Evaluating bath treatment effectiveness in the control of sea lice burdens on Atlantic salmon in New Brunswick, Canada

Authors: R Gautam[1,2], R. Vanderstichel[1], A. S. Boerlage[1], C. W. Revie[1], K. L. Hammell[1]

[1]Department of Health Management and Centre for Veterinary Epidemiology Research, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PEI, Canada. [2]Ottawa Hospital Research Institute, University of Ottawa, ON, Canada.

Corresponding author:
Raju Gautam
Department of Health Management
Atlantic Veterinary College
University of Prince Edward Island
550 University Avenue, Charlottetown, PEI C1A 4P3
Canada
Email: rgautam@upei.ca; ragautam@ohri.ca

Keywords: post-treatment, lag time, sea lice, treatment effect

Running title: Bath treatment effectiveness for sea lice

Abstract

The use of medicinal bath treatment for sea lice is becoming more common, due to increasing resistance to in-feed treatments with emamectin benzoate. Common treatment modalities in New Brunswick, Canada, include Salmosan administered by tarpaulin or wellboat, and Paramove administered by wellboat. In this study, we assessed the effectiveness of these treatment modalities in the field between 2010 and 2015 using a web-based sea lice data management system (Fish-iTrends©). Effectiveness was evaluated for adult female (AF) and for pre-adult and adult male (PAAM) life-stages separately. We also investigated the impact of variability in pre-treatment lead and post-treatment lag time on effectiveness measures. There were 1,185 treatment events at 57 farms that uniquely matched our pre- and post-treatment count criteria. The effectiveness of treatment modality was significantly influenced by season, pre-treatment level of sea lice, and by lead and lag times. In summer, Salmosan administered by tarpaulin had the greatest effectiveness on both AF and PAAM, when pre-treatment levels were above 10 sea lice; whereas in autumn, the performance of treatment modalities varied significantly, depending on the pre-treatment levels for the life-stages. Ignoring the lead or lag time effect generally resulted in an underestimation of treatment effectiveness.

Introduction:

The sea louse is a major ectoparasite of salmon aquaculture. The two main species of sea lice found on the east cost of Canada are *Lepeophtheirus salmonis* and *Caligus elongatus* (Boxaspen 2006). Of the two species, *L. salmonis* is most specific to salmonids (Øines et al. 2006), and is the predominant species in the Atlantic salmon aquaculture industry along the east coasts of Canada and the USA (Westcott et al. 2004).

Sea lice, and their treatment, can adversely impact the health and welfare of wild and farmed salmon (Costello 2009a, b, Øverli et al. 2014) requiring repeated control efforts. In addition to general husbandry methods, such as the use of single year-class sites and area management coordination of stocking and fallowing, chemotherapeutants are often required to manage sea lice infestations on farmed salmon in marine net pens. Depending on the therapeutant, the method of delivery is either topical application as a bath treatment or an in-feed treatment (Grant 2002). The increased resistance of sea lice to the only approved in-feed chemotherapeutant, emamectin benzoate, and subsequent decline in treatment effectiveness (Lees et al. 2008, Jones et al. 2012), has increased the reliance on bath treatments in recent years on salmon farms in the Bay of Fundy, New Brunswick (NB), Canada. Regulatory restrictions limit the sea lice treatment options, including bath treatments, in the study area to azamethiphos (Salmosan ®) and hydrogen peroxide (Interox®Paramove™ 50) (Burridge et al. 2010). Although Alpha Max (Deltamethrin®) was used in 2009-10, its use was discontinued and any treatments using that compound were excluded from the assessment of treatment effectiveness in this study.

During the study period (2010-2015), primarily two different methods were used to deliver bath treatments against sea lice on Atlantic salmon farms in NB: tarpaulin enclosures, and wellboats (Corner et al. 2008, Bravo et al. 2010, Ernst et al. 2014, Gautam et al. 2016). The most common practices over the study period were to use a temporary tarpaulin enclosure to deliver azamethiphos (Salmosan ®) or a wellboat to deliver either Salmosan or hydrogen peroxide (Paramove™ 50). The method of tarpaulin use was considerably different before 2013, and we have categorized this use as "Early-tarpaulin-Salmosan" (ETS), as opposed to "Late-tarpaulin-Salmosan" (LTS) for administration after 2013. The primary difference is that early tarpaulin applications were restricted to 30-minute maximal exposures, due to the time-consuming practice of setting up the tarpaulin, which decreased water circulation and aeration, creating less favorable conditions for the salmon and reducing the time available for bath treatment. There were also temperature restrictions as per label for use of Salmosan by tarpaulin and as such industry tried to limit the use of tarpaulin in 2011 and 2012. Tarpaulin setting practices, aeration, and drug delivery methods became more efficient after 2013, and the enhanced practice routinely enabled durations exceeding 60 minutes; providing a more appropriate exposure time and better effect, using similar concentrations of drug. Thus, combining the different drugs and delivery methods, the four bath treatment modalities in operation between 2010 and 2015 in eastern Canada were: ETS, LTS, Wellboat-Salmosan (WS), and Wellboat-Paramove (WP).

Previously, studies have used a pre- and post-treatment counting window of 1-2 weeks in assessing bath treatment efficacy (Whyte et al. 2014, Arriagada et al. 2014). Lag time affects the post-treatment sea lice levels (Arriagada et al. 2014, Gautam et al. 2016a), but there is limited information on how lead and lag time affect the estimation of treatment effectiveness. Ensuring that bath treatment modalities are assessed in comparable ways facilitates appropriate decisions regarding expected treatment responses for different parasite life stages in different seasons. Such information is also important when monitoring long-term trends in resistance development. It is, therefore, critical to assess how estimates of treatment effectiveness may be modified after accounting for the differences in lead and lag times for pre- and post-treatment counts under production conditions.

