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Protein carbonyls and antioxidant defenses in corkwing wrasse (*Symphodus melops*) from a heavy metal polluted and a PAH polluted site

Bethanie Carney Almroth^{a*}, Joachim Sturve^a, Eiríkur Stephensen, Tor Fredrik Holth^b and
Lars Förlin^a

^a Department of Zoology, Zoophysiology, Göteborg University, Box 463, SE 405 30
Göteborg, Sweden

^b Norwegian Institute for Water Research (NIVA) Gaustadalléen 21, N-0349 Oslo, Norway
and the Department of Biology, University of Oslo, P.O. Box 1066, Blindern, N-0316 Oslo,
Norway

*Corresponding author

Tel: int + 46 (0) 31 773 3449

Fax: int + 46 (0) 31 773 38 07

bethanie.carney@zool.gu.se

19 **Abstract:**

20 The use of fish in environmental monitoring has become increasingly important in recent
21 years as anthropogenic substances, many of which function as prooxidants, are accumulating
22 in aquatic environments. We have measured a battery of antioxidant defenses as a measure of
23 oxidative status, as well as protein carbonylation as a measure of oxidative damage, in
24 corksiding wrasse (*Symphodus melops*) captured near a disused copper mine, where water and
25 sediment are contaminated with heavy metals, and an aluminum smelter, a site contaminated
26 with PAHs. Results were compared to two different reference sites. Fish at the heavy metal
27 site had lower glucose-6-phosphate dehydrogenase activity and elevated protein carbonyls
28 (1.8 times) compared to fish from the reference site. At the PAH site, EROD was increased 2-
29 fold, while total glutathione and methemoglobin reductase concentration, were decreased. No
30 differences were seen in protein carbonyl levels at the PAH site. Measures of both antioxidant
31 defenses and oxidative damage should be used when assessing effects of xenobiotics on
32 oxidative stress in fish species.

33
34 **Keywords:** fish; oxidative stress; antioxidant enzymes; biomarker; protein carbonyls; PAHs;
35 heavy metals

1. Introduction

Coastal marine environments are often exposed to the influences of anthropogenic activities and consequentially, toxic chemicals. Many of these xenobiotics exert their effects through redox-cycling, resulting in the production of reactive oxygen species (ROS). ROS production resulting from xenobiotic redox-cycling is an important mechanism of pollutant toxicity and can lead to oxidative damage to proteins, lipids and DNA (Stadtman and Oliver 1991). This is linked to diseases such as carcinogenesis in fish (Livingstone 2001). Protein damage resulting from oxidative stress may be useful as a biomarker for exposure of fish to environmental contaminants (Carney Almroth et al., 2005, Shi et al., 2005, Bagnyukova et al., 2006). The form of oxidative damage to protein molecules that is most widely accepted as a biomarker is protein carbonylation (Shacter et al., 1994). The formation of carbonyl derivatives is irreversible and increases the susceptibility of proteins to proteases (Stadtman and Oliver 1991). A number of enzymes and molecules play important roles in detoxifying xenobiotics and in protecting the cell against the harmful effects of ROS. Many of these enzymes and molecules are well studied in mammals (Halliwell and Gutteridge 1999) and have been applied as biomarkers for environmental risks in fish (van der Oost, et al. 2003, Valavanidis 2006).

Oxidative stress is a very complex phenomenon, starting with the production of ROS and including oxidative damage or disruption as well as adaptive responses of antioxidant defense components. Thus, instead of choosing a single biomarker for oxidative stress, use of a battery of biomarkers will provide a more complete picture of the effects of xenobiotics on oxidative stress in the cells of an organism. This battery should preferably include both oxidative damage as well as changes to the antioxidant defense system. In the current study a

large battery of biomarkers for pollutant exposure was assessed in corkwing wrasse (*Symphodus melops*) caught at a PAH contaminated site and a heavy metal polluted site near Stavanger, Norway. The biomarkers included were the phase I enzyme CYP1A and the phase II enzyme glutathione S-transferase as were the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) and DT-diaphorase (DTD) in liver, and methemoglobin reductase (MtHB) and glucose-6-phosphate dehydrogenase in blood. In addition to these enzymes, total and oxidized glutathione and metallothionein concentrations in liver were measured. Also, as an example of oxidative damage, protein carbonyl formation was measured. Though protein oxidation products have been used as biomarkers for oxidative stress and damage in humans (Dalle-Donne et al., 2003), their usefulness has not been fully explored as a potential biomarker in fish. The species of wrasse used in the current study is known to possess a limited home range within the littoral zone, as it is a territorial species. Its benthic behavior should make it a suitable sentinel species for studies involving contaminants (Aas, et al. 2001). In addition, corkwing wrasse are ubiquitous throughout the northeastern Atlantic and Mediterranean (Quignard and Pras 1986), making them suitable for European biomonitoring campaigns.

