change due to the influence of numerous factors such as the environment (soil, water, climatic conditions, geography, and seasons), plant and animal husbandry, genetic resources (food biodiversity, breeds, and varieties/cultivars), and processing (fortification, storage, transport, and market share) (Greenfield and Southgate 2003). Thus, it is vital to provide reliable and up-to-date data for the users from the disciplines mentioned earlier.

Moreover, the major phreatomagmatic eruption and continuous active volcanism of the Taal Volcano threaten the food safety of aquatic resources caught in the lake. Active volcanism can introduce metals (trace and toxic) into the environment, which could destabilize the ecosystem and consequently harm the organisms (Parelho et al. 2014; Peña-Fernández et al. 2014; Sonone et al. 2020). Toxic heavy metals such as mercury (Hg), lead (Pb), and cadmium (Cd) can enter the food chain and may pose risks to human health. Immediate hazards from volcanic eruptions (lava flow, lahars, debris, toxic gases, etc.) occur in short periods, but the released ash, pyroclastic materials, and other eruption effects stay in the volcanic vicinity and nearby areas for months, years, or decades (Ma et al. 2019).

This study was conducted to determine the current proximate composition, fatty acid profile, and heavy metal (Hg, Pb, Cd) contents of the only freshwater sardine, considering the natural and anthropogenic changes that occurred in the Taal Lake over time, as well as the antiquated available nutrient data, and food system influences. Moreover, its potential nutritional significance and food safety were also assessed.

2. MATERIALS AND METHODS

2.1 Sample Collection

Four sampling sites, namely Poblacion, San Nicolas; Don Juan, Cuenca; Mahabang Buhangin, Tanauan City; and Bilibinwang, Agoncillo were selected and included in this study (see Figure 1). The sampling areas were patterned from the sampling points of the "Carrying Capacity for Aquaculture Production in Lake Taal" project conducted by the National Fisheries Research and Development Institute - Freshwater Fisheries Research and Development Center (NFRDI-FFRDC). Moreover, the researcher employed the convenience sampling method for the areas due to *tawilis* availability and accessibility in these areas. Fish samples were procured from local fisherfolk in January 2022 through the assistance of NFRDI-FFRDC fish enumerators. A total of seven kilos of *tawilis* fish were acquired per sampling site. Fish samples were placed in plastic bags and properly labeled.

On the other hand, four liters of lake water were collected per sampling site and were stored in plastic reagent bottles. Both fish and water samples were immediately preserved on ice (4°C). All fish samples and two liters of lake water were transported to the Lipa Quality Control Center Inc. Laboratory in Lipa City, Batangas for proximate and fatty acid composition analysis (fish samples) and heavy metal analysis (fish and water samples). The remaining two liters of lake water were submitted to the NFRDI-FFRDC Laboratory in Taal, Batangas for nitrite-nitrogen (NO_2-N), nitrate-nitrogen (NO_3-N), ammonia-nitrogen (NH_3-N), and phosphate (PO_4-P) analysis. No chemical preservations were added by the researchers.

2.2 Chemical Analysis

Fish samples (including the head, scales, bones, and viscera) were cut into small pieces,

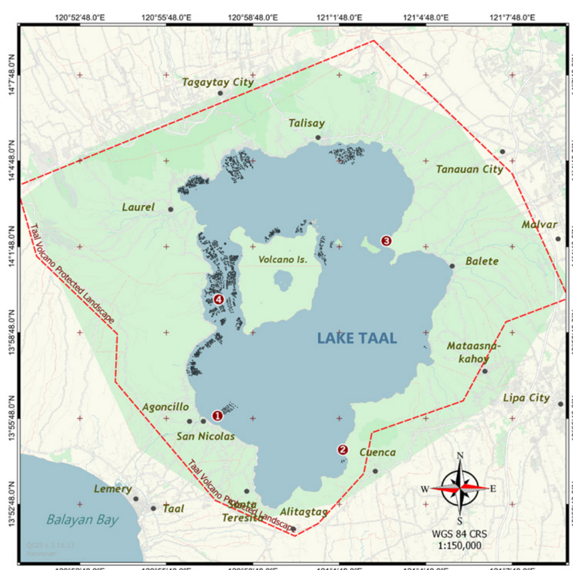

Figure 1. Sampling sites for this study (Map generated by Merilles MLD of NFRDI-FFRDC)

