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

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ABSTRACT

Sea lice (*Lepeophtheirus salmonis*) are the most costly parasitic infestation in the culture of Atlantic salmon (*Salmo salar*) with control strategies relying heavily on the use of a limited number of chemotherapeutants and traditional univariate analytical tools to evaluate and optimize their delivery. Azamethiphos (Salmosan®) is a powerful delousing agent, which has been administered as a topical treatment using three different modalities: skirt-style enclosures, fully-enclosed tarpaulin enclosures and wellboats. In this study, we analyzed and evaluated the efficacy of azamethiphos treatments between these three modalities against PAAM (pre-adult males and females and, adult males) and adult female stages using a multivariate approach. The exploratory analysis revealed efficacy in the fully-enclosed tarpaulin modality to be 2.2 times greater compared to skirt-style and wellboat modalities; whereas efficacy against adult females in the wellboat modality was two times larger than in the other modalities. Using the multivariate analysis, treatment efficacy in the fully-enclosed tarpaulin modality was greater than the skirt-style modality, but no significant differences were observed between the wellboat and skirt modalities. These results should be interpreted with caution as differences existed in the abundances and proportions of sea lice stages before treatment that may affect the observed treatment efficacies. For an evaluation and comparison of treatment modalities, multivariate techniques offer added advantages over the more traditional univariate methods, in that simultaneous analysis of multiple sea lice stages and any dependencies or correlation between these stages can be effectively addressed.

1. INTRODUCTION

Severe infestations by the sea louse *Lepeophtheirus salmonis* can incur significant health, production and market value costs for the Atlantic salmon (*Salmo salar*) aquaculture industry as a result of damage to the fish, treatment costs, potential impacts of treatments on non-target species, and public perceptions of aquaculture (Costello, 1993; Pike & Wadsworth, 1999;

Costello et al., 2001; Haya et al., 2005; Costello, 2009). To date, successful control of sea lice infestations rely heavily on effective delousing through the use of a variety of in-feed and bath-administered chemotherapeutants (Pike & Wadsworth, 1999; Boxaspen, 2006; Brooks, 2009). Inappropriate administration of therapeutants and reliance on individual therapeutants targeting specific life cycle stages can result in treatment failure and increase the risk of resistance developing in sea lice populations (Bloland & Ettling, 1999). Resistance has already been observed for chemotherapeutants in geographical areas where salmonids are intensively cultured (Jones et al., 1992; Tully and McFadden, 2000; Treasurer et al., 2000; Denholm et al., 2002; Sevatdal & Horsberg, 2003; Fallang et al., 2004; Bravo et al., 2008; Lees et al., 2008; Jones et al., 2013; Whyte et al., 2013; 2014; Grøntvedt et al., 2014).

In the Bay of Fundy aquaculture region of southwestern New Brunswick, Canada, the synthetic organophosphate pesticide, azamethiphos (Salmosan®) has been available for use, as a topical treatment for sea lice, for the greater part of 20 years. It was however, used sparingly between 2000 and 2009 due to the predominant use of emamectin benzoate. Salmosan® affects pre-adult and adult sea lice stages but is less effective against the juvenile stages. The active ingredient, azamethiphos, acts as an inhibitor of cholinesterase producing a continuous excitatory state in the parasite eventually causing irreparable nerve damage and death (Dutertre & Lewis, 2006). Fish are topically exposed via a bath treatment which is the most frequently used modality for delousing larger fish.

During the study period, three treatment modalities were available to administer bath treatments of azamethiphos at aquaculture cages sites in New Brunswick; skirt-style tarpaulin (semi-open) enclosures, fully-enclosed tarpaulin enclosures, and wellboats (closed system). The skirt-style enclosure was most utilized due to the relative ease and rapidity of access to equipment and materials. However, it was problematic for large cages or in strong tidal currents, produced variable and non-uniform distribution of the therapeutant in the cage and allowed therapeutant to escape through the open bottom. Not surprisingly, treatments performed with skirt style-enclosures have been shown to be much less effective than with fully-enclosed tarpaulins (SEARCH Consortium, 2006; Fridell, 2009; Corner et al., 2011). The fully-enclosed tarpaulin modality is often recommended or mandatory for bath treatments in many countries (Corner et al. 2007; Finne-Fridell et al., 2012; NASCO, 2010), providing a more uniform distribution of therapeutant due to the more defined treatment volume. Technical challenges, however, exist when used with large cages (90m - 120m in circumference), or at sites that maintain high current flow, and additional resources (personnel and time) are required for set up, with increased risk for potential complications (Corner et al., 2007). Both modalities can be stressful to the crowded fish, with a possible residual presence of the therapeutant within the cage once the tarpaulin has been removed, or absorbance of the therapeutant by organic debris in the water column and biofouling on the nets (Treasurer et al., 2000; Costello, 2009).