2. Materials and methods

2.1. Chemicals

Biotinylated anti-dinitrophenyl-KLH rabbit IgG fraction was purchased from Molecular Probes, streptavidin-biotinylated horse radish peroxidase (HRP) from Amersham Bioscience, UK, and the BCA Protein Assay Reagent Kit from Pierce. Tween 20 was obtained from BIO-

RAD. Copper (II) chloride dihydrate and EDTA (Titriplex III) were purchased from Merck, heparin from LEO Pharma AB, Sweden, and trasylol from Bayer, Germany. Trifluoroacetic acid, oxidized and reduced glutathione, glutathione reductase, reduced glutathione (GSSG), 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB), dichlorophenol indophenol (DCPIP), 2, 4-dinitrophenol hydrazine (DNPH) and rabbit metallothionein were obtained from Sigma. NADPH and NADH were purchased from Boehringer Mannheim and dicoumoral (DIC) was purchased from Aldrich. All other reagents were of analytical grade.

2.2. Field study sites

Corkwing wrasse (*Symphodus melops*) were caught at 4 sites using fyke nets during field sampling near Stavanger, Norway, in the fall of 2001. Fish were collected from the heavy metal site Visnes and the PAH site Høgvarde and the respective reference sites, Salvøy and Bokn. See figure 1 for site locations. The two polluted sites are well known as sinks for industrial pollution. Visnes is a village located outside Stavanger and is home to a copper mine which was in operation during several periods between 1865 and 1965. Tailings and slag were dumped into the sea and heavy metals, including copper, zinc and iron, contaminated the sea water and sediment as a result of dumping and land run off. There was no evidence of higher organisms close to the mine, where copper concentration in the water was $53.6 \mu\text{g l}^{-1}$. Andersen et al. (2003a) reported the contaminant levels in water, sediment and biota at Visnes as follows. The sediment content of metals at Visnes reaches up to 20000 mg Zn kg^{-1} , 13000 mg Cu kg^{-1} , 1500 mg Pb kg^{-1} and 35 mg Cd kg^{-1} . The site displays a sharp gradient in copper concentration, from the mouth of the mine to the sea. Copper concentration in the water at the catch site during this study was $8.59 \mu\text{g l}^{-1}$, zinc was $33.6 \mu\text{g l}^{-1}$, iron $96 \mu\text{g l}^{-1}$ and lead $0.135 \mu\text{g l}^{-1}$. Analyses of blue mussel (*Mytilus edulis*) tissue from Visnes indicated

48 mg Cu kg dry weight (d.w.)⁻¹, 257 mg Zn kg⁻¹ d.w., 470 mg Fe kg d.w.⁻¹ and 1.3 mg Pb kg d.w.⁻¹, levels which were 9.6, 4.0 and 4.2 and 2.6 fold higher than at the reference site.

Høgvarde is located outside an active aluminum smelter and is contaminated with a mixture of primarily pyrogenic PAHs (from the smelter) (Aas et al., 2001). In 2001, 0.6 tons of PAHs were discharged from the aluminum smelter into the sea. At the PAH site, both blue mussels and shore crabs (*Carcinus maenas*) were shown to contain high levels of PAHs in their tissues, totalling 38.3 mg kg⁻¹ d.w. in mussels and 7.8 mg kg d.w.⁻¹ in crabs. The levels in mussel were 1158 fold higher than at the control site and 16 fold higher in crabs (Andersen et al., 2003b).

The two polluted sites differed with respect to water salinity and current velocity (personal communication with Odd-Ketil Andersen, IRIS – Biomiljø, International Research Institute of Stavanger, Norway) and consequently required comparison with different reference stations. Water temperature at respective depths differed by no more than 0.5°C. Samples taken from Visnes were compared to the reference site Salvøy since both sites are exposed to the same water mass, the open ocean with a salinity of approximately 33‰. Samples taken at Høgvarde were compared to the reference site Bokn as these sites are located inside the archipelago with a lower average salinity of approximately 29.5‰.

