Assessment of heavy metal contaminants and consumption patterns of smoked sharks and rays in three regions of Java north coast

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Abstract. Sharks and rays, as predators, can accumulate heavy metals through the food chain. The human body can also accumulate toxic heavy metals, with subsequent health risks. Therefore, the frequency of public consumption of sharks and rays products is important to determine. The high utilization of endangered shark and ray species can affect their conservation status. This study aimed to determine heavy metal contamination in processed shark and ray products, identify the species by DNA barcoding, and determine consumption patterns and sales volume of processed shark and ray products in the three regions of Java north coast. The research method involved DNA barcoding, heavy metal testing, and distributing questionnaires to consumers of smoked sharks and rays as well as to producers. The species identified in processed smoked rays were *Neotrygon kuhlii* and *Maculabatis macrura*. In contrast, processed shark products were *Carcharhinus tjutjot, Carcharhinus sealei, Carcharhinus limbatus, Sphyrna lewini*, and *Rhynchobatus springeri*. All samples did not present any heavy metal contamination, except for mercury. Mercury contamination in smoked rays and sharks was generally below the safety limit, except for *C. limbatus* and *S. lewini*, which had mercury exceeding the maximum limit value. The consumption of smoked rays is correlated with age, education, monthly income, number of family members, the number of products purchased per week, and spending on buying per week. Shark sales volume is positively correlated with producers' education and income.

Key Words: DNA barcoding, human-health, mercury, risk, seafood.

Introduction. Shark is one of the most consumed fish in certain Indonesian regions. Based on data from the Ministry of Maritime Affairs and Fisheries for 2021, shark production in Indonesia reached 28148 tons in 2021 (MMAF 2021). Sharks have the potential to be used in various fields, with all parts of their body being utilized. Utilization is mainly into processed foods such as smoked, pindang (preserved fish in brine), and salted fish. Data obtained from the Ministry of Marine and Fisheries of the Republic of Indonesia (2011) shows that smoking is the preferred method of processing fish, fish processed through smoking reaching 30% of processed fish products. The data indicates that people like smoked fish products. Smoked fish products that are also popular besides sharks are smoked stingrays. Smoked stingrays have a distinctive aroma and taste, and many local people like them (Wicaksono et al 2014).

Indonesian people, especially people in the North Coast Java, like to consume processed products from sharks and rays, even though shark is one of the marine products prone to bioaccumulation or biomagnification of toxic metals from the environment into their bodies (Lara et al 2020). Examples of heavy metals that can accumulate in commodities include mercury (Hg), cadmium (Cd), lead (Pb), and copper (Cu). Heavy metals found in marine ecosystems, for example Hg, can accumulate through bacteria converting mercury into its organic form, methyl mercury (MeHg), and have the potential to accumulate and biomagnify in upper trophic level predators, such as sharks and rays (Barcia et al 2020). Research conducted by Burgess et al (2016) showed that the accumulation of heavy metals in stingrays showed different results for each species. For example, the manta ray (*Mobula birostris*) is a zooplankton, shrimp, and crab eater; this species does not accumulate as many pollutants as other ray species, which eat larger organisms in large quantities. Heavy metal contamination accumulates in fish that are processed and consumed, so consumers indirectly consume heavy metals from these organisms (Afe et al 2021).

Heavy metal contamination in processed shark and ray products distributed to the public can cause food safety problems. The maximum mercury level in the meat of large predatory fish for consumption, such as rays and sharks, is 1.0 μg g^{-1} (w/w) (OJEU 2006). It is necessary to pay attention to the frequency of consumption of this commodity, especially for pregnant and lactating women and children. Consumption of processed sharks and rays with high heavy metal contamination raises the risk of babies being born with defects, brain damage, mental retardation, swallowing disorders, blindness, and deafness (Pure & Widanarti 2019).

Fish products such as fins, skin, or other than original forms are difficult to identify morphologically. Biomolecular methods using nucleotide bases (DNA), such as DNA barcoding, can be used to identify shark species in processed products. DNA barcoding is a taxonomic method that uses short genetic markers from standard DNA genomic sections based on amplifying short DNA fragments and mitochondrial genomes. Cytochrome c oxidase subunit I (COI) gene markers are widely applied because they effectively identify all animal species (Abdullah & Rehbein 2017; Abdullah et al 2020). Species authentication of processed products is carried out to determine the species of shark and ray raw materials used and their conservation status on the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) and the International Union for Conservation of Nature (IUCN) red list. Data taken by IUCN (2023) shows that, of the 200 species of stingray fish in Indonesia, 49 species are endangered, 23 species are critically endangered, 28 species are near threatened, 55 species are vulnerable, 34 species are of least concern, and 11 species have deficient data.

This study aimed to determine heavy metal contamination in processed sharks and rays, identify species of processed sharks and rays using DNA barcoding, determine the correlation of several factors on sales of smoked shark production in the three selected regions of Java north coast, where most of the small-medium enterprises producing smoked fish are located, and determine the correlation between the frequency of public consumption and several variables.

Material and Method

Sample collection. The processed shark and ray product samples were collected from three regions in Java north coast, namely: Rembang, Tegal and Pati, between August 2021-December 2022. Samples were purchased from various locations selling processed shark and ray products, such as markets, restaurants, and traditional fish smoking enterprises. The samples obtained were five processed shark products and five smoked rays. Processed shark samples were coded KC2, KC3, CC3, CC4, and PKL4, while smoked rays were coded PKL 3 Pe, PKL 6 Pe, Da5a, Da1d, and NH1. Processed shark and ray meat were washed first with sterile mineral water to remove the spices, then stored in a 2 mL microtube. Ethanol p.a 96% was added to all samples in the microtube until the meat samples were submerged to keep the meat from being damaged during transportation to the laboratory. Processed meat (150 g) was stored in plastic zip-locks and transported to the laboratory for further heavy metal analyses.

