Assessment of sea lice (*Lepeophtheirus salmonis*) management in New Brunswick, Canada using Deltamethrin (AlphaMax®) through clinical field treatment and laboratory bioassay responses

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ABSTRACT

The control of the ectoparasite, sea lice (*L. salmonis*), on farmed Atlantic salmon in Eastern Canada was complicated by the development of resistance to Emamectin Benzoate, the primary in-feed medication used since 2000. Field efficacy and bioassay assessments were initiated to address the emergency authorization of Deltamethrin (Alpha Max®) used in limited circumstances in 2009-2010. Under farming conditions present in the Bay of Fundy, Deltamethrin consistently reduced pre-adult (male and female) and male adult lice stages in the range of 88-98% compared to pre-treatment levels. Cage-level reductions for both adult female and chalimus lice stages varied considerably with median reductions of around 50% or less commonly observed for either stage. *In vitro* bioassays using field collected mobile stages of sea lice generated average effective concentration (EC_{50}) values that were lower for combined stages of pre-adult and adult male lice compared to either pre-adult female or adult female lice stages. Stage ($p<0.001$) and temporal ($p<0.001$) differences were observed for EC_{50} values. Both field treatment observations and *in vitro* assessments of sea lice responses reflected greater reductions after Deltamethrin exposure for pre-adult and adult male lice compared to adult female lice stages. Variable response occurring in different lice categories is likely to affect the successful field application of this treatment and is an important factor to consider when deciding how best to report efficacy.

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1. INTRODUCTION

Sea lice (*Lepeophtheirus salmonis* and *Caligus* sp.) infestation of farmed salmonids, particularly Atlantic salmon (*Salmo salar*), represent a significant economic and health burden in regions where salmon are intensively cultured (Heuch et al., 2005; Costello, 2006). Infestation of cultured Atlantic salmon by *L. salmonis* has been reported in the North Atlantic and Pacific (Pike and Wadsworth, 1999; Revie et al., 2009). The more host indiscriminant *C. rogercreysii*, remains the predominant threat to the Chilean industry (Bravo et al., 2008) with the less well documented, but still economically impactful, *C.elongatus* and *C.orientalis* also reported in the North Atlantic (Tully, 1989; Hogans & Trudeau, 1989; Schram et al., 1998; Revie et al., 2002) and Japan, respectively (Nagasawa, 2004).

The use of long-term integrated biological and chemical strategies for managing sea lice is an important tool in the sustainability of salmonid aquaculture industries in many countries and the environment in which they operate. Generally, integrated pest management strategies use a combination of best management practices and treatment measures in an attempt to effectively and economically control sea lice infestations in an environmentally sustainable manner. Chemical treatments are used as a key component of these management plans and include a variety of agents (Pike and Wadsworth, 1999; Boxaspen, 2006; Brooks, 2009). Decisions as to the timing and choice of treatment should be based on a program of regular monitoring of lice species, stages and numbers. Monitoring the abundance of sea lice on farms and the development of resistance to chemotherapeutants are important factors in the successful management of this parasite (Treasurer & Pope, 2000; Denholm et al., 2002; Westcott et al., 2004; Sevatdal et al.,

2005; Heuch et al., 2005) providing decision support tools for delivery of evidence-based outcomes to all levels of policy makers. Unfortunately, reduced sensitivity in *L. salmonis* to a range of chemotherapeutants, from different geographical areas where salmonids are intensively cultured in sea water, have been reported (Jones et al., 1992; Tully and McFadden, 2000; Treasurer et al., 2000; Jones et al., 2002; Denholm et al., 2002; Sevatdal & Horsberg, 2003; Fallang et al., 2004; Bravo et al., 2008; Lees et al., 2008a).

Deltamethrin [(S)-α-cyano-3-phenoxybenzyl,(1R)-***cis***-3-(2,2-dibromovinyl)-2,2 dimethyl-cyclopropane carboxylate] is a synthetic type II pyrethroid insecticide and acaricide used for the topical control of ectoparasites in cattle, sheep and poultry. It is also available as Alpha Max® (Pharmaq) for use in Atlantic salmon aquaculture to treat sea lice infestations (Sevatdal & Horsberg, 2003; SEPA, 2008; Fisheries and Oceans Canada, 2012; Bravo, 2013). Several clinical treatment failures and reduced sensitivity to deltamethrin have been reported from Norway (Sevatdal & Horsberg, 2000; 2003) with unverified anecdotal reports of reduced sensitivity from Scotland and Ireland (Sevatdal et al., 2005). Reduced sensitivity has also been documented *in vitro* using bioassays (Sevatdal & Horsberg, 2003). Over the past decade, bioassay protocols have been adapted for use as a common tool to monitor sea lice resistance to chemotherapeutants globally (Sevatdal & Horsberg, 2003; SEARCH 2004; Sevatdal et al., 2005; Westcott et al., 2008; Bravo et al., 2008; Saksida et al., 2013; Whyte et al., 2013; Helgesen and Horsberg, 2013).

In July, 2009, a one-year emergency authorization, for the application of Alpha Max® (deltamethrin), was issued by the Pesticide Management Regulatory Agency, a branch of Health Canada, under the Pest Control Products Act (Department of Fisheries and Oceans, 2010) for use

in limited areas of southwestern New Brunswick, Canada (Limekiln Bay, Bliss Harbour, and Back Bay). The limited use of this product required documented evidence of efficacy in the field, together with *in vitro* bioassay data to identify trends in sea lice tolerance to this chemotherapeutant (Department of Fisheries and Oceans, 2010). A key component of an integrated pest management plan is monitoring treatment efficacy for each product to detect early indications of resistance development. Field treatment efficacy is evaluated by comparing sea lice counts (by stage) pre- and post-treatment of a sample of fish from treated cages. Precise measures of the clinical responses for different stages of sea lice performed in multiple cages at multiple sites, before and after field treatments, are required for optimization of control measures by farms and, to further justify continued access to therapeutic products. The lack of effective chemotherapeutic control options for sea lice infestations significantly impacted the New Brunswick salmon farming industry in 2010, in terms of financial losses.

The objective of this project was to provide critical evidence for sea lice control decisions at the government policy and farm management levels for the use of deltamethrin under the unique salmon culture conditions of the Bay of Fundy, New Brunswick, Canada. The information provided was primarily based on treatment efficacy as measured by the direct measurement of different stages of sea lice reduction post-treatment and through *in vitro* bioassays to inform the description of trends in tolerance patterns for deltamethrin in New Brunswick.

