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The relative importance of diet-related and waterborne effects of copper for a leaf-shredding invertebrate

J.P. Zubrod [a, *], D. Englert [a], R.R. Rosenfeldt [a], J. Wolfram [a], S. Lüderwald [a], D. Wallace [a], N. Schnetzer [a], R. Schulz [a], M. Bundschuh [a, b]

[a] Institute for Environmental Sciences, University of Koblenz-Landau, Fortstraße 7, 76829 Landau/Palatinate, Germany
[b] Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences, Lennart Hjelms väg 9, 75007 Uppsala, Sweden

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ABSTRACT

Copper (Cu) exposure can increase leaf-associated fungal biomass, an important food component for leaf-shredding macroinvertebrates. To test if this positive nutritional effect supports the physiological fitness of these animals and to assess its importance compared to waterborne toxicity, we performed a 24-day-bioassay in combination with a 2×2 factorial design using the amphipod shredder *Gammarus fossarum* and a field-relevant Cu concentration of 25 μg/L ($n = 65$). Waterborne toxicity was negligible, while gammarids fed leaves exposed to Cu during microbial colonization exhibited a near-significant impairment in growth (~30%) and a significantly reduced lipid content (~20%). These effects appear to be governed by dietary uptake of Cu, which accumulated in leaves as well as gammarids and likely overrode the positive nutritional effect of the increased fungal biomass. Our results suggest that for adsorptive freshwater contaminants dietary uptake should be evaluated already during the registration process to safeguard the integrity of detritus-based ecosystems.

1. Introduction

Copper (Cu) is an essential trace element to terrestrial and aquatic organisms, serving *inter alia* as a component of a variety of enzymes (e.g., the oxygen-carrier hemocyanin in crustaceans; Flemming and Trevors, 1989). In freshwater ecosystems, however, human activities such as mining and agriculture have led to elevated Cu-levels (e.g., Sridhar et al., 2000; Süss et al., 2006). As a result, Cu poses a high risk to aquatic life (e.g., Donnachie et al., 2014). Accordingly, numerous studies have focused on adverse effects of Cu on individual species up to whole freshwater food webs (see e.g. reviews by Eisler, 1998; Flemming and Trevors, 1989; Nor, 1987). However, implications of Cu in the colonization of leaf material by aquatic fungal decomposers (i.e., conditioning) and the resulting effects on the nutrition of leaf-shredding invertebrates, which rely heavily on the changes in leaf quality brought about by fungi (Bärlocher, 1985; Suberkropp, 1992), have largely been ignored. In this context, it was recently shown that Cu exposure can increase leaf-associated fungal biomass, which was sensed by the amphipod shredder *Gammarus fossarum* Koch (a species known to prefer food that allows high growth rates; Bärlocher and Kendrick, 1973), actively selecting Cu exposed over unexposed leaves (Zubrod et al., 2015b). It may hence be hypothesized that the presence of Cu during the conditioning of leaf material positively affects the physiological status of shredders over the long term (cf. Bärlocher and Kendrick, 1973), especially as metal toxicity via dietary uptake is commonly considered comparably insignificant (Abel and Bärlocher, 1988; Sridhar et al., 2001).

To test this hypothesis as well as its importance compared to waterborne toxicity, we employed a 24-day-bioassay in combination with a 2×2 factorial design: the first factor was the absence or presence of Cu during the conditioning of leaf material (used as food for our model shredder) and the second factor was the absence or presence of Cu in the medium used for culturing the shredders during the bioassay (=waterborne exposure). Despite its high baseline Cu content (e.g., Bourgeault et al., 2013), we chose *G. fossarum* as model shredder as it is a key species in leaf litter decomposition in many European streams (Dangles et al., 2004) and its effect thresholds in terms of Cu related feeding preferences and waterborne Cu toxicity are well established (Zubrod et al., 2014, 2015b). On this basis, we expected a Cu concentration of 25 μg/L,

* Corresponding author. Institute for Environmental Sciences, University of Koblenz-Landau, Fortstraße 7, D-76829 Landau, Germany.
E-mail address: zubrod@uni-landau.de (J.P. Zubrod).

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which is realistic for instance for streams impacted by wastewater treatment plant effluents or agriculture (e.g., da Silva Oliveira et al., 2007; Süss et al., 2006), to increase leaf-associated fungal biomass (cf. Zubrod et al., 2015b). We hypothesized further that, in consequence of long-term consumption of such Cu exposed leaves, the physiological fitness (judged by growth and lipid content) of *Gammarus* would increase. Since waterborne toxicity at the applied Cu concentration should be low (cf. Zubrod et al., 2014), an overall positive net effect on *Gammarus* was expected when subjected to combined effects from ingestion of Cu exposed leaves and exposure via water.

2. Materials and methods

2.1. Sources of leaves, microorganisms, and gammarids

Leaves of *Alnus glutinosa* (black alder), a species that features comparably low C:N ratios of <20 (e.g., Foucreau et al., 2013) and is decomposed in freshwater systems relatively fast (e.g., Schindler and Gessner, 2009), were collected in October 2012 near Landau, Germany (49°11′N; 8°05′E) and stored at −20 °C until further processing. Following the procedure described by Zubrod et al. (2011), leaves were deployed for 14 days in fine-mesh bags in the Rodenbach, Germany (49°33′N, 8°02′E), upstream of any agricultural activity, settlement, and wastewater inlet, to establish a natural microbial community. Back in the laboratory, unconditioned (i.e., previously not subjected to microbial colonization) leaves were added to the retrieved leaf material and the mixed leaves were kept in aerated conditioning medium (Dang et al., 2005) at 16 ± 1 °C in total darkness for at least another 12 days before use as microbial inoculum.

