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Morphological and Histological Analysis of Muscles and Liver Tissues in Selective Fish Species evidence based at Costal belt in Balochistan

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Abstract: *Histopathological changes in fish muscle and liver tissues serve as reliable bioindicators of aquatic pollution and ecological stress. This study analyzed seabream (Nemipterus japonicus) and Seabass (Lates calcarifer) from three coastal sites of Balochistan Gadani, Dam, and Kund Malir represented high, moderate, and low anthropogenic influence, respectively. Muscle and liver samples from posterior and caudal regions processed to use hematoxylin and eosin staining to assess fiber dimensions and tissue integrity. Muscle fiber thickness in Lates calcarifer from Dam (4.758 ± 0.813μm) was significantly lower than in Kund Malir (5.623 ± 0.739μm; $p < 0.01$), while fiber length showed no significant variation (24.76-5.195μm; $p > 0.05$). Histological observations revealed severe hepatic vacuolar degeneration, sinusoidal cells arrangement, and muscle necrosis in Gadani specimens, moderate tissue alterations in Dam fish, and normal architecture in Kund Malir samples. The findings demonstrate that environmental stress and pollution strongly influence fish tissue structure, confirming Gadani as the most impacted and Kund Malir as the least affected site. Additionally, the study emphasizes the importance of continuous biomonitoring and sustainable management of the Balochistan coast, support the histopathological biomarkers as sensitive indicators of environmental quality.*

Key words: *Seabass, Muscle Histology, Histopathological Alteration, Aquatic Environment, Water Quality*

1. INTRODUCTION

Examining the organs morphologically and histologically offers important information. This information described ecological adaptability, and stress responses of aquatic animals in particular environment. Coastal strip of Balochistan, an area known for its abundant marine biodiversity and ecological significance. Coast like Balochistan which lack proper monitoring system can have profound biological consequences. Understanding the physiological activities of fish, particularly in the ecologically sensitive areas along the Balochistan coast, requires examining muscle and liver tissues. These tissues are also important bioindicators of ecosystem health and environmental conditions (B. Mohanty et al., 2014). Skeletal muscle, which makes up 40-60% of a fish's body mass, is essential for development and movement and exhibits molecular alterations in response to environmental stress (Sáez-Arteaga et al., 2024). Myofibrillar, sarcoplasmic, and connective tissue proteins are the three types of muscle proteins; myofibrillar proteins, which include myosin and actin, make up 60–70% of all muscle proteins (Dara et al., 2021). Cheng et al. (2014) state that the myotome, which is made up of separate muscle fibers joined by myocommata, is in charge of muscular strength and structure. Fast-twitch (white) fibers allow for short, quick movements, whereas slow-twitch (red) fibers assist endurance swimming (Cediel et al., 2008; Nachtigall et al., 2015; Gabler-Smith et al., 2023). Fish muscles are composed of red, white, and intermediate fiber types. Because of these properties, muscle tissue is a crucial marker of both physiological well-being and the reaction to environmental stress. The liver, which is the biggest internal organ in fish, is essential for biosynthesis, detoxification, and metabolism (Long et al., 2022). It controls detoxification in the face of environmental stress, lipid metabolism, and nutrition storage (Long et al., 2021). The portal vein and hepatic artery supply 70 to 80% of the blood that the liver gets (Akiyoshi & Inoue, 2004). Plates of hepatocytes, sinusoids for nutrition exchange, and portal triads the hepatic artery, bile duct, and portal vein are its histological components (Sales et al., 2017). According to Qiao et al. (2016) and Hussein et al. (2023), the liver is a great model for evaluating the health of aquatic ecosystems because it facilitates the generation of vitellogenin, the metabolism of fat and carbohydrates, and detoxification. Fibrosis, necrosis, and vacuolation are examples of histopathological alterations that suggest infection or environmental stress (Wolf & Wolfe, 2005; Cao et al., 2023). The muscles and liver morphology under site specification in Asian seabass and seabream have not yet been described in Balochistan. However, this is identified with the influences by environmental stressors and promoted sustainable fisheries management. Histopathological examination is a sensitive and trustworthy method (Haredi et al., 2020; Shavalier et al., 2021).

