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Evaluation of architectural and histopathological biomarkers in the intestine of brown trout (*Salmo trutta* Linnaeus, 1758) challenged with environmental pollution

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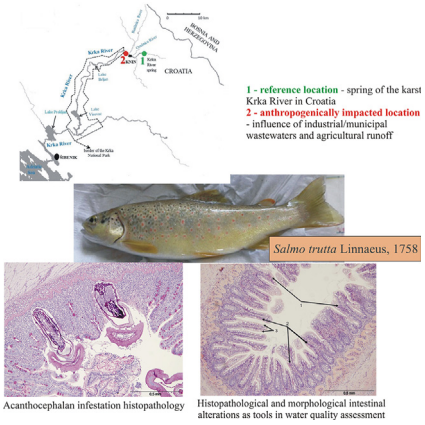
HIGHLIGHTS

- Pollution impact was assessed by fish intestinal histological/morphological changes.
- Decreased enterocyte supranuclear area indicated influence of water pollution.
- Fish infected with acanthocephalans exhibited specific histopathological anomalies.

GRAPHICAL ABSTRACT



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ABSTRACT

In the present study novel histopathological approach, using fish intestine as a sensitive bioindicator organ of pollution impact in the freshwater ecosystem, was proposed. Histopathological alterations were compared between native brown trout (*Salmo trutta* Linnaeus, 1758) from the reference (Krka River spring) and pollution impacted location (influence of technological/municipal wastewaters and agricultural runoff near the Town of Knin) of the karst Krka River in Croatia. In brown trout from both locations, severe parasitic infestation with acanthocephalan species *Dentitruncus trutae* was found, enabling evaluation of acanthocephalan infestation histopathology, which indicated parasite tissue reaction in a form of inflammatory, necrotic and hyperplastic response that extended throughout *lamina epithelialis mucosae*, *lamina propria*, and *lamina muscularis mucosae*. New semi-quantitative histological approach was proposed in order to foresee alterations classified in three reaction patterns: control tissue appearance, moderate (progressive) tissue impairment and severe (regressive and inflammatory) tissue damage. The most frequent progressive alteration was hyperplasia of epithelium on the reference site, whereas the most frequent regressive alterations were atrophy and necrosis seen on the polluted site. Furthermore, histopathological approach was combined with micromorphological and macromorphological assessment as an additional indicator of pollution impact. Among 15 observed intestinal measures, two biomarkers of intestinal

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tissue damage were indicated as significant, height of supranuclear space (hSN) and number of mucous cells over 100 μm fold distance of intestinal mucosa (nM), which measures were significantly lower in fish from polluted area compared to the reference site. Obtained results indicated that combined histological and morphological approach on fish intestinal tissue might be used as a valuable biological tool for assessing pollution impact on aquatic organisms. Therefore, semi quantitative scoring and multiparametric morphological assessment of intestinal tissue lesion magnitude should become a common approach to handle environmental pollution impact.

1. Introduction

The health of aquatic organisms and water quality are interconnected and directly proportional (Zimmerli et al., 2007). Due to their close contact with the environment, fish homeostatic mechanisms are highly dependent on existing conditions in their immediate surrounding, so even slight variations in water quality can cause a wide variety of biological responses (Authman, 2015; Nussey et al., 1995). For the evaluation of the impact of environmental contaminants on biota, various biochemical, molecular and histocytopathological biomarkers are usually applied. In contrast to commonly used biochemical and molecular biomarkers of pollutant exposure or effect, which still might be recovered or repaired at the molecular level (Authman, 2015; Gaber et al., 2014; Yeganeh et al., 2016), histopathological alterations represent more reliable indicators of specific influences of pollutants on aquatic organisms (Bernet et al., 1999). Since pollution caused histopathological changes in organs and tissues frequently occur before producing irreversible effects on the biota, histological methods are considered as a sensitive and early warning signs of pollution and therefore, have the advantage to be used in evaluation of potential risk for the species survival, as well as for the environmental protection (Gaber et al., 2014).

