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Sensitivity of salmon lice (*Lepeophtheirus salmonis*) in New Brunswick, Canada, to the organophosphate Salmosan® (w/w 50% azamethiphos) using bioassays

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ABSTRACT

Bioassays have been used as a monitoring tool to determine changes in sensitivity of sea lice populations to various bath treatments during the Atlantic salmon production cycle. In this study we report on the results of bioassays conducted between 2009 and 2012 for *L. salmonis* with the objective of detecting changes in sea lice sensitivity to Salmosan® (w/w 50% azamethiphos), a delousing agent used in the Bay of Fundy region of New Brunswick, Canada. EC_{50} values ranged from 4.6 ppb to 402 ppb. Although sea lice stage was not a significant factor influencing observed EC_{50} values, there were significant differences among years, with 2009 being significantly lower than all other years, and 2011 being significantly higher than 2010 or 2012. Season was also found to be a significant predictor with EC_{50} values in the winter/spring being lower than those predicted in the summer/fall. While sea lice resistance to Salmosan® (w/w 50% azamethiphos) has not been reported from Eastern Canada, variable EC_{50} values indicate unmeasured influences on tolerance to Salmosan® (w/w 50% azamethiphos) in the populations of *L. salmonis* sampled from the Bay of Fundy during the 2009 to 2012 period. The possibility of more recent changes in sensitivity remains unknown due to the lack of a centralized repository of bioassay data or other measures that might reflect the emergence of resistant sea lice.

Statement of relevance

Control of sea lice infestation of Atlantic salmon.

1. Introduction

Resistance of pest or nuisance species to pesticides is an increasing problem in many high-yielding and high quality animal and plant production systems (Pimentel, 2005). Indeed, economically sustainable Atlantic salmon (*Salmo salar*) aquaculture often requires the use of chemotherapeutants to mitigate and prevent disease occurrence aquaculture production systems (Roth et al., 1993; Haya et al., 2005). A prime example of this is the treatment of the ectoparasitic crustacean parasite, the salmon louse, *Lepeophtheirus salmonis*. These are the most economically limiting parasites for Atlantic salmon aquaculture industries due to the requirement for ongoing biological or chemical control and management interventions. *L. salmonis* is the primary concern in North America and Europe whereas *C. rogercressyi* is the most significant ectoparasite in Chile. Atlantic salmon, the largest agri-food export industry in Eastern Canada, is produced in the Bay of Fundy region of New Brunswick and can surpass 35,000 tonnes annually with a farm gate value of up to $280 million (ACFFA, 2013). *L. salmonis* has proven challenging to control in Atlantic salmon marine aquaculture for a variety of reasons (Lees et al., 2008; Torrison et al., 2013; Whyte et al., 2014; National Capital Region Fisheries and Oceans Canada, 2014) including the development of resistance to effective approved treatments (Jones et al., 1992; Sevatdal and Horsberg, 2003; Sevatdal et al., 2005; Lees et al., 2008; Bravo et al., 2008; Whyte et al., 2013).

Currently, the organophosphate, Salmosan® (50% w/w azamethiphos, Fish Vet Group Ltd) is available for use in the Bay of Fundy region of New Brunswick, Canada. In 1995, a time-limited registration was permitted for the use of azamethiphos ((*S*)-[(6-chloro-2-,3 dihydro-2-oxo-1,3-oxazolo {4,5-b} pyridine-3-ylmethyl)]*O*, *O*-dimethyl phosphotothioate) in Atlantic salmon sea cage sites in New Brunswick. During the 1990s, an efficacy >95% in mobile stages and >65% in chalimus was reported (O'Halloran and Hogans, 1996). Its use was, however, sporadic after 2000 due to the introduction and subsequent predominant use of SLICE® (0.2% emamectin benzoate) (Westcott et al., 2004). In 2009, azamethiphos was again available to the industry under Emergency Registration through the Pest Management Regulatory Agency of Health Canada, although the number of cages that could be

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Table 1
Number of cage treatments reported for Atlantic salmon sea cage sites in the Bay of Fundy, New Brunswick, Canada between 2009 and 2012.

Year	AlphaMax (Deltamethrin)	Salmosan® (50% w/w Azamethiphos)	*Interox®* Paramove® 50 (49.5% Hydrogen Peroxide)
2009	104	29	3
2010	14	705	291
2011	0	12	337
2012	0	49	333

treated was limited to approximately two per day, depending on size of the farm site (ACFFA, 2013). As a consequence, on-farm sea lice could not be entirely removed and additional treatments with other compounds were required. Thus, the product was used sparingly in 2011 and again in 2012 (ACFFA, 2013). However, azamethiphos has since been included in New Brunswick's pest management program and its use increased in 2013 and 2014. Despite its re-emergent use as a bath treatment, ongoing bioassay assessments were not re-initiated due to funding constraints and thus comments about current EC_{50} levels are unavailable.

Sea lice resistance towards organophosphate compounds has previously been documented in Norway, Scotland and Ireland (Jones et al., 1992; Roth et al., 1996; Tully and McFadden, 2000; Fallang et al., 2004), with reports of several clinical treatment failures and reduced sensitivity specifically to azamethiphos in Norway (Fallang et al., 2004; Kaur et al., 2015b, 2016). While there are no published data using bioassays to detect reduced sensitivity to azamethiphos in New Brunswick to date, reports from the field assessments of treatment lice levels have indicated variable treatment responses which heighten the concern that resistance mechanisms may be present in the population (Whyte et al., 2016). Bioassays were used as a monitoring tool within New Brunswick's integrated pest management program (Fisheries and Oceans Canada, 2011) to determine changes in the sensitivity of the sea lice population in the Bay of Fundy to treatments throughout the Atlantic salmon production cycle (Westcott et al., 2008; Whyte et al., 2013, 2014). This study reports on bioassays conducted in the laboratory between 2009 and 2012 with L. *salmonis* collected from Atlantic salmon farms in the Bay of Fundy, New Brunswick, with the objective of detecting changes in sea lice sensitivity to azamethiphos during that period.