Wellboat use was introduced into New Brunswick in 2010 (ACFFA, 2011). The espoused benefits of the wellboat include increased efficacy, reduced amount of, and increased precision in, the therapeutic dose administered and, reduced release of chemical that can affect non-target organisms (ACFFA, 2011; Page and Burridge, 2014). Multiple treatments may, however, be required to completely expose one cage to the therapeutant since wellboat applications are dependent on the well volume and the size and number of fish in the cage. While the concentration of chemotherapeutant reaching the environment is diluted as the well is flushed (Page et al., 2014; Ernst et al., 2014), the discharged effluent is often in the vicinity of the cages and surviving lice within the discharge could potentially increase the risk of transmission of sea-lice or other pathogens between and within farms. This risk is similar for the skirt-style and fully-enclosed tarpaulin modalities where the residual treatment disperses naturally via the current. Due to the limited availability and expense of operating these vessels, there is continual effort to enhance delivery of therapeutants via the fully-enclosed tarpaulin modality through improved dosing systems which provide more predictable and consistent exposure.

Analytical tools for evaluating and optimizing the delivery of chemotherapeutants are important for standardization and implementation of good management practices for the control of sea lice infections. The aim of this study was to utilize a multivariate approach to concurrently evaluate and compare the effectiveness of azamethiphos against all life cycle stages of the salmon louse between the three different modalities of treatments. This is important in studies using field data, where sampling (methods and type of data collected) does not always follow a standardized procedure and, as such, evaluation of treatment effect is often limited by the availability and the asymmetry of the collected data. The estimation of effects is therefore subject to sampling and misclassification biases and lack of control of confounders (e.g. water temperatures, salinity). Since many factors modulate the mortality, transmission (infectivity) and maturation (moulting) of sea lice (Ramette, 2007; Jimenez et al., 2013), the proportion of sea lice stages observed after treatment will vary under differing field conditions. Therefore, we suggest that it may be more meaningful to evaluate treatment effectiveness by considering all sea lice stages concurrently.

2. MATERIALS AND METHODS

2.1 Sea Lice Categorization

The sea lice monitoring program in New Brunswick requires lice to be categorized by stage (juvenile, pre-adult and adult) and sex (male and female). The sea lice categories used are: [1] Chalimus (stages I and II); [2] Pre-adult (stages I and II) (male and female) and adult male lice (PAAM) (Whyte et al., 2014); and [3] Adult female lice (gravid and non-gravid) (AF).

2.2 Sea Lice Monitoring and Treatment Efficacy

Sea lice abundance and response to azamethiphos treatment under field conditions of use were examined by performing pre-treatment and post-treatment counts. These counts have shown to be reproducible and were applied in a similar manner across the New Brunswick industry (Elmoslemany et al., 2013). Atlantic salmon (*Salmo salar*) aquaculture sites in the Bay of Fundy, which received treatments with azamethiphos during the period 2010 to 2013, were identified and assessed for sea lice numbers, as part of an integrated sea lice monitoring program. Sites receiving treatment were chosen by company managers and veterinarians based on sea lice abundance. The proportion of treated/non-treated cages was not specified and no randomized comparisons or untreated control cages / sites were available. Mean cage and treatment event levels of sea lice abundance were estimated based on samples of 5-10 fish per cage from at least 6 cages at each site by a generalized linear mixed effects model (GLMM) fit to counts of *Lepeophtheirus salmonis*. Counts were performed on each cage as close to the treatment day as possible, and at no point more than 5 days prior to treatment (pre-treatment count) as well as, potentially, multiple times within the 14 day period following each treatment (post-treatment counts). Pre- and post-treatment count data were available for treatment events involving a total of 178 cages from 24 separate sites within the Bay of Fundy. Counting of all cages would usually occur on the same day but as treatment days differed slightly cages were often measured at different days post-treatment depending on the day of treatment.

