



Confidence in assessing the effectiveness of bath treatments for the control of sea lice on Norwegian salmon farms

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ABSTRACT

The salmon louse *Lepeophtheirus salmonis* is the most important ectoparasite of farmed salmonids in the Northern hemisphere, having a major economic and ecological impact on the sustainability of this sector of the aquaculture industry. To a large extent, control of *L. salmonis* relies on the use of topical delousing chemical treatments in the form of baths. Improvements in methods for the administration and assessment of bath treatments have not kept pace with the rapid modernization and intensification of the salmon industry. Bath treatments present technical and biological challenges, including best practice methods for the estimation of the effect of lice treatment interventions. In this communication, we compare and contrast methods to calculate and interpret treatment effectiveness at pen and site level. The methods are illustrated for the calculation of the percentage reduction in mean abundance of mobile lice with a measure of confidence. Six different methods for the calculation of confidence intervals across different probability levels were compared. We found the quasi-Poisson method with a 90% confidence interval to be informative and robust for the measurement of bath treatment performance.

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1. Introduction

The salmon louse *Lepeophtheirus salmonis* constitutes a persistent parasite infection and a constant source of production losses in the culture of salmonids in the Northern hemisphere. Control of the parasite relies on the use of chemical treatments administered topically in bath treatments, and orally in the feed. In recent years, the use of bath treatments has increased due to the apparent development and spread of resistance to the widely used in-feed treatment, emamectin benzoate (Jones et al., in review; Lees et al., 2008; NFSA, 2010a). There is currently no standardized way to estimate and report treatment effectiveness in net pen fish farms, unlike the evaluation of efficacy of parasitocidal treatments in terrestrial species (Coles et al., 1992). In this paper, we propose a method and guidelines for evaluation and interpretation of the effectiveness of bath treatments. The method involves calculation of a point estimate of the percentage reduction in the number of mobile lice, with the associated uncertainty expressed as a confidence interval.

It is crucial to assess the true effect of bath treatments and thus provide a basis for detection and correction of intrinsic and external factors that limit the use of these treatments in the field.

However, current legislation neither provides grounds for the selected 90% margin, nor guidelines for the evaluation of treatment effect (NFSA, 2010b; NFSA, 2011). The proposed method is a more realistic approach to evaluation and interpretation of the true effect of treatment. It will be more sensitive in identifying those pens and sites which are below the recommended level of efficacy and decrease the associated risk of treatment resistance developing (Denholm et al., 2002).

2. Materials and methods

2.1. Study population

The dataset originates from a study conducted (Heuch et al., 2003) on salmon and rainbow trout farming sites located on the Norwegian coast. Sites with data on samplings performed the week prior to and after delousing treatment were selected. In this subset, a total of 1002 fish from five different sites and 18 pens were inspected on 24 treatment occasions between September 1997 and November 1999. For treatment evaluation, we extracted counts of mobiles within the week prior and after treatment. The majority of pens (21/24) had a single treatment and the three remaining pens in the same site were treated in three different occasions. The dataset included counts for chalimus, pre-adult, adult male and adult females of *L. salmonis* of 20 or more individual fish from each pen on each sampling occasion. Counts were performed at weekly, biweekly or monthly intervals

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depending on site and study period. Bath treatments were undertaken with deltamethrin (AlphaMax®, Alparma) using skirts. At the time, this was the most popular and effective delousing treatment available (Denholm et al., 2002).

We describe the distribution of mobile lice (pre-adult and all adult sea lice stages) both at pen and site level in the week prior to and 1 week after treatment. The distribution of mobiles tends to vary depending on mean abundance of *L. salmonis* (Heuch et al., 2011). For Poisson regression, goodness of fit of the model was tested using Pearson's chi-square. Empirical data were fit to the Poisson, quasi-Poisson and negative binomial distributions with maximum likelihood using the 'fitdist' and the 'vcd' packages available with R software (Friendly, 2000; Venables and Ripley, 2002; Vose, 2008). Overdispersion is characteristic of most parasitic infections (Anderson and May, 1992) including *L. salmonis* (Baillie et al., 2009; Heuch et al., 2011). Here, overdispersion refers to the variance of mobiles being larger than the mean. An indication of overdispersion is when the deviance measure of the goodness-of-fit for full model (standardized residuals) exceeds its degrees of freedom (Venables and Ripley, 2002).

2.2. Calculation of estimates and confidence interval for the percent reduction in count of mobiles

The percentage treatment effectiveness is calculated as $100 * \left(\frac{\mu_0 - \mu_1}{\mu_0} \right)$, where μ_0 and μ_1 are the mean abundances of *L. salmonis* before and after treatment, respectively.

