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Do weight loss drugs affect the survival and productivity of aquatic invertebrates?

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INTRODUCTION & AIM

Active pharmaceutical ingredients (API) can accumulate in the environment through industrial waste, municipal waste, and excreted drugs and drug metabolites in wastewater. APIs are recognized as a potential threat to aquatic ecosystems (Bouzas-Monroy et al. 2022), but more research is needed on the potential effects of APIs.

We studied the potential impact of a weight loss drug, orlistat, on aquatic invertebrates. We reasoned that since orlistat interferes with the enzymatic activity of lipases, this inhibitory activity could also in nature, if orlistat accumulates in the environment. We hypothesized that orlistat could affect lipid assimilation of aquatic invertebrates. Lipases facilitate assimilation of lipids, and if orlistat in nature interferes with the activity of the animal's lipases, then this could inhibit the animal's energy resources.

We selected *Daphnia magna* as our study organism. In nature, *D. magna* functions as a primary consumer. It occurs in freshwater lakes, ponds, and rivers. It grazes on phytoplankton, bacteria, and other microbes. Daphnia play a critical role in the transfer of energy between primary producers and higher trophic levels. Our objective was to measure the effects of orlistat at low concentrations. We tested the hypothesis that orlistat could affect the survival, growth, and reproduction of *D. magna*.

METHOD

D. magna females were isolated from aquarium stocks and placed in glassware. After 24 hours, females were removed from glassware. Newborn neonates were maintained for five days prior to the start of experiments. Precise volumes of spring water were measured into glassware and exact volumes of treatment and control solutions were added to the spring water. For the experimental treatments, Orlistat in pure ethanol (not denatured), was added with a micropipette. For the control treatments, the same volume of pure ethanol was added with a micropipette. The volumes of ethanol were very small, and they were presumed not to have a significant effect on Daphnia.
In Exp. 1 and 2, Spirulina was added in precise volumes. In Exp. 1, water was changed every 3 days. In Exp. 2, water was partially refreshed every two days by replacing 50 ml, followed by a complete change every seven days.
In Exp. 3 and 4, Nannochloropsis was added in precise volumes and water was changed daily.

Five-level Exp: Our first experiment was intended to identify the lowest concentrations at which we could observe effects of Orlistat with our methods. We used five concentrations of orlistat, ranging from 2.48 * 10^{-5} to 3.96 * 10^{-4} mg/ml, for small survival experiments.

Exp. 1 tested for survival. Individual neonates were placed in 50.0 ml synthetic spring water at 18°C. The experimental treatment (n=36) was 1.98 x 10^{-3} mg/ml Orlistat in spring water (controls in spring water, n=18).

Exp. 2 tested for survival. Individual neonates were randomly assigned to 200.0 ml natural spring water at 20°C. The experimental treatment (n=15) was 2.13 x 10^{-3} mg/ml Orlistat in spring water (controls in spring water, n=16).

Exp. 3 tested for effects on growth and productivity: Daphnia aged ~5 days were randomly assigned to individual glassware in 50.0 ml natural spring water at 20°C. The experimental treatment (n= 8) was 2.128 x 10^{-3} mg/ml Orlistat in spring water (controls in spring water, n=6).

Exp. 4 tested for a dose-dependent effect. Daphnia aged 8-12 days were randomly assigned to 50.0 ml natural spring water at 20°C to test for dose dependence. The high and low doses of Orlistat were 6.95 x 10^{-3} mg/ml and 2.38 x 10^{-3} mg/ml, respectively, and controls were spring water. Twelve Daphnia were placed in individual glassware for each treatment.

Survival was measured as the number of days the Daphnia lived in treatments. The growth rate was inferred from the intervals in days (d.) between body molts. Productivity was inferred based on days when Daphnia were observed to give birth to neonates.

Morphology and lipid/ovary indices: Moribund *D. magna* were immediately preserved in 95% ethanol upon discovery during daily checks. Body size was measured as the length (± 0.01 mm) from the head to the end of the body, excluding the apical spine using a calibrated digital microscope. Lipid and ovary indices were assigned by visual inspection, using Tessier and Goulden's (1982) 0-3 scale and a compound microscope with 40x magnification. According to this scale, a lipid index of 0 indicates starvation and poor health, while 3 represents a healthy individual with multiple lipid droplets. An ovary index of 0 suggests stress and inability to allocate lipids to ovary development, while 3 indicates ample resources and large opaque ovaries.