Skirt-style enclosures were open to limited water exchange, unlike the enclosed tarpaulin which completely enveloped the cage. Either method involved deployment of a tarpaulin barrier prior to administering the therapeutant into the net pen for the prescribed period. Following treatment, removal of the tarpaulin allowed the therapeutant to disperse into the surrounding water by the action of the tide, waves and currents. Wellboat treatments were conducted by pumping the fish from the cage into treatment wells filled with sea water in the wellboat. The prescribed amount of chemotherapeutant was added to each well and fish were monitored. Following the prescribed treatment time, the wells were flushed with clean sea water, to reduce and eventually eliminate the concentration of therapeutant, and the effluent was discharged into the surrounding water. After a rest period, the fish were returned to the sea cage. The prescribed treatment dose of Salmosan® for wellboat or enclosed tarpaulin treatments was 200 parts per billion (ppb) and 300 ppb for skirt treatments. The duration of exposure was 60 minutes when the water temperature was below 10°C, and 30 minutes when the water temperature was above 10°C, at the discretion of the prescribing veterinarian.

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

2.3 Exploratory Analysis

The dataset included cages for which matching pre- and post-treatment counts were available. In New Brunswick, post-treatment sea lice counts should be repeated on the same cages as for the pre-treatment count. In practice, however, management constraints sometimes result in counts from non-matched cages, (i.e. there exists either a pre-treatment count or a post-treatment count but not both). Only matched cage data was considered in this study.

The correlation of abundances among taxa data: scatter plot matrices of transformed abundances (before and after treatment) was also performed. Exploratory multivariate analysis using ordination methods were employed to simultaneously evaluate the effects of azamethiphos treatment in different sea lice stages. A diagrammatic representation of the different patterns representing the assemblages of sea lice stages for each cage, before and after treatment, was generated. To visualize pattern differences between sampling times for each treatment modality, we utilized principal component analysis (PCA), after standardizing abundances from all three sea lice stages to unit variance, to obtain a more balanced ordination. Principal component analysis excluded missing data for eight treatments (n=21); five skirt-style, one wellboat and one tarpaulin treatment where counts for chalimus stages had not been recorded.

2.4 Statistical Analysis

2.4.1 Exploratory analysis for treatment effectiveness

If more than two fish per cage had counts greater than 100 lice, the data for this cage were excluded from the analysis as there was no way to accurately estimate the actual number of lice on the fish (Elmoslemany et al., 2013). This circumstance occurred rarely and resulted in the exclusion of only two cage treatment records. The percentage knockdown value based on the pre- and post-treatment count values (i.e. the proportion of the difference in counts before and after treatment divided by the count before treatment), and the 95% confidence interval was estimated using the quasi-Poisson method (Jimenez et al., 2012) calculated in R using the *pairwise CI* package (R Development Core Team, 2008). For the purpose of the analysis, treatment effectiveness (% knockdown) for each sea lice stage was modeled. Treatment effectiveness was set to zero when percent knockdown values were negative.

2.4.2 Univariate (multivariable) statistical analysis

Two models were developed for the post-treatment counts of PAAMj and AFj, where j represents the cage treatment using a negative binomial model. The negative binomial model was selected to account for over-dispersion in the data. For modeling counts of PAAM and AF, we multiplied by the number of fish sampled (typically 10) and rounded to the nearest integer. Zero counts of PAAM or AF represented less that 1% of the data. The data included pre-treatment abundances of PAAM and AF, modality of treatment and the interval (in days) between treatment and sampling. Pre-treatment abundances were transformed with a natural logarithm as it best described how the model predicted/ explained the observed data. The covariates' pre-treatment abundances and count day interval were also centered. Random effects were tested to account for treatment event and locality and model selection was undertaken using Akaike's information criteria (Zuur, 2009). For model validation, standardized residuals were plotted against all the explanatory variables using the package glmmADMB (Fournier et al., 2012; Skaug et al., 2013) in R, version 3.1.2 (R Development Core Team, 2014). Post-treatment counts of chalimus were not included in the model, as no treatment effect was observed against chalimus stages in the skirt or wellboat modalities. Although there may be an influence of availability of each treatment modality in any given year (e.g. skirt treatments were discontinued in 2011), levels of sea lice were much higher in the skirt and wellboat modalities than in the tarpaulin modality, possibly suggesting a greater infection pressure. We were, however, unable to account for this in our model due to a lack of environmental data, e.g. water temperatures, infection pressure from neighboring cages or localities. In addition, chalimus stages are often overlooked due to their very small size, with field counts of chalimus stages often underestimated by up to 40% (Schram, 1993; Beamish et al., 2005; Elmoslemany et al., 2013).

2.4.3 Multivariate analysis

A generalized linear model with a multivariate extension (Warton, 2011) was utilized for the multivariate analysis. This included a negative binomial regression which is appropriate for this type of data since the mean-variance function of counts is often quadratic. The analysis was performed using the open source mvabund package in R (Wang et. al., 2012).

