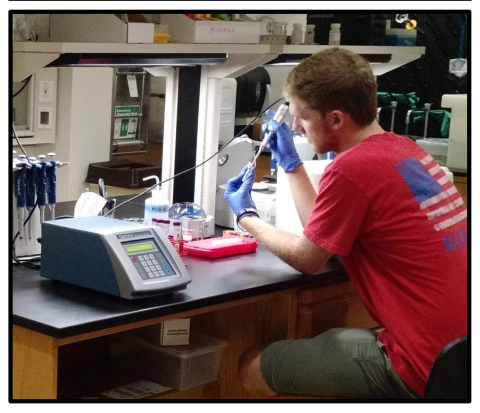

Cause for concern: biological implications of chemical contamination in Kazakhstan's Syr Darya river

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INTRODUCTION

Background and Study Goal.

- The Syr Darya River located in southern Kazakhstan is one of the two main water sources that feed the Aral Sea.
- During the Soviet era, water diversion from the Syr Darya to surrounding agricultural land caused the drying of the Aral Sea [1]. These irrigation practices, in conjunction with heavy industrial chemical use from uranium mining, created the opportunity for chemical contamination in the Syr Darya.
- Efforts to revive the Aral Sea are underway, yet few investigations have sought to identify the presence and effects of heavy metals in the Syr Darya.

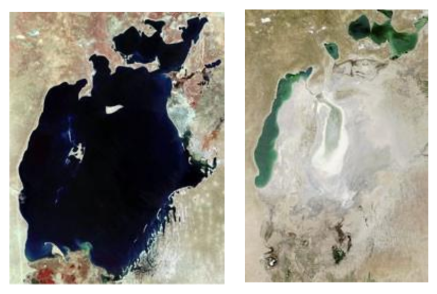

Figure 1. Satellite images of the Aral Sea taken in 1973 (left) and 2009 (right).
From www.nasa.gov

- **The goal of this study was to determine the potential ecological risks associated with heavy metal contamination in the Syr Darya River.**

Specific Study Objectives.

Objective 1: Determine the presence of heavy metals in the surface waters, sediments, and fish tissues collected from the Syr Darya River.

Objective 2: Evaluate the biological markers indicative of metal exposures and/or adverse effects in roach fish (*Rutilus rutilus*) from a subset of sampling sites.

METHODS

- Five field sites along the Syr Darya River were selected for inclusion in this study (Fig. 1).
- In early June of 2015, heavy metal contamination at each site was assessed using the three methods as outlined in Table 1.
- The presence of 14 heavy metals was evaluated at the selected field sites using the general methods outlined in Table 1. Heavy metals were assessed via inductively coupled plasma mass spectrometry.

Figure 2. A map of the Syr Darya watershed indicating the five field sampling sites.

Table 1. General methods utilized to assess chemical contamination at each of the five selected field sites.

Method		Description & Purpose
Water grab samples		Three 1 L grab samples were collected from each site to provide a "snapshot" of heavy metal load at the time of sampling
Tissue analysis of wild-caught fish		Muscle tissue was taken from roach (*Rutilus rutilus*) sampled from three of the field sites for heavy metal analysis

- To assess the potential biological impacts associated with chemical contamination in the Syr Darya, roach (*Rutilus rutilus*) were collected from three of the five field sites.
- Mass, length, condition factor, hepatosomatic index (HSI) and gonadosomatic index (GSI) were determined for each fish.
- Tissues (brain, gill, gonad and liver) were collected for subsequent gene expression analysis via quantitative PCR. A subset of the genes measured appears in Table 2.

Biological Markers

- Genes were chosen for their potential as biological signals of metal contamination. The pathway for SOD and CAT, two biomarkers of a response to metal contamination is shown below (Fig. 3).

