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Effect of acanthocephalan infection on metal, total protein and metallothionein concentrations in European chub from a Sava River section with low metal contamination

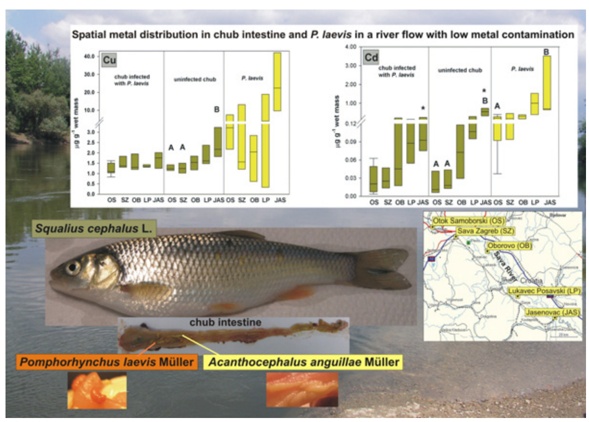
Vlatka Filipović Marijić *, Irena Vardić Smrzlić, Biserka Raspor

Division for Marine and Environmental Research, Ruđer Bošković Institute, P.O. Box 180, 10002 Zagreb, Croatia

HIGHLIGHTS

- Higher levels of Cu, Mn, Ag, Cd, Pb in acanthocephalans than in chub intestine.
- Bioconcentration factors for non-essential metals (Ag, Cd, Pb) ranged from 7 to 113.
- Bioconcentration factors for essential metals (Cu and Mn) ranged from 1 to 14.
- Chub infected with P. laevis had lower Cu, Cd, Pb levels than uninfected chub.
- Comparable spatial distribution of Cu and Cd levels in P. laevis and chub intestine.

GRAPHICAL ABSTRACT



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ABSTRACT

In the present study, the importance of considering fish intestinal parasites i.e. the acanthocephalans in metal exposure assessment was estimated under low metal contamination conditions. Two acanthocephalan species, *Pomphorhynchus laevis* and *Acanthocephalus anguillae* were examined in 59 specimens of European chub (*Squalius cephalus* L.) sampled at 5 locations along the Sava River, Croatia. Concentrations of essential (Cu, Mn) and non-essential (Ag, Cd, Pb) metals were higher in intestinal parasites than chub gastrointestinal tissue, but levels of essential metals Fe and Zn were comparable or lower in parasites, respectively. The highest accumulation in both acanthocephalan species was found for non-essential metals and followed the order: Ag > Pb > Cd. Higher infection intensity with *P. laevis* allowed us to present their spatial metal distribution and evaluate the influence of *P. laevis* on metal levels and sub-cellular biological responses (total protein and metallothionein levels) in the host infected with *P. laevis*. Even in the river section with low metal contamination, parasitism affected metal levels, resulting in lower Cu, Cd and Pb concentrations in chub infected with *P. laevis* than in uninfected chub. Although total protein and metallothionein levels remained constant in infected and uninfected chub, acanthocephalans should be considered a potential confounding factor in metal exposure assessments. Moreover, *P. laevis*-chub system can be suggested as an appropriate tool in biomonitoring, since in both species increased Cu and Cd concentrations towards the

* Corresponding author. Tel.: +385 1 468 0216; fax: +385 1 468 0242.
E-mail addresses: vfilip@irb.hr (V. Filipović Marijić), ivardic@irb.hr (I. Vardić Smrzlić), raspor@irb.hr (B. Raspor).

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downstream locations were found. Higher Cu and Cd levels in *P. laevis* suggest acanthocephalans to be sensitive bioindicators if low metal levels have to be detected.

1. Introduction

Animals such as bivalves, crustaceans and fishes are often used as bioindicators of metal pollution. Although there are common and well established indicator organisms and tissues, as well as biomarkers of metal exposure, we are still in need of reliable and sensitive bioindicators to monitor low levels of metal pollution in the environment (Sures, 2004a). Therefore, the need for new and more sensitive indicators of metal accumulation, than the established ones, still exists. In the past decades, the interrelation between parasites and contaminants has gained increasing interest, especially in aquatic ecotoxicology (Sures, 2001, 2003).

