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Comparative analysis of site-specific Intestinal Histological variations in Asian Seabass (Lates calcarifer) and Seabream (Nemipterus japonicus) along the Balochistan coast, Pakistan

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Abstract: Coastal marine ecosystems are increasingly exposed to anthropogenic pressures that can impair fish health and compromise ecosystem integrity. The intestinal tract of fish is particularly sensitive to environmental stressor making intestinal histology a valuable indicator of habitat quality and environmental impacts. The present study examined site-specific variations in the intestinal histology of Asian Seabass (*Lates calcarifer*) and Seabream (*Nemipterus japonicus*) collected from Dam, Gaddani, and Kund Malir along the Balochistan coast, Pakistan. Intestinal tissues were processed using standard histological techniques, and quantitative measurements of villi length and mucosal thickness were used to assess structural alterations among sites. The results revealed significant reductions in villi length (34.79 ± 7.29 µm, $p < 0.0001$) and mucosal thickness (8.33 ± 1.88 µm, $p < 0.05$) in *L. calcarifer* from Dam compared to species from Gaddani and Kund Malir, where no significant differences were observed. Similarly, *N. japonicus* collected from Dam showed significantly reduced villi length (34.79 ± 7.29 µm, $p < 0.001$) and mucosal thickness (7.11 ± 1.67 µm, $p< 0.05$), while individuals from Gaddani and Kund Malir remained normal. These findings indicate that Dam coastal area is subjected to greater environmental stress, highlighting intestinal histopathology as a reliable biomarker for assessing coastal ecosystem health.

Keywords: Balochistan Coast, Histological Analysis, Intestinal Tissues, Seabass, Seabream

1. INTRODUCTION

The sensitivity of fish to environmental changes and water quality positions them as important bioindicators for assessing the health of aquatic ecosystems. Variations in fish physiology and tissue structure often reflect exposure to environmental stressors and can provide early warnings to ecosystem degradation. Among commercially significant species, Asian seabass (*Lates calcarifer*) holds considerable ecological and economic importance due to its wide distribution, high market value, and adaptability to diverse environments (Shaw & Handy, 2011, Vij et al., 2020;). The Balochistan coast, extending approximately 770 kilometers along the Arabian Sea, represents one of Pakistan's most commercially and ecologically vital coastal regions, supporting rich biodiversity and productive fisheries (Qureshi et al., 2010). However, increasing anthropogenic activities such as industrial discharge, uncontrolled fishing, oil spills, and coastal development threaten the stability of this fragile ecosystem (Herrera-García et al., 2021). These environmental pressures can disrupt fish physiology, alter tissue structure, and pose potential risks to both ecological balance and human consumption (Kalantzi et al., 2016).

The fish intestine is a vital organ for digestion, nutrient absorption, and immunological defense, and is among the first organs to response to contaminants present in food and water (Salinas & Parra-Medina, 2019).

Histopathological changes in the intestine, such as goblet cell hyperplasia, villous atrophy, and inflammatory infiltration, serve as critical indicators of sub-lethal stress induced by pollutants, microplastics, and other environmental stressors (Walford & Lam, 1993). For seabass, these alterations not only compromise health and growth but also reduce resilience against diseases, thereby threatening their ecological role and aquaculture potential (Shaw & Handy, 2011).

Nemipterus japonicus is a benthic and demersal fish that is found in the western Pacific and Indian oceans. The species is frequently found in Pakistani water at depths of 5 to 80 meters, typically in schools, along sandy and muddy coastal areas. It is one of Pakistan's most prevalent and economically significant fish species, making a substantial contribution to the trawl fisheries along the shores of Sindh and Balochistan (Kalhoro et al., 2014).

Asian seabass (*Lates calcarifer*), also known as barramundi, is a widely distributed and economically significant Indo-West Pacific teleost fish inhabiting coastal, estuarine, and freshwater environments, including the waters of Balochistan, Pakistan (Shaha et al., 2020). It is highly valued for its rapid growth, high-quality flesh, and ability to tolerate a broad range of salinity, making it important for both aquaculture and wild fisheries (Pattarapanyawong et al., 2021; Mehak et al., 2025). Despite its adaptability, seabass is susceptible to environmental stressors such as pollution, habitat degradation, and salinity fluctuations (Maulu et al., 2021).