The clinical response of sea lice to bath treatments is typically assessed at the net pen level by comparing average pre- and post-treatment sea lice abundances (Jones et al. 2012, Bravo et al. 2014). In previous studies we illustrated that there can be considerable variation in the lead and lag times associated with pre- and post-treatment counts, and that this can significantly affect the estimate of sea lice abundances (Gautam et al. 2016a; Gautam et al. 2016b). Although pre- and post-treatment sea lice abundances were also shown to be influenced by season, the effect of season on estimates of the treatment effect is, to our knowledge, unknown.

The two fold objectives of this investigation were (i) to determine the effectiveness of different bath treatment modalities in different seasons under field conditions, and (ii) to evaluate the differences in effectiveness estimates of bath treatments after accounting for lead and lag time effects, compared to estimates derived using more typical methods that ignore such effects.

Materials & Methods:

Source and description of data

The study area in the Bay of Fundy region of southwestern New Brunswick, in eastern Canada, and the aquaculture Bay Management Areas (BMAs) covered in this study have been described previously (Gautam et al. 2016b). Data consisting of bath treatment events and net pen-level sea lice abundance records for six years (January 2010 through December 2015) were obtained electronically from the sea lice data management system, Fish-iTrends©. Briefly, the database consisted of weekly producer-reported sea lice counts on samples of five or more fish per net pen from at least six cages per event from farms in the study area. In the event of a bath treatment, regulations require both pre- and post-treatment sea lice abundances to be reported, but the counting window is not dictated and, in these data, varied from 5 to 0 (i.e. same day) days before treatment, and 0 to 14 days after treatment.

We restricted the maximum lead and lag times to five days before and eight days after treatment, respectively (Gautam et al. 2016a). On occasions when counts were performed on multiple days before or after treatment, within this defined window, the counts closest to the treatment date were selected. Based on standard industry practice, sea lice counts were classified in terms of the following three life stages: i) chalimus (Chal), ii) pre-adults (males and females) and adult males (PAAM), and iii) adult females (AF). The practice of combining counts of pre-adults (both sexes) and adult males is generally adopted in NB to reduce observation error (Elmoslemany et al., 2013). Only data from cages with sea lice counts on five or more fish per net pen, pre- and post-treatment, were included. All treated fish groups were uniquely identified using site, net pen, date, and treatment event identifier (TID). However, fish groups were often split or merged between the two counting events (pre- and post-treatment), usually to facilitate the practical constraints in the delivery of treatment. This could lead to the TIDs being linked to multiple fish groups in pre-treatment, post-treatment, and/or both counting occasions (Gautam et al. 2016a). To avoid potential bias, TIDs associated with the splitting or merging of fish groups were excluded, and only those TIDs that matched uniquely to the fish groups at pre- and post-treatment counts were included in the analysis.

Most counting events occurred between mid-April and late December. For most treatment events, water temperature (^{o}C) was recorded, but the depth or time of day associated with this recording varied. Therefore, the predicted temperature from a locally-weighted scatterplot smoothing (LOESS) analysis was used to create a new variable, “season,” with three levels (Spring, Summer, and Autumn), as described previously (Gautam et al. 2016a). Briefly, “season” was defined to have the following categories: (i) spring (< 10 °C before August 31), (ii) summer (≥ 10 °C), and (iii) autumn (< 10 °C after August 31). Other potential predictors obtained from the database were: treatment date, treatment delivery method (e.g. tarpaulin enclosure, wellboat), and the trade name of the drug used for the bath treatment. Although treatment concentration and duration were reported, there were many inconsistencies and missing data in the records, so these predictors were dropped from the analyses.

We combined information from two treatment delivery methods (tarping and wellboat), and two chemicals (Salmosan and Paramove) used during the study period, to generate the four possible treatment modalities: i.e. WP, WS, ETS, and LTS. The final dataset consisted of 1,185 treatment events derived over six years (2010 to 2015), using records from 57 farms in 5 BMAs.

Statistical analysis

Treatment effectiveness for the four modalities was assessed by estimating and comparing post-treatment sea lice abundance and determining the percentage change in sea lice abundance between pre- and post-treatment counting events.

Post-treatment sea lice abundance

The average pre- and post-treatment sea lice abundances for the three life stages (AF, PAAM, Chal) were aggregated at the net pen level. The distributions of the response variables, post-treatment AF and PAAM levels, were right skewed, and a Box-Cox analysis was performed to determine the appropriate power transformation to improve normality and homoscedasticity.

Based on lambdas (λ) of -0.02 and 0.1 for PAAM and AF, respectively, from Box-Cox analyses, a natural log transformation was considered appropriate and used to transform the response variables. To achieve natural logarithmic transformation, either 0.5 or 1 was added – these values being determined after exploring values between 0.1 and 1 in the Box-Cox analyses to optimize normality of the data. The average pen-level pre-treatment PAAM and AF levels were log transformed using $\log_e$ (sea lice number +1), while the pre-treatment Chal level was transformed using $\log_e$ (Chal number + 0.5). The dataset was divided into two subsets: (i) spring season only (n = 127), and (ii) summer and autumn seasons combined (n = 1,058). The reason for splitting the data was that most of the treatment events in the spring season were associated with only one treatment modality (Wellboat-Paramove), while all four treatment modalities were used for sea lice control during the remainder of the year. A composite variable, "year-BMA", was created, using the variables year and BMA because there were few levels within each of these random effects variables. We developed linear mixed-effects models for the log-transformed post-treatment sea lice abundance with the composite variable, year-BMA and farm (site) as random effects, while accounting for nesting of site within year-BMA, which is mathematically represented as:

$$Y_{ijk} = (X\beta)_{ijk} + u_k + v_{j(k)} + \varepsilon_{ijk}$$

where Y_{ijk} is the i^{th} response (sea lice) from j^{th} site and k^{th} year-BMA, X is the matrix of fixed effects variables, β is the vector of fixed effects coefficients, u_k is the k^{th} year-BMA, $v_{j(k)}$ is the j^{th} site within k^{th} year-BMA, and ε is the error term.