The Asian seabass and seabream are marine fish species used for food in Pakistan. However, few studies have indicated the importance of biodiversity. None of the previous work has been evident the liver and muscle morphology have been conducted on these species. This study aims to study structural investigation of the muscle and liver tissues of Asian seabass and seabream. In addition, support fish health monitoring, environmental evaluation, and sustainable fisheries management in Gadani, Dam and Kund Malir Balochistani. This study explored baseline data to evaluate the tissue morphological and histological parameters in the costal belt of Balochistan.

2. MATERIALS AND METHODS

2.1 Study Area

The research was conducted across three ecologically significant coastal sites at the costal belt in Balochistan which are Dam, Gadani, and Kund Malir (Figure 1 a-c). These locations were selected due to their environmental relevance and the presence of commercially important fish species. Samples were collected with the assist of local fishermen using traditional fishing methods. This approach not only ensured the authenticity and ethicality of the sampling process.

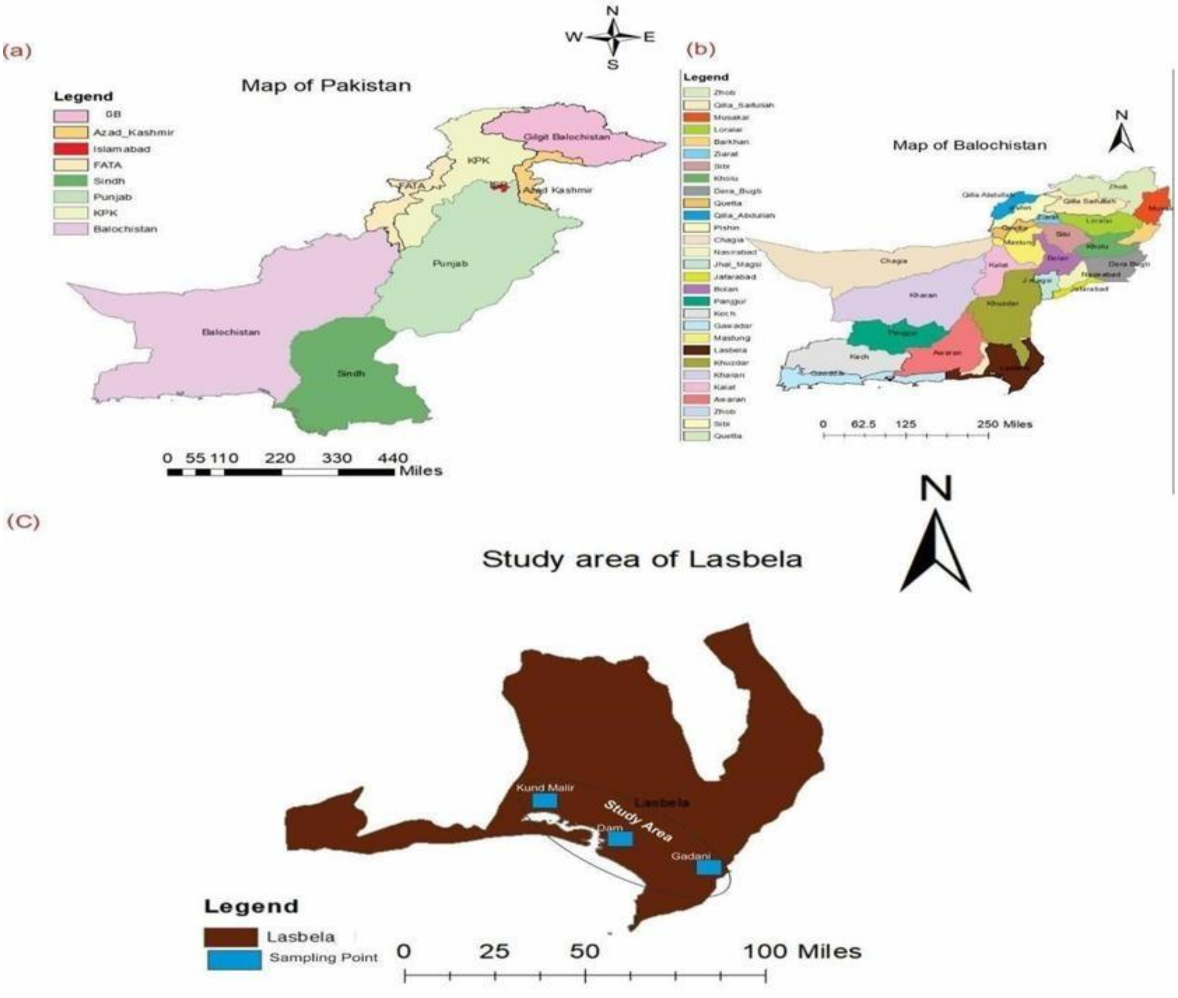

Figure 1: Map of the study area: (a) represents the map of Pakistan; (b) shows the map of Balochistan; and (c) illustrates the specific research area and sampling points

2.2 Sample Collection

Specimens of Asian seabass and seabream were collected from each site using gill nets with the assistance of local fishermen. Fish were euthanized following ethical guidelines. Morphometric measurements, including total length and body weight, were recorded prior to dissection.

2.3 Muscle Tissue Sampling

Muscle tissues were excised from the posterior and caudal regions, (Figures 2c,d and e) Samples were rinsed with deionized water to remove blood. For histological analysis, tissues were fixed in 10% neutral buffered formalin for 24-48 hours.