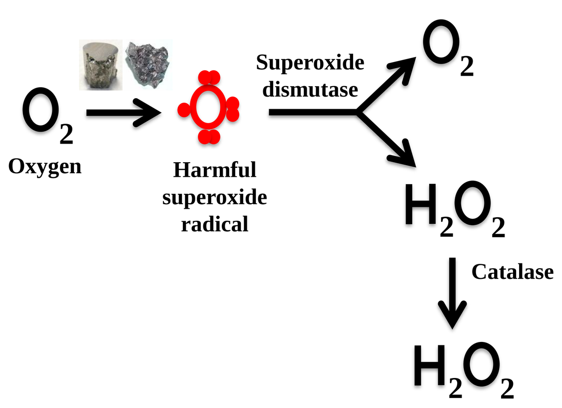

Figure 3. Detoxification of superoxide anion and hydrogen peroxide.

Table 2. Target genes analyzed in this study and their respective functions.

Target gene	Function
Metallothionein (*mt*)	Biomarker of heavy metal induced oxidative stress.
Heat shock protein 70 (*hsp70*)	Biomarker associated with a generalized "stress" response [2]
Superoxide dismutase (*sod*)	Biomarker of oxidative stress due to a variety of environmental factors including chemical contamination [3]
Catalase (*cat*)	Biomarker of oxidative stress (catalyzes the production of hydrogen peroxide)
Glutathione s-transferase (*gst*)	Biomarker of exposures to a diverse range of xenobiotics and exogenous toxicants.

RESULTS AND DISCUSSION

- Heavy metals were detected in water samples and fish tissues collected from each site (Fig. 4). **Concentrations of manganese, copper and vanadium exceeded local regulatory limits**. However, no differences were seen in total metal concentrations in fish tissues.

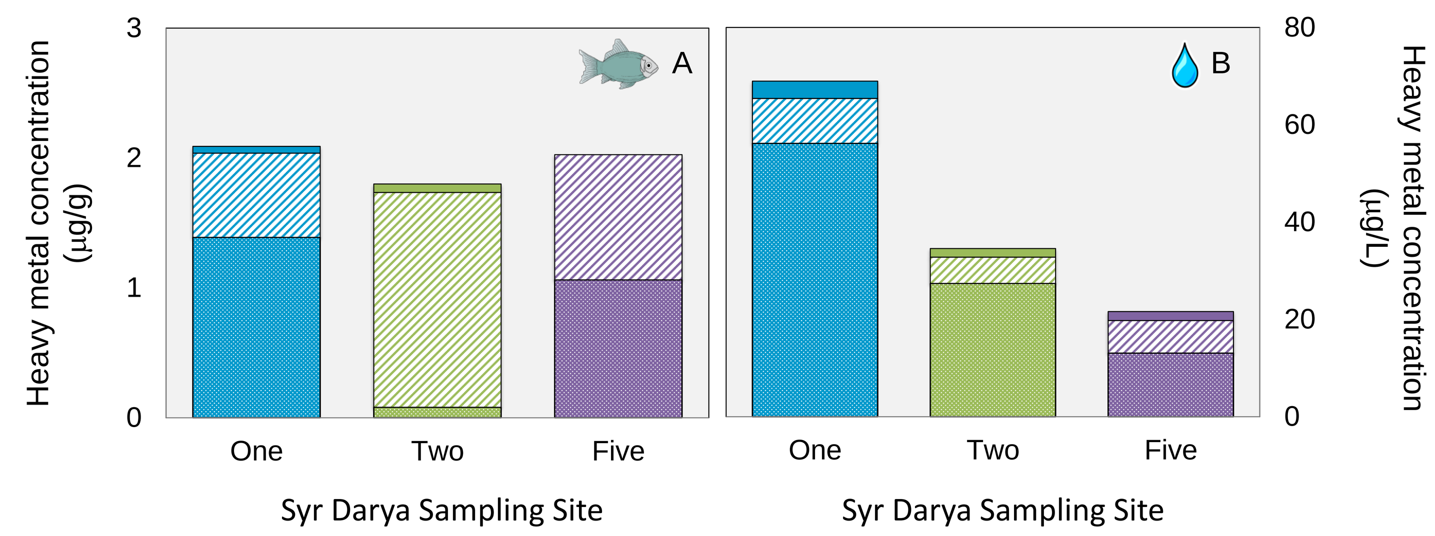

Figure 4. Concentrations of manganese (dotted bars), copper (lined bars) and vanadium (solid bars) in muscle from roach (A) and water samples (B) collected from the Syr Darya River.