Seabream (*Nemipterus japonicus*) is a lucrative commercial marine species that is widely spread throughout the Balochistan coast, supporting local fisheries and contributing significantly to the regional economy. Seabream, a benthic species that dwells in sandy and muddy settings, is very vulnerable to oscillations in water quality and sediment-bound contaminants, making it a suitable bioindicator of environmental health (Khan et al., 2017). Histological examinations of its primary organs, particularly the intestine, provide information regarding the effects of anthropogenic stress on fish physiology. Modulations in villi form, mucosal thickness, may reflect site-specific stress conditions.

Materials and Methods

2. Study Area

The research was conducted along the Balochistan coast of Pakistan, which stretches about 770 km along the Arabian Sea and supports diverse marine habitats and fisheries. Three sampling sites were selected as Dam, Gaddani, and Kund Malir. Dam is characterized by artisanal fishing activity with relatively low pollution; Gaddani is heavily influenced by ship breaking and industrial discharges; while Kund Malir, located within Hingol National Park, is considered comparatively pristine.

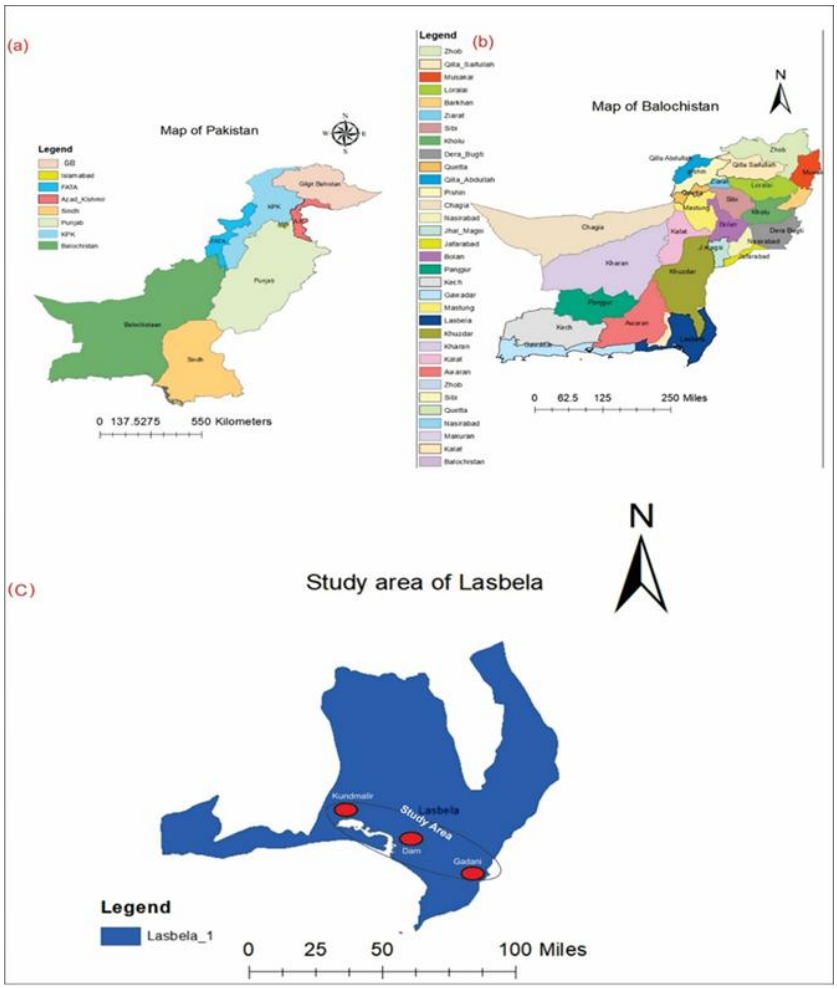


Figure 1: (a) Map of Pakistan showing its topographical setting, (b) Accessibility map of Balochistan highlight the regional linkage (c) Detailed map of the study area with sampling points

Five to eight samples of Asian seabass (*Lates calcarifer*) and Seabream (*Nemipterus japonicus*) were collected from each site, with weights ranging from 110 to 240 grames between September 2024 and January 2025 with the help of local fishermen using traditional gears. After capture, fish were transported in ice boxes to the Histology Laboratory, Department of Veterinary Medicine, Lasbela University of Agriculture, Water and Marine Sciences (LUAWMS). Standard morphometric data, including total length and weight, were recorded prior to dissection. Intestinal tissues were excised for histological analysis.