Initial screening of fixed effect variables was performed using bi-variable analyses in a mixed-effects model. All variables significant at $P \leq 0.2$ during screening (i.e. on bi-variable analyses) were considered when developing the multivariable mixed-effects model. In the model-building process, variable selection was performed manually using a stepwise-forward selection method. All biologically plausible two-way interactions between the fixed-effects variables were investigated. The final model was selected using Akaike Information Criterion (AIC) (Dohoo et al. 2009). Separate models were developed for each of the two life stages (AF and PAAM), using the two subsets of data. The predicted post-treatment sea lice abundances for different treatment modalities were compared to assess the effect of treatment modality on average sea lice abundance post-treatment. Model residuals were graphically evaluated at each level of clustering, and both normality and homoscedasticity assumptions were deemed acceptable. Effect of treatment on chalimus was not assessed because bath treatments tend to have a limited effect on this life-stage of sea lice (Jimenez et al. 2013).

Percentage change (% effectiveness) in sea lice abundance

We also performed analyses that compared the percentage change (% effectiveness) in sea lice abundance pre- and post-treatment. Percentage change in sea lice was defined as the proportion of difference in sea lice abundance between pre- and post-treatment (i.e. count before, minus the count after, divided by the count before treatment). The percentage change was set to zero if this calculation resulted in a negative value (Whyte et al. 2016). Approximately 5% and 10% of the treatments for PAAM and AF, respectively, were associated with negative percentage change values (i.e. no apparent effect of treatment). The model developed for percentage change was the same as that described above, except that the outcome variable was substituted with the percentage (proportion) reduction in sea lice levels between pre-treatment and post-treatment counts.

Effect of excluding lead and lag times when estimating treatment effectiveness

To investigate the effect of ignoring various lead and lag times between treatment and counting events before and after treatment, we used the models described above for post-treatment sea lice abundance and percentage change in sea lice levels, but excluded the pre-treatment lead time and post-treatment lag time parameters. Estimated post-treatment sea lice abundance values using these reduced models for different treatment modalities were then compared with the estimated post-treatment abundance from the full models (i.e., the models which included lead and lag time effects).

All statistical analyses were performed using R v3.1.1 (R Development Core Team, 2014), and statistical significance was set at $P < 0.05$.

Results

The variables retained in the final model were post-treatment lag time, pre-treatment lead time, season, pre-treatment sea lice abundance, pre-treatment chalimus level, and treatment modality; with significant interactions between pre-treatment sea lice level and treatment modality, and between season and treatment modality, and post-treatment lag time. All the variables and the interaction terms included in the final model for the two subsets of data are listed in Table 1.

Of the total treatment events (N = 1,185), 127 (10.7%) were performed in spring, 776 (65.5%) in summer, and 282 (23.7%) in the autumn. In the subset data for autumn and summer, there were no observations for LTS on day 6 and only a few observations after day 6; therefore, analysis for lag days > 5 for this treatment modality was not performed. The two-season subset data included 1,045 (776 + 282 - 13) observations. For this subset of the data (I), the distribution of pre-treatment sea lice levels associated with each of the treatment modalities by season is shown in Figure 1. The second subset of data for spring consisted of primarily of treatments delivered in wellboats using Paramove (over 90%, n=115); therefore, analysis for this season was restricted to only one treatment modality (i.e. WP).

Post-treatment sea lice abundance

There were significant interactions between season and treatment modality, treatment modality and pre-treatment sea lice levels, as well as between post-treatment lag time and season. Abundance of sea lice post-treatment was significantly influenced by the pre-treatment level of sea lice, season, treatment modality, and the lead and lag times before and after treatment. Figure 2 shows the variation in post-treatment sea lice levels in two seasons (summer and autumn) for different treatment modalities at different levels of pre-treatment sea lice levels. Little difference in the predicted post-treatment sea lice levels was apparent among the treatment modalities when pre-treatment sea lice levels were low (< 5). However, as pre-treatment sea lice levels increased, predicted post-treatment abundance varied greatly across the different treatment modalities. For instance, in summer, the LTS method achieved the lowest post-treatment sea lice levels whenever the pre-treatment sea lice level was above 10 (for both AF and PAAM).

The fixed-effects coefficients and variance components, along with intra-class correlation coefficients (ICC) for different levels of clustering and sea lice life stages in summer and autumn, are shown in Table S1 (supplementary table). The abundance of sea lice (both AF and PAAM) increased steadily from day 2 to day 8 after treatment in summer. This trend was consistent, in summer, across all treatment modalities for both sea lice life stages. However, in autumn the reduction in post-treatment sea lice abundance was prolonged and there appeared to be more stability of low abundance levels between the day of treatment and up to eight days after treatment, indicating perhaps a lower level of recruitment (or a more sustained treatment effect, although not very likely). Coefficients and variance components, along with ICC for the model from the second subset of data, from spring (Data sub-set II), are also given in supplementary table S2.

