Aquaculture 498 (2019) 464–469

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Evaluating the concentration of emamectin benzoate in Atlantic salmon tissues after sea lice treatments

Sophie St-Hilaire[a,b,*], Derek Price[a], Sarah Noftall[a], Brad Boyce[c], Diane Morrison[c]

[a] Department of Health Management, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PEI, Canada
[b] Department of Infectious Diseases and Public Health, College of Veterinary Medicine and Life Sciences, City University of Hong Kong, Kowloon, Hong Kong
[c] Marine Harvest Canada, Campbell River, BC, Canada

ARTICLE INFO

Keywords:
Emamectin benzoate
Sea lice
Treatment
Slice®
Tissue drug concentration

ABSTRACT

Pharmaceutical treatments are an important part of integrated sea lice control strategies in all major salmon producing countries in the world. Ensuring that in-feed medication is delivered appropriately, and that fish achieve an adequate tissue concentration of the medication is critical to a successful treatment. The muscle tissue concentration of emamectin benzoate was assessed post SLICE® treatment in 867 Atlantic salmon from 147 treatments. Approximately 90% of the muscle samples that included skin had emamectin benzoate levels above 60 ng/g (ppb); however, only 63.8% of muscle samples without skin were at or above this threshold. Further, we found a wide range of tissue concentrations across our samples, with some fish as high as 398 ng/g. The variation in the emamectin benzoate concentration in our samples was associated with the following factors: feeding rate, whether or not the muscle sample contained skin, year of the sample, laboratory analyzing the tissue, prescribed dose, and condition factor, after controlling for temperature, day of the sample post-treatment, and pen effect. Interestingly, the positive relationship between condition factor and the concentration of emamectin benzoate in the tissue sample was dependent on the dose of medication in the feed. In other words, increasing the dose of emamectin benzoate had a greater effect on the concentration of drug in tissues for fish with a high condition factor compared to fish with a low condition factor. Our findings suggest that the feeding rate and fish feeding behavior within a pen may be important factors to consider when applying medicated feed to fish populations to ensure a more even distribution of medication between animals.

1. Introduction

The annual value of aquaculture production in Canada has grown over the last 30 years, from $35 million to approximately $1 billion CAD, and this growth is predicted to continue and intensify (FAO, 2016). As the number and density of farms increases in fish farming areas disease control can become more difficult, especially in open net pen systems. Parasites, such as *Lepeophtheirus salmonis,* are host dependent, so the greater the number and density of hosts the more favorable conditions are for parasite replication. It is important to note that fish under aquaculture conditions can tolerate a certain level of sea lice without showing clinical signs, but once the lice levels exceed a certain threshold, which varies depending on the size and species of fish, as well as the species of sea lice, there can be direct and indirect health impacts (González et al., 2015, 2016; Wells et al., 2006).

In Norway and in British Columbia, Canada, regulations exist to reduce the exposure of wild juvenile salmonids to sea lice originating from farmed fish, regardless of whether the parasite is causing health issues in the farmed fish. In British Columbia (BC), the risk of transmission to wild juvenile salmonids is the primary reason to treat farmed fish, as the levels of sea lice never exceed the threshold for known health impacts on farmed fish. Because the sea lice species *L. salmonis* can be transmitted between farms (Rees et al., 2015; Adams et al., 2012; Salama et al., 2013; Jansen et al., 2012), integrated area management plans are required to control their numbers. These plans vary by regions; however, the basic premise of these plans is to maintain control of *L. salmonis* in farming areas using non-pharmaceutical measures (i.e. single year class farming, fallowing between year classes, reducing densities, mechanical or biological removal of sea lice, genetic selection for sea lice resistance), in addition to pharmaceutical treatments, when required.

In BC, chemical treatments for sea lice are limited by regulations to in-feed treatments with emamectin benzoate (SLICE®), and in some areas where permitted by the province, bath treatments with hydrogen

* Corresponding author at: Department of Health Management, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PEI, Canada.
E-mail address: ssthilai@cityu.edu.hk (S. St-Hilaire).

https://doi.org/10.1016/j.aquaculture.2018.08.071
Received 20 April 2018; Received in revised form 23 June 2018; Accepted 29 August 2018
Available online 03 September 2018
0044-8486/

peroxide. The limited options for chemical control of sea lice in BC means the aquaculture industry in the province must be judicious and strategic in their use of these two products to reduce the likelihood of developing resistance in the sea lice populations, which has been observed in other countries managing sea lice infestations (Jones et al., 2012; Aaen et al., 2015).