For calculation of treatment effectiveness, we have used mobiles (pre-adult and adult stages). Counts of chalimus stages were not included because this stage is known to be relatively insensitive to most chemical treatments used in baths (Burka et al., 1997). Due to their small size (0.7–2.7 mm) they are also more difficult to see when examining the fish. Hence, counts of chalimus are often underestimated on the farm (Beamish et al., 2005; Schram, 1993).

Calculation of treatment effectiveness was made both at individual pen and site level. At site level, we aggregated counts of mobiles for all treated pens to obtain a single estimate of treatment effectiveness. Confidence intervals for the ratio of two means were calculated using approximate methods (Chaudhary and Stearns, 1996; Cimermanová, 2007). Six methods were used to calculate confidence intervals with the 'pairwiseCI' package available in R (R Development Core Team, 2008). These included two methods for continuous data: a two-sample *t*-test with Fieller confidence intervals assuming heteroscedasticity (Hasler and Hothorn, 2008; Hasler et al., 2008), and the ratio of two means assuming a lognormal distribution (log-normal ratio) (Chen and Zhou, 2006). Three methods for count data were also evaluated, the Poisson, quasi-Poisson and negative binomial methods (Venables and Ripley, 2002; Zeileis et al., 2008). The last method is a percentile bootstrap confidence interval using the Harrell–Davis method (Harrell and Davis, 1982). The original dataset was modified for calculation of the confidence intervals for the two-sample *t*-test and the log-normal ratio. For the latter, 0.1 was added to all data; in the former a value of 0.1 was added to one fish if none of the fish in the sample were infected with mobiles (Tukey, 1977).

A two sided confidence interval was chosen to state the precision of the estimate of treatment effectiveness (Steiger and Fouladi, 1997). We examined confidence levels of 70%, 80%, 90% and 95% to study their effects on the interval widths. The confidence level is dictated by criteria which include confidence and informativeness or decisiveness (Senn, 2007; Smithson, 2003).

2.3. Treatment vs control abundance for effectiveness

The effects of synthetic pyrethroids have been previously investigated. Several studies have shown that adult and pre-adult

stages of *L. salmonis* are highly susceptible to synthetic pyrethroids (Hart et al., 1997).

The required minimum margin is decided by the user, although common practice is often used such as in trials of human antibiotics, where a 10% or 20% difference compared to the efficacy of the reference treatment is used (FDA, 2010). The same margin is recommended for the approval of new antiparasitic treatments in terrestrial animals (Burrige et al., 2010; CVMP, 2001; Schall and Luus, 2011). The Norwegian Food Safety Authority (NFSA), states that a 90% reduction is satisfactory (NFSA, 2010b; NFSA, 2011).

Treatment effectiveness is here measured relative to a chosen standard of treatment efficacy which could be any value. In the case of synthetic pyrethroids, prior studies have shown the average percent reduction in pre-adults and adult females was in the 95 to 99% range (Hart et al., 1997). In this case, we have followed the guidelines laid out by the NSFA. We classify the treatment effect as acceptable or unacceptable (failure). Treatments were considered acceptable when the calculated lower confidence limit is equal to or higher than 90%. When the calculated lower limit is below the 90% minimum margin of efficacy, the treatments were considered unacceptable.

3. Results

3.1. Descriptive statistics

In the extracted subset of data, average counts of mobiles per fish before treatment were 4.6 (0.1 to 16.5) at pen and 4.9 (0.3 to 10.1) at site level (Table 1). The calculated mean and variance were similar at mobile counts around 10–12 per fish (Fig. 1a). In 60% (6/10) of the sites and 75% (18/24) of the pens, the Poisson distribution fits the

Table 1
Mean and variance of the counts of *L. salmonis* mobiles (pre-adult and adult stages) before (t0) and after treatment (t1) according to site, treatment date (month, year) and pen.

Site	Treatment date	Pen number	Mean (t0)	Variance(t0)	Mean (t1)	Variance (t1)
1	7/1997	30	10.1	16.2	0	0
1	7/1997	Site average	10.1	16.2	0	0
1	8/1998	22	11.7	18.3	3.9	9.5
1	8/1998	27	5.3	13.5	1.9	2.8
1	8/1998	Site average	8.7	26.2	2.9	7
2	8/1997	1	16.5	297	0.1	0.1
2	8/1997	2	6.4	8.3	0.9	1.7
2	8/1997	3	12.6	75.5	0.6	0.9
2	8/1997	Site average	11.8	140.4	0.5	1
3	10/1998	1	0.4	0.2	0	0
3	10/1998	6	0.1	0.2	0	0
3	10/1998	7	0.5	0.5	0	0
3	10/1998	Site average	0.3	0.4	0	0
3	7/1999	1	4.3	10.7	0.3	0.4
3	7/1999	6	3.6	3.2	0.1	0.1
3	7/1999	7	3.7	6.1	0.2	0.1
3	7/1999	Site average	3.9	6.6	0.2	0.2
3	8/1999	1	1.8	2.3	0.1	0.1
3	8/1999	6	1.5	2.1	0.3	0.2
3	8/1999	7	4.4	5.7	0.3	0.2
3	8/1999	Site average	2.5	5	0.2	0.2
4	8/1998	5	1	1.3	0	0
4	8/1998	Site average	1	1.3	0	0
5	3/1999	7	0.9	0.9	0	0
5	3/1999	9	0.7	1.1	0	0
5	3/1999	12	0.8	0.6	0	0
5	3/1999	Site average	0.8	0.8	0	0
5	7/1999	21	4.6	5.4	0.9	3.3
5	7/1999	24	6.8	5	0.1	0.1
5	7/1999	26	4.7	3.4	0	0
5	7/1999	Site average	5.3	5.5	0.3	1.2
5	8/1999	20	4.2	2.3	0	0
5	8/1999	25	4.2	3.3	0.4	0.5
5	8/1999	Site average	4.2	2.7	0.2	0.3

