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# Effect of Diluted Bitumen on the Survival, Physiology, and Behaviour of the Zebra Finch (*Taeniopygia guttata*) and Relevance to Birds of the Salish Sea



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## Introduction

- Diluted bitumen is a mixture of 20-30% natural gas condensate and 70-80% bitumen; bitumen is mixed with condensate to decrease viscosity for transport by pipeline
- There is very little published on diluted bitumen (dilbit) toxicity to fauna in the literature
- The potential for expanded shipment of dilbit out of the Port of Vancouver means increased risk of impact on marine resources including birds in the Salish Sea

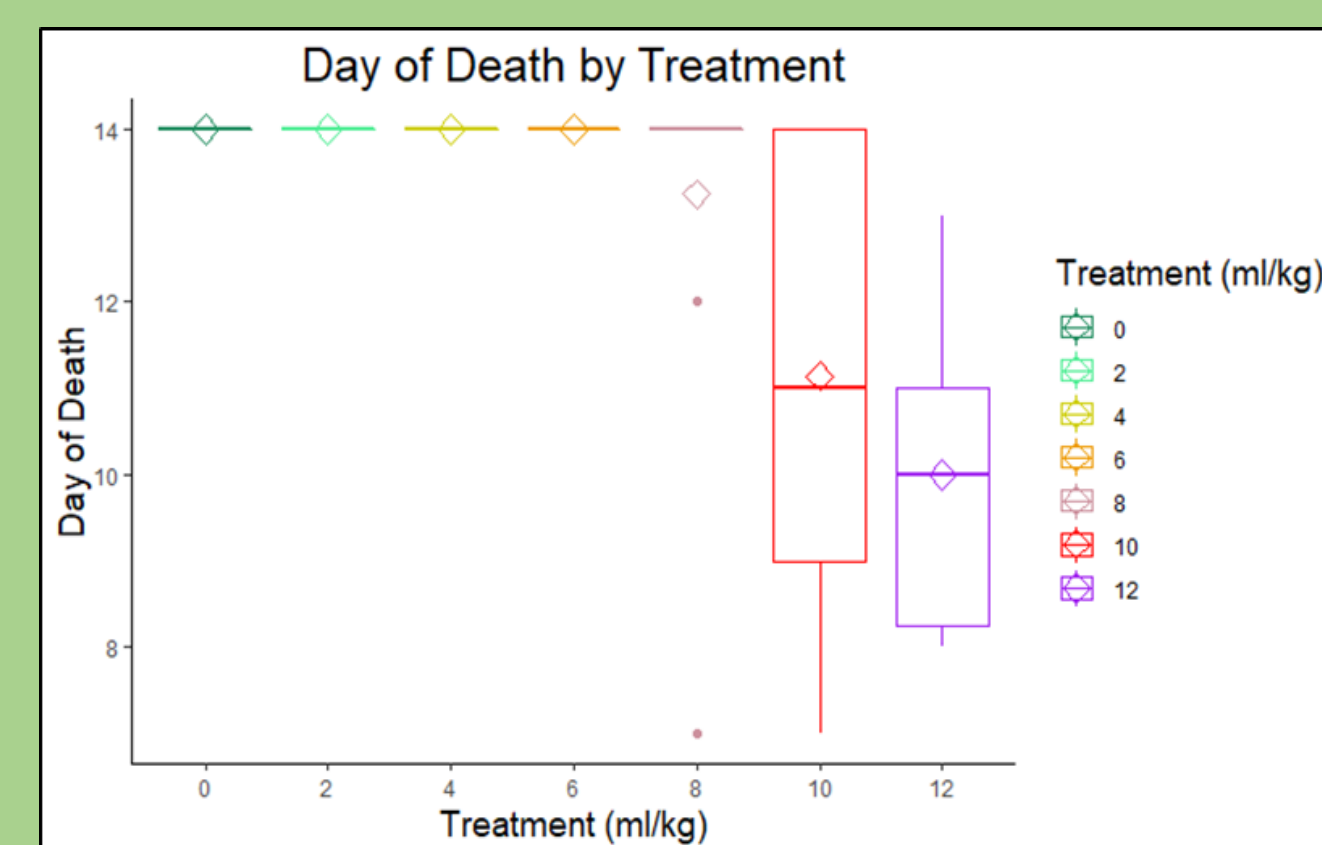
## Objectives

- Establish methods for evaluating toxicity of dilbit to adult birds
- Establish a range of doses at which sublethal effects occur, as well as LD<sub>50</sub> values
- Determine physiological and behavioural endpoints for use in future studies

