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METABOLOMIC AND IONOMIC STATUS OF HERBIVOROUS, CARNIVOROUS, AND OMNIVOROUS FISHES IN VELLAR ESTUARY, INDIA

*Pazhanivel Gowthami, Muthusamy Thangaraj**

Address(es):
Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai, Tamil Nadu, India-608 502.

*Corresponding author: correspondingauthor@mail.com

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ABSTRACT

This study investigated trophic-related variations in biochemical composition and elemental profiles of six estuarine fish species using Fourier Transform Infrared (FT-IR) spectroscopy and Inductively Coupled Plasma–Optical Emission Spectrometry (ICP-OES). Fish representing herbivorous, carnivorous, and omnivorous feeding habits were analyzed using liver tissue to assess metabolic fingerprints and mineral content. FT-IR analysis revealed clear differences in proteins, lipids, and carbohydrates among trophic groups, with significant species-specific variation confirmed by principal component analysis and one-way ANOVA ($p < 0.05$). Carnivorous species exhibited relatively higher protein and lipid signatures, whereas herbivorous species showed lower protein content and distinct carbohydrate-associated bands. Omnivorous species displayed intermediate metabolic profiles, reflecting dietary flexibility. ICP-OES results indicated that potassium and magnesium were the predominant elements across all species, while cadmium was consistently detected at minimal levels. Elevated accumulation of certain trace metals in carnivorous species suggests trophic transfer and potential implications for food safety.
The findings highlight the potential of FT-IR–based metabolic fingerprinting coupled with elemental analysis for food quality assessment, traceability, and safety evaluation of aquatic food resources.

Keywords: Aquatic food safety; Fish liver; Ionomics; Metabolomics; PCA; Trophic groups; Trace metals

INTRODUCTION

Fish exhibit diverse feeding strategies, broadly categorized as herbivorous, carnivorous, and omnivorous, which play a crucial role in shaping their metabolic processes and elemental composition. These trophic differences are reflected in biochemical pathways, nutrient assimilation efficiency, and overall physiological adaptations. In recent years, integrative approaches such as metabolomics and ionomics have emerged as powerful tools to investigate these variations at a systems level. Metabolomics enables the comprehensive profiling of low-molecular-weight metabolites within biological systems, providing insights into functional metabolic states, whereas ionomics focuses on the qualitative and quantitative assessment of elemental composition, including essential and trace elements (**Yoshida *et al*., 2014; Bayona *et al*., 2022**). Together, these approaches offer a holistic understanding of how dietary habits influence biochemical and elemental signatures in fish (**Rebenaque *et al*., 2024**). Such integrative analyses are increasingly applied in aquaculture research to evaluate nutritional physiology, health status, and environmental interactions. Compared to conventional platforms such as nuclear magnetic resonance (NMR) and mass spectrometry-based methods, FT-IR requires minimal sample preparation while providing reliable information on major biomolecular classes, including proteins, lipids, and carbohydrates (**Ellis *et al*., 2007**). In fish biology, FT-IR analysis of liver tissue is particularly valuable, as the liver serves as a central organ for metabolism and nutrient processing (**Liu & Naganuma, 2024**). Dietary specialization strongly influences metabolic pathways in fish. Herbivorous species rely on carbohydrate metabolism and possess specialized enzymatic systems for digesting plant-derived polysaccharides and fibers (**Clements *et al*., 2009**). In contrast, carnivorous fish predominantly utilize proteins and lipids, exhibiting metabolic profiles enriched in amino acid and fatty acid metabolism (**Du *et al*., 2022**). Omnivorous species display metabolic flexibility, enabling them to exploit both plant and animal food sources, resulting in more heterogeneous biochemical profiles (**Pang *et al*., 2024**). Comparative metabolomic studies across trophic groups can therefore reveal distinct biochemical adaptations associated with feeding ecology.
In parallel, ionomic profiling provides critical information on elemental accumulation and regulation in fish tissues, which may be influenced by both diet and environmental exposure. Techniques such as inductively coupled plasma–based spectrometry (ICP-MS/OES) have been widely employed to quantify macro- and trace elements in various fish species such as *Oreochromis mossambicus*, *Mugil cephalus*, *Cyprinus carpio*, and *Labeo rohita* (**Murtala *et al*., 2012; Bhuvaneshwari *et al*., 2012; Taweel *et al*., 2012**). These studies have highlighted the importance of elemental composition in assessing nutritional quality, ecological adaptation, and potential risks associated with metal accumulation (**Bouki *et al*., 2013; Nagato *et al*., 2013**). Furthermore, untargeted metabolomics approaches, which aim to capture the global metabolic fingerprint of a sample, offer significant advantages over targeted analyses, including the ability to discover novel biomarkers and perform retrospective data interpretation (**Chatterjee *et al*., 2019**). When combined with multivariate statistical tools such as principal component analysis (PCA) and analysis of variance (ANOVA), these approaches enable robust discrimination of species-specific metabolic patterns and dietary influences (**Wold *et al*., 1987**).
In this context, the present study employs FT-IR spectroscopy and inductively coupled plasma–optical emission spectrometry (ICP-OES) to investigate the metabolic and ionomic profiles of selected herbivorous, carnivorous, and omnivorous fish species from the Vellar estuary. Multivariate statistical analyses, including PCA and one-way ANOVA, were applied to elucidate dietary-driven variations and species-specific biochemical signatures, thereby providing insights into trophic adaptations and their implications for aquaculture and ecosystem management.