2.3. Sampling

In order to reduce the risk of measuring changes due to capture-related stress, fish were caged on site 2-3 days after capture before being sacrificed. After acclimatization, fish were killed by a blow to the head, measured and weighed. A blood sample was taken from the caudal vein using a syringe treated with heparin. Blood was centrifuged at 6 000 g for 2 minutes and

plasma samples were immediately frozen at -80°C where they were stored until measurement. The liver was excised, weighed, divided into pieces, and stored in liquid nitrogen until analysis. The numbers of fish sampled at each site were as follows: Salvøy – 10 males, 8 females; Visnes – 10 males, 10 females; Bokn – 10 males; Høgvarde – 10 males, 6 females. No female fish were caught at Bokn.

2.4. Protein carbonyl measurements

An ELISA method described by Winterbourn et al. (1999) was used to measure protein carbonyls in blood plasma of cormorant wrasse. In brief, plasma samples were derivatized with DNPH (10 mM DNPH in 6 M guanidine hydrochloride, 0.5 M potassium phosphate buffer, pH 2.5) which binds selectively to protein carbonyl groups. Detection was achieved using biotinylated anti-dinitrophenyl (anti-DNP) and streptavidin-biotinylated HRP. A standard curve of fully reduced and oxidized bovine serum albumin was used to quantify results, according to a colorimetric method described by Levine et al. (1990).

2.5. Enzymatic activities

Preparation of liver microsome and cytosol fractions was performed according to Förlin (1980). Ethoxyresorufin-*O*-deethylase (EROD) activity was measured in the microsomal fraction using ethoxyresorufin as a substrate and NADPH to provide reducing equivalents (Förlin et al., 1994). Hepatic antioxidant enzyme activities were measured in the cytosolic fraction. Superoxide dismutase (SOD) activity was measured using 6-hydroxy dopamine as a substrate. The assay was run with and without sample to distinguish between SOD-catalyzed activity and auto-oxidation reactions (Heikkilä and Cabbat 1976). Catalase (CAT) activity

was measured in liver cytosol with hydrogen peroxide as a substrate, according to Aebi (1985). Glutathione reductase (GR) activity was measured using GSSG as a substrate, with NADPH acting as an electron donor. The resulting reduced GSH then reacts with 5,5'-Dithio-bis-(2-nitrobenzoic acid) (DTNB), forming a glutathione-TNB conjugate and free TNB, the production of which is measured over time (Cribb et al., 1989). Glutathione *S*-transferase (GST) was measured using 1-chloro-2, 4-dinitrobenzene (CDNB) as a substrate as described by Habig et al. (1974) and adapted to a microplate reader by Stephensen et al. (2002). The formation of GST-catalysed CDNB-glutathione conjugate is measured spectrophotometrically. DT-diaphorase activity (DTD) was measured according to Sturve et al. (2005). Here, 2,6-dichloroindophenol (DCIP) was used as a substrate, and samples were analysed with and without the addition of dicumarol, a DTD inhibitor, to distinguish between DTD-catalysed reduction and the action of all other reductases. Red blood cells were lysed and cytosolic fractions prepared according to Beutler (1984). SOD activity in the red blood cells (rbc) was measured following the same method as was used in liver samples (Heikkila and Cabbat 1976). Methemoglobin reductase activity (MetHb) in the rbc was measured using $K_3Fe(CN)_6$ as a substrate according to Beutler (1984). Glucose-6-phosphate dehydrogenase (G6PDH) activity in the rbc was measured using glucose-6-phosphate and NADP⁺ in the reaction buffer, according to Deutsch (1987).

2.6. Molecular antioxidant measurements

Total glutathione (tGSH) and oxidized glutathione (GSSG) were measured according to Baker et al. (1990), adapted to a microplate reader by Vandeputte et al. (1994). This is an indirect method in which all GSH is reduced through the action of GR, followed by conjugation to DTNB as described above in section 2.5. To measure the GSSG portion of the

total glutathione pool in the sample, GSH was first derivitized by a reaction with 2-vinylpyridine.

2.7. Metallothionein

The concentration of metallothionein was measured using differential pulse polarography (DPP) according to Olafson and Olsson (1991). Briefly, cytosolic fractions of liver were prepared according to Förlin et al. (1980), diluted 10x in 0.9% NaCl and heat denatured at 95°C for 4 min. Heat-denatured samples were centrifuged 15 min at 10 000 g after which the supernatant was analysed with DPP electrolyte (2 mM hexamine cobalt chloride, 1M NH₄Cl, 1M NH₄OH 300 µl 0.025% Triton X-100 added per 10 ml electrolyte). The purging time with N₂ before measurements was 60 sec. A standard of 50.8 µg/ml rabbit MT in 0.9% NaCl was used to prepare a standard curve.

