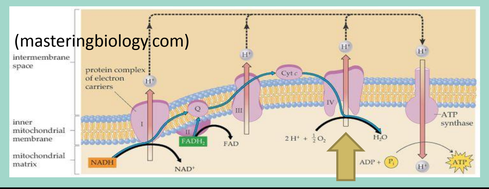
Effect of sulfide toxicity on cytochrome *c* oxidase in liver and muscle tissue in fish species

Nicole M. Martin, Yasuhiro Kobayashi, and Brian R. Maricle
Department of Biological Sciences, Fort Hays State University

Introduction

Toxic effects of sulfide have long been recognized in organisms (Beauchamp et al., 1984). However, specific physiological effects of sulfide have been investigated in few metabolic systems. Sulfide is a potent metabolic toxin; many of its effects come from a poisoning of a number of enzymes, especially cytochrome *c* oxidase (Bagarinao, 1992), which catalyzes the terminal step in aerobic respiration in eukaryotes. Commonly, micromolar concentrations of sulfide are enough to inhibit cytochrome *c* oxidase activity (reviewed by Bagarinao, 1992). Other toxic effects of sulfide have been recognized, including inhibition of other enzymes (Carlsson et al., 1988; Pearson and Havill, 1988). Previous work investigated cytochrome *c* oxidase activity in the presence of sulfide in numerous plant species and showed that estuarine species were more tolerant of sulfide compared to flooding sensitive species (Martin and Maricle, 2015). This suggested that varying sensitivities to cytochrome *c* oxidase exist across organisms as an adaptive mechanism to life in highly sulfidic salt marshes. But little is known whether fish species that live in sulfide-rich environments have a similar adaptive mechanism compared to those that live in pristine environments.



Sulfide toxicity comes from inhibition of cytochrome *c* oxidase, which is complex IV in the inner mitochondrial membrane.

Hypothesis

We hypothesized cytochrome *c* oxidase activity may be less sensitive to sulfide exposure in common carp, known to be highly tolerant of low water quality, compared to channel catfish or fathead minnow, species who are less tolerant to low water quality.

Acknowledgments

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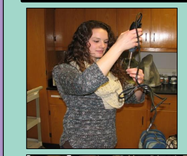
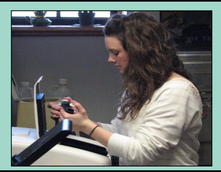
Materials and Methods

Three species of fish were compared to assess susceptibility to sulfide exposure. One species, common carp, is adapted to high levels of sulfide and is capable of tolerating a range of environmental conditions. Two species, channel catfish and fathead minnow, were not adapted to life in a sulfide-rich habitat. Liver and muscle tissues from all three species were measured to compare tolerances between species to sulfide.

Care and handling of fish used in this study was approved by the FHSU Institutional Animal Care and Use Committee (#13-0004). Samples (100 mg) of liver and muscle were collected from common carp (3 fish, 88.3 mm ± 4.0 mm), fathead minnow (3 fish, 75 mm ± 4.1 mm), and channel catfish (3 fish, 152.0 mm ± 12.8 mm) that were obtained from the Milford Fish Hatchery of the Kansas Department of Wildlife, Parks, and Tourism.



Effects of increasing sulfide concentrations were measured on activities of cytochrome *c* oxidase (CytOx; Complex IV in oxidative phosphorylation). Enzyme activities were measured in 0, 5, 10, 15, and 20 µM sodium sulfide. Cytochrome *c* oxidase assays were performed after the protocol of Maricle et al. (2006) and Martin and Maricle (2015).



Enzyme activities were assayed spectrophotometrically. Rates of cytochrome *c* oxidation were measured as a decrease in absorbance at 550 nm. The inhibition constant, K_i, is the sulfide concentration that resulted in a 50% decrease in enzyme activity. Enzyme activities were compared with repeated measures analysis of variance, with species and tissues as fixed effects and sulfide concentration as the repeated effect.

To detect genetic change among species, gene sequences for cytochrome *c* oxidase subunits were identified by screening common carp gene sequences available in GenBank using common carp cytochrome *c* oxidase subunit sequences as query.

Literature Cited

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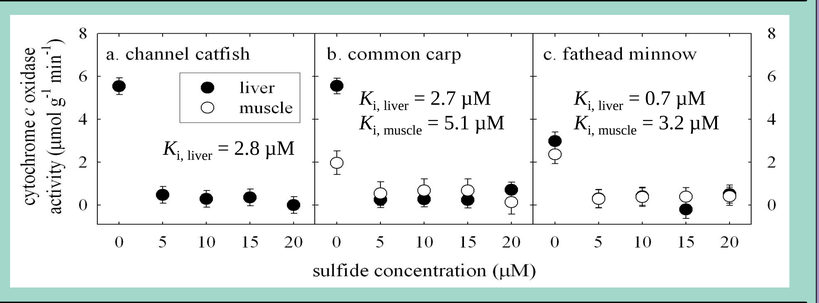
Results and Discussion

Figure 1: Cytochrome *c* oxidase activity across five concentrations of sulfide in liver and muscle tissue in three fish species.



Cytochrome *c* oxidase activities were significantly higher in liver tissues compared to muscle tissues in all species ($P < 0.001$). Activities of cytochrome *c* oxidase were very sensitive to sulfide in all tissues and were reduced to near zero at 5 µM sulfide. Cytochrome *c* oxidase activities in all three species and both organs were equally sensitive to sulfide, as K_i values were not different ($P \geq 0.095$). This illustrates the potent metabolic effects of sulfide. Human physiology is also sensitive to sulfide, making the results of this study potentially useful in several contexts.

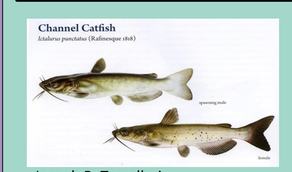


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Table 1: Nucleotide sequences of common carp cytochrome *c* oxidase subunit 1 in comparison with other fish species.

Species	Genbank Accession Number	Percent Similarity
Carp	JN6753560.1	100%
Fathead Minnow	KR092384.1	78%
Channel Catfish	-	-
Zebrafish	AY996924.1	84%

Table 2: Nucleotide sequences of common carp cytochrome *c* oxidase subunit 2 in comparison with other fish species.

Species	Genbank Accession Number	Percent Similarity
Carp	FJ655365.1	99%
Fathead Minnow	-	-
Channel Catfish	AF227811.1	77%
Zebrafish	-	-