2.1 Histological Procedure

Intestine samples were preserved in 10% buffered formalin for 24 48 h, dehydrated in ascending grades of ethanol, cleared in xylene, and embedded in paraffin wax. Thin sections (5–7 µm) were prepared using a rotary microtome and mounted on glass slides. Routine hematoxylin and eosin (H&E) staining was performed following standard protocols.

2.2 Microscopic and Morphometric Examination

Prepared slides were examined under a light microscope (Leica Diaplan) at 100 magnifications. Histological features such as villous height and mucosal thickness changes were assessed. Digital photomicrographs were

captured, and morphometric parameters were measured using ImageJ software (v1.51f).

2.3 Statistical Analysis

Data was analyzed using GraphPad Prism (version 6). Normality and homogeneity of variance were tested with Bartlett's test. One-way ANOVA followed by Tukey's post hoc test was applied to detect significant differences among sites. For non-parametric data, the Kruskal Walli's test followed by Dunn's multiple comparison test was used. Results are presented as mean ± standard deviation (SD), and statistical significance was set at $p < 0.05$.

RESULT AND DISCUSSION

RESULTS

The current study describes the results of a histological analysis of the intestines of two fish species, *Late calcarifer* and *Nemipterus. japonicus*, obtained from three coastal sites in Balochistan Dam, Gaddani, and Kund Malir (Fig.1). The Dam and Gadani is compared with Kund Malir and is shown by * (Kruskal- Walli's test followed by a Dunn's multiple comparison test, p<0.05, N=3-5, 21-25 per individual measurement. Data are expressed as mean ± SD.

4.1 Histological Analysis of Intestinal Tissues Using HE Staining

Hematoxylin and eosin (HE) staining was applied to the intestinal tissues of commercially important fish species, *Late calcarifer* and *Nemipterus. japonicus,* collected from different sites along the Balochistan coast. The histological examination revealed variations in mucosal thickness, goblet cell density, and villi length across different sampling locations.

In seabass, the intestinal tissues from Kund Malir (Figure 2A) displayed a balanced goblet cell count with well-maintained mucosal thickness in contrast, the intestinal tissues from Dam (Figure 2B) showed moderate mucosal thickness and slightly elongated villi.

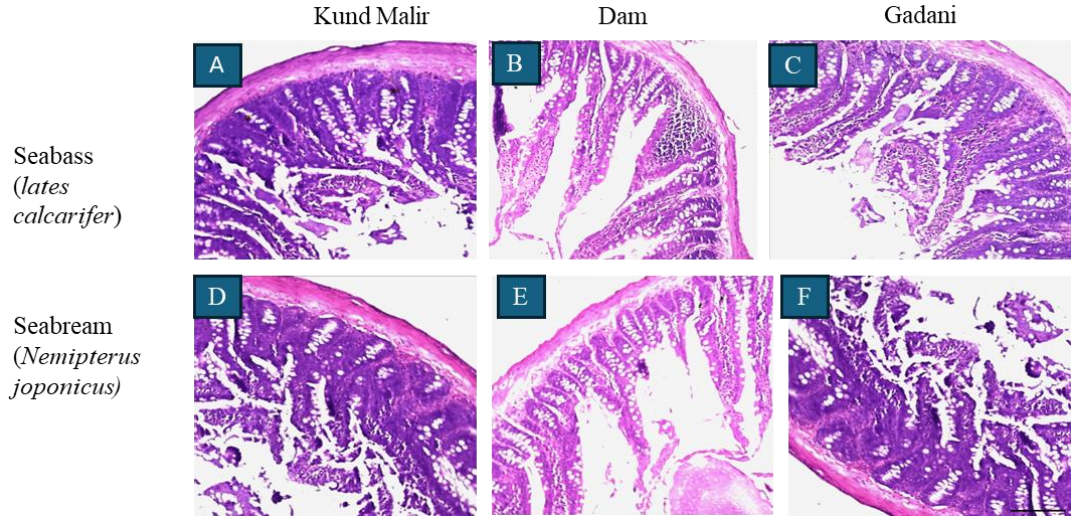


Figure 2: Comparative analysis of intestinal tissues of Seabass (*Lates calcarifer*) and Seabream (*Nemipterus japonicus*) across various sampling sites