2.8. Morphological indices

Morphological indices were calculated as described here. Condition factor (CF) was calculated according to the formula: $CF = (\text{weight (g) length (cm)}^{-3}) \times 100$. Liver somatic index (LSI), gonadosomatic index (GSI) and spleen somatic index (SSI) were calculated according to the formula: $(\text{tissue weight (g) body weight (g)}^{-1}) \times 100$.

2.9. Statistics

All data values are given as means $\pm$ standard error. Data from contaminated site and the respective reference site were tested for statistically significant differences using the t-test. All

data that did not meet requirements for heterogeneity of variance and normality were log-transformed prior to testing. Significance limit was set at $p < 0.05$. Data were tested using SPSS® 12.0 for Windows. False discovery rate (FDR) was calculated for each p-value. This is a correction for multiple testing, accepted values are normally set at <0.1 or <0.2 .

3. Results

3.1. Wrasse captured near the copper mine

Data from male and female fish were compared separately since little is known about variations between the sexes in corkwing wrasse in the parameters measured here.

3.1.1. Morphometric indices

Male fish caught at Salvøy weighed 126 ± 11 g and females weighed 93 ± 3 g. Males caught at Visnes weighed 93 ± 13 g and females weighed 93 ± 11 g. The mean SSI was significantly lower in all fish, both males and females, captured at Visnes compared to Salvøy. Hematocrit levels were significantly higher (122%) in male wrasse captured at Visnes compared to Salvøy. GSI was significantly decreased by 47% in male fish at Visnes. Both LSI and SSI were significantly higher in males compared to females at Salvøy. See table 1 for results and p values. The FDR for each significant p value in table 1 was <0.1 . The FDR for SSI and LSI comparisons between the two sexes was <0.2 .

3.1.2. Protein carbonylation ELISA

Protein carbonylation was significantly higher in the fish captured at Visnes, outside a copper mine, compared to Salvøy (table 2) in both males and females. Levels were 123% higher in males and 49% higher in females caught at Visnes than at Salvøy. FDR was < 0.01 in both sexes.

3.1.3. Enzyme activities and antioxidants in liver and red blood cells

Few of the antioxidant enzymes differed between Visnes and Salvøy. G6PDH levels were significantly lower in red blood cells of male fish from Visnes. Males also had significantly higher levels of both G6PDH (FDR<0.01) and SOD (FDR<0.1) than females at Salvøy. No other differences were seen between the two sites. Results and p values are shown in table 2.

3.2. Wrasse captured at the PAH contaminated site

Only male fish were available for measurements from the reference site Bokn. Data from female fish captured at Høgvarde were therefore not compared to the control site, but were analyzed statistically for sex-related differences at the PAH site.

3.2.1. Morphometric indices

Male fish living at Bokn weighed 85 ± 4 g. Male wrasse caught at Høgvarde weighed 107 ± 10 g and females weighed 67 ± 14 g. CF was significantly higher in male fish captured at Høgvarde compared to Bokn (FDR<0.2). Hematocrit LSI, GSI and SSI were not affected. See table 3 for results and p values.

3.2.2. Protein carbonylation

No difference was seen between Høgvarde, the PAH exposed site, and Bokn, the reference site. However, a comparison between males and females at Høgvarde shows that the female fish had higher levels of oxidized proteins in their blood ($FDR < 0.2$).

3.2.3. Enzyme activities and antioxidants in liver and red blood cells

EROD activity was significantly higher in male fish caught at the PAH contaminated site compared to the reference site ($FDR < 0.1$). tGSH was significantly decreased in male fish captured at Høgvarde ($FDR < 0.02$) but GSSG was unaffected. tGSH also correlated significantly to size of the fish (Pearson correlation, $p = 0.037$) where levels decreased with increasing weight. GR activity did not differ. Methb levels in red blood cells were significantly lower in fish captured at Høgvarde compared to Bokn ($FDR < 0.1$). No other parameters showed any significant effects when comparing the reference and polluted sites. See table 4 for results and p values.

4. Discussion

The definition of oxidative stress states that a disturbance in the prooxidant-antioxidant balance in favor of the former can lead to potential damage. Here, we have investigated a battery of antioxidant defenses, both molecular and enzymatic, and an oxidative damage product, protein carbonyls, and have found that a limited number of biomarkers are affected in corkwing wrasse captured at sites with high levels of heavy metals and PAHs. The information concerning heavy metal and PAH levels in water, sediment and biota, cited in the

field site descriptions in section 2.2, indicate that the contaminants are bioavailable and since this species of wrasse is known to feed on molluscs and crustaceans (Quignard and Pras 1986), they would have been receiving contaminants both through water exposure and through their food supply. Measurements of metals and PAHs in fish tissue should be conducted in future studies to determine the extent of actual exposure.