A model based approach was taken to test the effect of treatment modalities after controlling for pre-treatment abundances of sea lice and the interval of days between treatment and post-treatment sampling, using the function manyglm in the R package mvabund version 3.8.4.

(Wang et al., 2012). This approach allows us to make inferences on sea lice community and composition within the cage by fitting separate GLMs to each variable, using a set of explanatory variables, and testing significance through re-sampling-based hypothesis testing (Wang et al., 2012). Negative binomial GLMs were conducted with a two dimensional matrix of the PAAM and AF assemblage composition in the cages following treatment as the dependent variable, with the covariates, pre-treatment abundances of PAAM and AF, modality of treatment and post-treatment sampling interval as independent variables. The models were tested using the ANOVA function in the mvabund, providing a multivariate test for community and each louse stage (PAAM and AF) post-treatment. Wald test statistics (Nooten et al., 2014), were constructed, assuming correlation (matrix shrink by parameter 0.84), to allow for correlated responses among sea lice assemblages. P-values were calculated using 999 re-sampling iterations via trap re-sampling (Wang et al., 2012). The analysis was performed at the level of the cage.

3.0 RESULTS

This study included a total of 178 cage treatments comprised of 110 skirt-style enclosures, 20 fully-enclosed tarpaulin and 48 wellboat treatments conducted during the period 2010 to 2013. Approximately 90% of the locations (and 152 of the 178 cages) performed treatments using a single modality, but at five locations they simultaneously performed cage treatments using a mixture of skirts (n=19) and fully-enclosed tarpaulins (n=7). The number of cages sampled pre- and post-treatment at each site ranged between one and five for the wellboat and enclosed-tarpaulin modalities, and from 1 to 9 for the skirt-style modality.

During the period under study, the level of infection happened to be lower in cages using the fully-enclosed tarpaulin treatment modality, when compared to the wellboat and skirt-style modalities. Figure 1 illustrates the differences in proportions of sea lice based on the median values calculated for each stage within the three treatment modalities at pre-treatment, one week post-treatment and two weeks post-treatment. For example, the level of infection at any sampling time was larger in the wellboat modality compared to the other two treatment modalities, particularly for the chalimus and adult female stages. The proportion of PAAM is also larger compared to the chalimus and AF stages in the fully-enclosed tarpaulin and skirt-style modalities, whereas, in the wellboat modality, the proportions of all three sea lice stages are more alike. These differences in the initial levels and proportion across treatment modalities will likely influence the observed post-treatment levels of sea lice.

Treatment was observed to be effective at varying levels against mobile stages (PAAM and AF) in all three modalities. The greatest reductions in post-treatment abundances of PAAM and AF were observed in the fully-enclosed tarpaulin and wellboat modalities (Table 1). Azamethiphos treatment was not, however, effective against chalimus in either the skirt-style or wellboat modalities. Treatment effect also appeared to be greater for all stages in the fully-enclosed tarpaulin modality in the second week post-treatment compared to the first week (Table 1).

In contrast, chalimus and PAAM abundances increased in the second week post-treatment in the skirt-style and fully-enclosed tarpaulin modalities, which was reflected in a reduction of the treatment efficacy at week two post-treatment. In the wellboat modality, the treatment effect was sustained only for adult female sea lice, most likely as a result of the fact that at least two weeks are required for surviving chalimus to moult into adult females. Chalimus are difficult to predict since their arrival will at least partially depend on external infection pressure, which is not known.

Changes in sea lice stage composition from pre- to post-treatment for the three treatment modalities, analyzed using ordination plot analyses, revealed the largest spread in the fully-enclosed tarpaulin modality with the greatest spread being along the horizontal axis, in the chalimus and PAAM stages (Figure 2a). A lesser separation was observed between pre- and post-treatment counts in the wellboat modality, along the PAAM and AF planes (Figure 2b). In contrast, the large amount of overlapping in the skirt-style modality indicates fewer differences in the composition of sea lice assemblages before and after treatment (Figure 2c).

Results from the univariate statistical models indicate a 30% ($p < 0.05$) post-treatment reduction in abundances of PAAM lice and a 20% reduction in abundances of AF lice ($p = 0.09$) in the fully-enclosed tarpaulin, compared to the skirt-style modality, after controlling for pre-treatment levels and time interval (Table 2a & b). No significant differences were observed between the skirt-style and wellboat modalities. In comparison, the multivariate analyses indicated that the assemblage composition of sea lice stages (or 'pre-treatment profile') was significantly different between the fully-enclosed tarpaulin and the skirt-style modalities ($p = 0.02$), but not significantly different when comparing the wellboat and skirt-style modalities ($p = 0.344$) (Table 3). Pre-treatment abundances of PAAM and AF lice and the interval of days post-treatment when the sea lice count was taken were significant and positively associated with the post-treatment counts of PAAM and AF lice.

