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Toxicity testing of Atlantic salmon aquaculture chemotherapeutants on spot prawns and benthic invertebrates

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Introduction

Demand for protein sources is high in North America and rising globally. Aquaculture is one approach to meeting this demand. Intensive salmon farming practices result in periodic infestations with the naturally-occurring parasitic copepod *Lepeophtheirus salmonis*, commonly referred to as “sea lice”. To reduce productivity losses, chemical and physical treatments have been used to clear infestations.

To determine the level of risk aquaculture chemotherapeutic treatments pose to non-target marine annelids and crustaceans including spot prawns (*Pandalus platyceros*), king ragworms (*Alitta virens*), and amphipods (*Eohaustorius estuarius*). The sub-chronic toxicity of the sea lice pesticides Slice® (active ingredient: 0.2% emamectin benzoate, sediment half-life: 404-d, mode of action: chloride ion channel hyperpolarization)[1,2] was tested.

Objective

To identify lethal and sub-lethal effects of the chemotherapeutant treatment Slice® (active ingredient: 0.2% emamectin benzoate) at environmentally relevant concentrations to non-target native marine annelids and crustaceans using spot prawns (*P. platyceros*), king ragworms (*A. virens*), and amphipods (*E. estuarius*).

Methods

Screened marine sediment was spiked with Slice® to achieve nominal concentrations representative of environmentally-relevant levels. Test organisms were exposed to spiked-sediments in aerated, water quality-monitored static systems for sub-chronic durations of 30-days (*E. estuarius* and *A. virens*) or 60-days (*P. platyceros*) using protocols adapted from Environment Canada[3], ASTM[4], and Park, 2013. Concentration-response was assessed using the endpoints of: mortality and behavioural responses.

Mortality and growth was measured for all organisms. Daily burrowing/emergence and post-exposure re-burrowing rate were measured for *A. virens*. Molting incidence and post-exposure olfactory response were measured for *P. platyceros*.

Results

1-3: *A. virens* sub-lethal tests

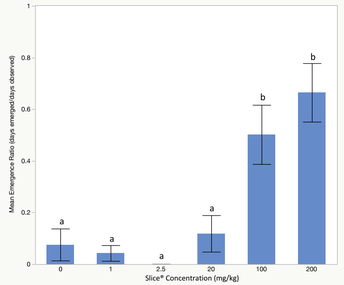

Fig 1. Mean daytime sediment emergence rate of *A. virens* during Slice® (AI: 0.2% emamectin benzoate) spiked sediment 30-d exposure. Nominal concentrations of 0, 1, 2.5, 20, 100, and 200 mg/kg Slice® with n=60. Error bars indicate 1SE. Letters indicate evidence of a difference of mean emergence rate by Tukey-Kramer HSD pairwise comparison (P<0.05).

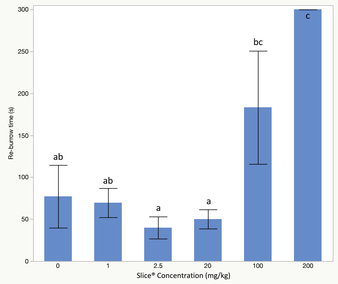

Fig 2. Mean time to re-burrow in clean sediment of *A. virens* post 30-d Slice® (AI: 0.2% emamectin benzoate) spiked sediment 30-d exposure. Nominal concentrations of 0, 1, 2.5, 20, 100, and 200 mg/kg Slice® with n=32. Error bars indicate 1SE. Letters indicate evidence of a difference of mean re-burrow time (s) by Tukey-Kramer HSD pairwise comparison (P<0.05).

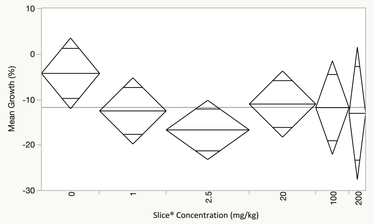

Fig 3. Mean growth (% change wet weight) of *A. virens* in response to Slice® (AI: 0.2% emamectin benzoate) spiked sediment 30-d exposure. Nominal concentrations of 0, 1, 2.5, 20, 100, and 200 mg/kg Slice® with n=39. Diamonds represent SE 95% CI. No evidence of a difference of mean growth was detected by way of CRD ANOVA, Tukey-Kramer HSD pairwise comparison with effect threshold P=0.05.