Percentage change (% effectiveness) in sea lice abundance

The performance of different treatment modalities varied significantly in the two seasons (summer and autumn). In summer, the highest effectiveness (%) between pre- and post-treatment counts were observed on day 1 post-treatment for all treatments (Figure 3). The highest reduction (approximately 80%) in post-treatment AF was achieved with LTS and WP, with lower reductions for WS and ETS (Figure 3 upper left). A greater than 80% reduction in PAAM was also observed with LTS, while the other modalities indicated no more than a 65% reduction (Figure 3 lower left). Apart from minor differences, which may be due to an interaction effect between treatment modality and pre-treatment sea lice levels, the performance of different treatment modalities in summer is consistent with the results from the abundance analysis above. In the autumn season, WP showed better performance on AF than other treatment modalities, closely followed by LTS (Figure 3 upper right), which is consistent with the model for sea lice abundance (Figure 2 upper right). For PAAM, highest reduction was achieved with LTS, followed by WS and WP with similar reductions (Figure 3 lower right), which appear to be different from the abundance model (Figure 2 lower right). The apparent inconsistency in performance between the abundance model and % effectiveness model (Figure 2 lower right and Figure 3 lower right) could be explained by the presence of significant interaction effect and partly due to the differences in the pre-treatment sea lice levels (Figure 1). For instance, WS performed better at lower pre-treatment sea lice levels, and LTS performed better at higher sea lice levels before treatment.

Effect of lead and lag time on treatment effectiveness

The model predicted that post-treatment levels of sea lice were relatively higher when associated lead and lag times were ignored, in comparison to the scenario in which they were accounted for, and this was true for all treatment modalities except for LTS, where no such difference was evident (Figure 4). Increased post-treatment levels were predicted in both seasons (autumn and summer), with a noticeably greater difference for ETS in summer between the adjusted and un-adjusted models for both life stages (Figure 4).

Discussion

Bath treatments represent an important component of sea lice management, particularly due to the development of resistance to the only in-feed treatment, emamectin benzoate, available in Atlantic Canada in recent years (Jones et al, 2012). In eastern Canada, two chemotherapeutants are available for topical sea lice treatment: Salmosan and Paramove. While treatment with Salmosan can be delivered by tarpaulin or wellboat, Paramove is administered only by wellboat so far, although there is no restriction for its delivery by tarpaulin. In this study, the objective was to assess the field effectiveness of various treatment modalities (combinations of chemical and treatment delivery methods) and compare their performance across different seasons and varying levels of pre-treatment sea lice abundance, after controlling for other factors (e.g. lead and lag time of counting before and after treatment). We found that treatment effectiveness varied considerably among treatment modalities, depending on the sea lice life-stage, season, and pre-treatment abundance levels.

The influence of pre-treatment sea lice levels on post-treatment abundance varied for different treatment modalities and seasons, and depended on the life-stage of sea lice being assessed. For instance, in summer, post-treatment sea lice levels were lowest in pens treated using LTS for both life stages (AF and PAAM), irrespective of the level of pre-treatment sea lice, except at very low levels (< 5 lice) of pre-treatment abundance when there was no appreciable difference in post-treatment levels among treatment modalities. However, in autumn, treatment modalities performed differently for the two life-stages and varied by pre-treatment levels of the respective life-stages. The lowest post-treatment levels for AF were achieved with WP. For PAAM, performance of treatment modalities depended more on pre-treatment levels of PAAM (i.e., WS performed better when pre-treatment levels were below 30, while LTS performed better at higher levels). This may be partly related to the differences in the pre-treatment levels of sea lice among treatment modalities, as the treatment modality WS was often used in cages with significantly lower pre-treatment levels of sea lice in autumn (Figure 1). However, it is also recognized that many decisions factors related to choosing the treatment modality cannot be addressed in this analysis, including the effect of certain applications on salmon survival anticipated by farm managers during periods of peak water temperature. The difference in treatment performances of ETS and LTS could be related to the duration of exposure to treatment. Prior to 2013, treatment using Tarpaulin and Salmosan was typically administered for a shorter duration (approx. 30 minutes), which was later optimized to 1 hour. Relatively weak performance of ETS compared to LTS potentially suggests that shorter treatments are less successful.

To facilitate interpretation, as previously described (Gautam et al 2016a), season was defined using a temperature cut-off and time of year variable to assess the influence of temperature and season on the post-treatment sea lice abundance and/or effectiveness. Performance of all treatment modalities were relatively better (i.e., achieved lower lice levels post-treatment) in the summer than in the autumn, which indicates the potential effect of temperature and season on treatment effectiveness. Though the variation in treatment efficacies against different life stages of sea lice has been described previously (Branson et al. 2000, Sevatdal et al. 2005, Whyte et al. 2014), we are not aware of previous reports on different treatment performances by season. The abundance of AF and PAAM post-treatment in summer was lowest after a lag of one day, after which sea lice abundance increased steadily. On the other hand, lice abundance appeared to remain at low levels for a prolonged period following treatment in the autumn. More rapid increases in sea lice levels in summer, following treatment, may be related to high temperatures facilitating faster development of chalimus to pre-adult life stages (Johnson & Albright 1991).

We used both the abundance model (i.e., using post-treatment lice abundance as the outcome variable) as well as a model of proportion change in lice abundance before and after treatment; our findings were broadly consistent between these two models. While some of the inferences from the two models were repetitive, complementary information and interpretations were generated. For instance, it was not possible to discern the interaction effect between treatment modality and pre-treatment lice levels from the proportion change model, because it does not allow pre-treatment lice level to be used as an explanatory variable as it is already part of the outcome variable (i.e. the change in proportion). Similarly, the abundance model alone would not be able to reflect the magnitude of knockdown effect of treatment modalities, which has practical significance for the aquaculture industry.