4. DISCUSSION

Research have shown that treatment efficacies vary against different lice stages (Wootten et al.,1982; Branson et al., 2000; Sevadtal et al., 2005; Whyte et al., 2014). The practice of monitoring clinical treatment response through the analysis of field-collected data is a key factor in the detection of changes in treatment effectiveness. Treating at the appropriate time, based on the assemblage of sea lice stages observed during monitoring, is a relevant consideration for controlling sea lice levels. In reality, however, these issues are difficult to resolve as the trigger for management action, usually treatment, in many farming regions is dependent on exceeding an established threshold of sea lice numbers (particularly adult female lice) and is independent of the composition of lice assemblages (SEARCH Consortium 2006; Norwegian Food Safety Authority. 2010*;* Code of Good Practice Management Group 2010; DFO, 2014). For this reason, we believe that multivariate analysis is a more appropriate approach when analyzing these data since the univariate test only evaluates the effect of treatment on one stage or subgroup of sea lice assemblages. Also, the results from separate tests cannot be correctly interpreted, as the effect of the therapeutant is correlated among the different sea lice stages. Multivariate analyses are now commonly utilized in the analysis of ecological data (Dray et al., 2012; Buttigieg & Ramette, 2014) and incorporate a generalized linear approach (Wang et al., 2012) to overcome the limitations commonly found with classical multivariate tests such as MANOVA.

In this study, a multivariate approach was used to highlight differences in the efficacy of azamethiphos across different bath modalities. All treatment modalities exhibited greater effectiveness against mobile stages of lice (PAAM and adult female) as compared to juvenile stages (chalimus). While this is similar to earlier studies (O'Halloran & Hogans 1996; Roth et al., 1996), more interestingly we report differences in efficacy for azamethiphos when using different treatment delivery modalities. Reduction in post-treatment lice levels was significantly larger in the fully-enclosed tarpaulin modality compared to the skirt-style modality, though no significant difference was observed between the wellboat and skirt-style modalities. This raises questions about the ability to objectively evaluate and compare efficacies of different chemotherapeutants under different treatment modalities. For example, treatment efficacy against PAAM in the fully-enclosed tarpaulin modality was twice that compared to the wellboat modality; whereas the reverse was observed for adult female stages.

The dichotomy in the resulting treatment efficacies between the fully-enclosed tarpaulin and wellboat modalities may be due to pre-treatment differences in the abundances and composition (proportion) of sea lice stages for each modality. The largest reduction in post-treatment counts of PAAM in the fully-enclosed tarpaulin modality is partially explained by the larger proportion of pre-treatment PAAM stages (73%) compared to the skirt-style (62%) and wellboat (43%) modalities. Lower abundances and proportions of chalimus stages in both pre- and post-treatment counts in the fully-enclosed tarpaulin modality (compared to the other two modalities) may further hamper post-treatment recovery of PAAM stages. This finding is also supported by a larger reduction of PAAM two weeks post-treatment in the fully-enclosed tarpaulin modality, in contrast to the other two treatment modalities. In contrast, the apparently greater effectiveness of azamethiphos against adult female lice in the wellboat modality is likely due to the relatively lower contribution of surviving pre-adult female lice within the PAAM group developing into post-treatment adult female lice, as evidenced by the lower (4-6 times) ratio of abundances of PAAM/adult female in the wellboat modality compared to the other two treatment modalities.

The skirt-style modality demonstrated effectiveness against only the PAM stage at one week post-treatment and this markedly declined, as expected, at week two post-treatment against all mobile stages. Post-treatment lice survival is perhaps the largest contributor to an apparent reduction in treatment efficacy. Faster rates of infections may result from higher pre-treatment infection levels of adult female and chalimus stages (Kristoffersen, 2013). This would suggest that re-infection occurred at a faster rate in the wellboat and skirt-style modalities. These results agree with the exploratory analysis using ordination plots which suggest that the largest change in the assemblages of sea lice following treatment was seen in the fully-enclosed tarpaulin modality, followed by the wellboat and, lastly, the skirt-style modality.

