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Relationship Between Mudskipper (Periophthalmus spp.) Protein Content and Environmental Factors in CMC Tiga Warna Mangrove

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ABSTRACT

Mudskipper is a brackish water fish with high protein content and potential as an alternative source of protein. One of its natural habitats is the Clungup Mangrove Conservation (CMC) Tiga Warna area. Therefore, this study aimed to analyze the protein content of mudskipper and examine its relationship with environmental factors and sediment organic carbon in the mangrove ecosystem. Samples of mudskipper (Periophthalmus gracilis and Periophthalmus modestus), environmental parameters, and organic carbon were collected using purposive sampling at five stations. Protein content was determined using the Lowry method, while organic carbon was analyzed using the Walkley-Black method. Data were analyzed using ANOVA, and relationships among variables were assessed using Principal Component Analysis (PCA). The results showed that protein content ranged from 11.55% to 18.55%. The highest pH was recorded at station 2 (7.437), temperature at station 1 (30.667°C), dissolved oxygen at station 3 (4.400 mg/L), salinity and total dissolved solids at station 5 (41 ppt; 27,833,333.33 ppm), and organic carbon at station 5 (1.995%). PCA indicated that protein content was positively associated with organic carbon and pH, but not with temperature, salinity, dissolved oxygen, or total dissolved solids. Furthermore, protein content did not significantly differ between species or sampling stations.

INTRODUCTION

Mudskippers are amphibious brackish-water fish that inhabit mangrove ecosystems and are capable of surviving on land for extended periods. Mudskippers belong to the subfamily Oxudercinae within the family Gobiidae, which comprises diverse species adapted to intertidal environments. In Indonesia, mudskippers are represented by several genera, including *Periophthalmus*, *Boleophthalmus*, *Periophthalmodon*, *Scartelaos*, and *Oxuderces* (Hidayat, 2020).

The protein content in mudskippers is influenced by both external and internal factors. External factors arise from environmental conditions, including habitat characteristics and food availability (Duggal *et al*., 2020). Mudskippers can survive in a pH range of 6–9 (Pramunandar *et al*., 2023). Salinity in brackish water typically ranges between 0.5 and 30 ppt (Purwaningtyas, 2020), and mudskippers are capable of tolerating salinity levels between 17 and 34.4 ppt (Irawan *et al*., 2020). According to Rajmohan *et al* (2021), The high salinity and ions in environment is affected by evaporation, irrigation return flow, and saline sources. Pramunandar *et al* (2023) reported that mudskippers thrive in water temperatures ranging from 28–38°C. Dissolved oxygen is a key indicator of biological activity, such as respiration, metabolism, growth, and reproduction, in aquatic organisms. Oxygen concentration influences the number of erythrocytes in fish (Maimunah *et al.,* 2023).

Another external factor is total dissolved solids (TDS). According to Muthmainnah & Mulyono (2022), total dissolved solids indicate the amount of dissolved material in water, which is generally in the form of solids that are slightly smaller in size when compared to suspended solids. TDS levels affect aquatic organisms inhabiting the ecosystem (Argianti *et al*., 2021). Food availability can be measured using carbon content analysis of organic substrates. Carbon, in combination with other elements, forms more complex compounds that serve as food sources for aquatic organisms, including mudskippers. According to Prihandana *et al* (2021), organic carbon content is categorized as follows: <1% (very low), 1–2% (low), 2–3% (moderate), 3–4% (high), and >5% (very high).

Internal factors include age, species, sex, seasonal variations, and feeding behavior (Kanejiya *et al*., 2017). Juvenile mudskippers typically have underdeveloped organs, while adult individuals possess fully developed organs. Such differences in organ maturity can influence the formation of biochemical compositions, including protein levels, in mudskippers (Graves, 1970). Both categories of factors may also influence the expression of Heat Shock Proteins (HSPs) in mudskippers. HSP expression typically increases in response to environmental and physiological stressors, such as elevated temperatures, heavy metal exposure, oxidative stress, and pathogenic infections (Jeyachandran *et al*., 2023).

Differences in habitat, species, and sex can affect the protein content of mudskippers. Duggal et al. (2020) reported that male *Boleophthalmus dussumeri* had a higher protein content than females. Similarly, Kanejiya et al. (2017) found that *Boleophthalmus dussumeri* contained 1.5 ± 0.47 mg/100 mg of protein, *Periophthalmus waltoni* contained 1.4 ± 0.45 mg/100 mg, and *Scartelaos histophorus* contained 0.45 ± 0.4 mg/100 mg. Furthermore, Duggal et al. (2020) showed that *Boleophthalmus dussumeri* had a protein content of 28.22 ± 8.6%, which is higher than that of *Euthynnus affinis* (20.9 ± 0.1%) and *Auxis rochei* (21.0%).