The second objective of the study was to assess how ignoring lead and lag time affected the estimated treatment effectiveness. We found that, in general, ignoring lead and lag time resulted in a decrease in the estimated treatment effectiveness for all treatment modalities in both seasons. Previously, studies have used post-treatment counts taken at a variety of lag times, from one week to several weeks, to evaluate treatment efficacy of several chemicals (Lees et al. 2008, Jones et al. 2012, Jimenez et al. 2013). Awareness of this effect is important when assessing the potential for changing treatment responses over time, which is an expected observation when resistance is developing. While the estimation of treatment efficacy for in-feed treatments may not be affected by the lag time because it is administered over a longer time and is expected to exert a prolonged effect, due to long lasting tissue concentrations of the drug, the effectiveness of bath treatment could be significantly underestimated. The magnitude of difference observed for LTS was relatively low compared to differences observed for other treatment modalities. The reason for this could be that LTS represented treatment on and after 2013, when most post-treatment lice counts were carried out on day 1 and most pre-treatment counts were carried out closer to the treatment day (Gautam et al. 2016a,b).

We used a historical cross-sectional approach in this study, with weekly data recorded by aquaculture farmers, as required by regulations to enumerate and report sea lice counts on salmon farms in NB, Canada. The study has limitations associated with cross-sectional study design, such as an inability to draw certain types of conclusions (Levin 2006, Dohoo et al. 2009). Other limitations may include inconsistencies in the reported counts, as the sea lice were enumerated by multiple counters, and potential selection bias due to non-random sampling, because convenience sampling was used to obtain fish, as has been described before (Gautam et al. 2016b). However, any potential selection bias, if present, was expected to be homogeneously present across all lag times and treatment modalities and, therefore, not expected to significantly alter the interpretation of the study findings. Nevertheless, we recognise that the findings of this study would benefit from verification through a longitudinal study.

In summary, our findings indicate that season and pre-treatment level of sea lice affect treatment effectiveness estimates for different treatment modalities, and that ignoring lead and lag time when estimating treatment efficacy generally underestimates the effect. In order to make valid comparisons between two or more treatments it is important to account for differences in any lead or lag time between treatments and sea lice counting events. Alternatively, a protocol for counting sea lice before and after treatment should be standardized and implemented across the aquaculture industry.

Acknowledgements

Funding for this study was provided by the Canada Excellence Research Chair (CERC). Data was submitted by the salmon farmers of the Atlantic Canada Fish Farmers Association and managed by Fish-iTrends©. The authors thank William Chalmers for editorial assistance with the manuscript.

References

Arriagada G.A., Stryhn H., Campistó J.L., Rees E.E., Sanchez J., Ibarra R., Medina M., St-Hilaire S. (2014) Evaluation of the performance of pyrethroids on different life stages of *Caligus rogercresseyi* in southern Chile. *Aquaculture* **426-427**:231–237.

Boxaspen K. (2006) A review of the biology and genetics of sea lice. *ICES Journal of Marine Sci*ence **63**, 1304–1316.

Bravo S., Sepulveda M., Silva M.T., Costello M.J. (2014) Efficacy of deltamethrin in the control of *Caligus rogercresseyi* (Boxshall and Bravo) using bath treatment. *Aquaculture* **432**, 175–180.

Bravo S., Treasurer J., Sepulveda M., Lagos C. (2010) Effectiveness of hydrogen peroxide in the control of *Caligus rogercresseyi* in Chile and implications for sea louse management. *Aquaculture* **303**:22–27.

Burridge L., Weis J.S., Cabello F., Pizarro J., Bostick K. (2010) Chemical use in salmon aquaculture: A review of current practices and possible environmental effects. *Aquaculture* **306**:7–23.

Corner R.A., Marshall J., Hadfield B., Gowrie K., Wallace C., Davies P., Price C., Telfer T.C. (2008) A review of the sea lice bath treatment dispersion model used for discharge consenting in Scotland. In: Final Report to the Scottish Aquaculture Research Forum. Project No. SARF 023.

Costello M.J. (2009a) The global economic cost of sea lice to the salmonid farming industry. *Journal of Fish Diseases* **32**, 115–118.

Costello M.J. (2009b) How sea lice from salmon farms may cause wild salmonid declines in Europe and North America and be a threat to fishes elsewhere. *Proceedings of the Royal Society B: Biological Sciences* **276**, 3385–3394.

Dohoo I., Martin W., Stryhn H. (2009) Mixed models for continuous data. In: *Veterinary Epidemiologic Research* (ed. by S. Margaret McPike), 2nd edn, pp. 553-577. VER Inc., Charlottetown, PE.

Ernst W., Doe K., Cook A., Burridge L., Lalonde B., Jackman P., Aubé J.G., Page F. (2014) Dispersion and toxicity to non-target crustaceans of azamethiphos and deltamethrin after sea lice treatments on farmed salmon, *Salmo salar*. *Aquaculture* **424-425**:104–112.

Gautam R., Vanderstichel R., Boerlage A.S., Revie C.W., Hammell K.L. (2016a) Effect of timing of count events on estimates of sea lice abundance and interpretation of effectiveness following bath treatments. *Journal of Fish Diseases* (**in press**).

Gautam R., Boerlage A.S., Vanderstichel R., Revie C.W., Hammell K.L. (2016b) Variation in pre-treatment count lead time and its effect on baseline estimates of cage-level sea lice abundance. *Journal of Fish Diseases* DOI: 10.1111/jfd.12460 [Epub ahead of print].

Grant A.N. (2002) Medicines for sea lice. *Pest Management Science* **58**, 521–527.

Jimenez D.F., Revie C.W., Hardy S.P., Jansen P.A., Gettinby G. (2013) Multivariate evaluation of the effectiveness of treatment efficacy of cypermethrin against sea lice (*Lepeophtheirus salmonis*) in Atlantic salmon (*Salmo salar*). *BMC Veterinary Research* **9,** 258.

Jones P., Hammell K., Dohoo I., Revie C. (2012) Effectiveness of emamectin benzoate for treatment of *Lepeophtheirus salmonis* on farmed Atlantic salmon *Salmo salar* in the Bay of Fundy, Canada. Diseases of Aquatic Organisms **102**, 53–64.