2.4 Liver Tissue Sampling

Liver tissues were carefully dissected from the ventral cavity of each specimen, (Figures 2c,d and e). Samples were washed in deionized water to eliminate blood residues and immediately fixed in 10% neutral buffered formalin for 24-48 hours. This fixation step ensured the preservation of hepatocyte morphology for subsequent histological evaluation.

Figure 2: This figure illustrates the sample collection and preparation: (a) samples being collected from the field, (b) dissection of the collected samples in the laboratory, and (c–e) display of the dissected samples prepared for further analysis.

2.5 Histological Analysis

Fixed tissues were dehydrated in a graded ethanol series, cleared in xylene, and embedded in paraffin wax. Sections of 4-5 μm thickness were prepared using a rotary microtome, mounted on glass slides, and stained with Hematoxylin and Eosin (H&E). Slides were examined under a light microscope (40x–100x) to evaluate muscle fiber arrangement, degeneration, necrosis, and liver histoarchitecture including hepatocyte organization, vacuolation, and sinusoidal dilation.

2.6 Morphometric Analysis

The morphometric analysis has been implemented in this study. Liver sinusoidal cells arrangement and Muscle fiber dimensions, including length, width, and height, were measured using ImageJ software.

2.7 Statistical Analysis

Data has resulted as mean ± standard deviation (SD). Differences among the sites were analyzed using one-way Analysis of Variance (ANOVA), followed by Tukey's post-hoc test where applicable. A significance level of $p < 0.05$ was used.

3. RESULTS

The result of this study provides a deep description of the histological examinations performed on the liver and muscle tissues. seabream *(Nemipterus japonicus)* and Asian seabass *Lates calcarifer*, which were obtained from three ecologically different coastal locations in Balochistan: Gadani, Dam, and Kund Malir (Figures 1b-c).

