The biological effects of the anti-sea lice pesticide Salmosan® on the Pacific spot prawn, *Pandalus platyceros*

Mill, K[1], Kennedy, C[1]

[1] Department of Biological Sciences, Simon Fraser University, Burnaby, BC, Canada, V5A 1S6

Introduction

Sea lice are ecto-parasitic crustaceans and are the most damaging parasite in salmonid aquaculture in Europe and North America [1,2]. The formulations designed to be effective against these parasitic crustaceans are subsequently released into surrounding waters and there is significant concern regarding their potential impact on proximal non-target crustaceans [2,3,4]. Salmosan® (active ingredient: azamethiphos) is an organophosphate chemotherapeutant currently approved for use by Health Canada [5]. Azamethiphos acts by inhibiting acetylcholinesterase, leading to the over-stimulation of the nervous system, paralysis, and eventually death [2,5]. Salmosan® is applied as a bath treatment at a target concentration of 100-150 μg/L, and may be applied in multiple pulses to treat multiple net pens in one area [2,5].

Prior research has shown that concentrations suggested by label protocols can have both lethal and sub-lethal impacts on non-target aquatic crustaceans including lobsters and mysid shrimp [2,3,4]. Molting stage and temperature have also been shown to affect the sensitivity of lobsters to Salmosan® [6,7]. To expand on these results, I will investigate the effects of realistic exposures in combination with a mixed stressor scenario (variable temperatures) and sensitive life stage (post-molt) on the Pacific spot prawn, *Pandalus platyceros*. This economically important crustacean is indigenous to the B.C coast and its habitat overlaps with net-pen areas, potentially putting it at risk.

The objectives of this study are: 1) to determine the effect of multiple pulse exposures of Salmosan® under varying temperature regimes on olfactory, feeding, and locomotory behavior; 2) to determine the effect of repeated exposures of Salmosan® on molting success in inter-molt prawns; and, 3) to determine the effect of repeated exposures on post-molt prawns.

This project is currently in progress and this presentation is a summary of data gathered to date. Methodology for future tests will be introduced and the implications of these results will be discussed.

Materials and methods

Behavioural effects. Prawns were placed individually in 9L glass aquaria contained within temperature controlled water baths at 11°C. Prawns were acclimated for 10 min before video recordings began. After 2 min, Salmosan® (Fish Vet Group Ltd, Inverness, Scotland) was introduced into the tanks and the prawns exposed for 1 h. Following exposures, prawns were transferred to uncontaminated water. Feeding tests were conducted at t=24, 48, 72 and 96 h. After a 10 min acclimation period, recordings were started for baseline behaviour and after another 2 min 3 mL of a sardine slurry food stimulant (Brunswick®, Vancouver, Canada) was introduced to the rank. Recording was terminated after another 5 min. Videos were analyzed for a series of olfactory, feeding and locomotory behaviours. This will be repeated under multiple stressor (T=5, 11, 17°C) and multiple pulse (3 1-h pulses in 12 h) conditions.

Inter-molt exposures. Prawns were individually exposed for 1 h to 0, 10, or 100 μg/L of azamethiphos (as Salmosan®) in a 9L glass aquarium 3 times at t=0, 4, 24 h (n=80). Between and after exposures prawns were held semi-communally in clean sea water, with 2 prawns per tank separated by a plexiglass divider. Prawns are currently being monitored daily for molting or mortality. Mortalities are inspected for molting stage. Molted prawns are monitored for 96 h for survival.

Post-molt exposures. Prawns are currently being held in semi-communal tanks (2 prawns per tank) at 5, 11 and 17°C (n=84) and are being monitored daily for molting. Once molted, prawns are exposed to 0, 10, 50, or 100 µg/L of azamethiphos (as Salmosan®) 3 times within 24 h (t=0, 4, 24 h). After exposure, the prawns are monitored for 96 h for survival.

Results and discussion

Behavioural effects: olfaction. Acetylcholine is a signalling molecule in the crustacean olfactory system, therefore there is the potential for organophosphates including azamethiphos to interrupt olfactory pathways (8,9)**.** A common metric of olfactory system function in prawns is antennule flicking, used to dislodge particles which allows new odorants to access the sensory neurons located on the antennules (8,9). Preliminary behavioural tests have indicated that no significant difference in antennular flicking rate occurs 96 h following a 1-h exposure to 200 and 500 µg/L (p>0.05) (Figure 1). These results suggest that this exposure scenario at concentrations 2 to 5-fold higher than environmentally-relevant do not result in detrimental effects on the olfactory system of the Pacific spot prawn.