DNA isolation. The DNA isolation used 200 mg of processed shark and ray meat samples. The DNA isolation process followed the procedure according to the manufacturer's instructions. Genomic DNA was extracted using a Presto™ Food DNA Extraction Kit (Geneaid Biotech Ltd, Taiwan). The concentration and purity of the DNA were estimated by 2% agarose gel electrophoresis (Mupid-eXu System, Takara Bio, US) and by using a spectrophotometer NanoDrop (Implen, Munchen, Germany). Extracted DNA was stored at −20°C until PCR amplification.

DNA amplification. DNA amplification was carried out using conventional Polymerase Chain Reaction (PCR) (ProFlex PCR System, United States) with cytochrome c oxidase I (COI) gene markers. The PCR mixture consisted of 12.5 μL of Taq Master Mix, 0.5 μL of each primer (20 μM), 2 μL of DNA template (10 ng μL^{-1}), and nucleic-free water (NFW) up to a final volume of 25 μL. PCR conditions were set with pre-denaturation at 95°C for 15 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 1 min, followed by final extension at 72°C for 10 min. Amplicon size with COI primer was 650 bp. The obtained amplicon was visualized using 2% agarose gel electrophoresis (Mupid-eXu System, Takara Bio, US) to ensure the success of the amplification process before proceeding with the sequencing stage.

Sequencing. The sequencing procedure was carried out using Sanger Sequencing. This method was carried out to obtain the nucleotide base sequence of a sample species. These nucleotide base sequences were compared with the GenBank National Center of Biotechnology Information (NCBI) data to identify shark and ray species.

Determination of heavy metal contamination. Hg, Pb, and Cd content was determined using the ICP-MS (Inductively Coupled Plasma-Mass Spectrometry). The reference used in Hg, Pb, and Cd testing was AOAC 2015.01.2015 (Briscoe 2015) about heavy metals in foods and AOAC 2011.19.2014 (Briscoe 2015) about the application of inductively coupled plasma/mass spectrometry for measuring chromium, selenium, and molybdenum in infant formula and adult nutritional products. A standard series of metal alloys comprise at least six concentration points. Sample digestion was done by adding HNO_3 solution and using a microwave digester. Interpretation of the results for testing Hg, Pb, and Cd was carried out by calculating the levels of metals/minerals in the sample using a standard calibration curve with the line equation: Y = bx + a, with the following formula:

$$A_{spl} = \frac{\text{Sample ratio}}{\text{IS ratio}}$$

$$\text{Metals content (ppm, mg L}^{-1}\text{, mg Kg}^{-1}\text{)} = \frac{\frac{\{(\text{Aspl} - \text{blank ratio}) - a\}}{b} \text{x V x fp}}{\text{Wspl or Vspl}}$$

Where: A_{spl} - sample ratio to internal standard ratio; a - intercept of the standard calibration curve; b - slope of the standard calibration curve; fp - dilution factor; V - final volume of the flask (mL); W_{spl} - weight of the test portion (g); V_{spl} - pipetting volume of the test portion (mL).

Cu content was determined using ICP-OES (Inductively Coupled Plasma-Optical Emission Spectroscopy). The reference for testing copper was AOAC 2011.14 (AOAC 2012) about calcium, copper, iron, magnesium, manganese, potassium, phosphorus, sodium, and zinc in fortified food products (microwave digestion and ICP-OES). A standard series of metal alloys comprise at least six concentration points. Sample digestion was done by adding HNO_3 solution and using a microwave digester. Interpretation of the results for the copper test were conducted by calculating the metal/mineral content in the sample using the standard calibration curve with the line equation: Y = bx + a, with the following formula:

$$\text{Metals content (ppm, mgL}^{-1}\text{, mgKg}^{-1}\text{)} = \frac{\frac{(\text{Aspl} - a)}{b} \text{x V x fp}}{\text{Wspl or Vspl}}$$

Where: A_{spl} - sample ratio to internal standard ratio; A - intercept of the standard calibration curve; b - slope of the standard calibration curve; fp - dilution factor; V - final volume of the flask (mL); W_{spl} - weight of the test portion (g); V_{spl} - pipetting volume of the test portion (mL).

Questionnaire on consumption patterns of smoked stingrays. The questionnaires were used to determine consumer consumption patterns of smoked ray products. Questionnaires were applied to 30 respondents who were in food stalls or restaurants from Rembang, Tegal, and Pati (North Java Coast Region), presumably being processed shark and ray meat consumers. The total number of respondents in this study was 90, with each region having 30 respondents. Questionnaires were made for consumers/households, which were then divided into several sections. Questionnaire for consumers/households were divided into: (a) identification of place and characteristics of respondents to obtain information on name, age, education, gender, city, condition of consumers (pregnant/lactating/children), number of family members, net income per month; (b) consumption patterns of consumers/households. The second part of the questionnaire consisted of 6 questions to determine the respondents' consumption patterns of smoked ray products. The answers are in multiple choices, with several choices for each question.

Determining the volume of processed shark production using a questionnaire. The questionnaires were used to determine production volume and factors affecting this volume of processed sharks on the north coast of Java. Questionnaires were distributed to 13 producers/traders of small and medium enterprises (SMEs) of processed shark products. The data was collected by filling out questionnaires by field enumerators in Rembang, Tegal, and Pati. The questionnaire was divided into: a - identification of place and characteristics of respondents, consisting of name, age, education, gender, city (place of sale), and total net income per month; b - sales, to obtain information related to sales of shark products which consisted of 11 questions with multiple choice answers: weekly sales volume, smoke-curing of fish, and packaging. Questionnaires were distributed in a Google form, and enumerators conducted direct interviews. The correlation between the variables was analyzed using correlation analysis. Spearman's correlation coefficient was used to measure the correlation between producer location, age, and income on the smoke-curing agent, packaging, and sales volume in a week.