4: *A. virens* lethal tests

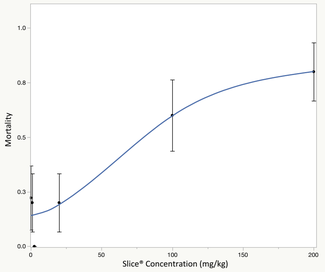

Fig 4. Mortality of *A. virens* in Slice® (AI: 0.2% emamectin benzoate) spiked sediment 30-d exposure. Nominal concentrations of 0, 1, 2.5, 20, 100, and 200 mg/kg Slice® with n=59. LC_{50} predicted as 101 mg/kg or 202µgEB/kg (95% CI = 65-165 mg/kg). Error bars indicate 1SE. Evidence of a concentration effect was found using binomial probit regression with P<0.0001 (SE 95% CI = 0.0053 – 0.015).

5: *E. estuarius* lethal tests

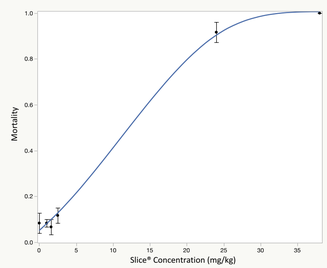

Fig 5. Mortality of *E. estuarius* in Slice® (AI: 0.2% emamectin benzoate) spiked sediment 30-d exposure. Nominal concentrations of 0, 1, 1.6, 2.5, 24, and 38 mg/kg Slice® with n=18. LC_{50} predicted as 12.5 mg/kg or 25 µgEB/kg (95% CI = 10.7-14.8 mg/kg). Error bars indicate 2SE. Concentration effect by probit with fit P<0.0001 (SE 95% CI = 0.10 – 0.144).

6: *P. platyceros* sub-lethal tests

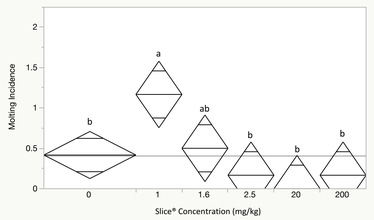

Fig 6. Molting incidence of *P. platyceros* in Slice® (AI: 0.2% emamectin benzoate) spiked sediment 60-d exposure. Nominal concentrations of 0, 1, 1.6, 2.5, 20, and 200 mg/kg Slice® with n=42. Diamonds represent SE 95% CI. Letters indicate evidence of a difference of mean emergence rate by Tukey-Kramer HSD pairwise comparison (P<0.05).

Conclusions

- At high concentrations, Slice® was acutely lethal to polychaete *A. virens* and resulted in sub-lethal effects to daily emergence and post-exposure re-burrowing rate.
- Slice® caused mortality of *E. estuarius* at concentrations recorded near actively-treated aquaculture pens.
- Further study should be conducted on sub-lethal effects to benthic marine invertebrates including synergistic toxic effects of Slice® and ivermectin. Ivermectin is another avermectin under investigation for use in British Columbia aquaculture facilities.

Future Research

Table 1. Outline of completed, ongoing, and future chemotherapeutant sediment bioassays

	E. estuarius	*A. virens*	*P. platyceros*
Slice®	• Sub-chronic spiked sediment • 30-d duration • 1, 1.6, 2.5, 24, and 38 mg/kg	• Sub-chronic spiked sediment • 30-d duration • 1, 2.5, 20, 100, and 200 mg/kg	• Sub-chronic spiked sediment • 60-d duration • 1, 1.6, 2.5, 20, and 200 mg/kg
Ivermectin	• Sub-chronic spiked sediment • 30-d duration • 3, 5, 8, 12, and 18 µg/kg	• Sub-chronic spiked sediment • 30-d duration • 3, 5, 8, 12, and 18 µg/kg	• Sub-chronic spiked sediment • 60-d duration • 3, 5, 8, TBD, and TBD µg/kg
Slice® + ivermectin	• Sub-chronic spiked sediment • 30-d duration • Additive concentration	• Sub-chronic spiked sediment • 30-d duration • Additive concentration	• Sub-chronic spiked sediment • 60-d duration • Additive concentration

Green shaded = focus of this poster
Blue shaded = other completed/ongoing bioassays

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References

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5. Park, A. 2013. The biological effects of emamectin benzoate (SLICE®) on spot prawn (*Pandalus platyceros*).

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