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## Oxidative damage in rainbow trout caged in a polluted river

**Bethanie Carney Almroth\*, Joachim Sturve, Lars Förlin**

*Department of Zoology, Göteborg University, Box 463, SE-405 30, Göteborg, Sweden.*

### **Abstract**

Sewage treatment works (STWs) are a common source of chemicals entering into the aquatic environment. In order to assess effects of these effluents on oxidative stress parameters in aquatic organisms, we caged rainbow trout at 5 sites: upstream, near an STW effluent, and three sites downstream in the river Viskan in western Sweden for 14 days during autumn, 2006. We then measured protein carbonyls in plasma as well as 20S proteasome activity and lipid peroxidation products, i.e. MDA and 4-HNE, in liver samples. Levels of both lipid and protein oxidative damage products were elevated in fish caged near the STW effluent while 20S activity showed no differences. This argues that complex mixtures of chemicals entering into the aquatic environment do have deleterious effects on fish. Additionally, oxidative stress parameters can serve as a biomarker in aquatic organisms.

*Keywords:* Oxidative stress; Protein carbonyls; 20S proteasome; Lipid peroxidation; Rainbow trout

Corresponding author:

*Email address:* bethanie.carney@zool.gu.se (B.C. Almroth)

Oxidative stress, an imbalance between oxidative and reductive processes in the cell, is relevant in many diseases and in the effects of aging. Reactive oxygen species (ROS) are produced naturally during metabolism, and they are normally prevented from having toxic effects by antioxidants, both molecular and enzymatic. Environmental pollutants can affect the balance between prooxidants and antioxidants in biological systems. When the production of ROS is greater than the cells' ability to remove them, damage can occur in DNA, lipids and protein molecules.

Several different forms of oxidative damage can occur in protein molecules, including thiolation, methylation and carbonylation. The first two mentioned are reversible and may serve some antioxidant function (Stadtman and Levine, 2000). Protein carbonyl formation, on the other hand, is irreversible and can lead to enzymatic degradation of the proteins, mostly via the 20S proteasome (Grune, 2000). The relative stability of protein carbonyls makes them suitable markers of ROS-mediated protein oxidation and several methods of detection have been developed (Levine, 1990, Winterbourn and Buss, 1999). Research has shown that protein carbonylation increases both with age and with oxidative stress (Grune, 2000). Activity of the 20S proteasome has also been implicated in oxidative stress (Reinheckel et al., 1998). Decreases in 20S activity result in the accumulation of oxidized protein agglomerates which is in turn responsible for a number of oxidative stress related pathologies. Lipid peroxidation products, i.e. MDA and 4-HNE, are also known to form as a result of oxidative stress and can interfere with protein metabolism (Valavanidis, 2006).

Sewage treatment works (STWs) are a common source of chemicals entering into the aquatic environment. Effluent water from STWs contains complex mixtures of chemicals, including for example PAHs, pharmaceuticals, solvents and flame retardants, originating from household use as well as industry. Little is known about how such complex mixtures of chemicals affect aquatic organisms. Therefore, we conducted a field study using rainbow trout as a model organism to investigate effects of environmental exposure to STW effluent on oxidative stress parameters in fish. Juvenile rainbow trout (*Oncorhynchus mykiss*) weighing  $59.4 \pm 13.4$  g were caged for 14 days in November at 5 different sites in the river Viskan in western Sweden. Water quality was turbid. The site called Öresjö, upstream of the STW and of the city downtown, served as a reference. Fish were also caged at Gässlösa, near the STW effluent, and at 3 stations downstream, called Djupasjön, Guttasjön and Rydboholm. At sampling, fish were killed by a blow to the head. Blood was removed via the caudal vein and then centrifuged to collect plasma which was frozen at  $-80^{\circ}\text{C}$ . Liver samples were stored in liquid nitrogen until homogenized and then stored at  $-80^{\circ}\text{C}$  until analysed.