Sea lice resistance to emamectin benzoate (EMB) in other areas has been demonstrated to be genetically conferred; however, the mechanisms for this selection are not confirmed. In general, antimicrobial resistance is thought to occur because of over use of a product and or dosing of antimicrobials at subtherapeutic levels. The latter is generally not done purposefully, but can occur inadvertently. For example, our research team has identified that when treating large populations of fish with antibiotics, a significant number of the feeding population of fish were under-dosed (Price et al., 2018).

The specific objectives of this study were to determine if fish treated with EMB under commercial aquaculture conditions attain therapeutic concentrations of EMB in their muscle and skin tissues, and identify factors associated with EMB tissue concentrations.

2. Methods

Retrospective data, from 2010 to 2017, for 43 Atlantic salmon farms in British Colombia that underwent EMB treatments for sea lice (7 days at different doses ranging from 50 μg/kg/day to 125 μg/kg/day) were analyzed for tissue levels of EMB. The data were provided to the research team for a statistical analysis in 2017. The industry also provided the level of EMB in the feed for 124 of the 151 treatments. All feed samples tested contained at least 80% of the prescribed dose and 50% of the treatments were above 90%.

The industry sampled five fish from each treated pen. Samples were taken within 1 to 5 days after the last day of each treatment. Muscle samples with or without skin attached were collected, transported on ice for < 24 h, and stored at −20 °C until they could be sent frozen to one of three commercial ISO/IEC 17025 certified laboratories for analysis. The laboratories used liquid Chromatography (LC) with mass spectrometry (MS or MS/MS) to determine the concentration of EMB β1a in the tissues. Two laboratories cited using the Canadian Food Inspection Agency emamectins residue detection protocol SOM-DAR-CHE-030, and the other laboratory used the protocol described in (Van De Riet et al., 2001). The level of EMB β1a was reported in ng/g (ppb) to the producers and subsequently provided to our research team for analysis.

Producers also shared with us information on the weight of the fish sampled, the EMB dose prescribed, the concentration of EMB in the feed for a selected number of treatments, the feed rate during the treatment, the water temperature at the time of the sample, the year of the treatment, the number of days that the fish were treated, and the day (post treatment) when the sample was collected. The latter variable was evaluated both as a linear term and as a dichotomous variable where the cut-off was set at equal to or > 3 days post treatment. Additional information collected on individual fish included weight, length, and whether or not skin was included in the sample. The condition factor was calculated for each fish by dividing the weight of the fish by its length cubed and then multiplying this number by 100.

We described the distribution of EMB tissue concentrations across the dataset and calculated the proportion of samples with levels above 60 μg/kg (ppb). This threshold was selected because it is the recommended level based on the SLICE® usage guidelines (MSD Animal Health, 2012a,b).

We conducted two statistical analyses. The first was a mixed-effects general linear regression model to assess whether water temperature, prescribed dose, feed rate, weight, condition factor, whether skin was included in the sample, and treatment duration, were associated with the concentration of EMB controlling for the laboratory where the sample was analyzed, the year, and the pen where the sample was collected. The second analysis conducted was a logistic regression to determine if the same predictors were associated with the probability of being at or above the 60 ppb threshold. For both the linear and logistic regression models, pen was included as a random effect to correct for clustering of data at the pen/treatment level. Only one pen was sampled per site treated. We tested whether there was an interaction between the effect of dose and the condition factor of the fish on the concentration of EMB. A Box-Cox analysis was used to determine whether the data needed to be transformed to meet the assumptions of a parametric test. Predictors were only maintained in the analysis if the probability value associated with their likelihood ratio test was < 0.05 or if the predictor was a known confounder. Data cleaning and statistical analyses were conducted using the lme4 package (Bates et al., 2015) in R 3.4.2 (R Core Team, 2017) and Stata 15 (StataCorp, College Station, TX).

3. Results

In total, this study included data on 867 fish samples from 147 treatments on 43 sites over an eight-year period. Fifty-three of the 867 samples in our study were excluded from the analysis because of laboratory testing issues identified by the company providing us the data. The range of EMB concentration in our samples was from 1.5 ng/g to 398.8 ng/g (Fig. 1). The interquartile range was from 54.2 to 102.7. Overall, only 68.1% of samples in the dataset were at or above the therapeutic dose of 60 ppb; however, 90% of the samples that included skin were at or above the 60 ng/g (ppb) threshold (Table 1). Inclusion of the 53 samples that were exclude from our analysis resulted in a reduction in the total proportion of samples above the threshold by approximately 3%.

