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Minerals and fatty acids profile of armored catfish *Pterygoplichthys pardalis* from Ciliwung River, Indonesia

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Abstract. *Wijayanti F, Lisdaniyah A, Hasanah M, Elfidasari D. 2023. Minerals and fatty acids profile of armored catfish* Pterygoplichthys pardalis *from Ciliwung River, Indonesia. Nusantara Bioscience 15: 58-67.* Fish is an important food source in human consumption due to its minerals and fatty acids needed for various body functions. One fish widely eaten by the people around the Ciliwung River, Indonesia is the armored catfish, *Pterygoplichthys pardalis* (Castelnau 1855). It has great economic value and is easily obtained by the residents along this river. Due to its high protein content, *P. pardalis* is a potential source of animal protein for humans. The unavailability of information detailing the minerals content and fatty acids in *P. pardalis* from the Ciliwung River makes it necessary to conduct this research to analyze the fish's minerals content and fatty acid profile. Analyses of minerals, fatty acid contents, and fatty acid profiles were conducted using Atomic Absorption Spectrophotometry (AAS), socletation, and Gas Chromatography-Mass Spectrophotometer (GC-MS) methods, respectively. The mineral content of *P. pardalis* from the highest order was calcium, phosphorus, potassium, magnesium, sodium, iron, and zinc. Based on *P. pardalis* body size, large fish had the highest calcium concentration content, and the lowest calcium content was found in the medium fish. The fat content in this fish was very low (<1%), and the content of Saturated Fatty Acids (SFA) was greater than unsaturated ones. Furthermore, palmitate acid was the dominant fatty acid in the fish, while the biggest ratio of omega-6: omega-3 is fatty acids.

Keywords: AAS, FFA, GC-MS, Indonesia, pleco, *Pterygoplichthys pardalis*

INTRODUCTION

Minerals and fatty acids are important fish nutrients needed by humans. These minerals are inorganic substances the body needs due to their various benefits. Nutrients play an active role in the physiological and structural functions of the body, as well as in preventing nutritional deficiencies (Chen et al. 2018; Muscaritoli 2021; Witkamp 2021). Minerals contained in the body of fish include sodium, potassium, calcium, magnesium, phosphorus, selenium, iron, iodine, cobalt, and manganese (Prabhu et al. 2014; Eti et al. 2019). Sodium and potassium help in regulating osmotic pressure in the body. While, iron helps in transporting oxygen, calcium aids in protecting bone health, and iodine plays a role in controlling normal growth mechanisms, as well as physical and mental development (Prashanth et al. 2015; Goff 2018; Alagawany et al. 2021). Some factors influence the minerals found in fish, such as body size, feeding, species, sex, age, reproduction phase, habitat, and the quality of the fish's waters (Prabhu et al. 2014; Paul et al. 2018).

In addition to the minerals, fatty acids in fish are needed by humans for some essential functions (Pal et al. 2018; Chasanah et al. 2021). For example, the human body needs omega 3; Eicosa-Pentaenoic Acid (EPA); Docosa-Hexaenoic Acid (DHA): Omega 6; and arachidonic acid. These essential fatty acids help form cells, regulate the nervous system, strengthen the cardiovascular system, build the immune system, and help the body absorb nutrients (Citil et al. 2014; Kaur et al. 2014). In addition, these are important for the health of brain and eye functions, improve vision by increasing photoreceptors in the eye, as precursors of several hormones, repair wall tissue of nerve cells, act as anti-inflammatory compounds, and prevent muscle breakdown for bodybuilders (Glick and Fischer 2013; Njinkoue et al. 2016).

The widely eaten fish by the people living around the Ciliwung River, Indonesia is the armored catfish, *Pterygoplichthys pardalis* (Castelnau 1855), which is only one genus of pleco in the river (Elfidasari et al. 2016; Rosnaeni et al. 2017). This fish has been identified in the river since the 1980s as an invasive species originating from South America and could live in various aquatic locations, such as bodies and estuaries of rivers, lakes, and ponds. It also adapts easily to water polluted with waste; hence, it is often called janitor fish. According to Hadiaty (2011), *P. pardalis* dominated the Ciliwung River and caused a decline in the population and species of other freshwater fish present in this river. That could be due to the availability of phytoplankton in the river, serving as natural food in sufficient quantities for *P. pardalis*. In addition, *P. pardalis* has no predators or competitors in this river; therefore, the fish are available in the high population (Elfidasari et al. 2020c).

The fish is used as a raw material for making various processed food products such as dumplings, meatballs, *otak-otak* (grilled fish cakes), and crackers in the community. The use of *P. pardalis* as a food ingredient is due to its economic value, and people living along the Ciliwung River easily obtain this fish. *P. pardalis* is a good source of animal protein for humans due to its high protein content (Elfidasari et al. 2019).

