Metallothionein Gene Expression in Liver Tissues of Yellow Perch Fish

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Introduction

Metal contamination can lead to destructive effects on aquatic life due to its strong toxicity and cause stressors that throw homeostasis off balance. A metalloid that contaminates lakes and the aquatic life balance is arsenic. Arsenic is an underlying toxic element that is spreading and polluting lakes and is a common pollutant in urban waters that can pose high risks to both environmental and human health (Barrett et al 2018).

The yellow perch fish have been collected from the three different lakes.

- **Pine Lake (less contamination)**
- **Steel Lake (moderate contamination)**
- **Lake Killarney (most contaminated).**

Objective: To conduct the hypothesis we will be using the yellow perch fish that were exposed to heavy metals from these three lakes and semi-quantitatively expressing the MT gene as a molecular biomarker which will allow us to examine the liver tissues and examine the gene expression assays.

Table 1: Primers used to amplify Metallothionein gene

Forward primer (5' to 3')	ATCCTGCACTTGCACGAACTG
Reverse primer (5' to 3')	ACATCCAGAGGCGCACTTG

Table 2: Primers used to amplify beta-actin gene

Forward primer (5' to 3')	GCCTCTCTGTCCACCTTCCA
Reverse primer (5' to 3')	GGGCCGGACTCATCGTACT

METHODS AND MATERIALS

•Trizol reagent will extract the RNA from the liver tissue of the fish

• A nanodrop was used to check the RNA concentration and purity. The RNA was reversed and transcribed to cDNA using Superscript III.

•PCR will be used to amplify the genes and the gel electrophoresis will be used to analyze the fragments of the target gene (MT gene) and the housekeeping gene (beta-actin) based on their sizes.

•Image J and the fold ratio will be calculated.

• Densitometry is used to quantitatively measure the optical density of the data.

•ANOVA will compare the three lakes and allow for variability inside the data set, the significance threshold was set at p = 0.05.

Discussion

It was concluded there was no significant upregulation of the target gene and no statistical significance in MT expression between the lakes. The yellow perch fish did not have an increased expression of MT.

Future Implications

- A different species for experiments such as phytoplankton and zooplankton in lake
- Different arsenic contaminants in sediments with different seasonal stratification in Lake Killarney can be used.

RESULTS

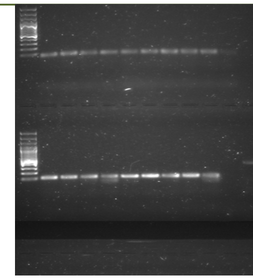

Fig 1. Gel electrophoresis in the target gene and the beta-actin. Row 1= Beta-actin gene (housekeeping gene) and the yellow perch PCR products. Row 2= Metallothionein gene (target gene) and the yellow perch PCR products. Each band is labeled for Pine Lake (P), Steel Lake (S), and Killarney(K).

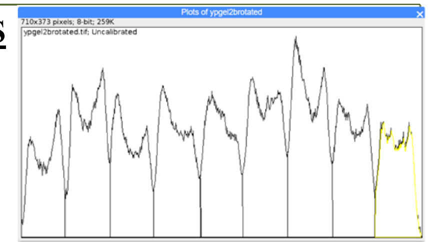

Figure 2A-B. Results of the housekeeping gene Beta-actin and target gene

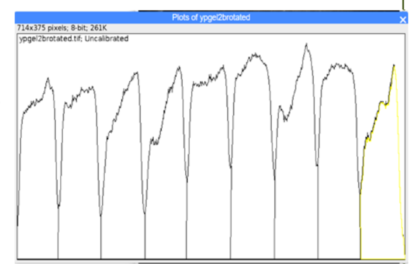

Figure 2B.

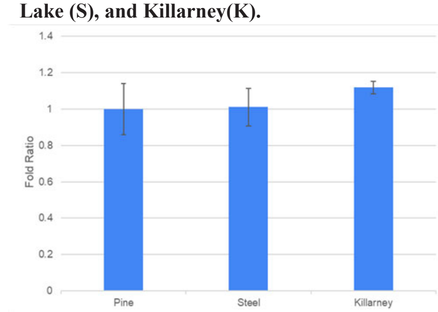

Figure 3. This shows the fold ratio results of each of the lakes. Pine lake's fold ratio was at 1 and Steel lake shows no change in gene expression from the control lake. Lake Killarney's fold ratio was 1.1 which is higher than the other lakes but not statistically significant.

REFERENCES

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