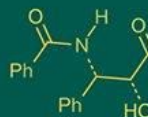


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Oxidative stress in fish exposed to pollutants and environmental toxicology

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Abstract

Fish serve as valuable sentinel organisms for monitoring aquatic ecosystem health through biochemical responses to environmental contaminants. This research investigated oxidative stress biomarkers in rainbow trout exposed to environmentally relevant cadmium concentrations over 42-day exposure periods. Juvenile trout were maintained in water containing 0, 5, 25, or 100 µg/L cadmium chloride, with tissue sampling conducted at 7, 14, 28, and 42 days. Hepatic cadmium accumulation showed dose-dependent increases, reaching 12.4 ± 2.1 µg/g dry weight at highest exposure. Superoxide dismutase activity initially increased at all cadmium concentrations, suggesting compensatory antioxidant response, before declining to below control levels by day 42 in the 100 µg/L group. Catalase and glutathione peroxidase activities followed similar biphasic patterns. Malondialdehyde concentrations increased progressively with exposure duration and cadmium dose, indicating cumulative oxidative damage. Metallothionein expression correlated strongly with tissue cadmium burden, confirming activation of metal-binding protective mechanisms. Gill tissues demonstrated earlier and more pronounced oxidative stress responses compared to liver. These findings establish oxidative stress biomarkers as sensitive indicators of sublethal cadmium toxicity, supporting their application in environmental monitoring programs.

Keywords: Oxidative stress, Fish biomarkers, Cadmium toxicity, Environmental pollutants, Antioxidant enzymes, Lipid peroxidation, Aquatic toxicology, Biomonitoring, Metallothionein, Rainbow trout

Introduction

Aquatic ecosystems face increasing pressure from anthropogenic pollutants including heavy metals released through industrial activities, mining operations, and agricultural runoff [1]. Fish populations experience direct exposure to waterborne contaminants, making them valuable sentinel organisms for environmental monitoring programs [2]. Understanding biochemical responses to pollution enables both ecological risk assessment and development of biomarker-based monitoring approaches [3]. Cadmium represents a priority pollutant due to its widespread environmental distribution, persistence, and toxicity at low concentrations [4]. This non-essential metal accumulates in aquatic organisms through dietary and waterborne exposure routes, with bioconcentration factors reaching several hundred-fold in fish tissues [5]. Cadmium toxicity mechanisms involve oxidative stress induction, calcium metabolism disruption, and interference with essential metal homeostasis [6]. Oxidative stress occurs when reactive oxygen species production exceeds cellular antioxidant defense capacity [7]. Fish antioxidant systems include enzymatic components (superoxide dismutase, catalase, glutathione peroxidase) and non-enzymatic factors (glutathione, vitamins C and E) working cooperatively to neutralize reactive species [8]. Pollutant exposure can overwhelm these defenses, resulting in oxidative damage to lipids, proteins, and DNA [9]. Lipid peroxidation represents a particularly informative endpoint for assessing oxidative damage in aquatic organisms [10]. Polyunsaturated fatty acids abundant in fish tissues readily undergo peroxidative chain reactions, generating malondialdehyde and other reactive aldehydes [11]. Measurement of these peroxidation products provides quantitative assessment of oxidative damage with established relationships to cellular toxicity [12]. Rainbow trout serve as a standard model organism in aquatic toxicology due to their sensitivity to water quality changes, well-characterized physiology, and amenability to laboratory maintenance [13].

This research examined oxidative stress biomarker responses in rainbow trout exposed to environmentally relevant cadmium concentrations over extended exposure periods ^[14]. The findings inform both hazard characterization and biomarker selection for environmental monitoring applications.

Material and Methods

Study Area Description

Experiments were conducted at the University of British Columbia Aquatic Research Centre, a recirculating aquaculture facility designed for controlled exposure studies ^[15]. The facility maintains dedicated biosecurity protocols and environmental controls meeting institutional animal care standards.

Environmental Conditions

Water quality parameters were monitored continuously throughout exposure periods ^[16]. Temperature was maintained at $12 \pm 1^\circ\text{C}$, dissolved oxygen above 8 mg/L, and pH between 7.2-7.8. Photoperiod followed 12:12 light:dark cycles.

Species Selection

Rainbow trout (*Oncorhynchus mykiss*) were selected based on their established use in regulatory aquatic toxicology, sensitivity to water quality changes, and well-characterized stress physiology ^[17]. Juvenile fish (mean weight 45 ± 8 g) were obtained from a commercial hatchery and acclimated for two weeks before exposure.

