Effect of timing of count events on estimates of sea lice abundance and interpretation of effectiveness following bath treatments

Authors: R Gautam[1], 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.

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

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

Running title: Post-treatment lag time effect on sea lice abundance

Abstract

Effectiveness of sea lice bath treatment is often assessed by comparing pre- and post-treatment counts. However, in practice the post-treatment counting window varies from the day of treatment to several days after treatment. In this study we assess the effect of post-treatment lag time on sea lice abundance estimates after chemical bath treatment using data from the sea lice data management program (*Fish-iTrends*) between 2010 and 2014. Data on two life-stages; (i) adult female (AF) and (ii) pre-adult and adult male (PAAM) were aggregated at the cage level and log transformed. Average sea lice counts by post-treatment lag time were computed for AF and PAAM, and compared relative to treatment day, using linear mixed models. There were 720 observations (treatment events) that uniquely matched pre- and post-treatment counts from 53 farms. Lag time had a significant effect on the estimated sea lice abundance, which was influenced by season and pre-treatment sea lice levels. During summer, sea lice were at a minimum when counted 1 day post treatment irrespective of pre-treatment sea lice levels, whereas in the spring and autumn low levels were observed for PAAM over a longer interval of time provided the pre-treatment sea lice levels were > 5-10.

Introduction

The sea louse is an ectoparasite of salmon aquaculture that causes severe production loss, while adversely impacting the health and welfare of farmed and wild salmon (Costello 2009a, b, Øverli et al. 2014). *Lepeophtheirus salmonis* and *Caligus elongatus* are the two main species of sea lice found on the east cost of the United States (US) and Canada (Boxaspen 2006). Between these two species, *L. salmonis* is the most predominant and problematic species in the aquaculture region along the east coast of Canada (Westcott et al. 2004). This species is also specific to Atlantic salmon, while *C. elongatus* has a wider host range (Øines et al. 2006).

Application of chemotherapeutant is an important component to the overall management of sea lice infestation on salmonid farms (Grant 2002). The chemotherapeutant is administered either as an in-feed treatment or applied topically as a bath treatment. Since the decline in effectiveness of in-feed treatments due to development of resistance by the sea lice to emamectin benzoate (Gustafson et al. 2006, Lees et al. 2008, Jones et al. 2012), there has been increased reliance on bath treatments in eastern Canada in recent years. Bath treatment is either delivered by tarp or well-boat, depending on the choice of pesticide (Corner et al. 2008). Skirt was another method of bath treatment delivery, used briefly in 2009 and 2010, by the aquaculture industry in eastern Canada. While tarp enclosures are only used to deliver azamethiphos (Salmosan Vet®), wellboats are used to deliver either azamethiphos or hydrogen peroxide (Interox®Paramove™ 50). Thus, there are essentially three bath treatment modalities using the combination of treatment delivery methods (tarp vs. wellboat) and drugs (azamethiphos vs hydrogen peroxide) currently in operation in eastern Canada.

Clinical responses to bath treatments are assessed at the cage level by comparing average pre-treatment with post-treatment sea lice abundance levels. In practice, there is a lead time of several days associated with pre-treatment counts (Gautam et al. in press 2016) or considerable lag time associated with the post-treatment counts due to logistical constraints of personnel and equipment during busy treatment periods. Previous studies assessing treatment efficacy have used a pre-treatment counting window of 16-21 days (Lees et al. 2008, Jones et al. 2012) and post-treatment count window of one to several weeks (Jones et al. 2012). We showed previously that lead time affects the estimate of pre-treatment sea lice count for different seasons (Gautam et al. in press 2016). To date, the effect of timing on the post-treatment estimated abundance remains untested. There could be similar effects of season, but in addition the pre-treatment levels of sea lice may also influence the optimum counting window for sea lice post-treatment abundance. To make appropriate treatment decisions and to monitor trends in resistance development over time, bath treatments should be regularly assessed for their effectiveness in a way that is comparable across all treatment events. It is therefore, critical to assess the effect of lead and/or lag time within which counting is expected to occur for pre- and post-treatment under production conditions.

Therefore, our main objectives were to (i) assess the effect of post-treatment lag time on the assessment of sea lice abundance, and (ii) determine the most appropriate post-treatment lag time for counting sea lice.

Materials & Methods

Source and description of data

The study area was the Bay of Fundy aquaculture region of southwestern New Brunswick, in eastern Canada. Aquaculture in this region follows a bay management approach, called Aquaculture Bay Management Areas (ABMAs), that was established in 2005 to manage location and stocking of fish (Beattie et al. 2005). Data consisted of bath treatment events and cage-level sea lice abundance estimates from January 2010 to December 2014. Data were obtained electronically from the sea lice data management system, *Fish-iTrends*. This data management system is a closed-access Web-based sea lice information system, developed by the Atlantic Veterinary College, University of Prince Edward Island. It facilitates the management of data from different producers (users) and is designed to generate real-time data visualization in the form of descriptive summary graphical outputs for participating industry partners and for agreed research purposes within the university. Participating industry partners use *Fish-iTrends* to enter fish-level sea lice count and treatment data as they become available. Regional regulations require producers to report sea lice counts on samples of 5 or more fish per cage from at least 6 cages every week (when water temperatures are above 5°C). In the event of a bath treatment, sea lice abundance for both pre- and post-treatment must be reported, but the timing around treatment-related counts is unregulated. The reported sea lice counts are enumerated under the following three life stage categories: chalimus (Chal), pre-adults (both male and female) and adult males (PAAM), and adult females (AF). It is the standard practice in the industry to combine counts of pre-adults (both sexes) and adult males to reduce observation errors (Elmoslemany et al. 2013).