One of the natural habitats of mudskippers in Indonesia is the mangrove forest of CMC Tiga Warna, Malang regency, East Java. This area is a conservation zone, where most mangrove vegetation has been established through reforestation programs, contributing to ecosystem stability and organic matter dynamics. The CMC Tiga Warna mangrove forest is also designated as an ecotourism site. Mu'tashim and Indahsari (2021) describe ecotourism as a form of conservation activity that integrates environmental preservation with tourism. Previous research by Nur (2015), identified three mudskipper species in this area, namely *Periophthalmus gracilis*, *Periophthalmus modestus*, and *Periophthalmus waltoni*. However, the study did not examine the nutritional composition of mudskippers. Therefore, this study was conducted to address this gap by: (1) analyzing the protein content of mudskipper meat, and (2) evaluating the influence of environmental factors on protein levels. This research provides important insights into the nutritional potential and ecological interactions of mudskippers in mangrove ecosystems.

METHOD

Materials

The instruments used in this study included raffia rope, plastic clips, surgical tools, thermometer, vortex mixer (Dlab 2012 Plus), plastic containers, analytical balance (Shimadzu ATX224), test tubes (Iwaki), volumetric flasks (Pyrex 10 mL and 100 mL), cuvettes, micropipettes 20–200 µL (Bio-Rad) with tips, UV-Vis spectrophotometer (WPA Biowave II), freezer (Thermo Scientific), labeling paper, Avenza Maps, graph paper, pH meter, portable refractometer, DO meter, and TDS meter (Water Quality Meter EZ 9908/686).

The reagents included Folin-Ciocalteu, reagent C (2% sodium carbonate in 100 mL 0.1 N sodium hydroxide, with the addition of 1 mL of 1% copper (II) sulfate and 2% sodium tartrate), Tris-HCl, Bovine Serum Albumin (BSA), ammonium sulfate, water and soil samples from the mudskipper habitat, and adult male mudskipper specimens.

Sampling Procedure

Adult male mudskippers were collected using purposive sampling from five stations in the CMC Tiga Warna mangrove forest: estuary, Clungup River, abandoned pond, educational mangrove area, and rehabilitated mangrove area. These five stations were selected due to their differing environmental characteristics. Samples were collected within a 100 meter radius from the central point of each station. The geographic coordinates of the sampling stations are presented in Table 1.

Table 1. Conditions and geographical coordinates of observation stations

Station	Geographic Condition	Coordinate
1	Estuary	8.437635 SL and 112.667113 EL
2	Clungup river	8.435547 SL and 112.666797 EL
3	Former fishpond area	8.435436 SL and 112.668806 EL
4	Mangrove planting education area	8.436873 SL and 112.669529 EL
5	Rehabilitation mangrove planting area	8.436857 SL and 112.670664 EL

Note: SL= South Latitude, EL= East Longitude

Sampling was conducted during low tide in April–May 2024. At each station, three individuals of each mudskipper species were collected to improve data accuracy (Hidayat *et al*., 2022).

Identification of Mudskipper Species

Mudskipper species were identified based on morphological characteristics following Nur (2015), Identification of Species and Community Structure of Mudskippers (Gobiidae) in the Mangroves of Clungup Beach, Tambakrejo Village, Malang Regency. Species identification was carried out on-site at the CMC Tiga Warna mangrove forest.