Genetic data analysis. The sequencing data obtained was analyzed using bioinformatics with alignment. Alignment was performed using MEGA 11 (Molecular Evolutionary Genetic Analysis) software. The alignment process was carried out using the ClustalW program on MEGA 11 to determine the level of homology between the DNA base sequences analyzed. Acquired sequences were compared using the Basic Local Alignment Search Tool (BLAST) on the National Center for Biotechnology Information (NCBI) website to determine the species in the sample. Analysis using nucleotide BLAST (Blast-n) was used to determine the level of the kinship of species based on nucleotide sequences from specific groups (nr/nt) and very similar sequences (Megablast). The kinship of the samples can be determined by constructing a phylogenetic tree in Mega 11 software. The method used in constructing phylogenetic trees is the Neighbor-Joining Tree (NJT) method, with a bootstrap value of 2000 times.

Statistical analysis. Respondents selected were 90 consumer respondents from the north Java coast region (represented by Tegal, Pati, and Rembang), where the questionnaire was distributed, presumably with a high consumption level of processed smoked stingrays. These data were processed using Microsoft Office Excel to obtain descriptive data on the consumer demographic category. Further analysis was carried out to determine the correlation between variables and the frequency of public consumption of smoked shark and ray products using IBM SPSS (Statistical Package for the Social Sciences) version 23 software assistance. For processed shark products, data from 13 respondents from producers/traders of Small and Medium Enterprises (SMEs) of processed shark products were also processed using Microsoft Excel to obtain descriptive data. The correlation level of the relationship between sales volume and certain variables was determined using the correlation matrix with IBM SPSS 23. The correlation between variables (Spearman correlation) was determined with the help of SPSS. The correlation coefficient (r) is a number that expresses the strength of the relationship between variables or the direction of the relationship between the variables. The value of the Spearman correlation coefficient (r) is between -1<0<1; if the value of r is 0 then there is no relationship between the two variables (Mohr et al 2021).

Results

DNA barcoding-based species determination. The species identification results in processed shark and ray products can be seen in Table 1.

Table 1

The results of species identification with the NCBI BLAST

Sample code	*Species*	*E-value*	*Homology (%)*	*Access code*	*IUCN Redlist*	*CITES*
Da5a	*Neotrygon kuhlii*	0.0	99.85	KU498010.1	DD	-
NH1	*Maculabatis macrura*	0.0	100.00	MG774914.1	EN	-
PKL3Pe	*Maculabatis macrura*	0.0	100.00	MG774914.1	EN	-
PKL6Pe	*Neotrygon kuhlii*	0.0	99.56	KU498012.1	DD	-
Da1d	*Neotrygon kuhlii*	0.0	100.00	KU498009.1	DD	-
KC2	*Carcharhinus tjutjot*	0.0	99.26	NC_026871.1	VU	Appendix II
KC3	*Carcharhinus sealei*	0.0	100.00	KF590375.1	VU	Appendix II
CC3	*Carcharhinus limbatus*	0.0	97.85	JQ654710.1	VU	Appendix II
CC4	*Sphyrna lewini*	0.0	100.00	MF508688.1	CR	Appendix II
PKL4	*Rhynchobatus springeri*	0.0	99.85	MG792088.1	CR	Appendix II

Note: DD - data deficient; EN - endangered; VU - vulnerable; CR - critically endangered.

The species identification results in Table 1 show that the smoked stingray samples with the codes Da5a, PKL6Pe, and Da1d are identical species to *Neotrygon kuhlii* or the blue spotted stingray. Sample codes NH1 and PKL3Pe were identified as identical species, namely *Maculabatis macrura*. Sample D1ad has yet to be identified. The results of the identification of processed shark samples showed different species. *Carcharhinus tjutjot* was identified in the KC2 sample. The KC3 sample was identified as the species *Carcharhinus sealei*. The species *Carcharhinus limbatus*, or blacktip shark, was identified in sample CC3 and *Sphyrna lewini*, with the local name hammerhead shark, was identified in sample CC4. The PKL4 sample was identified as *Rhynchobatus springeri*. All samples identified using BLAST have a homology percentage in the 97-100% range with an E-value of 0.0.

Sequence results of all processed samples of sharks and rays were compared with various other species of sharks and rays from the GenBank database to construct a phylogenetic tree. A phylogenetic tree was constructed to determine compatibility and kinship between species obtained from the BLAST sample results. The phylogenetic tree for shark species can be seen in Figure 1, and for stingray species in Figure 2.

The results of the phylogenetic tree analysis showed that the KC2 sample had a close match and close relationship with the *C. tjutjot* species with a bootstrap value of 97. The KC3 sample was identified as *C. sealei* because it had the highest kinship and closeness to *C. sealei* isolate FPCsea2 with a bootstrap value of 67 on the same branch. Another similar branching occurs for the CC3 sample, closely related to the *C. limbatus* SOSSRC voucher with a bootstrap value of 80. The PKL4 sample, closely related to the *R. springeri* voucher JRFR066-17 forms the same branch with a bootstrap value of 95. The closest match and relationship between the CC4 sample with several *S. lewini* sequences showed a bootstrap value of 100, so the CC4 sample was identified as *S. lewini*.

The results of the species kinship analysis in Figure 2 show that the Da5a, PKL6Pe, and Da1d species are closely related to the *N. kuhlii* species with a DNA bootstrap value of 74, 39, and 52 respectively. Last et al (2016) stated that *N. orientalis* is a complex species of *N. kuhlii*. The phylogenetic tree results indicate that Da5a, PKL6Pe, and Da1d samples are *N. kuhlii* species. The phylogenetic tree results indicate that Da5a and PKL6Pe samples are *N. kuhlii* species. The phylogenetic tree shows that NH1 and PKL3Pe are closely related to the *M. macrura* species with a bootstrap value of 100, indicating that the two samples are *M. macrura* species.