In this study, we only included data from cages that had sea lice levels on five or more fish per cage for both pre- and post-treatment counting events. In addition, pre-treatment lead time was restricted to a maximum of 5 days and post-treatment lag time to 8 days because current practices in New Brunswick use a maximum counting window of 5 days for pre-treatment counts, and we assumed that the maximum effect of bath treatment would not be observed beyond 8 days of treatment (this assumption is further explained under Discussion section). If farms had performed counts on multiple days either for pre- or post-treatment, within the selected window of time, then the count that was closest to the treatment date was selected.

The fish groups were tracked by unique identifiers using site, cage, date, and treatment event id (TID). Because there were lead times associated with pre-treatment counts and lag times associated with post-treatment counts, cages could be split or merged to facilitate treatment between the two counting events (pre- and post-treatment counts). In such situations, the TIDs were linked to multiple fish groups in pre-treatment, post-treatment and/or both counting events. An illustration of the possible ways fish groups could change during the treatment is shown in Figure 1. To reduce possible bias, all treatment events that led to a split or merge (Figure 1) were excluded from the analysis. Only the unique fish groups and TIDs that matched for pre- and post-treatment counts were retained in the final dataset.

Water temperature (^{o}C) was available for most of the post-treatment counting events. Because there could be site-to-site variation, introduced by measurement protocols (e.g. measurement depth, time of the day and person), locally weighted scatterplot smoothing (LOESS) was used to predict a general temperature trend. This predicted temperature was used to create a new variable, "season", using a combination of temperature cut-off (at 10 ^{o}C) and time of year (peak summer temperatures at the end of August). The new variable, season, was defined with the following categories: (i) spring (< 10 ^{o}C before August 31), (ii) summer (≥ 10 ^{o}C), and (iii) autumn (< 10 ^{o}C after August 31; see Figure 2). Due to natural sea lice infestation patterns, most of the counting events occurred between mid-April and late December in all the years (Gautam et al., in press 2016). The treatment with the lowest recorded temperature was 4 ^{o}C. The final dataset consisted of 720 treatment events derived from recorded treatments between 2010 and 2014.

Statistical analysis

The response variables were post-treatment average sea lice abundances per fish for two life stages (PAAM and AF). For these life-stages, data were aggregated at the cage level as mean sea lice per fish for all treatment events. The average sea lice abundances in the cages were right skewed. Therefore, Box-Cox analysis was performed to determine appropriate power transformation for improving the normality and to stabilizing the variance.

The Box-Cox analyses suggested a lambda (λ) of -0.02 and 0.1 for PAAM and AF, respectively, so a natural log transformation was considered appropriate and used to transform the response variables. The post-treatment cage-level mean sea lice abundance was log transformed using $\log_e$ (sea lice number + 0.5) for both life-stages. Pre-treatment cage-level sea lice abundances of PAAM and AF were log transformed using $\log_e$ (sea lice number +1), and abundance of Chal was transformed using $\log_e$ (Chal number + 0.5). Notice that for natural logarithmic transformation, 0.5 and 1 was added; these values differ because numbers between 0.1 and 1 were explored to optimize normality of the data. A composite variable, "year-ABMA", was created using the variables year and ABMA. We developed linear mixed-effects models for the log-transformed post-treatment sea lice abundance with the composite variable, year-ABMA and farm (site) as the random effects while accounting for the nesting of site within year-ABMA. The model 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-ABMA, X is the matrix of fixed effects variables, β is the vector of fixed effects coefficients, u_k is the k^{th} year-ABMA, $v_{j(k)}$ is the j^{th} site within k^{th} year-ABMA, and ε is the error term.

Fixed-effects variables were initially screened using bi-variable analyses in a mixed-effect model. All variables significant at P ≤ 0.2 in the bi-variable analyses were considered for developing of multivariable mixed-effects model. Variable selection was performed manually using a stepwise forward selection method. All possible two-way interactions between the fixed-effects variables were investigated. The final model was selected using the Akaike Information Criterion (AIC) and the likelihood ratio tests (Dohoo et al. 2009). The variables retained in the final model were post-treatment lag time, pre-treatment sea lice abundance and season, including all possible combinations of two way interaction between these variables. Separate models were developed for each of the two life-stages, together with an additional model for total mobiles (AF+PAAM). The predicted post-treatment sea lice abundances for different lag times (1 to 8 days, inclusive) were compared with that of no lag time (i.e. count performed on the day of treatment or day 0) to assess the effect of lag time on average sea lice abundance post-treatment. Model residuals were graphically evaluated at each hierarchical level and normality and homoscedasticity assumptions were deemed acceptable. Model for the Chalimus stage of sea lice was not developed because bath treatment is known to have a limited effect on the abundance of juvenile sea lice stages (Jimenez et al. 2013). All statistical analyses were performed using R v3.1.1 (R Development Core Team, 2014).