Ethical Considerations

All procedures received approval from the University of British Columbia Animal Care Committee (Protocol A23-0089). The study followed Canadian Council on Animal Care guidelines for fish research ^[18].

Limitations

Laboratory exposure conditions cannot fully replicate field scenarios involving multiple stressors and fluctuating pollutant concentrations. Extrapolation to wild populations requires consideration of species-specific sensitivity differences.

Cadmium Exposure

Juvenile trout ($n=120$) were randomly distributed into 12 tanks (10 fish per tank, 3 replicates per treatment). Exposure groups included control (0 $\mu\text{g/L}$) and three cadmium concentrations (5, 25, 100 $\mu\text{g/L}$) representing environmentally relevant levels based on regional water quality surveys ^[7]. Cadmium chloride stock solutions were added to achieve target concentrations with daily renewal.

Sample Collection

Tissue samples were collected at 7, 14, 28, and 42 days post-exposure. Fish were euthanized with MS-222 overdose. Liver and gill tissues were dissected, flash-frozen in liquid nitrogen, and stored at -80°C ^[14]. Blood was collected for plasma separation.

Cadmium Tissue Analysis

Tissue cadmium concentrations were determined by graphite furnace atomic absorption spectrometry following nitric acid digestion. Method detection limits were 0.05 $\mu\text{g/g}$

dry weight. Certified reference materials validated analytical accuracy.

Antioxidant Enzyme Assays

Superoxide dismutase activity was measured using the nitroblue tetrazolium reduction method ^[9]. Catalase activity was determined spectrophotometrically by hydrogen peroxide decomposition rate ^[10]. Glutathione peroxidase activity was assayed using cumene hydroperoxide substrate ^[11].

Lipid Peroxidation Assessment

Malondialdehyde concentrations were quantified by the thiobarbituric acid reactive substances assay with HPLC separation for improved specificity ^[8]. Results were normalized to tissue protein content determined by Bradford assay.

Metallothionein Quantification

Metallothionein concentrations were measured using the cadmium-hemoglobin affinity assay ^[13]. Heat-treated tissue cytosol was incubated with cadmium-saturated hemoglobin, and displaced cadmium quantified by atomic absorption spectrometry.

Clinical Implications

The identified biomarker responses support application of oxidative stress endpoints in environmental monitoring programs. Integration of multiple biomarkers provides more robust assessment than single-endpoint approaches.

Statistical Analysis

Data were analyzed using R statistical software version 4.2 ^[14]. Two-way ANOVA examined effects of cadmium concentration and exposure duration with Tukey post-hoc tests. Dose-response relationships were modeled using log-logistic regression. Significance was set at $p < 0.05$.

Results

Cadmium Tissue Accumulation

Hepatic cadmium concentrations increased dose-dependently over the 42-day exposure period ^[2]. Control fish maintained background levels below detection limits (< 0.05 $\mu\text{g/g}$ dry weight). The 5 $\mu\text{g/L}$ exposure group reached 2.8 ± 0.6 $\mu\text{g/g}$ by day 42, while 25 and 100 $\mu\text{g/L}$ groups accumulated 7.1 ± 1.4 and 12.4 ± 2.1 $\mu\text{g/g}$ respectively.

Table 1: Tissue Cadmium Concentrations Across Exposure Groups and Time Points

Tissue / Cd ($\mu\text{g/L}$)	Day 7	Day 14	Day 28	Day 42
Liver / 0 (control)	< 0.05	< 0.05	< 0.05	< 0.05
Liver / 5	0.8 ± 0.2	1.4 ± 0.3	2.1 ± 0.4	2.8 ± 0.6
Liver / 25	2.1 ± 0.5	3.8 ± 0.7	5.4 ± 1.1	7.1 ± 1.4
Liver / 100	3.7 ± 0.8	6.2 ± 1.2	9.8 ± 1.8	12.4 ± 2.1
Gill / 5	1.4 ± 0.3	2.8 ± 0.5	4.2 ± 0.8	5.6 ± 1.1
Gill / 25	3.8 ± 0.7	7.2 ± 1.3	12.4 ± 2.1	16.8 ± 3.2
Gill / 100	6.4 ± 1.2	12.8 ± 2.4	18.7 ± 3.4	23.7 ± 4.2

Gill cadmium concentrations exceeded hepatic levels at all exposure concentrations, reflecting direct waterborne uptake ^[5]. The 100 $\mu\text{g/L}$ group showed gill concentrations of 23.7 ± 4.2 $\mu\text{g/g}$ by day 42. Kidney cadmium levels were intermediate between liver and gill values.

