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Assessment of Sodium Perchlorate as Disrupter of Environmental Sex Determination in Daphnia Magna

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Introduction

The effects of perchlorate anion, ClO4-, has shown to be an inhibitor in the thyroid hormone synthesis process as well as an endocrine disruptor in the ecological context. The amount of perchlorate found in our waters has been on an incline since the increase of usage as an oxidizer found in rockets, ammunition, airbags, and fireworks (Park et al. 2006). Although it has been determined to be harmful at low levels, this unregulated chemical has been knowingly consumed by humans for approximately 15 years through produce, milk, and even drinking water (Bernhardt et al. 2006). The environmental sex determination (ESD) is the environmental notion that triggers an alternative genetic signal within the organism that regulates the male or female sex determining gene (Kato et al. 2011). Alternatively, in genetic sex determination (GSD) there genetically segregation of genes, often on the sex chromosomes that initiated the sexual determination pathway that that organism will undergo (Kato et al. 2011). In this study we are looking into the ocean crustacean Daphnia magna as our model organism and seeing the effects that perchlorate has as an endocrine disruptor on their ESD process. The endocrine system is comprised of glands that secrete hormones that interact with specific targets or receptors (La Merrill et al. 2020). The Double Sex or Dsx gene is crucial in controlling sexual dimorphism in genetic sex determining species, *D. Magna* has two Dsx genes that they can express; Dsx-1 and Dsx-2. The Dsx-1 mainly is the driving force behind the expression of male traits during ESD, whereas the Dsx-2 gene has a similar function but isn't as drastic in the exhibition of phenotypic changes within the species. The research objectives for this study are to evaluate the Dsx 1, both α & β variants, and Dsx 2 genes in the *D. magna* that are in different sodium perchlorate concentration solutions. We hypothesis that the *D. magna* will increase the regulation of Dsx1 gene, in both α & β variants, when exposed to perchlorate.

Methods

-Collect 180 juvenile *D. magna* in 3 separate jars, 60 per jar. Collect 30 *D. magna* after 2 hours and 24-hour time marks. After collection *D. magna* were snap frozen with liquid nitrogen and suspended in Trizol.

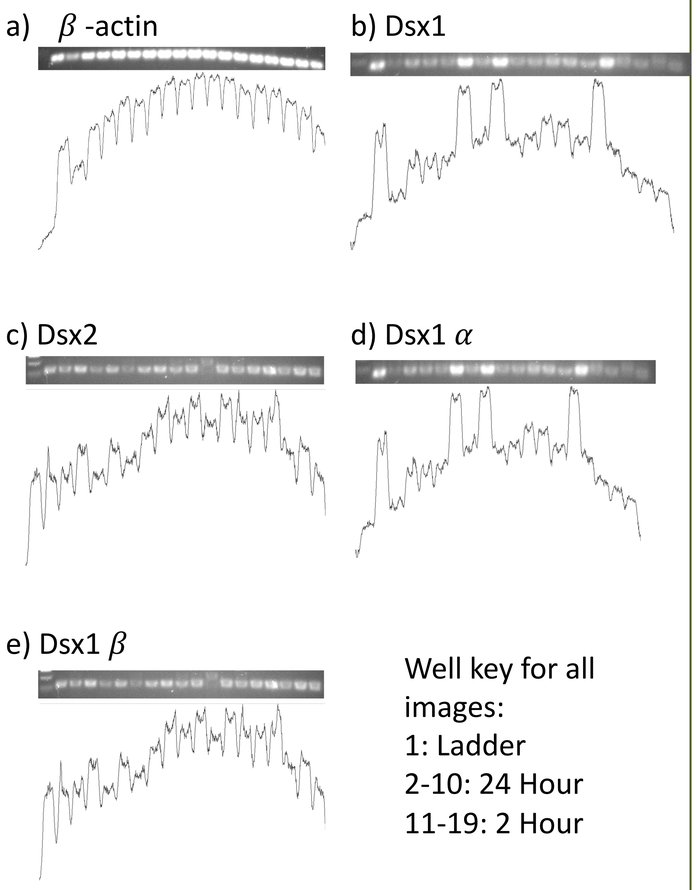
- Control group jar= 0 perchlorate
- Low treatment group= 10 mg/L
- High treatment group= 100 mg/L

-Extract RNA from *D.magna* and measure concentrations via spectrophotometry
-Reverse transcribe RNA into cDNA and prepare forward and reverse primers for:

- Dapma Dsx1 and α & β variants
- Dapma Dsx2
- β-actin as housekeeping

-Amplify cDNA with semi-quantitative PCR
-Utilize gel electrophoresis to measure gene expression
-Obtain imaging and perform densitometry analysis

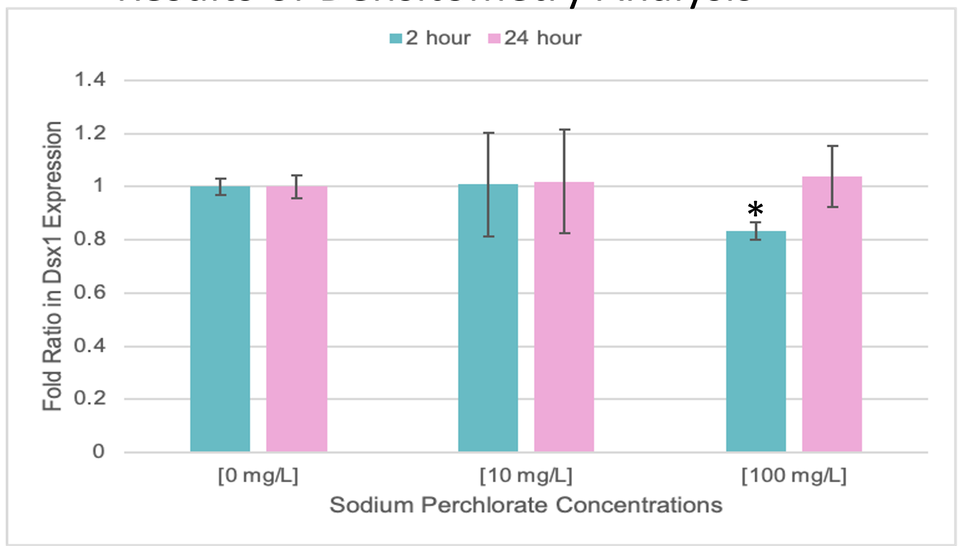
GEL Images



Well key for all images:
1: Ladder
2-10: 24 Hour
11-19: 2 Hour

Figure 1. Gel imaging and densitometry analysis plots of the respective Dsx and housekeeping genes. The peaks indicate expression of the target genes.

Results of Densitometry Analysis



Graphs of Fold Ratio Expression: Dsx 1:

- 2-Hour [0] vs. [100] p value is 0.0216, down regulated
- No other statistical significance was shown

- Other P values include:
  - [0] vs. [10] 2 Hour= 0.969
  - [0] vs. [10] 24 Hour= 0.930
  - [0] vs. [100] 24 Hour= 0.773

Conclusion

We only had one statistical evidence that pointed to down regulation of the 2-hour control group compared to the high treatment of 100 mg/L of perchlorate. There was no consistent statistical evidence within any gene or timepoints that supports our hypothesis. This down regulation suggests that perchlorate reduces the quantity of the Dsx1 gene. Additional studies are required to further understand the effects that perchlorate has on *D.magna* endocrine-related pathways.

Future Experiments

- Repeat of experiment with larger scale and more critical time points
- Additional concentrations of perchlorate could be utilized to see the scale effects it could have
- Future studies with perchlorate are warranted due to it being putative obesogenic

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