The results from the univariate (for PAAM and adult females) and multivariate analyses are similar and show a significantly greater reduction in post-treatment sea lice in the fully-enclosed tarpaulin modality, compared to the skirt-style modality; whereas no differences were seen between the skirt-style and wellboat modalities. The results should, however, be treated with caution given the differences in infection levels, pre-treatment proportions of sea lice and imbalances in the data between modalities. Furthermore, modality choices and sampling strategies, particularly timing of sampling before and after treatment, were subject to influences uncontrolled by the investigators. For example, if sea lice numbers increase at multiple locations in the New Brunswick industry, the availability of the three wellboats becomes a deciding criterion. Thus, it is difficult to conclude whether efficacies with the wellboat modality truly differ from the other two modalities. A better approach, where feasible, may be to model the levels of sea lice following treatment, for example, by modeling the counts of each stage following treatment based on surviving lice numbers and water temperatures, accounting for the effect of infection pressure in the environment (Kristoffersen et al., 2014). In our case, and given the limited availability of data, we followed a multivariate approach. The importance of this analytical tool is that it improves our interpretation and understanding of the efficacy of azamethiphos and its application method in the field. Furthermore, it may provide a novel and improved analytical tool to assess the increasing desensitization of sea lice to the most common delousing chemotherapeutants, including azamethiphos.

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Table 1. The mean (+/- 95% confidence intervals) percent effectiveness (% knockdown) of salmosan at week 1 and week 2 post-treatment.

Week 1

	Chalimus	PAAM	Adult Females
Skirt	5.9[-10.1,19.6]	50[44.6,55]	17[4.6,27.8]
Tarpaulin	58.8[49,66.9]	69.4[64.8,73.5]	20.9[6.3,33.2]
Wellboat	5.4[-20,25.6]	49.9[40.9,57.7]	45.9[36.6,53.9]

Week 2

	Chalimus	PAAM	Adult Females
Skirt	13.1[-1.1,25.4]	38.2[30.9,44.9]	-13.4[-28.1,-0.3]
Tarpaulin	59.9[49.7,68.2]	82.5[79.7,85]	26.9[12.3,39.2]
Wellboat	-23.4[-51.4,-0.6]	29.4[17.9,39.5]	42.8[33.8,50.7]

Table 2a. Negative binomial model (with random effect for treatment) for abundances of PAAM after treatment. Covariate PAAM before treatment (PAAM, pre) was log-transformed and centered. Days post-treatment were also centered.

Variable	**Value**	**Std.Error**	**p-value**
(Intercept)	4.846	0.07	<0.001
Log(PAAM ,pre)	1.072	0.06	<0.001
tarpaulin	-0.40	0.18	0.027
wellboat	0.223	0.13	0.101
Days after treatment	0.005	0.016	0.586

The variance for the random intercept (treatment) is 0.416 and standard deviation is 0.436.Negative binomial dispersion parameter is 3.66 for 178 treatment and 329 observations

Table 2b. Negative binomial model (with random effect for treatment) for post-treatment abundances of AF in three modalities. Covariate PAAM and AF before treatment (PAAM, pre) was log-transformed and centered. Days post-treatment were also centered.

Variable	**Value**	**Std.Error**	**p-value**
(Intercept)	4.469	0.06	<0.001
Log(PAAM ,pre)	0.532	0.04	<0.001
Log(AF,pre)	0.336	0.05	<0.001
tarpaulin	-0.25	0.15	0.09
wellboat	-0.03	0.12	0.772
Days after treatment	0.04	0.01	0.586

The variance for the random intercept (treatment) is 0.249 and standard deviation is 0.499. Negative binomial dispersion parameter is 4.30 for 178 treatment and 329 observations

Table 3. Summary of the negative binomial multivariate model assuming a shrink correlation matrix. The model shows the effect of treatment modalities after controlling for the abundances of PAAM and AF before treatment and the interval (in days) between treatment and sampling post-treatment.

Variable	**Wald value**	**Wald Pr(>wald)**
(Intercept)	106.9	
Log(AF,pre)	17.1	0.002
Log(PAAM,pre)	20.1	0.002
Tarpaulin	5.2	0.002
Wellboat	1.5	0.344
Interval (days)	4.3	0.002

Test-statistic : 29.41 , p-value=0.002

Test statistics calculated assuming a correlation matrix shrunk by parameter =0.85, P-value calculated using 499 resampling iterations via pit.trap.

Figure 1. The proportion of individuals in each sea lice stage.
Each bar represents the proportion of lice (from cage medians) between treatment modalities and sampling times.

