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Paper

Quality monitoring of fish through histological assessment of their health status: Proposal for a New Scoring System

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Abstract

The quality and health of fish are closely intertwined. This study aimed to unveil the effectiveness of histological techniques in characterizing fish health status and deducing their quality. A new classification scoring model was also proposed to provide a holistic assessment. A total of 80 silver carp (*Hypophthalmichthys molitrix*) specimens were collected from a freshwater dam, in two areas (Z1-Z2) at two periods (July and September), with twenty fish per sampling. The physico-chemical parameters of the water were measured, and a global pollution index (IGP) was calculated. Histological analysis was carried out on five vital organs (gills, kidneys, liver, intestines, and muscles) using a standard method, specifically the semi-quantitative system of Bernet et al. (1999), to determine the condition of each organ, enabling an organic index (OI) to be assigned (IG, IK, IL, II, IM). These indices were combined to obtain total scores for each specimen, named the IFish. The latter is explored as a biomarker of fish health status and quality to develop an innovative classification system. The results showed that the indices were generally notable in September in Z1, illustrating a significant spatio-temporal influence. A highly significant correlation was observed between the IFish and IGP (P = 0.0001). The proposed scoring system made it possible to classify fish according to their quality. To conclude, a healthy fish is a reliable indicator of its quality, and the use of histology, in particular through the IFish scoring, proved to be an effective biomarker to demonstrate this.

Keywords

Biomarker, Fish, Health, Histological techniques, Quality, Scoring

Introduction

Fish continue to be very popular with consumers. Over the last three decades, both their nutritional value and health benefits have been widely and effectively emphasized (Balami et al., 2019; Phogat et al., 2022a; Torfadóttir and Ulven, 2024). However, consumers may question their quality, especially that of freshwater fish. In some countries, the fisheries sector plays an important socio-economic role, particularly for those that are producers, consumers, and exporters of fish products (Cheikhna, 2009; Yaou et al., 2016). In Algeria, the Ministry of Fisheries and Aquatic Productions forecasts freshwater aquaculture production of fifty-three thousand tons by the end of 2025 (MPRH, 2008) and more than one hundred thousand tons by 2030 (BTR1 de l'Algérie, 2024). This goal is expected to be achieved largely through the exploitation of inland waters, including intensive fish farming, semi-intensive, and extensive livestock farming (MPRH, 2008). In fact, Algeria's freshwater fish fauna consists of at least sixty species, half of which are introduced (Kara, 2012). Production is mainly based on the capture of carp from restocking lakes and dams. This activity encompasses larvae and juvenile specimens of multiple species, notably including common carp (*Cyprinus carpio*), bighead carp (*Hypophthalmichthys nobilis*), silver carp (*Hypophthalmichthys molitrix*), zander (*Sander lucioperca*), black bass (*Micropterus salmoides*), nile tilapia (*Oreochromis niloticus*), and, to a lesser degree, bighead mullet (*Mugil cephalus*) (Naji et al., 2023). Meanwhile, consumer demand for high-quality, secure and healthy food is increasing worldwide (Alsubhi et al., 2023). Consumer studies suggest that quality remains the main criterion for fish purchases (Alasalvar et al., 2011; Bernardi et al., 2013; Les études, 2023). In fact, the high dietary value of fish offers the ideal food source. However, fishery products are extremely perishable, with a relatively high rate of spoilage after fishing (Yaou et al., 2016; Ali et al., 2022; Phogat et al., 2022b). Indeed, fish spoilage mechanisms are very intricate and linked to different intrinsic and extrinsic factors, which constitute a threat to both fish and consumers (Zhuang et al., 2021; Tahiluddin et al., 2022; Sequino et al., 2024). Thus, fish deterioration involves a range of physical, chemical and microbiological processes. These processes can be assessed by several methods, including sensory, chemical and microbiological (Nik Zad Sangari, 2013; Agüeria et al., 2016; Latifou et al., 2019; Castrica et al., 2021). Nevertheless, the overall effect of all these mechanisms is reflected in the histological state of an organ (Van der Oost et al., 2003; Van Dyk et al., 2009; Saraiva et al., 2015; Salamat and Zarie, 2016). In fact, the use of histological techniques is acquiring interest for fish quality control. Apart from a species-by-species approach, this work aimed to assess the relevance and effectiveness of using histological techniques, especially the semi-quantitative system proposed by Bernet et al. (1999), as a tool to untangle the health status of fish and to assess their quality. Thereby, it substantiates the hypothesis that asserts the correlation between fish health and quality. This system, applied for the first time in Algeria to the best of our knowledge, has undergone minor modifications in terms of nomenclature. Additionally, we are proposing an innovative classification model for a global assessment of fish health and quality, which represents the original contribution of our research. This approach is holistic in nature, as it considers both the intensity of the histopathological lesions and their multi-organ distribution. Furthermore, it provides an integrated analysis that synthesizes histological-quantitative and qualitative criteria to accurately convey the health and quality status of fish.

Materials and methods

Sampling area and period

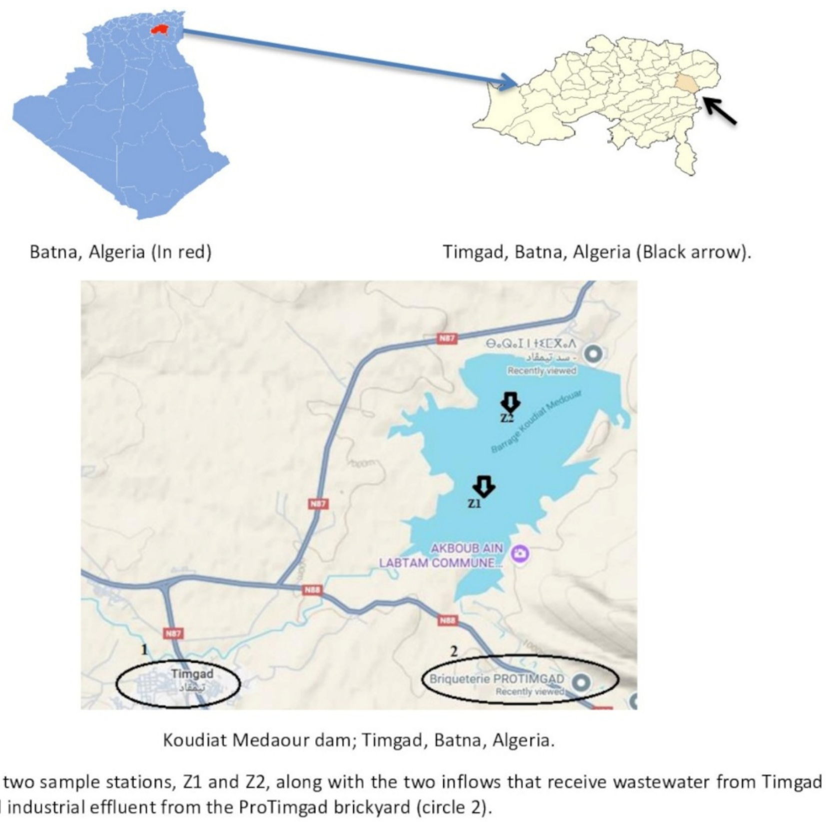

Figure 1. Geographical overview of the study area.

Our study was carried out at the Koudiat Medaour reservoir, a gravity dam in the Oued Chemora valley. It belongs to the Beni-Haroun complex system, with a surface area of 590 km^2. It is located about 35 km from Batna, in northeastern Algeria, at a longitude of 06°30'32.61" E and latitude of 35°31'27.03 " N. Fish and water were sampled during July and September 2024, from two zones, Z1 and Z2, where fishing activity is significant, to have a diversity of sampling (Figure 1). In fact, this is a retrospective study.

Water quality

Water quality parameters measured were of a physico-chemical and organic nature. Next, the global pollution index (IGP) was calculated according to Sargaonkar and Deshpande (2003); and James et al. (2013).

Fish sampling procedures

Silver carp, *Hypophthalmichthys molitrix*, is the major component of our study area. Fish were sampled in July and September (N = 20 per zone). They were captured using standard fishing techniques.