homogenized, and analyzed. The proximate composition, fatty acid content, and heavy metal content of the fish samples were analyzed according to the Association of Official Analytical Collaboration (AOAC) International official methods of analysis (AOAC International 2016). Following AOAC 934.01, moisture content was analyzed using the oven-drying method. The nitrogen contents of the fish samples were determined using the Kjeldahl method (AOAC 988.05) and the protein content was estimated by multiplying the nitrogen content by the protein factor 6.25. Fat was measured through the acid hydrolysis method (AOAC 954.02) with petroleum ether as the solvent. The ash contents of the samples were measured by heating the samples in a muffle furnace at 600°C until they became light gray in color (AOAC 942.05). Fatty acid (FA) contents of the fish samples were analyzed through gas chromatography in reference to the AOAC 938.09 method. For heavy metals, fish samples were homogenized and digested with concentrated acids, then analyzed Hg, Cd, and Pb contents using the atomic absorption spectrophotometric method (AOAC International 2016). The reference methods used were AOAC 971.21 for Hg and AOAC 968.08 for Cd and Pb.

2.3 Water Quality Data Collection

Physico-chemical water quality parameters were determined to characterize the water quality and conditions of the sampling sites. Dissolved oxygen (DO) and pH were measured on-site using a Horiba U-50 multi-parameter water quality checker. Water nutrients were analyzed by the NFRDI- FFRDC Laboratory using the spectrophotometric method, following the EPA-NERL: 350.2 method for ammonia-nitrogen, EPA-NERL: 354.1 method for nitrite-nitrogen, EPA-NERL: 352.1 for nitrate-nitrogen, and EPA-NERL: 365.2: method for phosphate (National Environmental Methods Index n.d). The heavy metal contents of lake water were determined by the Lipa Quality Control Center Inc. Laboratory through atomic absorption spectrophotometric methods, (SMEWW 3112B for Hg; SMEWW 3113B for Cd and Pb), following the Standard Methods for the Examination of Water and Wastewater (Baird and Bridgewater 2017). This study only included water quality data for January 2022.

2.4 Calculations

Carbohydrate content was computed by difference using Equation 1, while energy value (Equation 2) was estimated by multiplying the amount in grams of the food components: carbohydrate, fat, and protein, with their corresponding Atwater factors (4, 4, and 9, respectively).

$$Total\ Carbohydrate = [100 - (\%\ water + \%\ protein + \%\ fat + \%\ ash)] \quad (1)$$
$$Energy\ (kcal) = [(4 \times g\ Carbohydrates) + (4 \times g\ Protein) + (9 \times g\ Fat)] \quad (2)$$

The potential nutrient contribution of the *tawilis* fish was calculated in reference to the nutrient recommendations of the Philippine Dietary Reference Intakes (PDRI) for Filipino adults aged 19–59 if given 100 g of raw portion and a 100-gram portion of fried *tawilis* fish. A 100% dietary bioavailability and 100% edible portion were assumed in calculating the contribution values.

2.5 Statistical analysis

Descriptive statistics (mean and standard deviation) were performed for proximate analysis, fatty acid profile, heavy metal contents, and computations (carbohydrate and energy). One-way ANOVA was used to evaluate the significant differences in the mean values between the moisture, protein, ash, and fat values from the four sampling sites. The level of statistical significance was set at $p<0.05$.

3. RESULTS

3.1 Taal Lake Water Quality and its Heavy Metal Contents

Table 1 shows the water quality data of Taal Lake during the stated sampling period for the four sampling areas, along with the standard values for Freshwater Class B, according to the DENR Administrative Order No. 2016-08 (DENR 2016). Based on the data, the pH level of the four sampling sites exceeded the recommended range (6.5-8.5), with Agoncillo bearing the highest pH level. In terms of dissolved oxygen (DO), all sites surpassed the minimum acceptable level (5 mg/L). Phosphate concentrations in all sites exceeded the acceptable level (0.5 mg/L), with values ranging from 2.4719 to 2.6439 mg/L. The observed nitrite levels range from 0.0369 to 0.0785 mg/L. Ammonia and nitrate concentrations were below the detection limit, thus, did not exceed the maximum acceptable levels of 0.05 mg/L and 7 mg/L, respectively.