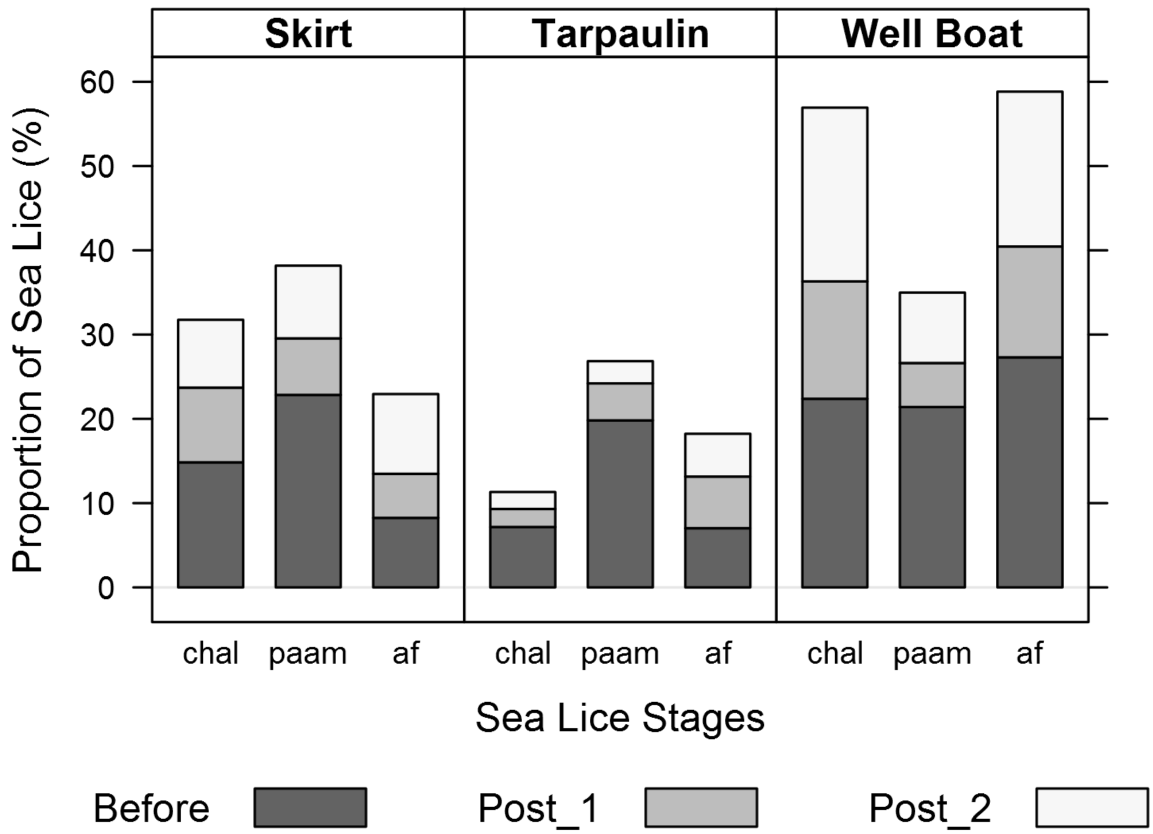
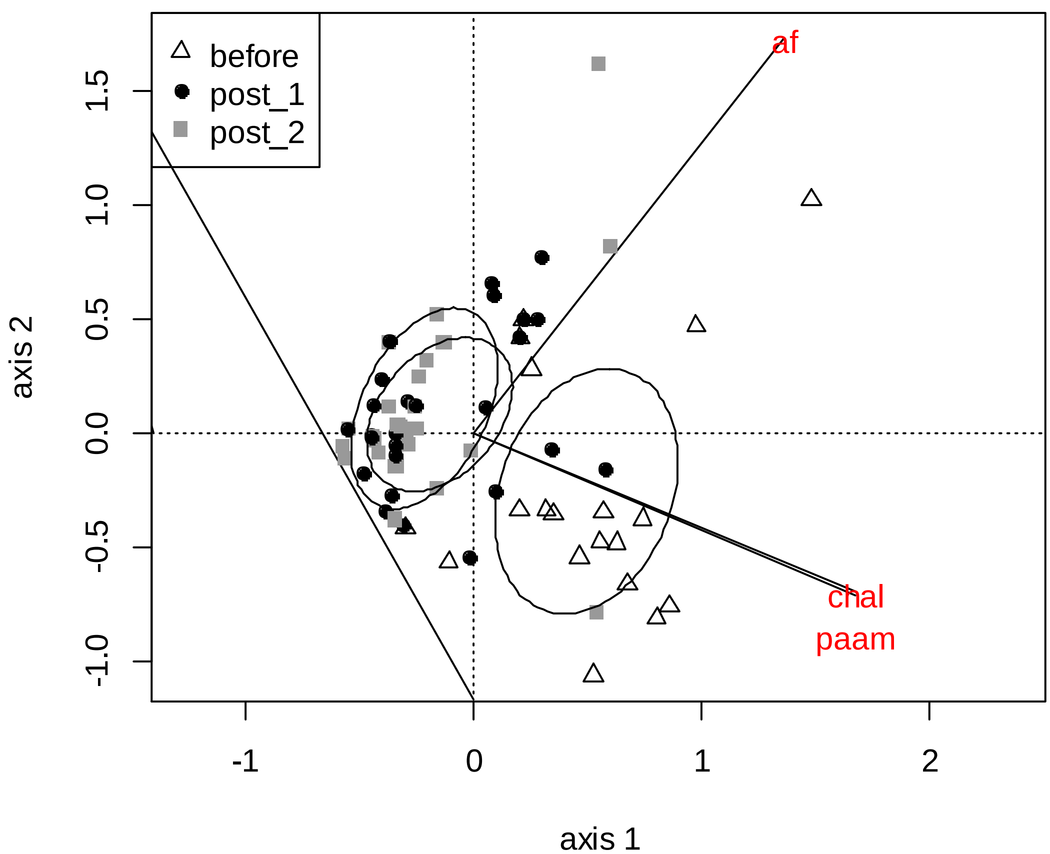
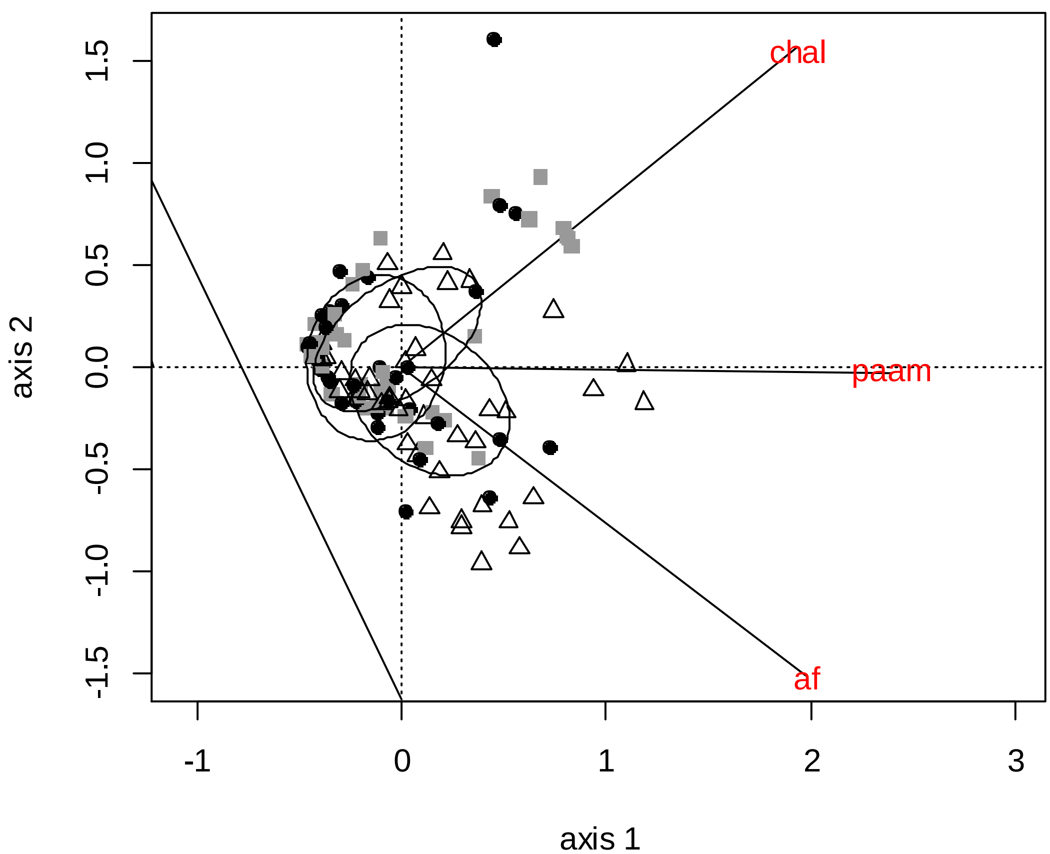


Figure 2a-c. Ordination plots using Principal Component Analysis median (ABC) abundances of lice stage groups by treatment modalities at pre and post-treatment (n=486). In total 21 treatments were not included because of missing data for chalimus. The proportion of the total variance explained by the two main principal components (first and second axes) is 0.85 (skirt), 0.94 (wellboat) and 0.79 (tarpaulin).

A) **Tarpaulin**



B) Wellboat



C) Skirt