The seabass samples from Gadani (Figure 2C) exhibited reduced mucosal thickness and an increased number of

goblet cells. For seabream, the intestinal tissues from Kund Malir (Figure 2D) presented the tallest villi and well-developed mucosal thickness. In contrast, the samples from Dam (Figure 2E) exhibited moderate mucosal thickness and villi length. The intestinal tissues from seabream collected at Gadani (Figure 2F) showed shorter villi and reduced mucosal thickness. Hematoxylin and eosin staining of the gut tissues of major commercial fish seabass (*Lates calcarifer*) and seabream (*Nemipterus japonicus*) of different locations in the Balochistan coast were conducted. In the histology, variations at the level of mucosal thickness, goblet cell density, and villus length among places of sampling were observed. Gadani intestinal tissue of seabass (Figure 2A) revealed reduced mucosal thickness and increased goblet cell number. Dam's intestinal tissue (Figure 2B) revealed moderate mucosal thickness and slightly hypertrophied villi. Seabass from Kund Malir (Figure 2C) revealed a well-balanced number of goblet cells and maintained mucosal thickness. Gadani seabream intestinal tissue (Figure 2D) contains short villi and a thin mucosa. Conversely, dam samples (Figure 2E) contained intermediate villi length and mucosal thickness. Intestinal tissues of seabream at Kund Malir recovered (Figure 2F) possessed the highest villi and well-developed mucosal thickness.

Table 1: Shows the assessment of intestinal villi length in Seabass

Seabass			
S. No	Study Site	Villi length (µm)	Significance
1	Kund Malir	43.89 ± 7.93	Not Significant
2	Dam	34.79 ± 7.29	(***p<0.0001)
3	Gadani	36.13 ± 8.46	(**p<0.001)

Table 1 shows site-specific variations in intestinal villi length of *Lates calcarifer* along the Balochistan coast. The villi were longest at Kund Malir (43.89 ± 7.93 µm), followed by Gadani (36.13 ± 8.46 µm) and Dam (34.79 ± 7.29 µm). The differences were highly significant in fish from Dam (***$p < 0.0001$) and Gadani ($p < 0.001$) compared to Kund Malir.

Table 2: Indicate comparison of mucosal layer thickness

Seabass			
S.NO	Study Site	Mucosal thickness (µm)	Significance
1	Kund Malir	8.33 ± 1.88	No difference
2	Dam	8.33 ± 1.88	p<0.05
3	Gadani	7.37 ± 1.86	No difference

Table 2 presents the comparison of intestinal mucosal layer thickness in *Lates calcarifer*. The mucosal thickness was similar at Kund Malir (8.33 ± 1.88 µm) and Dam (8.33 ± 1.88 µm), while a slightly reduced value was recorded at Gadani (7.37 ± 1.86 µm). Statistical analysis showed a significant difference ($p < 0.05$) at Dam compared to the other sites.

Table 3: Display the evaluation of intestinal villi length in *Nemipterus japonicus*

Seabream			
S. No	Study Site	Villi length (µm)	Significance
1	Kund Malir	43.89 ± 7.93	Not Significant
2	Dam	34.79 ± 7.29	(**p<0.001)
3	Gadani	36.13 ± 8.46	Not Significant

However, the findings provoked to associated with collected data from three coastal sites along the Balochistan coast. The villi were elongated at Kund Malir (43.89 ± 7.93 µm), followed by Gadani (36.13 ± 8.46 µm) and Dam (34.79 ± 7.29 µm) (Table 3). Statistical analysis revealed a significant difference ($p < 0.001$) in fish from Dam compared to Kund Malir, while the variation at Gadani was not significant.

Table 4: Illustrate the mucosal layer thickness in Seabream

Seabream			
S.NO	Study Site	Mucosal thickness (µm)	Significance
1	Kund Malir	8.33 ± 1.88	Not significant
2	Dam	7.11 ± 1.67	p<0.05
3	Gadani	7.37 ± 1.86	Not significant

Table 4 illustrates the mucosal layer thickness in *Nemipterus japonicus* from study sites. The mucosal thickness was highest at Kund Malir (8.33 ± 1.88 µm), followed by Gadani (7.37 ± 1.86 µm) and Dam (7.11 ± 1.67 µm). Statistical analysis indicated a significant difference ($p < 0.05$) at Dam compared to Kund Malir, while no significant difference was found at Gadani.