Results

There were 720 treatment events in which the fish groups in the pre-treatment and post-treatment were uniquely linked from 53 farms in five ABMAs over 5 years. Of these, 85 (11.8%) treatments were performed in the spring, 452 (62.8%) in the summer, and 183 (25.4%) in the autumn. During the earlier part of the study period (i.e. prior to 2013), post-treatment counts were performed any time over the lag window of 0 – 8 days; however, in the later years, there was a tendency to count more frequently closer to the time of treatment (Figure 3), in particular for 2013 and 2014 at Day +1.

Lice abundance by count date relative to treatment date

There were significant interactions between lag time and season, lag time and pre-treatment sea lice abundance, and pre-treatment sea lice abundance and season (Table 1). The predicted post-treatment sea lice abundance (for both life stages) was greatly influenced by the pre-treatment level of sea lice (Table 1). For every one $\log_e$ unit increase in pre-treatment sea lice level, post-treatment sea lice abundance decreased by $\log_e$ 0.26 (i.e. ~0.77 lice) on day 1, which was significantly lower ($P < 0.01$) when compared to day 0 (day of treatment). Figure 4 (a,b,c) show the interaction plots between lag time and pre-treatment abundance and their effect on post-treatment sea lice levels in different seasons for

PAAM. In the spring, post-treatment sea lice level was the lowest on day 3 and day 4 when pre-treatment sea lice levels were below 30 lice, but as the pre-treatment sea lice level increased above 35, lowest post-treatment sea lice was observed on day 1 and day 3 (Figure 4a). In the summer, lowest post-treatment sea lice (PAAM) was predicted on day 1 post-treatment consistently across all levels of pre-treatment sea lice abundance (Figures 4b and 5b). Similar predictions were observed for AFs in the summer (Figure 5a). In the summer, the abundance of post-treatment sea lice increased steadily after day 1, while in other seasons there was a leveling period after reaching low levels (Figure 5a, b). Figure 4d shows the effect of season on post-treatment PAAM abundance at different levels of sea lice pre-treatment. The fixed-effects coefficients and variance components along with intra-class correlation coefficients (ICCs) for different levels of clustering and sea lice life stages are shown in Table 1. Approximately 40% of the variation in AF and approximately 30% of the variation in PAAM was explained by differences between sites (farms).

Discussion

This is the first study describing the effect of lag time on the assessment of post-treatment sea lice abundance after topical treatment with chemicals. The objectives of this study were twofold: (i) to assess the effect of lag time between chemical bath treatment and post-treatment count of sea lice on the estimated abundance of sea lice for different pre-treatment levels of sea lice and season, and (ii) to evaluate the optimal temporal window for counting sea lice following topical treatment of sea lice in different seasons.

Lag time significantly affected the estimated post-treatment sea lice abundance, and the effect was influenced by both pre-treatment levels of sea lice and season. In the summer, sea lice levels were at a minimum one day after treatment, irrespective of the level of sea lice before the treatment. However, in the spring and autumn, the lag times (in days) at which sea lice levels were estimated to be at their lowest depended on the pre-treatment abundance of sea lice. Furthermore, in the summer, sea lice abundance increased immediately after reaching low levels, while in the spring and autumn there was a longer period of low levels before sea lice levels increased. This prolonged low leveling effect in the spring and autumn may be related to slower re-population of sea lice from earlier life stages due to cooler water temperatures (Stien et al. 2005, Boxaspen 2006). At lower temperatures, as seen in the spring and autumn, the development of eggs and planktonic stages of sea lice are prolonged (Johnson & Albright 1991, Boxaspen & Naess 2000), contributing to a slower increase in adult sea lice levels post-treatment.

The lack of understanding of the lag time effect, and other uncontrollable production management factors, led to the general use of various lag times for post-treatment counting events throughout the industry (Lees et al. 2008, Jones et al. 2012, Jimenez et al. 2013). Our finding that post-treatment sea lice levels are significantly influenced by season and pre-treatment sea lice levels is important to decision makers when developing protocols and standards for determining post-treatment abundance estimates of sea lice. Our data suggest that the appropriate time to count sea lice is 1 day after treatment in the summer, while there is some flexibility in the spring and autumn where counting could occur after a longer lag time, starting after day 1 and up to 4 days in the spring and 7 days in the autumn, provided the pre-treatment sea lice levels were high enough (e.g. >5-10 lice). However, for relatively low pre-treatment sea lice levels (e.g. approximately <5-10 lice), there were few differences between lag days up to 7 days. Having standard protocols in place to determine post-treatment abundance of sea lice is crucial if the treatment performance is to be monitored over time and compared between different treatments. However, recognising the limitations of cross-sectional data, it is most appropriate to verify the findings of this study with a longitudinal study.

To facilitate interpretation, as previously described by Gautam et al. in press (2016), season was defined using temperature cut-off values and set dates to assess the influence of temperature and season on the effect of lag time on sea lice abundance. Seasons were found to have a significant effect on post-treatment sea lice abundance (Figure 4d). Post-treatment sea lice levels in the spring and summer were consistently lower at all levels of pre-treatment sea lice abundances compared to autumn levels. Variation in treatment efficacies against different life stages of sea lice have been reported before (Branson et al. 2002, Sevatdal et al. 2005, Whyte et al. 2014), but we are not aware of any study reporting differences in treatment performances by seasons.