Lees F., Baillie M., Gettinby G., Revie C.W. (2008) The Efficacy of Emamectin Benzoate against Infestations of *Lepeophtheirus salmonis* on Farmed Atlantic Salmon (*Salmo salar L*) in Scotland, 2002–2006. *PLoS ONE* **3**, e1549.

Øines Ø., Simonsen J.H., Knutsen J.A., Heuch P.A. (2006) Host preference of adult *Caligus elongatus* Nordmann in the laboratory and its implications for Atlantic cod aquaculture. *Journal of Fish Diseases* **29**, 167–174.

Øverli Ø., Nordgreen J., Mejdell C.M., Janczak A.M., Kittilsen S., Johansen I.B., Horsberg T.E. (2014) Ectoparasitic sea lice (*Lepeophtheirus salmonis*) affect behavior and brain serotonergic activity in Atlantic salmon (*Salmo salar L.*): Perspectives on animal welfare. *Physiology and Behavior* **132**, 44–50.

Westcott J.D., Hammell K.L., Burka J.F. (2004) Sea lice treatments, management practices and sea lice sampling methods on Atlantic salmon farms in the Bay of Fundy, New Brunswick, Canada. *Aquaculture Research* **35**, 784–792.

Whyte S.K., Jimenez D., Revie C.W., Hammell K.L. (2016) Multivariate evaluation of the effectiveness of delousing treatment efficacy of azamethiphos (Salmosan®) against the salmon louse (*Lepeophtheirus salmonis*) in Atlantic salmon (*Salmo salar*) using wellboat, skirt and tarpaulin treatment modalities. *Aquaculture* **450**:301–307

Whyte S.K., Westcott J.D., Jimenez D., Revie C.W., Hammell K.L. (2014) Assessment of sea lice (*Lepeophtheirus salmonis*) management in New Brunswick, Canada using deltamethrin (AlphaMax®) through clinical field treatment and laboratory bioassay responses. *Aquaculture* **422-423**, 54–62.

Table Legends

Table 1: Variables for the fixed effects and interaction terms included in the final model for the two subsets of data.

Variables **Fixed effects**	Subset data I (summer and autumn)	Subset data II (for spring only)
Pre-treatment lead time	√	√
Pre-treatment lice level	√	√
Pre-treatment chalimus level	√	√
Season	√	
Post-treatment lag time	√	√
Treatment modality	√	
Interaction terms		
Pre-treatment level x Treatment modality		
Season x Treatment modality	√	
Post-treatment lag time x Season	√	

Figure legends

Figure 1. Distribution of pre-treatment sea lice levels by treatment modalities and season for the two life stages, adult females (AFs) in the upper panel and pre-adults and adult males (PAAM) in the lower panel.

Figure 2. Predicted interaction plots showing how the effect of the four treatment modalities, post-treatment, vary at different pre-treatment sea lice levels, while keeping other factors constant, for different life-stages and season: (a) adult female (AF) in summer, (b) AFs in autumn, (c) pre-adult and adult males (PAAM) in summer, and (d) PAAMs in autumn. Abbreviations used: ETS (Early tarping with Salmosan); LTS (Late tarping with Salmosan); WP (wellboat with Paramove); WS (wellboat with Salmosan).

Figure 3. Linear mixed model predicted average proportion change in sea lice levels between pre- and post-treatment counts by lag days and treatment modalities for different life-stages and season: (a) adult females (AF) in summer (b) AFs in autumn, (c) pre-adults and adult males (PAAM) in summer, and (d) PAAMs in autumn. Abbreviations used: ETS (Early tarping with Salmosan); LTS (Late tarping with Salmosan); WP (wellboat with Paramove); WS (wellboat with Salmosan). [*]LTS is terminated at day 5 because there were zero observations on day 6 and few observations on later days.

Figure 4. Mixed model predicted average sea lice reduction (%) with 95% confidence interval (error bars) post-treatment by treatment modality. The upper panel compares predicted adult female (AF), and the bottom panel pre-adult and adult males (PAAM) between the full (controlled for lead and lag time) and reduced (ignoring the effect of lead and lag time) models. The extension "-R" to the treatment modality refers to the reduced model.

Figure 1. Distribution of pre-treatment sea lice levels by treatment modalities and season for the two life stages, adult females (AFs) in the upper panel and pre-adults and adult males (PAAM) in the lower panel.

