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The impact of freshwater metal concentrations on the severity of histopathological changes in fish gills: A statistical perspective

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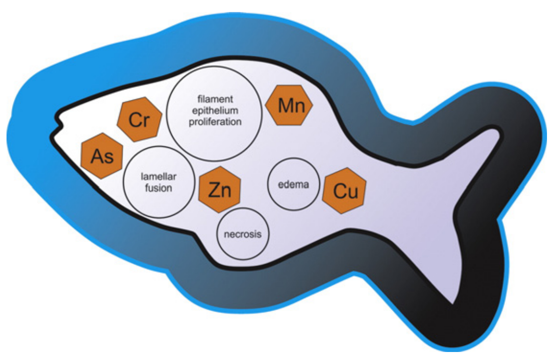
HIGHLIGHTS

- Gill histopathology of three fish species was evaluated in six Portuguese Rivers.
- Gill histopathological analysis revealed alterations in fish.
- Gill alterations act as a biomarker to toxicity of sub lethal concentrations of metals.
- Filament epithelium proliferation shows the highest correlation with metal concentrations.
- Boga is the most responsive species.

GRAPHICAL ABSTRACT



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ABSTRACT

The purpose of this study was to relate the severity of histopathological changes in fish gills with changes in metal concentrations of freshwater samples, and to use the relationships as premature warnings of impairment in aquatic fauna populations. The investigated species were the native barbel (*Luciobarbus bocagei*) and boga (*Pseudochondrostoma* sp.), and the introduced trout (*Oncorhynchus mykiss*), collected from 6 northern Portuguese rivers in a total of 249 individuals. The sampling sites have been linked to different ecological status by the official authorities. The sampling has been repeated 4 times to cover different hydrologic and environmental conditions. The analyzed metals were aluminum, arsenic, cadmium, cobalt, chromium, copper, manganese, nickel, lead and zinc. For each fish, 30 filaments of a gill arch were observed in a light microscope, and the histopathological changes evaluated according to a 6-degree gradation scale that combines the extent and severity of each lesion. The relationships between the histopathological and the chemical results were investigated by the non-parametric Goodman Kruskal gamma correlation and Partial Least Squares regression (PLS). The statistical results highlighted the importance of filament epithelium proliferation (FEP) as key biomarker to the toxicity of sub lethal concentrations of metals, because FEP was significantly correlated with all analyzed metals and explained through PLS regression by concentration changes of Cu, Zn, Mn, Cr and As. A refined regression analysis, where histopathological data on the 3 species were processed in separate, revealed that FEP severity is especially

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sensitive to changes in metal concentrations in boga. Thus, monitoring studies on the ecological status of northern Portuguese rivers would benefit in time and cost if FEP is used as biomarker and boga as species. Naturally, the option for this species depends on the availability of boga individuals along the stream reaches selected for the monitoring programs.

1. Introduction

Heavy metals, such as copper or zinc, are nutrients required for various biochemical and physiological functions (Rengel, 1999). However, their inadequate supply may result in a variety of deficiency diseases, while high concentrations may result in cell and tissue damage (Tchounwou et al., 2012). Pollution by heavy metals represents a major risk for public and wildlife health. The risk exists because some heavy metals may biomagnify through the aquatic food web or even bioaccumulate, with exceptions (Monroy et al., 2014). In general, the continuous exposure of lower organisms to reduced concentrations of heavy metals can expose predatory organisms, including humans, to potentially harmful concentrations (Brito et al., 2015; Stephansen et al., 2014).

