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.

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; Torrissen 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; Sevetdal & Horsberg, 2003; Sevedtal 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 greater than 95% in mobile stages and more than 65% in chalimus was reported (O'Halloran & 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 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 & 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.

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 24h of collection (sea

lice appeared to become more robust if stored at 10-12°C with air pumps for approximately 12 hours 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 minutes. 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 minutes periods. After the first 30 minutes post-exposure, the dishes were drained and re-submerged for the remaining 30 minute 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 minute 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 hours. Following the 24 hour 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 & 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 monitor fish health and sea lice pest management programs in Atlantic Canada, operated by the Atlantic Veterinary College, UPEI. The proportion of lice (based on each life stage) remaining on the fish after bath treatment was calculated based on the (mean post-treatment count) / (mean pre-treatment count). For example, 1 PAAM after treatment mean count following a 10 PAAM pre-treatment count on the same group of fish results in 0.1 *Relative Change (RC)* related to that particular treatment. All

treatment events with both pre-treatment and post-treatment records in the data management system for a calendar week were then used to calculate a median RC and these were graphically presented over time.

Results

A total of 795 cage treatments occurred between 2009 and the end of 2012 (Table 1), with most treatments occurring during 2010, between June and September (Figure 1). The median RC is presented by week of the year in Figure 2; A and B for AF and PAAM sea lice, respectively. An RC value close to 1 (or above), indicates there are as many sea lice remaining on the fish after a treatment as there were prior to the treatment. An RC value of ≥ 1.0 was observed in the summer months between weeks 30-35 for AF sea lice and peaked above 1.0 at approximately week 30 for PAAM.

The opportunistic way in which sea lice were collected from Atlantic salmon marine cages resulted in substantial variability in the number of bioassays conducted at different times during the observed period. A total of 91 bioassays were conducted between 2009 and 2012; approximately 75% of which were carried out in either 2010 or 2011. For each bioassay conducted, the proportion of moribund and dead sea lice in the control group for the stage under study was reported (Figure 3). Based on all bioassays (N=91), the overall mean mortality [95% CI] in the control groups was 19.3% [14.4, 24.3]. A multiple regression model indicated that sea lice stage was not a significant factor ($p = 0.90$) but that both year and season were highly significant ($p < 0.01$). When 2011 was used as the reference year, the model indicated that all other years had significantly lower control mortalities levels, and that bioassays conducted in the winter/spring have lower control mortalities ($p < 0.01$) than those that were conducted in the summer/autumn.

In all cases where the proportion of control mortalities was 20% or higher (N=27) it was considered that any EC_{50} estimate derived from the bioassay may be of questionable value, and so no such estimation was carried out. (Indeed for just over 50% of these 27 bioassays the probit model did not converge.) Of the remaining 64 bioassays, the probit regression model failed to

estimate a credible 95% confidence interval for a further 11, so these were also excluded from the analysis. This resulted in a total of 53 bioassays that were suitable for further analysis (Table 2). As only one of these was associated with the PAF sea lice stage, that bioassay was also excluded from the statistical modelling (only a total of 4 of the completed bioassays were conducted on PAF stage sea lice). The range of EC_{50} values spanned from a low of 4.6 ppb to a high of 402 ppb (Table 2) with a mean [95% CI] value of 97.2 [69.6, 124.7] ppb. In considering a linear model for the EC_{50} values it was found that a square-root transformation of the outcome variable improved model fit and also that three cases were clear outliers (studentised $R > 2$). The fitted GLM indicated that sea lice stage ($p = 0.45$) was not a significant factor influencing observed EC_{50} values. However, there were significant differences among years, with 2009 being significantly lower than all other years ($p < 0.02$), and 2011 being significantly higher than 2010 or 2012 ($p = 0.01$), in addition season was found to be a significant predictor ($p < 0.01$) with EC_{50} values in the winter/spring being lower than was predicted in the summer/fall (Figure 4A and B).

