

# Perchlorate Exposure and Its Effects on *Daphnia magna* Morphology, Hemoglobin Expression, and Lipid Metabolism



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## BACKGROUND

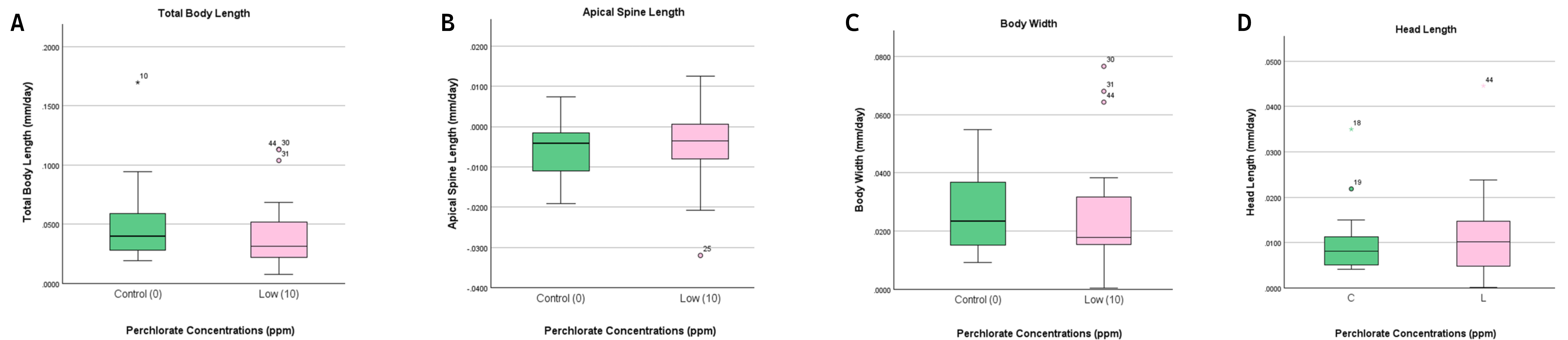
- **Perchlorate** contamination is an emerging concern in aquatic ecosystems.
- Perchlorate acts as an **endocrine disruptor** that can alter physiological processes.
- Effects of perchlorate on aquatic invertebrates are not well understood.
- *D. magna* used as model organism due to its sensitivity to environmental stressors.
- This study examined how **10 ppm** perchlorate exposure affects:
  - Morphology
  - Hemoglobin gene expression
  - Lipid synthesis

## HYPOTHESIS

- Perchlorate exposure will reduce body morphometrics, alter hemoglobin expression, and disrupt lipid homeostasis.