We restricted the lead time window to a maximum of five days before the treatment, the reason for which has been described before in the Methods section. The lag time effect was limited to 8 days after treatment which was based on the assumption that the bath treatment effect would not last more than 8 days. This assumption is supported by a study that reported treatment efficacy on adult lice in most farms at day 1 post-treatment was either greater or not different than at day 10 except for one farm that had slightly higher efficacy on day 10 (Bravo et al. 2014).

In this study we used historical data that was recorded weekly by producers, as required by the New Brunswick Sea Lice Monitoring Program. Although these data allowed us to assess the impact of lag time and pre-treatment sea lice abundance on post-treatment estimate of sea lice levels, the study is cross-sectional and, as such, has its inherent design limitations (Levin 2006, Dohoo et al. 2009). In addition to study design limitations, counting sea lice by multiple counters could have also introduced inconsistencies, as well as the use of convenience sampling techniques (potential selection bias), as described by Gautam et al. in press (2016). Should there be any potential selection bias, we assumed it was homogenously present across different lag times and season, and therefore its effect should be inconsequential to the interpretation of this study results.

In summary, we found that lag time between treatment and counting sea lice affects the post-treatment abundance of sea lice, which is influenced by season and the level of sea lice before treatment. In the summer, counting on day 1 after treatment had the lowest level of sea lice compared to before *or* after day 1 for both AF and PAAM but the effect was most pronounced for PAAM. In the spring, counting could be done over the longer interval of 4 days for PAAM, provided the pre-treatment sea lice levels were relatively high (e.g. >5-10 lice) and the day of treatment (day 0) was avoided (due to higher PAAM). In the autumn, counting between day 1 and day 7 post-treatment appeared to give similar estimates for PAAM abundance provided day 0 was avoided, while for AF each day up to day 8 was superior compared to day 0. For relatively low levels of sea lice (e.g. <5 lice), there were few count differences up to one week lag. As industry recommendations, post-treatment counts on day 0 should generally be avoided, while Days 1 to 4 provide similar estimates to each other, except in summer when PAAM and AF are optimally counted at day 1 post-treatment. The findings of this study provide evidence on which to move towards a standardized approach to counting intervals when monitoring sea lice abundance after bath treatment interventions with conditions observed in eastern Canada.

Acknowledgement

We thank Atlantic Canada Fish Farmers Association for their cooperation. Data was provided by the Fish-iTrends© program. Funding was provided for this study by the Canada Excellence Research Chair (CERC) programme.

References

Beattie M.J., McGeachy S.M., Chang B.D., Page F.H. (2005) Water movement and fish health in the salmon farming industry of southwestern New Brunswick. *Bull Aquacul Assoc Can* **105**, 34–43.

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

Boxaspen K., Naess T. (2000) Development of eggs and the planktonic stages of salmon lice (*Lepeophtheirus salmonis*) at low temperatures. *Contributions to Zoology* **69**, 51–55.

Branson E.J., Rønsberg S.S., Ritchie G. (2002) Efficacy of teflubenzuron (Calicide®) for the treatment of sea lice, *Lepeophtheirus salmonis* infestations of farmed Atlantic salmon *Salmo salar* at low water temperatures. *Diseases of Aquatic Organisms* **51**, 101-106.

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.

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.

Elmoslemany A., Whyte S.K., Revie C.W., Hammell K.L. (2013) Sea lice monitoring on Atlantic salmon farms in New Brunswick, Canada: comparing audit and farm staff counts. *Journal of Fish Diseases* **36**, 241–247.

Gautam R., Boerlage A.S., Vanderstichel R., Revie C.W., Hammell K.L. (2016) Variation in pre-treatment count lead time and its effect on baseline estimates of cage-level sea lice abundance. *Journal of Fish Diseases* (In Press).

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

Gustafson L., Ellis S., Robinson T., Marenghi F., Endris R. (2006) Efficacy of emamectin benzoate against sea lice infestations of Atlantic salmon, *Salmo salar L.*: evaluation in the absence of an untreated contemporary control. *Journal of Fish Diseases* **29**, 621–627.

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.

Johnson S.C., Albright L.J. (1991) Development, Growth, and Survival of *Lepeophtheirus Salmonis* (Copepoda: Caligidae) Under Laboratory Conditions. *Journal of Marine Biological Association of the United Kingdom* **71**, 425–436.

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.

Levin K.A. (2006) Study design III: Cross-sectional studies. *Evidence-Based Dentistry* **7**, 24–25.

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.

Sevatdal S., Copley L., Wallace C., Jackson D., Horsberg T.E. (2005) Monitoring of the sensitivity of sea lice (*Lepeophtheirus salmonis*) to pyrethroids in Norway, Ireland and Scotland using bioassays and probit modelling. *Aquaculture* **244**, 19–27.

Stien A., Bjørn P. A., Heuch P.A. & Elston D.A. (2005) Population dynamics of salmon lice *Lepeophtheirus salmonis* on Atlantic salmon and sea trout. *Marine Ecology Progress Series* **290**, 263-275.

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., 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 1. Variables associated with the mean abundance of different life-stages of sea lice in the final linear mixed-effects regression model (n=720) from 53 farms in 5 Aquaculture Bay Management Areas (ABMAs) over five years (2010 to 2014, inclusive) in the Bay of Fundy, New Brunswick, Canada.