Seven days prior to their introduction into the bioassay, *G. fossarum* were kick-sampled in the Hainbach, Germany (49°14′N; 8°03′E), upstream of any agricultural activity, settlement, and wastewater inlet, where the local population is exclusively composed of cryptic lineage B (Feckler et al., 2014). Only visually non-parasitized adult males (6–8 mm body length) were used. Throughout their acclimation to laboratory conditions, animals were kept in a temperature-controlled chamber at 16 ± 1 °C in total darkness and fed *ad libitum* with pre-conditioned black alder leaves, while they were gradually adapted to the nutrient medium used during the bioassay (i.e., SAM-5S; Borgmann, 1996).

2.2. Experimental design

Our experimental design, following in general Zubrod et al. (2011), consisted of two compartments – a leaf conditioning to provide gammarids with microbially colonized food and the bioassay with *Gammarus* (see below). The employed 2×2 factorial design, resulted in four treatments: (i) a control with uncontaminated leaf material and gammarids cultured in Cu free medium, (ii) leaf material exposed to Cu, gammarids not (i.e., indirect effect pathway), (iii) gammarids exposed to Cu, leaf material not (i.e., direct effect pathway), and (iv) leaf material and gammarids exposed to Cu (i.e., both pathways combined). All Cu containing media were dosed with Cu sulfate pentahydrate (Fluka, St. Gallen, Switzerland) at a nominal concentration of 25 μg Cu/L.

2.3. Experimental conditioning

Leaf strips of 3–5 cm × 5–9 cm were cut from unconditioned black alder leaves and placed in aerated circular aquaria ($n = 3$; 150 strips per aquarium) filled with 14 L conditioning medium together with 50 g fresh weight of the microbial inoculum. Conditioning took place at 16 ± 1 °C in total darkness. Three aquaria were dosed with Cu at 25 μg/L, while three represented control conditions. The conditioning medium (with the appropriate concentration of Cu) was renewed every 3 days to ensure a continuous exposure, accounting *inter alia* for losses due to potential adsorption to the test vessels (cf. Zubrod et al., 2014). After 12 days, two leaf discs (2.0 cm diameter) were cut from each strip, one disc from each side of the leaf's midrib. Discs were immediately introduced into the bioassay or frozen (−20 °C) for the analyses of leaf-associated fungal biomass and Cu content. To provide the gammarids during the bioassay every 6 days with food of constant quality, four conditionings were performed, each one starting 12 days before the respective leaves were introduced into the bioassay.

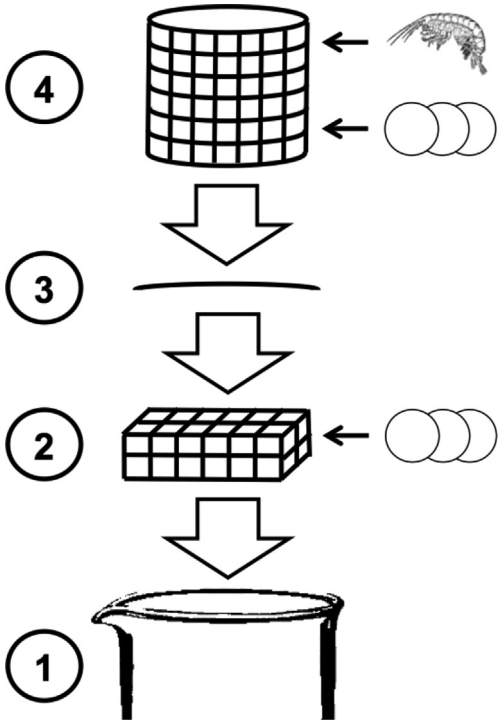

Fig. 1. Scheme illustrating the setup of the bioassay replicates. At the bottom of a (1) 250-mL glass beaker filled with 200 mL bioassay medium a (2) rectangular cuboid cage containing three leaf discs is situated. This cage is topped by a (3) watch glass on which a (4) cylindrical cage containing another three leaf discs and one gammarid is placed. For more details see text.

2.4. Bioassay

Each of the four treatments of the bioassay comprised 65 replicates consisting of 250-mL glass beakers filled with 200 mL bioassay medium (item 1 in Fig. 1). Beakers were equipped with cylindrical cages made from stainless steel mesh screen and within each cage one gammarid was placed together with three leaf discs originating from three separate leaf strips (item 4 in Fig. 1). These cages allowed a careful transfer of the animals to new vessels containing fresh bioassay medium (with the appropriate concentration of Cu) every 3 days (ensuring a continuous exposure; cf. Zubrod et al., 2014) and prevented the animals from coprophagy. In addition, a rectangular cuboid cage made from the same stainless steel mesh screen (item 2 in Fig. 1) was situated on the bottom of each glass beaker below the cylindrical one. This cage contained the corresponding three leaf discs, which originated from the same three leaf strips as the leaf discs offered as food to the gammarid and allowed to control for abiotic and microbial leaf mass losses in each replicate. To prevent that the feces produced by the animal interacts with the leaf discs within the rectangular cuboid cage, the