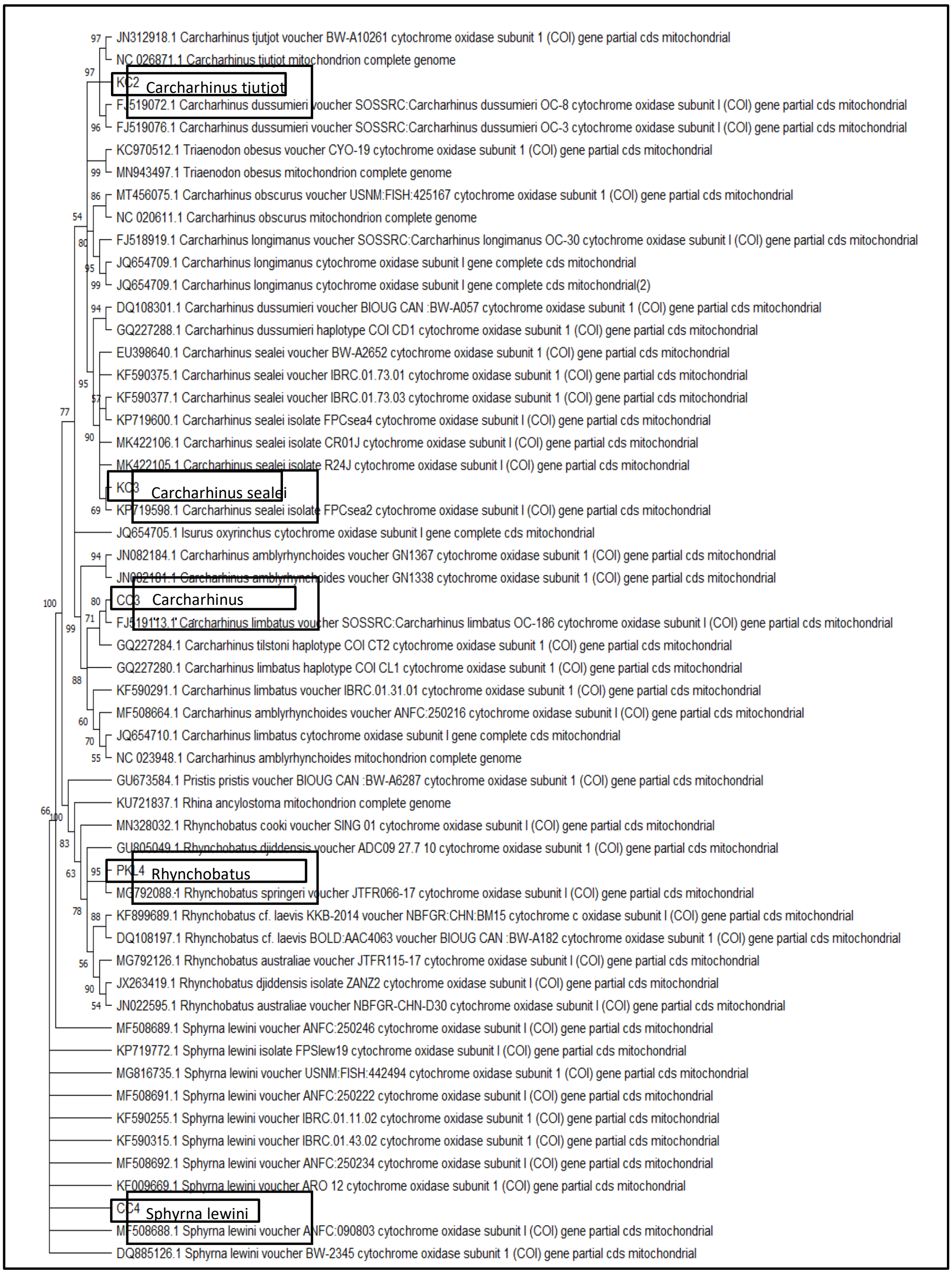

Figure 1. Phylogenetic tree of shark species.