Histological assessment

Histological techniques and tissue sampling

Samples of gill, liver, kidney, intestine, and axial muscle were taken and fixed in a 10% neutral buffered formalin solution for 48 hours. For the gills, a 12 hour decalcification was also performed 1. After fixation, the samples were washed with tap water and prepared for histological analysis using standard techniques 2. An overnight program was adapted in an automate system, and tissues were dehydrated in a series of increasing concentrations of ethanol (70, 80, 90, and 100%, one hour for each) and clarified with xylene. Then, ribbons of 5-7 µm in thickness were obtained using a paraffin microtome. Sections were stained with haemaatoxylin-eosin (H&E) according to a standard protocol (Luna, 1968). Finally, for microscopic observation, the slides were analysed at magnifications of x100, x400, x800, and x1000. The micrographs were obtained using a USB camera. The images were acquired and displayed on a monitor for bioimaging.

Histopathological analysis and diagnosis

The slides were diagnosed in a blinded manner, even repeating certain examinations to ensure that the same result would be obtained. Tissue alterations were identified on the basis of fish histology atlases (Hibiya et al., 1984; Genten et al., 2009; Mothtar, 2021) and other related articles which will be cited as the results are discussed.

For the histopathological analysis, the semi-quantitative histological system developed by Bernet et al. (1999) was used. Briefly, for each organ, histopathological alterations were classified into five lesion categories, which in turn comprise several alterations that are scored. Lesion patterns cover circulatory, regressive, progressive, inflammatory, and neoplastic lesions. Scores ranged from 0 to 6 according to severity. Next, two basic factors were assigned to each alteration individually. The importance factor (w) ranging from 1 to 3, according to the pathological significance of the alteration, and the score value (a) ranging from 0 to 6, according to its extension. Intermediate values were also considered. For each diagnosed alteration, the final value is obtained by multiplying the score value by the importance factor. The sum of these final values for a lesion category gives the index of the respective reaction pattern, i.e., the index of circulatory (ICir), regressive (IReg), progressive (IPro), inflammatory (IInf), and neoplastic or tumor (ITum) lesions. To obtain the organ index (IO), the calculated indices for each lesion profile are used. For instance, in the liver (L), these indices became IC-L, IR-L, IPro-L, II-L, and IT-L. The sum of these indices gives the total organ index (IL: Liver index). Finally, the sum of the IOs (gill index [IOG], liver index [IOL], kidney index [IOK], intestine index [IOI], and muscle index [IOM]) per fish gives the total fish index, IFish, indicating the combined histological response of the analysed organs for each fish. These index value assessment tools can be summarized as follows:

For a given alteration (X) the final value or index is IX= ax × wx. ax is the score value for a given alteration (X), wx is its importance factor.

The index for a given pattern (P) is IP = Σ IX = Σ (ax × wx). For instance, the index of circulatory pattern in the liver is ICir-L = Σ (aC-L × wC-L); where C and L refers to circulatory alteration and liver, respectively.

The organ index IO = Σ IPs. For example, in the liver, IOL = (IC-L) + (IR-L) + (IPro-L) + (II-L) + (IT-L). IFish = Σ IOs =

[IOG] + [IOL] + [IOK] + [IOI] + [IOM].

Proposed classification model

Different indices can be obtained and interpreted depending on how the researcher intends to discuss the results. The proposed classification is inspired by the scheme presented by Zimmerli et al. (2007). In brief, IO values were used to classify and distinguish fish with pathologically altered organs from non-altered ones into five categories, namely: Class I: IO ≤ 10; Class II: IO between 11-20; Class III: IO between 21-30; Class IV: IO between 31-40; Class V: IO > 40.

Here, we developed a new classification system for a holistic assessment of fish health and quality, integrating both lesion severity and their multi-organ distribution. It is based on the quantitative assessment of histopathological alterations that reflect IFish scorings. Five categorical patterns are identified:

Class I: IFish < (10 × n): fish of excellent quality, healthy, in good condition with absent or minimal alterations. Normal tissue structure and no impact on health.

Class II: (10 × n) ≤IFish < (30 × n): good quality fish, slightly affected with slight alterations. First signs of histopathological stress, but with limited impact on health.

Class III: (30 × n) ≤ IFish < (60 × n): fish of average quality, moderately altered with controlled changes affecting several organs. Incipient functional disturbance and potential health impact.

Class IV: (60 × n) ≤ IFish < (100 × n): fish of poor quality, deeply affected with pronounced alterations. Marked degradation of morphology significantly reducing organ functions.

Class V: IFish > (100 × n): fish of critical quality and condition with severe and diffuse lesions. High risk of multi-organ failure (fish is in imminent danger of death).

This classification is dependent on the number of studied organs (n). IFish values will necessarily be high, since they combine several organ indices. The classification must therefore be adapted to this requirement, given that IFish scores increase linearly with the number of analysed organs. Thus, the classification adopted in this study, for five organs, is defined as follows: class I: IFish < 50; class II: 50 ≤ IFish < 150; class III: 150 ≤ IFish < 300; class IV: 300 ≤ IFish < 500; class V: IFish > 500.

The IFish classification thresholds (10, 30, 60, 100) are defined based on statistical analysis using robust statistical approaches, primarily skewed data distribution analysis, given that our data follow an abnormal distribution (Shapiro test). According to this approach, the classification thresholds are:

A threshold of 10, close to the mean or the first standard deviation.

A threshold of 30, corresponding to 2σ (two standard deviations above the mean).

A threshold of 60, corresponding to 3σ (three standard deviations above the mean).

A threshold of 100, corresponding to 4σ (four standard deviations above the mean).

Statistical analysis

Results for all analysed parameters were processed using Microsoft Excel 2019 spreadsheet software. Statistics were carried out using MedCalc.Ink software version 22.030. Water analysis parameters were calculated using an independent two-sample parametric T-test. The various biological scores were tested for normality using the Shapiro-Wilk test. Correlation was assessed using the Spearman test. The different scores were compared by Mann-Whitney analysis, for two variants, and Kruskal-Wallis for a multi-comparison test. Significance levels were set at 0.05.

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Results

Water quality

Water quality parameters are presented in Table I. Apart from temporal fluctuations, these parameters revealed certain stability between the two sites for some parameters, while others showed significant differences. Overall, the most polluted water occurs in Z1 during September (IGP: 292.94).

		July		September	
		Z1	Z2	Z1	Z2
Temperature (°C)		25.28^{b**}	25.08^{b**}	23.28^{b**}	23.09^{b**}
pH		8.18^{b*}	7.48^{b*}	8.15^{b*}	7.45^{b*}
Turbidity (NTU)		26.28^{a**}	0.80^{b**}	47.02^{b*}	0.81^{a*}
Conductivity (µs/cm)		1326.35	1342.98	1229.72	1246.25
Dissolved oxygen (mg/l)		7.37^{b**}	7.46^{b**}	7.50^{b**}	7.76^{b**}
Ammonium (mg/l)	NH_4^+	0.14^{b**}	0.02^{b**}	0.21^{b**}	0.02^{b**}
	Nitrate (NO_3^-)	3.12^{a**}	3.56^{a**}	1.75^{a**}	1.81^{a**}
	Nitrite (NO_2^-)	0.03^{b**}	0^{b**}	0.07^{b**}	0^{b**}
Phosphates PO_4^{-3} (mg/l)		0.01^{b**}	0^{b**}	0^{b**}	0^{b**}
Sulfates SO_4^{-2} (mg/l)		264.1^{a*}	285.8^{a*}	200.31^{a*}	232.12^{a*}
Suspended matter SM (mg/l)		80^{b**}	78.07^{a**}	83.16^{b**}	85.09^{a**}
Organic matter MO (mg/l)		3.52^{b*}	1.60^{b*}	3.92^{b*}	1.99^{b*}
Copper Cu (mg/kg)		29.12^{b*}	23*	27^{b**}	29.76**
Chrome Cr (mg/kg)		106.88^{a**}	104.95^{a**}	110.09^{a**}	108.16^{a**}

Table I. Water physicochemical parameter analysis results. The symbols a and b denote temporal differences, while * and ** represent spatial differences. a and *: a significant differences; b nd **: no significant differences.