Moreover, before this study, there is less data explained the minerals and fatty acids contents of *P. pardalis* from the Ciliwung River. Study in 2018 explained that smaller-sized *P. pardalis* has the highest protein and fat contents (50.0517% and 1.1261%) (Elfidasari et al. 2018). Therefore, the aim of this study was to analyze the mineral contents and fatty acid profiles of *P. pardalis* originating from the Ciliwung River, thus, obtaining information on the potential use of the fish as sources of minerals and fatty acids.

MATERIALS AND METHODS

Study area

The fish sampling locations were at two points along the Ciliwung River Basin, namely the Kalibata area (S1) with coordinates of S 06.25830°-E 106.86040° and the Cawang area (S2) with coordinates of S 06.28599°-E 106.84717° in Jakarta, Indonesia (Figure 1). These two sampling locations are areas with a high *P. pardalis* population of about 58 individuals/m^2 (Elfidasari et al. 2020b).

Procedures

Sampling and sample preparation

The purposive sampling technique was used in this study, which involved choosing a sampling location for fish collection with the assumption that most people consume the fish. Sampling was conducted by catching the fish through a seine net that spread in Ciliwung, and after fishes were caught, they were placed in a container filled with ice cubes to maintain their freshness during transportation. Then, the samples were taken to the laboratory to measure the total length and weight. Based on the body measurements of the fish taken from the Ciliwung River in Jakarta, groupings were conducted concerning the method proposed by Tisasari et al. (2016). A total of 60 *P. pardalis* were measured and grouped into three sizes, large (295-391 mm), medium (193-294 mm), and small (91-192 mm), as shown in Table 1. The fish samples were then dissected, and the flesh separated. Subsequently, the flesh was weighed, placed in a petri dish, and dried in an oven at 105°C for 24 hours. The dried samples were pulverized using mortars and a pestle until smooth. Then, about 1 gram of the powdered samples was subjected to mineral testing with two repetitions per fish size. Finally, *P. pardalis* was compared with the control reared in the Ornamental Fish Research and Aquaculture Center or *Balai Riset dan Budidaya Ikan Hias* (BRBIH), Depok, Indonesia. The fish control reared in an aquarium and fed with pellets.

Minerals content analysis

The mineral content analysis used the Atomic Absorption Spectrophotometry (AAS) method. Hence, the concentration of minerals in the material was calculated using the following formula

$$\text{Mineral concentration (mg/kg)} = \frac{Sample\ concentration\left(\frac{mg}{l}\right) x\ Sample\ volume\ (L)}{sample\ weight} x\ FP$$