Fixed effects						
	AF		PAAM		Total mobile	
Variables	β	P value	β	P value	β	P value
Lag days after treatment						
Intercept	-0.31	0.30	-0.30	0.29	-0.37	0.26
Day 0	Ref		Ref		Ref	
Day 1	0.39	0.30	0.46	0.22	0.77	0.07
Day 2	0.34	0.39	-0.31	0.47	-0.33	0.49
Day 3	0.47	0.25	-0.18	0.65	0.15	0.74
Day 4	0.31	0.45	-0.33	0.42	-0.11	0.80
Day 5	0.51	0.21	0.08	0.84	0.05	0.92
Day 6	1.33	0.00	-0.34	0.52	-0.89	0.14
Day 7	-0.02	0.98	-0.31	0.59	-0.47	0.54
Day 8	0.19	0.74	-0.87	0.17	-0.31	0.67
Season						
Spring	Ref		Ref		Ref	Ref
Autumn	-0.02	0.96	-0.33	0.44	-0.54	0.25
Summer	-0.24	0.46	0.10	0.76	0.04	0.91
Pre-treatment sea lice level	0.52	0.00	0.68	0.00	0.70	0.00
Lag days after treatment: Season						
Day 0: Spring	Ref		Ref		Ref	
Day 1: Autumn	-0.01	0.98	0.16	0.69	0.13	0.75
Day 2: Autumn	-0.34	0.44	-0.26	0.58	-0.32	0.49
Day 3: Autumn	-0.61	0.16	0.27	0.60	-0.05	0.92
Day 4: Autumn	-0.41	0.36	0.05	0.93	-0.07	0.88
Day 5: Autumn	-0.78	0.11	-1.00	0.07	-1.14	0.04
Day 6: Autumn	-2.14	0.00	-1.14	0.03	-1.29	0.01
Day 7: Autumn	-0.85	0.17	-0.40	0.60	-0.72	0.30
Day 8: Autumn	-0.61	0.35	-0.11	0.87	-0.67	0.31
Day 1: Summer	-0.35	0.33	-0.16	0.66	-0.30	0.43
Day 2: Summer	-0.16	0.70	0.20	0.64	0.04	0.92
Day 3: Summer	-0.19	0.64	0.65	0.15	0.36	0.42
Day 4: Summer	0.04	0.92	0.90	0.06	0.66	0.16
Day 5: Summer	-0.27	0.52	-0.13	0.78	-0.29	0.53
Day 6: Summer	-1.11	0.01	-0.31	0.49	-0.28	0.52
Day 7: Summer	-0.16	0.77	0.54	0.41	0.26	0.67
Day 8: Summer	0.81	0.18	0.40	0.53	0.20	0.75
Season: Pre-treatment sea lice abundance						
Spring: Pre-treatment abundance	Ref		Ref		Ref	
Autumn: Pre-treatment abundance	0.29	0.02	0.25	0.02	0.30	0.01
Summer: Pre-treatment abundance	0.25	0.03	0.00	0.98	0.03	0.71
Post-treatment lag days: Pre-treatment sea lice abundance						
Day 0: Pre-treatment abundance	Ref		Ref		Ref	
Day 1: Pre-treatment abundance	-0.26	0.00	-0.36	0.00	-0.36	0.00
Day 2: Pre-treatment abundance	-0.14	0.15	-0.05	0.69	0.02	0.89
Day 3: Pre-treatment abundance	-0.13	0.29	-0.19	0.10	-0.16	0.20
Day 4: Pre-treatment abundance	-0.16	0.10	-0.13	0.29	-0.11	0.37
Day 5: Pre-treatment abundance	-0.01	0.94	0.06	0.60	0.12	0.34
Day 6: Pre-treatment abundance	0.00	0.99	0.24	0.08	0.37	0.01
Day 7: Pre-treatment abundance	0.06	0.71	-0.02	0.92	0.10	0.66
Day 8: Pre-treatment abundance	-0.19	0.15	0.26	0.09	0.16	0.34
Random effects						
Level	Variance	ICC	Variance	ICC	Variance	ICC
Year-ABMA	0.01	0.01	0.06	0.09	0.03	0.05
Site	0.25	0.37	0.18	0.27	0.23	0.33
Residual	0.41		0.42		0.43	