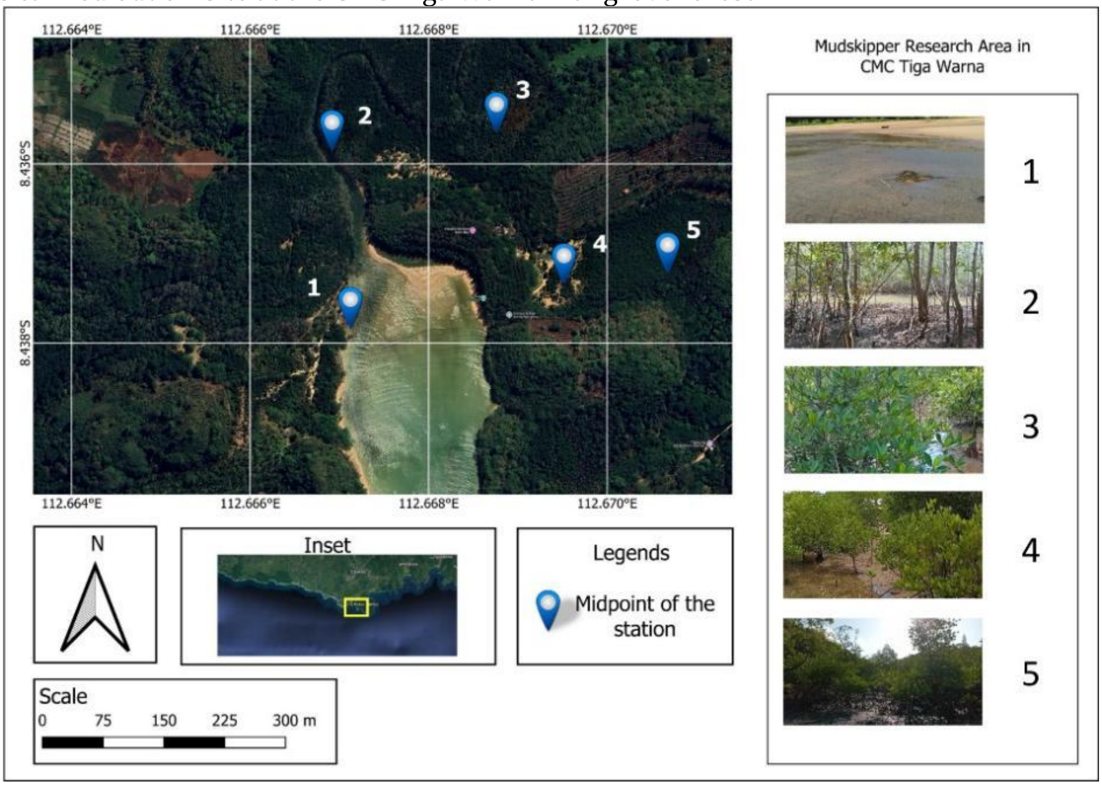

Figure 1. Sampling Station Point (QGIS 3.12.)
Source: Personal documentation (2024)

Protein Extraction

Muscle tissue samples were taken from the trunk and caudal regions (0.1 g in total). Samples were homogenized with Tris-EDTA buffer (0.5 M Tris, pH 8.3, and 1 mM EDTA) at a ratio of 1:3. Homogenates were centrifuged at 10,000 g for 10 minutes at 4°C. The resulting supernatants were collected and stored at −20°C (Hidayaturrahmah *et al.*, 2019).

Protein Precipitation

Protein purification was performed using ammonium sulfate precipitation (Nurhayati, 2013). A volume of 180 µL of ammonium sulfate was added to the protein extract to reach 60% saturation at 0°C, followed by incubation for 30 minutes. Samples were centrifuged at 15,000 g for 15 minutes at 4°C. The pellet was resuspended, and an additional 30 µL of ammonium sulfate was added to reach 70% saturation. After incubation for 30 minutes at 0°C, samples were centrifuged again under the same conditions. The final pellet was dissolved in 300 µL of 10 mM Tris-HCl buffer (pH 8) and stored at −20°C until Lowry protein assay was conducted (Hidayaturrahmah *et al.*, 2019).

Protein Quantification (Lowry Assay) and Standard Curve

Protein precipitates (100 µL) were diluted in 10 mL distilled water using a volumetric flask. From this, 1 mL of the diluted sample was mixed with 8 mL Lowry reagent C and incubated for 10 minutes. Subsequently, 1 mL Folin-Ciocalteu reagent was added, followed by distilled water up to the mark, mixed thoroughly, and left for 20 minutes.

The standard curve was prepared by dissolving 50 mg of Bovine Serum Albumin (BSA) in 50 mL distilled water to obtain a stock solution. Serial dilutions were prepared, and absorbance readings were obtained using a UV-Vis spectrophotometer. Protein concentration was calculated using the following equation (Botutihe, 2016):

$$\text{Protein Content (\%)} = \frac{\text{C. fp. V extract}}{\text{Weight sample (gr)}}$$

Protein Content (%)	=	(C.fp.V extract)/(Weight Sample (gr))
C	=	Protein Consentration in extract (mg/mL)
fp	=	Dilution factor
V extract	=	Extract volume (mL)
Sample Weight	=	Sample Weight (gram)

Soil Sample Preparation

Soil samples collected from mudskipper habitats were air-dried indoors for two weeks, sieved using a 0.5 mm mesh, and stored in plastic clips.

