

Effect of Mine Runoff Metals on the Cyclin-Dependent Kinase 3 (CDK3) Gene of *Tetrahymena thermophila*

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Introduction

- Heavy metal contamination in Montana streams via mine runoff represents a significant environmental and public health hazard.
- Cyclin-dependent kinase 3 (*CDK3*) is highly expressed in the early stages of conjugation during meiosis initiation. *CDK3* complexes with cyclin-dependent kinases and conjugation-specific cyclins to initiate meiosis, making it an essential enzyme in meiotic division.
- Hypothesis:** Exposure to mine runoff metals would result in significant changes both in the expression of *CDK3* and in the conjugation rate of *T. thermophila* cultures.

Materials and Methods

- Culturing** of stock *T. thermophila* was performed with NEFF media. Experimental and control *T. thermophila* (two flasks each) were cultured in starvation media to induce meiotic division. Experimental cultures were exposed for 12 hours to starvation media containing mine runoff metals consistent with environmental concentrations (see Table 1).
- RNA extraction** was performed after a 12-hour exposure period using RNeasy RNA extraction kit.
- Reverse transcription** was facilitated by RevertAid reverse transcription enzyme to produce cDNAs.
- Quantitative PCR** was performed using *CDK3* specific primers, *BTU1* positive control primers, and PowerUp Sybr Master Mix
- Conjugation counts** were determined via hemocytometer count every three hours during exposure.

Table 1: Heavy metal contaminant concentrations for treatment groups.

| Contaminant | Treatment 1 (ppm) | Treatment 2 (ppm) |
|-------------|-------------------|-------------------|
| Cadmium | 5 | 3 |
| Copper | 1000 | 320 |
| Lead | 160 | 80 |
| Zinc | 1000 | 800 |

Results

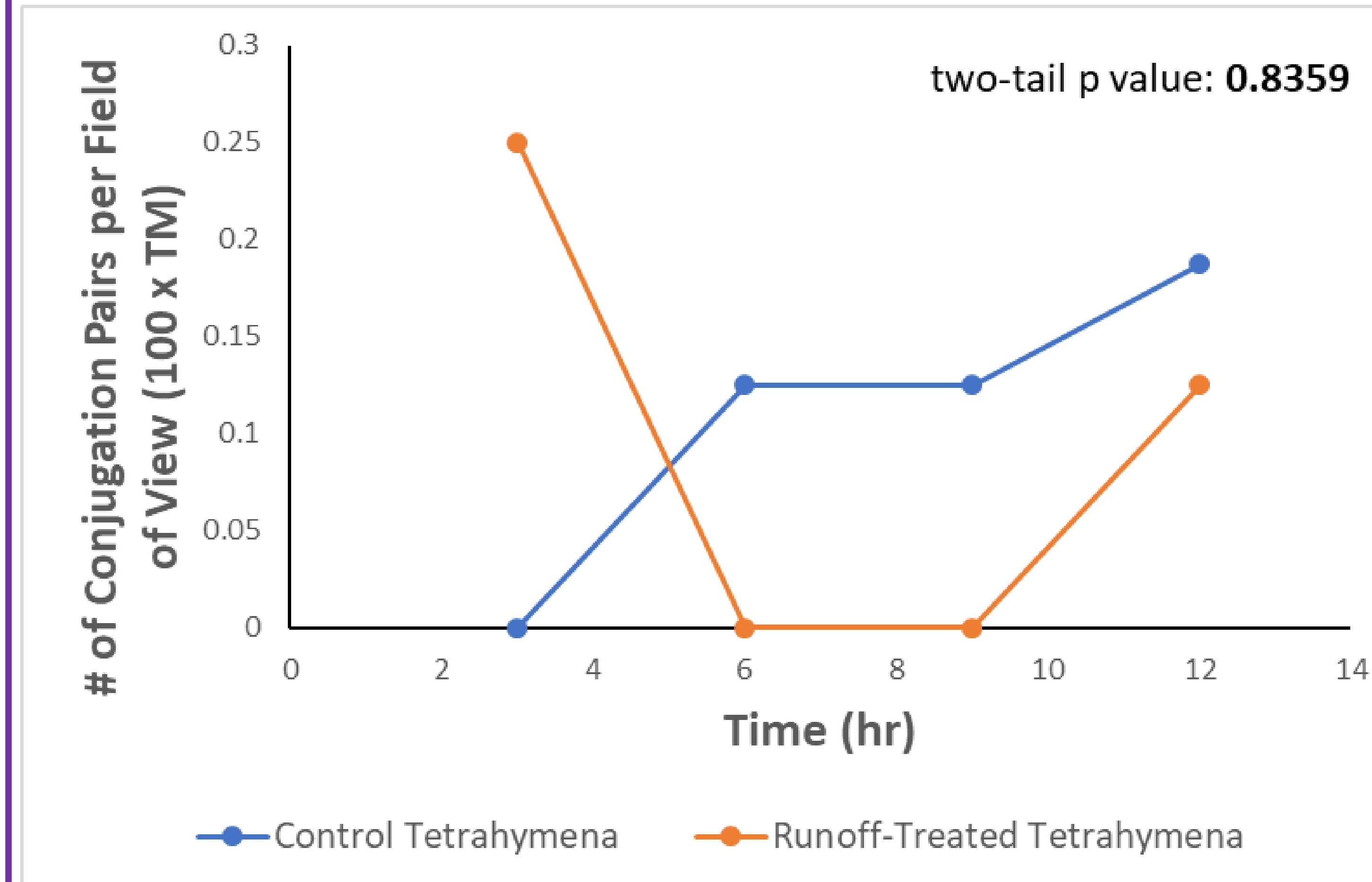


Figure 1: Number of *T. thermophila* conjugation pairs per field of view (100x TM) over a 12-hour exposure period to runoff and control media. Counts were recorded every three hours using a hemocytometer. $n = 4$ cultures per group.

