Variation in pre-treatment count lead time and its effect on baseline estimates of cage-level sea lice abundance

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Running title: Effect of lead time on sea lice baseline estimation

Abstract

Treatment efficacy studies typically use pre-treatment sea lice abundance as the baseline. However, the pre-treatment counting window often varies from the day of treatment to several days before treatment. We assessed the effect of lead time on baseline estimates, using historical data (2010-2014) from a sea lice data management program (Fish-iTrends). Data were aggregated at the cage level for three life-stages: (i) chalimus, (ii) pre-adult and adult male, and (iii) adult female. Sea lice counts were log transformed and mean counts by lead time relative to treatment day were computed and compared separately for each life stage, using linear mixed models. There were 1,658 observations (treatment events) from 56 sites in 5 Bay Management Areas. Our study showed that lead time had a significant effect on the estimated sea lice abundance, which was moderated by season. During the late summer and autumn periods, counting on the day of treatment gave significantly higher values than other days and would be a more appropriate baseline estimate, while during spring and early summer abundance estimates were comparable among counts within 5 days of treatment. A season-based lead time window may be most appropriate when estimating baseline sea lice levels.

Introduction

Sea louse, a major parasite of salmon aquaculture worldwide, poses a consistent threat to production loss, health and welfare of wild and farmed fish (Costello 2009b a; Øverli, Nordgreen, Mejdell, Janczak, Kittilsen, Johansen & Horsberg 2014). The two predominant species of sea lice found on salmon in Eastern Canada are *Lepeophtheirus salmonis* and *Caligus elongatus* (Boxaspen 2006, DFO 2014). A major component of sea lice management is the use of chemical therapeutants administered topically as bath treatment or orally in the feed (Grant 2002). The use of bath treatment for sea lice management has become increasingly important because sites have experienced varying levels of resistance to the widely used in-feed treatments (Jones, Hammell, Dohoo & Revie 2012; Lees, Baillie, Gettinby & Revie 2008). Clinical responses to bath treatments are assessed at the cage-level by comparing average sea lice abundance pre-treatment with post-treatment levels.

In practice, there can be a lead time of one or more days between pre-treatment counting and treatment events due to the limited availability of personnel and equipment during busy treatment periods or adverse weather conditions. Previously published in-feed treatment effectiveness studies have arbitrarily accepted sea lice abundance data from counting events anytime within a window of 16 days prior to treatment as the baseline abundance (Lees *et al.* 2008; Jones *et al.* 2012). These studies were published in times when in-feed treatments were effective and sea lice levels were expected to change at a slower rate. However, it is unknown whether such a window was valid then or in situations of rapidly increasing sea lice abundance. Current industry standards in Eastern Canada restrict the counting window to 5 days prior to administering a bath treatment. There is limited information on the effects of varying lead times between pre-treatment sea lice counting and bath treatment events on the actual pre-treatment sea lice abundance estimate that is used to evaluate a treatment. As the number of sea lice tend to increase over time before treatment, it can be hypothesized that a long length of lead

time between a counting event and treatment may result in significantly different sea lice abundance compared to a shorter lead time between a counting event and treatment. This difference may result in imprecise and/or bias in treatment evaluations due to increased variation unrelated to actual treatment responses.

The objectives of this study were to 1) determine whether the estimated sea lice abundance is affected by the length of lead time between pre-treatment counting event and bath treatment, and if so, 2) identify a suitable window of lead time between pre-treatment counting and treatment events that the estimated figure would not be significantly different if the counting was done on the day of the treatment. This will allow more appropriate comparison of treatment responses across treatments in aquaculture production settings.