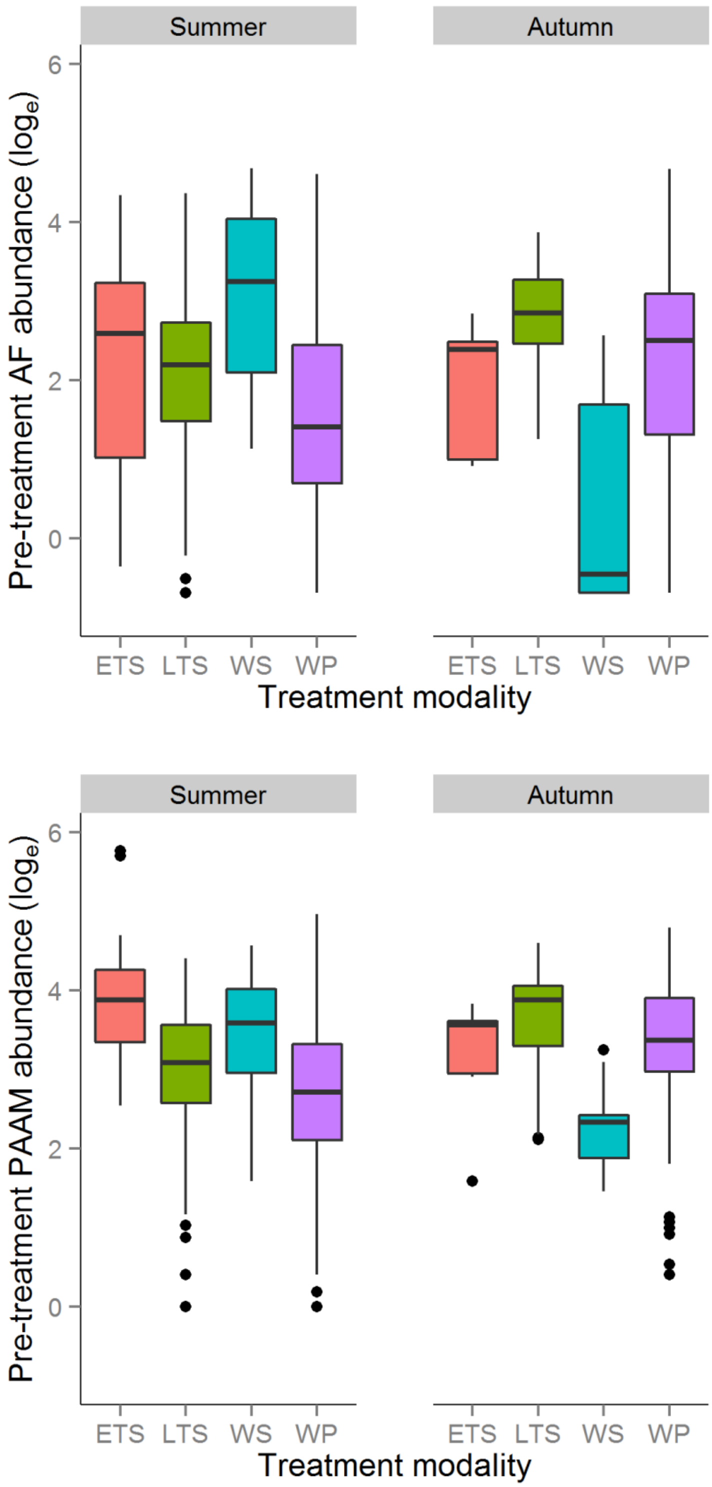

Figure 2. Predicted interaction plots showing how the effect of the four treatment modalities, post-treatment, vary at different pre-treatment sea lice levels, while keeping other factors constant, for different life-stages and season: (a) adult female (AF) in summer, (b) AFs in autumn, (c) pre-adult and adult males (PAAM) in summer, and (d) PAAMs in autumn. Abbreviations used: ETS (Early tarping with Salmosan); LTS (Late tarping with Salmosan); WP (wellboat with Paramove); WS (wellboat with Salmosan).