2. MATERIALS AND METHODS

2.1 Sea Lice Categorization

The sea lice monitoring program in New Brunswick requires lice to be categorized by species (*Lepeophtheirus* sp. and *Caligus* sp.), stage (larval, pre-adult and adult) and sex (male and female). Sea lice categories for field counts included: [1] Chalimus (Chalimus 1-IV); [2] Pre-adult (stage I and II) (male and female) and adult male lice (PAAM); [3] Adult female lice (gravid and non-gravid) (AF). Pre-adult (stage I and II) (male and female) and adult male lice stages were combined into one category to facilitate ease of counting as separating them by sex and stage is time-consuming, labour-intensive and potentially lead to unhealthy consequences for fish due to excessive handling when lice numbers are high. Categorization differed for the bioassay procedure whereby pre-adult lice were further separated by stage (stage II) and sex; pre-adult male (PAM) and pre-adult female (PAF). Differences in sex and stage responses to treatment have been observed in bioassays (Sevatdal et al., 2005; Westcott et al., 2008; Whyte et al., 2013) which suggest that mixing different sexes and stages of lice within a single bioassay may result in pertinent information being overlooked. Immature male lice are distinguished from mature male lice by the surfaces of the second antennae and the presence of a rough-surfaced pad (post-oral adhesions pad) located near the base of the first maxilla (Johnson & Albright 1991), a process that is facilitated by magnified inspection. To efficiently assign hundreds of field-collected lice of mixed ages to multiple bioassay containers with as little impact on their survival as possible, PAM lice were further combined with adult male lice to create a PAM-AM category.

2.2 Sea Lice Monitoring and Treatment Efficacy

Sea lice abundance and response to deltamethrin (AlphaMax®) treatment under field conditions of use were examined by performing pre-treatment and post-treatment counts, at least weekly. These counts were reproducible (similar counts by different trained personnel at the same site or the same trained personnel at different sites) and were applied in a similar manner across the industry (Elmoslemany et al., 2013). *In vitro* bioassays contributed descriptive information on the trend in tolerance patterns for deltamethrin in New Brunswick, Canada. Marine Atlantic salmon (*Salmo salar*) aquaculture sites in the Bay of Fundy (Figure 1), which received treatments with deltamethrin during the period July to September 2009, were identified and assessed for sea lice numbers during focused monitoring, as part of the integrated sea lice monitoring program coordinated by the New Brunswick Department of Agriculture, Aquaculture and Fisheries (NBDAAF). Recent development of resistance to emamectin benzoate (SLICE®) (Jones et al., 2013) necessitated rapid action and, at the time, no alternative treatments were permitted in the region. Sites receiving treatment were chosen based on conditions related to emergency permissions arranged by NBDAAF and as a result, no randomized comparisons or untreated control cages / sites were feasible.

Mean cage and treatment event levels of site-level sea lice abundance were estimated based on samples of between 10 and 15 fish per cage and from all cages at each site (3 to 9 cages per site). Counts were performed on each cage, as close to the treatment day as possible, at a point no more than 4 days prior to treatment (pre-treatment count) and multiple times within 14 days following each treatment (post-treatment counts). Pre and post count data were available for 6 treatment events involving a total of 41 cages from 4 sites within the same management area of the Bay of Fundy, New Brunswick (Figure 1). Counting of all cages would occur on the same

day but treatment days differed slightly, thus cages were often measured at different days post-treatment based on the day of treatment.

All treatments were performed on-site using skirt enclosure tarpaulins. Curtains of tarpaulin material were deployed to fully surround the cage to an appropriate depth (generally the lower end of the tarpaulin should be at least 2 meters deeper than the lowest depth of the net holding fish to be treated, as dictated by emergency permits), but without an enclosed bottom, and were open to limited water exchange (so called bottomless-bath treatments) (Corner et al. 2011). The prescribed treatment dose was 3 parts per billion (ppb) deltamethrin and an exposure duration of 40 minutes, prior to skirt removal. Supplemental aeration was added during the exposure period.

Fish sampled for sea lice abundance counts were collected using a sample procedure involving capture (with a dip-net) of fish attracted to the surface with feed, followed by anesthesia using tricaine methane sulphonate (TMS; Syndel Laboratories), at a dose of approximately 100 mg l^{-1}. Each stage of lice was counted and recorded on a per-fish basis. The percentage knock-down values for sea lice were estimated based on the number of lice recorded during the pre-treatment count conducted closest to the time of deltamethrin treatment (when there was more than one count) and the average count in the first 7 days post-treatment period.

2.3 Sea Lice Collections for Bioassay

Sea lice 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 deltamethrin using the skirt enclosure tarpaulin method during this period. Pre-

market sized fish were anaesthetized using TMS 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 allow them to acclimate prior to bioassay set-up the following morning.

2.4 Bioassay Procedure

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 personnel carried out the 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 12 hours to allow them to recover from handling and transport) (Westcott et al., 2008). A stock solution of deltamethrin (AlphaMax[7], Pharmaq, Overhalla, Norway) was prepared for each bioassay by dissolving 100µL of deltamethrin in 999.9 ml of sea water. This working solution was used to prepare experimental solutions with varying concentrations of deltamethrin (0.1 ppb, 0.3 ppb, 0.6 ppb, 1.0 ppb, 3.0 ppb). Control dishes (seawater only) were included in each trial. In all cases, the experimental solutions used seawater taken from the same site from which the sea lice were collected. All experimental solutions were maintained in an incubator at 10-12°C. The exposure protocol involved exposing ten apparently healthy sea lice, of the same stage and sex, according to categories previously described, in plastic Petri dishes, to the treatment and control solutions. 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 and to minimize the handling to transfer sea lice between containers. The Petri dishes were submerged in the solutions of deltamethrin dilutions for a period of 30 minutes. At 15 minutes post-exposure, the dishes were drained and submerged for the remaining 15 minute exposure period. The temperature was also recorded at this time. At the end of the 30 minute exposure period, the Petri dishes were drained and placed in a "rinse" bucket containing clean, control seawater. All dishes were rinsed before being placed into a container of clean seawater aerated with an electric air pump and incubated in a temperature-controlled chamber at 10-12°C for 24 hours. All dishes were blind-coded to reduce assessor bias. Following exposure, the condition (live, weak, moribund and dead) of the sea lice, as described in Westcott et al. (2008) was evaluated according to an adopted set of bioassay response criteria. As a rule of thumb, bioassays for which control mortality for a sea lice stage and sex category exceeded 20% were excluded from the analysis.

2.5 Statistical Analyses

Analyses of field data were conducted at the level of the treatment event (site) as well as for cages at a site within such events. Descriptive statistics were computed for each of the grouped life stages (chalimus, PAAM, and AF) over the study period. The percentage knock-down value was simply a ratio based on the pre- and post- count values, and the 95% confidence interval for this ratio was estimated using the quasi-Poisson method (Jimenez et al., 2012) calculated in R using the *pairwise CI* package (R Development Core Team, 2008). The data from the bioassays were analyzed using a probit regression model with natural responsiveness (Finney, 1971) with the software GraphPad (Graphpad Software Inc., La Jolla, CA). The effective concentration (EC_{50}) leading to a response of 50% of the lice not prone to a natural response (moribund + dead)

was used to determine sensitivity. Confidence limits (95 %) were also calculated for the EC_{50} estimates. Data from bioassay evaluations that fitted the probit regression model poorly and resulted in a failure to be able to estimate confidence limits, were not included in the analysis. Further analysis of bioassay data was performed using Stata version 12 (Stata Corp., College Station, TX). Cooks distance criterion was used to identify any outliers within the set of bioassays. The predictor variables for EC_{50} were year, stage of lice and season. Season in half year periods was defined as winter-spring for data collected from January to June and, summer−fall for data collected during July to December. Two-way analysis of variance (ANOVA) was performed. Data were subjected to F-tests for homogeneity of variances (Wilcox et al., 1986). Mean (± SD) water temperatures recorded during the study period was 4.6 (± 2.0) °C in winter−spring and 11.8 (± 2.3) °C in summer−fall.