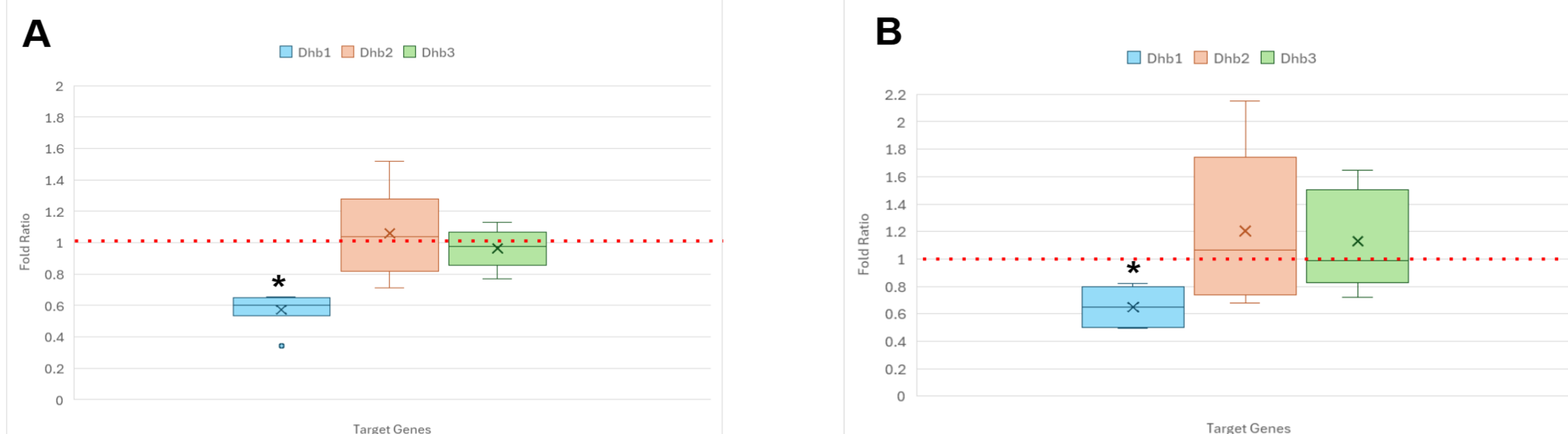
## RESULTS

**Morphology: No statistical significance was found between the control and 10 ppm groups over the 7-day period.**



**Fig.1 A-D: Morphological Growth Rate of *D.magna* Exposed to Perchlorate over a 7-Day Period**  
Growth of (A) Total Body Length, (B) Apical Spine Length, (C) Body Width & (D) Head Length in juvenile female *D. magna* following exposure to 10 ppm perchlorate for 7 days. Each individual daphnia were imaged on Day 0 & Day 7. Measurements of *D.magna* for Control (n=22) & 10 ppm (n=25) were obtained using ImageJ software. The growth rate was calculated and shown above as mm per day over the 7-day exposure period. No statistical significance was found between the control and 10 ppm groups for each morphometric. Survival rates were observed to be 48.9% for the Control Group & 62.5% for the 10 ppm treatment.