4.1 Wrasse living at the copper mine

Although copper and zinc are essential trace elements needed, for example, as cofactors in enzymes (i.e. SOD) they are also toxic heavy metals. Marine teleosts will take up heavy metals directly into the blood stream via the gills (Dethloff et al., 1999) as well as additional amounts via the gut, as they, contrary to fresh water species, drink large amounts of water (Filipovic Marijic and Raspor 2007). Redox cycling of copper and iron species can induce the formation of ROS which can lead to oxidative stress (Stadtman and Oliver 1991, Dean et al., 1997). The levels of protein carbonylation measured in this study were significantly higher at Visnes thereby indicating that the fish were suffering from oxidative stress as a result of heavy metal exposure. Metals are known to directly induce the formation of protein carbonyls via metal catalyzed oxidation reactions (MCO) (Stadtman and Oliver 1991). The resulting damaged protein is more susceptible to degradation and may lose some or all of its function. Interestingly, we did not see an induction of metallothionein in wrasse captured at Visnes, near the copper mine. Measurements of MT proteins have shown mixed results in field studies as levels can be influenced by both biotic factors (age, organ, reproductive status, season) and abiotic factors (temperature, pH, salinity). As a result, MT protein levels will reflect bioavailability, tissue pharmacokinetics of metal uptake or MT synthesis (Handy et al., 2003, Filipovic Marijic and Raspor 2007). Also, we have measured MT levels in liver, while

other studies have shown great induction in kidneys and intestines in marine teleost species (Filipovic Marijic and Raspor 2007). To our knowledge, there is no information available on MT levels in corkwing wrasse, but hepatic MT concentrations in a related species, goldsinny wrasse (*Ctenolabrus rupestris*) were lower than those seen here (median concentrations of 1.5-2.0 $\mu\text{g}/\text{mg}$ protein). In goldsinny wrasse, there was a clear positive correlation between hepatic concentrations of Zn/Cu and MT (Ketil Hylland, Department of Biology, University of Oslo, Norway, personal communication). Other binding sites may be involved in metal chelation in corkwing wrasse, thereby eliminating the need for MT induction. Kamunde and MacPhail (2007) found indiscriminate Cu binding in all subcellular fractions of rainbow trout liver suggesting that Cu-binding sites are ubiquitous in this organ. Also, base-line levels of MT in corkwing wrasse may be sufficient to counteract the metal exposure at this site.

G6PDH levels were significantly lower in RBC of male fish captured at Salvøy. Bagnyukova et al. (2006) report a decrease in G6PDH in goldfish exposed to iron. This decrease was negatively correlated to protein carbonyl levels and the authors suggest that this enzyme may be inactivated due to oxidative damaged. The indication that wrasse from the copper mine site might be suffering from oxidative stress, as is evident in the measured increase in oxidative damage to protein molecules, was not paralleled in results from the other measurements; GR, DTD, GST, tGSH, GSSG, metHb and SOD were unaffected. The fact that few of the antioxidant defense mechanisms were increased at the site near the copper mine indicates that these heavy metals may not have been able to induce antioxidant defenses or that fish may have acclimated to the exposure conditions in the field and become more tolerant to acute challenges of metal exposure, via regulation of uptake, detoxification, storage or excretion of metal ions (Kamunde and MacPhail 2007, Rainbow 2007). However, metal exposure that exceeds detoxification capacity may lead to toxicity (Hamilton and Mehrle 1986), i.e.

increases in ROS and accumulation of damaged protein molecules. Based on the data found in the current field study, it becomes more evident that oxidative damage products, and not just antioxidant defenses, are of importance to measure in order to determine effects on oxidative stress parameters.

SSI was lower in both male and female wrasse caught outside the copper mine, results that are mirrored in the higher hematocrit levels measured in these same fish, though this increase was only significant in males. This indicates that wrasse living in this environment were under a certain degree of stress, as release of red blood cells from the spleen is an adaptive response. Cyriac et al. (1989) showed that fish acutely exposed to copper showed an increase in both hematocrit as well as hemoglobin content in blood, possibly due to changes in blood parameters which result in erythrocyte swelling, or by release of large red blood cells from the spleen. GSI was significantly lower in male fish captured at Visnes although this parameter was not altered in female fish. Levesque et al. (2003) found that yellow-perch from metal-contaminated lakes had lower GSI values and that they possessed gonads at less mature stages compared to those from reference stations. Similar results were obtained by Sepúlveda et al. (2002) in a study conducted on largemouth bass. Chronic exposure to metals could alter the physiological functions of fish and delay reproduction.