Histological analysis and diagnosis

The mean lesion and organ index values are summarized in Table II. Additionally, the mean values of IFish, IGP indices and the percentage of lesion indices are illustrated in Table III. The class distribution of the IFish scores is shown in Figure 2. In general, I Reg was the dominant pattern, and there were no neoplastic lesions except for those observed in the liver. The IO values are all above 17, and they correlated very significantly with IGP (P=0.0001 for all organs). In terms of alteration, we note that the most significant values were almost always recorded in September in Z1. This observation is consistent with the results of the water analysis.

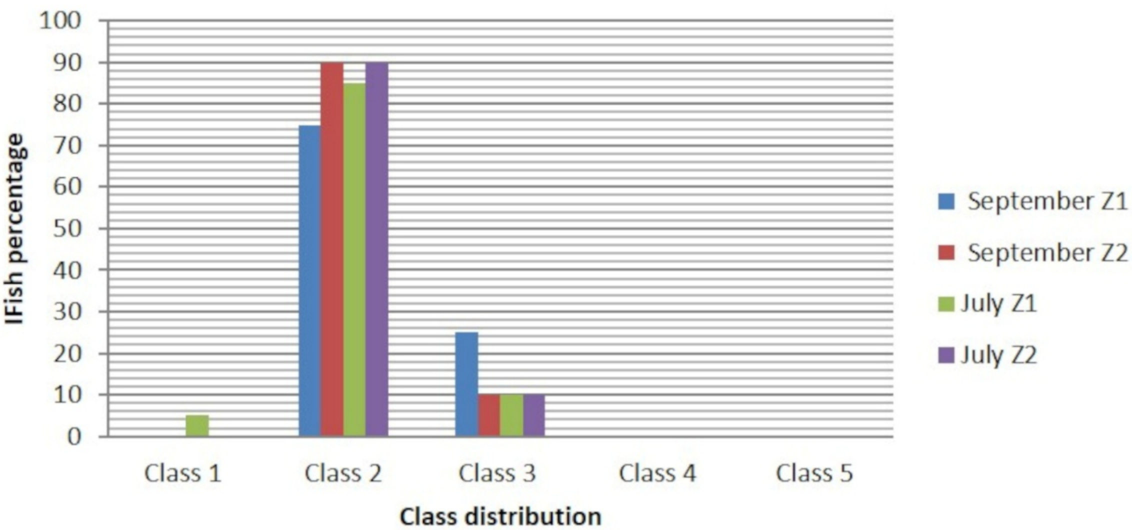

Figure 2. Class distribution of IFish values.

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Organ	Period	Zone	ICir	IReg	IPro	IInf	ITum	IO	
Grill	July	Z1	2,54	15,94	4,91	1,76	0	IB	25,1
		Z2	2,16	15,44	4,41	1,95	0		23.96
	September	Z1	3,96	17,44	6,40	2,89	0		30,68
		Z2	3,01	16,44	5,41	2,09	0		26,95
Kidney	July	Z1	2,28	22,95	1,88	1,03	0	IR	28,12
		Z2	1,80	22,45	2,13	1,03	0		27,37
	September	Z1	3,70	24,45	2,80	2,03	0		32,98
		Z2	2,75	23,45	2,15	1,33	0		29,67
Liver	July	Z1	1,95	19,15	1,65	1,89	0,2	IF	24,83
		Z2	1,50	18,65	1,88	1,73	0,25		24
	September	Z1	3,20	20,65	2,88	3,09	0,25		30,06
		Z2	2,35	19,65	1,93	2,29	0,2		26,41
Intestine	July	Z1	1,35	14,50	1,50	0,32	0	II	17,30
		Z2	1,20	13,25	1,33	0,65	0		17,17
	September	Z1	2,35	16,00	1,73	0,95	0		21,02
		Z2	1,70	15,00	1,33	0,40	0		18,42
Muscle	July	Z1	1,23	17,19	1,96	1,00	0	IM	21,37
		Z2	0,90	16,69	1,56	0,78	0		19,92
	September	Z1	2,20	18,69	3,35	1,75	0		25,98
		Z2	1,55	17,69	2,41	1,20	0		22,85

Table II. Mean values of lesion indices and IO indices. The circulatory index (ICir), regressive index (IReg), progressive index (IPro), and inflammatory index (IInf) are used to denote the respective pattern. The IOG: gill index, IOL: liver index, IOK: kidney index, IOI: intestine index, and IOM: muscle index, are used to denote the respective organs. The symbols a, b and c denote temporal differences, while *, ** and ** represent spatial differences. a and *: a very significant differences; b and **: a significant differences; c and ***: no significant differences.

Period	Zone	IFish	ICir	IReg	IPro	IInf	ITum	IGP
July [b]	Z1**	111.89^{ρ}	7.99^{++}	76.81^{++}	$9,88^{++}$	5.12^{+}	0.17	237.51^{ρ}
	Z2*	114.73^{ρ}	4.27^{+}	49.33^{+}	$6,39^{+}$	39.85^{++}	0.14	204.34^{ρ}
September [a]	Z1**	140.75^{ρ}	10.95^{++}	69.08^{++}	$12,18^{++}$	7.6^{++}	0.17	292.94^{ρ}
	Z2*	124.31^{ρ}	9.13^{+}	74.18^{+}	$10,63^{+}$	5.87^{+}	0.16	269.85^{ρ}

Table III. The mean values of the IFish, IGP, and lesion indices (represented as a percentage). The circulatory index (ICir), regressive index (IReg), progressive index (IPro), and inflammatory index (IInf) are used to denote the respective pattern. While IGP refers to global pollution index. The symbols a and b denote temporal differences, the * and ** represent spatial differences, while ρ denotes the correlation between the IFishs and the IGP. a and **: a significant differences; b and *: no significant differences. The symbols + (significant) and ++ (highly significant) represent the predominant lesions within a period.

Gills

Gills ranked second in terms of lesion sensitivity. The average IOG (gill index) in July, were lower, Z1 (25.1) > Z2 (23.96) with no significant difference ($P = 0.1194$). Whereas in September values reached 30.69 at Z1 and 26.95 at Z2, with a very significant difference ($P = 0.0019$). Comparing the two periods, there was a very significant difference in Z1 ($P = 0.0012$) compared to Z2 ($P = 0.009$). Regressive lesions dominated, with progressive lesions taking second place. Circulatory and inflammatory lesion indices were nearly similar (Figure 3-A).

Kidneys

Kidneys were the most affected organs, with higher average IOK (kidney index) scores in September, Z1 (32.98) > Z2 (29.67), expressing a significant difference ($P = 0.0104$). In July, values were lower, with Z1 (28.12) > Z2 (27.37) showing no significant difference ($P = 0.42$). Comparing the two periods, there was a very significant difference in Z1 ($P = 0.0057$) compared with Z2 ($P = 0.163$, no significant difference). As for lesions, regressive alterations were clearly dominant, with very high indices when compared with circulatory and inflammatory scores. Proliferative lesions, although rare, were more visible in September in both zones (Figure 3-B).

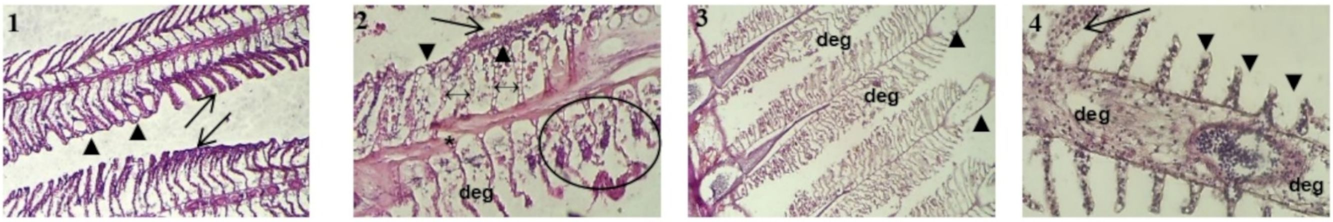

Figure 01-A: Gill: 1: epithelial lifting and hyperplasia (arrows), secondary lamellae fusion (arrowheads) ×100; **2**: rupture of the primary lamella (asterisk), epithelial hyperplasia(arrows) leading to a fusion and clubbing of the secondary lamellae (arrowheads), mild vascular dilatation congestion and haemorrhage (left-right arrow), secondary lamellae degeneration (deg) and necrosis (circle) ×200; **3**: haemorrhage, secondary lamellae appear irregular and disorganized showing degeneration (deg), terminal vasodilatation (arrowheads) ×100; **4**: hyperplasia (arrow), atrophy of the secondary lamella (arrowheads), degeneration of the interstitial tissue (deg) and leukocyte infiltration ×400.