Discussion

Azamethiphos has been reported to be effective in controlling pre-adult and adult stages of sea lice (both *L. salmonis* and *Caligus* sp.) in regions where resistance has not been reported (O'Halloran & Hogans, 1996; Roth et al., 1996; Armstrong et al., 2000; Fish Vet Group, 2011; Valenzuela-Munoz et al., 2015) but is reportedly less efficacious against chalimus stages (Roth et al., 1996). Development of a resistance mechanism to azamethiphos in *L. salmonis* was documented at a biochemical level in adult female sea lice collected from Eastern Canada and Norway over the period 1999-2002 (Denholm et al., 2002; Fallang et al., 2004). A geographical difference was observed in sea lice samples collected from marine Atlantic salmon aquaculture sites in Eastern Canada and Norway; with less resistance reported from Eastern Canadian sites when compared to Norwegian sites (Fallang et al. 2004). The authors contend that this was consistent with the greater and longer historical use of organophosphates in Norway than in Canada.

Increased use of chemotherapeutants can lead to the development of resistance through evolutionary adaptation, whereby random mutations create genetic heterogeneity within the population (Denholm et al., 2002). Genes conferring resistance may remain at a low frequency within the population in the absence of the chemical driver but increased exposure to the chemotherapeutant can result in favourable selection of these genes to the point at which resistance can be observed to have occurred. Resistance to azamethiphos has been shown to be mostly associated with mutations in AChE genes, specifically Phe362Tyr mutation (Kaur et al., 2015b).

The use of azamethiphos lapsed in New Brunswick in 2002 due to the predominant use of the in-feed medication, emamectin benzoate, and was only reinstated under an emergency registration in 2009, as a result of increasing resistance to emamectin benzoate (Burridge et al. 2010; Jones et al., 2013). A comprehensive monitoring and surveillance program was instituted at this time to document any negative impact of azamethiphos on the environment. There was substantial reliance on azamethiphos bath treatments (using tarpaulins to fully-enclose the cage of fish) during 2010 (see Table 1) however, the industry reduced its use in 2011 and 2012 due to the introduction of *INTEROX*® PARAMOVE® 50 hydrogen peroxide, applied using well boats. The Relative Change (RC), or proportion of sea lice (by stage) remaining on the fish following a treatment, indicates azamethiphos was effective at reducing the number of sea lice (both AF and PAAM) post-treatment (Figures 2A and 2B) although there is a period in the summer of 2010 when there appears to be a poor response to azamethiphos treatment. This may be due to many factors, including pre-treatment abundance of PAAM and AF and the interval of days post-treatment (Whyte et al., 2016), less experienced operators (lower pre-treatment counts (when the precision of the estimate for either pre-treatment or post-treatment counts is less reliable) (Elmoselmany et al., 2013), and environmental differences (e.g. water temperature, which is increasing between May and August) (Groner et al., 2014). As the year progresses, the treatment response appears to improve for both AF and PAAM life stages (Figures 2A and 2B).

There is limited information on the reported EC_{50} values for azamethiphos efficacy as a therapeutant for sea lice. It should also be noted that EC_{50} values can be reported in the literature as either azamethiphos or Salmosan®, but in some instance not distinguished at all. This makes it

challenging to compare values between regions and as such for greater clarity the formulation should be included. The EC_{50} values reported in New Brunswick were higher than those reported in other regions where Salmosan® (50% w/w azamethiphos) has been used. For example, in Scotland, Roth et al. (1996) reported EC_{50} values ranging from 0.06 to 0.21 ppb for adult female and pre-adult lice. This is in contrast to higher values reported in Norway by Helgesen & Horsberg (2013), for azamethiphos-Pestanal® where values of 1.0 ppb were recorded from sites where no azamethiphos had been used, to between 10.5 ppb and 13.1 ppb in sites with repeated azamethiphos use. Using the scale reported by Jimenez et al. (2011), EC_{50} values for Salmosan® (azamethiphos) between 10 and 40 ppb suggest a decreased sensitivity, while those greater than 50 ppb suggest the development of resistance. In New Brunswick, values less than 50 ppb were reported in 2009 for both adult female and pre-adult male and adult male lice, however by 2010 and later, the mean EC_{50} values for both stages were often greater than 50 ppb.