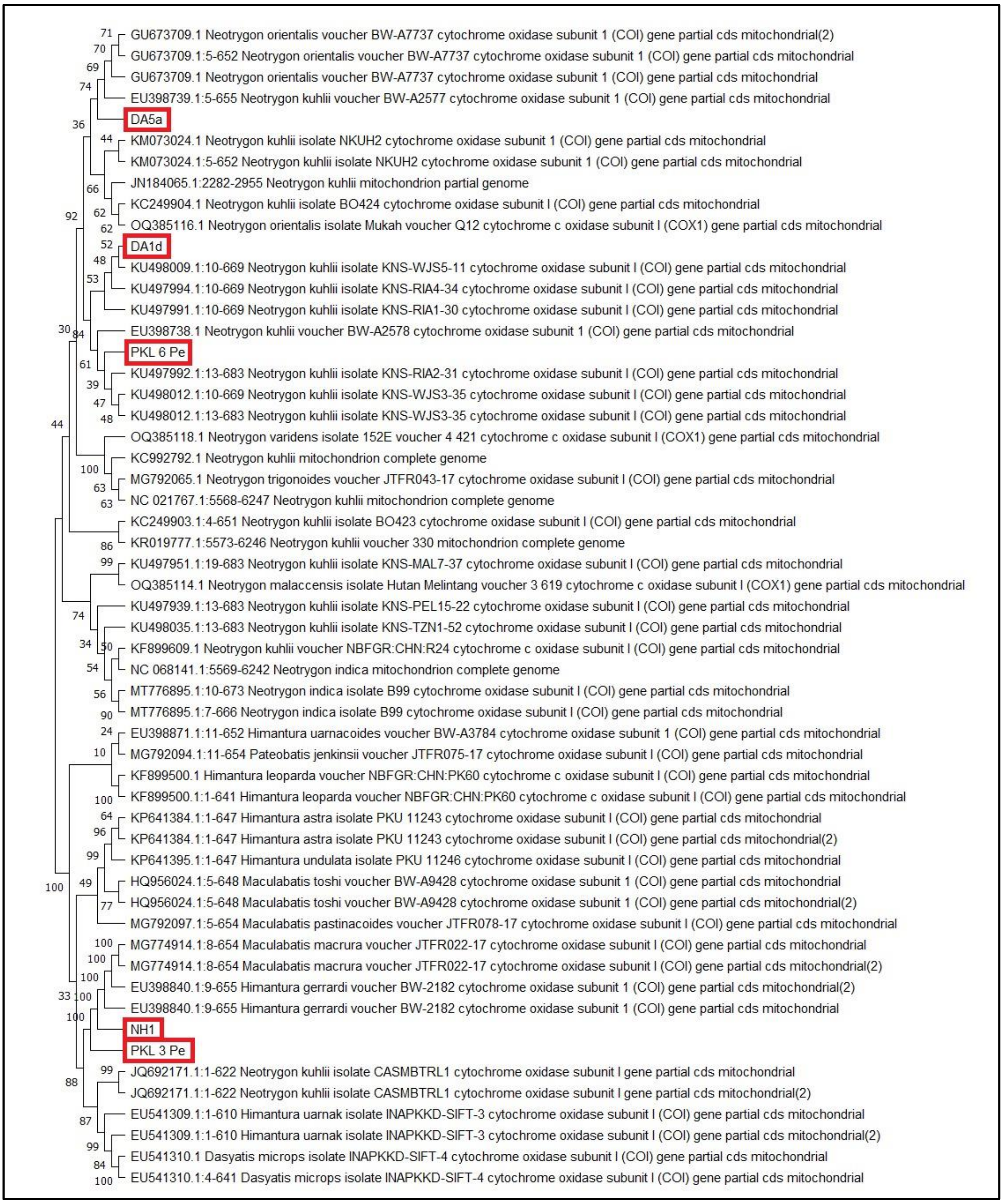

Figure 2. Phylogenetic tree of stingray species.

The concentration of heavy metal contamination. Heavy metals are toxic compounds that can cause damage to organisms. Hg, Pb, and Cd are examples of heavy metals that are toxic, while Cu is an essential heavy metal in small concentrations. The results of the analysis of heavy metal contamination in processed shark and ray products can be seen in Table 2.

Table 2

The concentration of heavy metal contamination in processed sharks and rays

Sample code	Species	Hg (mg kg^{-1})		Pb (mg kg^{-1})		Cd (mg kg^{-1})		Cu (mg kg^{-1})	
		mean ±SD	Max*	mean ±SD	Max*	mean ±SD	Max*	mean ±SD	Max**
Da5a	*Neotrygon kuhlii*	0.865±0.021	1.0	Nd	0.4	Nd	0.5	Nd	70
NH1	*Maculabatis macrura*	0.39±0.00	1.0	Nd	0.4	Nd	0.5	Nd	70
PKL3Pe	*Maculabatis macrura*	0.74±0.014	1.0	Nd	0.4	Nd	0.5	Nd	70
PKL6Pe	*Neotrygon kuhlii*	0.17±0.00	1.0	Nd	0.4	Nd	0.5	Nd	70
Da1d	*Neotrygon kuhlii*	0.2±0.00	1.0	Nd	0.4	Nd	0.5	Nd	70
KC2	*Carcharhinus tjutjot*	0.26±0.00	1.0	Nd	0.4	Nd	0.5	Nd	70
KC3	*Carcharhinus sealei*	0.305±0.007	1.0	Nd	0.4	Nd	0.5	Nd	70
CC3	*Carcharhinus limbatus*	1.105 ±0.007	1.0	Nd	0.4	Nd	0.5	Nd	70
CC4	*Sphyrna lewini*	1.485±0.007	1.0	Nd	0.4	Nd	0.5	Nd	70
PKL4	*Rhynchobatus springeri*	0.585±0.007	1.0	Nd	0.4	Nd	0.5	Nd	70

Note: Nd - not detected; * - maximum limit (SNI 2725:2013); ** - maximum limit (FAO 1983).

The results of the heavy metal testing showed that the samples only presented Hg contamination. Hg levels in all samples of processed rays are below safe limits. However, Hg contamination was detected in processed sharks exceeding the maximum limits specified in SNI 2725:2013, in samples CC3 and CC4. The standard setting for safe limits for Cu levels in fish meat was regulated by the FAO (1983), with a safe limit for copper consumption being 20-70 mg kg^{-1} body weight per week.

Smoked stingray consumption frequency. The frequency of consumption of smoked stingrays was observed in areas that presumably had a relatively high consumption level. The socio-demographic characteristics of respondents can be seen in Figure 3, and the consumption pattern characteristics of respondents can be seen in Figure 4.

The data in Figure 3 shows that the majority of smoked stingray consumers are women (65.56%), with education in elementary school (43.33%) and junior high school (31.11%). The age range that consumes the most processed smoked stingrays is 41-50 years (32.22%), and they do not have special conditions (93.33%). Pregnant consumers are 2.22% and breastfeeding consumers are 4.44%. The monthly income of most consumers is in the range of 129.7-194.6 USD per month, while a small proportion (2.22%) have their monthly income in the range of 259.5-324.3 USD per month and >324.3 USD per month. The number of family members in the <4 persons category had the highest percentage of 38.89%, but the family with four-person members had the lowest percentage of 25.56%.

Consumers from these three regions prefer to consume processed smoked stingrays (70%) compared to processed products and processed smoke sharks (Figure 4). Most people (45.56%) from the three regions tend to consume processed smoked rays daily. Meat is the most consumed part of the fish (100%). The people usually buy more than five products (>1 kg) per week and spend less than 2.6 USD per kg. The most popular places to buy fish are traditional markets and fish markets.