Where:
Fp : Dilution factor

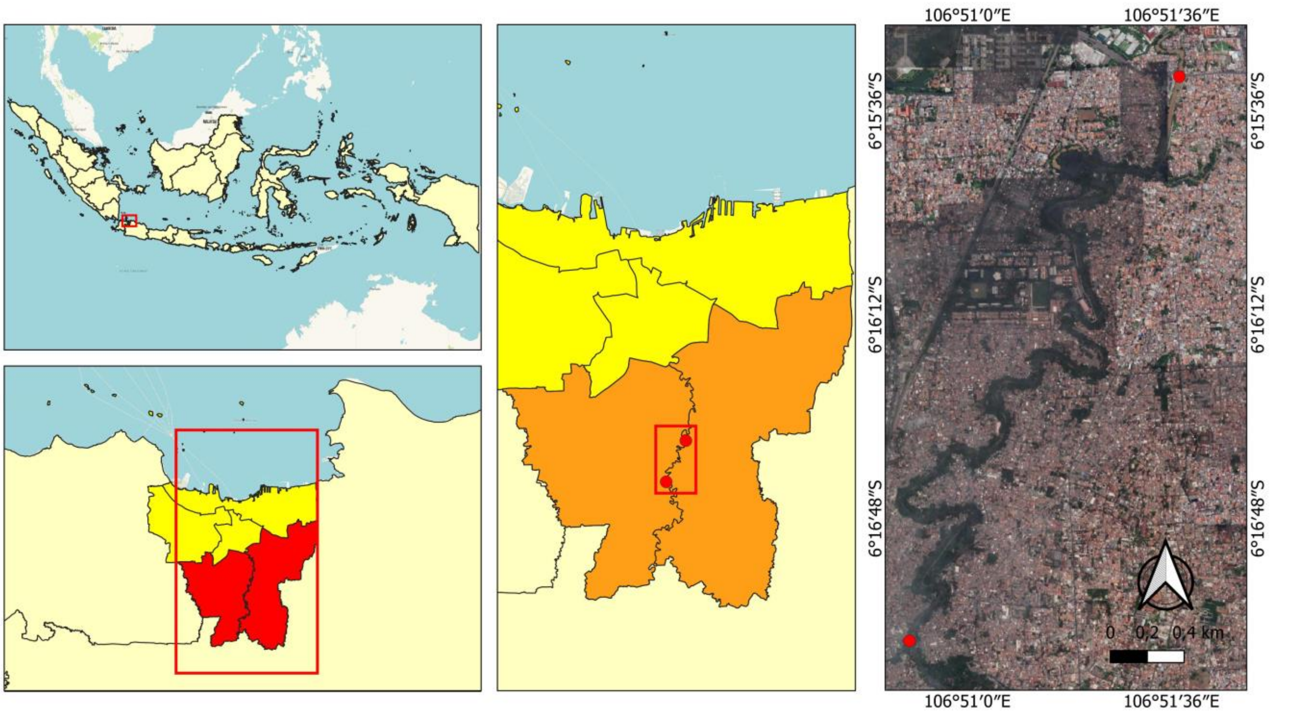

Figure 1. Map of sampling locations for armored catfish *Pterygoplichthys pardalis* in Jakarta, Indonesia

Table 1. Grouping of *Pterygoplichthys pardalis*' body size (Tisasari et al. 2016)

Body size category	Range of body length
Small	91-192 mm
Medium	193-294 mm
Large	295-391 mm

Analysis of fatty acids content

The fatty acid analysis was conducted using the socletation method and yield calculation. Fat content was calculated using the following formula:

$$\text{Fat concentration (\%)} = \frac{W3-W2}{W1} x\ 100\%$$

Where:
W1 : Sample weight (g)
W2 : Weight of an empty erlenmeyer (g)
W3 : Weight of erlenmeyer with fat (g)

Analysis of fatty acid profiles with Gas Chromatography-Mass Spectrophotometer (GC-MS)

This stage involved the extraction of fat from the samples. These were then subjected to the methylation process to form methyl esters. The resultant substances were injected into a chromatographic device (GC-MS) (AOAC 2005).

Analysis of Free Fatty Acids (FFA)

About 1 gram of the sample was placed in 20 mL of 96% alcohol in a 250 mL Erlenmeyer. These were shaken and titrated with 0.1 N KOH until a pink color appeared, which did not disappear in 15 seconds (BSN 1998). The FFA percentage was calculated using the following equation:

$$FFA\ (\%) = \frac{M \times A\ \times N}{10G}$$

Where:
A : Number of KOH titrations (mL)
N : Normality of KOH
G : Gram of sample
M : weight of fatty acid molecules

Data analysis

The data obtained is entered and grouped or tabulated into a table made under the aims and objectives of the research, then analyzed descriptively and elaborated in the form of charts.

RESULTS AND DISCUSSION

Mineral composition of *P. pardalis*

The highest concentration was calcium at 26,130.96 ppm in large fish, 21,546.44 ppm in medium fish, and 24,463.99 ppm in small fish. This was followed by phosphorus, potassium, sodium, magnesium, iron, zinc, and copper. The highest mineral content was found in the large fish compared with other sizes (Table 2).

The mineral content, such as calcium, magnesium, phosphorus, and iron, of *P. pardalis* from the Ciliwung River was higher than the control fish from the BRBIH pond (Table 3).

Minerals content of *P. pardalis* based on body size

Calcium and phosphorus are the most abundant minerals found in fish. The calcium content was in the range of 21,546.44-26,130.96 ppm (Table 4). Also, the highest calcium content of 26,130.96 ppm was found in the large fish, while the lowest concentration of 21,546.44 ppm was found in the medium fish.

In addition, the calcium content was higher than the phosphorus. The phosphorus content was 6,778.99-8,451.68 ppm, of which the highest content of 8,451.68 ppm was found in large fish, while the lowest concentration was 6,778.99 ppm, found in the small fish (Table 5).

The concentration of potassium in the fish was in the range of 2,942.07 - 4,480.16 ppm. The highest content was found in the small fish at 4,480.16 ppm, while the lowest was in the large fish at 2,942.07 ppm (Table 6).

The sodium content of the fish was in the range of 824-949.99 ppm. The highest concentration was found in the small fish, at 949.99 ppm, while the lowest was found in the medium fish, at 824 ppm (Table 7).

The magnesium concentration of *P. pardalis* was in the range of 748.53 - 913.19 ppm. The highest concentration was found in small fish at 913.19 ppm, while the lowest concentration of 748.53 ppm was found in large fish (Table 8).