A further 158 samples were dropped from the regression models because these samples were missing fish weight. The residual diagnostics on our non-transformed regression model suggested a slight violation of the normality assumption, and the Box-Cox analysis suggested a square root transformation to improve model assumptions. However, the overall conclusions from the analyses on the transformed and non-transformed datasets were similar (see Table 2), so for simplicity we discuss the trends based on our non-transformed models.

Our initial general linear model contained the following predictors: inclusion of skin in the sample, dose, condition factor, weight of fish, year of treatment, feed rate, temperature, duration of treatment, day of sample post treatment, and laboratory conducting the analysis (Table 2). The final models (square root transformed data and raw

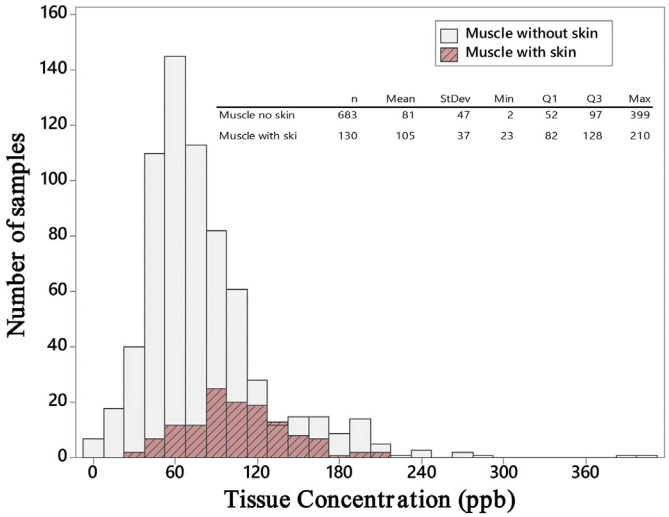

Fig. 1. Distribution of emamectin benzoate tissue concentration in Atlantic salmon post sea lice treatment.

Table 1
Proportion of emamectin benzoate sea lice treatments in Atlantic salmon at or above the therapeutic concentration of 60 μg/kg (ppb) (n = 813).

Tissue type	Frequency	Proportion (%)
Muscle only samples	683	63.8
Muscle and skin samples	130	90.8
All samples	813	68.1

data), where only predictors with P-values above 0.05 were retained, with the exception of temperature and day of sample, which were included to control for confounding, suggested that the feeding rate, whether or not the muscle sample had skin attached, year of the sample, and laboratory analyzing the tissue were associated with the level of EMB in tissues, after controlling for pen effect (Table 2; Fig. 2a–c). Our analysis also suggested that prescribed dose had an effect on the concentration of EMB, but this effect was dependent on the condition factor of the fish (Fig. 2d). Increasing the dose for fish with low condition factors did not have as large of an effect on the EMB concentration as it did for fish with higher condition factors (Fig. 2d). Temperature and day-of-sample collection post treatment were not significantly associated with EMB tissue levels, but these were maintained in the model to correct for confounding and because of their biological significance. Day-of-sample collection was included in our model as a dichotomized variable with a threshold of greater than or equal to 3 days and < 3 days post treatment.

The same predictors that were significant in our linear model were also significant in our logistic regression model with the exception of the condition factor and its interaction term (Table 3).

4. Discussion

After a metaphylactic treatment with an in-feed therapeutant it would be expected all fish have relatively similar levels of medication in their tissues, and preferably that level should be at or slightly above the therapeutic threshold. Our study suggests that approximately 90% of

Table 2
Table of coefficients and P-values for our linear mixed model fitted with 656 observations from 121 treatments. (158 values excluded because weigh and/or length was not recorded).