3. RESULTS

3.1 Field-Based Efficacy

As part of this study, a total of 1268 fish were sampled from 41 cages on four sites that administered deltamethrin treatments during the period July to September, 2009. The sample points were associated with six treatment 'events'. Such events consisted of 3 to 9 cages being treated at the same site over a period of 1 to 3 days. Each count involved 8 to 15 fish from a given cage, with more fish typically being sampled during the pre-treatment period, as can be seen from Table 1. This table also indicates that one cage did not have a count carried out in the first seven days following treatment ("Week +1"), while 10 cages were missing counts during the period of 8-14 days following treatment ("Week +2"). The levels of lice on fish prior to

treatment were, on average, approximately 80 mobiles per fish, though this varied greatly at the cage level, from approximately 11 to over 175. These data were right-skewed, as can be seen from Table 1, and also tend to be more peaked subsequent to treatment (note the increase in kurtosis values). Despite these variations it is clear that overall sea lice numbers were reduced following treatment, as would be expected. However, this was much more evident for the PAAM stages, where the median abundance of 55 was reduced to 2 and 4 lice, for Week+1 and Week+2 respectively. This compared to an overall reduction of approximately 50% in the case of both the chalimus and AF stages. The largest reductions in sea lice levels were observed the first week following treatment. This, together with the fact that there were no cage counts from one of the events (E6) during the second week post-treatment, informed the decision to use data from only Week+1 when estimating the treatment knock-down values.

There were significant differences among the treatment events. Figure 2 illustrates the sea lice levels on all 1268 fish assessed, in terms of the AF and PAAM stages, with fish samples grouped according to treatment event (E1 to E6) and time of sampling (Before / Week+1 / Week+2). What is evident from this graph is that the effect on the PAAM stages is relatively consistent and marked with a noticeable flattening of the data down the y-axis as you move from “Before” to “Week+1”. The same cannot be observed for the AF stage where a much more modest reduction (on the x-axis) is evident. Shifts in chalimus stage infestation tended to be similar to those observed for AF (data not shown).

A more formal set of comparisons can be found for each treatment event in Figure 3. The efficacy of deltamethrin in reducing PAAM stages was obviously much higher (ranging from an

average of 98% to 88% knock-down across the events) than was the case for the other stages. This reduction was also fairly consistent across cages, as demonstrated by the relatively narrow 95% confidence intervals for PAAM knock-down. In the case of the AF stage, one event reached an average knock-down of almost 70% but the median was closer to 50% and two events (E2 and E4) indicated no effective reduction (with their 95% confidence intervals spanning the 0% knock-down level). The situation for chalimus stages was similar to that of AF with most of the knock-down values estimated at below 50%; indeed for two cases the mean number of chalimus post treatment was actually higher than had been observed prior to the event (as indicated by negative knock-down values for E2 and E6).

3.2 Bioassay Outcomes

Since sea lice were collected opportunistically from marine aquaculture sites, irrespective of a deltamethin treatment event occurring on site, there was a difference in the number and type of bioassay conducted during 2009-2011. Similarly, bioassays were eventually discontinued as deltamethrin use by the industry lapsed (i.e. after 2010). A total of 74 bioassays were conducted between 2009 and 2011. Sixteen bioassays (AF: 12; PAM-AM: 3; PAF: 1) fitted poorly in the probit regression model, resulting in a failure to estimate the EC_{50} and/or 95% confidence limits. Of the 58 remaining bioassays, 50.0% were conducted in 2009, 22.4% in 2010 and 27.6% in 2011. In addition, the Cook's distance criterion identified two PAM-AM bioassays and one AF bioassay to be outliers; these were also excluded from the analysis. For each bioassay conducted and each sea lice stage tested in the bioassay (i.e. adult females, pre-adult/adult males and pre-adult females) the proportion of dead and moribund sea lice was calculated. In total, twelve bioassays (AF: 7; PAM-AM: 5) had control mortality greater than 20% and it was decided not to

include these in the analysis. A total of 43 bioassays were included in the analysis, the majority (58.1%) of which were derived from sites receiving deltamethrin in 2009 (these sites contained fish that were harvested before 2010 and so were unavailable for sampling in future years). Bioassays conducted in subsequent years, (10 in 2010 and 8 in 2011) involved lice collected from fish undergoing harvests or during counting procedures. Deltamethrin use was discontinued in New Brunswick in 2010. Sea lice stages and sexes for bioassays, dictated by availability, were represented by PAM-AM (56.1%), PAF (29.3%) and AF (19.5%). EC_{50} values ranged from 0.20 ppb to 3.03 ppb (Table 2).

The overall mean mortality in the control groups for adult female lice (18.8%) was higher than either PAM-AM (12.3%) or PAF (7.0%) lice for all trials included in the analysis. Season was demonstrated to be a significant factor ($p=0.005$) with more bioassays conducted between January to June excluded from the analysis due to natural mortality occurring in the control lice compared with bioassays conducted between July to December (Figure 4). Approximately 39% of bioassays conducted during 2009-2011 were excluded due to unacceptable levels of mortality occurring in the control lice; 24% conducted between January-June and 15% between July-December.

The target dose of deltamethrin in the field was 3 ppb for 40 minutes in sea cages with skirt enclosures. In general, the average EC_{50} values for all stages were below the field target dose of 3 ppb (Figure 5). Stage ($p< 0.001$) and temporal differences ($p<0.001$) were, however, observed in EC_{50} values. While the mean EC_{50} values for all lice stages appeared to increase in 2010 compared with 2009, sample size for PAF and AF were too small to detect differences. However,

the mean EC_{50} values for PAM-AM, for which more bioassays were performed over multiple years, were significantly lower in 2009 (0.56 ± 0.31,0.81) compared with 2010 (1.80 ±1.16,2.44) ($p < 0.001$). Bioassays conducted during 2009 when deltamethrin was being administered in the field, indicated a 2-fold higher EC_{50} value for PAF and AF compared with PAM-AM. The EC_{50} values for PAM-AM were significantly lower compared with PAF lice (1.20 ± 0.74, 1.66) ($p<0.01$), and while no significant difference was observed between PAM-AM and AF (1.29 ± 0.45, 2.12), the mean EC_{50} values for AF lice in 2009 were similar to those reported for pre-adult female lice but the sample size (n=4) for AF bioassays in 2009 did not enable reliable comparisons.