Behavioural effects: feeding. There were no significant differences in behaviour before and after the food stimulant was added in any treatment group including the controls, suggesting that the stimulant itself was not sufficiently attractive to the prawns (Figure 2). Therefore, conclusions cannot be drawn from these feeding tests and alternative food stimulants are currently being compared for use in future tests.

Behavioural effects: locomotion. The prawns exposed to 200 µg/L spent significantly more time moving during the 1-h exposure as compared to the controls (p>0.008) (Figure 3). While the 500 µg/L treatment group was not significantly different than the control group, the prawns in this treatment group ranged widely in response. This may suggest that at lower concentrations the prawns experience a level of agitation or avoidance, while at higher concentrations some individuals may experience physiologically toxic effects of the chemical.

To further elucidate the risks of environmentally-relevant exposures to non-target crustaceans, these behavioural tests will be quantified under multiple pulse (3 1-h exposures in a 12-h period) under multiple stressor scenarios (temperature: 6, 11 and 16°C).

Inter-molt exposures. Preliminary results are summarized in Table 1. A spike in molting occurred in the 11°C 10 µg/L group, suggesting that this treatment may be sufficiently stressful to induce molting while the 100 µg/L treatment may express inhibitory effects (Figure 4). Most molts were successful, with mortality following molting occurring in 20% of the 17°C 10 µg/L treatment group and 17% of the 17°C 100 µg/L treatment group. Data analysis is in progress and this presentation will summarize the final results including a temporal analysis of molting frequency as well as considerations for carapace length and mass.

Post-molt exposures. Preliminary tests suggest that multiple pulse exposures of Salmosan® that are non-lethal to inter-molt prawns are lethal to post-molt prawns. Experiments are currently in progress and this presentation will summarize updated results including considerations for carapace length and mass.

Conclusion. These preliminary results suggest that inter-molt prawns do not experience significant sub-lethal effects (olfactory, feeding, locomotory) after 1-h exposures to environmentally-relevant concentrations of Salmosan®, however they may experience effects under mixed stressor (temperature) and multiple pulse (t=0, 4, 24 h) scenarios. These exposure scenarios may also result in mortality when molting in a small portion of the population. Further, these scenarios may cause higher rates of mortality in prawns exposed within 24 h of molting. Understanding the toxicity of pollutants released from aquaculture sites is essential to developing evidence-based regulations that prioritize environmental health while meeting the needs of the industry.

Acknowledgements
This work was funded by the Department of Fisheries and Oceans Canada National Contaminants Advisory Group. All experiments followed Canadian Council for Animal Care Guidelines.

References

1. Costello MJ (2006) *Trends in Parasitology*, **22** 475-483.
2. Burridge LE and Van Geest JL (2014) *Canadian Science Advisory Secretariat Research Documents,* 2014/002.
3. Burridge LE, Hamilton N, Waddy SL, Haya K, Mercer SM, Greenhalgh R, Tauber R, Radecki SV, Crouch LS, Wislocki PG and Endris RG (2004) *Aquaculture Research,* **35** 713-722.
4. Abgrall P, Rangely RW, Burridge LE and Lawton P (2000) *Aquaculture,* **181** 1-10.
5. Health Canada: Pest Management Regulatory Agency (2016) *PMRA Public Registry*, accessed 26 July 2017.
6. Burridge LE, Haya K and Waddy SL (2005) *Ecotoxicology and Environmental Safety,* **60** 277-281.
7. Burridge LE, Haya K and Waddy SL (2008) *Ecotoxicology and Environmental Safety,* **69** 411-415.
8. Blinova NK and Cherkashin SA (2011) *Journal of Evolutionary Biochemistry and Physiology*, **48** 155-165.
9. Barker DL, Herbert, E, Hildebrand JG and Kravitz EA (1972) *Journal of Physiology,* **226** 205-229.

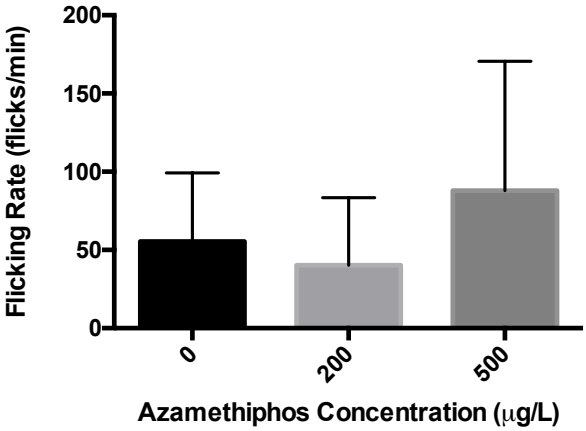

Figure 1. Flicking rate in the Pacific spot prawn, *Pandalus platyceros*, 96 h after a 1-h exposure to Salmosan® (active ingredient: azamethiphos) at 11°C. Neither treatment group is significantly different than the control.