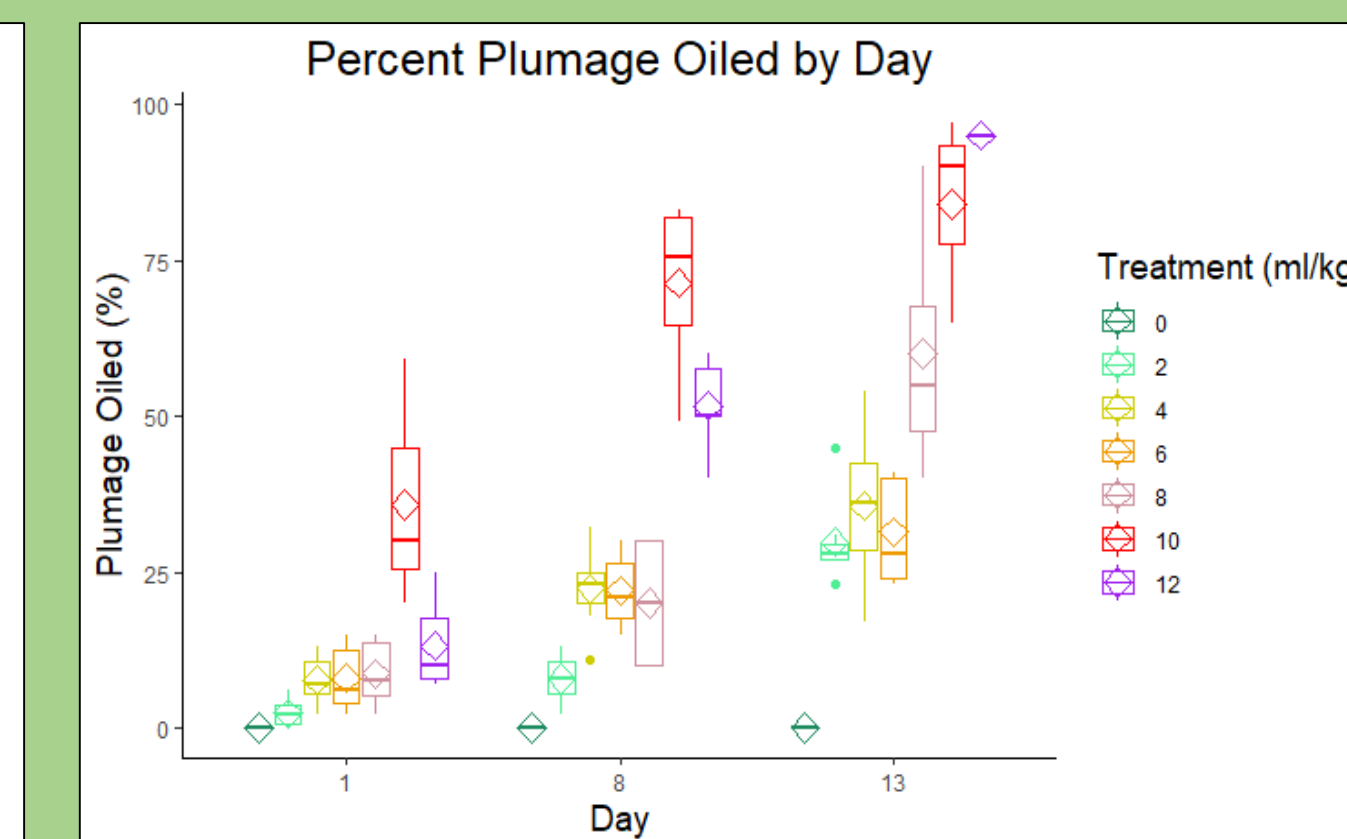
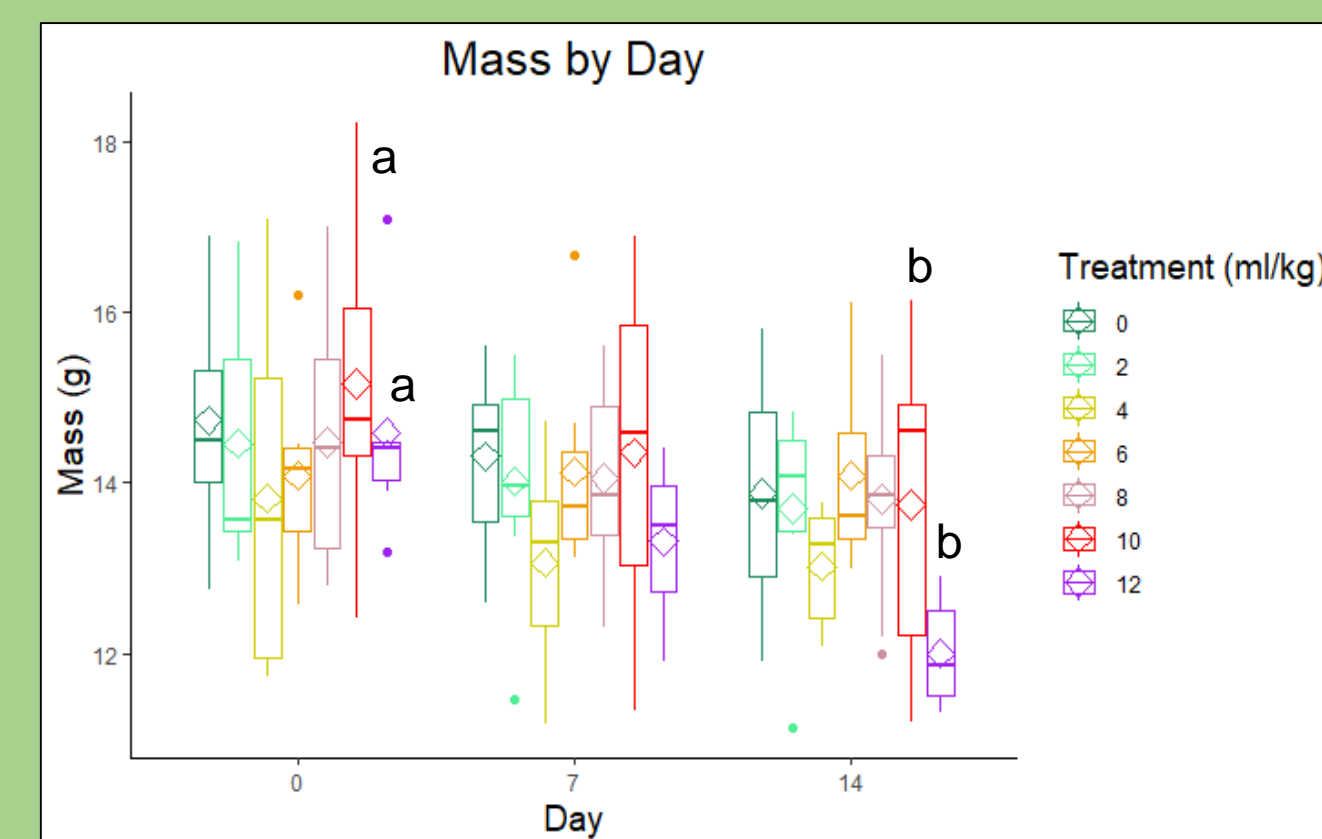
## Materials and Methods