The highest concentration of iron was found in the medium *P. pardalis* at 129.82 ppm, while the lowest was found in the large fish at 91.48 ppm (Table 9).

The highest zinc concentration was found in the large fish at 28.32 ppm, while the lowest was found in the small fish at 24.48 ppm (Table 10).

Fatty acids profile of *P. pardalis* from Ciliwung River

The fatty acid profiles for all the fish sizes and control using GCMS showed that the highest fatty acid content was found in the large fish at 84.97% and unidentified fatty acids at 15.03%. On the other hand, the lowest total identified fatty acids were found in medium fish at 62.86% and unidentified fatty acids at 37.14%. Also, the control fish had the second highest fatty acid content at 76.47% and unidentified fatty acids at 25.53% (Table 11).

Analysis of free fatty acids in *P. pardalis* from Ciliwung River

The analysis of free fatty acids from the *P. pardalis* oil sample showed that the highest percentage was found in large fish, while the lowest was in the control (Table 12).

Table 2. The concentration of mineral elements in *Pterygoplichthys pardalis* from the Ciliwung River, Indonesia based on the difference in size (ppm)

Element mineral	Mineral concentration in *P. pardalis* from Ciliwung River			
	Control	**Large fish**	**Medium fish**	**Small fish**
Ca	160.52 ± 0.30	26130.96 ± 902.72	2154.44 ± 670.37	24462.99 ± 2346.79
P	5176.02 ± 41.81	8451.68 ± 27.27	7320.75 ± 41.98	6778.99 ± 82.55
K	5258.86 ± 81.04	2942.07 ± 28.82	3450.95 ± 431.17	4480.16 ± 385.82
Mg	510.74 ± 3.40	748.53 ± 23.48	854.85 ± 11.93	913.19 ± 37.36
Na	1293.97 ± 5.13	828.04 ± 7.71	824.00 ± 38.44	949.99 ± 28.21
Fe	21.89 ± 2.51	91.48 ± 4.04	129.82 ± 2.74	94.86 ± 1.42
Zn	16.48 ± 0.69	28.32 ± 1.70	27.29 ± 0.17	24.48 ± 2.05

Table 3. Concentrations of Ca, P, Mg, and Fe minerals in *Pterygoplichthys pardalis* from Ciliwung River and BRBIH, Indonesia

Mineral	Mineral concentration (ppm)	
	***P. pardalis* from Ciliwung River**	**Control**
Ca	24463.99 ± 2346.79	160.52 ± 0.30
P	6778.99 ± 82.55	5176.02 ± 41.81
Mg	913.19 ± 37.36	510.74 ± 3.40
Fe	94.86 ± 1.42	21.89 ± 2.51

Table 4. The measurement of calcium mineral in *Pterygoplichthys pardalis* from the Ciliwung River, Indonesia

Nth-Repetition Measurement	The Ca content in *P. pardalis* (ppm)		
	Large fish	**Medium fish**	**Small fish**
1	25492.63	22020.45	22804.55
2	26769.28	21072.41	26123.42
Total	52261.92	43092.89	48927.98
Average	26130.96	21546.44	24463.99
Standard deviation	902.72	670.37	2346.79

Table 5. The measurement of phosphorus minerals in *Pterygoplichthys pardalis* from the Ciliwung River, Indonesia

Nth-repetition measurement	The P content in *P. pardalis* (ppm)		
	Large fish	**Medium fish**	**Small fish**
1	8470.97	6808.68	7257.42
2	8432.39	6749.30	7384.08
Total	16903.35	13557.98	14641.50
Average	8451.68	6778.99	7320.75
Standard deviation	27.27	670.37	2346.79

Table 6. The measurement of potassium mineral in *Pterygoplichthys pardalis* from the Ciliwung River, Indonesia

Nth-repetition measurement	The K content in *P. pardalis* (ppm)		
	Large fish	**Medium fish**	**Small fish**
1	8470.97	6808.68	4207.34
2	8432.39	6749.30	4752.98
Total	5884.14	6901.90	8960.33
Average	2942.10	3451.00	4480.20
Standard deviation	28.83	431.18	385.83

Table 7. The measurement of sodium minerals in *Pterygoplichthys pardalis* from the Ciliwung River, Indonesia

Nth-repetition measurement	The Na content in *P. pardalis* (ppm)		
	Large fish	**Medium fish**	**Small fish**
1	833.49	796.82	969.94
2	822.58	851.18	930.04
Total	1656.08	1648.00	1899.99
Average	828.04	828.04	949.99
Standard deviation	7.71	38.44	28.21