4. DISCUSSION

The primary finding when monitoring field efficacy of deltamethrin treatments in New Brunswick using pre- and post-treatment sea lice counts was the variability of its efficacy in reducing different sea lice stages following treatment. When considering only the pre-adult male and adult male sea lice stages, this compound was found to be effective, with only around 5-15% of this stage of lice being observed to survive one week after treatment despite relatively high levels prior to treatment. While not quite so consistent or effective, these results were similar to those reported by Hart et al. (1997) where pre-adults were reduced by 95 to 99% following treatment with a structurally similar pyrethroid, cypermethrin. However, that study also reported similar levels of clearance for adult female lice (Hart et al., 1997), something which was in sharp contrast to the present study. Here, a much lower level of efficacy was observed on both adult female lice and chalimus stages following deltamethrin treatment. Based on a total of 6

deltamethrin treatment events examined, 4 showed a knock-down for adult females of just over 50% while 2 indicated no effective reduction. The results were similar for chalimus stages with, if anything, slightly less successful treatment effects. Treasurer & Wadsworth (2004) also reported limited reduction in chalimus stage lice, with levels of between 26-69% of the starting values following treatment with cypermethrin.

The effect of deltamethrin on adult female and chalimus lice stages was highly variable, unlike the pre-adult male and adult male lice which showed more consistency in their response to deltamethrin. Differences in sensitivity patterns have been noted in previous studies where adult female lice were found to be less sensitive than other life stages to bath treatment products (Roth et al., 1993, Treasurer et al., 2000) and some in-feed parasiticides, such as emamectin benzoate (Jones et al., 2013). It is possible that during this study period, there were differences in the concentration of deltamethrin in the treated cages due to an inability to reach the intended target dose. One of the perceived problems of using the skirt tarpaulin method is difficulty in maintaining the appropriate prescribed dose of therapeutant, whilst concurrently dealing with losses of therapeutant from the open bottom (Corner et al., 2011). It is also possible that the concentration of deltamethrin varied in different locations within cages during the treatment procedure due to inadequate mixing of the product, loss of product escaping through the bottom of the skirt, or binding with organic material in the water or biofouling on nets of the treated cages. These factors likely varied by cage and by site, affecting the observed effect for some, but not all, treatment events.

Anatomical and biological differences between the different stages of the parasite life cycle could also theoretically account for variation in response to deltamethrin treatment. It has been postulated that the composition of the sea louse cuticle might explain the variation in sensitivity to pesticides (Boxaspen, 2006). Deltamethrin is known to be absorbed predominantly through the cuticle on the extremities of the ventral surface of the sea louse body (Sevadtal et al., 2005). The cuticle of chalimus larvae has been shown to be very similar to that of free-living copepods, but with some modifications associated with a parasitic existence (Gonzalez-Alanis et al., 2001), while the larger size or thicker cuticle of the dorsal body surface might result in adult female sea lice being less responsive to bath treatments. As reinfection of farmed fish within the site can be an important determinant of ongoing sea lice pressures (Krkosek et al.,2010; Aldrin et al., 2013), the variability in response to chalimus could also be attributed to the highly variable intensity of new copepodids settling on the fish following treatment. Deltamethrin does not prevent post-treatment copepodid settlement. Therefore new settlements occurring after the deltamethrin treatment event and prior to the post-treatment count would obscure any evidence of a treatment-related chalimus reduction. Hart et al. (1997) noted that cypermethrin treatment resulted in significant reductions in numbers of all stages of the *L. salmoni*s except early chalimus, and the development of any later chalimus stages was significantly reduced or halted. While a reduction in number of pre-adult male and adult male lice, and pre-adult female lice was observed following treatment with deltamethrin, only pre-adult female lice demonstrated a statistically significant reduction in numbers. In contrast, a similar magnitude of effect was not observed in adult female and chalimus stages. Chalimus stages are believed to be less affected by most chemical treatments used in baths compared with other life stages (Burka et al., 1997), with the exception of cypermethrin (Jakobsen & Holm, 1990; Roth et al., 1993).The reasons for this are

unclear although consideration should be given to the possibility that although chalimus may be observed after post-treatment, they may subsequently die or fail to moult into the next stage of development. Enumeration of different sea lice life stages and regularly timed counts would indicate a treatment effect on chalimus by the number moulting to pre-adult stages, provided a post-treatment sea lice count is conducted within 4-5 days of treatment to ensure that new chalimus settlements have not occurred and do not distort the post-treatment count. The ability to distinguish early chalimus stages in the field is also challenging due to their small size (0.7–2.7 mm), thereby often resulting in underestimation of these stages during field counts (Schram, 1993; Beamish et al., 2005; Elmoslemany et al., 2013). As such, all chalimus stages are purposefully grouped into one category in the New Brunswick integrated sea lice monitoring program (Fisheries and Oceans Canada, 2011). Therefore, it was not possible to quantify the range (I through IV) of chalimus stages present. While separating the chalimus counts into newly settled versus older chalimus stages could indicate if the majority of chalimus identified immediately following treatment were new settlements, this was not practical in this field monitoring situation.

The reason for the poor response of adult female lice to deltamethrin compared to pre-adult male and adult male lice is unlikely to have been due to any changes in sea lice tolerance since the population of sea lice in New Brunswick had not been previously exposed to this compound, and we did not demonstrate any definitive reduction in efficacy in the use of deltamethrin over the time period monitored. It is possible that a reduction in response could develop over short time periods if treatments did not result in high mortality of lice or, if there were large numbers of mobiles and repeated cycles of treatment were performed within a short interval of time; this

would result in an increase in the proportion of less responsive individuals in the population. It is important to consider the possible implications of individual host selection during sampling as this may be relevant for many parasitic diseases where variation in parasite abundance and predilection of certain parasite life stages on individual hosts may be evident (Churcher & Basáñez, 2009). Sea lice abundance on the host fish can vary by age or size of fish, as well as temporally and spatially (Saksida et al., 2007, Lees et al., 2008b). While sea lice counts were conducted in a consistent manner in the same region of the Bay of Fundy, some spatial differences likely existed between sites. Similarly, the temperature varied between the beginning of sampling in July (8.6°C ± 4.4 (mean ± SD)) and the end of sampling in September (11.4°C ± 3.0 (mean ± SD)). The variation in response to deltamethrin is most likely a function of metapopulation structure, recruitment dynamics and dispersal dynamics of the lice within each site.