MATERIAL AND METHODS

Sample collection

Fish species representing different trophic groups were collected from the Vellar estuary, Parangipettai, on the southeast coast of India. The selected species included *Leiognathus russellii*, *Drepane punctata*, and *Chelmon sexfasciatus* (carnivores); *Siganus javus* (herbivore); and *Gerres erythrourus* and *Terapon puta* (omnivores) (**Plate 1**). A total of 10 individuals per species were obtained as bycatch from local fishing activities and transported to the laboratory under chilled conditions for further processing.

Sample preparation

Liver tissues were carefully dissected from each specimen, rinsed with deionized water to remove adhering contaminants, and subsequently lyophilized. Approximately 2.5 mg of freeze-dried liver tissue was finely ground with 75 mg of spectroscopic-grade potassium bromide (KBr) using an agate mortar and pestle.

The homogenized mixture was transferred to a pellet die and compressed under a pressure of approximately 6 × 10^6 Pa for 8 min to obtain a ~1 mm thick transparent pellet suitable for infrared analysis (**Akkas *et al*., 2007**). Pure KBr pellets were used as blanks.

Plate 1 Fishes used in this study (A) *G. erythrourus;* (B) *D. punctata;* (C) *L. russelli;* (D) *C. sexfasciatus;* (E) *S javus;* (F) *T. puta*

FT-IR spectroscopy analysis

Fourier Transform Infrared (FT-IR) spectra were recorded using a Shimadzu IR Affinity-1S spectrometer. Spectral acquisition was performed in the mid-infrared (MIR) region (4000–400 cm^{-1}) at a resolution of 4 cm^{-1}, with 50 scans per sample. This spectral range captures characteristic vibrational bands corresponding to major biomolecular functional groups, including proteins, lipids, and carbohydrates. All measurements were performed in triplicate for each species to ensure reproducibility.

ICP-OES analysis

For ionomic analysis, 2 mg of lyophilized liver tissue was digested with 6 mL of aqueous nitric acid (6.9% v/v) using a thermomixer, following the method described by **Walting (1981)**. The digested samples were filtered through a 0.22 µm Millex-GS filter (Millipore) to remove particulate matter. Elemental analysis was carried out using an Agilent Technologies 5100 ICP-OES system according to the manufacturer's protocol. The concentrations of essential and trace elements were determined based on calibrated standards.

Statistical analysis

FT-IR spectral data were processed and analyzed using the MetaboAnalyst 5.0 platform (**Pang *et al*., 2021**). Data normalization was performed using Pareto scaling, followed by mean-centering and division by the standard deviation to minimize technical variability. Metabolite patterns were visualized using hierarchical clustering and heat map. Multivariate analysis, including principal component analysis (PCA), was conducted to identify patterns and discriminate among trophic groups. One-way analysis of variance (ANOVA) was applied to determine statistically significant differences among species. Additional statistical analyses were performed using the *vegan* package in R software (**R Core Team, 2024**).