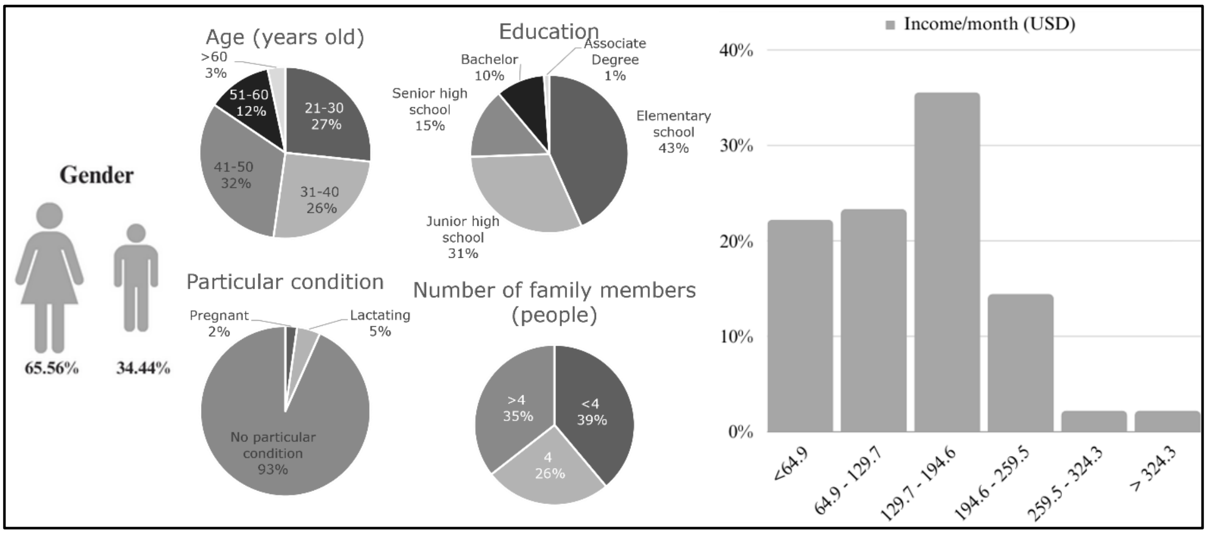

Figure 3. The socio-demographic characteristics of respondents.

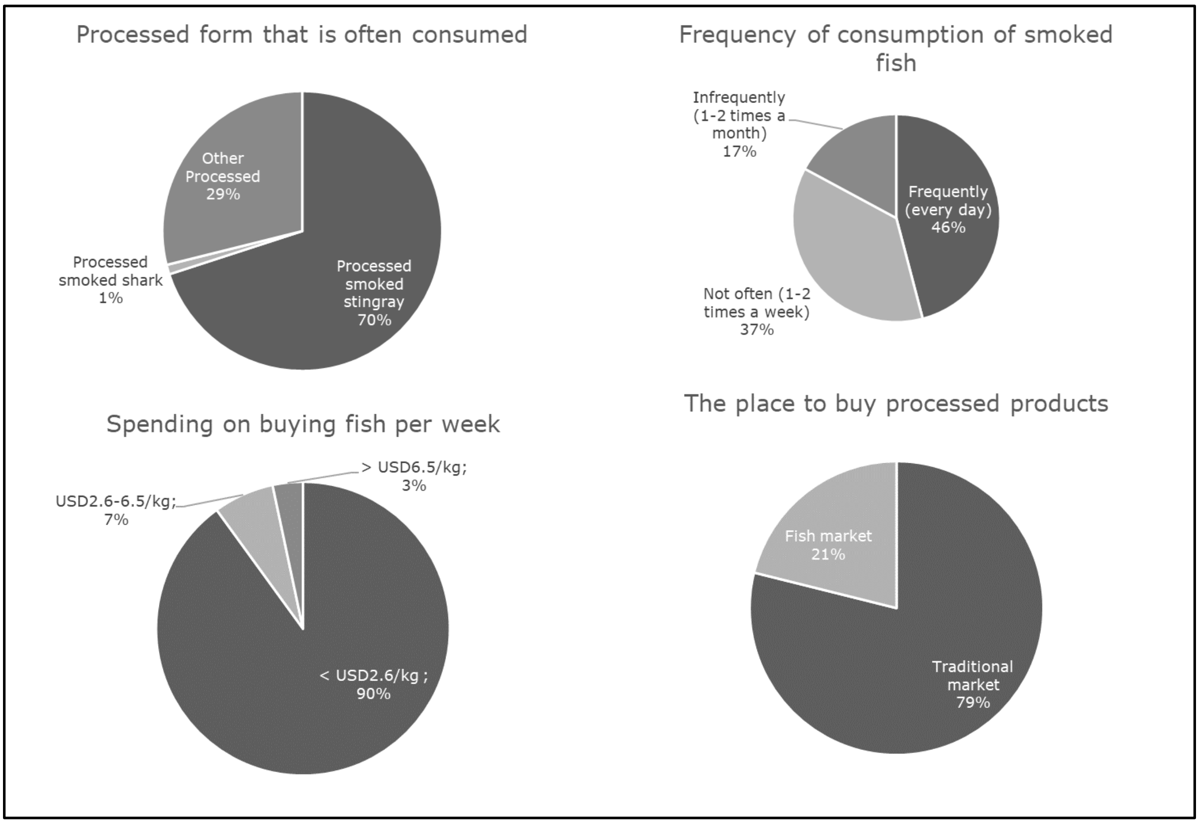

Figure 4. Characteristics of consumption patterns of smoked rays in the north Java coast region.

Socio-demographic characteristics and consumption patterns are considered independent variables that correlate with the frequency of public consumption, namely the dependent variable on processed smoked stingrays. The level of correlation and the strength of the relationship between variables can be seen in Table 3.