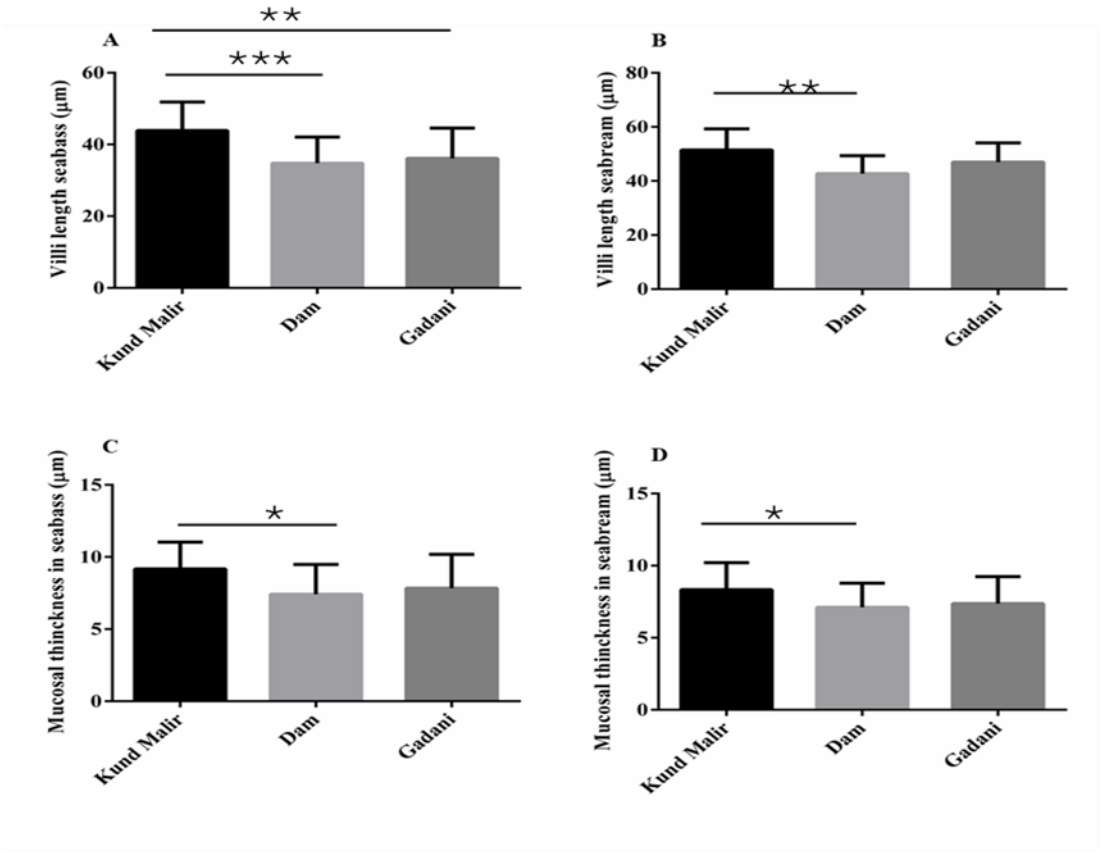
5. DISCUSSION

The current study evaluated the intestinal histology of (*Lates calcarifer)* and (*Nemipterus japonicus)* taken from three coastal sites in Balochistan Dam, Gadani, and Kund Malir to identify potential structural differences. Such studies are very rare from the coast of Balochsitan. There are several studies that have analyzed the seabass histology and physiological parameter (Coz-Rakovac et al., 2005; Purushothaman et al., 2016; Thophon et al., 2003) & seabream (Kerdgari et al., 2013; Ibrahim et al., 2025).

In the current findings fish from Kund Malir showed the longest intestinal villi and the thickest mucosal layer, reflecting a healthier gut condition (Liu et al., 2025). A similar pattern was described in several studies under experimental conditions who found that seabass exposed to minor challenge (0.22ppm of heavy metal) had long villi and a thin mucosal layer. Fish exposed to dietary changes showed villi shortening and tissue alterations. Likewise, Sklan, Prag, and Lupatsch (2004) observed tall and well-organized villi lined with columnar epithelial cells bearing a brush border in healthy *Tilapia*. In the same way, Vélez-Calabria et al. (2023), also reported that gilthead seabream (*Sparus aurata*) fed with hydrolyzed porcine mucosa developed thicker villi and mucosal layers than those in the control group (0 dose), which indicates better intestinal structure and nutrient absorption capacity. Fish collected from Dam and Gadani showed shorter intestinal villi length of seabass. a similar observation was made by Pedà et al. (2016), who found that European seabass (*Dicentrarchus labrax*) exposed to contaminated conditions developed shortened and swollen villi along with epithelial damage. Their findings