A comparison of the muscle fiber lengths of seabream (*Nemipterus japonicus*) in Dam, Gadani, and Kund Malir showed no significant differences (p=>0.05). Additionally, no morphological variation was found across the research sites, and the muscle fiber length in Kund Malir was (24.76±5.195 μm). The muscle fiber in Dam is 25.49±2.650μm in length. However, the muscle fiber in Gadani findings has been measured as 24.32±3.166 μm which are slightly closer to Kund Malir site but lower to Dam.

Using Hematoxylin and Eosin (H&E) staining the muscle fiber thickness of seabass (*late calcarifer*) revealed a significant difference between Dam, Gadani, and Kund Malir (Figure 3), (**p<0.01). For seabream no morphological variation was found among the sites. However, Kund Malir's muscle fiber thickness was measured as 5.612±0.760 μm). Dam's muscle fiber has a thickness of 5.385±0.688 μm. The muscle thickness in Gadani site was measured as 5.069±0.612 μm) in which fish mentioned name, this has bit confusion. Seabass (*late calcarifer*) muscles fiber thickness analysis shows significant difference between Dam and Kund Malir (**p<0.01), there was no morphological difference between Kund Malir and Gadani. observed in which muscle fiber thickness of Kund Malir is (5.623±0.739 μm). The thickness of muscle fiber Dam is (4.623±0.813μm), in Gadani this observed with (5.661±0.852μm).