In general, the average EC_{50} values for sea lice stages increased over time between 2009 and 2012, although the majority remained below the field target dose suggested for azamethiphos of 100 ppb (200 ppb Salmosan®) and relatively few assessments were completed in 2012. Treatment of marine cages with azamethiphos during this period was observed to be generally effective in the field against mobile stages (PAAM and AF) in all available topical treatment modalities (skirt, tarpaulin, wellboat) (Whyte et al., 2016). While sea lice resistance to azamethiphos has not been reported from Eastern Canada, increasing EC_{50} values indicate possible changes in tolerance to azamethiphos in the population of *L. salmonis* sampled from the Bay of Fundy between 2009 and 2012. A similar observation was reported for use of azamethiphos in Norway where indications of increasing resistance appeared following its initial use between 1994 and 1999 and again from 2008 to 2013 (Grontvedt et al., 2014). It is possible that this observation is linked to an increased expression of the azamethiphos-resistant enzyme acteylcholinesterase reported by Fallang et al. (2004), and more recently by Kaur et al. (2015a; 2015b; 2016). Also, the EC_{50} values obtained in this study exhibited elevated levels in 2011 and 2012 despite minimal use of azamethiphos in the region during that time, suggesting the organophosphate-resistant mechanism was still present in the population. Fallang et al. (2004) noted that the organophosphate-resistant acetylcholinesterase could be present in sea lice

populations three to five years subsequent to any lapse in use of organophosphates. More recently, Glover et al. (2011) and Besnier et al. (2014) have shown that *L. salmonis* has significant potential to disseminate new mutations across regions within a few generations or years, thereby strengthening concerns that resistance to anti-parasiticides can develop and spread rapidly over large areas on an ecological time-scale. Although early bioassays (i.e. 2009) had lower EC_{50} values than later years, the evidence for the emergence of resistance to this treatment is weakened by the fact that bioassays were discontinued and field response measures of potential resistance continue to be inconclusive. However, continued intense monitoring using sea lice bioassays is warranted.

While season and gender of sea lice have been shown to influence the results of bioassays involving sea lice for other chemotherapeutants, e.g. emamectin benzoate (Westcott et al., 2008; Whyte et al., 2013) and deltamethrin (Whyte et al., 2014), no such effects were observed for azamethiphos. However, assessments were insufficiently distributed across the year to enable a full analysis of these effects. An increase in control mortality was observed for the period July to December for those years in which sea lice were collected across both seasons, thus reducing the utility of bioassays throughout the year and greatly increasing the unit cost. Development of more cost effective tests, such as genetic indicators of resistance, would facilitate more detailed descriptions useful for integrated management of sea lice populations.

Acknowledgements

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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

Table 2 Stage and gender-specific EC_{50} estimates (effective concentration leading to a response of 50% of sea lice not prone to natural mortality) used to determine differences in susceptibility to Salmosan® (w/w 50% azamethiphos) of sea lice (*L. salmonis*) collected from marine Atlantic salmon aquaculture sites in the Bay of Fundy, New Brunswick, Canada between 2009 and 2012. (%D+M: percent dead and moribund).

Adult Females (AF)				Pre-adult / Adult Males (PAAM)			
Date	**EC_{50}**	**95% CI**	**%D+M**	**Date**	**EC_{50}**	**95% CI**	**%D+M**
28-Oct-09	15.9	[11.4, 22.2]	6.7	28-Oct-09	12.6	[0.48, 327]	0
28-Oct-09	14.8	[12.3, 17.9]	6.7	2-Nov-09	28.2	[17.3, 45.9]	6.7
2-Nov-09	16.7	[9.87, 28.4]	10	16-Nov-09	13.8	[7.30, 26.1]	10
19-Jan-10	9	[7.73, 10.5]	0	9-Dec-09	6.2	[4.09, 9.25]	0
27-Jan-10	272	[199, 370]	3.3	19-Jan-10	14.3	[11.7, 17.6]	0
3-Feb-10	64.1	[50, 82.1]	0	19-Jan-10	11.7	[9.82, 13.9]	3.3
8-Feb-10	190	[146, 246]	3.3	27-Jan-10	73.4	[23.4, 230]	0
10-Mar-10	12.6	[10.2, 15.5]	0	3-Feb-10	24.1	[20.1, 28.9]	3.3
22-Mar-10	4.56	[3.94, 5.29]	6.7	8-Feb-10	17.5	[12.3, 24.9]	0
7-Jul-10	209	[144, 304]	6.7	10-Mar-10	17.6	[17.2, 18]	0
11-Aug-10	51.4	[23.8, 111]	13.3	17-Mar-10	6.92	[2.51, 19.1]	3.3
8-Sep-10	42.5	[23.1, 77.9]	10	11-Aug-10	301	[155, 586]	13.3
8-Sep-10	179	[101, 316]	0	8-Sep-10	126	[70.8, 223]	13.3
22-Sep-10	32.7	[11, 97.2]	12	8-Sep-10	215	[173, 268]	6.7
29-Sep-10	8.15	[2.51, 26.5]	6.7	15-Sep-10	138	[68.8, 276]	6.7
29-Sep-10	124	[100, 153]	13.3	29-Sep-10	22.7	[12.4, 41.7]	0
22-Feb-11	77.3	[32.4, 184]	10	29-Sep-10	134	[62.4, 287]	3
23-Feb-11	46.1	[27.3, 77.7]	10	5-Oct-10	109	[79.7, 150]	10
29-Mar-11	18.7	[11.9, 29.2]	7	22-Feb-11	93.5	[59.5, 147]	7
6-Apr-11	15.6	[11.7, 20.7]	4	23-Feb-11	297	[192, 459]	3
10-May-11	67.7	[17.7, 259]	10	29-Mar-11	106	[93.7, 120]	0
22-Jun-11	141	[80.5, 248]	10	6-Apr-11	108	[35.2, 331]	17
28-Jun-11	357	[2.23, 2876]	7	28-Jun-11	402	[42.4, 3808]	10
12-Sep-12	40.6	[32.3, 51]	16.7	12-Sep-12	173	[130, 231]	10
17-Sep-12	182	[123, 270]	16.7	17-Sep-12	77	[9.62, 616]	6.7
21-Nov-12	193	[160, 234]	13	21-Nov-12	141	[108. 184]	17
Pre-adult Females (PAF)							
16-Nov-09	25.8	[8.25, 80.6]	10				