Materials & Methods

Source and description of data

The study population is the Bay of Fundy aquaculture region of southwestern New Brunswick, Canada. Producers in this region use a bay management system (Aquaculture Bay Management Area or ABMA) for location and stocking of fish (Jones et al. 2012). Bath treatment events and cage-level sea lice abundance data from January 2010 to December 2014 were obtained electronically from the sea lice data management system, Fish-iTrends. This closed-access web-based sea lice information program was developed by the Atlantic Veterinary College, University of Prince Edward Island (UPEI) to manage data from different users and to generate real-time data visualization and descriptive summary graphical outputs for participating industry partners and authorities.

Participating industry partners enter fish-level sea lice count data as it becomes available. Regional regulations require weekly samples of 5 or more fish per cage from at least 6 cages. In the event of a bath treatment, both pre-treatment and post-treatment sea lice abundances must

be reported and include classification of the following life stages: 1) chalimus (Chal), 2) pre-adult (male and female) and adult male (PAAM), and 3) adult female (AF). Standard industry practice is to combine pre-adult (both sexes) and adult male (PAAM) abundances into one category to improve classification– these categories are difficult to distinguish visually in the field, and are influenced by the counter's experience level (Elmoslemany, Whyte, Revie & Hammell 2013). In keeping with the 5-day window counting restriction imposed in eastern Canada, we included counts with a maximal lead time of 5 days prior to a bath treatment event. If a counting event on a site was performed multiple times within the 5-day window, only the count closest to the treatment event was included. Unique identifiers tracked fish groups using site, cage, and treatment event. Any group that was mixed or merged with fish from another group was considered a new group with missing data prior to the merging event.

Water temperature (^{o}C) was recorded on site for most of the counting events. However, due to the site-to-site variation in measurement protocols (e.g. measurement depth or time of day) we predicted temperatures, using locally weighted scatterplot smoothing (LOWESS), to estimate general temperature trends. A new variable, "season", was created using a combination of water temperature cut-off (at 10 ^{o}C) and time of the year (peak summer temperatures at the end of August) to define the following categories: (i) spring (temperatures ≥ 4 ^{o}C and < 10 ^{o}C before and including August 31), (ii) early summer (≥ 10 ^{o}C before and including August 31), (iii) late summer (≥ 10 ^{o}C after August 31) and (iv) autumn (< 10 ^{o}C after August 31). Figure 1 illustrates the characterization of season using temperature data for 2010 – note that most of the counting events occurred between mid-April and late December year after year, representing approximately 9 months per year. A similar approach was applied to each year (only 2010 is shown as an example).

Average sea lice abundance per fish for the three life stages (Chal, PAAM, and AF) were analysed as outcome variables. For each life stage, data were aggregated at the cage level

(mean sea lice abundance per fish) from a sample of 5 or more fish for each sampling event. Cage records with fewer than 5 fish per event were excluded from the analyses. Cage-level means of sea lice abundance per fish for each life stage were transformed using the natural log (i.e. $\log_e$(sea lice number + 1)) to improve the normality and homoscedasticity of model residuals (Dohoo, Martin & Stryhn 2009). The final dataset consisted of 1,658 treatment events derived from 5 years of records. All data management and statistical analyses were performed using R v3.1.1 (R Development Core Team, 2014).

Statistical analysis

Linear mixed-effects models were developed for the log-transformed sea lice abundance with lead time of pre-treatment sea lice abundance and season as fixed effects. Year and ABMA were combined to form a composite variable (year-bay management). This composite variable and site were included as random effects to account for the nested structure of the data. Interaction between lead time and season was investigated when developing the models. Model selections were performed by comparing Akaike Information Criterion (AIC) and using the likelihood ratio test (Dohoo *et al.* 2009). Separate models were developed for each of the three sea lice life stages, and an additional model was developed for total mobiles (AF+PAAM). The predicted average sea lice abundance per cage per fish for different lead times (by days 1 to 5, inclusive) were compared with those with no lead time (i.e. a count performed on the day of the treatment event was recorded as day 0) to assess the effect of lead time on average sea lice abundance. The normality and homoscedasticity assumptions of the model residuals were assessed graphically for each level of clustering and deemed acceptable.