The heavy metal concentration of lake water samples is shown in Table 2. All sites had <0.01 mg/L lead (Pb) concentrations and <0.003 mg/L cadmium (Cd) concentrations, which conformed to the set maximum concentration, 0.01 mg/L, and 0.003 mg/L, correspondingly. Mercury (Hg) levels in the lake water range from 0.00018 to 0.00039 mg/L, which did not surpass the maximum allowable level (0.001 mg/L).

3.2 Proximate Composition and Energy Content of Raw Freshwater Sardine

Table 3 summarizes the mean proximate values for the raw *tawilis* fish samples from the four sampling sites. Moisture values vary from 66.26–72.84%, while protein content ranges from 16.89–18.49%. Fat values were 6.39–10.57%, and ash contents were 3.02–3.61%. There is no significant difference (p>0.05) in the moisture, protein, fat, and ash content among the fish samples from the four sites. Carbohydrate values range from 0.00–3.87%, with Agoncillo bearing the least amount, while San Nicolas *tawilis* samples had the greatest carbohydrate value. The mean carbohydrate value for all *tawilis* samples is 2.19±1.61%. Fish samples from Agoncillo resulted in a negative calculated carbohydrate value (-1.32%), which was then assumed to be zero (0).

In terms of energy (kcal), computed values range from 131 to 174 kcal per 100g. All *tawilis* samples were estimated to contribute a mean energy value of 158±19 kcal per 100g raw fish sample. Among the four sampling sites, samples from Agoncillo yielded the least mean energy value (131 kcal/100g raw *tawilis*). In contrast, samples from Cuenca had the greatest mean energy value (174 kcal/100g raw *tawilis*).

Table 1. Water quality data for pH, dissolved oxygen, phosphate, ammonia-nitrogen, nitrite-nitrogen, and nitrate-nitrogen of the four sampling sites in Taal Lake.

Parameters	Standard Level*	Sampling Site			
		San Nicolas	Cuenca	Tanauan City	Agoncillo
pH	6.5-8.5	8.94	8.98	8.95	9.04
Dissolved Oxygen, mg/L	5.0 (minimum)	9.62	11.67	7.95	6.89
Phosphate, PO_4-P, mg/L	0.5 (maximum)	2.4768 ± 0.0102	2.4719 ± 0.0091	2.6439 ± 0.0103	2.5471 ± 0.0043
Ammonia-Nitrogen, NH_3-N, mg/L	0.05 (maximum)	<0.0444	<0.0444	<0.0444	<0.0444
Nitrite-Nitrogen, NO_2-N, mg/L	N/A	0.0665 ± 0.0251	0.0369 ± 0.0155	0.0785 ± 0.0290	0.0712 ± 0.0266
Nitrate-Nitrogen, NO_3-N, mg/L	7.0 (maximum)	<0.2155	<0.2155	<0.2155	<0.2155

Notes:
*Standard level set for Freshwater Class B based on the DENR Administrative Order No. 2016-08 on Water Quality Guidelines and General Effluent Standards of 2016
Ammonia method detection limit= 0.0444 mg/L; Nitrate method detection limit= 0.2155 mg/L

Table 2. Mean heavy metal concentration (mg/L) of Taal Lake water samples from the four sampling sites.

Heavy Metal (mg/L)	**Standard Level***	**Sampling Site**				**Mean**
		San Nicolas	**Cuenca**	**Tanauan City**	**Agoncillo**	
Mercury (Hg)	0.001 (maximum)	0.00032	0.00018	0.00039	0.00021	**0.00028 ± 0.00010**
Lead (Pb)	0.01 (maximum)	<0.01	<0.01	<0.01	<0.01	**<0.01**
Cadmium (Cd)	0.003 (maximum)	<0.003	<0.003	<0.003	<0.003	**<0.003**

Notes:
*Standard level set for Freshwater Class B based on the DENR Administrative Order No. 2016-08 on Water Quality Guidelines and General Effluent Standards of 2016
Pb method detection limit = 0.01 mg/L; Cd method detection limit = 0.003 mg/L

Table 3. Proximate composition and energy content of raw freshwater sardine (*tawilis*) from the four sampling sites per 100g raw basis.