- Given the absence of data on dilbit toxicity to birds we used information from conventional crude oiling studies to determine our dosing levels
- Adult, male zebra finches (*Taeniopygia guttata*) were used as our model laboratory species
- We decided on doses of 0, 2, 4, 6, 8, 10, and 12 ml dilbit per kilogram bodyweight per day over a 14 day exposure
- Dilbit was mixed with boiled egg yolk and safflower oil to form a 'slurry' prior to administration of dosage. Birds were dosed via oral gavage into the crop twice a day for 14 days
- Pre-treatment (day -7), day 7 and day 14 blood samples (~100 µl) were obtained from the brachial vein of each bird

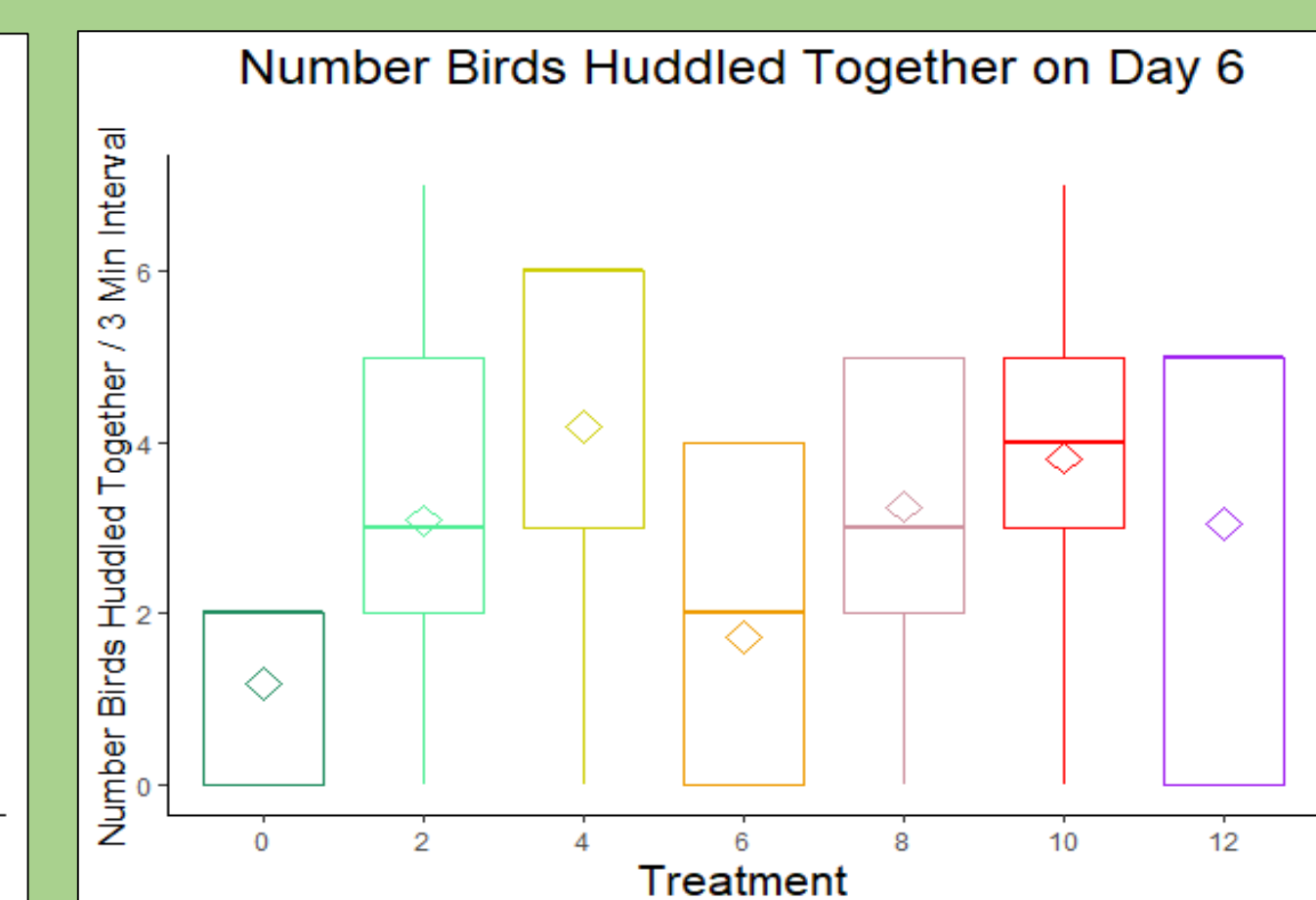
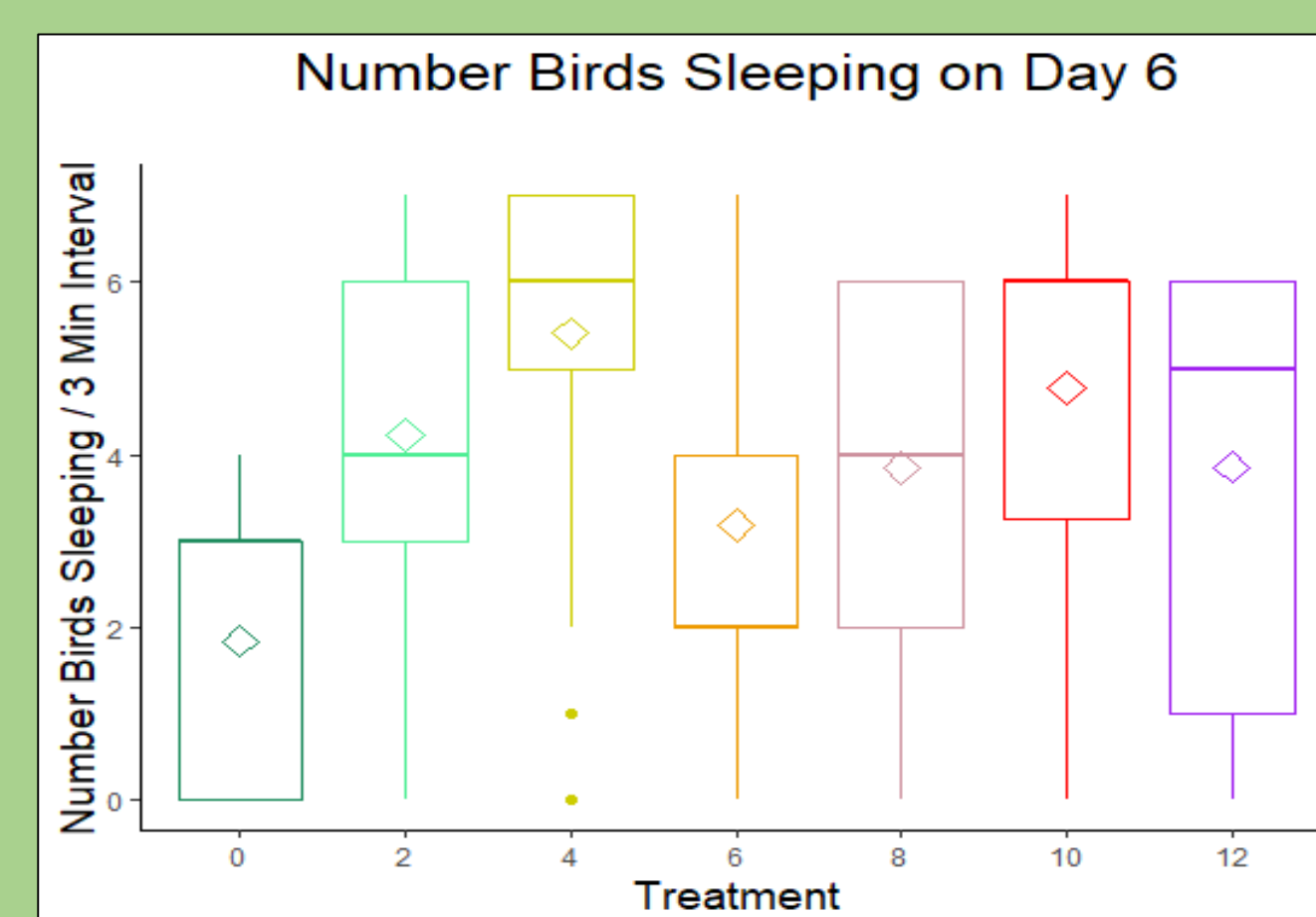
## Results