Table 8. The measurement of magnesium minerals in *Pterygoplichthys pardalis* from the Ciliwung River, Indonesia

Nth-repetition measurement	The Mg content in *P. pardalis* (ppm)		
	Large fish	**Medium fish**	**Small fish**
1	765.14	846.41	939.61
2	731.93	863.29	886.87
Total	1497.07	1709.71	1826.39
Average	748.54	854.86	913.19
Standard deviation	23.48	11.93	37.36

Table 9. The measurement of iron minerals in *Pterygoplichthys pardalis* from the Ciliwung River, Indonesia

Nth-Repetition Measurement	The Fe content in *P. pardalis* (ppm)		
	Large fish	**Medium fish**	**Small fish**
1	94.34	127.87	93.84
2	88.63	131.76	95.87
Total	182.98	259.64	189.72
Average	91.48	129.82	94.86
Standard deviation	4.04	2.74	1.42

Table 10. The measurement of zinc minerals in *Pterygoplichthys pardalis* from the Ciliwung River, Indonesia

Nth-repetition measurement	The Zn content in *P. pardalis* (ppm)		
	Large fish	**Medium fish**	**Small fish**
1	29,53	27,17	23,03
2	27.12	27.42	25.94
Total	56.65	54.58	48.96
Average	28.32	27.29	24.48
Standard deviation	1.70	0.17	2.05

Table 11. Fatty acid profile of *Pterygoplichthys pardalis*

Fatty acid	Small *P. pardalis* (%) n = 10	Medium *P. pardalis* (%) n = 10	Large *P. pardalis* (%) n = 10	Control *P. pardalis* (%) n=2
Saturated Fatty Acids (SFA)				
Lauric Acid (C12: 0)	0.58	0.51	0.45	0.19
Tridecanoic Acid (C13: 0)	-	-	-	0.11
Myristic Acid (C14: 0)	0.43	0.52	-	0.32
Pentadecanoic Acid (C15: 0)	2.54	2.58	1.89	2.76
Palmitic Acid (C16: 0)	14.27	29.73	24.95	27.75
Heptadecanoic Acid (C17: 0)	-	1.00	0.72	-
Stearic Acid (C18: 0)	13.52	10.10	13.37	-
Behenic Acid (C22: 0)	-	-	-	10.27
Trichosanoic Acid (C23: 0)	-	-	-	0.34
Lignoceric Acid (C24: 0)	-	-	0.66	0.67
Total Saturated Fatty Acids	31.34	44.44	42.04	42.41
Mono Unsaturated Fatty Acids (MUFA)				
Palmitoleic Acid (C16: 0)	6.48	8.61	3.49	1.09
Oleic Acid (C18: 1n9c)	20.69	-	22.87	21.15
Glyceric Acid (C22: 1n9)	2.30	-	2.34	0.54
Nervonic Acid (C24: 1)	-	1.26	-	-
Total Mono Unsaturated Fatty Acids	29.47	9.87	28.70	22.78
Poly Unsaturated Fatty Acids (PUFA)				
Linoleic Acid (C18: 2n6c)	6.83	5.12	7.78	5.42
γ-Linolenic (C18: 3n6)	1.21	1.34	-	-
Linolenic Acid (C18: 3n3)	1.71	1.04	1.49	0.75
Arachidonic Acid (C20: 4n6)	3.42	-	3.22	2.00
EPA (C20: 5n3)	0.89	0.32	0.70	1.11
DHA (C22: 6n3)	1.29	0.73	1.04	2.00
Total Poly Unsaturated Fatty Acids	15.35	8.55	14.23	11.28
Total Fatty Acids	76.16	62.86	84.97	76.47
Total Unidentified Fatty Acid	23.84	37.14	15.03	23.53
Ω6/Ω3	2.94	3.09	3.40	1.92

Table 12. Percentage of free fatty acids of *Pterygoplichthys pardalis* oil

	Body size			
	Large (%) n=10	**Medium (%) n=10**	**Small (%) n=10**	**Control (%) n=2**
	7.15	3.47	5.23	3.73
	1.92	5.13	3.70	3.70
$\bar{x}$	4.53±3.69	4.30±1.17	4.46±1.08	3.71±0.02

Discussion

The mineral content in P. pardalis *from the Ciliwung River*

Variations in the mineral composition of fish could occur due to seasonal and biological differences, such as species, size, dark/white muscle, age, sex, sexual maturity, area of catch, processing method, food source, as well as environmental conditions such as water chemistry, salinity, temperature and contamination (Nurmadia et al. 2013; Debnath et al. 2014)

