



GÖTEBORGS UNIVERSITET

**From Proteomic Analysis to Biomarker
Application
-Studies of Carbonyl Reductase in Fish**

Eva Albertsson
Zoologiska institutionen
Naturvetenskapliga fakulteten

Akademisk avhandling för filosofie doktorsexamen i Zoofysiologi, som med tillstånd från Naturvetenskapliga fakulteten kommer att offentligt försvaras kl.10.00, fredagen den 14:e oktober, 2011, i föreläsningssalen, Zoologiska institutionen, Medicinargatan 18, Göteborg.

ISBN: 978-91-628-8348-5



GÖTEBORGS UNIVERSITET

Abstract

Many anthropogenic substances are present in the aquatic environment, but there is limited information on how this combination of chemicals affects exposed wildlife. To assess the impact of chemicals, a biomarker approach is frequently applied in environmental biomonitoring programmes.

In this thesis, proteomic analysis was used as a hypothesis generator for biomarker development in fish. Two-dimensional gel electrophoresis was performed on a liver fraction from rainbow trout (*Oncorhynchus mykiss*) that were caged downstream of a sewage treatment plant, and it was found that a protein identified as carbonyl reductase was induced. This was confirmed with qPCR, indicating that carbonyl reductase mRNA expression levels were markedly induced by the same effluent and also by a second investigated sewage treatment plant effluent of a similar character. More advanced sewage treatment technologies reduced carbonyl reductase mRNA expression, indicating that fish that were exposed to effluents of conventionally treated sewage have induced hepatic carbonyl reductase expression.

To obtain more information regarding the possible inducers of carbonyl reductase, several different single-substance exposure studies were performed with rainbow trout in controlled aquaria systems. The results demonstrated that hepatic carbonyl reductase expression was not induced by the steroids that were tested but by an aryl hydrocarbon agonist and a pro-oxidant. Others have observed substances with the same mode of action in the effluents of Swedish sewage treatment plants.

To promote the use of carbonyl reductase as a biomarker, a method for measuring enzymatic activity was developed in the sentinel coastal fish species eelpout (*Zoarces viviparus*), which is included in the Swedish national integrated fish-monitoring program. The protocol for carbonyl reductase enzymatic activity combined with qPCR analyses in eelpout were applied on a comprehensive field material, including sites with different grades of pollution among the Swedish, Danish and German coasts. Carbonyl reductase activity and mRNA did not show any site-specific differences between the polluted and reference sites. Therefore, it can be concluded that the induction of carbonyl reductase appears to be a promising biomarker for assessing exposure and the possible impact of sewage treatment plant effluents in caged fish. However, more research is required before and if carbonyl reductase can be used in biomonitoring programmes as a biomarker to assess the impact of toxic chemicals in wild fish populations.

Keywords: Fish, proteomics, biomarker, carbonyl reductase, AbR agonists, oxidative stress, biomonitoring