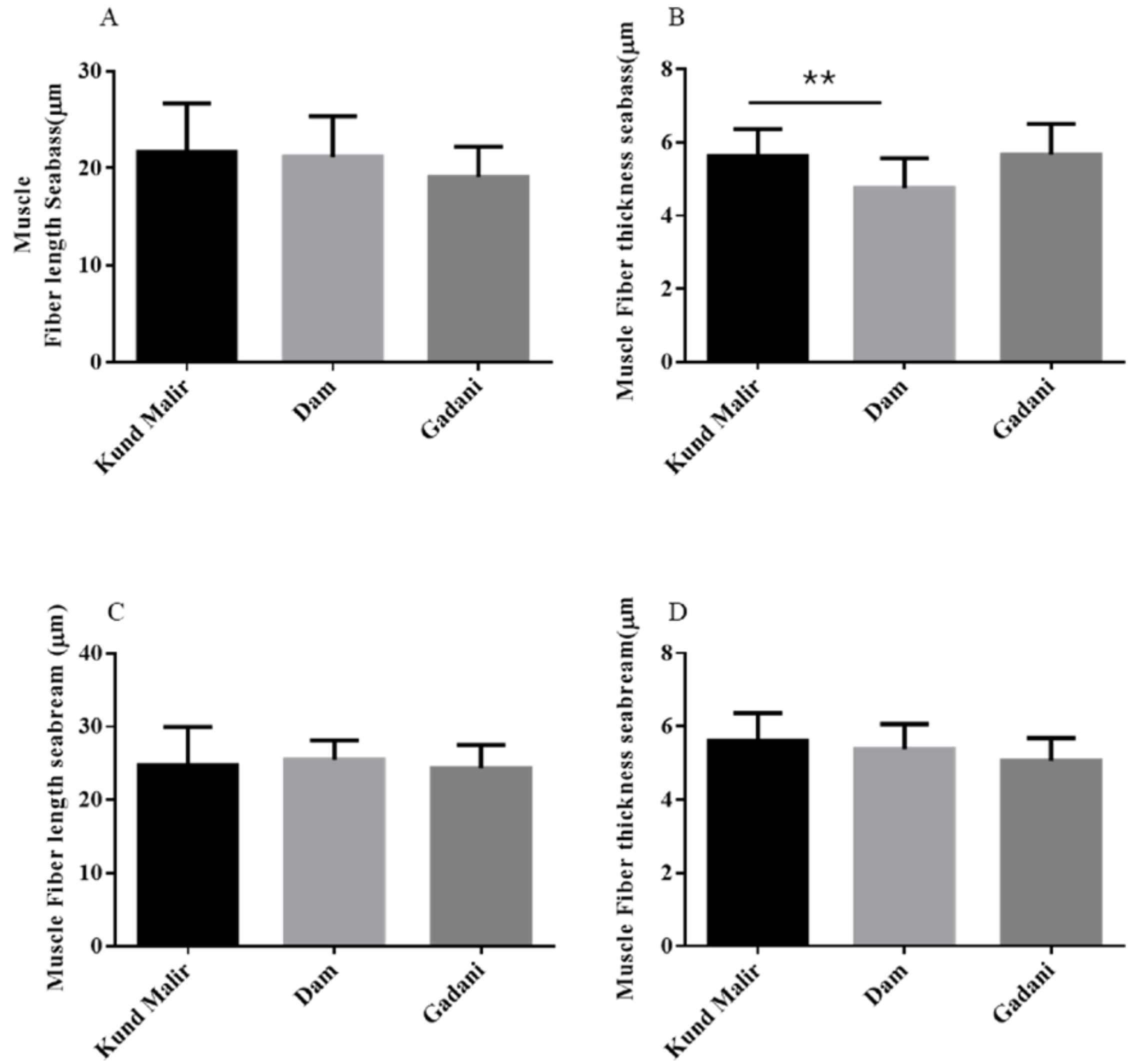

Figure 3: Muscles Fiber Length and Thickness in Seabass (A & B) and Seabream (C &D)

The measurements of muscle fiber thickness and fiber length in Seabass *(Lates calcarifer)* and seabream *(Nemipterus japonicus)* collected from Dam, Gadani, and Kund Malir showed no significant variations in fiber length across the three sampling sites. However, a notable reduction in muscle fiber thickness was observed in specimen collected from Dam when compared to those from Kund Malir.

Table 1: Comparison of Muscle Fiber of Length and Thickness Across the sites

Species	Sites	Length (µm)	Thickness (µm)	Significance (length)	Significance (Thickness)
Seabass (*lates calcarifer*)	Kund Malir	21.67±4.997	5.623±0.739	p>0.05(not significant	p>0.05(not significant)
	Dam	21.15±4.214	4.758±0.813	p>0.05(not significant	p<0.01(significantly different
	Gadani	19.08±3.147	5.661±0.852	p>0.05(not significant	p>0.05(not significant)
Seabream (*Nemipterus japonicus*)	Kund Malir	24.76±5.195	5.612±0.760	p>0.05(not significant	p>0.05(not significant)
	Dam	25.76±2.650	5.385±0.688	p>0.05(not significant	p>0.05(not significant)
	Gadani	24.32±3.166	5.069±0.612	p>0.05(not significant	p>0.05(not significant)

The muscle fibers in fish from the Dam may be attributed to environmental stressors, including potential pollution or suboptimal water quality conditions that can negatively impact muscular development. Muscle fiber thickness is a recognized indicator of fish health and growth performance, reduced thickness often reflects chronic exposure to environmental contaminants, reduced food availability, or metabolic stress. The findings highlight the potential influence of site-specific ecological conditions on muscle physiology, further investigation into environmental factors such as water chemistry, pollutant load, and habitat quality in the Dam area.

3.1 Histological Analysis of Muscles

The muscle tissues of Seabass *(Lates calcarifer)* and seabream *(Nemipterus japonicus)* were collected from various study sites and subjected to HE staining. *Lates calcarifer* from Gadani showed severe muscle fiber degeneration with extensive fragmentation and large gaps as shown in (Figure 4A). *Lates calcarifer* from Dam showed moderate muscle fiber disorganization with visible gaps and signs of muscle atrophy (Figure 4B). *Lates calcarifer* from Kund Malir had very clear organization of muscle (Figure 4C). *Nemipterus japonicus* from Gadani showed severe muscle damage with fragmented fibers and large empty spaces inflammation (Figure 4D). *Nemipterus japonicus* from Dam showed moderate muscle degradation and fiber misalignment (Figure 4F). *Nemipterus japonicus* from Kund Malir, which closely resembles healthy muscle histology due to its well-organized muscle structure (Figure 4F).