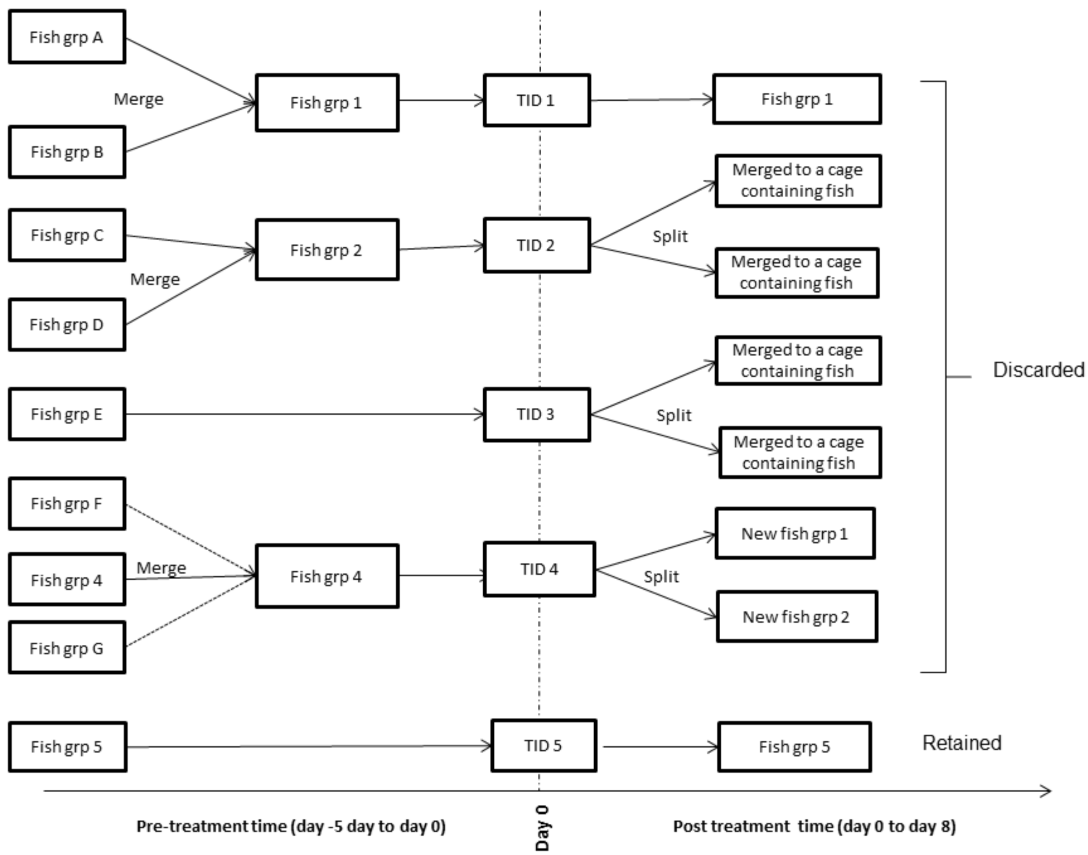

Figure 1. A schematic of the possible ways in which fish groups could be handled between pre- and post-treatment counting events, and the inclusion criteria for final analyses. Notice that the x-axis represents the time interval (in days) between both (pre- and post-treatment) counting events.

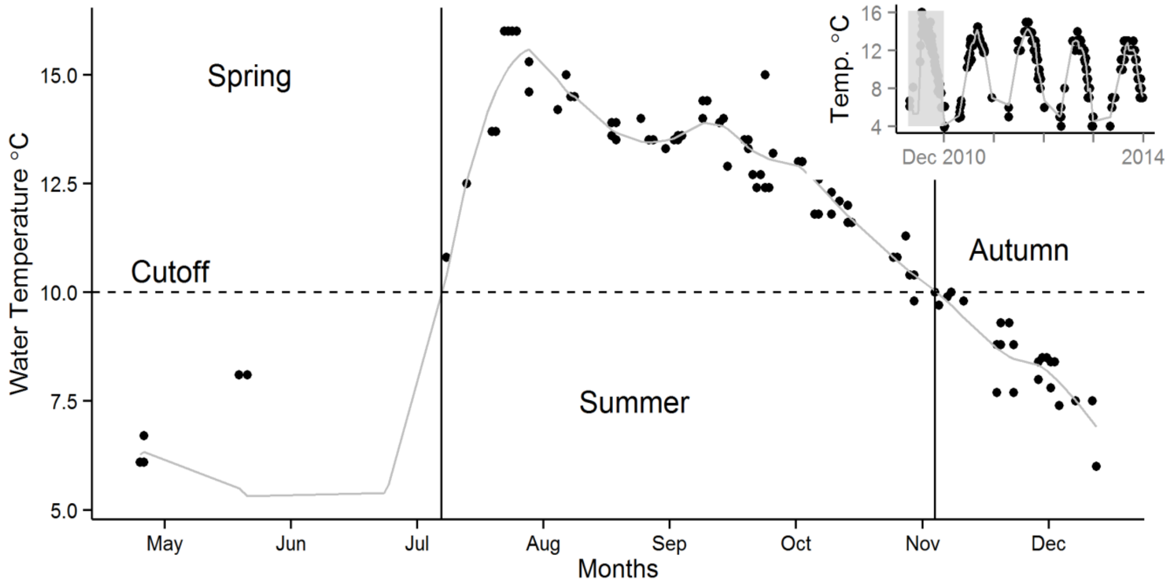

Figure 2. A diagrammatic representation of the criteria used to categorize season, using locally weighted scatterplot smoothing (LOESS) techniques to water temperatures and time of year. The recorded water temperatures (solid circles) and LOESS curve (solid line) are shown for 2010, with an inset data for the entire study period (upper right). Seasons were determined with a temperature cut-off value of 10 °C (Spring and Autumn < 10 °C before and after August 31, and summer as ≥ 10 °C). Notice that the x-axis label in the figure represents months from April to December, which is because most of the counting events occurred between mid-April and late December in all the years with missing months lacking the counting events.