We used Independent-Samples Median and Kruskal-Wallis Tests to assess if lipid and ovary indices differed across treatments, because the data were not normally distributed. We tested for a relationship between lipid index and ovary index using a linear regression. Statistical calculations were performed with IBM SPSS 29.0.2.0. Welch's test was used when equality of variances could not be assumed. This test calculates degrees of freedom with decimals.

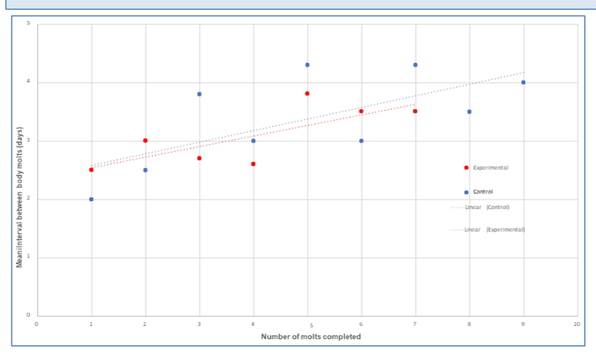

Figure 1. Average interval in days between exoskeleton body molts 1-9, Experiment 3.

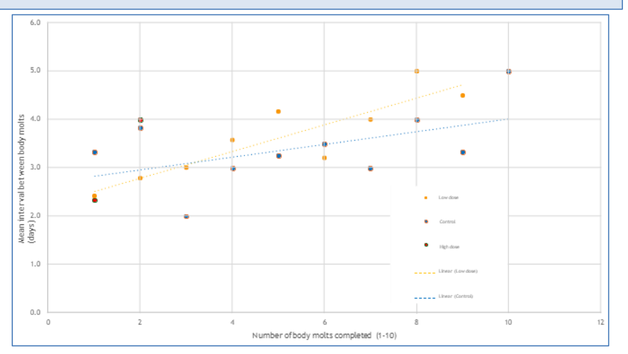

Figure 2. Average interval in days between exoskeleton body molts 1-10, Experiment 4.

RESULTS & DISCUSSION

Survival did not differ across the five levels of orlistat, One-Way ANOVA, F (5,29) =1.37, p=0.26. This informed our decisions about the concentrations we used in subsequent experiments.

In Exp. 1, *D. magna* neonates in the orlistat treatment survived fewer days (x=4 d., SD=2) than controls (x=8.6 d., SD=4.7), according to Welch's t-test, t(20.2) = -3.9, p<0.001. (Effect size as estimated by Cohen's d = -1.422).

In Exp. 2, *D. magna* neonates in orlistat survived fewer days (x=1.6 d., SD=0.7) compared to the control (x=4.6 d., SD=3), according to Welch's t-test, t(16.9)= -3.8, p=0.001 (Cohen's d = -1.34).

Growth rates were compared based on exoskeleton molts. In Exp. 3, Daphnia in the orlistat treatment completed fewer molts over the same time, suggesting there could be a cumulative effect of orlistat (Fig. 1).

Differences in rates of molt appeared to occur in Exp. 4 based on divergent trend lines (Fig. 2). Daphnia in the high orlistat dose completed very few molts. Daphnia in the low orlistat dose appeared to complete molts at a slower rate than controls, and the divergent trend lines suggest a cumulative effect.

Dose dependence was observed in Exp. 4. Survival differed between treatments (one-way ANOVA, F(2,33)=5.40, p=0.009). Daphnia in the higher dose survived fewer days (x= -13 d., S.E.=3.6) than Daphnia in the low dose (p=0.01, Dunnett's T-3, post hoc test).

Productivity was lower for orlistat treatments. In Exp. 3, fewer neonate births were recorded for the orlistat treatment. By contrast, the cumulative neonate births were higher in controls despite there being fewer females in this treatment (Fig. 4). In Exp. 4, very few neonates were born to females in the high dose treatment. Also, as the experiment progressed, the females in the low dose treatment gave birth to fewer neonates (Fig. 4). By contrast, as the experiment progressed, more neonates were born in the control treatment. Daphnia in the orlistat treatment retained their eggs for a prolonged period. The eggs turned white and opaque and failed to develop (Fig. 5).