RESULTS AND DISCUSSION

In this study, the major biomolecules in the liver tissue of six fish species were investigated using FT-IR spectroscopy to monitor different functional groups. The intensity and area of absorption bands in the FT-IR spectrum are directly related to the concentration of molecules **(Cakmak *et al*., 2006; Damian *et al*., 2007)**. **Figure 1** displays the FT-IR spectra of the six fish liver tissues within the 4000-500 cm^{-1} range. The spectrum is complex and contains several bands arising from different functional groups associated with proteins, lipids, and carbohydrates. The absorption bands and their corresponding assignments are presented in **Table 1**. Annotated and unannotated metabolite peak values were subjected to ANOVA with to assess species variation ($p < 0.05$). All the liver tissues displayed significant metabolite abundance shifts influenced by both species and food habit. PCA again confirmed significant species-based differences and emphasized both species-specific patterns and clear distinctions between omnivore fish and carnivore fish (**Figures 2, 3 & Table 2**). Interestingly, the herbivore fish samples spotted along with carnivore fish sample in PCA and showed minimal intra-species variability. Carnivorous species exhibited relatively higher protein and lipid-associated peaks, consistent with their reliance on amino acid and fatty acid metabolism. The same pattern of PCA observation was made by **Marie *et al*. (2026)** in two freshwater fishes, *Squalius cephalus* and *Gobio gobio*. In contrast, the herbivorous species (*Siganus javus*) showed reduced protein and lipid intensities but distinct carbohydrate-related bands, reflecting adaptation to plant-based diets as reported by **Karthikeyan (2012)**. Omnivorous species displayed intermediate metabolic characteristics, indicating dietary flexibility.

Table 1 FTIR spectral peaks and their functional assignments in the liver tissue of six fish species

S. No	*L. russelli*	*D. punctata*	*S. javus*	*C. sexfasciatus*	*G. erythrourus*	*T. puta*	Peak assignments
1.	3292.49(s, b)	-	3292.42 (s, b)	3294.42 (s, b)	3290.56 (s, b)	3286.70 (s, b)	Amide A: N-H stretching of proteins
2.	-	3020.53 (m)	3020.53 (m)	3022.45 (m)	3078.39 (m)	3078.39 (m) 3010.88 (m)	C-H stretch of aromatics: mainly carbohydrates
3.	2924.09 (s)	2924.09 (s)	2924.09 (s)	2926.09 (s)	2926.09 (s)	2924.09 (s)	C-H stretch of alkane: mainly lipids
4.	2856.58 (s)	2856.58(s)	2856.58 (s)	2858.58 (s)	2858.58 (s)	2854.65 (s)	C-H stretch of alkane: mainly lipids
5.	1741.72 (s)	1734.01 (s)	1735.93 (s)	1735.93 (s)	1735.93 (s)	1743.65 (s)	C=O stretch of ester: mainly lipids
6.	1643.35 (s)	1651.07 (s)	1649.94 (s)	1645.28 (s)	1649.14 (s)	1649.14 (s)	Amide I: C= O stretching of proteins
7.	1541.12 (m)	1544.98 (m)	1543.05 (m)	1539.20 (m)	1543.05 (m)	1541.12 (m)	C=C stretch of aromatic: mainly carbohydrates
8.	1456.26 (m)	1458.18 (m)	1456.26 (m)	1456.26 (m)	1456.26 (m)	1458.18 (m)	C-H bend of alkane: mainly lipids
9.	1398.39 (m)	1400.32 (m)	1400.32 (m)	1400.32(m)	1398.39 (m)	1396.46 (m)	C-H bend of alkane: mainly lipids
10.	1309.67 (m)	1309.67 (m)	1313.52 (m)	1344.38(m) 1307.74 (m)	1307.74 (m)	1305.81 (m)	Amide II: C-N stretching of proteins
11.	1155.36 (s)	1153.43 (s)	1151.50 (s)	1153.43 (s)	-	1168.86 (s)	C-F stretch of alkyl halide: mainly carbohydrates
12.	1085.92 (m)	1080.14 (m)	1078.21 (s)	1080.14 (m)	1082.07 (m)	1089.78 (m)	Amide II: C-N stretching of proteins
13.	1043.49 (s)	1039.63 (s)	1039.63 (s)	1035.77 (s)	-	-	C-F stretch of alkyl halide: mainly carbohydrates

Note: (s – strong; b- broad; m- medium)