Certain fish intestinal parasites, such as acanthocephalans, can accumulate metals at concentrations that are orders of magnitude higher than those in the host tissues or in commonly used indicator organisms like bivalves or in the aquatic environment. Accordingly, attempts have been made to utilize acanthocephalans as biological indicators of metal exposure in environmental risk assessment studies (Sures, 2004a, 2004b; Sures et al., 1999a; Vidal-Martinez et al., 2010). Acanthocephalans are widespread and common parasites of fishes and, although their lifespan is generally shorter than that of their host, their metal concentrations are likely to respond rapidly to changes in environmental exposure (Kennedy, 1985; Siddall and Sures, 1998). By using acanthocephalans in environmental impact studies very low concentrations of metals can be detected, owing to the enormous accumulation capacity of these parasites. In addition, parasites might be used to pre-concentrate metals of their surrounding environment to concentrations high above the detection limit of an analytical method (Sures, 2004b). Numerous reports have been published on the uptake and accumulation of metals by parasites. Sures (2004a, 2004b) and Vidal-Martinez et al. (2010) demonstrated that acanthocephalans can be successfully used for monitoring purposes. The overview of selected recent field studies on metal accumulation in parasites such as Acanthocephala, Cestodes, Digenea and Nematoda and their hosts, has been presented by Sures (2004b). Analysis of the available literature on parasites as sentinels reveals that a few parasite species could be promising indicators of metal accumulation. Among the variety of aquatic parasites that have been investigated so far, the acanthocephalan *Pomphorhynchus laevis* particularly meets some of the criteria commonly suggested for sentinels (Sures, 2003). This parasite shows very high metal accumulation capacity (Sures and Siddall, 2003; Sures et al., 2003) and it is a common and widespread parasite of chub and barbel, both of which are distributed on a global scale. The genus *Pomphorhynchus* appears to be a promising sentinel in many aquatic areas of the world. The data on metal accumulation of intestinal parasite *P. laevis* in the host such as barbel from Danube River in Hungary (Thielen et al., 2004) and Bulgaria (Nachev et al., 2013), as well as in chub from the Ruhr River in Germany and the respective bioconcentration factors (Taraschewski and Sures, 1996) are of specific interest for our study.

Vidal-Martinez et al. (2010) assessed the usefulness of parasites as bioindicators of environmental impact, compiling selected studies published during the past decade. By means of a factorial meta-analysis they have demonstrated significant effects and interactions between parasite levels and the presence and concentration of various pollutants and/or environmental stressors. The authors address study design and make recommendations for future studies to minimize sampling effort and maximize useful biomonitoring information. Considerable amount of information on: i) the use of parasites as indicators; and ii) the effects of parasites on common bioindication procedures such as the analysis of biomarkers is still lacking (Sures, 2004b). In that sense, our field study, accomplished in the river water with low level of metal contamination, presents metal distribution in two acanthocephalan species, *P. laevis* and *Acanthocephalus anguillae*, and their definitive host, freshwater fish European chub (*Squalius cephalus* L.). With respect to *P. laevis* a number of papers have already addressed its accumulation capacity particularly for non-essential metals, under laboratory conditions as well as in field studies (Nachev et al., 2013; Siddall and Sures, 1998; Sures and Siddall, 1999, 2001, 2003; Sures et al., 2003). In most of these studies chub host was involved, although previously named *Leuciscu cephalus* a synonym for the valid name *S. cephalus*. On the other hand, field studies dealing with *A. anguillae* are scarce.

Selected essential (Cu, Fe, Mn, Zn) and non-essential (Ag, Cd, Pb) trace metals were analysed in intestinal parasites and chub gastrointestinal tissue, and the respective bioconcentration factors calculated. The bioconcentration factor is considered to be a potential tool for the evaluation of environmental metal exposure in freshwater areas of low level of metal contamination. Spatial distribution of metal concentrations in fish and acanthocephalans was analysed at 5 sampling locations along a 150 km long section of the Sava River, characterized as containing low total dissolved metal concentrations (Dragun et al., 2009). In addition, metal concentrations in gastrointestinal tissue of chub infected with *P. laevis* and uninfected chub were compared, since it was reported that acanthocephalans might alter metal uptake and accumulation, resulting in reduced metal levels in tissues of infected compared to uninfected hosts (Sures, 2008). There are relatively few investigations examining the interaction between ecotoxicology and parasitology in freshwater ecosystems. So far these studies investigated the interactions between parasitism and biological responses, such as cellular biomarkers against oxidative stress, antitoxic responses, energy reserves, immune defense, involving bivalves (Heinonen et al., 2001; Minguez et al., 2009, 2012), crustaceans (Gismondi et al., 2012a, 2012b) and fishes (Marcogliese et al., 2005). In our study possible impact of acanthocephalans was extended on chub biological response to metal exposure, regarding total cytosolic proteins, as indicators of general stress, and metallothioneins (MT), as proteins involved in metal homeostasis and detoxification (Langston and Bebiano, 1998).

2. Materials and methods

2.1. Fish and parasite collection

A total of 59 specimens of European chub (*S. cephalus* L., Cyprinidae) were collected in September 2006 at the 150 km long section of the Sava River in Croatia. The selected area involved 5 sampling sites, from the Croatian–Slovenian state border (Otok Samoborski) to the state border between Croatia and Bosnia and Herzegovina (Jasenovac) (Fig. 1). The Sava River water quality status was characterised by the average total dissolved metal concentrations, based on grab water samples taken at the same section of the Sava River during 2006. The average total dissolved metal concentrations in the Sava River water followed the order (in $\mu g\ l^{-1}$): Fe (12.6) > Mn (3.44) > Zn (2.27) > Ni (0.59) > Cu (0.54) > Pb (0.06) > Cd (0.01) (Dragun et al., 2009). According to Gaillardet et al. (2004) who reported the world average levels of total dissolved metals for so-called "natural" river systems, based on the metal concentrations in major world rivers (excluding the heavily polluted ones), total dissolved trace metal concentrations in the Sava River were not significantly above the "natural" level.

Fish were caught by electro-fishing method, according to the Croatian Standard HRN EN, 14011: 2005, and the minimum number of animals