In most recent years, the contamination of aquatic systems by heavy metals triggered public health and ecological apprehension. The growth of human exposure to heavy metals has been recognized and related to an expanding use of these substances in industrial, agricultural, domestic and technological products (Bradl, 2005; Duffus, 2002; Fergusson, 1990). Most environmental contamination and animal exposure to heavy metals were found to result from anthropogenic activities such as mining or production and use of fertilizers in cropland (Goyer and Clarkson, 1996; He et al., 2005; Herawati et al., 2000). Heavy metal concentrations were also the focus of European Union (EU) regulatory legislation. In that context, the Water Framework Directive - WFD (Directive, 2000/60/EC) stipulates predefined concentrations for some metals in receiving water masses. Coupled with improved control and abatement techniques, the action of EU resulted in the general reduction of heavy metal emissions (Pacyna et al., 2007). Northern Portuguese rivers are subject to important anthropogenic pressures, derived from direct discharge of domestic and industrial effluents into stream water, leachates from livestock excreta and dressings of cropland fertilizers, environmental land use conflicts, human induced wildfires, among others (Oliveira et al., 2005; Pacheco and Sanches Fernandes, 2016; Pacheco et al., 2015b; Santos et al., 2015a, 2015b; Valle Junior et al., 2015; Vieira et al., 2012, 2013). It is therefore essential to monitor heavy metal emissions in these rivers, to ensure their reduction as well as the harmful ecological effects derived therefrom. The periodic analysis of heavy metal concentrations in a river allows determining the water chemical status through chemical tests, but is not adequate to determine the river's ecological status. Early methods to assess the ecological condition of a river were based on indices of biotic integrity (IBIs), which frequently link a good ecological condition to a high abundance and diversity of aquatic fauna, namely macroinvertebrates (Hauer and Resh, 1996; Santos et al., 2015c; Valle Junior et al., 2015) or fish (Fame, 2005; Hermoso and Clavero, 2013; Simpson et al., 2000). Although IBIs are easy to use, they can only detect impairment when there is a switch in the structure of aquatic fauna communities. To prevent the occurrence of local extirpations, it essential to develop and implement early warnings as regards impairment of fauna populations. The need for such rapid and sensitive tools to reveal sub-lethal effects in aquatic organisms has led to the use of biomarkers (e.g. Fonseca et al., 2016). A biomarker is a change in a biological response, either at molecular, cellular, histological, physiological or behavioral level, that can be related with the exposure to toxic environmental elements (Colin et al., 2016).

Fish communities are key elements to evaluate the ecological condition of rivers based on biomarkers (Fonseca et al., 2016; Hermoso and Clavero, 2013; Scardi et al., 2008), while histopathology is the standard method for assessing both short- and long-term xenobiotic effects on fish (Hinton and Lauren, 1990). Histopathological changes in fish organs are fundamental indicators of a prior exposure to environmental stressors, probably being the result of adverse biochemical and physiological changes. Fish gills are among primary target organs of environmental pollutants because gills are in direct contact with water and are characterized by large surface area and absorption rates of chemicals (Pandey et al., 2008). Pathological alterations of gill epithelium are frequently the consequence of exposure to contaminants, especially to heavy metals (Fonseca et al., 2016), with the severity being dependent on the pollutant concentration and exposure time span (Tchounwou et al., 2012).

The association of high metal concentrations to severe injuries in fish gills may be investigated from various stand points, namely the statistical perspective (Cappello et al., 2016; Nowak and Lucas, 1997). Statistical techniques are generally useful because they can handle large amounts of data. Some techniques are particularly interesting because they can distinguish a subset of predictors relevant for a specific phenomenon, among a larger group of variables. In the sequel, these algorithms can alert for predictors not accounted for in the analysis. The multiple histopathological changes usually observed in fish gills (the “phenomenon”), as well as the large number of metals responsible for their development (the “predictors”), render attraction to these approaches, especially if the dataset is substantially large. The non-parametric Goodman and Kruskal's gamma rank order correlation (Goodman and Kruskal, 1954) or the Partial Least Squares (PLS) regression (Wold, 1966) are two among the methods that may eventually set up robust associations between metal concentrations and histopathological changes, because these techniques impose few assumptions on the dataset or the underlying theory. The Goodman and Kruskal coefficient is commonly used in all sorts of studies for long. Following the precursory work by Engle et al. (1986), numerous papers based on the PLS regression model have also been published (Aneiros-Pérez et al., 2004; Chen, 1988; Ferreira et al., 2017; Pacheco and Sanches Fernandes, 2016; Pacheco et al., 2015b; Schick, 1996; Speckman, 1988). The general purpose of this study was therefore to assess the relationship between the severity of fish gill histopathological changes and exposure to heavy metals in freshwaters, using the Goodman and Kruskal correlation and the PLS regression methods. To achieve the proposed goal, a number of specific objectives had to be attained through execution of predefined tasks: 1) collection of 249 fish specimens from species representing native and introduced fauna of northern Portuguese rivers, namely the native barbel (*Luciobarbus bocagei*) and boga (*Pseudochondrostoma* sp.) and the introduced trout (*Oncorhynchus mykiss*). The purpose of collecting native and introduced species was to compare the sensitivity of fish species to xenobiotics according to origin. The sampling comprised four campaigns and nine locations, which accounted for seasonality and differences in ecological status among sites; 2) analysis of fish gills for identification of lesions and their severity; 3) collection of 36 freshwater samples in the same sites as those used for fish capture, for analysis of heavy metal concentrations (ten substances in total); 4) preparation of a dataset with histopathological and chemical parameters, properly combining the fish species, the campaigns and the sampling sites, to be used in STATISTICA, version 7 (Statsoft Inc., 2004), with the correlation and regression methods; 5) Identification and interpretation of statistically significant associations between specific injuries and metals, in general or considering the species involved.