

Liver Histopathological Assessment in Wild Mullet

Chronically Exposed to Cu and Zn

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Abstract:

Mullet (*Liza saliens*) is the dominant fish to inhabiting polluted waters as observed in a small coastal lagoon (Northwest Portugal coast). Previous studies showed that Cu and Zn are potential metal stressors and that Cu metabolism is mostly related whit liver, achieving this organ high levels and showing age-dependent concentrations, whereas Zn is well regulated. In this study, hepatic tissue ultrastructure revealed the presence of general diagnostic categories and additionally an extensive area of heterogeneous parenchyma, composed of hepatocytes with different spectrum of vacuolization. Despite this lesion is scarcely recorded in literature, their presence was associated with Cu and Zn. Data obtained in this study may be useful to compare biomarker fish responses from other polluted sites.

Introduction:

Histopathology used as indicator of environmental stress provides a definitive biological end-point of historical exposure [2]; as a result liver histopathology has been used as an indicator of environmental stress and the kind of injury is often dependent upon time of exposure to pollutants, such as metals. High metal concentrations in the liver of *Liza saliens* from a portuguese coastal lagoon were reported [3] and their relation with fish hepatic alterations were examined.

Materials and methods:

- ❖ Lagoon mullets were captured by gill net, anaesthetised and livers randomly taken and immediately fixed in 10% buffered formalin. Fish age was determined by reading the annual ring structure of scales. Liver Cu and Zn levels were analysed elsewhere [3].
- ❖ Liver tissue were routinely prepared for light microscopy and sagittal sections were stained with hematoxylin-eosin (HE). Alterations were classified in general diagnostic categories : non-neoplastic lesions; foci of necrosis; granuloma and melanomacrophage centres [4]. Additionally an extensive area of heterogeneous parenchyma, composed of hepatocytes with different spectrum of vacuolization, was classified and scored based in lesion severity, as follows: **0** = no pathological alterations, **1** = focal mild pathological alterations, **2** = moderate pathological alterations, **3** = severe pathological alterations.
- ❖ Relationships between measured parameters were tested with Pearson's correlations, and significance level (α) was set at 0.05.

Results & Discussions:

Livers majority showed 1 to 2 different types of lesions and heterogeneous parenchyma was the most prevalent lesion (Tab 1). This alteration was characterized by areas of heterogeneous parenchyma vacuolization, consisting of hepatocytes, either with a large spectrum of vacuolization contributing to parenchyma heterogeneity, or poorly vacuolated showing moderate to strong basophilia (Fig 1A). Some foci of necrosis spread over large areas (Fig 1B). Non-neoplastic lesions included cell and nuclear polymorphism, focal hepato and nuclear hypertrophy (Fig 1C) and frequent focal cell death (Fig 1D).

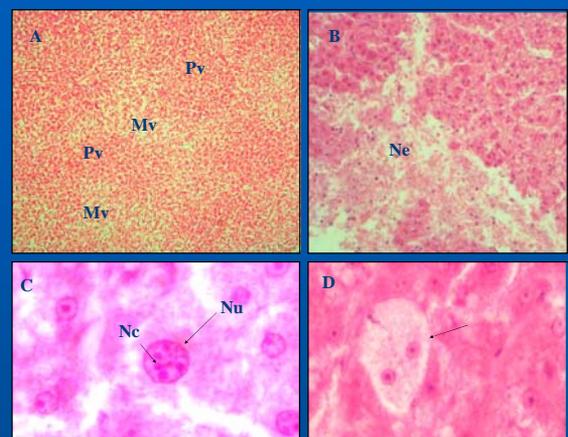


Fig 1 Histological sections of *Liza saliens* liver: (A) extensive heterogeneous parenchyma with large spectrum of vacuolization (Mv) and poorly vacuolated (Pv) 40x; (B) large area of lytic necrosis (Ne) 100x; (C) nuclei hypertrofia (Nu) and simultaneous nucleolus (Nc) 250x; (D) focal hepatocyte death (arrow) 250x;

Tab. 1 Sample prevalence of the number and categories of hepatic alterations in lagoon mullets (N = 35).

Variables		Prevalence (%)
number of lesions	0	43
	1 - 2	48
	3 - 4	9
categories	Heterogenous parenchyma	34
	Foci of necrosis	31
	Granulomas	14
	Non-neoplastic lesions	9
	Melanomacrophagic centres	6

Number of lesions = number of different types of liver lesions found simultaneously.
Prevalence = % of fish presenting a number of lesions or a specific categories of lesion.

Tab. 2 Liver metal levels, liver heterogeneous parenchyma severity and fish age from lagoon mullets (N = 35)

Variables	Mean \pm standard deviations
Zn-liver (mg.kg ⁻¹ d.w.)	88.64 \pm 32.00
Cu-liver (mg.kg ⁻¹ d.w.)	262.08 \pm 140.71
Age (years)	8.85 (range = 6 - 13)
Heterogenous parenchyma severity	0.56 \pm 0.85

Lagoon mullets, with 6 to 13 years, had high metal concentrations in liver ranging from 51 to 547 mgCu.kg⁻¹ d.w. and 26 to 190 mgZn.kg⁻¹ d.w. (Tab 2). Average heterogeneous parenchyma show a mild severity, still achieving most severe score (Tab 2). The severity of this alteration increases with Cu and Zn levels ($r=0.429$, $p=0.01$; $r=0.402$, $p=0.017$, respectively) suggesting that lesion severity was induced by metals. No relationships were observed between this lesion and fish age.

Conclusions:

- ❖ Heterogeneous Parenchyma was associated with Cu and Zn accumulation in fish liver, rather than a pathology depending on fish age.
- ❖ Mulletts from the lagoon seem to be well adapted to stress conditions since no pre-neoplastic nor neoplastic lesions were found.
- ❖ Considering that mullet is living in the lagoon for its life span, a long-term evaluation of the stress responses was achieved which are more realistic than acute toxicity laboratory tests.
- ❖ Data obtained in this study may be useful to compare biomarker fish responses from other polluted sites.

References

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