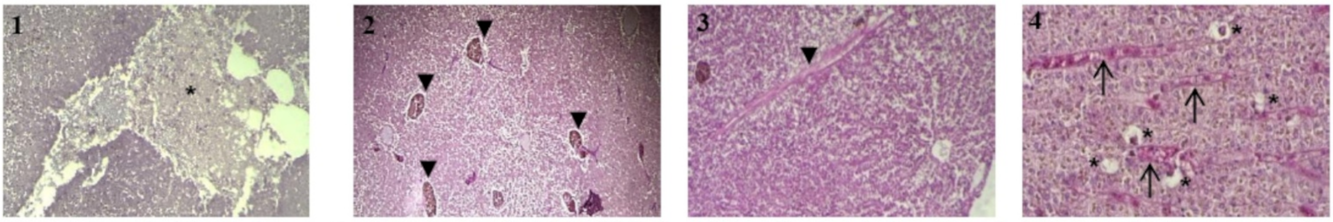

Figure 01- B: Liver: **1** hepatic necrosis (asterisk) ×100; **2**: mildly affected liver with cellular vacuolisation and MMC (arrowheads) ×40; **3**: hepatic steatosis, note extensive fibrosis (arrowheads) ×100; **4**: sinusoidal dilatation with hyperaemia (arrows), cellular vacuolisation and early-stage degeneration of hepatocytes (asterisks) ×400.

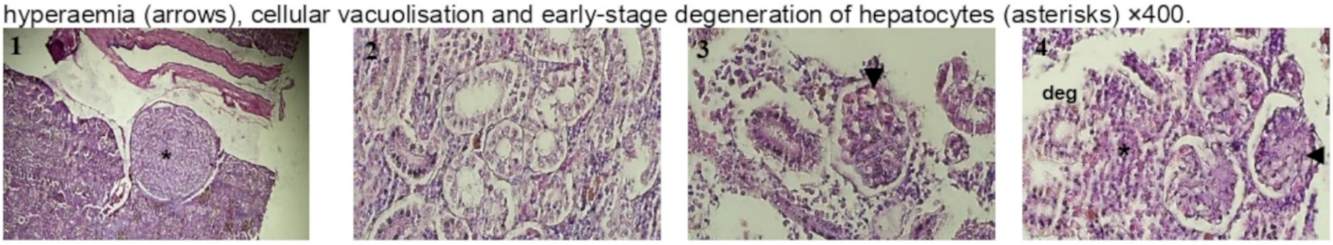

Figure 01- C: Kidney: **1**: nephrocalcinosis (asterisk) ×40; **2**: Interstitial and tubular degeneration: with epithelial cell vacuolization and loss of brush border, epithelial cell desquamation ×400; **3**: Glomerular telangiectasia (arrowheads), tubular necrosis, interstitial necrosis and infiltration: a clear sign of interstitial nephritis ×400; **4**: glomerular alteration with glomerulosclerosis (arrowheads), interstitial necrosis (asterisk), tubular degeneration (deg) ×4

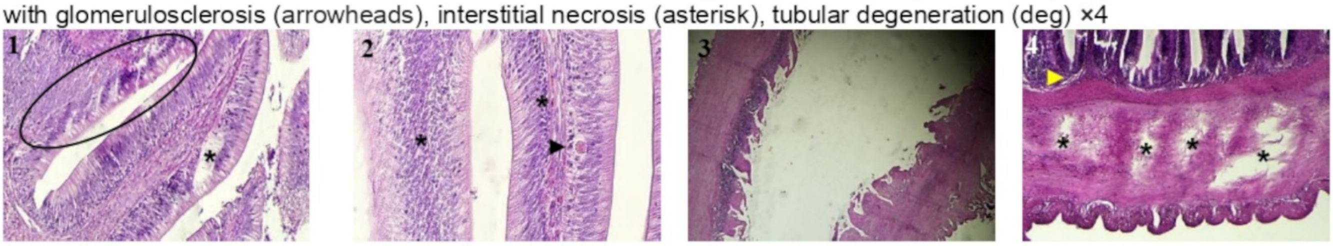

Figure 01- D: Intestine: **1**: separation of the epithelial layer (asterisk), epithelial alteration an increase in goblet cells ×200; **2**: leukocyte infiltration of epithelial layer (asterisk), note the coccidian-type oocysts (arrowhead) ×400; **3:** sever destruction of villi ×40; **4:** sever mucosal layer disintegration (yellow arrowheads) and muscular degeneration (asterisks) ×100.

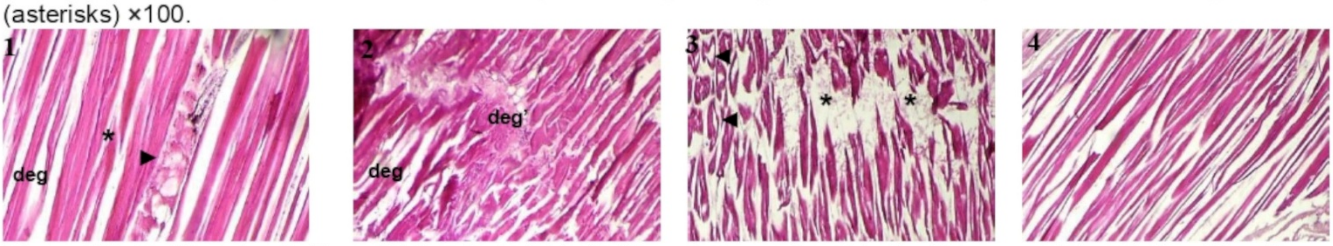

Figure 01- E: Muscle: **1**: fibre division (asterisk), fibrillary degeneration (deg) with segmental and discoid patterns within plaques form (arrowhead) ×200; **2**: disintegration and rupture of fibres, degeneration of fibres (deg) and the connective tissue (deg') ×100; **3**: fibre size variability with degeneration fragmentation, and eosinophilic inflammatory exudate (asterisks) ×100; **4**: muscle fragmentation, splitting and atrophy ×100.

Figure 3. Histopathological alterations in the examined organs (H & E).

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Liver

Livers, in third place, stood out with IOL (Liver index)) values ranging from 24 (Z2 July) to 30.06 (Z1 September). Comparing the two areas there was a significant difference in Z1 (P = 0.0423). While in Z2 there was no significant difference (P = 0.1472). Otherwise, in July, values were more homogeneous (P = 0.4164), Z1 (24.83) > Z2 (24.00), while September showed higher indices in Z1 (30.06) > Z2 (26.41) with no significant difference (P = 0.101). There was a notable predominance of regressive alterations and a uniform distribution of other reaction patterns. Tumour lesions were only recorded in this organ (Figure 3-C).

Intestines

As for the intestines, the organs least affected, moderate intestine index (IOI) were observed, oscillating between 17.17 (Z2 July) and 21.02 (Z1 September). In fact, no significant difference was observed for the IOI_s. Yet, regressive damages were more prevalent, while circulatory and inflammatory lesions tended to be more frequent and homogeneous than proliferative damages, which were rare (Figure 3-D).

Muscles

Muscles were relatively spared, with indices (IOM: muscle index) ranging from 19.92 (Z2 July) to 25.98 (Z1 September). When comparing the two periods, a very significant difference was reported in September (P=0.0038) compared to a significant difference in July (P = 0.0238). Meanwhile, comparing the two zones, in Z1 there was a significant difference (P = 0.0207) compared with Z2, where there was no significant difference (P = 0.1847). Regressive lesions remained the most common. A lesion pattern similar to that noted in the kidneys and liver was also recorded for other reaction patterns, while proliferative lesions were rarely recorded (Figure 3-E).