Fixed effects

Model	Initial (untransformed data)		Final (untransformed data)		Final (Transformed data)	
Term	Estimate	P-value	Estimate	P-value	Estimate	P-value
(Intercept)	−1.87		116.45		10.76	
Year		< 0.01		< 0.01		< 0.01
2010 (ref)						
2011	1.89		2.43		0.10	
2012	−7.62		−6.48		−0.45	
2013	1.14		4.58		0.06	
2014	34.30		47.25		1.71	
2015	1.59		14.54		−0.35	
2016	−15.27		−6.20		−1.36	
2017	−51.59		−44.10		−3.22	
Treatment duration	−0.23	0.93				
Temperature	−2.10	0.07	−1.48	0.20	−0.07	0.21
Day of sample (post treatment)[a]		0.70				
1 (ref)						
2	−0.45					
3	1.97		−2.18[a]	0.73	−0.14[a]	0.67
5+	−10.20					
Weight (kg)	1.35	0.28				
Condition factor (K)[b]	27.30	< 0.01	4.27[b]		0.23[b]	
Cond. fact10 x Dose				< 0.01		< 0.01
Cond. fact10: Dose			120.11		4.95	
Cond. fact10: Dose^2			98.41		5.17	
Dose (μg/kg/day)	1.32	< 0.01	627.39		32.77	
Dose^2			−11.02		−3.98	
Feed rate	−18.94	0.04	−28.45	< 0.01	−1.54	< 0.01
Lab		< 0.01		< 0.01		< 0.01
Lab 1 (ref)						
Lab 2	−51.90		−62.24		−2.57	
Lab 3	−21.08		−30.75		−0.59	
Samples skin-on		< 0.01		< 0.01		< 0.01
No (ref)						
Yes	35.22		40.93		2.09	

Random effects

Model	Initial		Final		Final (Transformed)	
Term	Variance	P-value	Variance	P-value	Variance	P-value
pen treatment ID	100.29	0.07	103.30	< 0.01	0.32	< 0.01
Residual	878.26		877.80		2.84	

[a] In the final model this variable was dichotomized into two groups: 1) fish that were collected < 3 days after the completion of the treatment, and 2) fish collected 3 or more days after the treatment was completed.

[b] In the final models we multiplied the condition factor by 10, so this coefficient reflects the change in tissue concentration of EMB when the condition factor K is increased by 0.1 units.

