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Dispersion and toxicity to non-target crustaceans of azamethiphos and deltamethrin after sea lice treatments on farmed salmon, *Salmo salar*

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ABSTRACT

Since 2009 infestations of sea lice, *Lepeophtheirus salmonis* and *Caligus elongatus* on farmed salmon *Salmo salar*, in New Brunswick, Canada have been controlled by pesticides applied in bath treatments. Given the potential for effects on non-target organisms, a study was conducted to determine the dispersion from bath treatments of Salmosan® (active ingredient azamethiphos) and AlphaMax® (active ingredient deltamethrin) solutions, either in enclosed net pens or in well boats. The toxicity to *Eohaustorius estuarius*, *Crangon septemspinosa*, and *Mysis stenolepsis* of water samples taken in the dispersing plume was also assessed. A dye, sodium fluorescein, was added to the treatment solutions and a fluorometer was used to track the plume at various times after release of the pesticide solution after treatment. A strong correlation between dye and pesticide concentrations demonstrated the utility of real time dye measurements for following dispersing plumes. In water samples, azamethiphos was measured in greater concentrations in the aqueous phase than in the particulate phase. Deltamethrin, however, was in greater concentrations in the particle phase. The residues of azamethiphos measured after net pen treatments were approximately 3 times than those measured after the well boat treatment. Although 100% of exposed *E. estuarius* were affected (mortality and paralysis combined) in short term exposures (1 h) to ambient water samples taken in the net pen during treatments with Salmosan®, there was no substantive effect after the treatment solution was released, even when samples were taken directly adjacent to the net pen. Longer exposures (48 h), however, produced toxicity in samples taken up to 850 m from the net pens. Compared with Salmosan® treatments, the plume from AlphaMax® net pen treatments was more toxic with samples producing an EC50 (mortality plus paralysis) to *E. estuarius* in short term (1 h) exposures up to 350 m from the edge of the net pen.

1. Introduction

The farming of Atlantic salmon, *Salmo salar*, is an important contributor to the economy of the maritime provinces of Eastern Canada and currently is the highest value crop in the New Brunswick agri-food sector (Canadian Aquaculture Industry Alliance, 2013). The economic returns from salmon production can be substantially reduced by infestations of sea lice, *Lepeophtheirus salmonis* and *Caligus elongatus*. Various operational control methods have been employed in Canada to manage sea lice infestations including; drugs added to feed (ivermectin, emamectin and teflubenzuron), as well as by bath treatments with pesticides (azamethiphos, hydrogen peroxide, dichlorvos and pyrethrins) (Burridge et al., 2011). Until recently, in-feed treatments with emamectin benzoate had provided acceptable control however reduced efficacy by that drug resulted in a need for treatment alternatives (McGladdery, 2011).

Azamethiphos is an organophosphate pesticide which has been used operationally in Canada and throughout the world for sea lice control (Burridge et al., 2011). Deltamethrin has been used operationally for sea lice control in other parts of the world (Haya et al., 2005), and although not currently authorized for use in Canada, was proposed for use at the time of this study. Both pesticides are known to be toxic to a wide range of non-target marine organisms (Burridge et al., 2011; Ernst et al., 2001), moreover crustaceans such as American lobster (*Homarus americanus*), a species which is also locally important commercially (Burridge et al., 2008), are most sensitive. In eastern Canada the highly lucrative lobster fishery takes place in the same bays and inlets where salmon aquaculture is located. This has historically led to questions and concerns regarding the potential for anti-louse treatments to affect lobsters. Deltamethrin is a pyrethroid pesticide, and this class of pesticide has been shown to be highly toxic to lobster and sand shrimp (Burridge et al., 2000; Clark et al., 1989; Hill, 1985;

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McLeese et al., 1980). Although the environmental hazards of the pyrethroid pesticides have been identified using lab-based bioassays, there have been few field studies documenting their environmental impacts. At least one study (Ernst et al., 2001) suggests that environmental effects from bath pyrethroid use could be extensive.

The objective of this study was to determine the potential for environmental effects on non-target crustaceans of waste treatment solutions of azamethiphos and deltamethrin after their discharge to the marine environment and to quantify the distances from the treatment site over which such effects could be expected. We herein describe the toxicity to several species of marine invertebrates and residue concentrations of azamethiphos and deltamethrin present in dispersing plumes of pesticide solutions used operationally to treat sea lice on farmed salmon.

2. Materials and methods

2.1. Study sites

Sites were selected in the Lower Bay of Fundy, near St. Andrews New Brunswick, Canada, where operational treatments for sea lice control on marine farmed salmon were taking place (Fig. 1). A total of seven treatment events were sampled: three net pen treatments with Salmosan® (50% azamethiphos, Fish Vet Inc, Portland, Maine, USA); three well boat treatments with Salmosan® and one net pen treatment with AlphaMax® (1% deltamethrin, Pharmaq, Oslo, Norway). The sites, in our experience, represent the range of environmental conditions, particularly depth and current speed, typical of commercial operations in the region. Treatments occurred during September and October 2010. Specifics of treatment conditions are contained in Table 1.