The total fish indices: IFish

IFish values had a very significant correlation with IGP (P = 0.0001). In September, the average IFish scores increased, reaching 140.75 in Z1 and 124.31 in Z2 showing a significant difference (P = 0.0094). Most of the fish were categorized in class II (75% in Z1 and 90% in Z2), while class III individuals were more frequent in Z1 (25%) than in Z2 (10%). In July, the trend revealed a relative homogeneity; yet the indices were generally lower, corresponding to 111.89 for Z1 and 114.73 for Z2, with no significant difference (P = 0.5074). The distribution of classes indicated a dominance of class II, which constituted 85% of the fish in Z1 and 90% in Z2, closely followed by class III (10% for both zones) and a few specimens in class I (5%, exclusively in Z1). Comparing the two zones, there was a significant difference in Z1 (P = 0.0012) compared with Z2 (P = 0.0482, significant limit). In terms of quality, the best fish were those from Z1 in July, but they only represented a small proportion (5%). They were followed by those from Z2 during both periods, then by those from zone Z1 in July, and finally in September.

Spatiotemporal influence on the different histopathological indices

In September, the recorded indices were generally elevated in comparison with July, and more important values were seen in Z1. In terms of the overall lesion indices, the data indicated a large preponderance of regressive lesions. In July, the IReg was significantly higher in Z1 (76.81% peak value) than in Z2 (49.33%). Conversely, IInf was much higher in Z2 (39.85%) compared with Z1 (5.12%). IPro and ICir were relatively similar between the two zones (with 9,88% in Z1 vs. 6.39% in Z2 for IPro and 7.99% in Z1 vs. 4.27% in Z2 for ICir). In September, the situation differed slightly; IReg was higher in Z2 (74.18%) than in Z1 (69.08%), contrary to what was observed in July. Regarding the other indices, the IInf was higher in Z1 (7.60%) compared to Z2 (5.87%). Meanwhile, the IPro and ICir were also more pronounced in Z1 (12.18% and 10.63%) than in Z2 (10.95% and 9.13%). In sum, In Z1, IReg were dominant, followed by IPro, ICir, and IInf scores, whereas in Z2, the pattern by order of lesions was maintained, yet with lower values. While in September IReg became predominant, IInf were markedly higher in July. Additionally, across the two periods, the overall mean values of the IOs and IFish indices were elevated in Z1 compared to Z2. This trend was more noticeable in September. In essence, Z1 consistently showed higher scores, particularly in September, the mean value of the IOs indices for Z1 were higher than for Z2, for all the examined organs (see Table 2). On the other hand, the IFish index in Z1 showed a mean value of 140.75 compared to 124.31 in Z2. In contrast, the results showed that the two zones were more coherent in July, as the average values were comparable (111.89 for Z1 and 114.73 for Z2). Table 3 presents the full comparison of the IFish indices. The predominant classification of the fish was within class II, signifying an intermediate state of health and quality. Classes I and III, although present, remained marginal. Finely, it is noteworthy that classes IV and V were not registered.

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Discussion

The analysis of our results has confirmed the significant interaction between water quality, environmental conditions, health, and fish quality in the studied areas.

Water quality and spatiotemporal influence

The Qudiat-Medouar dam represents an optimal site for ecotoxicological studies related to fish. In fact, this site is exposed to pollution due to anthropogenic activities (Balla et al., 2017), receiving untreated inflows from wastewater, domestic and industrial discharges, waste, and those from agricultural practices (Balla, 2019; Smatti-Hamza et al., 2020). Meanwhile, this infrastructure harbours fish that constitute an essential part of its trophic network, and support small-scale commercial fishing. In our study, water analyses revealed that the fish were potentially exposed to various pollutants. Simultaneously, most of the biological indices calculated were higher in September than in July and were more significant in Z1 compared with Z2 due to clearly visible spatiotemporal variations, as also mentioned by Adamu et al. (2024). In fact, the higher values observed in Z1 could be attributed to its proximity to several anthropogenic factors. Z1 is located near inflows receiving wastewater from Timgad city, as well as industrial water discharge from the ProTimgad brickyard, as illustrated in Figure 1. These sources may contribute to the elevated values in this zone, potentially influencing the observed differences. Indeed, as reported by Silva et al. (2008), Hoomehr et al. (2018), and Dewangan et al. (2023), it is possible that the effects of external factors on the water are better justified by indicators of temperature, pH, and turbidity, which were more significant at Z1 than at Z2. The recorded temperatures, between 23.09 and 25.28 °C, were typical of temperate waters, although extended increases could reduce dissolved oxygen and increase the toxicity of certain compounds like ammonia (Qiu et al., 2024). For pH, slightly alkaline values (between 7.45 and 8.18) are generally suitable for most freshwater fish species; they support a good chemical balance and reduce the toxicity of ammonia. In fact, a stable pH between 7 and 8 promotes the balance between the toxic and non-toxic forms of ammonia. While extreme variations can affect the toxicity of metals and nitrogen compounds (Zambrano et al., 2021). However, turbidity, especially in Z1 (47.02 NTU in September; 26.28 NTU in July), can limit light penetration, affect fish respiration, and indicate organic pollution (Duque et al., 2020; Onwona Kwakye et al., 2021). In addition, the conductivity, relatively high (between 1229.72 and 1342.98 µS/cm), may indicate high mineralization (Nihlgård et al., 2020), which is potentially stressful for the fish (Zambrano et al., 2021). Regarding the concentrations of dissolved oxygen (O_2), between 7.37 and 7.76 mg/l, it should be noted that they were satisfying, in both areas, and above critical thresholds, ensuring good oxygenation for the fish (Zambrano et al., 2021; Qiu et al., 2024). In fact, oxygen poses no toxicity to fish, even at very high concentrations. It has been demonstrated that concentrations as high as 40 mg/L pose no disadvantage to Salmonids (Morin, 2012). Overall, the lower levels of ammonium, nitrates, nitrites and phosphates were acceptable according toHakkim et al. (2024). Nevertheless regarding NH_4^+, the low concentrations observed (0.02 to 0.21 mg/l) indicated good management of organic matter and a reduced toxic risk (Zambrano et al., 2021; Qiu et al., 2024). The same applies to nitrates (1.75 to 3.56 mg/l), present at moderate levels which, although non-toxic to adult fish, could promote eutrophication if they increase (Duque et al., 2020; Onwona Kwakye et al., 2021) as observed at the two zones in July. Yet, chronic exposure, even at moderate concentrations, can affect fish health (Camargo and Alonso, 2006). Nitrites, on the other hand, although present at low concentrations (0 to 0.07 mg/l), indicating a good functioning of the nitrogen cycle, which is reassuring since this compound is very toxic even at low doses (Zambrano et al., 2021; Qiu et al., 2024). Despite that, the slight increase in nitrites in September at Z1 (0.07 mg/l) could indicate a potential problem requiring closer monitoring. In reality, even low concentrations of nitrites can cause chronic stress, thereby affecting the fish health (Lewis and Morris, 1986; Shen et al., 2024). The situation remains favourable for phosphates, whose values are very low (0 to 0.01 mg/l). On the other hand, sulphates, although having little direct effect, showed significant concentrations (200.12 to 285.8 mg/l), contributing to the total mineralization load. In fact, the high levels of sulphates can contribute to the release of toxic metals from the sediments, which can affect fish health (Morford and Emerson, 1999; Zak et al., 2021). Suspended solids were high (78.07 to 85.09 mg/l), especially in September, they can cloud the water, irritate fish gills, and harm their development (Duque et al., 2020; Onwona Kwakye et al., 2021). Moreover, organic matter, although moderate (1.60 to 3.52 mg/l), can become problematic when it degrades by consuming oxygen and promoting bacterial proliferation (Suari et al., 2019). Finally, heavy metals, particularly copper (23.00 to 29.76 mg/kg) and chromium (104.95 to 110.09 mg/kg), pose a risk of bioaccumulation and lead to chronic toxic effects for fish (Job et al., 2023; Dippong et al., 2024). Their presence revealed pollution due to industrial activities, which is to be expected given the results of Xu et al. (2024).

In sum, our results highlight the spatiotemporal influence on the histological states of the fish. Higher values observed in September than in July, and the predominance of different scores in Z1 compared with Z2, confirm temporal and environmental variations influencing fish quality and health. The dominance of one lesion pattern over another can be justified by elevated concentrations of a given pollutant. Similarly, in July, the IReg was significantly higher in Z1 (76.81% peak value) than in Z2 (49.33%), which may be due to the relatively high levels of copper, conductivity, and, to a lesser extent, nitrates. In fact, the predominance of regressive lesions suggests sub-chronic stress exerted on the fish, since these pollutants exist during both periods, although with peaks in July. As for the higher IOs and $IFish_s$ in Z1, they indicate a stronger pollution pressure in this area. This was justified by a very significant correlation between the GPI and the different IOs and $IFish_s$ scores (practically P = 0.0001 for all indices). Finally, in terms of quality, more than 85% of the fish were within class II, signifying an intermediate state of health and quality, while the absence of classes IV and V indicates a favourable situation.

