Legacy Arsenic Contamination and its Effects on Gene Expression of the Heat Shock Protein in Sunfish

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INTRODUCTION

This study aimed to look at the effects of arsenic from three different lakes with varying levels of arsenic contamination and the effects it can have on the *hsp-70* gene expression of the liver on Bluegill (*Lepomis macrochirus*) and Pumpkinseed Sunfish (*Lepomis gibbosus*). Our proposed hypothesis was:

- The lake with the highest contamination (Lake Killarney) was expected to have a greater *hsp-70* expression than fish with no arsenic exposure from Pine Lake.

Arsenic contamination has been a significant problem in the South Puget Sound area for a long time, a major way arsenic has contaminated the soil and water in Tacoma is from the Ruston copper smelter (Arsenic, 2022).

We chose to focus on the liver in study because it is a major site for detoxification in the body.

METHODS AND MATERIALS

Samples were taken from 3 lakes- Lake Killarney, Steel Lake, and Pine Lake.
Bluegill and Pumpkinseed Sunfish were collected from the lakes.
Fish were euthanized and target tissues were collected (liver)
Tissue extracted using Trizol reagent and homogenized with liquid nitrogen
Purity and concentration was taken- threshold of purity concentrations was >1.7
Genes were amplified and ran through the gel electrophoresis unit
Target gene was *hsp-70*
Housekeeping gene was *EF1-α*
Gels were analyzed under UV light and looked at the densitometry
Used Image-J to find area of each band
Used ANOVA using the lakes as the single factor