- Fish from sites one and five had significantly lower body masses and condition factors relative to fish from site two (Fig. 5). These fish also had significantly lower lengths, HSI and GSI than fish from site two (data not shown).
- These findings, in conjunction with the gene expression analysis, suggest that **fish from sites one and five are less robust, and perhaps, less healthy than those from site two.**
- Although it was not significant, there was an upregulation of *mt* in fish from two compared to sites one and five in both the liver and gill (Fig. 6), suggesting that **fish from site two may be responding to heavy metal contamination.**
- Fish at site two may be overexpressing *mt* to protect themselves from heavy metal toxicity and thus avoid the oxidative stress that fish from sites one and five may be experiencing.

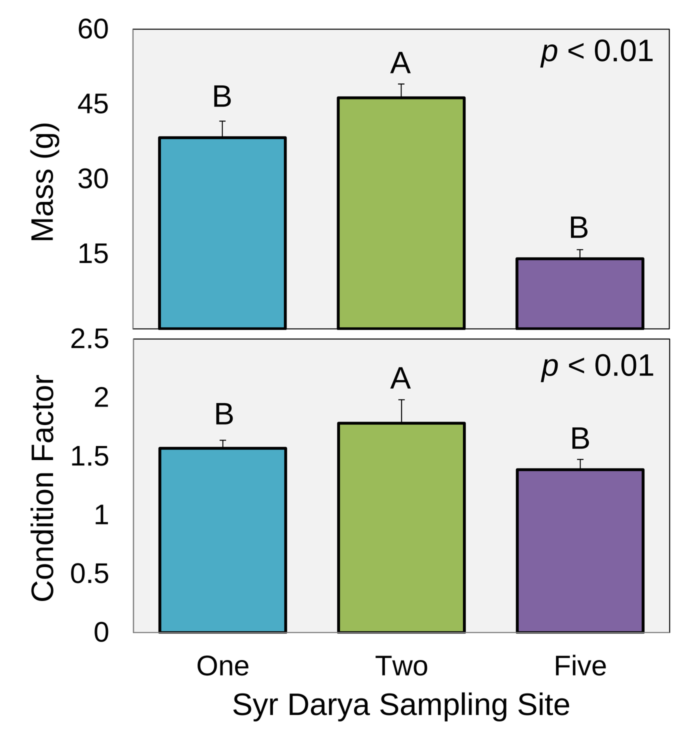

Figure 5. The mean (± standard error) mass and condition factor of Syr Darya roach.

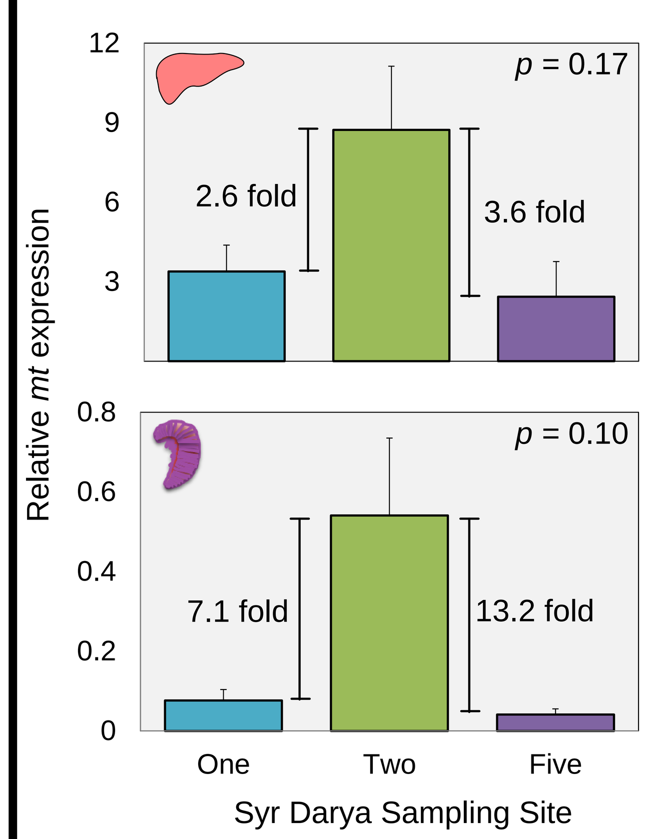

Figure 6. Hepatic and gill metallothionein (*mt*) expression of roach collected from the Syr Darya River.