Organic Carbon Determination (Walkley-Black Method) and Standard Curve

Dried soil samples (<0.5 mm) weighing 0.5 g were placed in a 50 mL volumetric flask. Then, 5 mL of 1 N $K_2Cr_2O_7$ was added, followed by shaking. Next, 7.5 mL concentrated H_2SO_4 was added, shaken, and left for 30 minutes. Distilled water was added up to the mark, and samples were left overnight.

A standard curve was prepared using 250 ppm glucose dissolved in a 50 mL volumetric flask. Absorbance readings were obtained using a UV-Vis spectrophotometer, and organic carbon content was calculated as follows (Sulaeman *et al.,* 2015)

$$\text{C Organic \%} = \frac{\text{ppm Curve . ml extract}}{\dfrac{1000}{\text{100. mg sample. fk}}}$$

C Organic (%)	=	(ppm Curve . ml extract)/(1000/(100.mg sample.fk))
Ppm Curve	=	The sample concentration obtained from the relationship between the concentrations of the standard series after blank correction
Correction factor	=	100/(100- Water Content)
ml extract	=	The amount of protein extract from the sample (ml)
mg sample	=	weight of the sample used

Data Analysis

Comparisons of protein content and environmental factors were analyzed using ANOVA when data met homogeneity assumptions. The Brown-Forsythe test was applied for non-homogeneous data as heteroscedastic alternative to the ANOVA F test. If significant differences were observed ($p < 0.05$), post hoc tests such as DMRT or Games-Howell were performed. Statistical analyses (ANOVA, Brown-Forsythe, DMRT, and Games-Howell) were conducted using IBM SPSS 25. The relationships between environmental factors, organic carbon content, and mudskipper protein levels were further analyzed using Principal Component Analysis (PCA) with PAST version 4.03.

RESULT AND DISCUSSION

Protein Content of *Periophthalmus gracilis* and *Periophthalmus modestus* in the Mangrove Forest of CMC Tiga Warna Using the Lowry Method

The highest protein content was observed in *Periophthalmus modestus* at Station 3 (18.55%), while the lowest was found in *Periophthalmus gracilis* at Station 2 (11.55%) (Table 2). However, statistical analysis using ANOVA indicated no significant difference in protein content between *Periophthalmus gracilis* and *Periophthalmus modestus* ($p > 0.05$). Therefore, no post-hoc test was conducted for protein analysis. These findings contradict Kanejiya *et al.* (2017), who reported that protein content in mudskippers varies among species.

Table 2. Protein content (%) of mudskippers in the mangrove forest of CMC Tiga Warna measured using the Lowry method.

Place	Protein Content (%)		
	Periophthalmus gracilis	*Periophthalmus modestus*	Average±SD
Station 1	15,48	15,08	15,280±4,461
Station 2	11,55	13,55	12,547±1,468
Station 3	14,88	18,55	16,713±2,866
Station 4	13,81	16,48	15,147±2,386
Station 5	16,21	16,75	16,480±1,859
Average ±SD	14,387±3,22	16,080±2,622	

Source: Processed data (2024)
Note: SD= Standard Deviation

The similarity in protein content between *Periophthalmus. gracilis* and *Periophthalmus modestus* is likely due to both species belonging to the genus *Periophthalmus*. Members of this genus share similar diets, primarily crustaceans and zooplankton (Gosal *et al.,* 2013). They also spend approximately 90% of their lives on land (Hidayat, 2020). For this reason, it is suggested that their protein content is relatively comparable.

Protein content across stations ranged from 11.55% to 18.55%; however, no significant differences were observed. This result may be attributed to the high adaptive capacity of mudskippers. According to Polgar and Lim (2011), *P. modestus* may possess a similar adaptation to *Periophthalmodon schlosseri*, namely the ability to excrete toxic compounds such as ammonia through detoxification via alanine synthesis, which simultaneously provides energy for terrestrial locomotion. Additionally, Davenport (2001) reported that mudskippers reduce protein catabolism by accumulating trimethylamine oxide (TMAO) during terrestrial activity. TMAO is known to protect marine organisms from adverse effects of temperature, salinity, urea concentration, and high hydrostatic pressure (Velazquez *et al.*, 2016).

Relationship Between Environmental Factors, Organic Carbon, and Protein Content in Mudskippers

Station 2 exhibited the highest pH (7.437), whereas Station 5 had the lowest pH (7,213) (Table 3). ANOVA results showed significant differences in pH ($p < 0.05$) and post-hoc DMRT tests were performed. All stations have alkaline water because it has a pH greater than 7.