Figure 1. Frequency of Salmosan® (w/w 50% azamethiphos) cage bath treatments per week in 2010 in the Bay of Fundy, New Brunswick, Canada

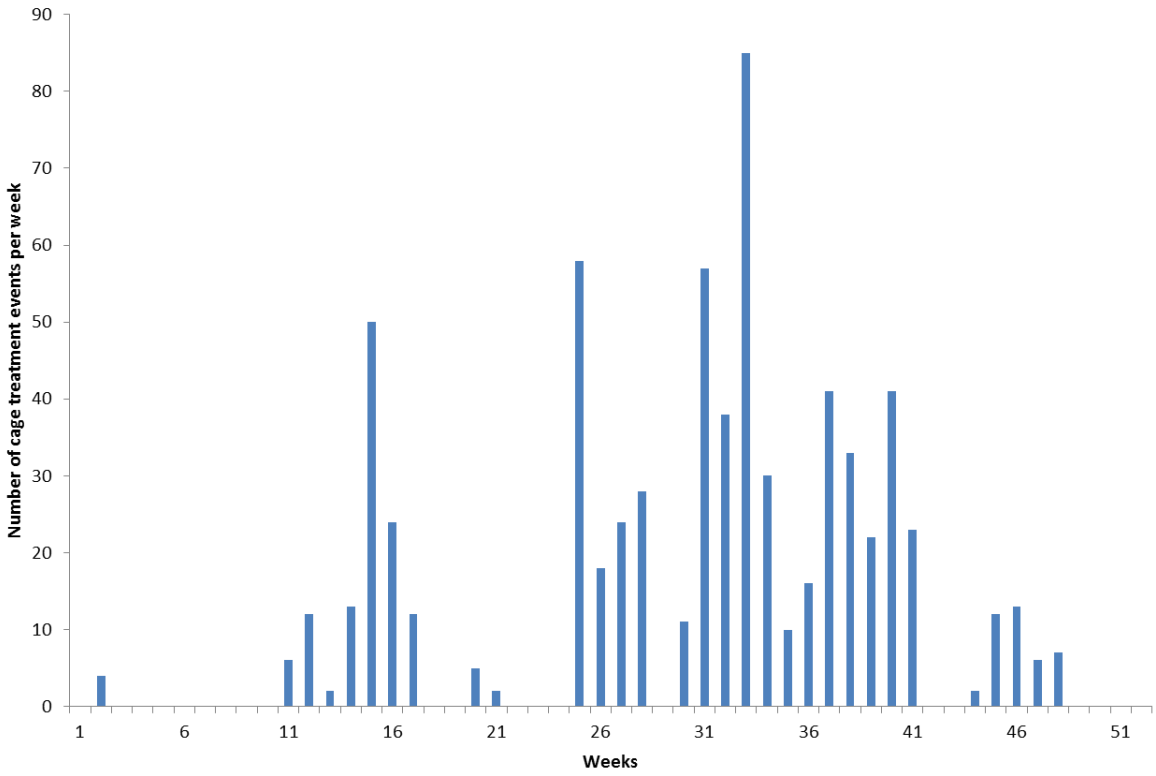


Figure 2A: The weekly median of the proportion of Adult Female (AF) sea lice remaining following Salmosan® (w/w 50% azamethiphos) treatments in 2010, in the Bay of Fundy, New Brunswick. Circle size is dependent on the number of sites treated during that particular week.

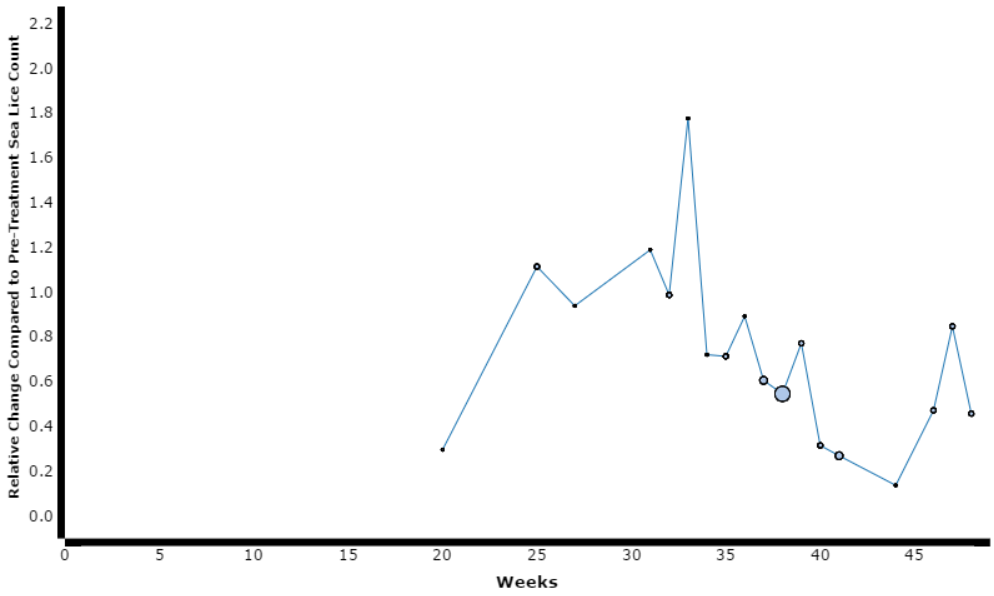


Figure 2B: The weekly median of the proportion of Pre-Adult (male and female) and Adtul Male (PAAM) sea lice remaining following Salmosan® (w/w 50% azamethiphos) treatment in 2010, in the Bay of Fundy, New Brunswick, Canada. Circle size is dependent on the number of sites treated during that particular week.