Histopathological Analysis

The histology of fish is closely linked to their ecosystem, which is affected by several environmental pollutants and physicochemical factors. These elements can cause various histopathological changes in fish tissues, which influence their survival, overall well-being, and consequently, their quality. We provide here a detailed assessment of these effects, supported by data from our results as well as related research. However, it is important to emphasize that one cannot attribute a given lesion to a specific pollutant with absolute certainty. Our study was conducted on fish in their natural environment, and the reported lesions may be attributable to the cumulative effect of many contaminants; however this necessitates additional experimental validation.

Gills are among the most delicate structures in *Teleosts* and are very sensitive to environmental conditions. In the long term, prolonged exposure to heavy metals, especially copper and chromium, leads to regressive lesions, which were the most frequent in our results. Epithelial degeneration, intraepithelial oedema, and lamellar desquamation were consistent with the effects reported in *Cyprinus carpio* and *Oreochromis mossambicus* exposed respectively to copper, chromium and manganese (Jabeen and Chaudhry, 2013; Saied et al., 2022). Our results also aligned with the observations ofVan Dyk, et al. (2009) in *Clarias gariepinus*, which exhibited degenerative changes due to organic matter, albeit moderate, and those of Yu et al. (2009) linking turbidity to irregular spaces in the gill lamellae and epithelial deformations. The fusion of secondary lamellae and epithelial necrosis, especially observed in September at Z1, may reflect subacute exposure to nitrites known to cause such lesions, as reported by Abdel-Warith et al. (2020). Furthermore, the necrosis of the gill epithelium can also be attributed to high concentrations of ammonia, as seen in the Siberian sturgeon (Nonnotte et al., 2018), nitrite (Abdel-Warith et al., 2020), low pH, as in the brown trout (*Salmo trutta fario*) (Orso et al., 2023), however, these observations did not align with our results, or to high levels of suspended solids as in *Mystus cavasius* (Karim et al., 2022), which were consistent with our own results. Regarding inflammatory lesions, our results were also consistent with those of Abdel-Warith et al. (2020) and Mahboob et al. (2020), showing inflammatory infiltrations related to heavy metals and nitrites. In addition to these, the effect of nitrogen and phosphates leads to algal blooms, thereby releasing toxins responsible for gill inflammation, as reported in *Glossogobius giuris*, by an oedematous separation of the basal membrane and cellular hypertrophy (Venkataraman et al., 2007). Nevertheless, these results did not completely fit with our data. Our findings for circulatory lesions, such as aneurysms, telangiectasia, and capillary congestion matched the bibliographic information that connected these changes to heavy metals, particularly copper and chromium (Atabati et al., 2015; Baiomy, 2016; Marvin et al., 2017; Mahboob et al., 2020; Saied et al., 2022). The severity of these lesions seems to have been amplified in July, a period when high temperatures can enhance the toxic effects of these metals, as demonstrated in Mozambique tilapia (*Oreochromis mossambicus*) also exposed to copper (Ribeiro et al., 2024).Moreover, the identified oedema and epithelial lifting correspond to those described following exposure to suspended matter and nitrogenous pollutants (Van Dyk et al., 2009; Venkataraman et al., 2007). As for hyperaemia, particularly observed in autumn, it was also compatible with the reported effects of nitrite (Abdel-Warith et al., 2020). According to Wu et al. (2023), ammoniac nitrogen is an important factor in hyperaemia and gill diseases. Lastly, the progressive lesions observed, such as epithelial hyperplasia, excessive mucus production, and cell displacement, were coherent with the alterations described by Atabati et al. (2015) in the herbivorous carp (*Ctenopharyngodon idella*), exposed to copper 3, and *Oreochromis niloticus* exposed to lead or carbon nanotubes (Barbieri et al., 2016).

Liver, the central organ of detoxification, is very sensitive to pollutants. The regressive lesions observed, such as vacuolisation, hydropic degeneration, necrosis, pyknotic nucleus, and karyolysis, were also reported in fish exposed to heavy metals (notably: Cu, Cd, Pb, Ni) 4. Furthermore, there was a significant increase in melanomacrophage centres (MMC) in the examined sections. This lesion may be linked to industrial and agricultural activities that contribute to the accumulation of copper and chromium according to Thabet et al. (2019), and as reported in several species of carp (Khan et al., 2024). The severity of these lesions is often correlated with the concentration of pollutants and environmental parameters, particularly pH (Paul et al., 2014). Nevertheless, we have reported certain stability for this parameter. All of these lesions were also linked to nitrates as observed in the African catfish *Clarias gariepinus* (Abdel-Warith et al., 2020) and to ammonia as in the Nile tilapia (*Oreochromis niloticus*) (Motamedi-Tehrani et al., 2025).

However, there is some discrepancy between these observations and our results. Meanwhile, the combined effect of ammonia and nitrite has been linked to haemorrhage, necrosis and liver vacuolisation in *Gangetic Mystus* and *Mystus cavasius* by Karim et al. (2022). In addition, nitrates can interact with other pollutants, such as heavy metals, and exacerbate their toxicity. For example, co-exposure to nitrates and cadmium led to hepatic vacuolar degeneration and sinusoid dilatation in Japanese flounder (*Paralichthys olivaceus*) (Yu et al., 2025). These findings reinforced our own observations. Similarly, phosphates and sulphates, although the latter were non-hypoxic in our study, can induce liver lesions but through other mechanisms (Venkataraman et al., 2007) as they can interact with other pollutants, such as heavy metals, thereby increasing their toxicity (Hamad et al., 2024). Nevertheless, pesticides are also involved in hepatic degeneration (Shahid et al., 2021). As for the circulatory lesions, they included dilation and congestion of the sinusoids and haemorrhages. These lesions may be due to the accumulation of heavy metals (Thabet et al., 2019; Mahboob et al., 2020) or, at a lesser extent according to our results, to nitrates known to cause such lesions (Abdel-Warith et al., 2020). Overall, polluted waters have often led to inflammatory lesions in the liver (Ogundiran and Fawole, 2021). In fact, heavy metals can cause the infiltration of inflammatory cells into the hepatic parenchyma (Mahboob et al., 2020) and the formation of MMC (Viana et al., 2021). These lesions were very widespread on the examined slides. While leukocyte infiltration in the portal tissues, which is often linked to nitrites (Abdel-Warith et al., 2020), was less frequently observed. Inflammatory and proliferative lesions, particularly hypertrophy and an increase in the number of Kupffer cells, were generally reported in fish exposed to suspended matter (Thabet et al., 2019) and heavy metals (Mahboob et al., 2020), which aligned with our observations; although to a lesser extent according to our results, organic matter recognized for inducing such lesions (Thabet et al., 2019) or nitrates (Abdel-Warith et al., 2020) can also cause such alterations. In conclusion, despite the histopathological alterations in fish livers caused by pollutants being well documented, It is crucial to take into account the role of bioremediation and other mitigation strategies, such as the use of microorganisms to reduce toxic contaminants, which have proven promising for improving water quality and reducing the impact of pollutants on aquatic life (Khan et al., 2024).