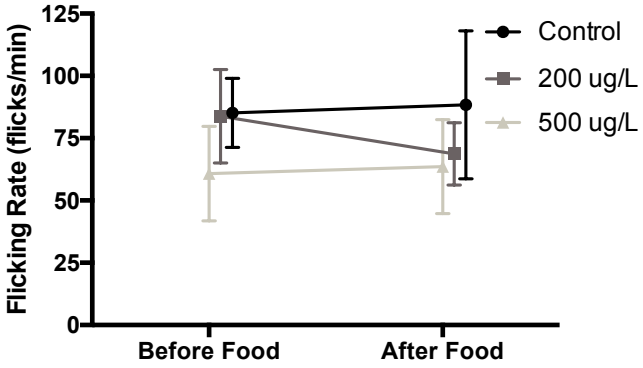

Figure 2. Response to a sardine slurry food stimulant by the Pacific spot prawn, *Pandalus platyceros*, 96 h after a 1-h exposure 11°C to Salmosan® (active ingredient: azamethiphos) at 11°C. No flicking rates were significantly different after introduction of the food stimulant.

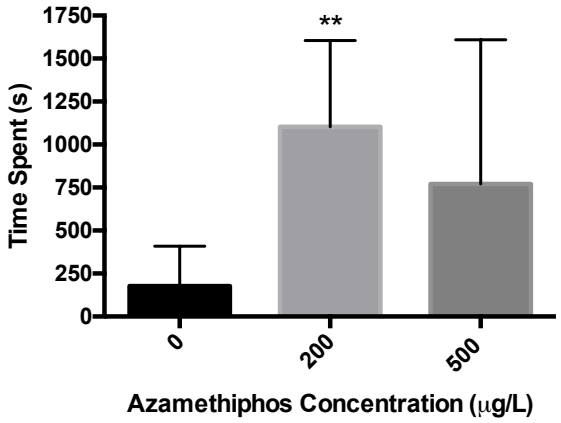

Fig. 3. The average time the Pacific Spot Prawn, *Pandalus platyceros,* spent in locomotion (walking or swimming) during a 1-h exposure to Salmosan® (active ingredient: azamethiphos) at 11°C. **Indicates significant difference from control.

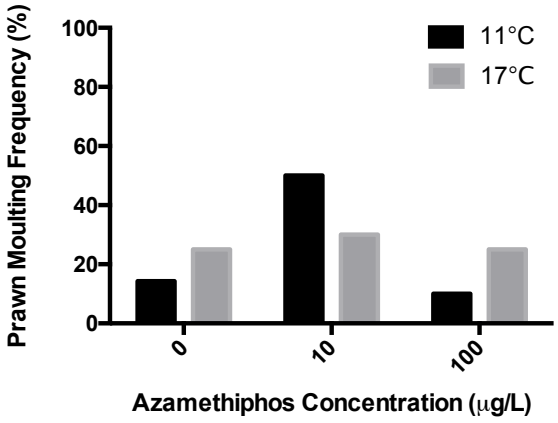

Fig. 4. Molting frequency of the Pacific spot prawn, *Pandalus platyceros*, within a 3-week observation period following a multiple pulse exposure (1-h exposures at t=0, 4, 24 h) to Salmosan® (active ingredient: azamethiphos) at 11°C and 17°C (n=70).

Table 1. Frequency of successful molts, mortalities and mortalities-upon-molting in the Pacific spot prawn, *Pandalus platyceros*, in a 3-week observation period following exposure to Salmosan® (active ingredient: azamethiphos) at varying temperatures.

	Azamethiphos Concentration	Molting (%)	Mean Time to Molt (days ± SE)	Mortality (%)	Mortality upon Molting (%)	n
11°C	0 μg/L	14.2	17.5 ± 0.5	0	0	14
	10 μg/L	50.0	17.4 ± 2.1	0	0	12
	100 μg/L	10.0	9.0 ± 3.0	0	0	10
17°C	0 μg/L	25.0	9.3 ±2.6	0	0	12
	10 μg/L	20.0	13.0 ± 2.8	20.0	20.0	10
	100 μg/L	25.0	14.2 ± 2.7	0	16.7	12