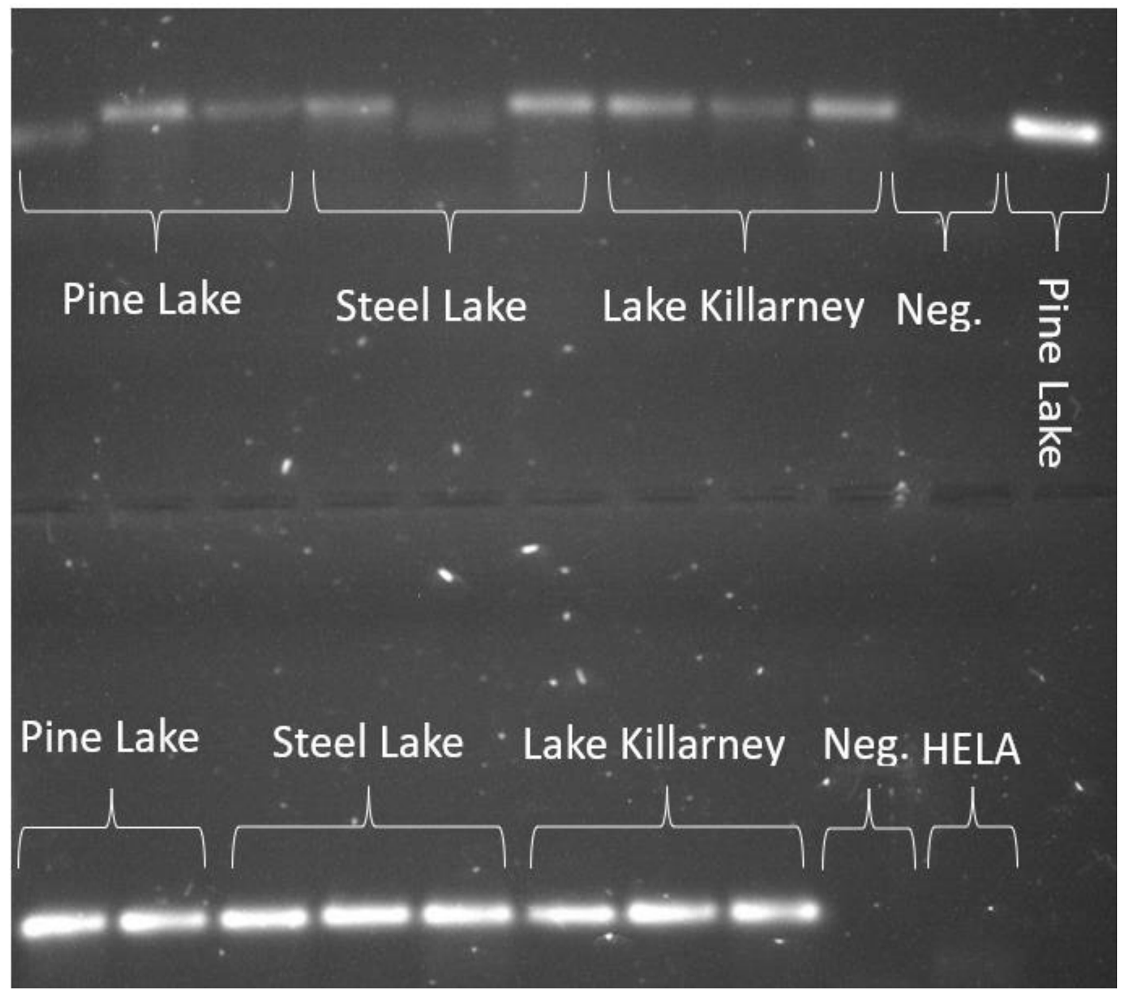


Figure 1: This image shows PCR amplicons of the fish collected from each of the lakes, Pine Lake, Steel Lake, and Lake Killarney. Both the target (*hsp-70*) and housekeeping (*EF1-α*) genes are shown. There was no significant difference in the *hsp-70* gene expression (Figures 1 and 2) across the three lakes. The first fish replica area from Pine Lake was not included in the calculations because the area was not able to be calculated accurately, as seen in the figure above the first replica sample was in the top row with the target gene samples so an accurate area was not able to be taken.