**Hemoglobin Expression: Only the dhb1 gene displayed a significant downregulation with 48hr perchlorate exposure**

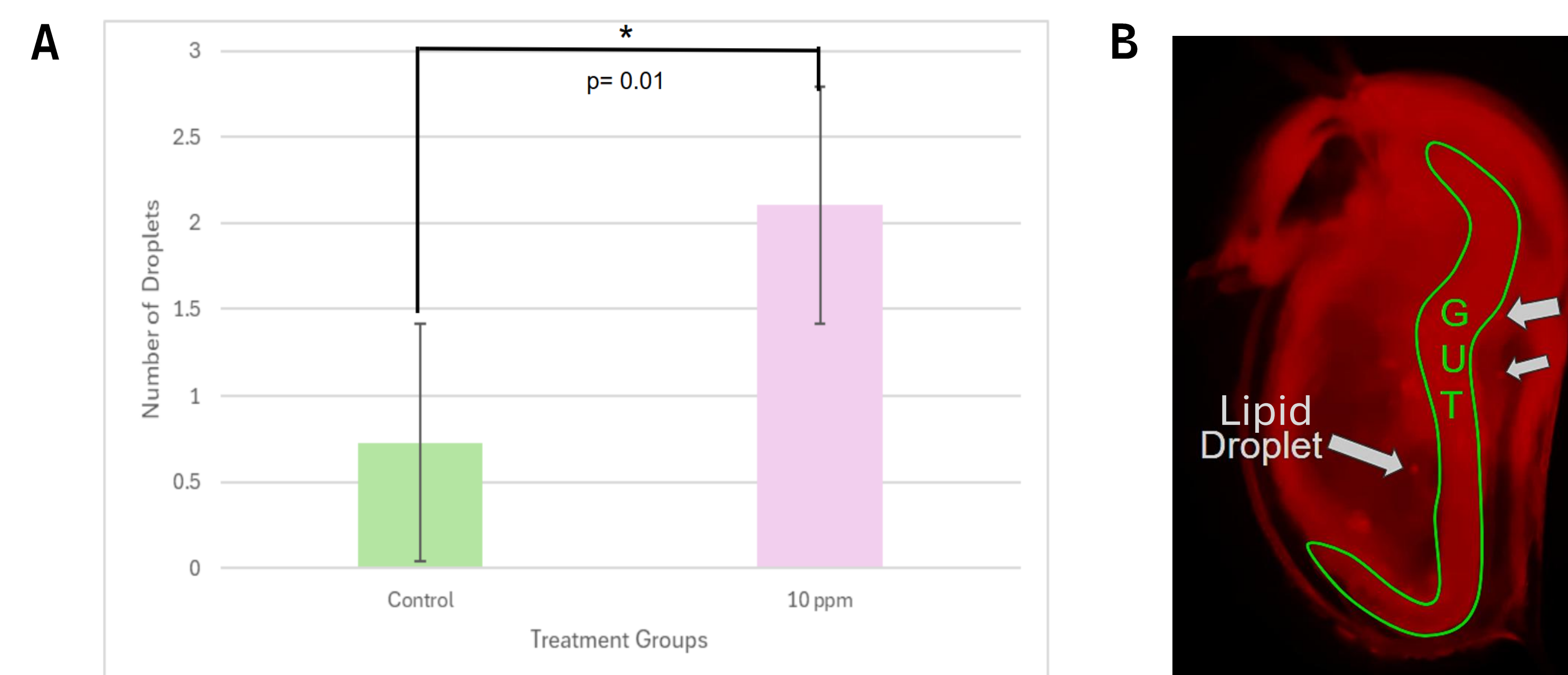


**Fig.2 A-B: Relative Expression of Hemoglobin Genes in *D.magna* Following Exposure to 10 ppm Perchlorate**  
Fold ratio change was calculated for our target hemoglobin genes (dhb 1, dhb2, & dhb3) of daphnia exposed to 10 ppm perchlorate over 48 hours. The red dotted line in both panels represents the fold ratio change of our control group, which is one. Each target gene consisted of 6 replicates for both the control and treatment groups. Asterisks (\*) denote statistically significant differences compared to the control group, for which a One Way ANOVA test was run where  $p < 0.05$ .

(A) Dhb genes normalized with B-actin. Only Dhb1 displayed a significant down regulation. Dhb 2 & Dhb 3 were very close to the baseline so there was no change in gene expression.

(B) Dhb genes normalized with GAPDH. Only Dhb1 displayed a significant down regulation. Dhb 2 & Dhb 3 were very close to the baseline so there was no change in gene expression. GAPDH data consists of only 5 replicates for perchlorate treatment and 6 replicates for the control due to a very faint band.

**Lipid Synthesis: Perchlorate exposure had a statistically significant increase in lipid droplets around the gut**



**Fig.3 A-B: Lipid Accumulation in Female *D.magna* exposed to 10 ppm Perchlorate**  
Juvenile female daphnia were exposed to 10 ppm perchlorate over a 7-Day period with (n=25) for each treatment group. Daphnia were stained with Nile Red and visualised under a fluorescent microscope. A two-tailed (unpaired) t- test was run for statistical analysis,  $p < 0.05$  is noted by an asterisk (\*).

(A) The number of lipid droplets were visualized under fluorescence microscopy and imaged with a cell phone. Each image was manually counted by five different researchers and then the averages were collected. The overall average for each treatment is displayed on the bar graph. Female daphnia exposed to perchlorate had a statistically significant increase in lipid droplets around the gut compared to the control.

(B) Image of a female daphnia exposed to 10 ppm perchlorate visualized under fluorescence. Each white arrow points to a single droplet. The green outline highlights the daphnia's gut.

## DISCUSSION/CONCLUSION

- Exposure to 10 ppm perchlorate caused measurable molecular and metabolic effects in *D.magna*.
- **No significant morphological changes** were observed in body size, body width, apical spine length, or head length.
- The **hemoglobin gene dhb1 was significantly downregulated** after 48-hour perchlorate exposure & suggests perchlorate may **impact oxygen transport**.
- Female *D. magna* exposed to perchlorate showed **increased lipid accumulation** & indicates **disruption of lipid metabolism**.

## NEXT STEPS

- Include **larger sample sizes**
- Examine **long-term** perchlorate exposure
- Further investigate **molecular** and **metabolic impacts** on aquatic invertebrates

## ACKNOWLEDGEMENTS

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## METHODS