Bioassays performed on sea lice collected from the same area as the deltamethrin-treated sites demonstrated average EC_{50} values for all sea lice stages, in general, below 3 ppb, however, EC_{50} values for all lice stages appeared to increase in 2010. Adult female and pre-adult female lice appeared to be less responsive to deltamethrin in 2009 during the period when the therapeutant was being administered in the field, as evidenced by the 2-fold increase in EC_{50} values compared with pre-adult and adult male lice. Pre-adult female lice, in particular, were significantly less responsive than pre-adult and adult male lice. While the response of adult female lice in 2009 was similar to pre-adult female lice, the small sample size of adult female bioassays in 2009 precluded any further interpretation. More than twice the numbers of adult female bioassays were excluded from the analysis (based on an inability to obtain an EC_{50} value and/or control

mortality exceeding an acceptable level) compared with the other stages. Although the reasons for this are unclear, we have previously observed and documented this lack of consistent robustness in adult female lice (Whyte et al., 2013). Thus, we can only speculate that if more EC_{50} data points for pre-adult female lice were available, the difference in sex response observed in 2009 may have been repeated in 2010. Sevatdal et al. (2005) also reported occasional reduction in sensitivity to pyrethroids in Norway where EC_{50} values ranged from of 0.09 ppb (95% CI: 0.02-0.20) to1.03 ppb (95% CI: 0.57-1.82). The EC_{50} values reported in New Brunswick are higher in comparison, ranging from 0.20 ppb (95% CI: 0.14-0.28) to 2.45 ppb (95% CI: 1.80-3.30). While a direct comparison with Sevatdal et al. (2005) is limited due to the fact that only pre-adult male II lice, derived from standardized laboratory-generated studies, were included in their studies, the field-derived pre-adult male and adult male groups assessed in our study comprised pre-adult II male lice and adult male lice. The reasons for this variation in reported EC_{50} values between the two regions are unclear but potentially related to differences in the metapopulation structure and treatment history of the two regions sampled.

In Norway, pyrethroids were used extensively from the mid-1990s and clinical treatment failures and reduced sensitivity of sea lice to these therapeutants were subsequently reported (Sevatdal & Horsberg 2003; 2005). While deltamethrin use is documented for only a short time period in New Brunswick, emamectin benzoate was used extensively for more than 9 years, and clinical treatment failures and reduced sensitivity to that therapeutant had been reported (Jones et al., 2013). While both classes of pesticide are potent neurotoxicants acting on various neuroreceptors and ion channels, development of resistance to pyrethroids and avermectin compounds is thought to be associated with increased degradation activity by detoxification enzymes in addition to the

insensitivity of target sites in the neural system or knockdown resistance (Kang et al., 2006). Other insect and mite agricultural pests express higher levels of detoxifying enzymes, leading to cross-resistance to other pesticide classes (Wostenholme & Kaplan, 2012). For example, bioassays performed in some insect pest species have demonstrated that oxidative degradation plays a critical role in resistance development to both the avermectin, fipronil, and cypermethrin (Kang et al., 2006). In addition, the efflux transporter, P-glycoprotein has been shown to transport a wide variety of pesticides, including the pyrethroid, cypermethrin (Clark & Di Giulio, 2013), and has also been linked to macrocyclic lactones resistance (Lespine et al., 2008; Carcamo et al., 2011). It is possible that the limited efficacy of deltamethrin to adult female sea lice in New Brunswick is indicative of non-specific mechanisms acquired through repeated generations of lice exposed to one drug (emamectin benzoate) thereby affecting the potential for resistance to develop to other drugs to which the pathogen has not been exposed (deltamethrin). Multiple resistance in sea lice to therapeutants of different chemical classes has been reported from Norway (Horsberg, 2013). Regulatory permission for deltamethrin use was discontinued in New Brunswick in 2010, preventing any further observations regarding field efficacy.

In conclusion, although there are many limitations in attempting to directly relate sensitivity data generated from *in vitro* bioassays to treatment concentrations used in the field (Denholm et al., 2002), the bioassay data in this study does appear to support the evidence from the field that pre-adult male and adult male lice are more responsive to deltamethrin treatment compared to adult female lice. To continue using deltamethrin when faced with the apparent lack of control of AF lice would have required modified treatment strategies, such as changing the treatment concentration, managing treatment mechanics to optimize exposure of all fish to the desired

concentration, or selecting an altogether different control method directed at AF. Furthermore, assessments of this chemical using fully enclosed tarpaulins or well-boats may have produced more optimal exposure and effect on all sea lice stages. Lastly, the clinical efficacy measures used in the analysis of this project provide scientifically valuable refinements for on-farm sea lice investigation.

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Table 1 Descriptive statistical summary of total sea lice, *Lepeophtheirus salmonis* counts for four sites that administered deltamethrin treatments during the period July to September 2009, aggregated by time of count (Before/Week+1/Week+2) and summarized by sea lice stage (PAAM: Pre-Adult (male and female) and Adult Male; AF: Adult Female).

Time of Count	Sea Lice Stage	Number of Cages Sampled	Number of Fish Sampled	*L. salmonis* counts			
				Lice per fish (mean)	Lice per fish (median)	Skew	Kurtosis
Before [a]	Chalimus	41	507	22.5	9	2.1	4.7
	PAAM	41	518	70.1	55	0.7	-0.5
	AF	41	518	11.3	9	0.9	0.7
Week +1 [b]	Chalimus	40	434	11.2	7	3.3	14.1
	PAAM	40	440	4.5	2	3.1	12.2
	AF	40	440	6.3	4	1.9	5.8
Week +2 [c]	Chalimus	31	308	13.7	9	2.8	9.6
	PAAM	31	310	9.3	4	2.7	7.4
	AF	31	310	6.8	5	1.6	4.1

[a] Before = sea lice counts were performed on each cage at a point no more than 4 days prior to treatment (pre-treatment count).
[b] Week +1 = sea lice counts were performed on each cage up to 7 days following each treatment (post-treatment count).
[c] Week +2 = sea lice counts were performed on each cage between 8 to 14 days following each treatment (post-treatment count).

Table 2 The sensitivity of different sea lice, *Lepeophtheirus salmonis* stages to deltamethrin as measured by EC_{50} values obtained through completion of bioassays conducted between 2009-2011 (where control mortality did not exceed 20%). PAF: Pre-Adult Female; PAM-AM: Pre-Adult Male and Adult Male; AF: Adult Female.