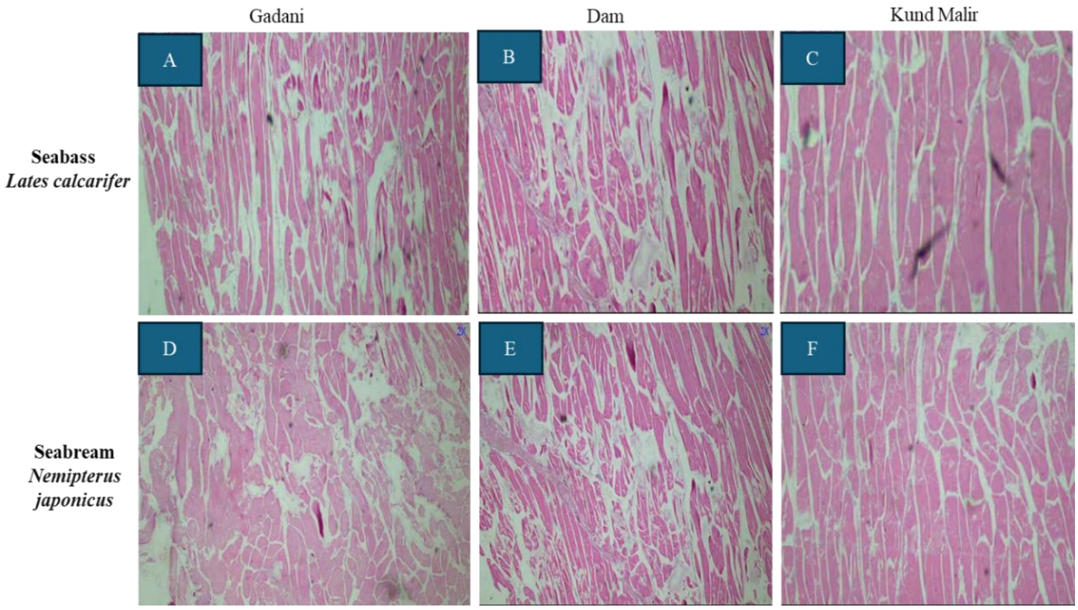

Figure 4: Hematoxylin and Eosin (H&E) staining of muscle tissues of Seabass *(Lates calcarifer)* and seabream *(Nemipterus japonicus)*. (A) Indicates *Lates calcarifer* from Gadani (B) Represents the *Lates calcarifer* from Dam (C) Shows the *Lates calcarifer* from Kund Malir, (D) Indicates the *Nemipterus japonicus* from Gadani (E) Represents the *Nemipterus japonicus* from Dam. Arrows; indicate gaps between the muscle fibers. 40X magnification.

3.2 Histological Analysis of Liver Tissue

Hematoxylin and Eosin (H&E) staining was applied on liver tissues to examine histomorphology. Seabass (*Lates calcarifer*) from Gadani (Figure 5A) displaying hepatocytes with vacuolar degeneration and sinusoidal dilatation (Figure 5B); Dam-Seabass (*Lates calcarifer*) from study site of Dam displaying inflammatory cell infiltration and necrotic hepatocytes (Figure 5C). Kund Malir Seabass (*Lates calcarifer*), normal hepatic tissue with well-organized hepatocytes and clear sinusoids; (Figure 5D) Gadani Seabream (*Nemipterus japonicus*) displaying hepatocellular disorganization, necrosis, and inflammatory infiltration; (Figure 5F) Kund Malir Seabass (*Nemipterus japonicus*) normal hepatic tissue with clear sinusoidal spaces and well-arranged hepatocytes.