Table 3

The correlation analysis of consumption frequency

Variables	*A*	*B*	*C*	*D*	*E*	*F*	*G*	*H*
A	1	0.051	-0.384**	-0.073	0.171	-0.025	0.660**	0.211*
B	0.051	1	-0.683**	0.211*	-0.130	-0.012	-0.012	0.086
C	-0.384**	-0.683**	1	-0.098	-0.019	-0,015	-0.314**	-0.083
D	-0.073	0.211*	-0.098	1	-0.184	0.008	-0.238*	-0.089
E	0.171	-0.130	-0.019	-0.184	1	0.131	0.266*	0.208*
F	-0.025	-0.007	-0.015	0.008	0.131	1	0.276**	0.150
G	0.660**	-0.012	-0.314**	-0.238*	0.266*	0.276**	1	0.328**
H	0.211*	0.086	-0.083	-0.089	0.208*	0.150	0.328**	1

Note: ** - correlation is significant at the 0.01 level (2-tailed); * - correlation is significant at the 0.05 level (2-tailed); A - consumption frequency; B - age; C - education; D - particular condition; E - income/month; F - number of family members; G - the number of products purchased/week; H - spending on buying products/week.

The results of the r value in Table 3 show that consumption frequency correlates significantly with dependent variables in various significant levels and correlation values. The dependent variables correlating with the stingray species' sustainability are education, the number of products purchased weekly, and the shopping budget. Other factors such as age, special conditions, monthly income, and the number of family members have no significant correlation with consumers' consumption.

The consumption frequency variable has the highest positive correlation with the variable number of processed products purchased by consumers per week, with a very strong relationship category (0.660). Another positive and significant correlation is the consumers' spending (0.211). Consumers tend to buy more stingray species when they can buy more products and have a higher budget to buy fish products. On the contrary, the highest negative correlation of consumption frequency occurs in the education variable. It means that consumers with higher education have a low intention to consume stingrays. The highest negative correlation relationship is with the education variable (-0.384).

Processed shark demand. In Indonesia, especially in the north coast region, high quantities of shark are processed by the local community into smoked, boiled, and salted products. Shark sales volume data obtained from three north Java coast areas show demand for shark meat. The processed shark producer questionnaire results can be seen in Table 4.

Table 4

Results of a questionnaire for processed shark producers in the north coast region of Indonesia

Local name	*Scientific name*	*Sales volume/week*	*% Species*	*IUCN Red List*	*CITES*
Hiu martil	*Sphyrna lewini*	1 quintal	11.1	*CR*	Appendix II
Hiu sirip hitam	*Carcharhinus limbatus*	1 quintal	22.2	*VU*	Appendix II
Hiu sirip putih	*Triaenodon obesus*	>1 quintal	11.1	*VU*	Non Appendix II
Hiu totol	*Stegostoma varium*	>1 quintal	22.2	*EN*	Non Appendix II
Hiu kikir	*Rhynchobatus djiddensis*	>1 quintal	11.1	*CR*	Appendix II
Hiu lanyam	*Carcharhinus obscurus*	>1 quintal	22.2	*EN*	Appendix II

Note: CR - critically endangered; VU - vulnerable; EN - endangered.

Based on the data obtained (Table 4), six species of shark are widely traded by producers of processed smoked shark, including *S. lewini*, *C. limbatus*, *Triaenodon obesus*, *Stegostoma varium*, *Rhynchobatus djiddensis*, and *Carcharhinus obscurus*. The percentage of the number of species from the 13 producers was 22.2% for *C. limbatus, S. varium, and*

C. obcurus. This percentage was higher than that of the other three species, namely *S. lewini, T. obesus*, and *R. djiddensis*, which was 11.1%.

The sales volume of sharks and processed shark species in the North Coast region is influenced by several variables (Figure 5; Table 5). All the connections between the producers' demographic parameters and sales volume are insignificant (Table 5). The relationships only occur between the producers' demographic parameters and smoked agents or packaging. This result describes that fish producers use their local wisdom for smoke-curing and packaging.

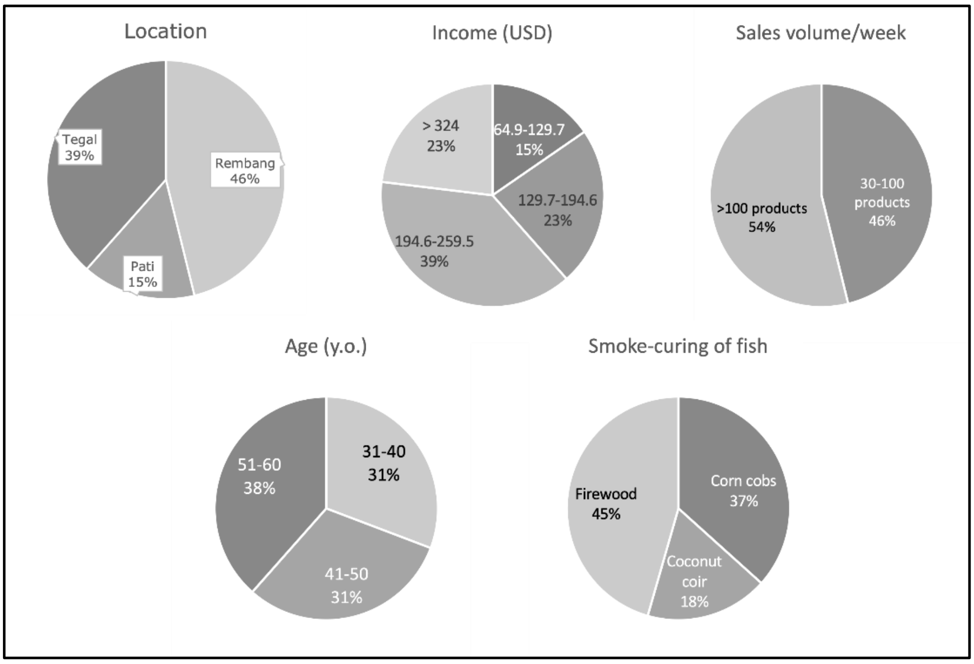

Figure 5. Producer profile.