Based on this study, calcium was the highest mineral content found in the fish (Figure 2). This was suspected to be due to the calcium derived from food sources containing a lot of clam shells and crustaceans on the edge of the Ciliwung River. Also, high calcium levels are only found in the bodies of fish from the sea. Therefore, marine fish could absorb more calcium from their environment, unlike freshwater fish requiring higher calcium content in their feed (Lilly et al. 2017; Monhaty et al. 2017; Islam et al. 2018). The analysis of calcium in mackerel fish (*Scomber scombrus*) and cork fish (*Chana striata*) showed the presence of high calcium content in fish living in water with high salinity. The mackerel habitat is in seawater with a fairly high salinity of 32°/₀₀. Therefore, it has a fairly high calcium content of 29,197.66 mg / 100 g ± 17.77 (Susanti et al. 2016).

However, *P. pardalis* in this study, which is a freshwater fish, has a fairly high calcium content. Also, it is suspected that this fish absorbed more calcium than phosphorus. According to Mogobe et al. (2015), phosphorus absorption could be hindered due to increased calcium in its feed. The feed of *P. pardalis* is a fragment of plants, algae, and detritus. In addition, the clam shells and crustaceans, such as crabs that have died or gone through molting, contribute calcium to water (Samat et al. 2016; Monhaty et al. 2017; Elfidasari et al. 2020c; Ismail et al. 2022).

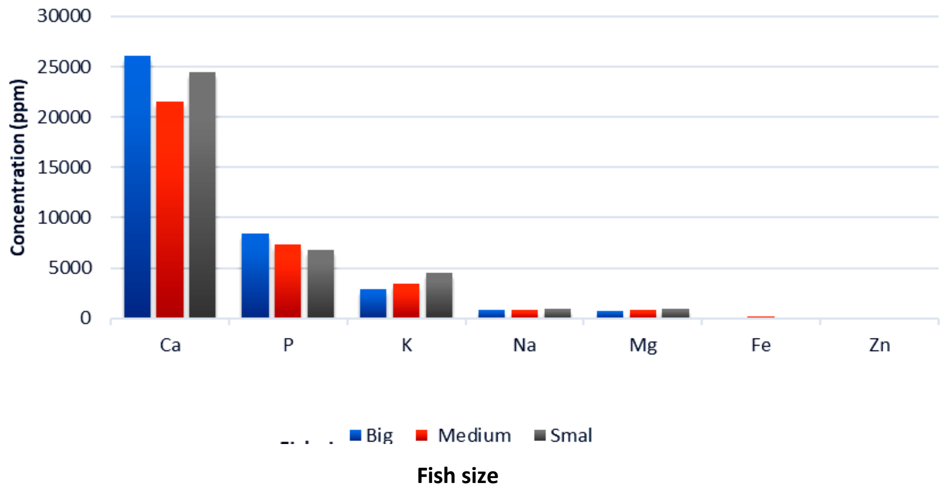

Figure 2. The concentration of minerals in *Pterygoplichthys pardalis* from the Ciliwung River, Indonesia

The high calcium levels in *P. pardalis* could also result from its gills containing chloride secretory cells, also found in other fish. Mitochondrial-rich "chloride" cells in seawater and freshwater fish are the sites for the absorption of Ca^{2+}. These cells in fish which live in a low-calcium freshwater environment adapt by increasing the number or density of cells and increasing cell size to facilitate the optimal transportation of Ca^{2+} (Guh et al. 2015; Leguen et al. 2015; Adam et al. 2019). In addition, the calcium contained in fish helps form bones and scales. According to Ebenstein et al. (2015), the inside of *P. pardalis* scales has a sturdy texture and comprises 58% carbon, 14% oxygen, 7% phosphorus, and 20% calcium.

The second highest mineral content of this fish was phosphorus. High phosphorus levels found in its flesh could result from factors such as food sources. Previous research showed the presence of Loricariidae in the digestive system of fish, a group of algae-eating freshwater fish, consuming several algae living on the bottom surface, such as *Bacillariophyta* algae (Samat et al. 2016; Monhaty et al. 2017; Elfidasari et al. 2020c). The more the presence of phytoplankton in the waters, the higher its phosphorus content. Hence, *P. pardalis* absorbs most of the nitrogen and phosphorus into its body's protective system. In addition, water's phosphorus content affects phytoplankton abundance (Ahmed et al. 2017; Wisha et al. 2018; Marsela et al. 2021; Nindarwi et al. 2021).

Potassium and sodium were the next abundant minerals in the fish. The sodium content of *P. pardalis* in this study was lower than its potassium content. There is a relationship between these two minerals; the higher the sodium, the lower the potassium concentration, and vice versa. According to Debnath et al. (2014) and Ahmed et al. (2017), this is related to the function of both minerals in maintaining the balance of osmotic pressure in the fish's body.