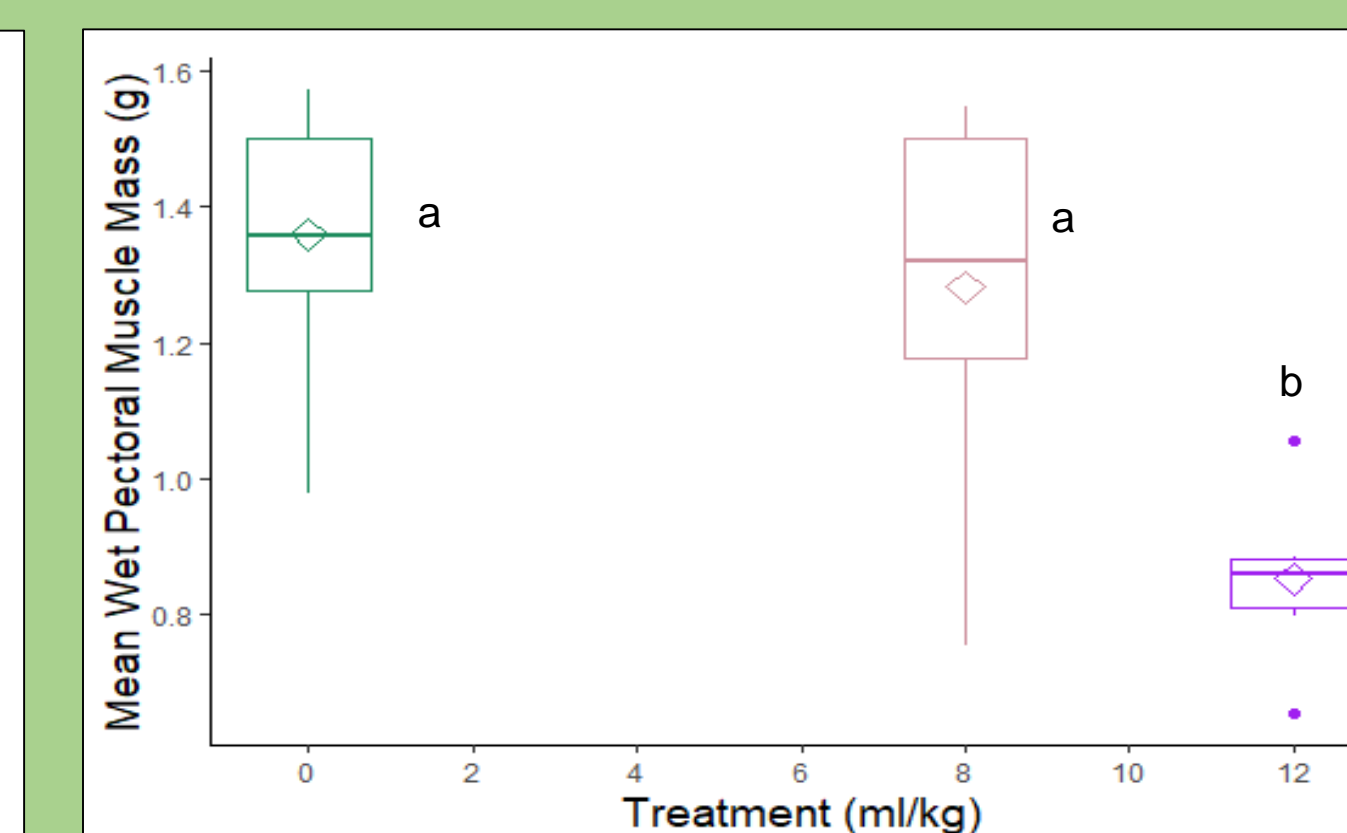
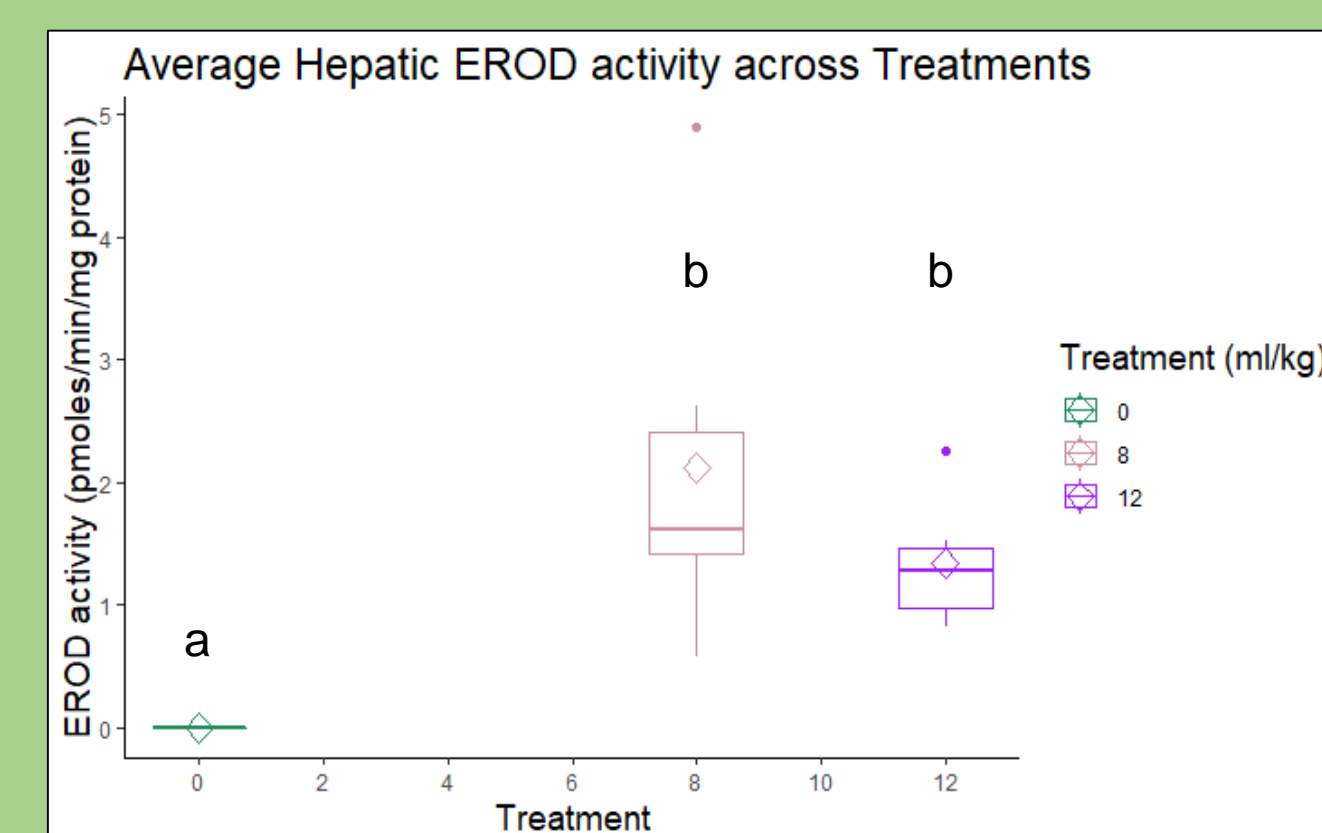
**Figure 1 (left)** Survival to day 14 differed significantly. While all birds were euthanized on day 14 (end of exposure), mortality occurred in the 8, 10, and 12 ml/kg groups starting as early as day 7. Before termination, 100% mortality had occurred in the 12 ml/kg group, 50% in the 10 ml/kg, and 8% in the 8 ml/kg group. Diamonds indicate the mean, mid lines in boxplots indicate the median.