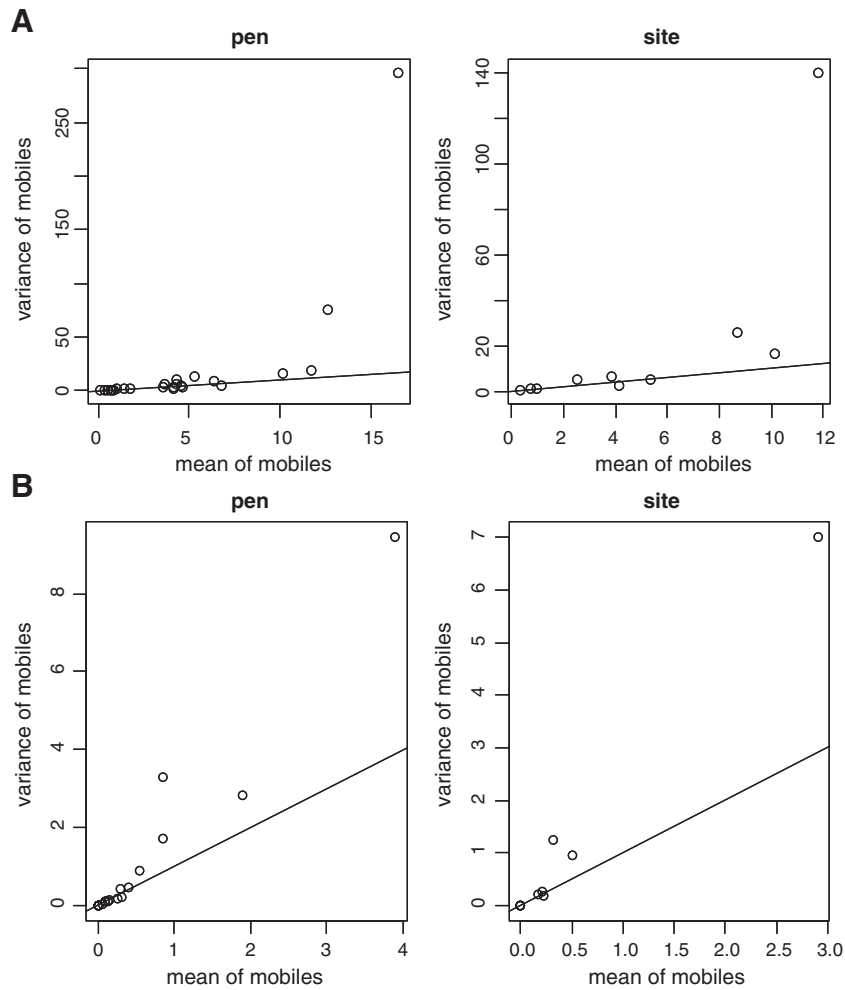


Fig. 1. Mean to variance relationship in the data, for the counts of *L. salmonis* mobiles before (A) and after treatment (B) at pen ($n=24$) and site ($n=10$) level. The black line represents a linear function with a slope of 1 and 0 intercept (mean = variance). Points above the black line indicate records for fish where the variance of mobiles is larger than the mean (overdispersion).

empirical data well (chi-square goodness of fit test for a Poisson distribution, $p>0.05$). After treatment (Fig. 1b), counts of mobiles are dominated by zero values and neither the Poisson nor negative binomial fits the empirical data well due to the excess of zeros present.

