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Does fish reproduction and metabolic activity influence metal levels in fish intestinal parasites, acanthocephalans, during fish spawning and post-spawning period?

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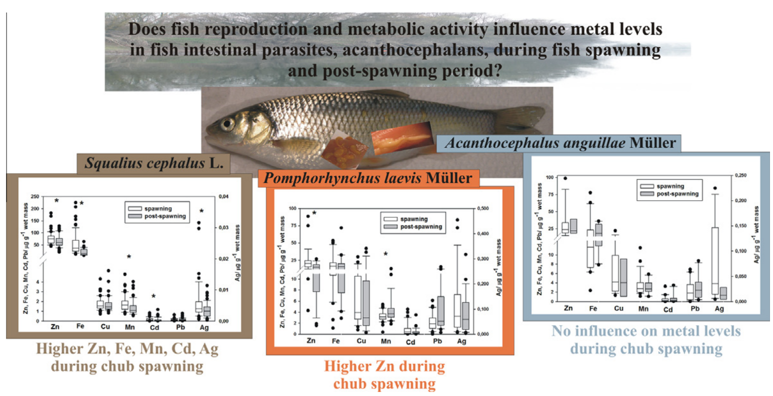
HIGHLIGHTS

- Higher Zn, Fe, Mn, Cd, Ag levels in chub gastrointestinal tissue during spawning.
- Possible influence of fish physiology on acanthocephalan metal levels is unknown.
- In parasites during chub spawning only Zn was higher in *Pomphorhynchus laevis.*
- Bioconcentration factors were higher in the post-spawning period for Fe, Mn, Ag, Pb.
- Chub physiology affected gastrointestinal metal levels and bioconcentration factors.

GRAPHICAL ABSTRACT



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ABSTRACT

Application of fish intestinal parasites, acanthocephalans, as bioindicators in metal exposure assessment usually involves estimation of their metal levels and bioconcentration factors. Metal levels in parasite final host, fishes, are influenced by fish physiology but there is no data for acanthocephalan metal levels. Gastrointestinal Zn, Fe, Mn, Cd, Ag levels in European chub (*Squalius cephalus* L.) from the Sava River were significantly higher during chub spawning (April/May) compared to the post-spawning period (September). In acanthocephalans (*Pomphorhynchus laevis* and *Acanthocephalus anguillae*) significantly higher metal levels during chub spawning were observed only for Zn in *P. laevis*. Bioconcentration factors were twice as high for Fe, Mn, Ag, Pb in the post-spawning period, probably as a consequence of lower gastrointestinal metal levels in fish rather than metal exposure. Therefore, bioconcentration factors should be interpreted with caution, due to their possible variability in relation to fish physiology. In addition, gastrointestinal Cu, Cd and Pb levels were lower in infected than uninfected chub, indicating that metal variability in fishes might be affected by the presence of acanthocephalans.

1. Introduction

Comprehensive approach in assessing metal exposure in the aquatic environment usually involves chemical analysis of water, which primarily comprises the total metal concentrations, and the application of different bioindicators in order to estimate

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possible adverse effects of metals (Dallinger, 1994). Various organisms have been investigated for their potential as bioindicators of metal exposure in the aquatic environment; among them bivalves, crustaceans and fish are the most commonly used (Phillips and Rainbow, 1993; Rinderhagen et al., 2000; Dragun et al., 2013; Kolarević et al., 2013; Vuković-Gačić et al., 2013). Recently, there has been an increasing interest in the relationship between parasitism and metal pollution due to the high metal accumulation potential and rapid response to the environmental metal exposure of fish intestinal parasites acanthocephalans (Sures, 2003). It was reported that acanthocephalans have a potential to serve as bioindicators of effect and accumulation (Sures, 2001). The indication of effect might be evident from the parasite population changes, while indication of accumulation from the parasite capability to accumulate metals to orders of magnitude higher values than those in the host tissues or the environment (Sures, 2001). Interactions between the environment and host-parasite systems are complex and not easy to interpret as they are dependent on a wide variety of factors. There are some papers reporting the influence of parasites on host immunology, antitoxic defence capacities or structural damage of intestine (Dezfuli et al., 2002; Đikanović et al., 2010; Gismondi et al., 2012). To our knowledge, there are no studies on metal variations in acanthocephalans in relation to the physiological state of their host. In this research, we have assumed that acanthocephalans are affected by the host physiology and therefore, the first data on acanthocephalan metal fluctuations regarding fish physiology are presented.