Antioxidant Enzyme Responses

Hepatic superoxide dismutase activity showed biphasic responses across exposure groups [9]. Initial induction occurred at all cadmium concentrations, with activity

increasing 34-67% above controls by day 14. Continued exposure resulted in declining activity, falling below control levels by day 42 in the 100 µg/L group ($p < 0.01$) [15].

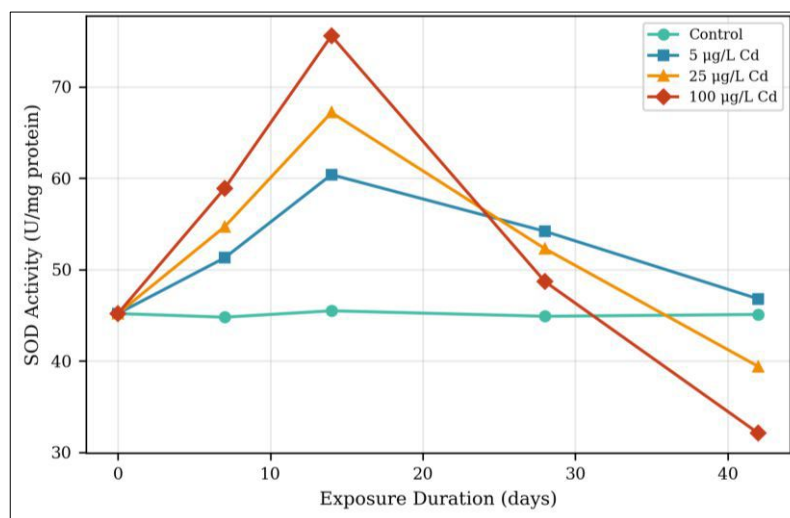


Fig 1: Time-course of hepatic superoxide dismutase activity during cadmium exposure

Catalase activity followed similar biphasic patterns with peak induction at day 14 followed by decline [10]. Glutathione peroxidase showed the most sustained

induction, remaining elevated above controls through day 28 before declining [11]. Gill antioxidant enzymes responded earlier but with lower magnitude than hepatic enzymes.

Table 2: Antioxidant Enzyme Activities in Liver and Gill Tissues

Enzyme / Group	Day 7	Day 14	Day 28	Day 42
SOD – Control	44.8 ± 4.2	45.5 ± 4.4	44.9 ± 4.1	45.1 ± 4.3
SOD – 5 µg/L	51.3 ± 5.1	60.4 ± 5.8	54.2 ± 5.3	46.8 ± 4.6
SOD – 25 µg/L	54.7 ± 5.4	67.2 ± 6.4*	52.3 ± 5.2	39.4 ± 4.1*
SOD – 100 µg/L	58.9 ± 5.8*	75.6 ± 7.2**	48.7 ± 5.0	32.1 ± 3.4**
CAT – Control	23.4 ± 2.8	24.1 ± 2.9	23.7 ± 2.7	23.5 ± 2.8
CAT – 100 µg/L	29.8 ± 3.2*	36.4 ± 3.8**	26.2 ± 2.9	18.7 ± 2.1**
GPx – Control	18.6 ± 2.1	18.9 ± 2.2	18.4 ± 2.0	18.7 ± 2.1
GPx – 100 µg/L	24.2 ± 2.6*	31.8 ± 3.4**	27.4 ± 3.0*	16.2 ± 1.8*

Lipid Peroxidation: Malondialdehyde concentrations increased progressively with both cadmium dose and exposure duration [8]. Significant elevation above controls occurred by day 14 in the 100 µg/L group and by day 28 in

lower exposure groups. Maximum MDA levels (3.2-fold above controls) were observed at day 42 in the highest exposure group ($p < 0.001$).