According to **Benedetti *et al*. (1997)**, and **Jagadeesan *et al*. (2005)**, variations in the FT-IR peak intensity ratios of amide bands at 1540 cm^{-1} and 1650 cm^{-1} indicate changes in the overall protein composition. In this study, the herbivorous fish *S. javus* exhibited lower protein and lipid content compared to other species, consistent with findings in the gill tissues of *Ctenopharyngodon idella* **(Ananth, 2015)** and *Cirrhinus mrigala* **(Karthikeyan, 2012)**. The decreased intensities of amide bands suggest alterations in protein synthesis and structure **(Webb, 1966)**. Additionally, shifts in the peak position of amide bands may quantitatively reflect changes in protein composition and secondary structure **(Susi & Byler, 1983)**. In this study, the omnivorous fish *G. erythrourus* also exhibited reduced protein peak intensity, similar to observations in *Labeo rohita* (**Palaniappan & Renju, 2009**). Similar trends were reported in muscle tissues of *Cyprinus carpio* **(Senthamilselvan & Chezhian, 2014)** and liver tissues of *Oreochromis mossambicus* **(Venkatramana *et al*., 2010)**. **Silva *et al*. (2014)** found that hepatic metabolites, particularly carbohydrates and proteins, exhibited decreased peak intensity in *Sparus aurata*. **Yoshida *et al*. (2014)** observed a reduction in tissue metabolites of *Acanthogobius flavimanus* and *Lateolabrax japonicus*, which they attributed to environmental changes.

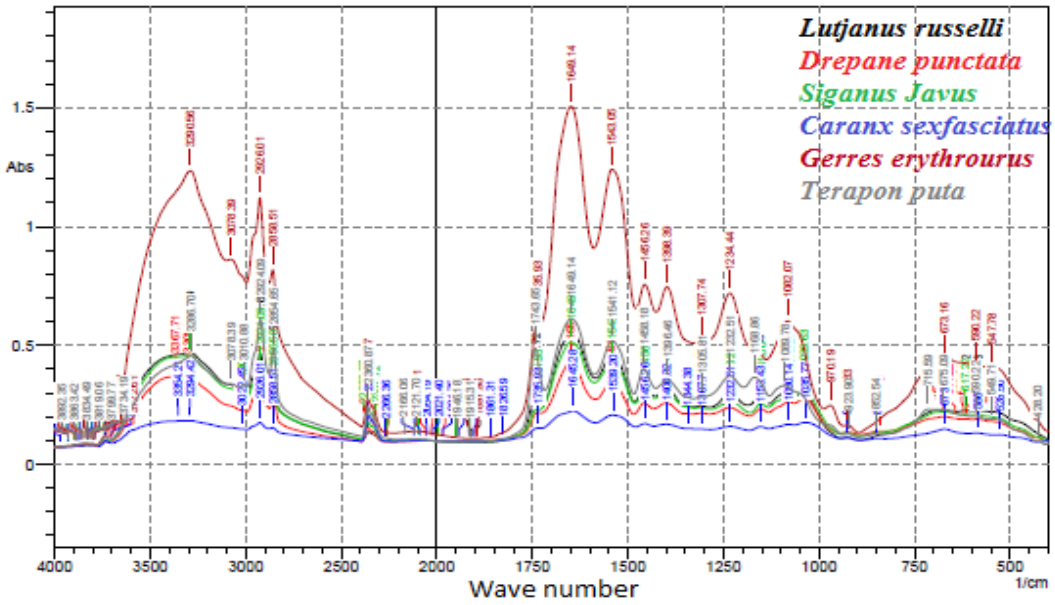

Figure 1 Comparative FT-IR spectra of liver tissues of six fish species in the range of 4000- 500 cm^{-1}

Table 2 ANOVA result of FT-IR spectrum of the liver tissue of six fish species

Between species	*p* - value	Significance difference
Caranx sexfaciatus Vs *Drepane punctatus*	0.0000	Yes
Caranx sexfaciatus Vs *Gerres erythrorus*	0.0000	Yes
Caranx sexfaciatus Vs *Lutjanus russelli*	0.0000	Yes
Caranx sexfaciatus Vs *Siganus javus*	0.0000	Yes
Caranx sexfaciatus Vs *Terapon puta*	0.0000	Yes
Drepane punctatus Vs *Gerres erythrorus*	0.0000	Yes
Drepane punctatus Vs *Lutjanus russelli*	0.0000	Yes
Drepane punctatus Vs *Siganus javus*	0.0000	Yes
Drepane punctatus Vs *Terapon puta*	0.0000	Yes
Gerres erythrorus Vs *Lutjanus russelli*	0.0000	Yes
Gerres erythrorus Vs *Siganus javus*	0.0000	Yes
Gerres erythrorus Vs *Terapon puta*	0.0000	Yes
Lutjanus russelli Vs *Siganus javus*	0.7945	No
Lutjanus russelli Vs *Terapon puta*	0.0352	Yes
Siganus javus Vs *Terapon puta*	0.0003	Yes