Results

There were 1,658 treatment events uniquely linked to the treated fish groups, from 56 sites in 5 ABMA over a 5 year period for all three life stages of sea lice. Most of the cage means for sea

lice were obtained from 10 fish samples (57%), followed by 5 fish samples (34%); the maximum number of fish sampled in a cage was 40 (one cage). In 2010 – 2012, the number of counting events was evenly distributed across the observed lead times (0 to 5 days pre-treatment). However, in 2013 and 2014, there was a tendency to count more frequently on the same day of treatment (i.e. day 0) with fewer counts as the length of lead time increased (Figure 2). Sea lice abundance per fish decreased in 2011, 2013 and 2014 compared to 2010 (Figure 3). Table 1 shows the fixed effect and the variance component along with the intra-class correlation coefficient for each level of clustering.

The interaction term between the lead time and season significantly improved the models for each sea lice life stage ($P_{Likelihood} < 0.01$), implying that temperature (season) affects the relationship between lead time and sea lice abundance. During spring, the average AF abundance per fish was not different between the lead days. In the same period, PAAM and total mobile abundance per fish were not different if counted within three days of treatment, but were significantly lower ($P < 0.001$) when counted on day 4 to treatment (Figure 4a, Table 1). During early summer, the average PAAM abundance per fish was not different between lead times. Differences between lead times for AF and Chal abundances in early summer were significant, but the magnitude of the difference was relatively small (< 0.425 $\log_e$ (1.52 lice) for AF and < 0.67 $\log_e$ (1.95 lice) for Chal) (Figure 4b, c). During late summer, average sea lice abundances for most lead times were significantly ($P < 0.05$) different than sea lice abundances at the day of treatment (Figure 4c), but the magnitude of difference was again relatively small (< 0.37 log (1.4 lice)). During autumn when water temperatures were below 10 ^{o}C, sea lice abundance at lead times of five to one day were significantly different ($P<0.05$) from sea lice abundance at the day of treatment, and in addition there was a trend of decreasing sea lice with increasing lead time length (Figure 4a,b,c). The average AF abundance is greater than other

two life stages (PAAM and Chal) in spring low temperatures, while it is less than PAAM during other seasons (Figure 4a b,c).

Discussion

To our knowledge, this is the first study reporting the effect of lead time on the estimated abundance of sea lice before chemical treatment. Pre-treatment estimation of sea lice abundance has been used as the baseline measure to evaluate the treatment efficacy of several chemicals (Gustafson, Ellis, Robinson, Marenghi & Endris 2006; Lees *et al.* 2008; Saksida, Morrison & Revie 2010; Jones *et al.* 2012). The majority of these studies used pre-treatment counts recorded up to 16-21 days before the treatment event as the baseline for comparison and/or evaluation of treatment efficacy.

Obviously, estimates of sea lice abundance done as close (but before) a bath treatment as possible would be optimal. However, the logistics and timing are often constrained by uncontrollable production management factors, lead to a variety of lead time periods for pre-treatment counting events being used. Our study objective was to determine if there was an effect of lead time length between the pre-treatment sea lice count and the time of treatment on the sea lice estimates and to quantify that influence on the baseline abundance estimate of sea lice. The study showed that there was an effect of lead time length on the estimated sea lice and the effect was moderated by season (here defined by a temperature cut-off and time of the year). The findings suggest that in spring pre-treatment sea lice counts within three days of the treatment event will provide comparable estimates, while in other seasons, especially in autumn, it would be more appropriate to count on the same day of treatment to determine the baseline estimate of sea lice. The longer length of lead time required to show a significant difference in abundance estimate during spring low temperature may be explained by the

increasing duration of the sea lice lifecycle at low environmental temperatures, which slows the development of their life stages (Stien, Bjørn, Heuch & Elston 2005; Boxaspen 2006).

