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From catchment to fish: Impact of anthropogenic pressures on gill histopathology

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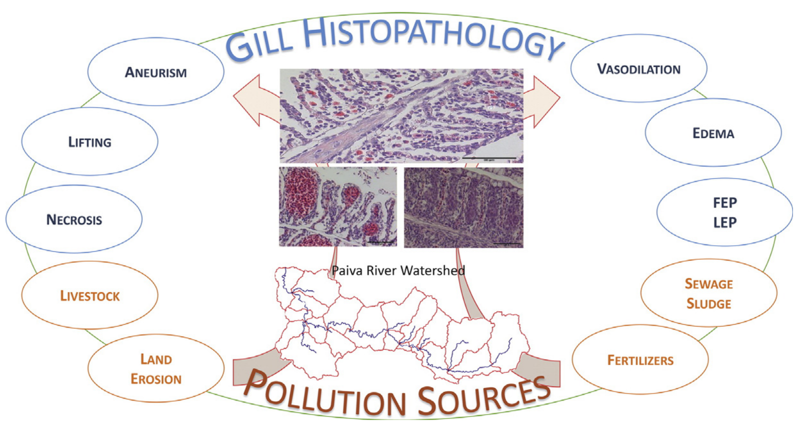
HIGHLIGHTS

- Gill histopathology of two fish species was evaluated from Paiva River;
- Gill histopathological analysis revealed alterations in fish.
- At reference location, mild disturbances were found in fish gills.
- Gill histopathology is an important biomarker of metal contamination.
- Paiva River suffers contamination from different sources of pollution.

GRAPHICAL ABSTRACT



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ABSTRACT

Gill histopathology was investigated in barbel (*Luciobarbus bocagei*) and nase (*Pseudochondrostoma* sp.) in sub-catchments of Paiva River (Portugal) located upstream and downstream of a Waste Water Treatment Plant (WWTP). Multivariate statistical analyses were performed to set up correlations between the species sample ($n = 24$) and injury types (8). The results discriminate well edema and vasodilatation between reference (upstream) and disturbed (downstream) samples. Using a watershed model, time series of physico-chemical parameters and heavy metal concentrations were calibrated and validated for the entire Paiva River basin as to investigate the relationship between water quality and the gill histopathology results. Increased concentrations of heavy metal downstream, specifically of zinc and lead, coincided with a higher severity of histopathological alterations in the fish gills. Significant but less evident relationship between water quality parameters and severity of gill injuries in the analyzed fish species were also observed for fecal coliforms, water temperature and manganese. Notwithstanding the location of the samples upstream and downstream of the WWTP, contamination of Paiva River and its effect on gill injuries cannot be disconnected from other punctual and diffuse pollution sources acting in different sectors within the watershed, namely agriculture and forest management. The severity of histopathological alterations in the fish gills reflected differences in the type and concentration of contaminants in Paiva River, and consequently can be viewed as valuable indicator of water quality.

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1. Introduction

The protection and conservation of river resources has never been more important than it is today. Loss of habitat and the increase of anthropogenic pressures on freshwater are leading to water quality deterioration endangering many aquatic species (Malmqvist and Rundle, 2002; Santos et al., 2015a; Valle Junior et al., 2015). Global freshwaters are experiencing a general decline in biodiversity due to overexploitation, water pollution, river domestication and habitat degradation (Dudgeon et al., 2006). According to Collen et al. (2014), the global population of freshwater fish has declined by 76% since the 1970s. This trend is expected to worsen in the course of increased human water demand and reduced rainfall promoted or amplified by anthropogenic climate change (Vörösmarty et al., 2010).

The rapid development of industry and agriculture has resulted in increasing pollution, especially by heavy metals which are a significant environmental hazard for invertebrates, fish and humans (Uluturhan and Kucuksezgin, 2007). The combination of these threats resulted in fish population declines, leading many freshwater species towards extinction (Darwall et al., 2008). To face this problem, international laws, such as the Water Framework Directive (WFD), have driven the need to assess the ecological status of the water bodies through an integrative ecosystem approach using elements of biotic quality, such as bioindicators (Directive, W.F., 2000). Along with diatoms, macroinvertebrates and macrophytes, fish are key elements to assess the ecological status of rivers. While diatoms have fast growth rates, and consequently respond quickly to variations in their environment, macroinvertebrates and macrophytes have a lifespan of over a year. Fish, in turn, respond to longer term (years) variations in the environment, being sensitive to changes in a wide array of environmental factors. In addition, assessing the health status of fish is a conservation priority given the marked decline of freshwater fish across the globe.