Samples	Proximate Composition					
	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Carbohydrate (%)	Energy (kcal)
San Nicolas	67.82 ± 1.67[a]	16.89 ± 0.99[a]	8.13 ± 0.88[a]	3.29 ± 1.22[a]	3.87	156
Cuenca	66.80 ± 5.12[a]	17.34 ± 1.14[a]	10.57 ± 1.83[a]	3.02 ± 0.23[a]	2.27	174
Tanauan City	66.26 ± 2.23[a]	18.08 ± 0.44[a]	9.71 ± 1.94[a]	3.31 ± 0.93[a]	2.63	170
Agoncillo	72.84 ± 5.23[a]	18.49 ± 0.99[a]	6.39 ± 1.88[a]	3.61 ± 0.71[a]	0.00	131
Mean ± SD	**69.15 ± 3.01**	**17.48 ± 0.72**	**8.98 ± 1.84**	**3.32 ± 0.24**	**2.19 ± 1.61**	**158 ± 19**

Notes:
Data are expressed as mean ± SD of triplicate, n =3.
The same superscript letters show no significant differences (p>0.05)
Carbohydrate values were calculated by difference, wherein negative value results were assumed as zero (0). Agoncillo had a calculated carbohydrate content of -1.32%.

3.3 Estimation of Potential Nutrient Contribution

The Daily Value % (% DV) for protein was computed through Equation 3:

$$\%\,Daily\,Value = \frac{amount\ of\ nutrient\ in\ a\ serving}{total\ recommended\ daily\ amount} \times 100 \quad (3)$$

Protein adequacy was computed for Filipino adults aged 19–59 if given 100 grams portion of raw *tawilis* fish and 100 grams of fried *tawilis* fish. Calculated values are shown in Figure 2. A 100% dietary bioavailability was assumed in the calculations. According to the Philippine Dietary Reference Intakes (DOST-FNRI 2015), Filipino males and females aged 19–59 need 71 g and 62 g of protein daily, respectively. For Filipino males, a serving of 100g raw *tawilis* (mean protein value = 17.48 g) covers 24.62% of their daily protein requirement, while 28.19% for females.

On the other hand, the protein contribution of 100 grams of fried *tawilis* fish was calculated using the cooked-to-raw (C-R) yield factor of 2.032 (Sardine, bombon, fried) based on the Philippine Food Composition Tables 1997 User's Guide (DOST-FNRI 1997). A 100-g portion of fried *tawilis* would be equivalent to 203.20 grams of raw *tawilis* fish. Based on the Philippine Food Exchange Lists developed by the DOST-FNRI (2019), one exchange of *tawilis* fish (30 g cooked), when fried, absorbs one exchange of oil; hence, 100 g of the fried *tawilis* fish would have absorbed approximately 3.33 exchanges of oil. If quantified, 3.33 exchanges are equivalent to 16.67 g of oil. It was also assumed that the fish was fried, without breading, and had a 100% edible portion (eaten whole). Thus, 100 grams of fried *tawilis* has a protein value of 35.52 g and meets 50.03% and 57.29% of the protein needs of male and female Filipino adults, correspondingly.

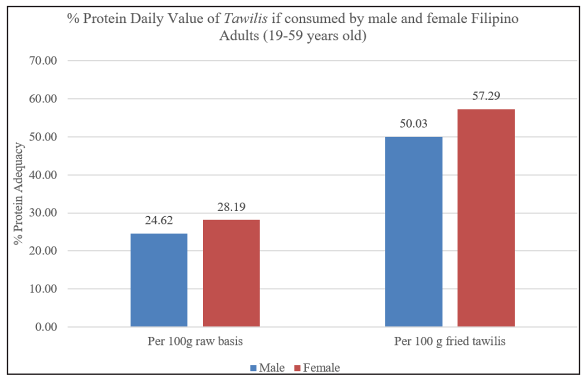

Figure 2. Protein daily value (%) of freshwater sardine (*tawilis*) if consumed by male and female Filipinos adults aged 19–59 per 100 g raw basis and per 100 g fried *tawilis* fish.

3.4 Fatty Acid Profile of Raw Freshwater Sardine

Weight percentages (% w/w) of the detected fatty acids (Table 4) were converted to g fatty acid per 100 g food using Equation 4:

$$FA\ content\ (g/100\ g) = \ weight\%\ FAME\ x\ FACF\ x\ lipid\ content\ (g\ lipid/100\ g\ food) \quad (4)$$

where FACF is the fatty acid conversion factor/ lipid conversion factor (g FA/g lipid). For fatty finfish (>5% total lipid), the FACF to be used is 0.90 (Compilers' Toolbox™ 2023). Fatty acid profile of the raw tawilis fish (g/100 g) is shown in Table 5.