Sea lice abundance for all life stages during spring low temperature was generally low as opposed to the other seasons. This finding is consistent with previously published report that sea lice persist over winter on farmed salmon, but at reduced prevalence and intensity (Chang, Page, Beattie & Hill 2011; Jones & Johnson 2015). At low temperatures, the development of eggs and planktonic stages of sea lice is significantly prolonged (Johnson & Albright 1991; Boxaspen & Naess 2000), which may also contribute to lower abundance of sea lice in the spring compared to the other seasons. The predominance of AF in spring (compared to PAAM and Chal) reflects their long life span and ability to overwinter (Mustafa, Conboy, Burka, Hendry & McGladdery 2000; Boxaspen 2006). Most AF in the spring are likely older lice that have survived the winter period when few, if any, early life stages are attached. Therefore, changes in lice abundance were less dynamic during this period.

Previously, Lees et al. (2008) had used pre-treatment counts within 16 days of treatment as baseline for evaluating treatment efficacy. We restricted our temporal window to within five days of treatment because industry practice in the Bay of Fundy during this study period limits the counting window to within five days of a treatment event.

This study utilized historical cross-sectional data recorded weekly by the fish farmers who were required to enumerate and report sea lice counts on Atlantic salmon. The study therefore, may have inherent limitations associated with cross-sectional studies (Levin 2006; Dohoo *et al.* 2009), including for example, a lack of consistency due to counting of sea lice by many counters. Additionally, fish were typically sampled using convenience sampling technique (i.e. attracting fish to the water surface with feed and capturing them using dip nets), and potential non-random sampling, as present in this study, may introduce selection bias. However, the

potential selection bias was assumed to be homogenously present across different lead times, and therefore should be inconsequential to the interpretation of the effect of lead time length on the estimated mean abundance of sea lice across different lead times. Overall, this study benefited from the use of a large dataset to improve the statistical power.

For the purpose of this study and ease of interpretation, season was defined using both a specific cut-off value for the water temperature and an annual date to assess potential moderation of both temperature and season on the effect of lead time on sea lice abundance. Although, spring and autumn were defined by the same temperature cut-off value, there was a clear difference in the effect of lead time on sea lice abundance between the two seasons. This difference is likely due to the contrast of the initial spring sea lice load and exposure of sea lice to warming temperatures after a period of overwintering, compared to higher sea lice loads carried over from the sustained warmer summer temperatures, immediately before start of autumn. These differences between the two seasons could affect the maturation rate for the developmental stages of sea lice and the abundance of earlier life-stages, which in turn would influence the effect of lead time.

In conclusion, increased lead time between counting and treatment events affects the estimated baseline value of pre-treatment sea lice abundance in Atlantic salmon. Since this baseline value is used to calculate the treatment response by comparing pre-treatment to post-treatment counts, it is important that the estimates at different lead times be as close to the estimate of sea lice if there was no lead time. This effect depends on seasonal variation of temperature; therefore a season-based maximum lead time length may provide the best balance between comparable treatment evaluation and practical considerations surrounding shortened length of lead times. In our study area, counting events that occurred on the day of treatment provided the highest baseline estimate of sea lice abundance during late summer and autumn than other days. During the spring and early summer, the timing of pre-treatment counting event within a 5

day window did not appear to influence the estimated mean abundance to vary significantly from what would be estimated if the counting was done on the day of treatment.

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Table Captions

Table 1: Final multivariable mixed effects model with variables associated with the mean abundance of different life-stages of sea lice in farmed Atlantic salmon (N = 1,658) from 56 farms in 5 Aquaculture Bay Management Areas (ABMAs) over five year (2010 through 2014) in the Bay of Fundy, New Brunswick.