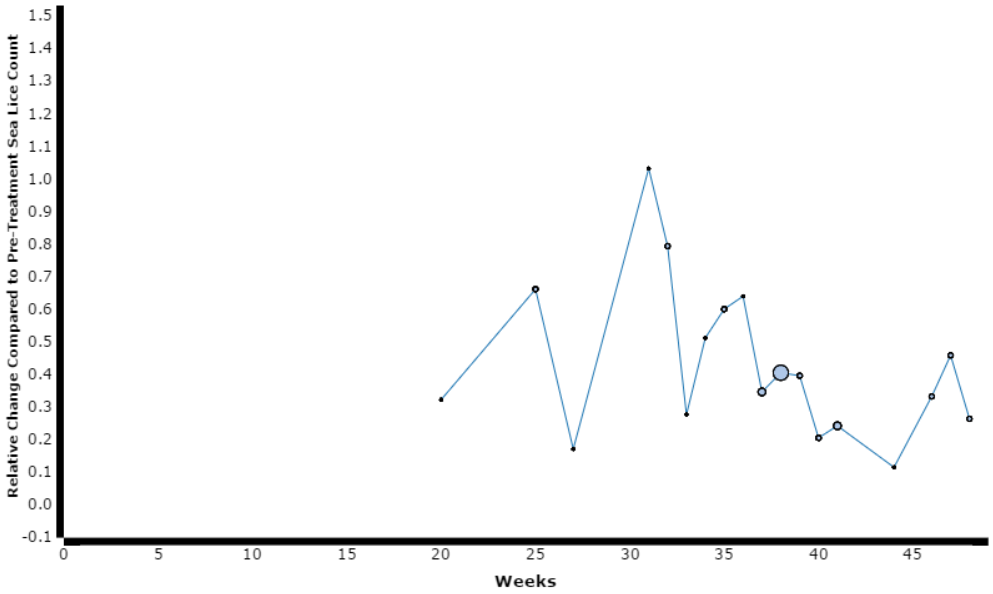


Figure 3 Mortality (moribund and dead) of control sea lice (*L. salmonis*) collected from the Bay of Fundy, New Brunswick, Canada during the period 2009 to 2012 in bioassays testing susceptibility to Salmosan® (w/w 50% azamethiphos). (Dotted line indicates rule of thumb, whereby bioassays with control mortality exceeding 20% were excluded from subsequent statistical analysis.)