The saturated fatty acids detected were caprylic acid (C8:0), capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), and stearic acid (C18:0). Caprylic (C8:0) and capric acid (C10:0) were only observed in Tanauan City *tawilis* samples (0.13 g/100 g and 0.01 g/100 g, respectively). All *tawilis* samples contain lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), and stearic acid (C18:0). The total mean saturated fatty acid (SFA) value for all *tawilis* samples is 5.88 ± 0.74

g/100 g. Palmitic acid (C16:0) is the most predominant saturated fatty acid among the fish samples. Oleic acid (C18:1) is the only monounsaturated fatty acid detected in the *tawilis* samples, with a mean content of 1.24±0.77 g/100 g. On the other hand, the observed polyunsaturated fatty acids were linoleic acid (C18:2) and α-linolenic acid (C18:3), amounting to 0.30 ± 0.14 g/100 g and 0.41 ± 0.45 g/100 g, respectively. The

Table 4. Fatty acid profile (% w/w) of the raw freshwater sardine (*tawilis*) from the four sampling sites.

Fatty Acids (%)	Samples				Mean (%)
	San Nicolas	Cuenca	Tanauan City	Agoncillo	
Caproic Acid (C6:0)	<0.1	<0.1	<0.1	<0.1	<0.1
Caprylic Acid (C8:0)	<0.1	<0.1	1.48	<0.1	1.48
Capric Acid (C10:0)	<0.1	<0.1	0.08	<0.1	0.08
Lauric Acid (C12:0)	1.04	0.25	0.66	3.08	1.26
Myristic Acid (C14:0)	24.21	11.54	10.12	8.55	13.61
Palmitic Acid (C16:0)	21.23	29.72	41.21	34.64	31.70
Stearic Acid (C18:0)	29.53	21.83	24.69	42.34	29.60
Total SFA	**76.01**	**63.34**	**78.24**	**88.61**	**76.55**
Palmitoleic Acid (C16:1)	<0.1	<0.1	<0.1	<0.1	<0.1
Oleic Acid (C18:1)	18.78	22.95	12.2	5.74	14.92
Total MUFA	**18.78**	**22.95**	**12.2**	**5.74**	**14.92**
Linoleic Acid (C18:2)	4.56	2.63	5.39	2.46	3.76
α-Linolenic Acid (C18:3)	0.64	11.09	4.17	3.20	4.78
Total PUFA	**5.20**	**13.72**	**9.56**	**5.66**	**8.54**

Notes: Detection limit: 0.1%
SFA= Saturated FA; MUFA= Monounsaturated FA; PUFA= Polyunsaturated FA

Table 5. Fatty acid profile (g/100g) of the raw freshwater sardine (*tawilis*) from the four sampling sites.

Fatty Acids (g/100g)	Samples				Mean ± SD (g/100g)
	San Nicolas	Cuenca	Tanauan City	Agoncillo	
Caproic Acid (C6:0)	-	-	-	-	0.00
Caprylic Acid (C8:0)	-	-	0.13	-	0.13
Capric Acid (C10:0)	-	-	0.01	-	0.01
Lauric Acid (C12:0)	0.08	0.02	0.06	0.18	0.08±0.07
Myristic Acid (C14:0)	1.77	1.10	0.88	0.49	1.06±0.54
Palmitic Acid (C16:0)	1.55	2.83	3.60	1.99	2.49±0.91
Stearic Acid (C18:0)	2.16	2.08	2.16	2.43	2.21±0.16
Total SFA	**5.56**	**6.03**	**6.84**	**5.10**	**5.88±0.74**
Palmitoleic Acid (C16:1)	-	-	-	-	0.00
Oleic Acid (C18:1)	1.37	2.18	1.07	0.33	1.24±0.77
Total MUFA	**1.37**	**2.18**	**1.07**	**0.33**	**1.24±0.77**
Linoleic Acid (C18:2)	0.33	0.25	0.47	0.14	0.30±0.14
α-Linolenic Acid (C18:3)	0.05	1.05	0.36	0.18	0.41±0.45
Total PUFA	**0.38**	**1.31**	**0.84**	**0.33**	**0.71±0.46**
TOTAL	**7.32**	**9.51**	**8.74**	**5.75**	

Notes: “-“= below detectable limit, thus no computed value
SFA= Saturated FA; MUFA= Monounsaturated FA; PUFA= Polyunsaturated FA

Table 6. Comparison of nutrient data values of the freshwater sardine and tilapia per 100 g raw basis.