Biochemical measurements were conducted as follows. Protein carbonyls were measured in plasma using an ELISA (Winterbourn and Buss, 1999) and quantified using a colorimetric method (Levine, 1990). Chemotryptic activity of the 20S proteasome was measured in liver homogenate according to Reinheckel et al. (2000) in sodium phosphate buffer containing 0.5 mM DTT. MDA and 4-HNE in liver tissue were assayed using the LPO-586 kit from Bioxytech, USA, according to the manufacturer's instructions.

Results indicated that fish caged at Gässlösa, nearest to the STW effluent, were affected by pollutants, as seen in the oxidative stress parameters presented here. These fish had significantly higher levels of protein carbonyls (Fig. 1). No differences were seen in 20S activities in livers of

the fish caged at the 5 different sites (data not shown). However, lipid peroxidation, measured as MDA and 4-HNE was elevated at Gässölsa (Fig. 2). 4-HNE is an oxidative damage product known to bind to and inhibit 20S activity (Esterbauer et al., 1991), which could in turn have confounding effects on the accumulation of oxidatively damaged proteins. Additionally, an accumulation of oxidatively damaged proteins has been shown to result in the formation of protease-resistant aggregates which in turn have been argued to bind to and inhibit 20S proteasome activity (Grune et al., 2004). So, a regulatory increase in 20S activity may have been masked by inhibition of enzymatic activity.

The river Viskan in which the rainbow trout were caged is known to contain polluted sediments at the sites located downstream of the city (Djupasjön, Guttasjön and Rydboholm). Many of these pollutants, including for example PAHs, nonylphenol and heavy metals, originate to a large extent from textile industries that have been active in the city since the mid 1800s as well as from non-point source runoff. The results presented here indicate, however, that oxidative stress parameters in the fish were most likely affected by current release of chemicals, probably originating from the STW, since the fish caged at Gässlösa were the only ones to show significant changes in oxidative damage, and that this damage is not likely to originate from 'historical' pollutants in the sediment further downstream. It is difficult to assess the degree of exposure to 'historical' chemicals without chemical data on both sediment and fish tissue, but turbid water would indicate some degree of availability of particle-bound pollutants to biota.

The use of oxidative stress parameters in biomonitoring of environmental pollution has increased in recent years (Valavanidis, 2006) and has great potential as an early warning sign, especially in combination with other biomarkers. Consequently, more work is needed to clarify effects of different compounds as well as complex mixtures on oxidative stress in teleost species.

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**Figure captions**

Fig. 1. Protein carbonyls in plasma of rainbow trout caged in a polluted river for 14 days.  $n = 10$ . Bars indicate mean  $\pm$  standard error. \* indicates statistical difference compared to reference site Öresjön,  $p=0.027$ , Dunnet's T-test.

Fig. 2. Lipid peroxidation in liver tissue of rainbow trout caged in a polluted river for 14 days.  $n = 10$ . Bars indicate mean  $\pm$  standard error. \* indicates statistical difference compared to reference site Öresjön,  $p=0.040$ , Dunnet's T-test.



Fig. 1

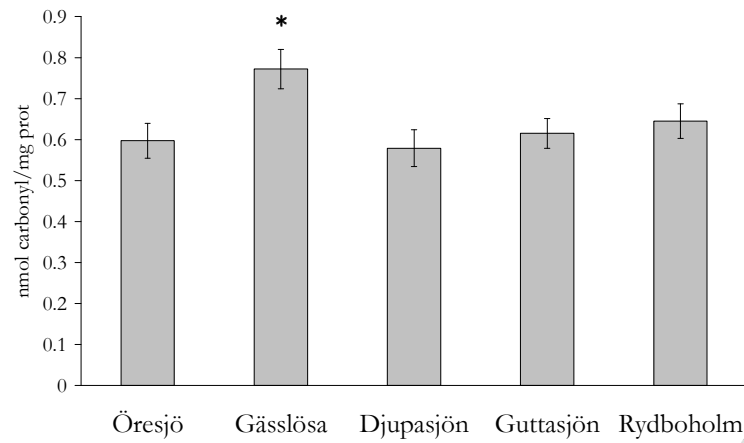


Fig. 2