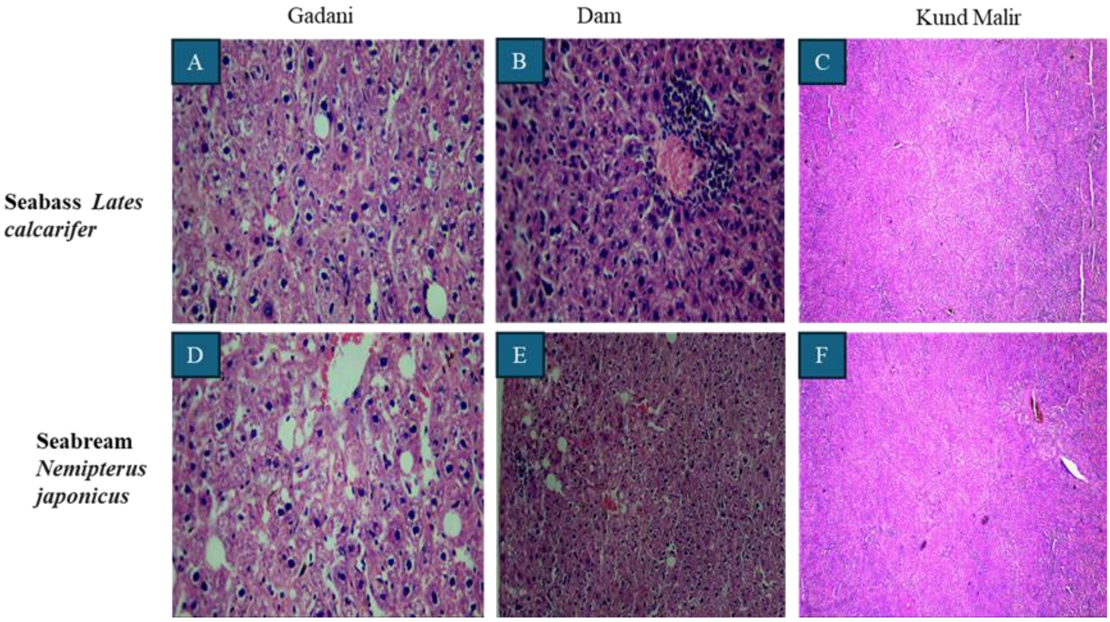

Figure 5: Hematoxylin and Eosin (H&E) staining was used on liver tissues. (A) Indicates *Lates calcarifer* from Gadani (B) Represents the *Lates calcarifer* from Dam (C) Shows the *Lates calcarifer* from Kund Malir, (D) Indicates the from *Nemipterus japonicus* Gadani (E) Represents the *Nemipterus japonicus* from Dam (F) Indicates the from *Nemipterus japonicus* Kund Malir. Arrows; indicate gaps between the muscle fibers. 40X magnification.

Table 2: Histological Observation of Liver Across the Three Study Sites Summarized.

Species	Site	Liver histology observation
Seabass (*Lates calcarifer*)	Kund Malir	Normal liver histology with clear sinusoids and well arrangement hepatocytes
	Dam	Presence of inflammatory cell infiltration and necrotic hepatocytes observed
	Gadani	Hepatocytes showed vascular degeneration and sinusoidal dilatation.
Seabream (*Nemipterus japonicus)*	Kund Malir	Liver tissue appeared normal with well-organized hepatocytes and minimal pathological changes
	Dam	Moderate hepatic changes such as inflammatory signs and mild cell degeneration observed
	Gadani	Hepatocellular disorganization, necrosis, and inflammation were prominent