4.2 The PAH site

PAHs are persistent chemicals and many studies have been conducted on their effects on marine animals (van der Oost, et al. 2003). The induction of cytochrome P450, a phase I detoxification enzyme, and its catalytic activity (EROD) have been used as biomarkers of PAH exposure for many years and are known to be sensitive to PAH exposure (Goksøyr and

Förlin 1992). An increase in EROD activity was measured at the PAH-contaminated site, Høgvarde, with no differences between the two sexes. EROD induction seen in this study was relatively low, approximately 2-fold. Though there are large amounts of PAHs in the sediment at Høgvarde, water flow is quite high in the strait and this may result in low waterborne PAH exposure for the fish. On the other hand, the fish would also be exposed to PAHs via their diet, as is evident in PAH levels in crab and mussel tissues (Andersen et al. 2003b). The somewhat modest elevated EROD activity at the PAH site compared to the reference site might indicate that fish at the PAH site have acclimated to the chronic PAH exposure, as has been demonstrated in brown bullhead (McFarland et al., 1999) and mummichog (Weis 2002).

Little work has been done on how PAHs affect protein oxidation in fish as most studies have focused on lipid peroxidation as a biomarker for oxidative damage (van der Oost, et al. 2003). A previous study showed increased levels of protein carbonyls in eelpout (*Zoarces viviparus*) exposed to bunker oil containing high levels of PAHs, both in the field following an oil spill and in a laboratory exposure study (Carney Almroth, et al. 2005). In the current study, protein carbonyl levels were not elevated at the PAH site compared to the reference site. The low EROD induction measured in these fish may indicate a low level of PAH exposure which may not have been sufficient to induce an accumulation of protein carbonyls. On the other hand, the fish may have acclimated to the chronic PAH exposure, resulting in mechanisms preventing accumulation of protein carbonyls, for example an increase in activity of the 20S proteasome (Grune et al., 2001). A comparison between male and female wrasse at Høgvarde revealed higher levels of protein carbonyls in the plasma of female wrasse, possibly due to differences in the plasma protein profile. Future work needs to be done in fish to ascertain

which proteins are most susceptible to protein carbonylation, the mechanisms by which this damage occurs, and whether baseline variations caused by i.e. season, sex, nutrition, exist.

We saw a significant decrease in amounts of tGSH at Høgvarde. The GSSG/tGSH ratio remained unchanged as GSSG also decreased, though not significantly. The activity of GR and GST did not differ between the PAH and control site, thereby indicating that the decrease in tGSH may result from reactions other than phase II conjugation, i.e. reduction of lipid peroxides. The method used to measure GST in the current paper measures several isoforms so it is not possible to say whether another isoform may be responsible for the observed decrease in tGSH. This decrease in tGSH could also be due to the action of ATP-dependent efflux pumps that export GSSG and glutathione-conjugates from liver cells during oxidative stress (Keppler 1999). We also found a significant negative correlation between tGSH and weight. These results may reflect changes in synthesis of new glutathione molecules as transcription of these genes can be affected by environmental factors as well as age (Lu 1999, Carney Almroth et al., 2008).

MetHb levels were significantly lower in the red blood cells of male wrasse captured at Høgvarde compared to those captured at Bokn. This indicates a decreased need for the reparatory activity of the enzyme and therefore a decrease in oxidation of hemoglobin molecules in red blood cells. CF in fish caught at Høgvarde was significantly higher, which probably reflects differences in food availability and nutritional content between sites rather than an effect of PAH exposure.

5. Conclusions

Results from the current study show that protein carbonyl levels serve as a good biomarker for oxidative perturbations in corkwing wrasse, especially in cases of heavy metal contamination. However, more studies are needed to confirm the mechanisms through which this damage occurs for specific pollutants and if these mechanisms differ between male and female fish. Knowledge concerning which proteins are most susceptible in different exposure situations is also needed. Further studies should investigate toxicological relevance of protein carbonyl accumulation in different species of fish.

The other parameters measured here, those involved in detoxification and antioxidant functions, were shown to be only lightly affected or unaffected in fish captured at both the heavy metal and PAH contaminated sites. Antioxidant defences do not appear to provide a good biomarker for chronic exposure in corkwing wrasse and other biomarkers should also be measured.

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Figure legends.