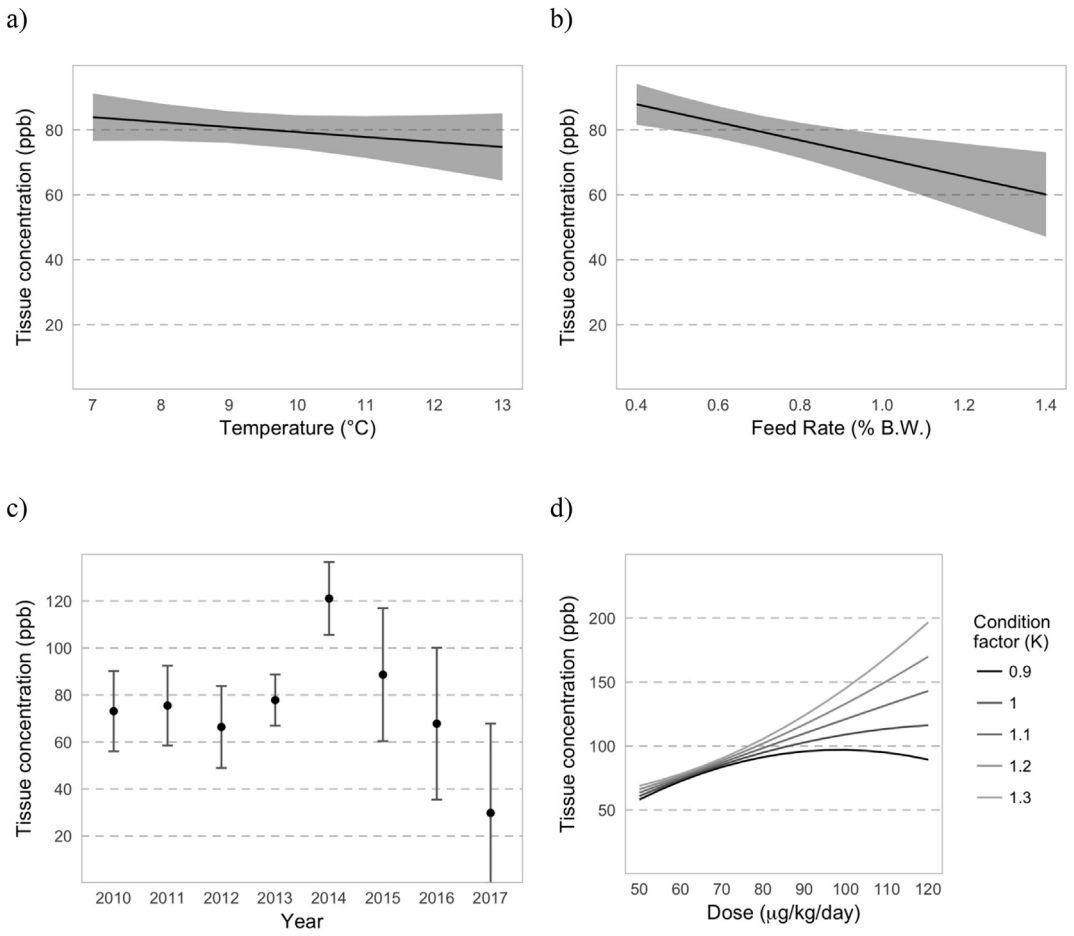

Fig. 2. Graphical summary of effects of a) temperature (°C), b) feed rate (% of body weight), c) year, and d) the interaction between condition factor (K) and dose (µg/kg/day) over the concentration of emamectin benzoate (ppb) obtained from our linear mixed model. The values for condition factor in panel d) roughly represent the 10, 25, 50, 75, and 90th percentiles. Figures derived from models using untransformed data.

the fish on salmon farms treated with a 7-day EMB treatment for sea lice in British Columbia had concentrations in the muscle (with skin attached) at or above 60 ppb, which is the threshold level recommended in the SLICE® Usage Guidelines (MSD Animal Health, 2012a). We observed a higher level of EMB in tissues if skin was included in the muscle sample (Table 2) likely because skin tissue and mucus are known to concentrate EMB (Sevatdal et al., 2005).

The proportion of fish in this study at or above our EMB threshold was similar to what has been reported for in-feed oxytetracycline treatments in Chile (Price et al., 2018), and considerably higher than what was reported for florfenicol treatments in the same study. Interestingly, oxytetracycline (Elema et al., 1996) and EMB (Sevatdal et al., 2005) have relatively long half-lives compared to florfenicol (Martinsen et al., 1993), which may increase the likelihood that a fish within a population on medicated feed attains the desired threshold during the course of a multi-day treatment. This could be explained by the fact that the drug accumulates faster than it is eliminated during the treatment period. A longer half-life is ideal for a sea lice product, such as EMB, as it may offer protection for an extended period of time, post treatment, and provide protection during the planktonic stages that can continue to infect fish after the adult sea lice have been eliminated. The disadvantage of using a drug with a long half-life is that it persists within the fish at levels below the therapeutic range for an extended period of time, which could increase the selection pressure for antimicrobial resistance.

Although there was a high proportion of fish above the 60 ng/g (ppb) threshold, there was also a wide range of EMB tissue concentrations across and within populations. This was also reported by Berg and Horsberg (2009). Treatments would be optimized if the numbers of fish with insufficient and very high levels of EMB in their tissues were reduced. If farmers were able to do this it would reduce sea lice exposure to sub-therapeutic levels of EMB, and make it easier to predict withdrawal times for treated fish.

Several factors explained the variation in EMB concentration in this study. Our analysis suggested that increasing the dose of EMB up to approximately 70 µg/kg/day increased the tissue concentrations in fish; however, increasing the dose beyond this level seemed to disproportionally increase the concentrations in fish with high condition factors (Fig. 2d). These fish were significantly more likely to have higher levels of EMB than fish with lower condition factors when they were treated with high doses (Table 2). It is possible that fish in good body condition had higher fat levels in their muscle, and this resulted in higher concentrations of EMB in this tissue.

Our logistic regression analysis also suggested that the higher the dose, up to approximately 70 µg/kg/day, the more likely fish would be above the 60 ng/g (ppb) threshold. Once the dose was > 70 µg/kg/day the effect on the probability of being at or above the threshold diminished (Fig. 3c), after controlling for all other factors in the model, including pen effect. In the logistic regression model, the dose effect on the probability of being at or above the threshold was not dependent on the condition factor.

It is possible that fish in better condition may disproportionately consume feed, relative to the fish with a lower condition factor. It was not possible to determine why there was a different relationship between dose and tissue concentration at higher doses in this study. Perhaps this should be further investigated with a targeted study to

Table 3
Table of odds ratios and P-values for our logistic mixed model fitted with 814 observations from 147 treatments.