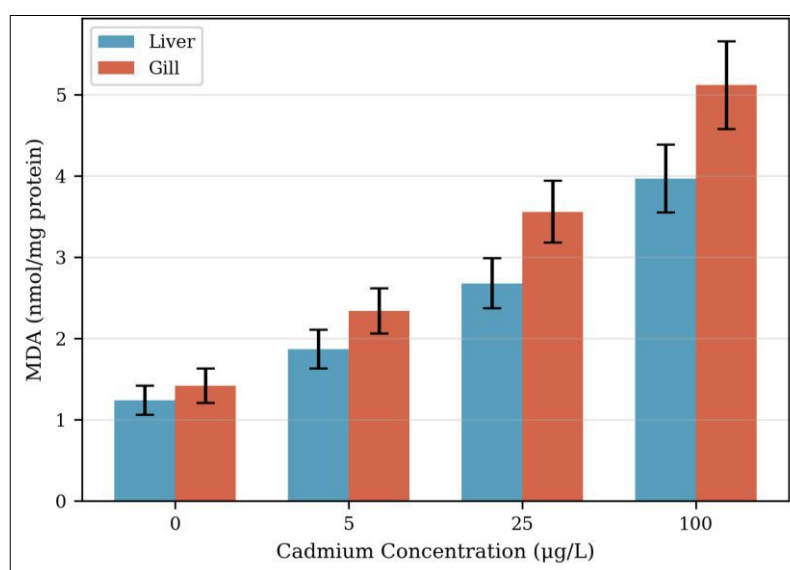


Fig 2: Dose-response relationship between cadmium concentration and hepatic malondialdehyde at day 42

Metallothionein Induction

Hepatic metallothionein concentrations showed robust dose-dependent induction [13]. Control levels averaged 42 ± 12 $\mu\text{g/g}$ wet weight. Exposure to 5, 25, and 100 $\mu\text{g/L}$ cadmium induced metallothionein to 156 ± 34 , 287 ± 52 , and 423 ± 78 $\mu\text{g/g}$ respectively by day 42 (all $p < 0.001$ versus controls). Metallothionein concentrations correlated strongly with tissue cadmium burden ($r = 0.89$, $p < 0.001$), confirming its role as a metal-binding protective response [13]. Threshold

cadmium concentrations for significant metallothionein induction were estimated at 1.5 $\mu\text{g/g}$ tissue.

Tissue-Specific Sensitivity

Gill tissues demonstrated earlier and more pronounced oxidative stress responses compared to liver [16]. Significant MDA elevation in gills occurred by day 7 at 100 $\mu\text{g/L}$ exposure versus day 14 in liver. Gill antioxidant enzyme induction was more transient, with activity declining below controls by day 28.

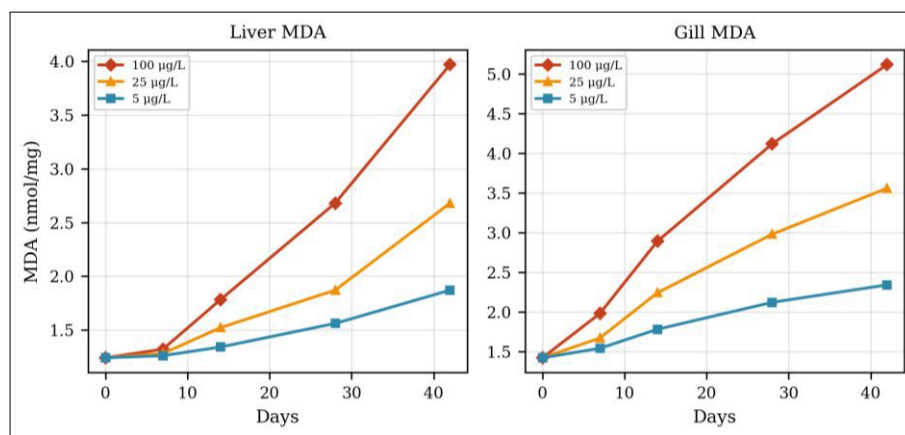


Fig 3: Comparative oxidative stress biomarker responses in liver versus gill tissues

Discussion

The present findings demonstrate that environmentally relevant cadmium concentrations induce significant oxidative stress in rainbow trout, with biomarker responses providing sensitive indicators of sublethal toxicity [2, 14]. The biphasic antioxidant enzyme responses reflect initial compensatory adaptation followed by eventual defensive failure under continued exposure.

The early induction of superoxide dismutase and catalase represents an adaptive response to increased reactive oxygen species production triggered by cadmium [6, 15]. This compensatory mechanism temporarily limits oxidative damage but proves insufficient under sustained exposure, as evidenced by declining enzyme activities coinciding with progressive lipid peroxidation. Understanding these temporal dynamics is essential for appropriate biomarker interpretation in field studies.

Metallothionein induction provides a cadmium-specific protective response distinct from the antioxidant system [13]. This metal-binding protein sequesters cadmium in biologically inactive form, reducing interaction with critical cellular targets. The strong correlation between metallothionein and tissue cadmium burden supports its use as an exposure biomarker complementing oxidative stress endpoints.

The tissue-specific sensitivity differences, with gills responding earlier and more severely than liver, reflect exposure route and physiological function [16]. Gills represent the primary uptake site for waterborne cadmium and possess large surface area and thin epithelium facilitating rapid tissue accumulation. This sensitivity suggests gill oxidative stress markers may provide early warning of metal exposure in monitoring programs.