Nutrient	**Analyzed**	**PhilFCT 2019**	
	Freshwater Sardine[a]	**Freshwater Sardine[b]**	**Tilapia[b]**
Edible Portion (%)	-	-	46
Moisture (g/100g)	69.15	75.0	77.2
Protein (g/100g)	17.48	19.3	18.1
Fat (g/100g)	8.98	4.1	3.8
Ash (g/100g)	3.32	1.6	1.2
Carbohydrate (g/100g)	2.19	0	0
Energy, calculated (kcal/100g)	158	114	106
Fatty acids, saturated, total (g/100g)	5.88	1.08	0.84
Fatty acids, monosaturated, total (g/100g)	1.24	0.80	0.49
Fatty acids, polysaturated, total (g/100g)	0.71	1.04	0.81

Notes: a= Analyzed; b= Nutrient data obtained from the Philippine Food Composition Tables (PhilFCT) 2019 (DOST-FNRI 2019)

total mean PUFA value for all *tawilis* samples is 0.71 ± 0.46 g/100g. Among the *tawilis* samples from the four sampling sites, Agoncillo had the lowest total fatty acid content (5.75 g/100 g), while samples from Cuenca had the highest fatty acid amount (9.51 g/100g).

3.5 Comparison of Fish Nutrient Data

Table 6 presents the fish nutrient data of the analyzed freshwater sardine, compared to the nutrient values for freshwater sardine and tilapia flesh published in the Philippine Food Composition Tables (PhilFCT) 2019 by DOST-FNRI (2019). The analyzed nutrient composition results of the *tawilis* are similar to the data published by DOST-FNRI but differ in fat, ash, SFA, and MUFA content.

Moreover, it was observed that the protein content for the analyzed *tawilis* and tilapia fishes was relatively close. On the other hand, values for fat, ash, carbohydrate, energy, saturated fatty acids (FA), monounsaturated FA, and polyunsaturated FA were higher in *tawilis* fish.

3.6 Heavy Metal Contents of Raw Freshwater Sardine

Table 7 shows the heavy metal concentration results for the *tawilis* samples.

For mercury (Hg), results range from 0.19 to 0.26 mg/kg, with a mean of 0.23±0.03 mg/kg. *Tawilis* samples from Agoncillo have the least detected mercury (0.19 mg/kg), while San Nicolas samples have

Table 7. Mean heavy metal concentration (mg/kg) of freshwater sardine samples from the four sampling sites and the maximum allowable concentrations (mg/kg) of for mercury (Hg), lead (Pb), and cadmium (Cd) for fish as set by DA-Bureau of Agriculture and Fisheries Standards (2017) and the European Union (2006).

Heavy Metal (mg/kg)	**Maximum allowable concentrations (mg/kg)**		**Samples**				**Mean ± SD (mg/kg)**
	DA-BAFS PNS/BAFS 205: 2017	**European Union (EC) No 1881/2006**	**San Nicolas**	**Cuenca**	**Tanauan City**	**Agoncillo**	
Mercury (Hg)	0.50	0.50	0.26	0.25	0.21	0.19	**0.23 ± 0.03**
Lead (Pb)	0.30	0.30	<0.06	<0.06	<0.06	<0.06	**<0.06**
Cadmium (Cd)	0.50	0.10	<0.02	<0.02	<0.02	<0.02	**<0.02**

Notes: Pb method detection limit = 0.06 mg/kg; Cd method detection limit = 0.02 mg/kg the highest mercury content (0.26 mg/kg). Moreover, lead (Pb) and cadmium (Cd) were not detected among all samples from the four sampling sites.

4. DISCUSSION

The nutrient composition of foods is greatly influenced by numerous factors, such as the environment (e.g., soil, water, climatic conditions, geography, and seasons) and other components in the food system (Greenfield and Southgate 2003). Furthermore, it is necessary to sustain good water quality to ensure the quality and safety of our aquatic foods and attain high yields and income. Most of the physicochemical parameters and heavy metal content (Hg, Pb, Cd) results of the lake water samples were within the prescribed standards set by the DENR (2016), except for pH and phosphate. Moreover, similar values were observed in the study of Merilles et al. (2021), which is 7.27–9.73 for pH, and 0.25–5.04 mg/L for phosphate. It was also stated by Zafaralla (1999) that volcanic lakes tend to have naturally high levels of phosphorus.