Fixed effects				
Model	Initial		Final	
Term	Odds Ratio (Coef.)	P-value	Odds Ratio (Coef.)	P-value
(Intercept)	0.17 (−1.78)		24.97 (3.22)	
Year		0.52		
2010 (ref)				
2011	0.84 (−0.18)			
2012	0.64 (−0.44)			
2013	0.88 (−0.13)			
2014	1.14 (0.13)			
2015	0.22 (−1.53)			
2016	0.10 (−2.35)			
2017	0.02 (−3.75)			
Treatment duration	1.08 (0.08)	0.55		
Temperature	0.95 (−0.05)	0.58	0.92 (−0.08)	0.39
Day of sample (post treatment)[a]		0.21		
1 (ref)				
2	1.48 (0.39)			
3	0.68 (−0.39)		0.38[a] (−0.97)	0.02
5+	0.37 (−0.99)			
Weight (kg)	0.94 (−0.06)	0.51		
Condition factor (K)	1.80 (0.59)	0.43		
Dose (ug/kg/day)	1.05 (0.05)	< 0.01	2.08×10^{11} (26.06)	
$Dose^2$			1.35×10^{-7} (−15.82)	< 0.01
Feed rate	0.13 (−2.02)	< 0.01	0.17 (−1.75)	< 0.01
Lab		0.11		0.14
Lab 1 (ref)				
Lab 2	0.46 (−0.78)	0.54	0.31 (−1.17)	
Lab 3	3.22 (1.17)	0.49	0.44 (−0.82)	
Samples skin-on		0.05		0.03
No (ref)				
Yes	9.49 (2.25)		3.19 (1.16)	

Random effects				
Model	Initial		Final	
Term	Variance	P-value	Variance	P-value
pen treatment Id	0.38	0.04	0.58	< 0.01

[a] In the final model this variable was dichotomized into two groups: 1) fish that were collected < 3 days after the completion of the treatment, and 2) fish collected 3 or more days after the treatment was completed.

provide insight into the optimal dosing level.

A factor that was negatively associated with EMB tissue concentration was the feed rate within the pen. Fish fed at higher feed rates, controlling for dose and condition factor and other factors in the models, had lower EMB tissue concentrations, and had a lower probability of being at or above the 60 ppb threshold than fish fed at lower feed rates (Tables 2 and 3). This suggests the more concentrated the medication in the feed, the higher the levels in the fish. The presence of a three-way interaction between the feed rate, the condition factor, and the dose of EMB could not be assessed due to the limited size of our dataset, but it may be interesting to evaluate whether the feed rate effect is dependent on the condition of the fish and or dose, in future studies with larger datasets.

The findings from the logistic and general linear models were relatively consistent, suggesting that feed rate and dose could be manipulated to change the distribution of EMB concentration in fish populations and increase the number of fish at or above the 60 ng/g (ppb) threshold.

We evaluated and controlled for a number of likely confounders in our models, such as temperature, the laboratory conducting the analysis, and sampling day post treatment. Although of these predictors, only the laboratory was significantly associated with the level of EMB in tissues and the probability of being at or above the threshold, the other two were forced in the final models to ensure they did not distort the relationships between the other predictors and the outcome. Laboratory 1 accounted for a significant amount of variation in the tissue concentration likely because they used a different method of analysis than the other laboratories. Pen was included as a random variable in the models to control for clustering within the pens. We also evaluated whether correcting the prescribed dose based on the level of EMB in the feed changed the conclusions of our study and it did not (data not shown). Although many potential predictors of EMB tissue concentration were included in the initial model, it is likely that some unknown confounders were missing from our analyses. For this reason, we have chosen not to over interpret the coefficients and odds ratios estimated in our models as these could be distorted if significant confounders were missing from the analyses.

Despite the limitations to this study, the trends observed such as the association between EMB tissue concentration and feeding rates, as well as the relationship between dose, condition factor, and drug tissue concentrations, are likely real and should be further investigated to improve treatment distribution during metaphylactic treatments with in-feed medications. Further research should also obtain empirical data on the length of time the levels of EMB in tissues persist in fish populations to enable farmers to better plan treatments and understand duration of therapeutic effect.