Studies on carnivorous fish, such as *Lates calcarifer*, have shown decreased protein peak absorption in muscle tissue **(Senthamilselvan *et al*., 2012)**. Similarly, the present study indicates a decrease in protein peak intensity in *L. russelli*, *D. punctata*, and *C. sexfasciatus*. The depletion of protein content suggests energy diversification to meet increasing metabolic demands under toxic stress **(Jagadeesan & Mathivanan, 1999)**. The reduction in protein content may also be attributed to its conversion into amino acid residues to enhance the amino acid pool. Additionally, protein depletion in fish tissues may result from non-selective inhibition of phosphorylation processes in gill tissues, primarily due to the suppression of protein synthesis in the endoplasmic reticulum **(Falco *et al*., 2020)**. Lipids play a crucial role in maintaining membrane fluidity and influence the conformation of membrane proteins, which govern exposure and diffusion of membrane components **(Cakmak *et al*., 2006)**. The absorption bands observed around 2923 cm^{-1} and 2853 cm^{-1} are attributed to the asymmetric and symmetric stretching modes of methylene chains in membrane lipids **(Haris & Servercan, 1999)**. Furthermore, carbohydrate metabolism is often disrupted in fish subjected to environmental stress, as stress affects glycogen levels and metabolic activity **(Jagadeesan & Mathivanan, 1999)**.

The concentration of metal ions in the liver tissue of the six fish species was determined using ICP-OES, and the results are presented in **Table 3**. To assess levels of trace metals and common metal ions the concentrations in the examined fish samples were compared to standard values reported in the literature (**Table 3**). The metal content in fish muscle varied considerably based on the species and feeding habit. International guidelines propose allowable levels for certain elements (e.g., Cd, Cr, Cu, Fe, Mn and Pb), while no specific limits have been established for other elements such as, B, Mg and K. In our study, almost all the elemental concentrations were not exceeded the recommended food safety guidelines (FSG) for fish as defined by international organizations (**Table 3**).

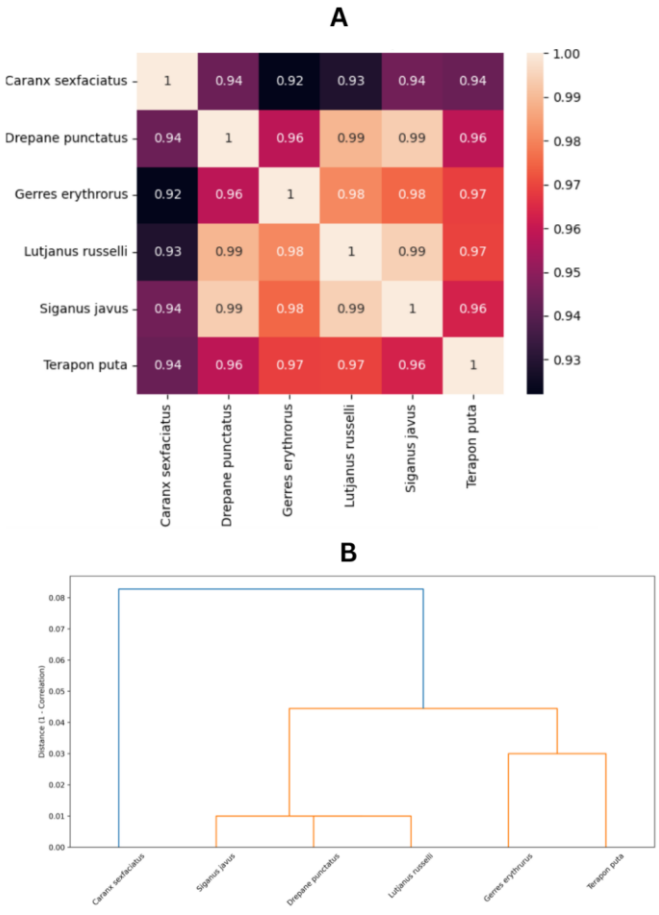

Figure 2 (A), heat map with correlation classification (B), correlation similarity cladogram based on FT-IR spectra of liver tissues in different fish species