Table 5

Relationship between producer behavior and demographic parameters

	Asymptotic significance (2-sided)		
	P value		
	Smoke-curing of fish	Packaging	Sales volume/week
The location of the producer	0.001	0.000	0.374
Age	0.000	0.000	0.347
Income	0.003	0.016	0.220

Discussion. The species identification results by DNA barcoding give a more significant percentage of homology, indicating a higher level of kinship. The BLAST results' maximum value (max score) and expected value (E-value) can determine the degree of kinship to a species. The sample species' lower E-value is more similar to or closely related to the target species (Marin et al 2009). The percentage level of sequence similarity with GenBank is significant if it has a value of 97-100% (Sarhan et al 2021). The E-value for all identified samples is 0.0. An E-value of 0 indicates that all samples have identical comparisons. These results are in accordance with the research of Sahaba et al (2021), who identified fresh shark products using DNA barcoding resulting in an E-value of 0.0 in all identified samples.

Identification of species *N. kuhlii* and *M. macrura* in four samples show that these species live in tropical waters. *N. kuhlii*, known as blue spotted stingray, is a commodity that has ecological and economic value and an important role in Indonesian fisheries (Abubakar et al 2015). The blue spotted stingray can be found in almost all Indonesian waters (White & Dharmati 2007). COI gene is commonly used as species identification target for most fish species and has been widely applied for seafood authentication. We believe that we correctly identified the shark and ray species from which the smoked samples were obtained. Most of the shark species being processed in the three regions of Java north coast are from the Carcharhinidae and Sphyrnidae families. Both fish groups have large total length and weight, therefore in high risk of toxic heavy metal bioaccumulation. In Indonesia, sharks and rays are not intentional target species for meat, being mostly by-catches of the tuna fishing industry and shark-finning industry. The systematic untraced use of Carcharhinidae and Sphyrnidae families could further affect sustainability and food safety.

The heavy metal contamination test results shown in Table 2 showed that heavy metal contamination of Cd, Pb, and Cu was not detected in all samples, which is presumably due to the low contamination of Cd, Pb, and Cu metals in the waters. The heavy metal contamination detected in all samples was Hg. Research by Abboah-Offei (2016) regarding heavy metal contamination in smoked fish products in the Accra district, Ghana, showed the same results: freshwater and seawater smoked fish products were tested positive for Hg, with high contamination detection in seawater fish. Contamination occurs due to the aquatic environment that has been polluted. In this study, Hg contamination in *S. lewini* and *C. limbatus* exceeded the Indonesian national standard for processed smoked sharks SNI 2725:2013, a maximum of 1 mg kg^{-1}. These results are similar to those obtained by Liu et al (2023) related to methyl mercury contamination in shark species, averaging above 1 mg kg^{-1} in processed smoked sharks. This research indicates that processes such as smoking can increase Hg contamination in fish, probably due to the product's loss of water and fat. Hg content was also found by Bwala et al (2023), in smoked fish samples from several unidentified smoked fish species sold in the markets in the Borno region, Nigeria. In that case, Hg was detected in amounts from 0.035 to 0.052 μg kg^{-1}. Hg is a heavy metal very toxic to aquatic organisms and humans. Hg can enter the water through factory waste, fungicides, pesticides, household waste, and others (Pratiwi 2020).

The frequency of consumption and the consumer age shows a positive correlation. Most consumers of smoked fish are 41-50 years. However, based on the survey, there are consumers of smoked stingrays of more than 50 years. This result is similar to those of Sokib et al (2012), who obtained a high frequency of fish consumption in people over 45 years. The other research also noted that people aged between 45 and 64 have the highest fish expenditure (Morales & Higuchi 2018). Thus, awareness of meeting safe nutritional intake increases in the age over 45. However, some respondents chose not to eat protein from fish due to health factors, such as allergies.

A positive strong correlation (0.266) was observed between income per month and spending on buying products per week. No correlation was observed between income and consumption frequency. It is because some consumers have low monthly incomes and a high frequency of consumption. The frequency of consumption is not only influenced by income and the ability to buy products, but also by factors such as food preferences and priorities, and other factors (Gittelsohn & Vastine 2003). The frequency of consumption strongly correlates with the number of products purchased per week. This result means that a more significant number of products purchased weekly shows a high frequency of product consumption. Based on the survey, the producers of smoked stingray products tend to use local wisdom agents to preserve the fish products. Their socio-demographics do not influence the producer's sales volume.

Frequency of consumption is negatively correlated with education. A negative correlation with the education variable may occur because most smoked stingray consumers have an elementary school education. Knowledge about nutritional intake, safe consumption limits, and other knowledge about fish consumption, especially fish belonging to the upper food chain, commodities with restricted use, can significantly affect the frequency of consumption.

Conclusions. Smoked stingray products are identified as *N. kuhlii* and *M. macrura*. According to the IUCN redlist, *M. macrura* has the endangered status. The smoked shark belonged to the species *C. tjutjot*, *C. sealei, C. limbatus, S. lewini,* and *R. springeri*. All identified shark species are listed in CITES Appendix II 2023, except for *R. springeri*. Smoked shark and ray products presented Hg contamination. Hg contamination in smoked rays is below the safe limit for consumption. Hg contamination in smoked shark products identified as *C. limbatus* and *S. lewini* exceeds human consumption's maximum safety limit value. The high consumption frequency of processed smoked sharks and stingrays correlated to the age, education, monthly income, number of family members, number of products purchased per week, and expenditures to buy products per week.

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Conflict of Interest. The authors declare that there is no conflict of interest.

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