Our previous studies revealed seasonal variations of metal levels in different fish tissues due to fish biometric parameters, age and spawning, which were regarded as the confounding biotic factors in metal exposure assessment (Filipović and Raspor, 2003; Filipović Marijić and Raspor, 2007, 2008, 2010, 2012, 2014). In the present paper basic epidemiological characteristics of acanthocephalans hosted in European chub, *Squalius cephalus* L., from the Sava River in Croatia were assessed. In addition, metal concentrations in chub gastrointestinal tissue and acanthocephalans were evaluated as the respective bioconcentration factors, comprising two for chub specific seasons, period of spawning, intense feeding and metabolic activity (April/May) and the post-spawning period (September). Presented approach will enable us to define possible sources of metal variability in acanthocephalans, not just regarding metal exposure in the environment but also physiological differences in metal concentrations of their host. Such knowledge is essential for proper assessment of metal exposure in the freshwater ecosystem when using acanthocephalans as bioindicators. The sampled section of the Sava River was characterized as low metal contaminated (Dragun et al., 2009) and therefore, in such freshwater ecosystem the influence of biotic factors has been assumed to be predominant over metal contamination. This statement was confirmed in our previous research, which indicated for the same chub specimens comprised in the present study, that site-specific differences in gastrointestinal metal concentrations were related to the fish spawning and age, and not to anthropogenic contamination (Filipović Marijić and Raspor, 2012, 2014). Such environmental conditions allowed us to estimate the impact of chub spawning and physiological status on selected essential (Zn, Fe, Cu, Mn) and non-essential metals (Cd, Pb, Ag) in two acanthocephalan species, *Pomphorhynchus laevis* and *Acanthocephalus anguillae* and in gastrointestinal tissue of their host in comparison to the post-spawning period. Gastrointestinal tissue was selected as an indicator organ, since it represents a site of dietborne metal exposure in fish, which is considered to be of equal of greater importance than the waterborne for native fish (Campbell et al., 2005). Bioconcentration factors and possible protective role of parasites against metal accumulation in chub were also studied in relation to the fish reproductive period and related physiological changes.

2. Materials and methods

2.1. Sample collection and preparation

Collection of European chub specimens (*S. cephalus* L., Cyprinidae) was conducted at the 150 km long section of the Sava River in Croatia, from the Croatian–Slovenian state border to the state border between Croatia and Bosnia and Herzegovina (Filipović Marijić et al., 2013). The fish were caught during 2006 in April/May, a period of chub spawning and enhanced feeding (76 specimens) and in September, chub post-spawning period (59 specimens). Sampling was performed by electro-fishing, according to the Croatian Standard HRN EN 14011: 2005. Total length and age range of fish individuals sampled in April/May was 14.7–27.0 cm and 2–5 years and in September 13.5–31.5 cm and 1–5 years. The captured fish were kept alive till further processing in the laboratory in 50 L aerated water collected at the same location as fish individuals. After the specimens were anesthetized with MS 222 (tricaine methane sulphonate, Sigma Aldrich) and sacrificed, the gastrointestinal tract (stomach, anterior, middle and posterior intestine) was removed and cut along its entire length and carefully checked for the presence of intestinal parasites. Due to the limited number of acanthocephalans, few individuals were collected, counted and fixed in 75% ethanol for morphological identification, few of them were stored at −80 °C for molecular analysis and the rest of acanthocephalans found in intestinal segment were stored at −80 °C for metal determination. After the acanthocephalans and the whole gut content were removed, gastrointestinal tissue was weighed and stored at −80 °C for subsequent metal determination.

The Sava River water quality status was characterized by the average total dissolved metal concentrations, based on grab water samples taken at the same section of the Sava River during 2006. Despite several wastewater inputs from urban/industrial sources, total dissolved metal concentrations in the Sava River water were low and followed the order (in $\mu g\,L^{-1}$): Fe (12.6) > Mn (3.44) > Zn (2.27) > Cu (0.54) > Pb (0.06) > Cd (0.01) (Dragun et al., 2009). According to Gaillardet et al. (2004), who classified the world average levels of total dissolved metals for so-called "natural" river systems, based on 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.

2.2. Morphological and molecular identification of acanthocephalan species

Morphological identification of parasites was done under the light microscope after clearing the fixed specimens in methyl salycilate or staining in Semichon's acetocarmine (Petrochenko, 1956; Heil, 2009). Additionally, nucleotide sequence analysis of the three molecular markers: cytochrome c oxidase subunit I (COI), ITS region (internal transcribed spacer) and 18S rRNA was used to confirm parasite species identification as described previously (Filipović Marijić et al., 2013). The sequence analyses by GenBank BLAST program (Altschul et al., 1997) confirmed the identification of the two acanthocephalan species: *P. laevis* and *A. anguillae*.

2.3. Acid digestion of chub gastrointestinal tissue and acanthocephalans

Chub gastrointestinal tissue was digested using a microwave oven (Anton Paar 3000, Austria) under the following conditions: power 1000 W, temperature 200 °C, ramp time 20 min, hold time