- While not significant, the upregulation of *hsp70* among fish from sites one and five (Fig. 7) suggests that **fish from these sites may have been exposed to one or more environmental stressors.**
- No differences were seen in the expression of *gst* (data not shown). Hepatic *sod* was significantly higher in fish from sites one and five compared to those from site two (Fig. 8), indicating the **possible presence of a chemical contaminant capable of inducing oxidative stress.**

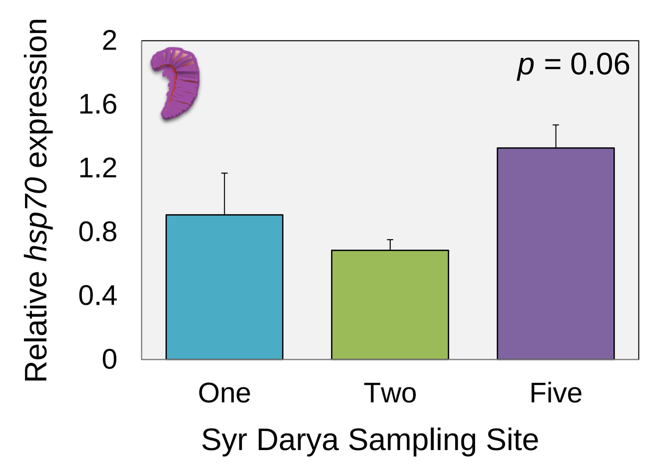

Figure 7. Heat shock protein 70 (*hsp70*) expression in the gills of roach collected from the Syr Darya River.

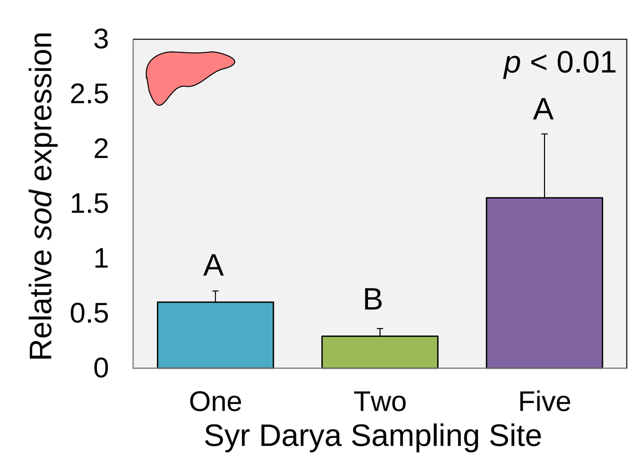

Figure 8. Hepatic superoxide dismutase (*sod*) expression of roach collected from the Syr Darya River.

CONCLUSIONS & FUTURE DIRECTIONS

- Chemical testing of water, sediment and fish tissues revealed the presence of heavy metals in the Syr Darya. Multiple heavy metals were observed above local regulatory limits suggesting that **fish in some stretches of the Syr Darya may be at high risk of heavy metal exposure.**
- Though no obvious correlations between heavy metal load and biomarker responses in roach were apparent, the presence of heavy metals above local regulatory limits and the differential expression of biomarker genes (*i.e., sod, cat, mt*) suggests that there are **spatial differences in environmental quality along the Syr Darya** watershed. The overexpression of *sod* and *cat* in roach from sites one and five illustrate the potential biological impacts of these contaminants in the environment.
- Future studies will explore the temporal variation in heavy metal load and biomarker responses in the Syr Darya River.
- Data from this study can be used to conduct an ecotoxicological risk assessment of the Syr Darya watershed, which may aid efforts to restore the health of the Syr Darya and the Aral Sea.

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