		PAF			PAM-AM			AF		
Trial	**Date**	**EC_{50}**	**CI**	**% Control Mortality**	**EC_{50}**	**CI**	**% Control Mortality**	**EC_{50}**	**CI**	**% Control Mortality**
1	Jul 2009	0.83	0.59, 1.18	10	0.50	0.34,0.75	13			
2	Jul 2009	0.75	0.55, 1.02	7	0.25	0.17, 0.37	13			
3	Jul 2009	0.83	0.67, 1.05	0	0.37	0.32, 0.43	0			
4	Jul 2009	0.91	0.72, 1.15	17	0.37	0.30,0.47	15			
5	Jul 2009				0.46	0.36, 0.58	4	1.50	1.03, 2.20	10
6	Jul 2009				0.35	0.24, 0.51	13			
7	Aug 2009				0.40	0.34,0.46	3			
8	Aug 2009	0.61	0.17, 2.25	0	0.61	0.17, 2.25	0			
9	Aug 2009	0.98	0.76, 1.27	3	0.33	0.23, 0.46	10	0.59	0.47, 0.75	7
10	Sept 2009	1.34	0.93, 1.94	13						
11	Sept 2009	2.71	1.00, 7.34	17	0.71	0.67,0.76	0	1.22	0.95, 1.57	3
13	Sept 2009	2.20	1.84, 2.64	0	1.51	1.18,1.93	3	1.83	0.89, 3.76	13
16	Oct 2009	1.43	0.86, 2.38	0.	0.91	0.80, 1.03,	7			
17	Oct 2009	1.68	1.17, 2.40	4	2.01	1.57, 2.56	0			
19	Mar 2010				0.90	0.48, 1.69	10	2.58	1.93, 3.44	0
21	Nov 2010				1.66	1.29, 2.14	7	3.03	2.57, 3.57	0
22	Nov 2010	2.64	0.99, 7.00		1.36	0.87, 2.13	7			
23	Nov 2010				2.45	1.80, 3.30	17			
24	Dec 2010				2.42	2.06, 2.85	3			
25	Jan 2011				0.20	0.14, 0.28	13			
26	Jan 2011				0.59	0.36, 0.96	3	1.87	1.07, 3.25	0
28	Feb 2011				0.72	0.04, 11.69	3	2.13	1.37, 3.32	7
32	Apr 2011				1.15	0.54, 2.44	10			
34	May 2011				1.84	1.12, 3.02	3			

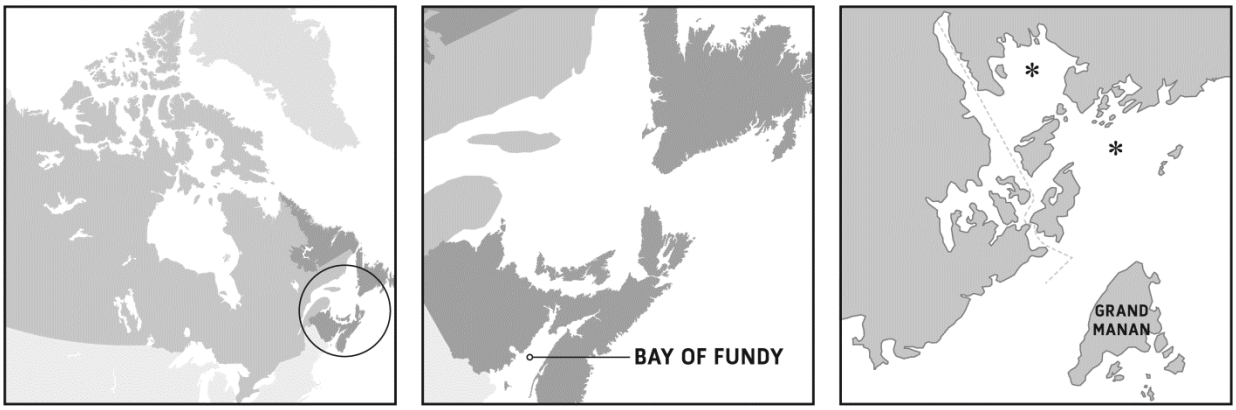

Figure 1 General location (*) of farm sites in the Bay of Fundy, New Brunswick where Sea Lice Counts and Collections were performed.

