Effects of Chitin-Synthase Inhibitors on Acute Stress in Copepods
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Background and Hypothesis

In order to combat parasitic copepods, fish farmers have been utilizing antiparasitics since the 1970s (Aaen & Horsberg, 2016). Over the years, farmers have been increasing the frequency of antiparasitic medication such as chitin-synthase inhibitor, dimilin (diflubenzuron), to combat resisting parasitic copepods, leading to a rising concern for toxic runoff and potential harm to non-parasitic copepod populations. In tandem with these effects, natural copepod populations are also being adversely affected by increasing temperatures and decreasing oxygen concentrations. This study focuses on the effects of thermal and hypoxic stress on Bodega Bay (38.3332° N, 123.0481° W) T. californicus copepods subjected to chitin-synthase inhibitor, dimilin. The question that our group is exploring is: Does increasing concentrations of dimilin change the ability for T. californicus copepods to withstand extreme conditions? We hypothesized: Increasing concentrations of dimilin will have negative effects on the survival of T. californicus in hypoxic and thermal stress conditions.

Methods

Thermal & Hypoxia Tolerance Control Assays
- For each experimental condition, 4 petri dishes with no dimilin, 25%, 50%, and 100% of the literature recommended dosage of 5 mg/L (Aaen & Horsberg, 2016), were created containing 10 copepod each.
- Copepods were subjected to normoxic conditions in the incubator (20°C) for 24 hours.
- 1 hour and 24 hour checkpoints to analyze behavior and potential knockdowns were conducted.
- A knockdown is classified as a copepod not swimming.

Thermal & Hypoxia Tolerance Experimental Assays
- For each experimental condition, 4 petri dishes, each containing 10 BB population copepods, were treated with no dimilin, 25%, 50%, and 100% of the literature recommended dosage of 5 mg/L were created.
- Thermal
  - Experimental groups were introduced to a 37°C water bath for an hour and then put into a 20°C water bath for 10 minutes afterwards for acclimation.
- Hypoxia
  - Experimental groups were subjected to 24 hours of hypoxic conditions.
  - Knockdowns were observed after 6 and 24 hours.

Figure 1: Percentage concentration is derived from a literature value of 5 mg/L. 100% concentration is 5 mg/L, 50% is 2.5 mg/L, 25% is 1.25 mg/L, and 0% has no Dimilin. (A). This plot shows the control trials for the adult male copepods. This control was conducted in 20°C with normoxic conditions for a total of 24 hours. (B). This plot shows the hypoxic treatment results conducted with adult male copepods for a total of 24 hours. Copepods were exposed to dimilin for 1 hour prior to the 24 hour hypoxic period. (C). This shows the adult male copepod thermal assay results. Copepods were introduced to dimilin for 1 hour, then placed in a 37°C water bath for 1 hour, then placed in 20°C conditions for the remainder of the 24 hours. (D). This plot shows the control trials for the nauplii. This control was conducted in 20°C with normoxic conditions for a total of 24 hours. (E). This plot shows the hypoxic treatment results conducted with nauplii for a total of 24 hours. Nauplii were exposed to dimilin for 1 hour prior to the 24 hour hypoxic period. (F). This shows the nauplii thermal assay results. Nauplii were introduced to dimilin for 1 hour, then placed in a 37°C water bath for 1 hour, then placed in 20°C conditions for the remainder of the 24 hours.

Results & Conclusion

- Adult male experiments resulted in varying knockdown indicating no visible correlation between Dimilin and copepod knockdown.
- Nauplii experiments revealed that Dimilin dosage has the potential to effect thermal tolerance long term and hypoxia tolerance in a short period with those results more consistent with the hypothesized outcome, however results still varied across all treatments.
- 50% and 100% concentrations of Dimilin had the greatest effect on nauplii knockdown.
- We cannot support our hypothesis given the inconsistent results in both the adult male and nauplii trials, therefore, we reject it.
- Future experiment ideas:
  - Looking at long-term effects of chitin-inhibitor exposure on larger experimental groups of nauplii and monitor growth into mature adults, then testing the hypoxia and thermal tolerances of the mature copepods.
  - Other measurements of the effects of Dimilin could be gene expression levels of chitin synthesis genes.

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References