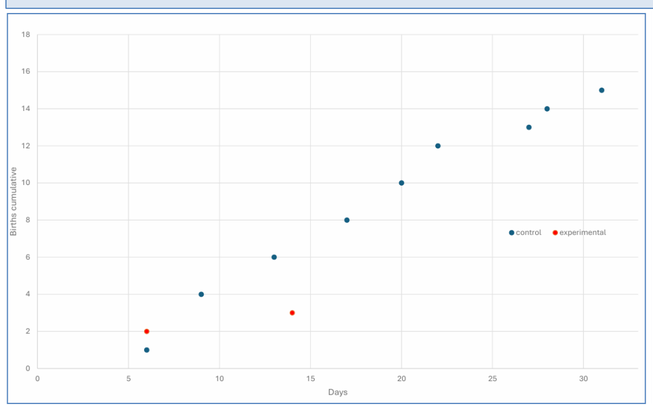

Figure 3. Cumulative neonate births in controls compared to experimental treatments, Exp. 3.

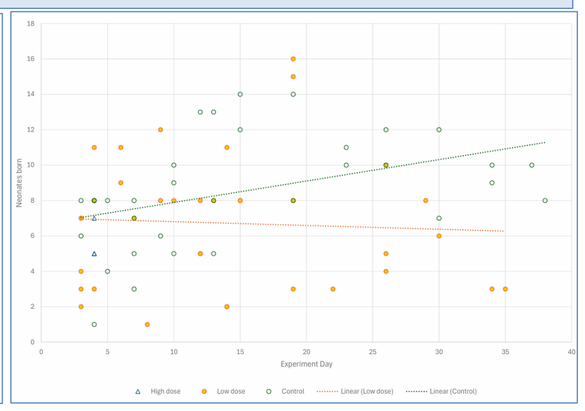

Figure 4. Numbers of neonate born to individual females by experimental day and by treatment, Exp. 4.

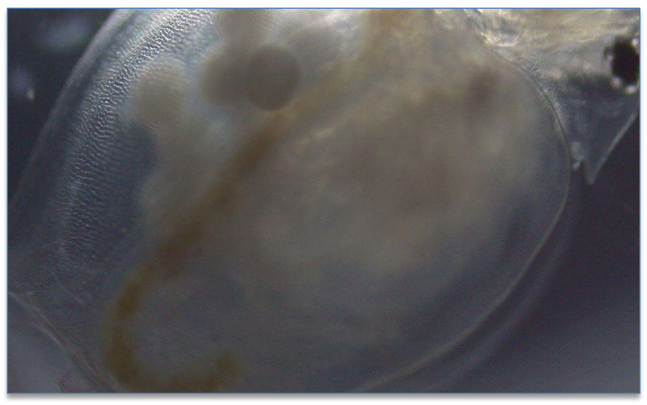

Figure 5. Eggs became opaque and white and were retained in the brood chamber for a prolonged period without additional development (Exp. 3, orlistat treatment).

The median values for the lipid index differed across treatments (X^2 = 7.8, df = 2, p = 0.02). The post-hoc tests showed that the medians of the high treatment differed from the control (p = 0.02). No significant differences were found between treatments in the ovary index.
The regression analysis showed the lipid index predicted the ovary index with a strong positive relationship (Y = -0.51 + 0.788x, R^2 = 0.62, p < 0.001).
Assimilation of lipids would have been critical to reproduction, and in the orlistat treatment the resource allocation was limited, particularly at higher concentrations.

CONCLUSION

Orlistat can have effects on *D. magna* that may reduce reproductive success, growth and survival. Although orlistat is hydrophobic, it may accumulate at ecological interfaces such as the surface microlayer, littoral zones, and sediments.

FUTURE WORK / REFERENCES

Tessier, J. A. and Goulden, C.E. 1982. Estimating food limitation in cladoceran populations. Limnology and Oceanography 27(4): 707-717.

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