4. Discussion

The current study examined the morphological and histological characteristics of the liver and muscle tissues in *Lates calcarifer* and *Nemipterus japonicus* from three coastal locations of Balochistan: Kund Malir, Dam, and Gadani. Seabass and seabream are widely studied species (Rosati et al., 2025). Seabream has shown strong development and flexibility in a range of environmental conditions (Bánó et al., 2024). At a 6% body weight feeding rate, Asian seabass had excellent development and physiological health, demonstrating the need for proper nutrition in preserving normal tissue structure (Ul Hassan et al., 2021). Seabream demonstrated that better dietary and environmental circumstances increased physiological performance and overall tissue health (Matadamas-Guzman et al., 2019). Seabream from the coast of Sindh (Ali et al., 2023) showed good growth under appropriate nutritional and climatic circumstances, supporting the current findings for seabream from the coast of Balochistan, which have shown similar histological changes in seabream tissues linked to dietary and environmental variables.

In the present studies seabass and seabream both species, myofibrillar organization and myotomal architecture stayed essentially normal. Particularly, Kund Malir samples had dense fiber packing and characteristic fascicular patterns. Only modest deviations from this baseline were seen in the specimens from Dam and Gadani: very slight fiber misalignment, a slight decrease in packing density, and small space between fibers. Significantly, there were no signs of pathogenic features including necrosis, widespread fragmentation, inflammatory infiltration, or noticeable atrophy.

These findings are consistent with a number of comparative studies that demonstrate how marine teleost muscle tissue frequently maintains structural integrity in the face of mild or temporary disruptions. According to Shahid et al. (2022), while maintaining overall myofibrillar organization, fish muscle can exhibit only minor architectural alterations (mild fiber separation, slight atrophy) across sites with varying water quality. In a similar vein, (Goralskyi et al., 2024) discovered that in fish populations that experience regular seasonal and dietary change, normal muscle histology continues to be the predominant type. Recent morphological and histological descriptions of Lates calcarifer show the species' strong muscular architecture under a variety of handling circumstances and life stages (Malila et al., 2024; (Takahashi et al., 1985). The little muscle abnormalities found in Dam and Gadani are comparable with low-magnitude, non-pathological variability seen elsewhere, according to these comparative studies.

Kund Malir's liver slices showed clear sinusoids, polygonal hepatocytes with identifiable nuclei, and well-organized hepatic cords. Although there were sporadic moderate vacuolization and minor cytoplasmic changes in the Dam and Gadani samples, the general parenchymal architecture was still identifiable and functioning. The specimens under examination did not exhibit broad inflammatory infiltrates, significant sinusoidal collapse, or extensive necrotic zones.

According to the literature, marine fish populations frequently exhibit modest hepatic vacuolization and slight alterations in cytoplasmic texture, which may indicate normal metabolic or nutritional dynamics rather than obvious disease (Santos et al., 2022; Topić Popović et al., 2023). For instance, Santos et al. (2022) found that seasonal patterns of moderate hepatocellular vacuolization in freshwater fishes were associated with metabolic cycles rather than permanent damage. Similarly, hepatocyte appearance varies with food composition or feeding history, and liver shape can revert to baseline once circumstances alter (Donadelli et al., 2024; Caballero et al., 2004). Small hepatic vacuoles, mild sinusoidal dilatation, or minimal cytoplasmic clearance are typical and should be evaluated in light of numerous lines of evidence, according to recent reviews and multi-marker studies (Jerald, 2024; Pramanik et al., 2024).

5. CONCLUSION

In the current study, tissue integrity and environmental quality were shown to be strongly correlated in seabass (*Lates calcarifer*) and seabream (*Nemipterus japonicus*) obtained off the coast of Balochistan. Due to industrial and human-caused contamination, fish from Gadani showed significant muscle degeneration and liver damage, but those from Dam showed modest changes. On the other hand, the fish from Kund Malir displayed normal liver and muscle histoarchitecture, suggesting that there was little environmental stress. These results demonstrate that fish histopathological alterations are reliable indicators for evaluating aquatic pollution and ecosystem health. The findings show how urgently sustainable fisheries management, efficient pollution control, and ongoing biomonitoring are needed to preserve the biological integrity of Balochistani coastal ecosystems.

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