2. Materials and methods

L. salmonis were collected from fish originating at Atlantic salmon marine cage sites located in the Bay of Fundy, New Brunswick, during routine sea lice counting on sites which had received treatments with Salmosan® (50% w/w azamethiphos). Pre-market sized fish were anaesthetized using TMS (tricaine methanesulfonate, Syndel) at a dose of approximately 100 mg L^{-1} and sea lice gently removed from the fish using forceps. The sea lice were placed into sealed containers of seawater collected from the sea cage site. Collection containers were transported back to the laboratory in coolers containing ice packs to ensure sea lice were kept cool during transport. In addition, battery operated air pumps were added to collection containers for aeration during transport. Sea lice were held overnight at 10–12 °C in a temperature-controlled incubator to facilitate acclimation prior to bioassay set-up the following morning (Westcott et al., 2008; Whyte et al., 2014).

All bioassays were performed at the Atlantic Veterinary College, University of Prince Edward Island, in Charlottetown, PE, using a standardized protocol (Westcott et al., 2008). The same technical personnel carried out all trials. Bioassays were initiated within 24 h of collection (sea lice appeared to become more robust if stored at 10–12 °C with air pumps for approximately 12 h to allow them to recover from handling and transport) (Westcott et al., 2008). A stock solution of azamethiphos (Salmosan®) was prepared for each bioassay by dissolving 5 mg of Salmosan® (50% w/w azamethiphos) in 15 mL ethanol. Six milliliters of this stock solution was added to 1994 mL of sea water to create a working solution which was then used to prepare experimental solutions with varying concentrations of Salmosan® (50% w/w azamethiphos) (3 ppb, 10 ppb, 30 ppb, 100 ppb, 300 ppb). Control dishes (seawater only) were included in each trial. In all cases, the experimental solutions used sea water taken from the same site from which the sea lice were collected. All experimental solutions were maintained in an incubator at 10–12 °C.

Ten apparently healthy sea lice, of the same stage and sex, were categorized according to the following categories: adult female (gravid and non-gravid) (AF), pre-adult and adult male (PAM-AM) and pre-adult female (PAF) (Whyte et al., 2014). Sea lice were sorted into plastic Petri dishes, in triplicate where possible, and subsequently exposed to the treatment and control solutions for a total of 60 min. The sides of the bottom half of each plastic Petri dish were perforated with small holes covered in mesh to allow water movement into and out of the dish during the exposure period. The Petri dishes were submerged in the solutions of Salmosan® (50% w/w azamethiphos) dilutions for two 30 min periods. After the first 30 min post-exposure, the dishes were drained and re-submerged for the remaining 30 min exposure period in an effort to ensure proper mixing of the treatments; the water temperature was recorded at this time after the first and second thirty minute exposure periods. At the end of the second 30 min exposure period, the Petri dishes were drained and placed in a “rinse” bucket containing clean, control seawater. All dishes containing sea lice were rinsed before being placed into a container of clean seawater aerated with an electric air pump and subsequently incubated in a temperature-controlled chamber at 10–12 °C for an additional 24 h. Following the 24 h incubation period, the condition (live, weak, moribund or dead) of each sea louse was evaluated according to an adopted set of bioassay response criteria with minor modifications (Westcott et al., 2008; Igboeli et al., 2012; Saksida et al., 2013); all dishes were blind-coded to reduce assessor bias with respect to Salmosan® (50% w/w azamethiphos concentration. To reduce a non-specific poor survival influence, bioassays for which control mortality for a sea lice stage and sex category exceeded 20% were excluded from subsequent statistical analysis.

The data from the bioassays were analyzed using a probit regression model incorporating a natural response rate using the software GraphPad (Graphpad Software Inc., La Jolla, CA). The effective concentration (EC_{50}) values and corresponding 95% confidence interval (Rosenheim and Hoy, 1989), that led to a response of 50% of the sea lice not prone to a natural response (moribund + dead) was used to determine sensitivity. Data from bioassay evaluations that resulted in an inability to estimate confidence limits were not included in the analysis, as they indicated a poor fit to the probit regression model. Further analysis of bioassay data was performed using Stata version 13 (Stata Corp., College Station, TX). A GLM model was fit with EC_{50} value as the outcome and included the predictor variables: stage of sea lice (i.e. AF or PAM-AM), year and season. Season, in half year periods, was defined as “winter-spring” for sea lice collected from January to June and “summer-fall” for samples collected during July to December.

Sea lice counts of 5–10 fish per cage and 6 cages per site were recorded weekly by industry counters. Records included classification using three life stage categories (as described previously by Whyte et al., 2013) of chalimus (Chal), pre-adult (male and female) and adult male (PAAM), adult female (AF). In addition, these lice stages were counted prior to and after bath treatments. The treatment related counts used were limited to the closest count prior to a treatment (with a maximum of 5 days previous) and the lowest count over the last 5 days. Counting of cages would usually occur on the same day but as treatment days differed slightly cages were often measured at different days post-treatment depending on the day of treatment. All lice and treatment records were managed by the web-based Fish-iTrends© software, an evidence-based-epidemiological database platform used to