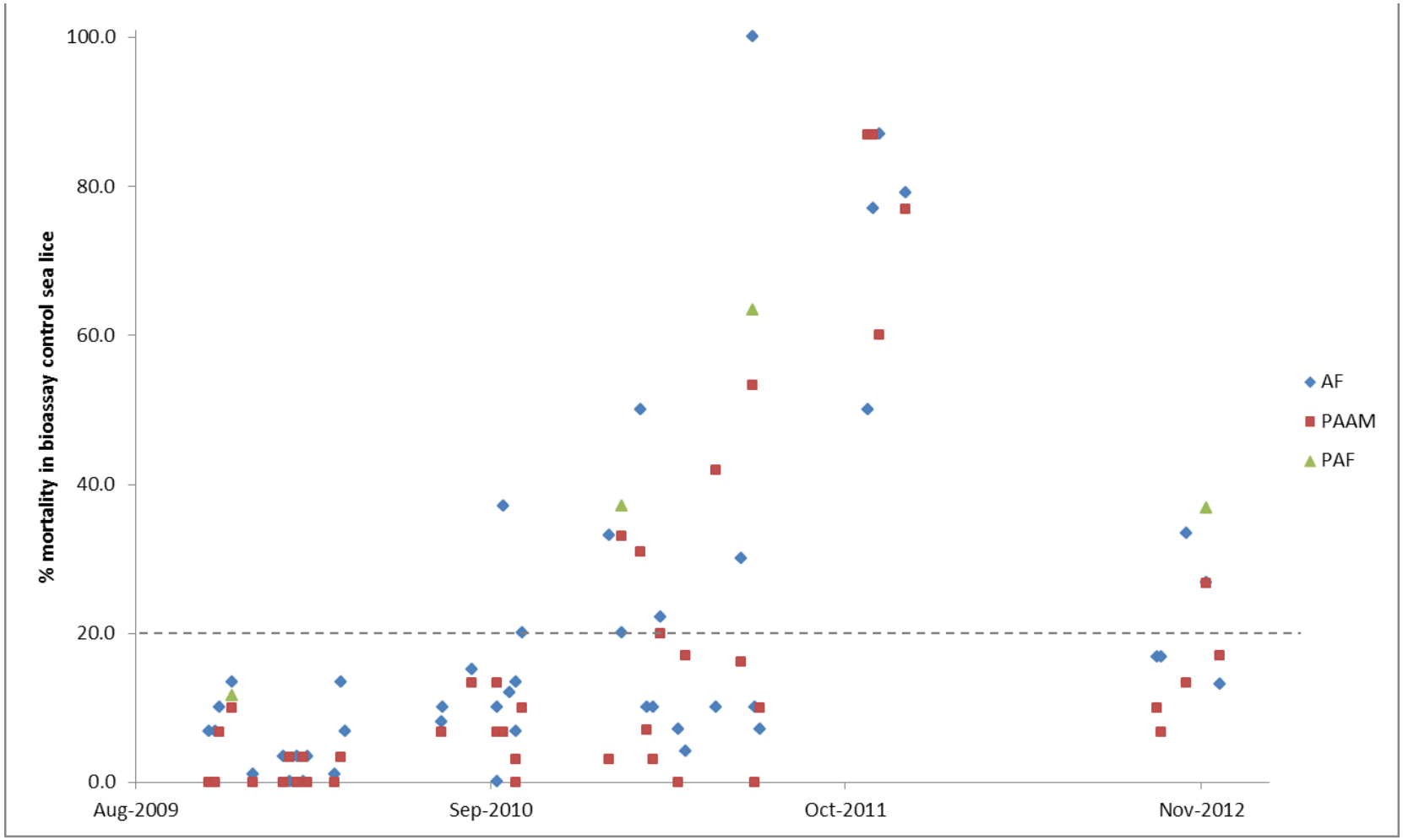
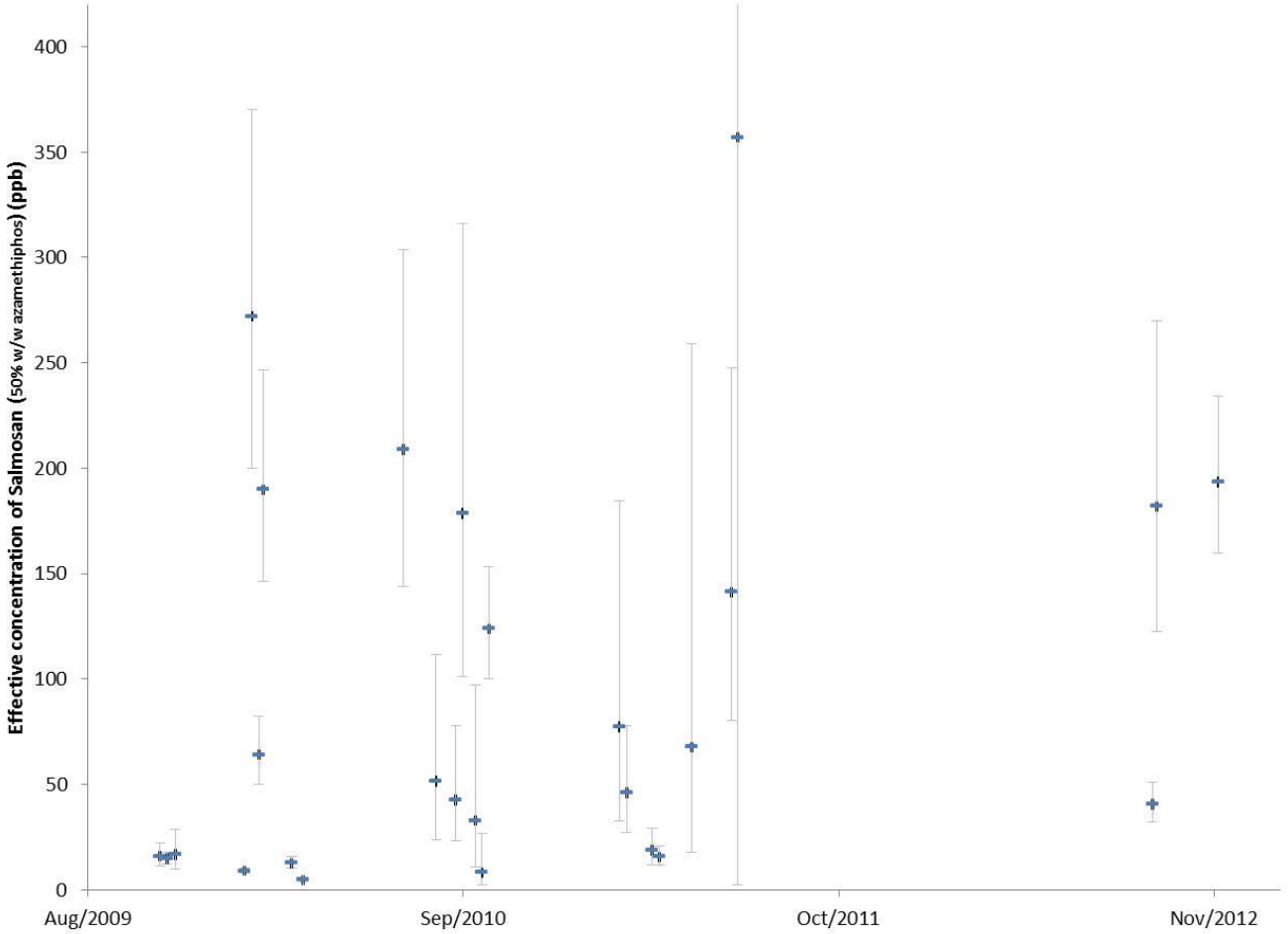
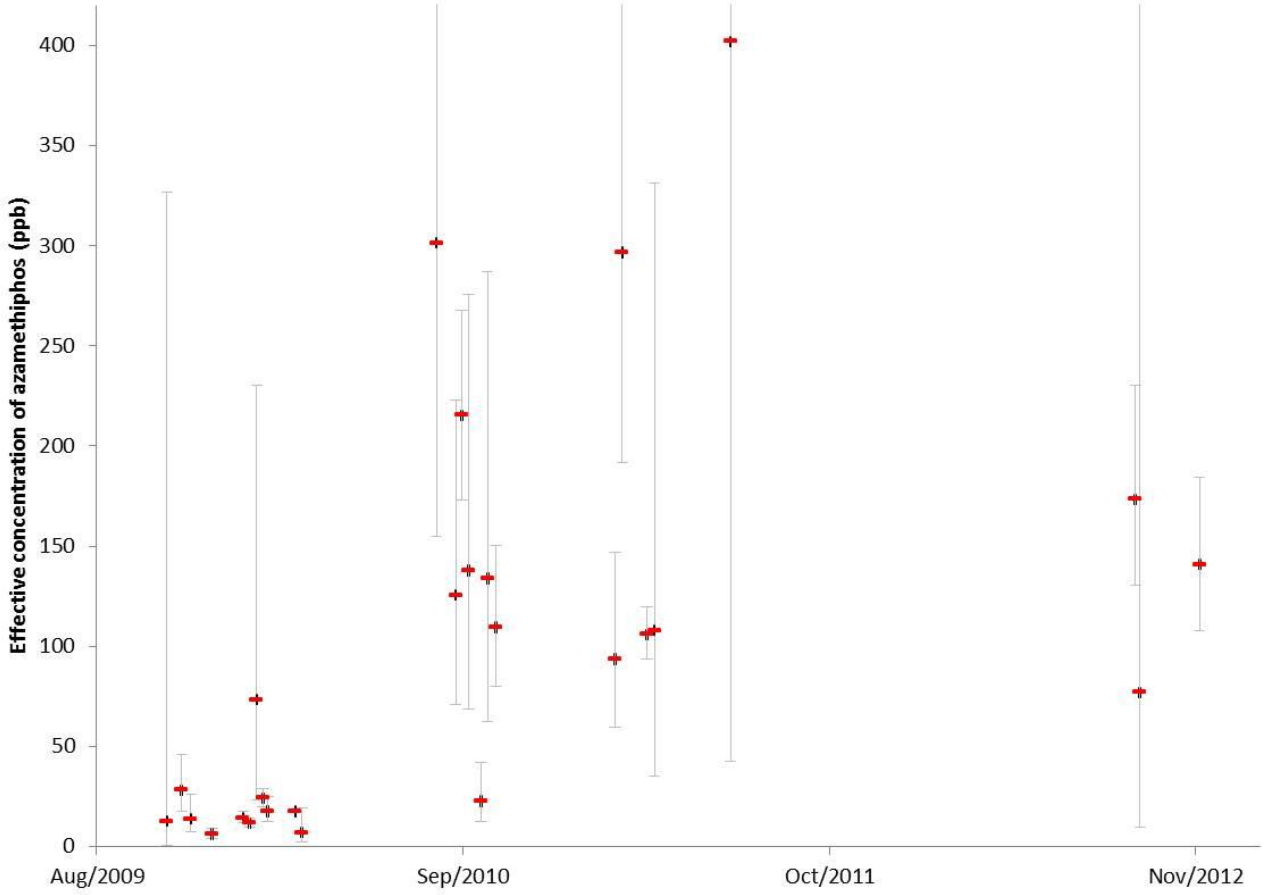


Figure 4 Azamethiphos bioassay EC_{50} values for sea lice (*L. salmonis*) collected from the Bay of Fundy, New Brunswick, Canada during the period 2009 to 2012. EC_{50}: effective concentration of Salmosan® (w/w 50% azamethiphos) leading to a response of 50% of sea lice not prone to natural mortality.

A) Adult Female



B) Pre-Adult Male and Adult Male



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