Fixed effects

	PAAM		AF		Chal		Total mobile	
Variables	β	P value	β	P value	β	P value	β	P value
Lead days before treatment								
Day 0	Ref		Ref		Ref		Ref	
Day -1	-0.24	0.029	-0.09	0.349	-0.24	0.045	-0.26	0.009
Day -2	-0.76	< 0.001	-0.13	0.236	-0.19	0.180	-0.64	< 0.001
Day -3	-0.66	<0.001	-0.46	< 0.001	-0.21	0.169	-0.63	< 0.001
Day -4	-0.95	< 0.001	-0.54	0.001	-0.70	0.001	-0.92	< 0.001
Day -5	-0.32	0.086	-0.62	< 0.001	-0.17	0.400	-0.47	0.007
Season								
Autumn	Ref		Ref		Ref		Ref	
Spring	-2.54	< 0.001	-1.64	< 0.001	-1.18	< 0.001	-2.29	< 0.001
Early summer	-1.15	< 0.001	-1.07	< 0.001	-0.57	< 0.001	-1.19	< 0.001
Late summer	-0.46	<0.001	-0.40	< 0.001	0.29	< 0.001	-0.46	< 0.001
Lag days : Season interaction								
Day 0 : Autumn	Ref		Ref		Ref		Ref	
Day -1 : Spring	0.18	0.325	0.04	0.799	0.31	0.119	0.21	0.203
Day -2 : Spring	0.81	0.001	0.27	0.209	-0.24	0.364	0.76	0.001
Day -3 : Spring	0.35	0.128	0.22	0.284	-0.52	0.044	0.29	0.176
Day -4 : Spring	0.28	0.291	0.29	0.233	0.08	0.791	0.26	0.291
Day -5 : Spring	-0.06	0.840	0.46	0.083	-0.16	0.617	0.14	0.615
Day -1 : Early summer	0.42	0.005	-0.19	0.165	0.62	< 0.001	0.32	0.021
Day -2 : Early summer	0.81	0.000	-0.29	0.063	0.50	0.010	0.59	< 0.001
Day -3 : Early summer	0.74	0.000	0.36	0.043	0.69	0.002	0.68	< 0.001
Day -4 : Early summer	1.19	0.000	0.43	0.057	1.40	< 0.001	1.05	< 0.001
Day -5 : Early summer	0.60	0.023	0.42	0.075	0.57	0.051	0.63	0.009
Day -1 : Late summer	0.00	0.998	-0.22	0.067	0.17	0.242	0.02	0.892
Day -2 : Late summer	0.53	0.000	-0.16	0.239	0.13	0.421	0.39	0.005
Day -3 : Late summer	0.36	0.023	0.14	0.316	0.01	0.946	0.30	0.041
Day -4 : Late summer	0.78	0.000	0.29	0.131	0.65	0.006	0.74	< 0.001
Day -5 : Late summer	0.16	0.449	0.25	0.180	0.15	0.516	0.27	0.162

Random effects

Level	*Variance*	*ICC*	*Variance*	*ICC*	*Variance*	*ICC*	*Variance*	*ICC*
Year-BMA	0.38	0.35	0.96	0.59	0.41	0.26	0.54	0.48
Site	0.22	0.20	0.29	0.18	0.60	0.38	0.17	0.15
Residual	0.48		0.38		0.58		0.41	

Figure legends

Figure 1: An illustration of the criteria used to categorize season using water temperature and time of the year showing study year 2010 as an example. The solid red line represents the locally weighted scatterplot smoothing (LOWESS) for water temperatures and the solid circles are the recorded water temperatures. Spring and autumn were determined with a temperature cut-off value at 10 ^{o}C, while early and late summer were categorized as before or after (and including) September 1st.

Figure 2: Distribution of cage-level observations of the (a) number of treatment events and (b) percentage of treatments, both by lead time (in days) and stratified by year.

Figure 3. Distribution of mean sea lice abundance per cage per fish (in $\log_e$ scale) by lead time, and stratified by year, for three sea lice life stages: (a) pre-adult and adult male (PAAM), (b) adult female (AF), and (c) chalimus (Chal).