The kidneys were the most affected organs. Our data were consistent with several studies that showed the predominance of regressive renal alterations. Lesions such as vacuolisation, tubular degeneration, necrosis, and glomerular alterations (reduction of glomeruli, dilation of Bowman's space) were common in fish exposed to various pollutants, including industrial effluents (Thangam, 2014; Dane and Şişman, 2015; Ullah et al., 2017; Jabeen et al., 2022; Rani, 2023)5 6 7 8 9heavy metals notably copper in *Channa punctatus*(Afaghi, 2020; Kumari et al., 2024), chromium, particularly its hexavalent form (Kumar et al., 2023), or suspended matter, as in *Oreochromis niloticus*(Shahid et al., 2022). However, even to a moderate degree, according to our results, organic substances such as pesticides and polychlorinated biphenyls (PCBs) could induce regressive alterations of the tubules and interstitial tissue as reported by Sula et al. (2020). Whereas nitrites, nitrates, and ammonium were associated with glomerular hyalinization according to Bin-Dohaish et al. (2004), and Karim et al. (2022), which may support our findings. Environmental adverse conditions, including pH or temperature variations, can also exacerbate these toxic effects by increasing contaminant bioavailability and toxicity (Kaur and Dua, 2016; Abdel-Moneim et al., 2019; Karim et al., 2022). Sulphates, in turn, can interact with other pollutants, such as heavy metals, and thus increase their toxicity. For example, environments characterized by high sulphate concentrations significantly enhanced the bioaccumulation of heavy metals, such as lead and zinc, in *Mugil cephalus*, resulting in renal impairment (Hamad et al., 2024). As noted by Badiane (2022), and Smart et al. (1979), the identified nephrocalcinosis was attributed to elevated levels of CO2, a parameter that was not investigated in the current study. Concomitantly, this lesion was also observed in certain conditions, including hyperoxia and hypercapnia (Minarova et al., 2023), or exposure to environmental toxins, such as sulfamerazine (Mchugh et al., 2013), or following nutritional deficiencies. Indeed, diets low in essential vitamins can exacerbate nephrocalcinosis (Béland et al., 2024). Circulatory disorders such as glomerular capillary congestion and haemorrhage, although secondary, are also consistent with damage caused by heavy metals, notably copper (Afaghi, 2020) and chromium, nitrogen compounds (Kumar et al., 2023), or due to the effect of PCBs as in *Crucian carp* (Sula et al., 2020), although the latter were low in our analyses. Suspended matter, on the other hand, accentuates these disorders through severe congestion and haemorrhages, as reported in *Oreochromis niloticus* (Shahid et al., 2022). Inflammatory responses, such as lymphocytic or mononuclear infiltration around the tubules, although less pronounced, were also reported in similar contexts, such as chronic exposure to chromium, copper, and nitrites (Abdel-Warith et al., 2020; Afaghi, 2020; Kumar et al., 2023; Kumari et al., 2024), pesticides, or PCBs, which were responsible for lymphocytic infiltrations and interstitial inflammation (Sula et al., 2020). Finally, the rare proliferative lesions detected in September, in both areas, are also part of the effects of prolonged exposure to heavy metals, such as cadmium causing hyperplasia of renal tubular cells (Jayakumar, 2017), or chromium, which also induces proliferation of interstitial cells (Kumar et al., 2023). Similar modifications including tubular hyperplasia were observed in fish exposed to pesticides and PCBs (Sula et al., 2020), or suspended matter, as in *Oreochromis niloticus* 10.

For the intestines, regressive lesions such as epithelial cell degeneration and necrosis were noted, paralleling findings in fish subjected to heavy metals notably copper and chromium (Jegede, 2013; Khan et al., 2024), leading to severe

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mucosal layer disintegration as reported by Begum et al. (2024). The effects of heavy metals, including copper and chromium, have also been documented in the work, of Khan et al. (2024), and Yacoub et al. (2023) as for mercury in *Cirrhinus mrigala*(Chavan and Muley, 2014), lead in herbivorous carp (Du et al., 2022), and cadmium in Nile tilapia (Younis et al., 2013; Younis et al., 2015). Furthermore, after chronic exposure to copper and chromium desquamation of the mucosal epithelium was reported by Chavan and Muley (2014), separation of the lamina propria was reported in marsh eels (Lin et al., 2023), and disruption of tight junctions in the intestinal epithelium which can compromise the intestinal barrier and lead to inflammation (Fatima and Usmani, 2013; Lin et al., 2023). These findings were not observed in our study. Other pollutants, such as suspended solids and organic compounds, have been linked to the destruction of villi and abnormal formation of the intestinal lumen, impairing nutrient absorption (Ahmed et al., 2015); these observations could be in agreement with our results, as these pollutants are recognized for leading to the occurrence of such lesions. In fact, intestinal villi are often affected by water pollutants, leading to their fusion and a loss of structural integrity (Chavan and Muley, 2014). As for the inflammatory lesions observed, they were characterized by eosinophilic secretions and leukocyte infiltration, and are in concordance with the results of Huerta-Aguirre et al. (2019), and Orso et al. (2023). This lesion can be linked to nitrogenous pollutants notably ammonia, nitrites and nitrates, according to Huerta-Aguirre et al. (2019), and Satkar et al. (2024), despite their low intensity; or to heavy metals such as copper (Lin et al., 2023). For example, in common carp exposed to lead, an increase in inflammatory cells in the lamina propria and thickening of the muscle layer were observed, (Zhang et al., 2021) which could be in agreement with our results but perhaps for copper and chromium. Circulatory alterations correlated with those of the inflammatory pattern. Indeed, congested blood vessels were frequently observed in the intestines of fish exposed to polluted waters, notably from nitrogenous pollutants: ammonia, nitrites and nitrates (Huerta-Aguirre et al., 2019), but in a restrained manner according to our results. These lesions were associated with an increase in the number of caliciform cells and blood congestion in the lamina propria, as observed in Mozambique tilapia exposed to cadmium (Yang et al., 2024), which may potentially be in line with our results for copper and chromium. As for proliferative lesions, such as hyperplasia and increased goblet cells, these were indicators of chronic exposure to pollutants, particularly heavy metals, which are recognized for inducing such lesions, as already mentioned. These observations could corroborate our findings and were also reported after exposure to copper and chromium (Fatima and Usmani, 2013; Lin et al., 2023) and to cadmium in Nile tilapia (Younis et al., 2015), with these authors also reporting disruption of tight junctions in the intestinal epithelium, compromising the intestinal barrier and leading to inflammation. Finally, all these modifications are often linked to the concentration and duration of exposure. According to Chavan and Muley, (2014) intestinal histopathology was positively correlated with concentration and duration of exposure, underlining the dose-dependent nature of the lesions.

Muscle, representing around 80% of edible fish tissue (Shahid et al., 2021), is relatively less exposed to pollutants compared to internal organs. However, our study revealed notable histopathological alterations in muscle tissue. Indeed, regressive lesions dominated. Muscle fibre division, degeneration, and necrosis were seen. These results are consistent with previous research showing their relationship with organic pollutants like pesticides and heavy metals like copper, lead, zinc, and cadmium (Bhuvaneshwari et al., 2015; Shahid et al., 2021; Yacoub et al., 2023; Shaalan, 2024). In a lesser order and subsequently, it can be considered that chemical pollutants such as ammonia, nitrites, nitrates, orthophosphates and sulphates could be potentially the cause of such lesions (Koca et al., 2005). Other studies have also mentioned muscle fibre fissions linked to copper and chromium exposure (Kaur et al., 2018). Conversely, species like *Oreochromis niloticus* exhibit hypertrophy myofiber swelling and muscle degeneration caused by pollution (Shahid et al., 2022; Yacoub et al., 2023). While the phenomenon of muscle fragmentation and atrophy has been correlated with the impact of nitrates (Abdel-Warith et al., 2020), or elevated concentrations of nitrogenous waste (Jabeen et al., 2022). This correlation may provide a rationale for our findings, potentially attributable to the repercussions of prolonged exposure. Among other damages, muscles may show changes indicating biological stress, such as vacuolar degeneration, hyalinized necrosis, or muscle atrophy and fragmentation supported by the presence of parasitic granulomas (Koca et al., 2005; Haredi et al., 2020). Furthermore, inflammatory lesions, which encompass leukocyte infiltration and the occurrence of Zenker necrosis, were also noted and found to be associated with the accumulation of heavy metals, including copper, chromium, zinc, lead, and cadmium (Kaur et al., 2018; Al-Mayahi et al., 2021; Yacoub et al., 2023; Shaalan, 2024) , or with nitrates (Abdel-Warith et al., 2020); although our findings can be considered as suggesting a comparatively lesser significance. Nitrogen pollutants can induce oxidative stress and promote muscle inflammation (Jabeen et al., 2022; Siraj et al., 2022). Furthermore, the circulatory lesions observed, notably interfibrillar haemorrhages, testified to acute stress induced by unfavourable environmental conditions, among which was the effect of nitrates (Abdel-Warith et al., 2020). As for proliferative lesions, they were rarely reported in the literature, according to Uçar and Atamanalp (2009), suggesting a poor ability for muscle regeneration in contaminated environments. Shahid et al. (2022) assert that these lesions may be seen in specific situations involving pollution exposure. They often involve muscle fibre hypertrophy. Finally, physico-chemical factors influence the intensity of histological alterations, and this has been well reported in several studies. Indeed, histopathological alterations in muscle tissue are correlated with pollutant levels, such as heavy metals and

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hydrocarbons (Osman et al., 2010; Reddy and Waskale Kusum, 2013).