Pumpkinseed Sunfish

Bluegill Sunfish

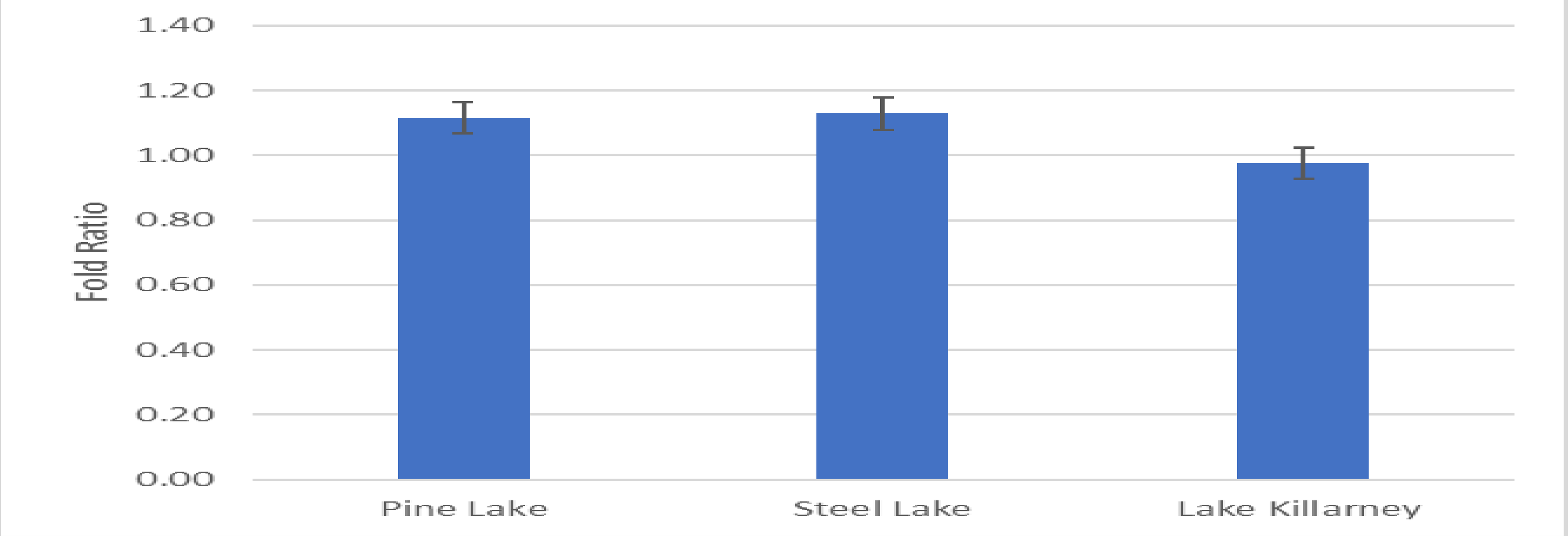


Figure 2: This graph shows the fold ratio between the three lakes, Pink Lake, Steel Lake, and Lake Killarney. The fold ratio was calculated by dividing the areas between the target and housekeeping genes of each of three lakes to get the normalized area. And the normalized area of each area was divided by the average of the reference lake (Pine Lake) to find the fold ratio.

RESULTS

There was no significant difference seen between the gene expression in the liver from any of the lakes in the Bluegill Sunfish and Pumpkinseed Sunfish with varying levels of arsenic contamination
P-value was 0.873, this is greater than 0.5 which also supports this claim
Error bars on the graph for fold ratio have significant overlap
One area for the housekeeping gene from Pine Lake was not included in the calculations to find these results
The results that came out did not support our hypothesis

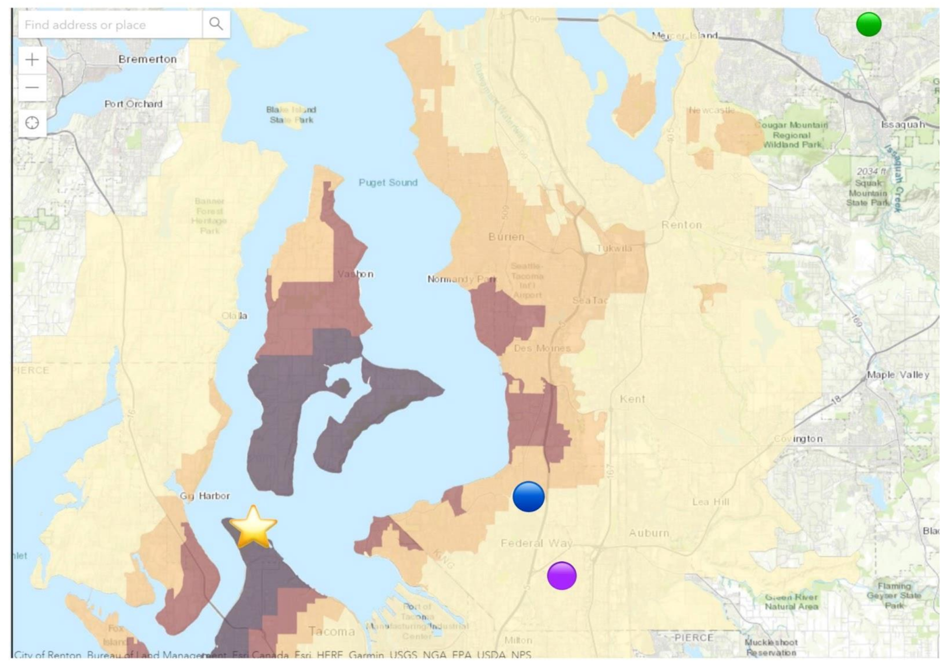
DISCUSSION

We expected there to be a difference in the gene expression of the liver in the fish that were in the lake with higher levels of arsenic compared to the lakes with less or no arsenic contamination

There are still many other studies that can be performed to further study the effects of legacy contamination on fish, including analyzing biomarker responses in other tissues as well as other species.

Genes	Forward Primer	Reverse Primer
Hsp-70	AGA GAC TGA TTG GGA GAA AGA TG	TCC GTC TCC GAC CAC CTT G
EF1-α	AAG CCT GGT ATG GTT GTG ACC TT	GCA TCT CAA CAG ACT TGA CCT CAG T

Primer sequences acquired from (Dennis et. Al., 2015)



Under 20 ppm
20-40 ppm
40.1-100 ppm
Over 100 ppm
Green: Pine Lake
Blue: Steel Lake
Purple: Lake Killarney
Star: Old Smelter Location

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