Figure 4. Linear mixed-effects model prediction for cage-level mean sea lice abundance per fish ($\log_e$ scale) by lead days before treatment, stratified by season, over the sea lice life stages: (a) pre-adult and adult males (PAAM) (b) adult females (AF) and (c) chalimus (Chal). The vertical bars represent standard errors of the mean. The asterix star (*) represents a significant difference in average number of sea lice at different lead times compared to the number recorded in the day of treatment (day 0).

Figure 1

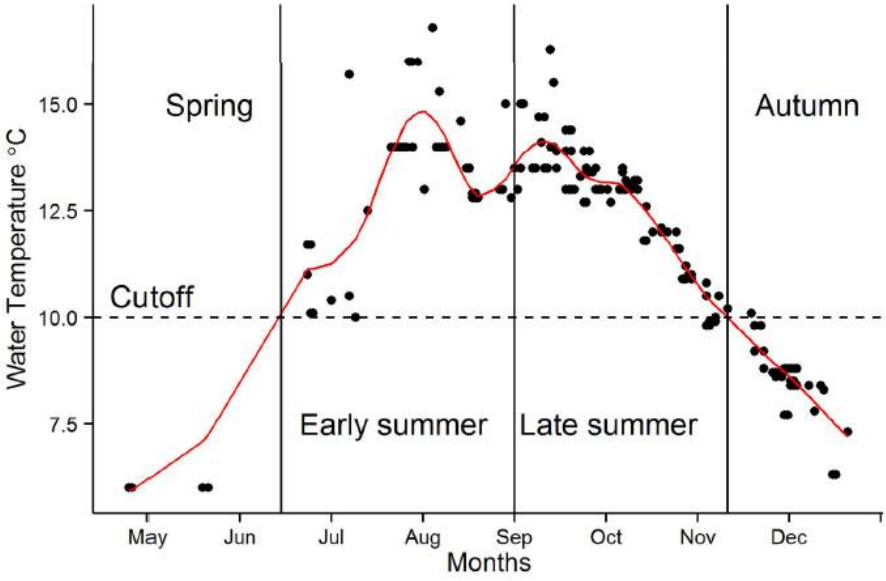

Spring
Autumn
Cutoff
Early summer
Late summer
Water Temperature °C
15.0
12.5
10.0
7.5
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Months