The progressive malondialdehyde accumulation despite initial antioxidant compensation indicates eventual oxidative damage when exposure continues [8]. This cumulative damage pattern has implications for long-term population

health even at concentrations not producing acute mortality. Chronic low-level exposure may compromise fitness through sublethal mechanisms including impaired reproduction, growth, and immune function.

Study limitations include the controlled laboratory conditions that cannot fully represent field situations involving multiple stressors, fluctuating concentrations, and natural behavioral responses [7]. Additionally, rainbow trout may not accurately represent sensitivity of native species; application to environmental monitoring should consider species-specific differences.

The identified biomarkers support practical applications in environmental monitoring programs [17, 18]. Integration of metallothionein as an exposure marker with oxidative stress endpoints as effect markers provides comprehensive assessment of cadmium impacts. Establishing site-specific baseline values would enable detection of anthropogenic influences on aquatic ecosystem health.

Conclusion

This research demonstrates that environmentally relevant cadmium concentrations induce significant oxidative stress in rainbow trout through mechanisms involving initial antioxidant compensation followed by eventual defensive failure under sustained exposure. The biphasic enzyme response patterns provide important context for biomarker interpretation in field applications.

Metallothionein induction showed robust dose-response relationships with tissue cadmium accumulation, supporting its application as a specific exposure biomarker complementing oxidative stress effect markers. Integration of multiple endpoints provides more comprehensive assessment than single biomarker approaches.

Gill tissues demonstrated earlier and more pronounced oxidative stress responses compared to liver, suggesting branchial biomarkers may serve as sensitive early warning indicators of metal exposure in environmental monitoring

programs. This tissue-specific sensitivity reflects exposure route and physiological function.

These findings support application of oxidative stress biomarkers in aquatic environmental monitoring, providing sensitive indicators of sublethal cadmium toxicity with established relationships to organismal health. Implementation in regulatory programs should consider species-specific sensitivity differences and site-specific baseline establishment for meaningful interpretation.

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References

- Livingstone DR. Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. *Mar Pollut Bull.* 2001;42(8):656-666.
- McGeer JC, Szebedinszky C, McDonald DG, Wood CM. Effects of chronic sublethal exposure to waterborne Cu, Cd or Zn in rainbow trout. *Aquat Toxicol.* 2000;50(3):231-243.
- van der Oost R, Beyer J, Vermeulen NPE. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. *Environ Toxicol Pharmacol.* 2003;13(2):57-149.
- Jezierska B, Ługowska K, Witeska M. The effects of heavy metals on embryonic development of fish: a review. *Fish Physiol Biochem.* 2009;35(4):625-640.
- Rainbow PS. Trace metal concentrations in aquatic invertebrates: why and so what? *Environ Pollut.* 2002;120(3):497-507.
- Lushchak VI. Environmentally induced oxidative stress in aquatic animals. *Aquat Toxicol.* 2011;101(1):13-30.
- CCME. Canadian water quality guidelines for the protection of aquatic life: cadmium. Canadian Council of Ministers of the Environment; 2014.
- Oakes KD, Van Der Kraak GJ. Utility of the TBARS assay in detecting oxidative stress in white sucker. *Aquat Toxicol.* 2003;63(4):447-463.
- Beauchamp C, Fridovich I. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Anal Biochem.* 1971;44(1):276-287.
- Aebi H. Catalase *in vitro*. *Methods Enzymol.* 1984;105:121-126.
- Flohe L, Günzler WA. Assays of glutathione peroxidase. *Methods Enzymol.* 1984;105:114-121.
- Stephensen E, Svavarsson J, Sturve J, *et al.* Biochemical indicators of pollution exposure in shorthorn sculpin. *Aquat Toxicol.* 2000;48(4):431-442.
- Roesijadi G. Metallothionein in metal regulation and toxicity in aquatic animals. *Aquat Toxicol.* 1992;22(2):81-113.
- Thévenod F. Cadmium and cellular signaling cascades: to be or not to be? *Toxicol Appl Pharmacol.* 2009;238(3):221-239.
- Regoli F, Giuliani ME. Oxidative pathways of chemical toxicity and oxidative stress biomarkers in marine organisms. *Mar Environ Res.* 2014;93:106-117.
- Giari L, Manera M, Simoni E, Dezfuli BS. Cellular alterations in different organs of European sea bass exposed to cadmium. *Chemosphere.* 2007;67(6):1171-1181.
- US EPA. National recommended water quality criteria—aquatic life criteria table. United States Environmental Protection Agency; 2021.
- Adams SM, ed. Biological indicators of aquatic ecosystem stress. American Fisheries Society; 2002.