**Figure 2a (left)** By day 14 or day of death mean body mass was significantly lower in both the 10 ml/kg ( $P=0.021$ ) and 12 ml/kg treatments ( $P<0.001$ ) as compared to day 0. **Figure 2b (right)** Incidental external oiling occurred after oral gavage in each exposure group. Percent external coverage of oil was highest in the 10 and 12 ml/kg groups throughout study timepoints; by day 13 average external oiling in these groups was > 80%



By day 6 of the exposure treated birds were more lethargic; more treated birds were exhibiting sleep-like behaviour ( $P<0.001$ ) (**Figure 3a left**) and huddling together ( $p<0.001$ ) (**Figure 3b right**) than control birds.



**Figure 4a (left)** By day 14 or day of death, EROD activity, an indicator of hepatic CYP1A activity rate was elevated in the 8 and 12 ml/kg groups ( $p=0.002$ ), and wet pectoral muscle mass had decreased in the 12 ml/kg group as compared to 8 ml/kg ( $p=0.038$ ) and controls ( $p=0.016$ ) **Figure 4b (right)**.

## Summary

- 100% mortality occurred at the 12 ml/kg dosage
- Using the Spearman Karber method, the lethal dose that resulted in 50% mortality (LD<sub>50</sub>) was 9.67 ml/kg (95% confidence interval: 8.82 - 10.60 ml/kg)
- Mortality was associated with >80% incidental external oiling by day 13 of the exposure, mass loss, and decreased wet pectoral mass
- Sublethal effects included increased lethargic behaviour in treated birds by day 6 of the exposure, and elevated EROD activity by day 14 or day of death.

## Discussion

- A similar exposure (Goodchild et al., unpublished) dosed zebra finches with light conventional crude oil at 10 ml/kg via oral gavage daily for a similar length of time, but reported no mortality. However, we found 50% mortality after exposure to dilbit at the same dosage

## Conclusions

- Oral ingestion of dilbit in adult male zebra finches causes mass loss, lethargy, decreases in (wet) pectoral muscle mass, changes in behaviour such as lethargy, and increased EROD activity. Incidental external oiling of plumage may have contributed to the lethargy
- The LD<sub>50</sub> for oral ingestion of dilbit in the adult male zebra finch is 9.67 ml/kg bodyweight/day
- Dilbit may be more toxic to zebra finches than conventional light crude oil, at least via oral ingestion
- If birds in the Salish Sea are exposed to dilbit via oral ingestion, they may experience the sublethal effects we observed in our laboratory model species such as lethargy. Lethargy may reduce time spent feeding and breeding in the wild, or increase probability of capture by predators, adversely affecting overall fitness.

## Acknowledgements



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Climate Change Canada

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