The results revealed that potassium (K) and magnesium (Mg) were the most abundant elements in all species, while cadmium (Cd) was present at the lowest concentration. Elemental composition varied among species, with *L. russellii* showing higher accumulation of Mg, Cu, Pb, Cr, and Cd, whereas *T. puta* exhibited higher levels of K, Fe, and Mn. Boron (B) was notably higher in the herbivorous species *S. javus*. The findings indicate that potassium was highly accumulated in *S. javus* (herbivore). **Bhuvaneshwari *et al*. (2012)** detected high copper levels in the liver of *Mugil cephalus* and muscle tissue of *Scardinius erythrophthalamus*. Omnivorous fish also exhibited significant metal accumulation of potassium and magnesium. **Yoshida *et al*. (2014)** reported high calcium and magnesium

concentrations in *Acanthogobius flavimanus* and *Lateolabrax japonicus*. **Ozturk *et al*. (2009)** identified elevated copper level in the liver, chromium, iron, and lead in the stomach-intestine of *Cyprinus carpio*. **May *et al*. (2009)** found high calcium concentrations in *Pomoxis nigromaculatus*, *Hypentilium nigricans*, and *Scaphirhynchus platorynchus*. **Uwem *et al*. (2013)** observed high copper concentrations in *Oreochromis niloticus*. Copper and potassium level was noted in *Oreochromis mossambicus* and *Nemipterus bipunctatus* **(Saravanamurugan *et al*., 2013)**. **Taweel *et al*. (2012)** also reported high lead levels in *Oreochromis niloticus*.

In the present study, the carnivore fish, *G. erythrourus* exhibited high potassium and magnesium concentrations and this result is similar to the observations by **Saravanamurugan *et al*. (2013)** in *Nemipterus bipunctatus.* **Yoshida *et al*. (2014)** found high calcium and magnesium levels in *Glossogobius olivaceus*. Across trophic groups, both omnivorous and carnivorous fishes showed relatively higher accumulation of trace metals, suggesting the influence of dietary intake and environmental exposure. The liver, being a central organ for metabolism and detoxification, reflects these accumulation patterns. Similar findings have been reported in earlier studies, highlighting the role of anthropogenic inputs in elevating metal concentrations in aquatic organisms (**May *et al*. 2009; Murtala *et al*., 2012; Choudhury *et al*., 2025)**. Carnivorous species, particularly *L. russellii*, showed higher levels of several heavy metals, possibly due to biomagnification through the food chain. In contrast, herbivorous and omnivorous species exhibited comparatively lower accumulation of toxic elements, although essential minerals such as K and Mg remained dominant across all groups. Overall, potassium was the most abundant element, while cadmium consistently showed the lowest concentration in all species.

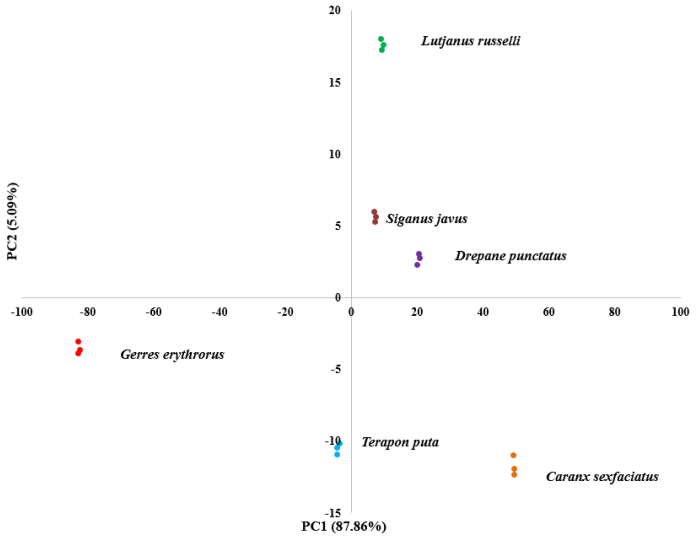

Figure 3 PCA plot of different fish species based on FT-IR spectra of liver tissues