Figure 2

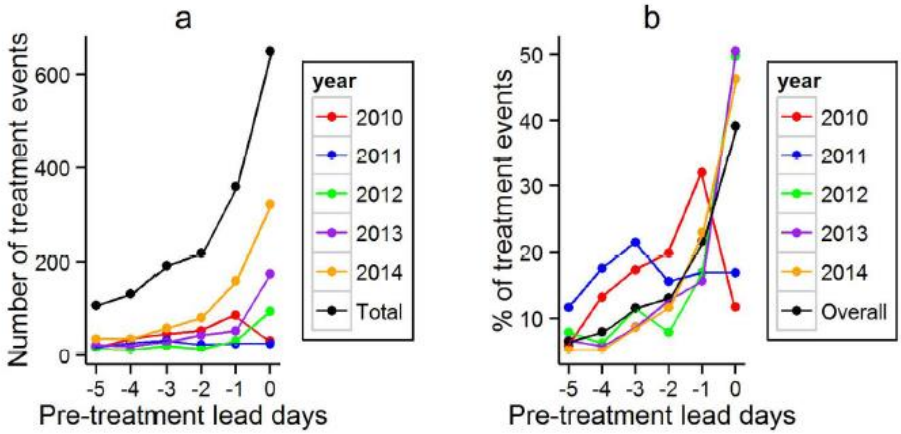


Figure 3

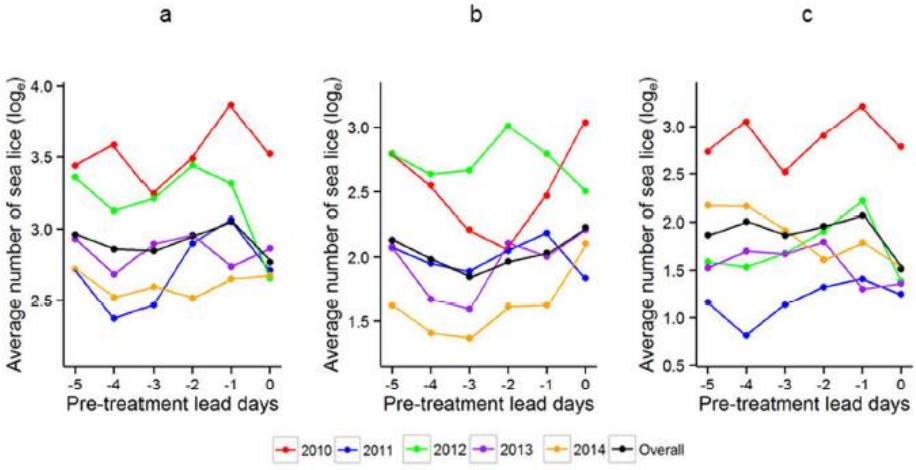
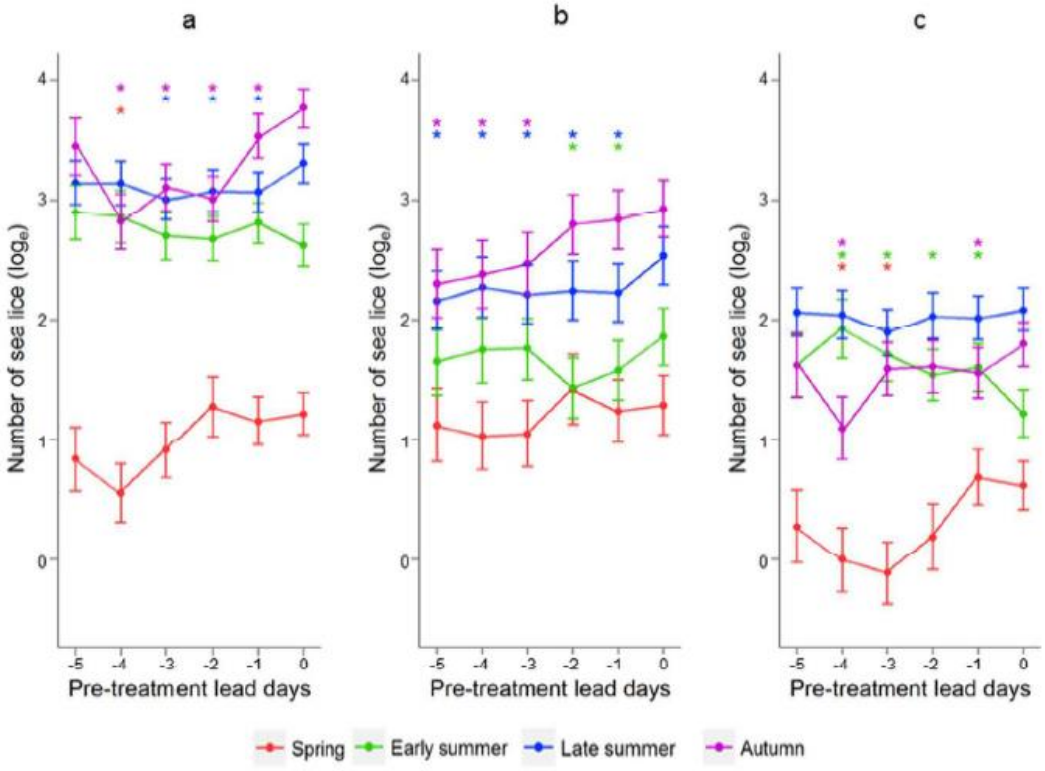


Figure 4


a
b
c
Number of sea lice (log$_e$)
Pre-treatment lead days
Spring
Early summer
Late summer
Autumn