There is no significant difference in the proximate composition of the *tawilis* fish from the four sampling sites. In a study by Mohanty et al. (2016), fish can be classified according to their fat content, wherein lean meat fishes have <2% fat, low-fat fish contains 2–4% fat, medium fat fish has 4–8% fat, and high-fat fish contains >8% fat. Based on the analyzed results, the freshwater sardine has a mean fat value of 8.98±1.84%, thus, it classifies as a high-fat fish. Small pelagic fishes, such as small tuna, mackerel, and sardines, are established as fatty fish species. The fish use fat as energy storage and mainly accumulates in the liver and adipose tissues (Sankar et al. 2010). This study analyzed the *tawilis* samples with its head, scales, bones, and viscera intact; thus, the fat stores from the viscera were accounted. Carbohydrate was calculated by difference, and the fish samples from Agoncillo had a negative calculated value (-1.32%) but was assumed zero. A negative calculated value for carbohydrates is acceptable for meat and fish (except for offal (e.g., liver) and mollusks, wherein it can be assumed as zero if computed values range from 0 to -5 g/100 g EP (edible portion) (FAO/INFOODS 2012). It was considered that the fish has a 100% edible portion since small pelagic fishes (< 25 cm at maturity) are usually consumed as whole (head, bones, and skin intact) (Thilsted 2012).

Based on the % Daily Value calculations, 100 g raw *tawilis* provides 24.63% of the daily protein requirement of Filipino males and 28.20% for females. Furthermore, consuming 100 g of fried *tawilis* fish satisfies 50.03% and 57.29% of the protein needs of male and female Filipino adults, respectively. As stated by the Codex Alimentarius Commission (2013), a solid food item is considered a "source" of protein if it provides not less than 10% of the nutrient reference values (NRV) per 100 g and is considered a "high protein" if its content is twice the value for "source" (20%). Upon calculation, the *tawilis* fish is considered a "high protein" food item. A hundred grams of this item provide more than 20% of the daily protein requirements for both Filipino adult males and females.

Fatty acids (FAs) can be classified according to the presence of double bonds. Saturated fatty acids are those which have no double bond. Meanwhile, fatty acids bearing one double bond are monounsaturated fatty acids, and if there are two or more bonds, these are called polyunsaturated fatty acids. These compounds are involved in many other vital processes in the body (e.g. structural components of cell membranes, precursors for bioactive molecules, regulators of enzyme activities, and regulation of gene expression) (EFSA 2010). Some FAs are noted to be essential, which human bodies cannot synthesize and thus should be procured from food or supplementation. Examples of these are linoleic acid (C18:2), α-linolenic acid (C18:3), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), among others. EPA and DHA were not specifically detected and quantified in this study. While it was observed that the total saturated FA had the highest value (5.88 ± 0.74 g/100 g) among other fatty acid groups, total saturated FA in raw *tawilis* is still relatively lower compared to red meats such as lean pork Boston butt (8.12 g SFA/100 g), pork belly (13.55 g SFA/100 g), and beef brisket (6.08 g SFA/100 g) (DOST-FNRI 2019). Variations in the fatty acid profile of fishes rely on different factors such as species, diet, and environmental factors like salinity, temperature, season, geographical location, and if the fish is farmed or in the wild. In the case of freshwater fishes, Taşbozan and Gökçe (2017) detailed that those microalgae in freshwater environments contain higher levels of linoleic acid (C18:2) and α-linolenic acid (C18:3) than EPA and DHA, compared to marine microalgae. These microalgae serve as food for the fish. Furthermore, this is linked to the observed presence of linoleic acid (C18:2) and α-linolenic acid (C18:3) in the raw *tawilis* samples.

The food composition data of the analyzed *tawilis* samples were compared to the data published by DOST-FNRI (2019). Values were relatively close but varied in fat, ash, SFA, and MUFA composition.

However, the food composition data of the *tawilis* fish entry from the PhilFCT 2019 can be traced back and is the same as the data from its 1980 edition; thus, it is outdated. Fatty acid data for the sardine is included in the 2019 Philippine FCT, though these data were borrowed and generated from other sardine entries from foreign food composition databases. Furthermore, FA values were expressed as total saturated fatty acids (FAs), total monounsaturated FAs, and total polyunsaturated FAs. It shall also be noted that seasons, environmental conditions, sampling, and analytical techniques may cause variations in the data.