Variations in pH are influenced by oxidation processes, rainfall, and terrestrial inputs (Patty & Akbar, 2018). Mudskippers thrive within a pH range of 6–9 (Pramunandar *et al*., 2023). Deviations from this range may hinder survival and affect protein metabolism for mudskipper.

Table 3. pH, temperature, DO, TDS, salinity, and organic carbon levels in the mangrove forest of CMC Tiga Warna.

Place	Measurement results±Standard Deviation					
	pH	Temperatur (^{o}C)	DO (mg/L)	TDS (ppm)	Salinity (ppt)	Carbon (%)
Station 1	7,430 ±0,070[b]	30,667 ±0,351[d]	4,333 ±0,058[a]	4379,667 ±631,432[a]	29,333 ±0,577[b]	0,385 ±0,051[a]
Station 2	7,437 ±0,080[b]	29,033 ±0,153[b]	4,267 ±0,208[a]	1422,667 ±66,861[b]	28,667 ±0,577[ab]	1,298 ±0,195[b]
Station 3	7,340 ±0,046[b]	27,400 ±0,265[a]	4,400 ±0,346[a]	7354,000 ±253,750[c]	28±0[a]	1,708 ±0,386[c]
Station 4	7,407 ±0,015[b]	30,000 ±0,173[c]	3,333 ±0,115[b]	$27{,}333 \times 10^{6}$ ±288675,135[d]	36,333 ±0,577[c]	0,376 ±0,045[a]
Station 5	7,213 ±0,091[a]	28,967 ±0,306[b]	4,167 ±0,153[a]	$27{,}833 \times 10^{6}$ ±288675,135[d]	41±1[d]	1,997 ±0,186[c]

Source: Processed data (2024)

Station 1 recorded the highest average water temperature (30.67 °C), while Station 5 had the lowest (28.97 °C). Significant differences were detected ($p < 0.05$), confirmed by DMRT tests. The elevated temperature at Station 1 is attributable to its estuarine location with sparse mangrove cover, allowing direct sunlight exposure, whereas Station 5 is shaded by dense mangroves, reducing solar penetration. Extreme temperature fluctuations disrupt circulation and stratification patterns of aquatic biota (Amalia & Budijastuti, 2022).

Station 3 exhibited the highest dissolved oxygen (DO) level (4.400 mg/L), while Station 4 had the lowest (3.333 mg/L). DO levels differed significantly ($p < 0.05$, Brown-Forsythe test), with further analysis using Games-Howell. Organic material such as decomposed waste, metabolic residues, and plant matter can reduce DO levels (Oghenetekevwe *et al.,* 2024). DO availability directly affects erythrocyte counts in mudskippers, influencing locomotor activity (Maimunah *et al.,* 2023).

Salinity levels ranged from 28 ppt (Station 3) to 41 ppt (Station 5). Significant differences were observed ($p < 0.05$), verified by DMRT. Salinity is affected by suspended sediment (Sun *et al.,* 2018). High salinity at Station 5 is attributed to passive tidal processes, where evaporation leaves salt deposits and ions contributing to high salinity (Rajmohan *et al* (2021). In contrast, Station 3 is adjacent to the Clungup River, which maintains lower salinity and experiences minimal human activity. The change in seawater salinity is closely related to natural phenomena (Yang *et al.,* 2024). Anthropogenic factors such as agricultural runoff also contribute to salinity changes (Oghenetekevwe *et al.,* 2024).

Total dissolved solids (TDS) also varied significantly ($p < 0.05$, Brown-Forsythe test). Station 5 exhibited the highest mean TDS (27,833,333.33 ppm), whereas Station 2 recorded the lowest (1422.67 ppm). Elevated TDS levels are associated with human activities, sediment deposition, and agricultural practices (Oghenetekevwe *et al.,* 2024). In addition, high TDS levels in water also indicate the influence of rock weathering (Sari & Huljana, 2019). Organisms persisting in high TDS environments often exhibit specialized adaptations, such as excreting chemical compounds like ammonia from their body (Polgar & Lim, 2011).

Organic carbon content ranged from 0.376% at Station 4 to 1.995% at Station 5, with significant differences ($p < 0.05$, ANOVA). Higher carbon levels correspond to greater food availability. Station 5, characterized by muddy soil, is rich in nutrients and has high water-retention capacity, whereas Station 4, with sandy soil, is nutrient-poor and retains little water (Rahman *et al.,* 2021).