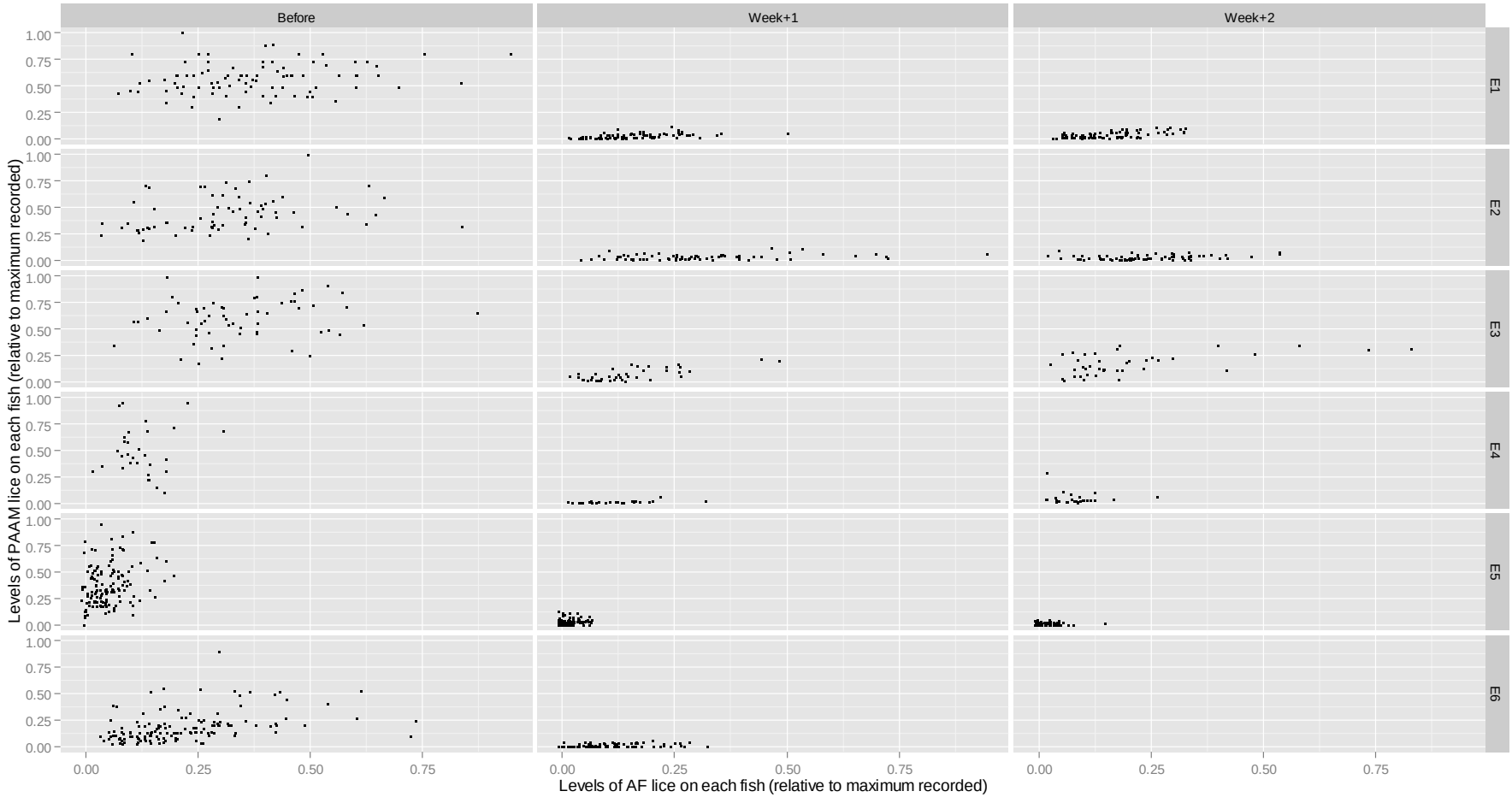

Figure 2 Graphical representation of *Lepeophtheirus salmonis* levels recorded on sampled fish organized by deltamethrin treatment event (row-level panels) and summarized by times at which fish were sampled (column-level panels). In all cases the value 1.0 on the interior x- and y-axes represents the maximum number of lice recorded for the relevant stage in the given treatment event.
PAAM: Pre-Adult (male and female) and Adult Male lice; AF: Adult Female lice. Before: Sea lice counts performed on each cage at a point no more than 4 days prior to treatment; Week +1: Sea lice counts performed up to 7 days following each treatment; Week +2: Sea lice counts performed between 8 to 14 days following each treatment.

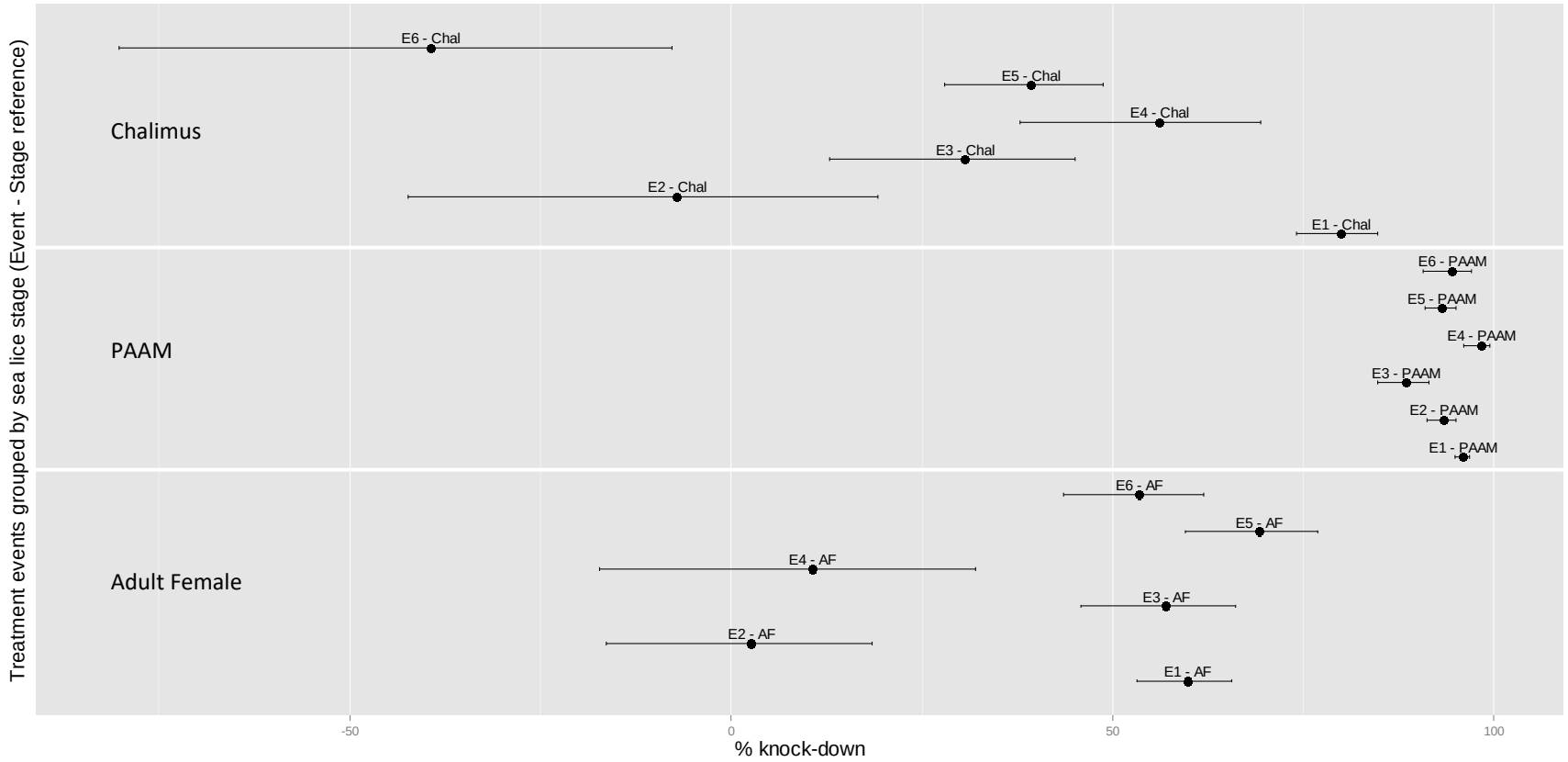

Figure 3 Graphical summary of percentage knock-down (mean and 95%CI) of *Lepeophtheirus salmonis* achieved for each of the six deltamethrin treatment events (E1 – E6) organized according to sea lice stage Chal: Chalimus; PAAM: Pre-Adult (male and female) and Adult Male; AF: Adult Female.