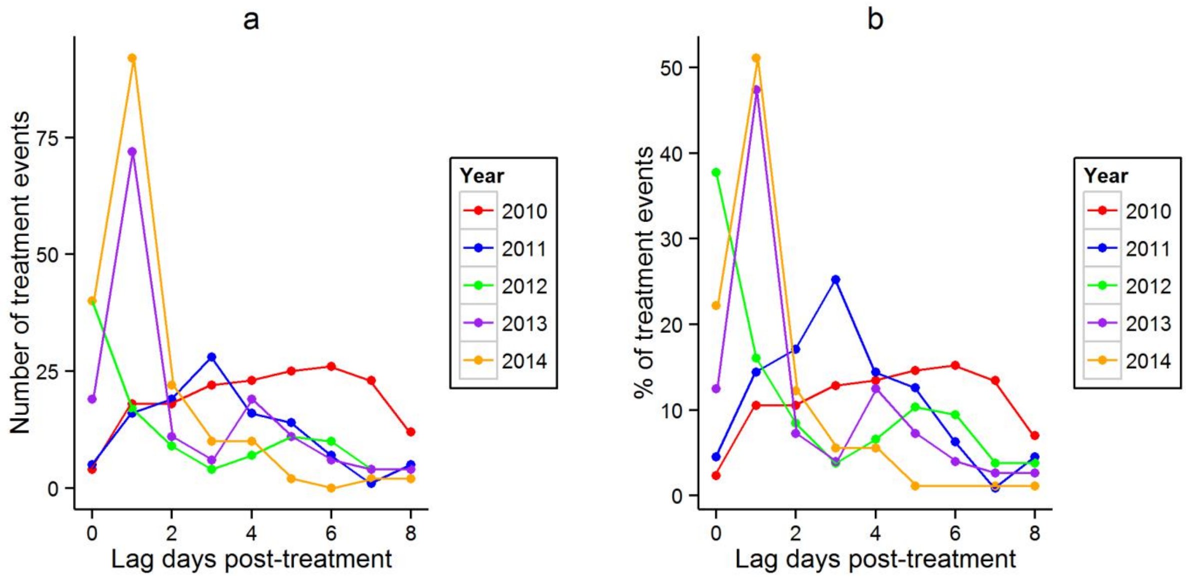

Figure 3. Distribution of the (a) number of cage-level treatment events and (b) corresponding percentage of treatments by lag time (in days), both stratified by year.