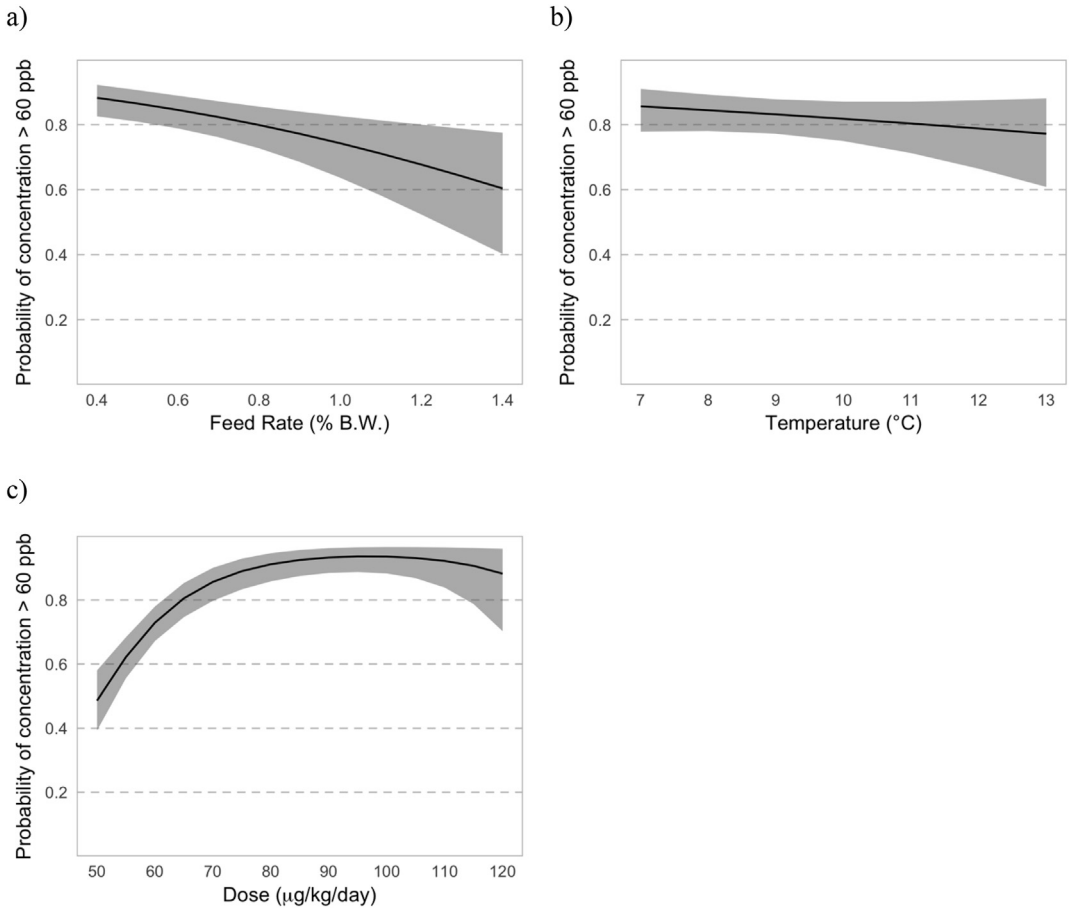

Fig. 3. Graphical summary of effects of a) feed rate (% of body weight), b) temperature (°C), and c) the second order polynomial of dose (µg/kg/day) over the concentration of emamectin benzoate (ppb) obtained from our logistic mixed model.

Acknowledgments

This research was undertaken, in part, thanks to funding from the Natural Sciences and Engineering Research Council of Canada(CRC programme). We would like to thank William Chalmers for editorial assistance in the preparation of the manuscript, and our industry partners for insightful discussions and access to their data.

References

Aaen, S.M., Helgesen, K.O., Bakke, M.J., Kaur, K., Horsberg, T.E., 2015. Drug resistance in sea lice: a threat to salmonid aquaculture. Trends Parasitol. 31 (2), 72–81. https://doi.org/10.1016/j.pt.2014.12.006.

Adams, T., Black, K., MacIntyre, C., MacIntyre, I., Dean, R., 2012. Connectivity modelling and network analysis of sea lice infection in Loch Fyne, west coast of Scotland. Aquacult. Environ. Interact. 3, 51–63. https://doi.org/10.3354/aei00052.

MSD Animal Health, 2012a. SLICE® Usage Guidelines. Intervet International B.V., a subsidiary of Merck & Co. Inc., Whitehouse Station, NJ, USA (MSD-SSP-07, p22).

MSD Animal Health, 2012b. The SLICE® Sustainability Project. Intervet International B.V., a subsidiary of Merck & Co. Inc., Whitehouse Station, NJ, USA, pp. 1–21 MSD-SSP-05.

Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67 (1), 1–48. https://doi.org/10.18637/jss.v067.i01.

Berg, A.-G., Horsberg, T.E., 2009. Plasma concentration of emamectin benzoate after SLICE™ treatments of Alantic salmon (*Salmo salar*): differences between fish, cages, and seasons. Aquaculture 288, 22–26.