Fish's health and quality are intimately associated with the environmental parameters, which significantly impact their overall well-being. These elements affect fish through multiple mechanisms and can interact in complex manners (Leblanc et al., 2009; Michel, 2018b; Visciano, 2024; K. Zhang et al., 2024). This correlation has been extensively highlighted. For instance, the FAO and WHO (OMS and FAO, 1974) along with Kenneth (2000), have explored the connections between the environmental context and the quality of fish and seafood. They underscored the necessity of considering certain substances present in these products, which could pose risks to human health or compromise their acceptability. A particularly concerning illustration is the bioaccumulation of heavy metals, especially within fish muscle, as previously mentioned. Consequently, the management of water quality is imperative for safeguarding the health and quality of fish (Michel, 2018a). Indeed, the presence of chemical pollutants, heavy metals (Zeitoun and Mehana, 2014; Ali et al., 2024), spatiotemporal variations in temperature, pH and dissolved oxygen (Recabarren-Villalón et al., 2024), as well as pathogens favoured by environmental stress (Sures and Nachev, 2022; Garibay-Valdez et al., 2024), weaken fish, impairing their growth, immunity and biochemical composition. These factors lead to a decrease in their nutritional and health value, proving that the quality of fish depends above all on the quality of their habitat. Histology, on the other hand, is relevant for its ability to control the quality of food such as meat and meat products, even in fish, and has always been satisfactory and well documented, giving reliable and abundant results (Popelka et al., 2014; Strateva and Penchev, 2021; Maghami et al., 2022; Abd-Elhafeez et al., 2024; Pchelkina, 2024). In fact, the IFish scoring serves as a powerful tool for monitoring the health status of fish. It aids in comprehending the ecotoxicological consequences on fish (Samuel et al., 2023), by integrating the responses of various organs (IOs) into a comprehensive score that reflects the histological severity of damages and the impacts of diverse contaminants on fish, and consequently on their quality. Dar et al. (2022) underlined the importance of regular assessments using this index to guide assessment, pollution control and ecosystem restoration strategies, thus promoting sustainable practices. Indeed, higher scores on this index were always linked to more affected fish and several studies have documented this positive correlation (Schmidt et al., 1999; Au, 2004; Bernet et al., 2004; Pieterse et al., 2006; Zimmerli et al., 2007; Zadinelo et al., 2020), which is also conceivable to be in corroboration with our results. The highest IFish value, 140.75, was recorded at Z1 in September where conditions were most unfavourable IGP: 269.85 (P = 0.0001). This correlation confirms the sensitivity of IFish, as a histopathological biomarker, to environmental quality variations. However, more than 85% of the fish were within class II; classes I and III, although present, remained marginal, while classes IV and V were completely absent. This signifies an intermediate state of fish health and quality, which is acceptable. Rigorous management of water quality could thus significantly improve these results.

To our knowledge, the bibliography does not provide an exhaustive definition of classes for IFish scores. Only McHugh et al. (2011) used IFish values based on the Zimmerli et al. (2007) classification system applied to IOs. There were no exact numerical thresholds provided for these IFish classes. However, this applies to IOs and not directly to IFish, and since the latter is a combined index, it is more logical that similar classification principles should be applied, yet not the same ones. Thus, in our study, we have developed a classification scheme and proposed the use of IFish to assess fish quality, since quality fish are those that come from a healthy environment. In fact, this system provides a clear and relevant classification of fish health, and consequently of their quality, based on the multi-organ extent of histological lesions observed. It enables rapid identification of individuals in good health, those in moderate distress and those in critical condition. This classification offers a comprehensive assessment, not least because it is applicable to different organs. Ultimately, the obtained classes can be interpreted in terms of quality and monitoring as follow: class I: fish of excellent quality, ready or clean for consumption, class II: good quality fish, where monitoring is recommended, classes III-IV: fish of medium and poor quality, respectively, requiring corrective interventions (mainly water quality), and finally class V: fish of critical quality, for which the prognosis is reserved.

Finally, the proposed classification system is at a preliminary stage, and we emphasize the need for future validation studies to confirm its effectiveness and robustness in different ecological and health contexts as well as according to biological variables such as size and sex. According to Bernet et al. (1999), sex and stage of sexual maturity should be noted since these two factors are known to potentially influence the histological appearance of certain organs. However, this would be possible if we were working on fish during the spawning period. The silver carp, *Hypophthalmichthys molitrix* , reproduces in late spring and early summer (García Gómez et al., 2023; Tucker et al., 2020). Typically, in the wild, spawning occurs between May and June. Whereas our study took place in July and September. Moreover, the importance of conducting such a study over a longer period and covering all four seasons will deepen our understanding of the temporal, even seasonal, dynamics of fish health concerning environmental conditions.), sex and stage of sexual maturity should be noted since these two factors are known to potentially influence the histological appearance of certain organs. However, this would be possible only if we were working on fish during the spawning period. The silver carp, *Hypophthalmichthys molitrix* , reproduces in late spring and early summer (Tucker et al., 2020; García Gómez et al., 2023). Typically, in the wild, spawning occurs between May and

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June, whereas our study took place in July and September. Moreover, the importance of conducting such a study over a longer period and covering all four seasons would deepen our understanding of the temporal, even seasonal, dynamics of fish health concerning environmental conditions.

Contribution and originality of the study

Our research introduces an innovative classification model that provides a holistic assessment of fish health and quality, using IFish scores. This comprehensive method associates the severity of lesions with their multi-organ distribution, offering an integrated monitoring approach based on both qualitative and quantitative histological criteria.

Conclusion

Fish histology is significantly affected by water quality, which can cause several abnormalities that lead to an inevitable impact on their quality. According to our research, fish undergo significant tissue damage as a result of water pollution, which makes the IFish index a very accurate biomarker for evaluating fish quality. Therefore, this study emphasizes the significance of histopathology and biological scores for judging the quality and health of aquatic environments, as well as of fish. In fact, the application of Bernet et al. (1999) semi-quantitative method, combined with the analysis of physico-chemical water characteristics, provided a comprehensive and accurate perspective on the status of fish in relation to spatio-temporal fluctuations of water quality. In fact, for effective fish quality management, it is essential to understand the relationship between IFish, health, and quality. Our findings validate that water contamination directly affects fish health. These data support the idea that water quality is crucial for the protection of aquatic resources, and that histopathology represents an indispensable procedure for identifying the impact of pollutants. Fish are considered good bioindicators of the quality of aquatic environments. Studying their condition is therefore very important if we are to maintain a viable fishery and a safe product for human consumption.

In other words, the assessment of fish histology will not only indicate the state of their health in terms of water condition, but also provide a better understanding of the quality characteristics associated with fish taken from an apparently polluted or unpolluted natural environment. This includes the impact of ecological aspects, temporal differences and biological stress factors that are not part of the controlled conditions associated with laboratory-reared fish. Finally, the general health threats to fish and consumers continue to be a significant concern, necessitating continued study and preventative measures. Ultimately, research is an ongoing process of refining techniques and analyzing measures. Nevertheless, healthy fish are synonymous with quality fish, and the use of histology, principally the IFish index, is proving to be a relevant biomarker in demonstrating this.

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Ethical approval

The study does not require any ethical approval.

Conflict of interest

There are no potential conflicts of interest.

Author Contributions

R.G. ensured all stages of the research, including conceptualization, formal analysis, investigation, writing the original manuscript, revising it, editing, and visualizing the results. R.E. continuously supervised the work, along with O.B., who managed the administrative aspects of the project. N.A. contributed to the development of the methodology. All authors have read and agreed to the published version of the manuscript.

Data availability

All the data are available upon request to the corresponding author.

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