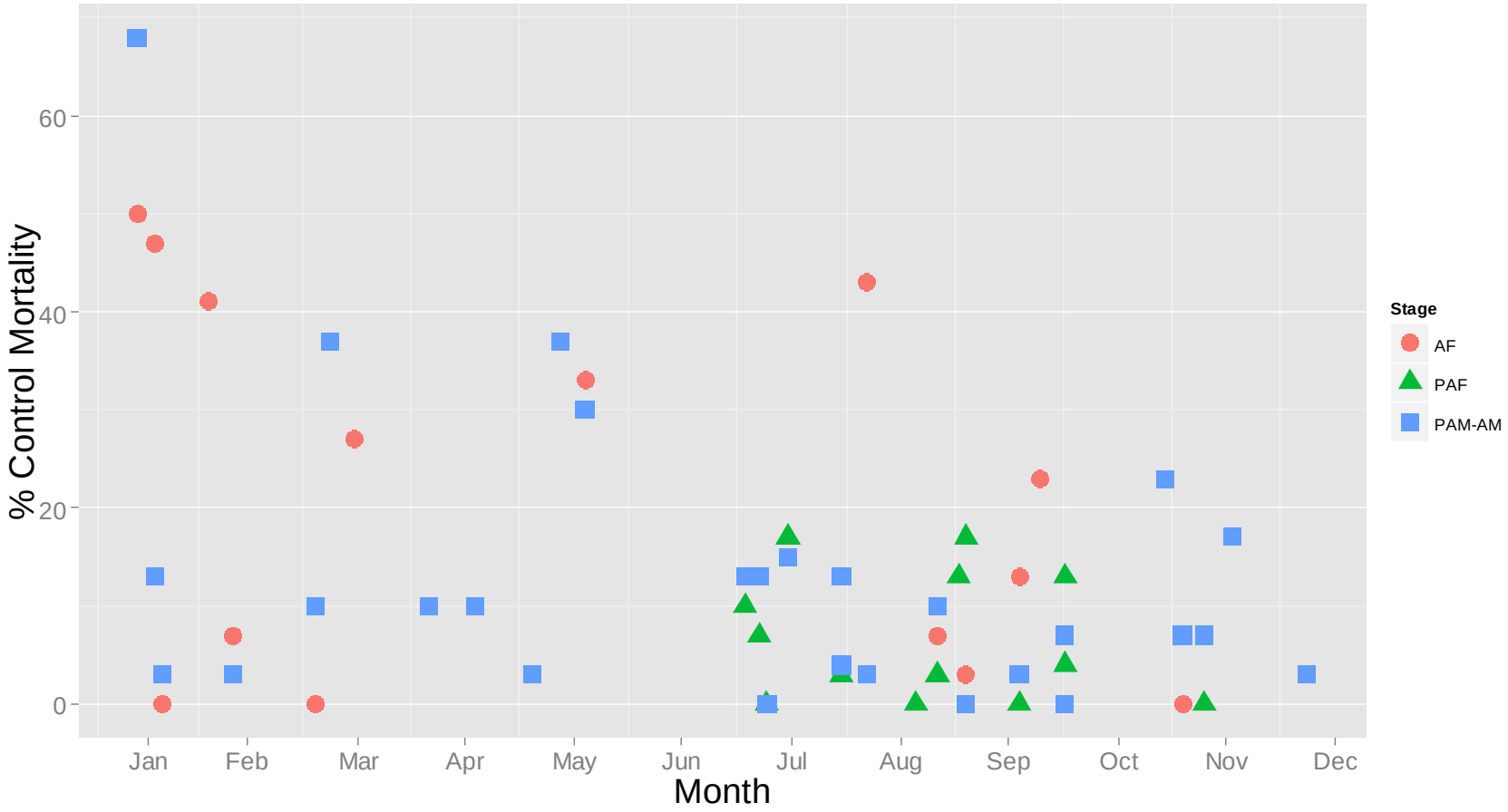

Figure 4 Percent control mortality of *Lepeophtheirus salmonis* by stage in deltamethrin bioassays conducted between 2009- 2011.
AF: Adult Female; PAF: Pre-Adult Female; PAM-AM: Pre-Adult Male and Adult Male.

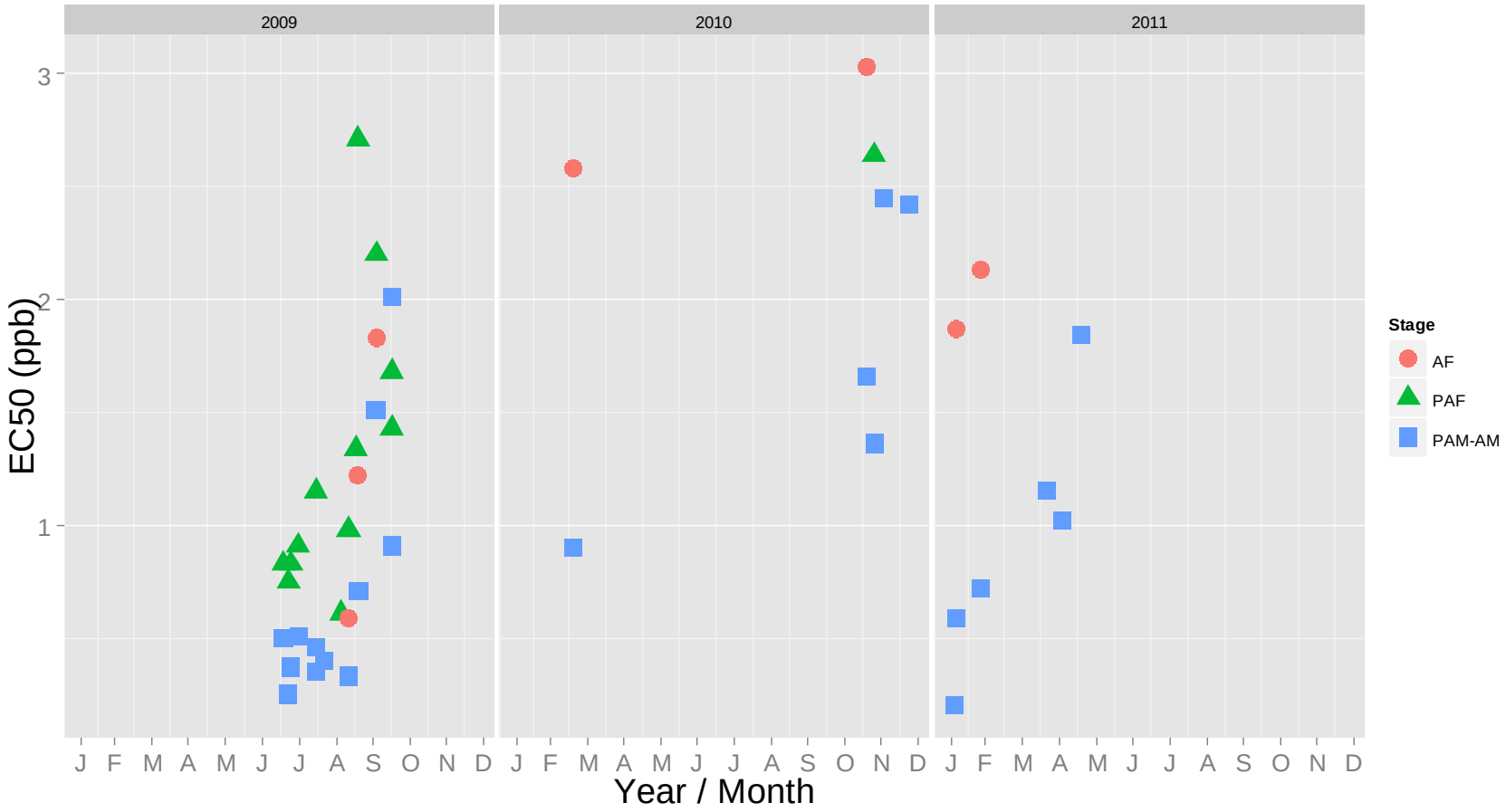

Figure 5 Deltamethrin bioassay EC_{50} values (ppb deltamethrin) for *Lepeophtheirus salmonis* collected from NB during 2009 to 2011. EC_{50} is the effective concentration of deltamethrin leading to a mortality of 50% of sea lice. PAF: Pre-Adult Female; PAM-AM: Pre-Adult Male and Adult Male; AF: Adult Female.