The net pens were at operating farms and contained fish which required treatment because of unacceptable lice infestation. The pens were Polar Circle cages that were 100 m in circumference. At the time of treatment, nets were drawn up to an approximate depth of 4 m and enclosed by an impervious tarpaulin. The pesticide was mixed in a separate container and pumped into the tarped net pen via two perforated pipes which transected the pen just below the surface. The quantities of pesticide added were intended to produce a treatment concentration of 100 μg/L for azamethiphos and 2 μg/L for deltamethrin. A photoreactive dye, sodium fluorescein (Key Acid Uranine Concentrate, Keystone Aniline Corp., Chicago. Ill. I.D. 801-073-52, CAS 518-47-8), was added to the pesticide solution prior to its introduction to the net pen in sufficient quantity to provide an approximate 1:6 pesticide: dye ratio. Dye was added to aid in identifying the location of the pesticide plume after its release for sampling purposes, a technique which has been shown to be effective in the past (Dobson and Tack, 1991; Ernst et al., 2001). Air bubbling into the enclosure, as well as the swimming movement of fish, were possible contributors to dispersion of the treatment solution in the enclosure. Treatments were generally timed for slack tide and release of treatment solutions, by removal of the tarpaulins, occurred as the tide began to ebb, which was approximately 45 min after the introduction of the treatment solution.

For the well boat treatments, procedures were similar in that pesticide and dye were pre-mixed and added to the well which contained fish; however circulation was greater because of the use of in-line circulation pumps. After approximately 45 min of treatment, the water in the wells was gradually exchanged with clean seawater and discharged via a port directing the stream perpendicular to the bow/stern axis.

2.2. Sample collection

At several times during the exposure period within the tarped net pen or in the well boats, water samples were obtained at various depths by pumping directly into 4 L amber bottles which had been certified clean by the supplier (IChem, Fisher Scientific, Toronto, Ontario). Samples were taken using the same methods at various times, depths and distances after the release of the pesticide/dye treatment solution at locations where the plume was either visible due to the dye or where fluorometric readings indicated there was dye present. Sampling continued until dye could not longer be measured, which was approximately 2–3 h after release. The locations of all sample collections were

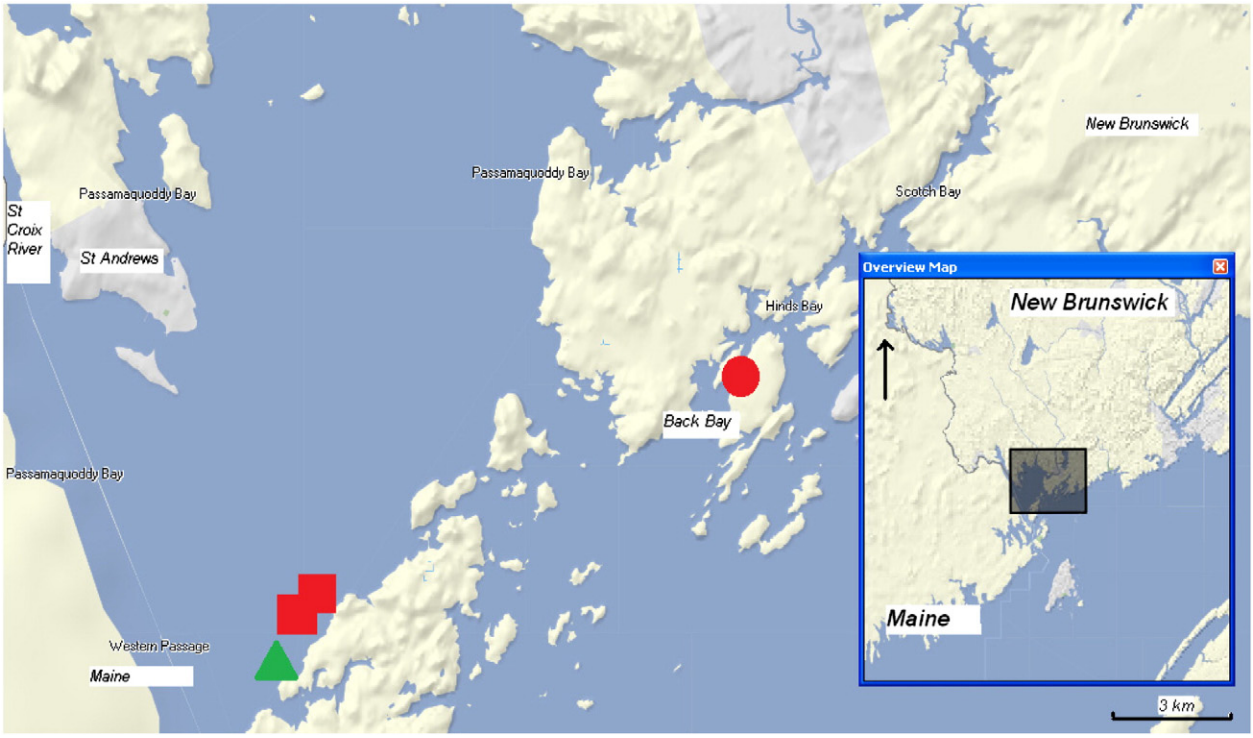

Fig. 1. Study sites in Lower Bay of Fundy where dispersing sea lice treatment plumes were tracked. Squares represent the net pen treatments with Salmosan®, triangle represents the well boat treatments with Salmosan® and circle represents the net pen treatment with AlphaMax®.