The magnesium content in *P. pardalis* was quite low. This is aligned with previous research, which showed the relatively low magnesium content in fish compared with land animals, and a concentration of ± 65% found in the fish bones (Monhaty et al. 2017; Romharsha and Sarojnalini 2018; Kiliç et al. 2019).

Then, iron content was next abundant. The iron content of *P. pardalis* was quite high compared with other freshwater fish, such as Nile tilapia (*Oreochronis niloticus*), with an iron content of around 0.835-2756 mg (Ramlah et al. 2016). High iron levels in *P. pardalis* flesh could result from the Ciliwung River waters environment. The iron levels in this river exceeded the specified quality standard, set at 2 mg/L. This could be due to the domestic waste produced by the residents on the river's edge (Vincent-Akpu and Obi 2014; Gemaque et al. 2019; Elfidasari et al. 2020a).

Zinc was the lowest mineral content in the samples of *P. pardalis* fish. This is in line with some previous studies which showed the relatively low presence of zinc mineral in several freshwater fish; 0.44 mg in white snapper, 0.45 mg in tilapia, and 0.36 mg in cork fish. That is because zinc is an essential micromineral that catalyzes the work of enzymes. In addition, it plays a role in building the structure of proteins and cell membranes and acts as a transcription factor in the process of gene expression (Sarma et al. 2014; Monhaty et al. 2016; Eti et al. 2019; Paul et al. 2019).

The difference in mineral content in *P. pardalis* from the Ciliwung River and the ones reared in BRBIH (control) could be due to the differences in habitat. The control fish were reared in a pond with the water coming from an underground well at BRBIH, Depok. The water pumping process was channeled to the settling water tank to deposit mud, dirt, parasites, and undesirable organisms, so they do not enter the rearing pond. The hardness of water could also result in the differences in mineral concentrations of fish from both habitats. The water used in rearing the control fish in BRBIH was cleaner than the one from the Ciliwung River, contaminated with various household wastes like soap. Hard water's calcium, magnesium, carbonate, and sulfate contents are usually high (Sengupta 2013; Reksten et al. 2020). Furthermore, differences in mineral content could result from food sources, one of which is the phosphorus mineral. According to Elfidasari et al. (2020c), Ciliwung waters have abundant phytoplankton, with the discovery of Bacillariophyta algae (82.03%) in the digestive tract of *P. pardalis*. However, the phosphorus concentration of the control *P. pardalis* was low. That could be due to the clean condition of the water in the pond.

The fatty acid content of P. pardalis

The fatty acids obtained from *P. pardalis* through the GC-MS process were classified into: Saturated Fatty Acid (SFA); Mono-Unsaturated Fatty Acid (MUFA); And Poly-Unsaturated Fatty Acid (PUFA). Overall, fish's various sizes contained more SFA than MUFA and PUFA; freshwater fish generally contain more C16 and C18 carbon chain fatty acids, which are included in SFA and MUFA (Kaur et al. 2014; Kandyliari et al. 2020).

In general, SFA is found to be higher in freshwater fish. The results of the study conducted by de Morais et al. (2016) on *P. pardalis* from Brazil showed the presence of palmitic acid (35.71%) as the dominant fatty acid and oleic acid (24.87%) in the fish. According to Bavi and Khodadadi (2017), palmitic and oleic acids are the dominant fatty acids in freshwater fish due to their function as energy ingredients. Therefore, palmitic acid is high in the flesh and liver of freshwater and seawater fish (Babatunde et al. 2020).

Differences in fatty acid content in freshwater fish are influenced by body size, age, sex, habitat, type of food (herbivores, carnivores, omnivores), and other abiotic factors affecting the overall fat content in fish. The high palmitic acid content is a general characteristic of the fatty acid profile of freshwater fish with more saturated fatty acid than unsaturated ones. However, Rodrigues et al. (2017) reported that oleic acid is sometimes the dominant fatty acid in freshwater fish rather than palmitic acid. Furthermore, to maintain the balance of phospholipid membranes in human body cells, there is a need for high levels of palmitic acid, as an SFA, present in freshwater fish flesh, as a daily food portion in the form of omega-3 and omega-6 fatty acids (Carta et al. 2017).