Based on the study conducted by Mutia et al. (2018b), *tawilis* is the top species caught and landed in Taal Lake, followed by tilapia. Tilapia is an introduced species and is now widely raised for aquaculture in the lake. Both *tawilis* and tilapia are common, accessible, and affordable fish due to their abundance and availability. Thus, the two species were compared in this study. Tilapia was also reported as the 3rd most consumed fresh fish in the Philippines (DOST-FNRI 2016). The protein content for *tawilis* and tilapia are relatively close, while nutrient values for fat, ash, carbohydrate, energy, saturated fatty acids (FA), monounsaturated FA, and polyunsaturated FA were higher in *tawilis*. It shall be noted that the reflected nutrient data for tilapia represents its flesh (edible portion) while assuming that *tawilis* was analyzed with its head, scales, bones, and viscera intact. This, therefore, could explain the difference in the ash content since fish bones are high in calcium (Ca) and phosphorus (P), constituting 2% of the whole fish (Toppe et al. 2007). It shall also be mentioned that variations in the obtained food composition data depend on numerous factors, such as their feeding habits, behavior, and conditions, reflecting their nutrient composition. Tawilis consumes zooplankton, while tilapia is usually farmed and fed with commercial aquatic feeds. Fatty acid levels can also be influenced by factors such as the season, behavior, location, etc. (Metillo and Apiras-Eya 2014; Taşbozan and Gökçe 2017). Furthermore, it could be concluded that freshwater sardine is an equivalent source of protein and other nutrients compared to tilapia.

In terms of heavy metal concentrations (Hg, Pb, and Cd), *tawilis* samples from all sampling sites were below the maximum allowable concentrations (Table 7) according to the standards set by the Department of Agriculture-Bureau of Agriculture and Fisheries Standards (DA-BAFS 2017) and the European Union Commission (EC No 1881/2006). Byrd et al. (2021) stated that small-sized fish species have relatively lower levels of heavy metals. Studies by Chen et al. (2009) and Piraino and Taylor (2009) investigated the association of fish body size and trophic level with methylmercury accumulation. Mercury accumulation tends to be higher in larger fish (higher trophic levels). However, it is emphasized that fish mercury concentrations still depend on environmental conditions, especially if they favor increased mercury methylation (e.g., low pH, low dissolved oxygen, high organic matter) (BFAR-PhilMINAQ 2013; Gribble et al. 2016).

5. CONCLUSION

The results obtained from this study reveal the proximate composition and fatty acid profile of the *tawilis* fish, along with its heavy metal concentrations. Raw *tawilis* fish is considered a "high fat" and a "high protein" food item. Total saturated FA had the highest value (5.88 ± 0.74 g/100 g) among fatty acid groups, followed by monounsaturated FA (1.24 ± 0.77 g/100 g), then the polyunsaturated FA (0.71±0.46 g/100 g), wherein palmitic acid is the most predominant FA. Both fish and water samples conform to the set maximum concentration levels for Hg, Pb, and Cd, hence, there is no heavy metal contamination. Parallel studies concerning seasonal variations and other environmental conditions could be conducted to validate the results further. This study is limited to the determination of the proximate and fatty acid composition of *tawilis* in its raw form, hence, future studies may focus on the composition of *tawilis* if cooked or processed and may include other significant nutrient components and data (e.g., minerals, essential amino acids, edible portion) to establish its significance to diets. Moreover, the food composition of other native and endemic fish species may be investigated, along with their potential contribution to food and nutrition security.

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AUTHOR CONTRIBUTIONS

Serrano JD: Conceptualization, Methodology, Investigation, Writing - Original Draft. **Barrion ASA**: Conceptualization, Methodology, Writing - Original Draft. **Abacan SF**: Methodology, Writing - Original Draft. **Mopera LE***:* Methodology, Writing - Original Draft. **Regalado JHP**: Methodology, Formal analysis. **Mutia MTM:** Resources, Writing-Reviewing and Editing.

CONFLICTS OF INTEREST

To the best of our knowledge, no conflict of interest exists.

ETHICS STATEMENT

No animal or human studies were carried out by the authors.

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