Figure 1: Map of Norwegian west coast, showing site locations. Site 1 = Salvøy, a reference site exposed to the open ocean, Site 2 = Visnes, located outside a copper mine, Site 3 = Høgvarde, a PAH contaminated site, Site 4 = Bokn, a reference site located in a strait.

Table 1: Levels of biometric parameters calculated in corkwing wrasse captured at a heavy metal contaminated site (Visnes) and reference site (Salvøy). Statistical differences between sites are shown in bold. * represents statistically significant difference, $p < 0.05$, between males (M) and females (F) at the same site.

Table 2: Activities or levels of various liver and red blood cell (rbc) detoxification and antioxidant defenses measured in corkwing wrasse captured at a heavy metal contaminated site (Visnes) and a reference site (Salvøy). Statistical differences between sites are shown in bold. * represents statistically significant differences, $p < 0.05$, between males (M) and females (F). EROD= ethoxyresorufin-O-deethylase, GST= glutathione-S-transferase, GR= glutathione reductase, tGSH= total glutathione, GSSG = oxidized glutathione, DTD= DT-diaphorase, SOD= superoxide dismutase, MT= metallothionein, metHb= methemoglobin reductase, G6PDH= glucose-6-phosphate dehydrogenase.

Table 3: Levels of biometric parameters calculated in corkwing wrasse captured at a PAH contaminated site (Høgvarde) and reference site (Bokn). Statistical differences between sites are shown in bold. * represents statistically significant difference, $p < 0.05$, between males (M) and females (F). Only male fish were available at Bokn.

Table 4: Activities or levels of various liver and red blood cell (rbc) detoxification and antioxidant defenses measured in corkwing wrasse captured at a PAH contaminated site (Høgvarde) and a reference site (Bokn). Statistical differences between sites are shown in bold. * represents statistically significant differences, $p < 0.05$, between males (M) and females (F). Only male fish were available at Bokn. See Table 2 legend for abbreviations.

640
641 Fig.1

642

Heavy metal site				
		Salvøy	Visnes	p values
LSI	M	2.39 ± 0.28	1.93 ± 0.21	0.074
(liver weight/body weight) x100	F	1.61 ± 0.15 *	1.87 ± 0.07	0.109
GSI	M	0.19 ± 0.05	0.10 ± 0.01	0.009
(gonad weight/body weight) x100	F	1.079 ± 0.08	0.93 ± 0.03	0.053
CF	M	1.52 ± 0.04	1.59 ± 0.04	0.237
(weight g/(length cm) ³) x100	F	1.58 ± 0.03	1.63 ± 0.03	0.317
SSI	M	0.13 ± 0.01	0.07 ± 0.01	0.0001
(spleen weight/body weight) x100	F	0.10 ± 0.01 *	0.06 ± 0.01	0.019
Hematocrit	M	28.11 ± 0.88	34.44 ± 1.29	0.001
(%)	F	30.71 ± 1.86	32.50 ± 1.81	0.504

643

644 Table 1

645

Heavy metal site				
		Salvøy	Visnes	p value
EROD (liver)	M	0.021 ± 0.002	0.029 ± 0.003	0.056
(nmol mg ⁻¹ min ⁻¹)	F	0.019 ± 0.002	0.025 ± 0.004	0.307
GST (liver)	M	0.860 ± 0.022	0.844 ± 0.037	0.065
(μmol mg ⁻¹ min ⁻¹)	F	0.714 ± 0.074	0.801 ± 0.033	0.233
GR (liver)	M	28.35 ± 1.93	20.82 ± 3.28	0.070
(nmol mg ⁻¹ min ⁻¹)	F	28.69 ± 3.73	31.72 ± 1.79	0.446
tGSH (liver)	M	2333.7 ± 137.3	2109.6 ± 104.0	0.216
(nmol g liver ⁻¹)	F	2083.7 ± 71.2	2158.8 ± 56.4	0.414
GSSG (liver)	M	58.77 ± 5.85	58.01 ± 4.56	0.998
(nmol g liver ⁻¹)	F	63.92 ± 6.11	60.70 ± 7.67	0.574
%GSSG (liver)	M	2.55 ± 0.24	2.81 ± 0.27	0.480
(%, ratio GSSG/tGSH)	F	3.07 ± 0.28	2.77 ± 0.30	0.479
Catalase (liver)	M	280.10 ± 30.91	291.93 ± 31.78	0.526
(nmol mg ⁻¹ min ⁻¹)	F	289.08 ± 23.50	288.18 ± 26.54	0.981
DTD (liver)	M	13.57 ± 1.22	16.49 ± 1.64	0.167
(nmol mg ⁻¹ min ⁻¹)	F	12.38 ± 1.90	18.18 ± 3.11	0.156
SOD (liver)	M	94.71 ± 11.01	93.56 ± 19.74	0.470
(ng mg ⁻¹)	F	65.67 ± 8.73	78.40 ± 7.60	0.238
MT (liver)	M	5.12 ± 0.24	5.83 ± 0.31	0.210
(μg mg prot ⁻¹)	F	4.63 ± 0.28	5.63 ± 0.38	0.693
SOD (rbc)	M	388.38 ± 45.21	419.32 ± 34.57	0.593
(ng mg ⁻¹)	F	376.92 ± 33.41 *	381.52 ± 61.78	0.949
metHb (rbc)	M	252.97 ± 8.63	248.00 ± 9.79	0.708
(D abs mg ⁻¹)	F	238.32 ± 18.18	225.78 ± 6.35	0.526
G6PDH (rbc)	M	125.72 ± 4.77	97.96 ± 7.41	0.004
(D abs mg ⁻¹)	F	84.14 ± 6.86 *	87.21 ± 6.75	0.709
Protein carbonyls	M	1.59 ± 0.17	3.55 ± 0.43	0.001
(plasma) (nmol/mg prot)	F	2.24 ± 0.44	3.34 ± 0.14	0.026