support the present results, indicating that unfavorable environmental conditions can lead to weakened intestinal structure and reduced absorptive capacity. These findings may represent our results from Gaddani and Dam site. These sites have been widely used for industrial activity. Pedà et al. (2016), species from both sites showed thinner mucosa just like, Purushothaman et al. (2016), study who observed about a 60% reduction in mucosal height in stressed seabass (*Lates calcarifer*), indicating weakened intestinal health. Fish from Dam and Gadani showed shorter villi and thinner mucosa, while those from Kund Malir had a healthier intestinal structure.

This morphological character promotes effective digestion and absorption of feed. In the current study, the well-preserved villi at Kund Malir may indicate stable physiological conditions. Besides this, shorter villi and thinner mucosal layers at Dam and Gadani may reflect adaptive or physiological responses to differences that can be linked with habitats, eating habits, or physical variable (temperature & salinity) stress. (Younis et al., 2013) observed degeneration and shortening of intestinal villi in *Oreochromis niloticus* exposed to sublethal cadmium levels. Al-Niaeem, Al-Hamadany, and Al-Tameemi (2010) observed villi atrophy and mucosal thinning in *Cyprinus carpio* during starvation stress. While the causes in previous investigations (Younis et al., 2013) were deliberately created, the type of tissue variation (shorter villi) found is like the modifications seen in the current study. This shows that fish intestines are extremely susceptible to environmental or physiological changes, but it needs to be analyzed more in depth with other molecular applications. Natural habitat changes, such as water temperature, salinity, or food availability, which are known to alter fish intestinal morphology, could explain the observed variation among sites. Previous research has found that fish intestine structure adjusts to eating conditions and habitat factors, influencing villi form and mucosal thickness (Sklan et al., 2004). The slightly reduced villi length and mucosal thickness seen in Dam and Gadani fish could be attributed to normal structural modifications caused by environmental conditions rather than pathogenic abnormalities. *Lates calcarifer* and *Nemipterus japonicus* had comparable patterns of site-wise variation, however the degree of variance differed each species. *Lates calcarifer* exhibited more significant villi shortening, but *Nemipterus japonicus* had slightly thinner mucosa, indicating interspecific heterogeneity in intestinal responses. These differences could be due to dietary habits, metabolism, or environmental preferences. In conclusion, the histological investigation demonstrated that intestine morphology varied between places along the Balochistan coast, presumably due to natural environmental changes or adaptive physiological responses. The morphological parameters seen at Kund Malir indicate acceptable habitat conditions, whereas slight structural variations in fish from Dam and Gadani indicate possible site-related impacts. The findings are comparable with previously recorded intestinal features in teleosts, supporting the notion that histological properties of fish intestines are sensitive markers of their health and habitat circumstances (Al-Niaeem et al., 2010; Sklan et al., 2004; Younis et al., 2013). Regular histological examinations of commercially important fish species can thus provide useful information about the biological state and habitat quality of the Balochistan coastal ecosystem.



6. CONCLUSION

The current study compares the histological assessment of the intestine in *Lates calcarifer* (seabass) and *Nemipterus japonicus* (seabream) taken from three coastal sites in Balochistan. The findings revealed significant differences in intestinal villi length and mucosal thickness across sites. Overall, fish from Kund Malir had longer villi and thicker mucosal layers, indicating well-developed intestinal systems, whereas fish from Dam and Gadani had shorter villi and thinner mucosa. These differences imply that local environmental or physiological factors may influence intestine shape in these animals. Although the specific causes of these changes were not investigated in the current study, the observed structural differences could be natural adaptations to habitat conditions, eating patterns, or other physiological influences. The results show that histological parameters like villi height and mucosal thickness can be effective indicators of fish intestines' overall health and functionality. Overall, the findings provide essential baseline information on the intestinal histology of two commercially important fish species from the Balochistan coast. Such baseline data is vital for future comparative and monitoring studies to understand how environmental and biological factors influence fish tissue shape and general health.

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