Table 3 Mean concentration (ppm ± SD) of minerals in the liver tissue of six fish species

Elements	*L. russelli*	*D. punctata*	*S. javus*	*C. sexfasciatus*	*G. erythrourus*	*T. puta*	Recommended Value (FAO, 1983)	Elemental function
B	0.020±0.001^{a}	0.040±0.001^{b}	0.050±0.001^{c}	0.030±0.001^{d}	0.040±0.001^{b}	0.040±0.001^{b}	-	Pleiotropic effect
Cd	0.020±0.002^{a}	0.010±0.001^{b}	0.010±0.001^{b}	0.010±0.001^{b}	0.010±0.001^{b}	0.010±0.001^{b}	0.05	No biological function
Cr	0.040±0.001^{a}	0.020±0.001^{b}	0.020±0.001^{b}	0.010±0.001^{b}	0.010±0.001^{b}	0.030±0.001^{c}	12.0-13.0	Enzymatic element
Cu	2.220±0.022^{a}	2.070±0.021^{a}	2.060±0.021^{a}	2.090±0.021^{a}	2.060±0.012^{a}	2.090±0.021^{a}	30.0	Enzymatic element
Fe	12.210±0.020^{a}	11.460±0.020^{a}	12.180±0.020^{a}	11.450±0.010^{a}	12.100±0.020^{a}	12.220±0.020^{a}	100.0	Enzymatic element
K	21562.96±1150^{a}	19857.06±1120^{a}	17865.53±1210^{b}	15945.23±1250^{b}	19580.88±900^{a}	22478.50±1320^{a}	-	Electrolytic element
Mg	180.50±12.50^{a}	190.70± 13.80^{a}	190.40±12.30^{a}	180.20± 12.20^{a}	105.40±12.10^{b}	150.30±13.70^{c}	-	Electrolytic element
Mn	0.80±0.02^{a}	0.50±0.01^{b}	0.50±0.01^{b}	0.40±0.01^{c}	0.50±0.01^{b}	0.90±0.01^{d}	1.0	Enzymatic element
Pb	0.090±0.002^{a}	0.070±0.001^{b}	0.070±0.001^{b}	0.085±0.001^{a}	0.090±0.001^{a}	0.090±0.001^{a}	0.3	Oxidative stress

Note: Values along the column sharing same superscript are not significantly different (p<0.05); ppm=parts per million

Authentication of species using metabolomics relies on the fact that each fish species exhibits a unique pat tern of endogenous metabolites, shaped by its genetic makeup, physiology and ecological niche (**Bhalavey *et al*., 2026**). Targeted and untargeted metabolomic analyses support the discrimination of closely related species, including those that are morphologically similar or frequently substituted in the market (**Sidira *et al*., 2024**) and it can be use full to improve the fish nutrition (**Roques *et al*., 2020).** Further, studies showed that metabolites identified in Atlantic salmon or various sturgeon species reveal clear differences at the molecular level depending on species, with NMR, FT-IR and MS-based methods enabling robust classification based on amino acids, lipids and other small molecules (**Ivanova *et al*., 2024; Liu & Naganuma, 2024**).

CONCLUSION

This study demonstrates that metabolomic and ionomic approaches effectively reveal trophic-specific biochemical and elemental variations in estuarine fish species. FT-IR spectroscopy highlighted distinct metabolic signatures associated with proteins, lipids, and carbohydrates, clearly differentiating herbivorous, carnivorous, and omnivorous fishes. Concurrently, ICP-OES analysis showed that essential elements such as potassium and magnesium dominate across all species, while variations in trace metal accumulation reflect dietary habits and possible environmental exposure. The integration of multivariate analyses further confirmed that feeding ecology plays a significant role in shaping both metabolic composition and elemental distribution. Notably, reduced biomolecular intensities suggest metabolic adjustments that may be linked to environmental stress or energy allocation strategies. This study emphasis the value of combining metabolomics and ionomics as a comprehensive framework for understanding fish physiology, trophic adaptations, and ecosystem health. These approaches also hold potential for applications in aquaculture nutrition, environmental monitoring, and species authentication.

Conflict of interest statement: Authors are not affiliated with or involved with any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this paper.

Data availability statement: The primary research data that support the findings of this study are in the possession of the authors of this manuscript only and are not available online other than for publication in this journal.

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