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647 Table 2

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PAH site				
		Bokn	Høgvarde	p values
LSI	M	1.40 ± 0.09	1.60 ± 0.09	0.118
(liver weight/body weight) x100	F		1.46 ± 0.11	
GSI	M	0.13 ± 0.02	0.23 ± 0.10	0.132
(gonad weight/body weight) x100	F		1.05 ± 0.05	
CF	M	1.47 ± 0.05	1.60 ± 0.03	0.034
(weight g/(length cm) ³) x100	F		1.50 ± 0.05	
SSI	M	0.09 ± 0.01	0.07 ± 0.01	0.200
(spleen weight/body weight) x100	F		0.06 ± 0.01	
Hematocrit	M	30.56 ± 1.00	27.67 ± 1.79	0.178
(%)	F		30.00 ± 2.22	

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650 Table 3

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PAH site				
		Bokn	Høgvarde	p value
EROD (liver)	M	0.013 ± 0.002	0.029 ± 0.006	0.008
(nmol mg ⁻¹ min ⁻¹)	F		0.024 ± 0.009	
GST (liver)	M	0.898 ± 0.043	0.842 ± 0.038	0.371
(μmol mg ⁻¹ min ⁻¹)	F		0.870 ± 0.079	
GR (liver)	M	30.33 ± 1.61	28.35 ± 1.51	0.382
(nmol mg ⁻¹ min ⁻¹)	F		24.57 ± 3.88	
tGSH (liver)	M	2530.4 ± 172.0	1855.1 ± 209.2	0.023
(nmol g liver ⁻¹)	F		2099.9 ± 233.0	
GSSG (liver)	M	61.81 ± 4.57	47.86 ± 9.70	0.056
(nmol g liver ⁻¹)	F		48.97 ± 6.25	
%GSSG (liver)	M	2.55 ± 0.26	2.53 ± 0.29	0.954
(%, ratio GSSG/tGSH)	F		2.38 ± 0.27	
Catalase (liver)	M	376.11 ± 26.04	349.75 ± 37.77	0.293
(nmol mg ⁻¹ min ⁻¹)	F		270.77 ± 22.77	
DTD (liver)	M	14.07 ± 2.00	14.84 ± 1.68	0.823
(nmol mg ⁻¹ min ⁻¹)	F		8.39 ± 3.38	
SOD (liver)	M	134.23 ± 11.50	147.11 ± 20.22	0.918
(ng mg ⁻¹)	F		105.40 ± 25.70	
MT (liver)	M	6.15 ± 0.60	5.52 ± 0.32	0.345
(μg mg prot ⁻¹)	F		5.37 ± 0.45	
SOD (rbc)	M	328.56 ± 39.13	330.42 ± 51.40	0.977
(ng mg ⁻¹)	F		406.99 ± 47.98	
metHb (rbc)	M	248.35 ± 12.63	192.17 ± 10.35	0.003
(D abs mg ⁻¹)	F		222.35 ± 10.71	
G6PDH (rbc)	M	106.16 ± 13.67	85.21 ± 5.95	0.208
(D abs mg ⁻¹)	F		88.57 ± 8.62	
Protein carbonyls	M	2.02 ± 0.14	2.39 ± 0.22	0.207
(plasma) (nmol/mg prot)	F		3.39 ± 0.35*	

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Table 4