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Natural organic matter (NOM) induces oxidative stress in freshwater amphipods *Gammarus lacustris* Sars and *Gammarus tigrinus* (Sexton)

Maxim A. Timofeyev [a,b], Zhanna M. Shatilina [b], Aleksey V. Kolesnichenko [b,c], Darya S. Bedulina [a], Viktoria V. Kolesnichenko [b,c], Stephan Pflugmacher [d], Christian E.W. Steinberg [e,*]

[a] *Irkutsk State University, Ul. Karl-Marx 1, 664003, Irkutsk, Russia*
[b] *Baikalian Research Center, Ul. Lenina 21-13, 664003, Irkutsk, Russia*
[c] *Siberian Institute of Plant Physiology and Biochemistry SB RAS, Lermontova 132, 664033, Irkutsk, Russia*
[d] *Leibniz Institute of Freshwater Ecology and Inland Fisheries, Müggelseedamm 310, 12587 Berlin, Germany*
[e] *Humboldt University at Berlin, Institute of Biology, Freshwater Ecology, Arboretum, Späthstraβe 80/81, 12437 Berlin, Germany*

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Abstract

Humic substances comprise the majority of natural organic matter (NOM) on Earth, including dissolved organic matter in freshwater systems. Recent studies show that these substances directly interact with aquatic organisms as chemical stressors. The aim of the present study was to investigate the mode of action of dissolved NOM on the freshwater amphipods *Gammarus lacustris* Sars and *Gammarus tigrinus* (Sexton), and in particular, to determine if NOM induces or promotes internal oxidative stress. NOM was isolated by reverse osmosis from a brown-water lake in Brandenburg State, Germany. Oxidative stress markers, such as lipid peroxidation, cell internal hydrogen peroxide concentration, as well as peroxidase, catalase and glutathione *S*-transferase activities, were quantified. Exposure of both amphipod species to NOM caused a significant increase in lipid peroxidation, hydrogen peroxide concentration, catalase, peroxidase and glutathione *S*-transferase activities. Both species showed a two-stage antioxidant response: the first stage allowed the organisms to effectively eliminate ROS and to protect cells from damage, whereas the second stage leads to H_2O_2 accumulation in combination with destruction of lipid structures in the cells and, finally, functional damage or even death of the organism.

Keywords: Amphipods; NOM; Humic substances; Catalase; Peroxidase; Glutathione *S*-transferase; Hydrogen peroxide; Oxidative stress; Lipid peroxidation

1. Introduction

Natural organic matter (NOM) is part of all aquatic ecosystems and mainly comprises humic substances (HS), which represent 60–80% of the total dissolved organic carbon (Steinberg and Münster, 1985; Thurman, 1985). Despite the fact that NOM is mainly formed as a

* Corresponding author.
E-mail address: christian_ew_steinberg@web.de (C.E.W. Steinberg).

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result of decomposition of terrestrial plants, NOM plays a crucial role in all aquatic ecosystems (Wetzel, 2001). The functions of NOM in aquatic systems include influence on energy regimes (Steinberg, 2003), modulation of concentration and toxicity of xenobiotics and metals, and decrease of pollution and aquatic organisms' exposure to potentially toxic substances (Gjessing, 1981; Campbell et al., 1997; Haitzer et al., 1999).

Relatively little is known about direct adverse impact of NOM on freshwater organisms. Pioneering studies by Petersen and Persson (1987) showed direct adverse effects of dissolved NOM on *Daphnia magna*. As potential mode of action, they discussed an irritation of membranes by the lipophilic NOM fraction, which was already postulated by Münster (1985) based on electrophoretic studies of HS. It is also known that NOM can modulate the activity of biotransformation enzymes of organisms, and also directly inhibit photosynthetic oxygen production of plants (Pflugmacher et al., 1999, 2001). Further biomarkers indicating weak chemical stresses imposed by NOM have been identified: For instance, Wiegand et al. (2004) found elevated levels of the chaperon HSP70 in fishes and amphipods, and Timofeyev et al. (2004) reported modulation of peroxidase activity in amphipods after exposure to HS. A comprehensive perspective of the ecophysiological function of dissolved HS is given by Steinberg et al. (in press).