The omega-6 fatty acids (Linoleic Acid, γ-Linolenic acid, and arachidonic acid) from the GC-MS analysis of *P. pardalis* were relatively small. According to Powell et al. (2017), omega-6 fatty acids such as Arachidonic Acid are needed by juvenile fish as an important element during growth and in the immune system. This arachidonic acid also plays an important role in pigmentation and cell growth. Based on previous studies, juvenile *Clarias* given omega-6 fatty acids-rich feed showed a better growth rate (Abaho et al. 2016; Enyidi et al. 2017; Effiong and Yaro 2020). All of these essential fatty acids play a vital role in regulating the osmotic pressure in the fish's body and also in the metabolism process.

The large control of *P. pardalis* contained higher EPA and DHA than large fish from the Ciliwung River, as shown in Table 11. It shows that commercial feed for cultured fish contains high levels of omega-3. That is aligned with the study of Rodriguez-Barreto et al. (2014), which showed that *Seriola dumerili* fish cultivated with commercial feed contained higher EPA in the flesh than wild fish. It also showed that commercial feeds of cultivated fish are generally made from marine ingredients, hence, rich in essential fatty acids.

The ratio between omega-6 and omega-3 fatty acids in the control fish was the smallest, at 1.92, compared with the remaining group. Generally, a greater ratio was produced by cultured fish, but in this case, the opposite results are observed. Therefore, it is assumed that cultured fish given commercial feed contain more complex nutrition than *P. pardalis* from the Ciliwung River. In addition, cultured fish tend to eat feed given regularly (Powell et al. 2017).

The biggest ratio between omega-6 and omega-3 fatty acids was shown by the large *P. pardalis* samples, at 3.4: 1. However, the smallest ratio was shown by the control fish at 1.92: 1. The results of these comparisons are still in the range of the omega-6: omega-3 ratio recommended by the UK Ministry of Health, set at 0.45-4.0 (Sheppard and Cheatham 2018; Shahidi and Ambigaipalan 2018; Djuricic and Calder 2021). The recommended dietary intake ratio needed to reduce obesity in adult humans and prevent coronary heart disease is a balanced ratio of 1 to 2:1 (Eilat-Adar et al. 2013; Liu et al. 2017). Linoleic acid is an omega-6 fatty acid, which after being consumed, parts of it are converted to γ-Linolenic acid and then to Arachidonic Acid (Simopoulos 2016). Therefore, reducing the intake of Linoleic Acid reduces the Arachidonic Acid levels in tissues, which are substrates needed for synthesizing molecules that cause inflammation if taken excessively (Jandacek 2017).

Free fatty acids in P. pardalis

Free fatty acids are an early indicator of bad oil. The content of free fatty acids, even though little, also results in bad taste. Factors responsible for forming free fatty acids include air humidity, light, high temperatures, and destructive bacteria, which cause rancidity (Handayani et al. 2013).

The large *P. pardalis* showed the highest percentage of free fatty acids, while the control fish showed the smallest. Fish cultured with organic food always show fewer free fatty acids. This is because organic food does not change the activity of lipolytic enzymes, which hydrolyze fat. Feed composition plays a very influential role in the fatty acid content of fish. Cultured fish usually eat more uniform feed and fewer microalgae, an important fat source (Balev et al. 2017; Hossain et al. 2018). In addition, they were fed with pellets that are easy to digest for fish. Generally, pellets are made from a mixture of fish meal, plant protein, vitamins, and minerals.

The medium fish showed a small percentage of free fatty acids. This is because fish have the lowest fat content. The free fatty acids content of fish with higher fat content is usually higher than those with lower fat. High levels of free fatty acids in large fish samples were also influenced by the total fatty acids identified by GC-MS (Table 3). This study showed that large fish samples had the largest total fatty acid content, at 84.97%. That contributed to the high percentage of free fatty acids in the large fish samples. The greater the fat content in the fish, the higher the percentage of free fatty acids (Arai et al. 2015; Rodrigues et al. 2017; Tramice et al. 2021).

The content of free fatty acids in the fish oil sample does not cause nutrient loss but only affects the taste and aroma. The content of free fatty acids also does not affect essential fatty acids, but the amount of fatty acids in the sample determines the percentage of free fatty acids, either high or low (Rodrigues et al. 2017). However, analysis of free fatty acid content is important to determine the quality of the oil (Citil et al. 2014; Suseno et al. 2014; Islam et al. 2018; Reksten et al. 2020). The mineral content of *P. pardalis* from the highest order was calcium, phosphorus, potassium, magnesium, sodium, iron, and zinc. Based on *P. pardalis* body size, large fish had the highest calcium concentration content, and the lowest calcium content was found in the medium fish. The fat content in this fish was very low (<1%), and the content of saturated fatty acids (SFA) was greater than unsaturated ones. Furthermore, palmitate acid was the dominant fatty acid in the fish, while the biggest ratio of omega-6: omega-3 was fatty acids.

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