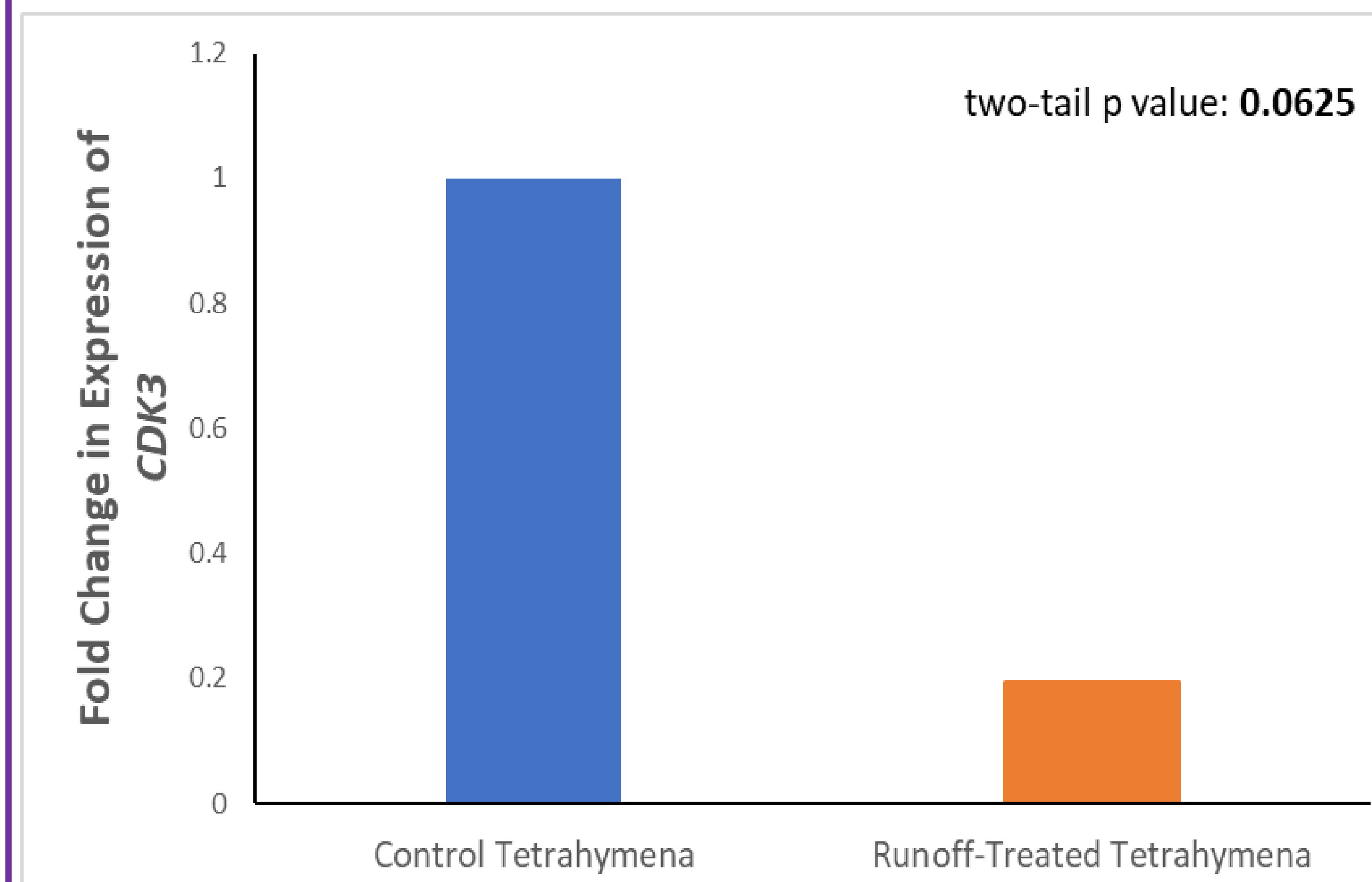


Figure 2: Comparison of fold change in expression of *CDK3* between control and runoff-treated *T. thermophila* cultures. $n = 4$ cultures per group.

Discussion

- Mine runoff metal exposure decreased conjugation pair count, as seen in Figure 1. The difference was insignificant and therefore inconclusive, but suggested a decrease in successful meiosis when paired with the qPCR analysis data ($p = 0.8359$).
- There was a statistically insignificant decrease in the quantitative relative expression of *CDK3*, as shown in Figure 2 ($p = 0.0625$).
- Given the statistical insignificance of the data, the results do not conclusively agree with our prediction that the conjugation rate of experimental *T. thermophila* cultures and their expression of *CDK3* would both significantly change as a result of mine runoff exposure.
- Future researchers should consider a reduced concentration of heavy metals in the simulated mine runoff, a wider diversity of metals used, and alterations in temperature and pH to more closely approximate natural Montanan water sources.

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References

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