In response to the generalized trend in water quality deterioration, the development of tools to evaluate the ecological and chemical status of freshwater has been a major priority for the management of riverine ecosystems in the last decades (Hermoso and Clavero, 2013). After the first attempt by Karr (1981), indices of biotic integrity (IBIs) have been widely used to assess the ecological status of water bodies using fish as bioindicators (Hermoso and Clavero, 2013), namely the European Fish Index (EFI) (Fame, 2005) and the Australian River Assessment System (AUSRIVAS) (Simpson et al., 2000). Overall, these methods rely on the use of biological indicators combined, in a final score, with a set of other independent metrics (Hermoso and Clavero, 2013). Although IBIs are easy to use, they can only detect impairment when there is a switch in the structure of fish communities. Hence, there is a need to use early warning signs of impairment in fish populations, before local extirpations occur. The need for such rapid and sensitive tools to reveal sub-lethal effects in aquatic organisms has led to the use of biomarkers. A biomarker is known to be 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). One advantage of biomarkers relative to bioindicators is that the former allow examining specific target organs and cells, including gills, kidney and liver, that are affected by exposure to environmental chemicals and are responsible for vital functions such as respiration, excretion and the accumulation and biotransformation of xenobiotics (Van der Oost et al., 2003). The histopathological changes are easily recognizable alterations that ideally indicate alone a pathological condition, also reflecting the severity and duration of pollution events. Although they cannot reveal toxic identity, the histopathological biomarkers can identify the pollution type through its mode of action, further supporting the use of histopathological biomarkers as faithful indicators of environmental pollution (Colin et al., 2016).

Fish gills are highly sensitive to chemical and physical changes in the aquatic environment. Due to their large surface area and direct contact with water, the gills are easily damaged by numerous xenobiotics, even at low concentrations, being the first target organ of environmental pollutants (Karlsson, 1983; Pandey et al., 2008). Fish gill histopathological changes were previously considered as valuable biomarkers of water ecosystem stressors (Barišić et al., 2015; Cruz et al., 2015; Pereira et al., 2013).

Paiva River (North of Portugal) basin is subject to various anthropogenic pressures, namely leachates of farmland fertilizers along with domestic effluents around Castro Daire City, being also affected by discharges from a waste water treatment plant. The aim of this study is to evaluate the relationship between gill histopathological changes and water quality. The evaluation extends to the catchment scale, in contrast to most studies addressing this topic (Ameur et al., 2015; Barišić et al., 2015; Cruz et al., 2015), which limit the impact assessment to the environment around the water quality threats. Catchments are important elements to consider in ecology because as water flows over the ground and along rivers it can pick up nutrients, sediment and pollutants (Pacheco et al., 2015). To achieve the proposed goal, gills from two native fish species, representative of the northwestern Portuguese rivers ichthyofauna, namely barbel (*Luciobarbus bocagei*) and nase (*Pseudochondrostoma* sp.), were collected at two different locations, upstream and downstream of the Castro Daire wastewater treatment plant (WWTP). These two species typify the conservation status of other freshwater species in the Iberian Peninsula, which are also affected by common threats (Maceda-Veiga, 2013). The impact analysis was based on spatially distributed simulation models (HSPF and BASINS), which define the watershed as representative spatial unit. The novelty introduced by this study is therefore the presentation of a holistic assessment that couples watershed pollution with fish health symptoms, by structuring the watershed pollution sources at the sub-basin and site levels, where it is possible to create links between the impacts of water quality on fish health.

2. Materials and methods

2.1. Study area

The hydrographic basin of Paiva River, which is a sub-catchment of the Iberian Douro River basin, covers an area of approximately 780 km^2, being located in the north of Portugal (Fig. 1). Topography is characterized by a craggy relief where altitudes vary between 1360 m (at the headwaters) and 40 m (at the river's mouth). Precipitation is high across the catchment, but is much higher around the river's spring (2650 mm/yr) than around the basin's outlet (1240 mm/yr). Land use is mostly characterized by shrub land (47%), while agriculture covers 30%, forests 15% and urban land 3% of the basin area.

The Paiva River is subject to continuous discharges of wastewater sourced from the Castro Daire City treatment plant as well as direct inputs of domestic effluents where sewage systems are inexistent or ineffective. Waste water is a major contributor to pollution, but there are other sources to be accounted for, namely those identified in the Paiva River Watershed Plan (PRWP) (ARH-Norte, 2012) such as agriculture and forest management. The yearly loads of nitrogen, phosphorus and the biochemical oxygen demand (BOD) for agricultural and forest areas are shown in Table 1. According to the PRWP, the manure loads are distributed equally between agriculture and forest areas. Metal loads were not specified in the PRWP and no information could be compiled from elsewhere. Thus, when modeling metal concentrations across the catchment (next section) default (automatically set by the software) calibration and threshold values had to be used.

2.2. Hydrology and water quality modeling at catchment scale

The Hydrologic Simulation Program (HSPF, in FORTRAN) coupled with the Better Assessment Science Integrated point and Nonpoint