Can Fish Adopt Same Defence Strategy in Gill and Liver to Face Chronic Metal Exposure?

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Abstract:

Among fish, mullet is one of the most resistant inhabiting polluted waters. *Liza saliens* is the dominant species in a coastal lagoon (Northwest coast - Portugal), which has been under investigation concerning histological and biochemical issues in response to environmental Cu and Zn. In this study, branchial and hepatic ultrastructure showed that lesions prevalence and number of simultaneous lesions was larger in gill than in liver. *L. saliens* proved to be able to increase CAT and SOD activities in these organs suggesting a compensatory tissue response mechanism to face metal exposure.

Introduction:

Several studies revealed that fish exposure to heavy metals could trigger oxidative stress [1, 2], by reactive oxygen species (ROS) generation [3, 4]. ROS can be detoxified by an enzymatic defence system, that includes CAT and SOD [5]. The dominant fish in a contaminated lagoon (Northwest coast of Portugal is mullet (*Liza saliens*) that showed bioaccumulation of Cu in liver and Zn in gill over age [6]. In this study gill and liver were compared in relation to ultrastructure changes and antioxidant enzymes activity (CAT and SOD).

Materials & Methods:

- Gill and liver samples were taken for biochemical essays (frozen in liquid nitrogen) and for histological examination (fixed in buffered formalin-10%) after lagoon mullets (N= 13) were captured and anaesthetised. Tissues Cu and Zn concentrations were analysed elsewhere [6, 7].
- Tissues were homogenized in ice-cold buffers and mitochondrial fractions were obtained by centrifugation at 15 000 X *g* (20 min, 4ºC). SOD activity was determined (550 nm) by the inhibition of cytochrome *c* reduction [8]. The reaction volume was according to [9]. CAT activity was determined (240 nm) by the consumption of H_2O_2 [10].
- Gill and liver tissue were routinely prepared for light microscopy and sagital sections were stained with hematoxylin-eosin (HE). Histological lesions in gill [11] and liver (heterogenous parenchyma) were scored based in lesion severity as follows: 0 = no pathological alterations, 1 = focal mild pathological alterations, 2 = moderate pathological alterations, 3 = severe pathological alterations.
- Differences between measured parameters were tested with Mann-Whitney U-Test and the relationships between them were tested with Spearman´s correlations. A 5% significance level was employed throughout.

Tab 1 Lesions prevalences in liver and gill from lagoon fish (mean ± sd).

	Prevalences (%)	
	Liver [14]	
lesions	Heterogenous parenchyma	54
	Foci of necrosis	38
	Non-neoplastic lesions	15
	Granuloma	8
	gill	
	Hyperplasia	92
	Vasodilation	92
	Lifting	69
	Aneurism	62
Number of lesions	**liver**	
	0	23
	1-2	69
	4	8
	gill	
	1-2	15
	3-4	85

Results & Discussion:

Gills showed higher prevalence of 3 to 4 simultaneous lesions and high prevalence for each lesion (Tab 1). Average gill lesions show a mild severity; still aneurism and lifting were the lesions that achieved the most severe score (Fig 1A).

Highest Cu content was found in liver, range 125 to 547 mg.kg^{-1}, while highest Zn content was observed in gill, range 88-134 mg·kg^{-1} (Tab 2). A decrease CAT activity in gill was observed with copper levels and with lifting severity (r=-0.678, p=0.015; r=-0.618, p=0.032, respectively). Osmoregulatory disturbances occur by gill permeability and cell integrity changes [12] and lifting constitutes a typical defence mechanism by increasing diffusion distance. CAT has an unusual rapid turnover rate [4] and could be affected by osmotic stress, thus reducing its activity. Lower branchial CAT activity than in liver was also observed (Tab 2).

The higher SOD activity in gill than in liver (Tab 2) and the increase SOD activity with fish age founded (r= 0.571, p=0.042) could be an indicator of compensatory tissue response to face metal exposure. Previous study was demonstrated an age-dependent Cu gill concentrations [6].

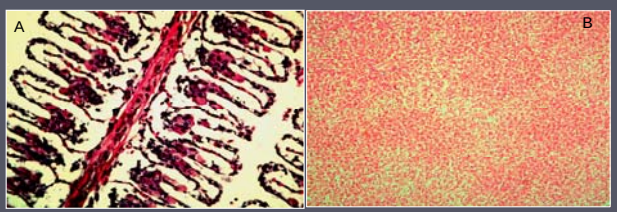

Fig 1. Histological sections of lagoon mullet: A ,Gill filament with lifting of filamentary and lamellar epithelium, 200X. B, Liver heterogenous parenchyma, 40X.

Some of fish livers showed non-existence of lesions, yet the majority showed 1 to 2 simultaneous lesions (Tab 1). Heterogenous parenchyma was the main lesion observed by prevalence (54%), with a mild severity average (0.88 ± 0.98) (Tab 1 and Fig 1B). No relationships were observed between lesions and CAT and SOD activities.

CAT liver increases activity with Cu levels (r=0.606, p=0.028) suggesting that a metabolic increase was induced to cope with Cu oxidative stress. SOD activity in liver was lower than in gill and also lower than mullets from polluted environments [9,13] suggesting activity decrease, possibly due to high copper content that we found in liver.

Tab 2 – Enzymatic activities and metal concentrations in gill and liver of lagoon mullet (mean ± sd).

Parameters	***Gill***	***Liver***
SOD (U/mg pr)	10.1 ± 4.3**	3.8 ± 0.9
CAT (mmol/min/mg pr)	2.9 ± 2.6	39.2 ± 16.6**
Cu (mg.kg $^{-1}$ d.w.)	9.6 ± 1.6	378.9 ± 122.5**
Zn (mg.kg $^{-1}$ d.w.)	118.8 ± 26.5*	99.9 ± 30.9

*p<0.01; **p<0.05

Conclusions:

- ✓ Induction of antioxidant enzymes is a mechanism of adaptive response in fish and this study reveals tissue-specific changes in SOD and CAT activities.
- ✓ Liver achieved higher Cu levels but that do not compromise biotransformation enzymes response. In fact, lesions assessment was lower in liver than in gill and high Cu levels may have led to induction of CAT activity in order to reduce oxidative stress.
- ✓ In gill the presence of epithelial lifting could reduced CAT activity
- ✓ *Liza saliens* seems to have developed an adaptive response to metal-induced stress by increasing CAT-liver and SOD-gill activites.

References

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