Elema, M., Hoff, K., Kristensen, H., 1996. Bioavailability of oxytetracycline from medicated feed administered to Atlantic salmon (*Salmo salar* L.) in seawater. Aquaculture 143, 7–14. https://doi.org/10.1016/0044-8486(96)01253-7.

FAO. 2016. The State of World Fisheries and Aquaculture 2016. Contributing to food security and nutrition for all. Rome. 200 pp.

González, M., Marín, S., Vargas-Chacoff, L., 2015. Effects of *Caligus rogercresseyi* infestation on physiological response of host *Salmo salar* (Linnaeus 1758): establishing physiological thresholds. Aquaculture 438, 47–54. https://doi.org/10.1016/j.aquaculture.2014.12.039.

González, M., Muñoz, J., Valerio, V., Vargas-Chacoff, L., 2016. Effects of the ectoparasite *Caligus rogercresseyi* on *Salmo salar* blood parameters under farm conditions. Aquaculture 457, 29–34. https://doi.org/10.1016/j.aquaculture.2016.01.027.

Jansen, P.J., Kristoffersen, A.B., Viljugrein, H., Jimenez, D., Aldrin, M., Stien, A., 2012. Sea lice as a density-dependent constraint to salmonid farming. Proc. R. Soc. Lond. B Biol. Sci. 279 (1737), 2330–2338. https://doi.org/10.1098/rspb.2012.0084.

Jones, P.G., Hammell, K.L., Dohoo, I.R., 2012. Revie CW. Effectiveness of emamectin benzoate for treatment of *Lepeophtheirus Salmonis* on farmed Atlantic salmon *salmo salar* in the Bay of Fundy, Canada. Dis. Aquat. Org. 102 (1), 53–64. https://doi.org/10.3354/dao02517.

Martinsen, B., Horsberg, T.E., Varma, K., Sams, R., 1993. Single dose pharmacokinetic study of florfenicol in Atlantic salmon (*Salmo salar*) in seawater at 11C. Aquaculture 112, 1–11. https://doi.org/10.1016/0044-8486(93)90153-P.

Price, D., Sanchez, J., McClure, J., McConkey, S., Ibarra, R., St-Hilaire, S., 2018. Assessing concentration of antibiotics in tissue during oral treatments against piscirickettsiosis. Prevent. Vet. Med. 156, 16–21.

R Core Team, 2017. R: A Language and Environment for statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.gbif.org/tool/81287/r-a-language-and-environment-for-statistical-computing/, Accessed date: 13 April 2018.

Rees, E.E., St-Hilaire, S., Jones, S.R.M., Krkošek, M., Dedominicis, S., Foreman, M.G.G., Patanasatienkul, T., Revie, C.W., 2015. Spatial patterns of sea lice infection among wild and captive salmon in western Canada. Landsc. Ecol. 30 (6), 989–1004. https://doi.org/10.1007/s10980-015-0188-2.

Salama, N., Collins, C., Fraser, J., Dunn, J., Pert, C., Murray, A., Rabe, B., 2013. Development and assessment of a biophysical dispersal model for sea lice. J. Fish Dis. 36 (3), 323–337. https://doi.org/10.1111/jfd.12065.

Sevatdal, S., Magnusson, Å., Ingebrigstsen, K., Haldorsen, R., Horsberg, T.E., 2005. Distribution of emamectin benzoate in Atlantic salmon (*Salmo salar* L.). J. Vet. Pharmacol. Ther. 28, 101–107. https://doi.org/10.1111/j.1365-2885.2004.00629.x.

Van De Riet, J., Brothers, N.N., Pearce, J.N., Burns, G., 2001. Simultaneous determination of emamectin and ivermectin residues in Atlantic salmon muscle by liquid chromatography with fluorescence detection. J. AOAC Int. 84, 1358–1362.

Wells, A., Grierson, C.E., MacKenzie, M., Russon, I.J., Reinardy, H., Middlemiss, C., Bjørn, P.A., Finstad, B., Bonga, S.E., Todd, C.D., 2006. Physiological effects of simultaneous, abrupt seawater entry and sea lice (*Lepeophtheirus salmonis*) infestation of wild, sea-run brown trout (*Salmo trutta*) smolts. Can. J. Fish. Aquat. Sci. 63 (12), 2809–2821. https://doi.org/10.1139/F06-160.