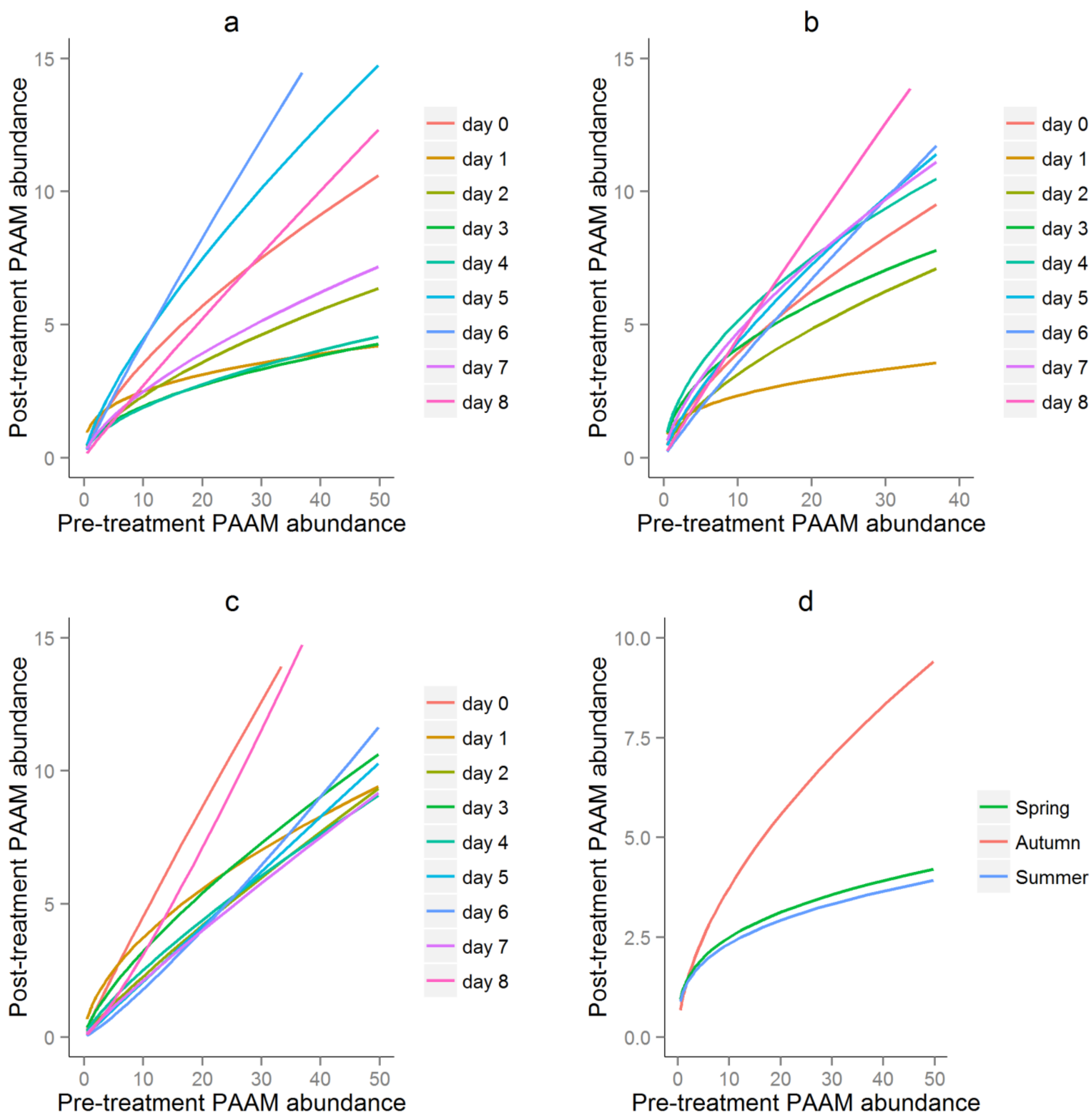

Figure 4. An interaction plot showing the predicted post-treatment sea lice abundance (for PAAM) at different levels of pre-treatment sea lice abundance (PAAM), when counted after different lag times in different seasons; (a) spring, (b) summer, and (c) autumn. The final interaction plot (d) shows the predicted post-treatment sea lice abundance between season and pre-treatment sea lice abundance, while holding the lag time constant at day 1 post-treatment.

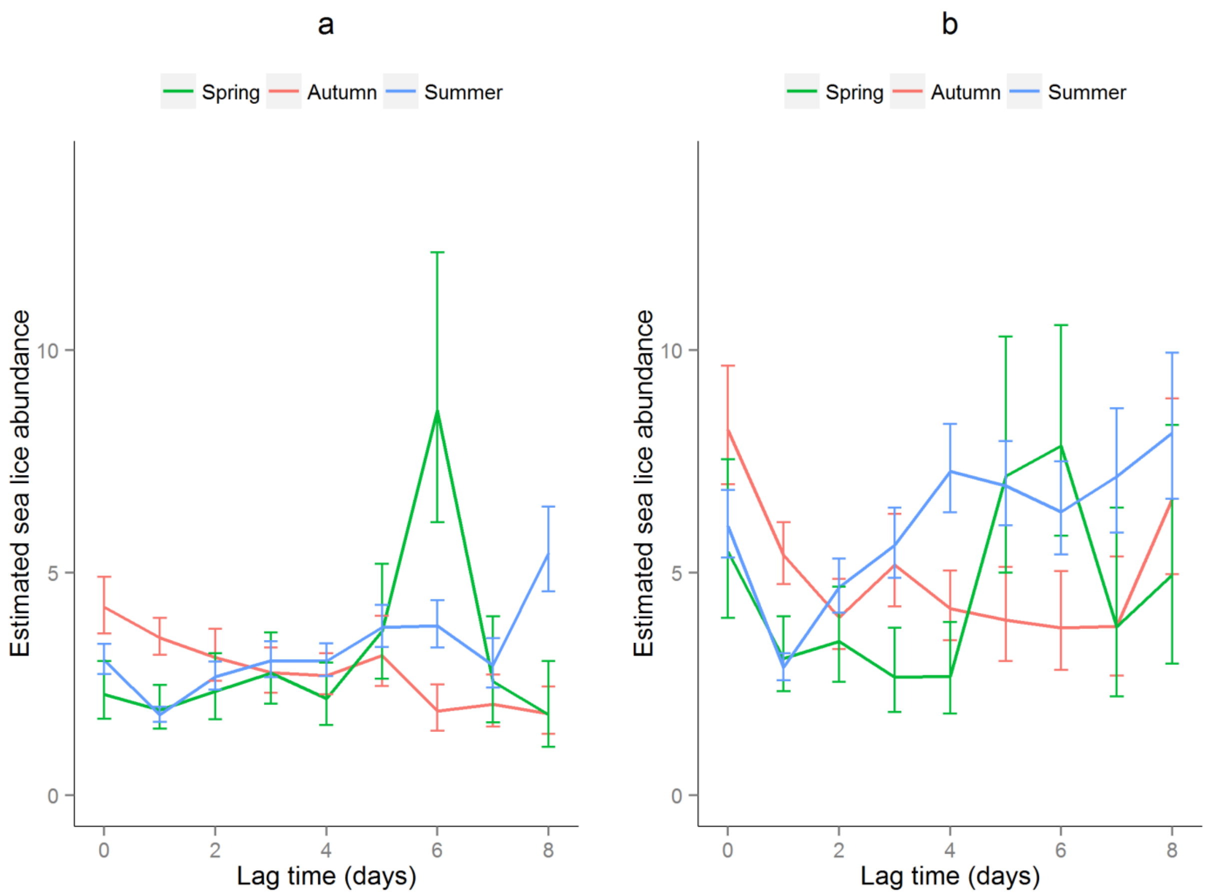

Figure 5. Linear mixed-effects regression model predictions of cage-level average sea lice abundance post-treatment by lag days and season for the different sea lice life-stages by seasons: (a) adult females (AF) and (b) pre-adult and adult males (PAAM), after controlling for pre-treatment sea lice abundance at their average (i.e. $\log_e$ 2.169 (approx. 9 lice) for AF, and $\log_e$ 2.939 (approx. 19 lice) for PAAM). The vertical bars represent standard errors of the estimated means.