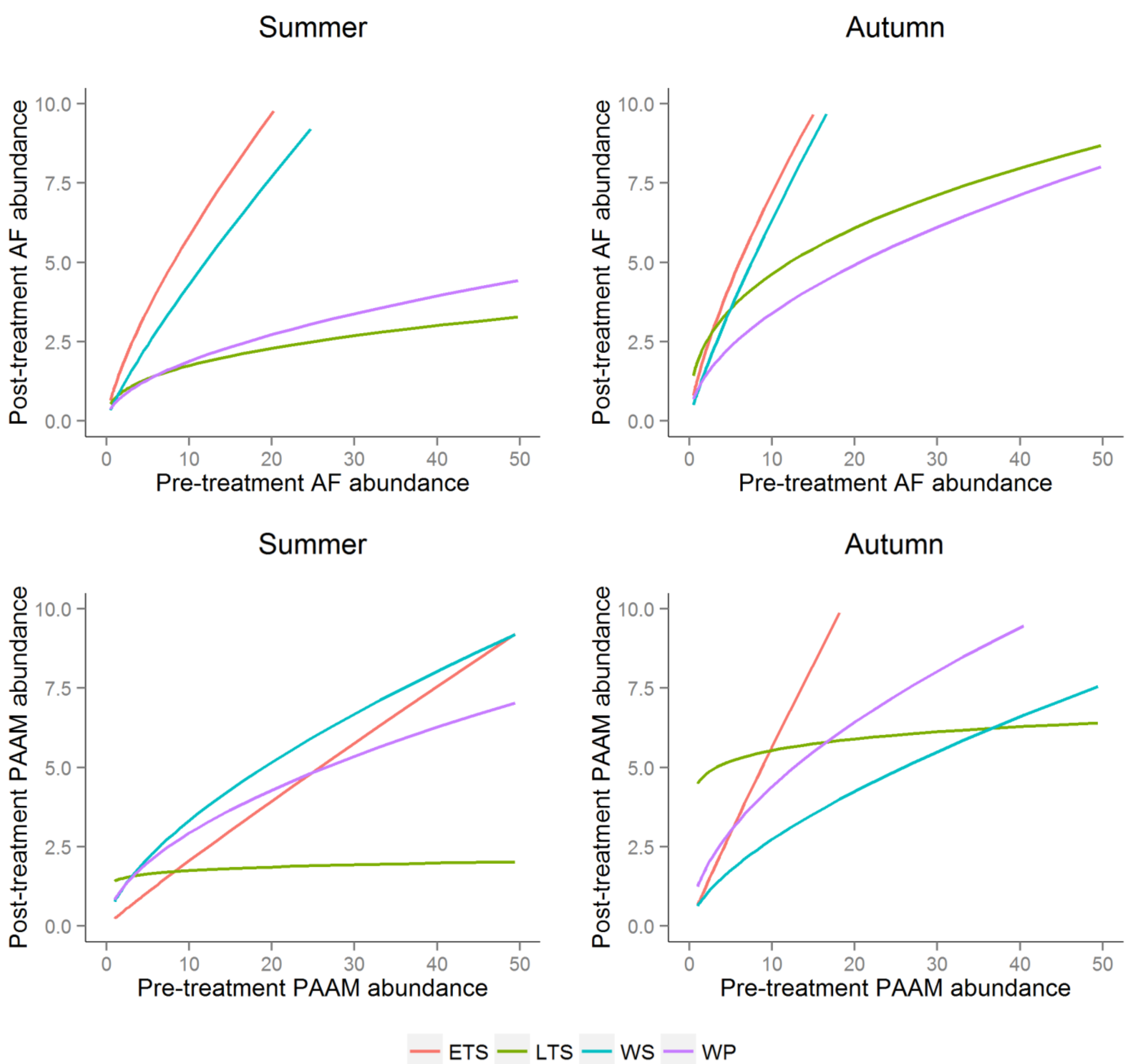

Figure 3. Linear mixed model predicted average proportion change in sea lice levels between pre- and post-treatment counts by lag days and treatment modalities for different life-stages and season: (a) adult females (AF) in summer (b) AFs in autumn, (c) pre-adults and adult males (PAAM) in summer, and (d) PAAMs in autumn. Abbreviations used: ETS (Early tarping with Salmosan); LTS (Late tarping with Salmosan); WP (wellboat with Paramove); WS (wellboat with Salmosan). *LTS is terminated at day 5 because there were zero observations on day 6 and few observations on later days.

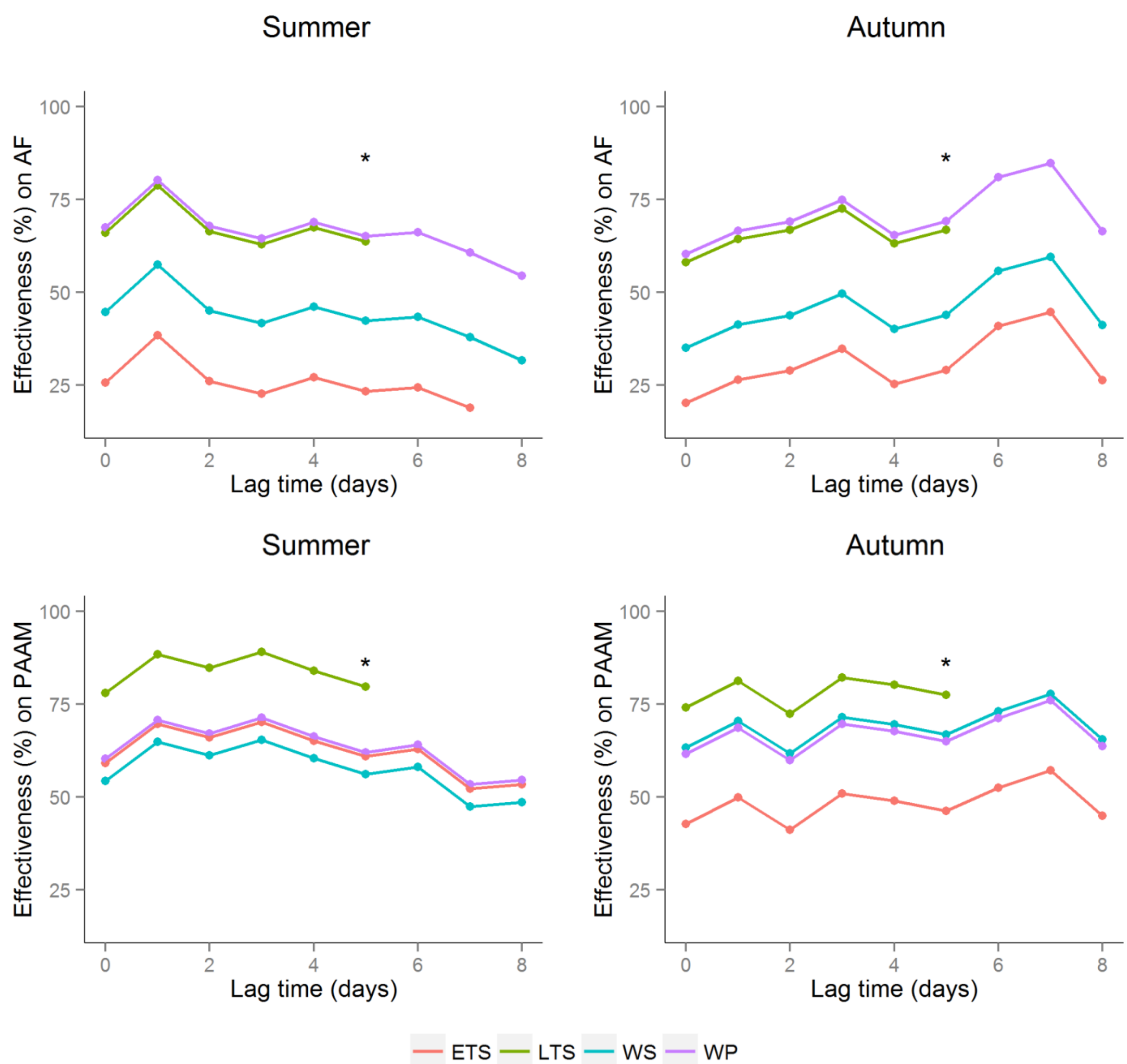

Figure 4. Mixed model predicted average sea lice reduction (%) with 95% confidence interval (error bars) post-treatment by treatment modality. The upper panel compares predicted adult female (AF), and the bottom panel pre-adult and adult males (PAAM) between the full (controlled for lead and lag time) and reduced (ignoring the effect of lead and lag time) models. The extension "-R" to the treatment modality refers to the reduced model.