Recent studies have revealed a significant impact of NOM on the physiological condition and sex ratio in fishes and amphibians (Meinelt et al., 2004; Lutz et al., 2005). Applying oligonucleotide-based whole genome DNA microarray experiments to the nematode *Caenorhabditis elegans*, it has been shown that an artificial humic substances, as well as a NOM source, induce transcriptional changes, which were identified in chemosensors and olfactory receptors, and enzymes of the biotransformation system (CYP, UGT, GST). The results confirmed that HS are recognized as environmental signals and weak chemical stressors (Menzel et al., 2005). Very recently, also the induction of CYP1A in fishes has been identified (Matsuo et al., in press). Furthermore, the latter paper showed that also organic transporters were activated in *C. elegans*, indicating that HS are taken up, as reported previously based on ^{14}C-uptake studies with ^{14}C-labeled HS-like substances (Steinberg et al., 2003).

Several mechanisms of the direct impact of NOM on aquatic organisms are still unclear. One of the mechanisms under discussion is the potential of dissolved NOM to form various external reactive oxygen species (ROS), such as superoxide, hydroxyl radical and hydrogen peroxide (Paul et al., 2004) on photoexicitation. These ROS may subsequently be taken up by freshwater organisms. Another mechanism is internal ROS production. Due to the apparently low-molecular mass of the water-soluble and ionizable HS fraction (Hoque et al., 2003; Reemtsma and These, 2003; Cooper et al., 2004; Hatcher et al., 2004; Seitzinger et al., 2005), NOM (including HS) can easily be taken up by freshwater organisms (Steinberg et al., 2003). Metabolism of NOM was shown to generate ROS (Timofeyev et al., 2004). Among ROS, hydrogen peroxide and superoxide anions are main initiators of a number of cellular reactions, including the iron-catalyzed Fenton reaction (Droge, 2002). The main cellular components susceptible to damage by ROS are lipids with subsequent peroxidation of unsaturated fatty acids in membranes, proteins with subsequent denaturation, as well as carbohydrates and nucleic acids (Blokhina et al., 2003).

The aim of the present paper has been to study the mechanisms of NOM impact on freshwater organisms and, in particular, to assess the possible role of NOM in the promotion of oxidative stress in freshwater amphipods and to identify their response to the oxidative stress on the biochemical level. As test organisms, the amphipods *Gammarus lacustris* and *G. tigrinus* were used. The responses of the amphipods to NOM exposure include internal H_2O_2 level, modulation of enzyme activities, such as that of catalase, peroxidase, glutathione *S*-transferase and lipid peroxidation.

2. Materials and methods

2.1. Amphipods

Two amphipods (Crustacea, Amphipoda), considered to be typical inhabitants of diverse continental water bodies, were chosen for this study: *G. lacustris* Sars, which is a cold water inhabitant widely spread in Palaearctic lakes (Barnard and Barnard, 1983), and *Gammarus tigrinus* (Sexton), which has recently been introduced to Germany from North America (Szaniawska et al., 2003). Specimens of *G. tigrinus* were collected from Lake Müggelsee (Berlin, Germany) during October to November 2003 and 2004. Specimens of *G. lacustris* were collected from several shallow lakes in eastern Siberia (Russia) during August to September 2003 and 2004. The size of the collected individuals was 1–1.5 cm (*G. lacustris*) and 1.5–2 cm (*G. tigrinus*). Age and sex of amphipods were not determined (in order to minimize handling of the delicate organisms); however, we assumed that most of the amphipods were sub-adults because of the clearly sub-maximal body size.

All amphipods were reared for a few days prior to experiments in aerated 5-L tanks with distilled water