Principal Component Analysis (PCA)

The data analysis technique for the influence of environmental factors and the amount of food on the protein of the mudskipper fish was carried out using the Principal Component Analysis (PCA) method. This technique is used when research results must be linked to more than two factors. This method transforms the original data variables into new, uncorrelated variables called principal components (Sriningsih *et al.,* 2018). The relationship between protein content, environmental factors, and organic carbon in mudskippers is illustrated in Figure 2. Vectors represent the influence of each factor, while colored areas indicate similarities among mudskipper samples, environmental factors, and organic carbon content across stations.

Protein content was positively correlated with organic carbon but negatively correlated with pH (Figure 2). DO, salinity, TDS, and temperature contributed differently to protein content variation, aligning more closely with the first two principal components (PC1 and PC2). Organic carbon reflects food availability in the habitat, with higher levels ensuring sufficient nutrition and consequently higher protein content in mudskippers. This aligns with Dewianti *et al*. (2022), who stated that adequate food supply supports mudskipper activity, growth, and development.

PCA results also revealed an inverse relationship between pH and protein content. Lower pH values corresponded to higher protein content. Randall *et al*. (1999) noted that alkaline conditions may increase ammonia concentrations beyond mudskippers' detoxification capacity, reducing protein levels and potentially causing mortality.

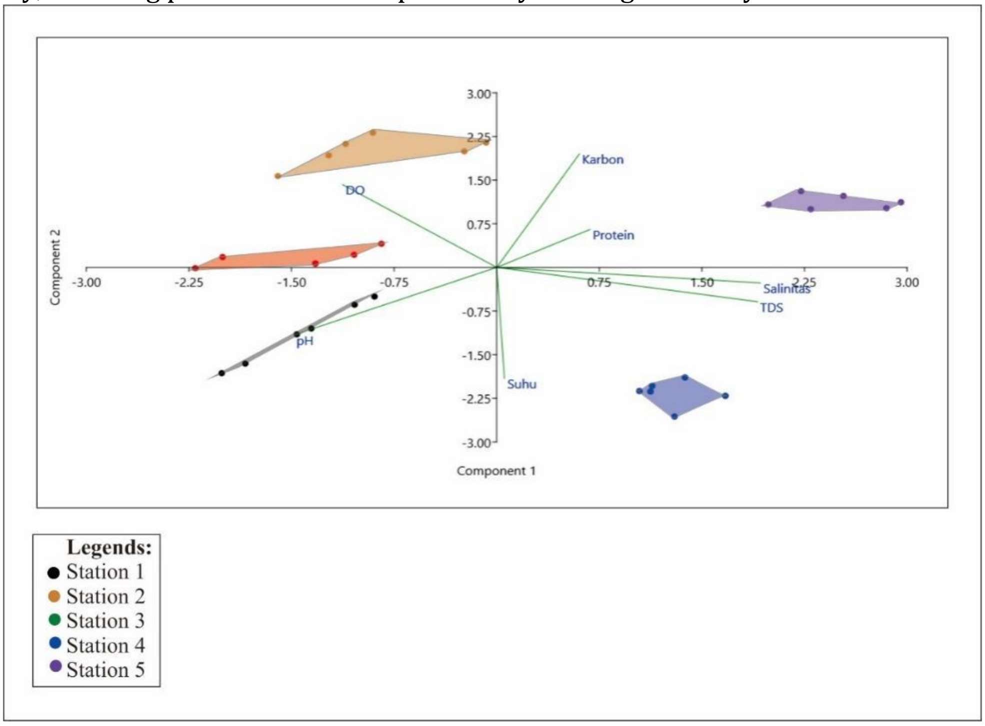

Figure 2. Relationship between mudskipper protein content and environmental factors.
Source: Processed data (2024)

CONCLUSION

The protein content of Periophthalmus gracilis and Periophthalmus modestus did not differ significantly across sampling stations. Periophthalmus modestus at Station 3 exhibited the highest protein content, at 18.55%, while Periophthalmus gracilis at Station 2 showed the lowest, at 11.55%. The organic carbon content, which indicates the availability of food in a habitat, showed a positive correlation with fish protein content, whereas pH exhibited a negative correlation. The higher the food availability in the mudskipper's habitat, the higher the protein content in the fish due to sufficient nutritional intake. Moreover, lower environmental pH levels were associated with higher protein content, as lower pH (acidic to neutral) tends to reduce ammonia levels, thereby minimizing the excretory burden on the mudskippers.

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