After prolonged exposure of fish to water contaminants, even in very low concentrations, various morphological and histological alterations occur in different organs, of which the mostly investigated were liver (Adams et al., 2010; Bernet et al., 2000; Giari et al., 2007; Girdoniya, 2016; Jordanova et al., 2016; Poleksic et al., 2010; van Dyk et al., 2009; Zeitoun et al., 2014; Zimmerli et al., 2007), and gills (Barišić et al., 2015; Fonseca et al., 2016, 2017; Lujić et al., 2015; Rašković et al., 2015). To date, only few studies reported histological alterations in the fish intestinal tissue, most of them in relation to heavy metal exposure and bioaccumulation preferences (Ajima et al., 2015; Bandowe et al., 2014; Ebrahimpour et al., 2011; Liu et al., 2012; Poleksic et al., 2010; Pyle et al., 2005), while only few of them reported the histomorphological endpoints of the intestine in relation to pollution impact (Ferrando et al., 2006; Gaber et al., 2014; Kaoud and El-Dahshan, 2010). Digestive tract represents one of the primary pathways of contaminant input to the fish body, as well as the organ for excretion processes, which is therefore exposed to surrounding contaminants and may reflect pollution impact. Toxicant effects upon the gastrointestinal tract of fishes were described in range from mild changes in motility, secretion, and absorptive functions to more severe effects associated with mucosal integrity, blood flow or neuromuscular control. These effects may in turn influence mucosal barrier function, biochemical reactions, microflora, nutrition, and, ultimately, the ability of the organism to thrive (Kleinow and James, 2005).

In the present study, fish intestine was selected as an indicator tissue of water pollution due to its importance in fish digestion and nutrient absorption, as well as considering limited data on pollution impacted histological alterations in intestine. Fishes are generally considered as an excellent pollution bioindicators, since they are organisms on the top of the aquatic food chain, long living, easy to collect, and of optimum size for analyses (Chovanec et al., 2003). Moreover, Bernet et al. (1999) have introduced the standardized histopathological assessment tools for fish liver, gills, kidney and skin, but standardized method for describing and assessing histological lesions in the intestine of freshwater fishes was not described yet. Therefore, the main goals of the present study were: a) to propose appropriate adjustments of highly accepted procedure of semiquantitative histopathological approach described by Bernet et al. (1999), regarding intestinal tissue; b) to evaluate micromorphological and macromorphological parameters, including introduction of new measures and calculations, as additional tools in the water quality assessment, following the method described by Escaffre et al. (2007).

Application of histopathological and architectural biomarkers in fish intestine was estimated in native fish population from the Krka River, a typical karst river in the Dalmatian karst area of the Republic of Croatia. Previous investigations of the wastewater influences on the river water quality involved chemical, microbiological and metal analyses of the river water or sediment and pointed to disturbed ecological water status downstream of the wastewater impact and surrounding agricultural soils near the Town of Knin (Cukrov et al., 2008, 2012; Filipović Marijić et al., 2018). Comprehensive assessment of the pollution impact on the aquatic organisms dwelling in the Krka River near the Town of Knin was not conducted yet. Accordingly, brown trout (*Salmo trutta* Linnaeus, 1758) was selected as an indicator organism since it is a typical representative of the Krka River ichthyofauna and moreover, widely spread species in European rivers, what provides the possibility for comparison between geographically distant regions. Water contamination was related to its possible effects on brown trout by novel approach using semiquantitative histopathological and morphological alterations in fish intestinal tissue exposed to mixture of contaminants under realistic, environmental conditions.

2. Materials and methods

2.1. Study area

Due to specific karst topography and extremely low natural levels of nutrients and metals, karst rivers are especially vulnerable to anthropogenic impact. Krka River is 72.5 km long karst river with an average flow rate of 50 $m^3\ s^{-1}$. Thanks to the constant process of tufa-deposition which built unique tufa cascades and waterfalls, as well as to high biodiversity and many endemic species, it represents a unique karst phenomenon, whose lower part was proclaimed national park in 1985 (Cukrov et al., 2012). However, specific pollutant sources, agricultural runoff from the surrounding rural area, as well as technological wastewater of the screw factory and municipal wastewater of the Town of Knin (11,000 inhabitants) are released without proper purification in the river water only few kilometres upstream of the beginning of the Krka National Park and represent a serious threat to the tufa barriers and overall living world. Previous investigations pointed to deteriorated ecological status of the Krka River, below good water quality status according to conductivity, chemical oxygen demand, levels of ammonium, total nitrogen, total phosphorus, nitrate and bacteria counts, even near the border of the Krka National Park (Filipović Marijić et al., 2018). Concentrations of majority of the 25 measured total dissolved metals/metalloids were significantly increased at locations impacted by the wastewater outlets compared to the river source, but their levels pointed to still moderate pollution impact. This was supported by the fact that concentrations of the most measured metals were again decreased downstream of wastewater sources, except of Al, Co, Fe, Li, Mn, Ni, Sr, Ti, and Zn whose levels remained increased for 2–400