3.2. Calculation of confidence intervals using different methods width CI

Six methods were used to calculate the 95% confidence intervals for the estimates of treatment effectiveness at pen (Fig. 2) and site levels (Fig. 3): the t -test, the log-normal ratio, the Harrell–Davis (bootstrap), the Poisson, the quasi-Poisson and the negative binomial. The widths of the confidence intervals varied depending on the method used. The Harrell–Davis method produced the narrowest interval (Figs. 2 and 3). The widest intervals were obtained with the log-normal method. The widths of the confidence interval were similar for the Poisson, the t -test, the quasi-Poisson and the negative binomial. For any of these methods the widths of the confidence interval fell between those of the log-normal ratio and the bootstrap method. The point estimates of treatment effectiveness calculated with the t -test, log-normal and Harrell–Davis methods were slightly different (Figs. 2 and 3) as a result of modification of the original dataset (t -test and log-normal method) or the method for calculation (Harrell–Davis).

Calculations using the log-normal method produced a large number of treatment failures, indeed 29.1% (7/27) of the pen-level

treatments were acceptable. In contrast, bootstrapping results gave the largest number of acceptable treatments (75%) and the least number of failures (25%) (Tables 2 and 3).

Methods for count data (Poisson, quasi-Poisson and negative binomial) produced comparable results. At pen level, the quasi-Poisson produced less failed treatments compared to the Poisson and negative binomial (Tables 2 and 3). At site level 70% (7/10) to 80% (8/10) of treatments were acceptable. One more site failed the treatment with the quasi-Poisson compared to the Poisson and negative binomial. The three sites where treatment failed had a large proportion of treatment failures at pen level ranging from 67% (2/3) to 100% (2/2 and 3/3) (data not shown).

We compared the width of the confidence intervals calculated with the quasi-Poisson method at four different confidence levels: 70%, 80%, 90% and 95%. Obviously smaller confidence levels reduced the width of the confidence interval (Figs. 4 and 5) and therefore tended to reduce the number of failed treatments (Tables 4 and 5).

Evaluation of treatments was carried out using the quasi-Poisson method with a 90% confidence level. At individual pen level, 21% of the pens had an estimate of effectiveness below 90%, and this increased two-fold (46%) when the evaluation was made using confidence intervals. A threefold increase (from 10% to 30%) in the number of failed treatments was shown at site-level when using confidence limits compared to the point estimate of treatment effectiveness.

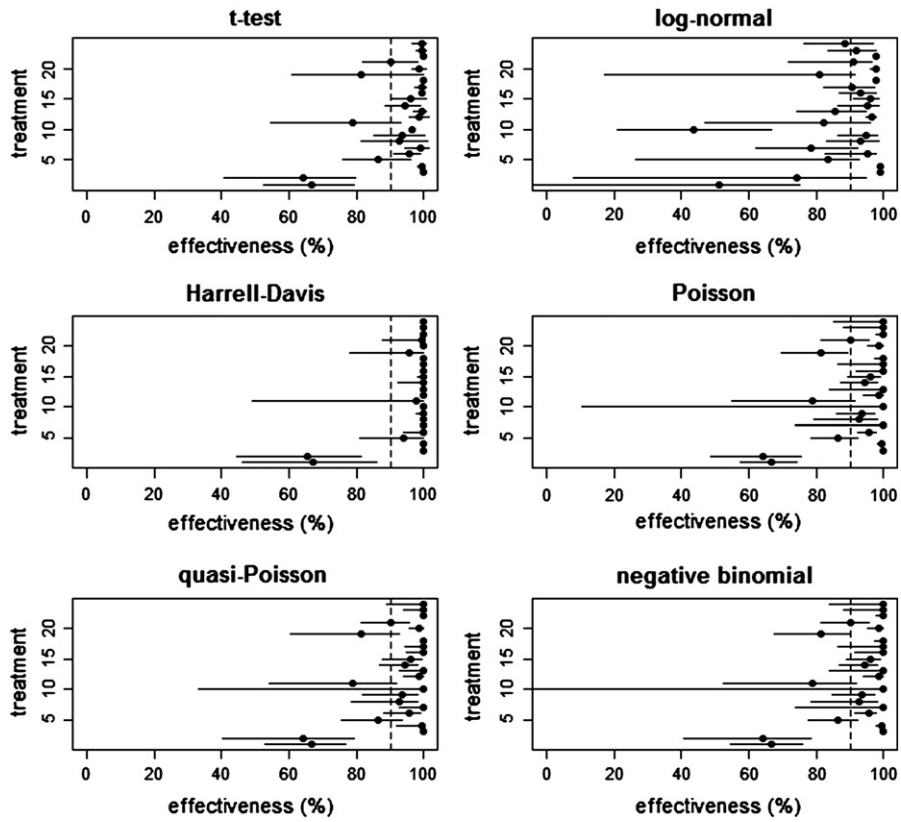


Fig. 2. Estimates and 95% CI of treatment effectiveness against *L. salmonis* in 24 salmon pens. The confidence intervals were calculated using six different methods: *t*-test, log-normal, Harrell–Davis, Poisson, quasi-Poisson and negative binomial.

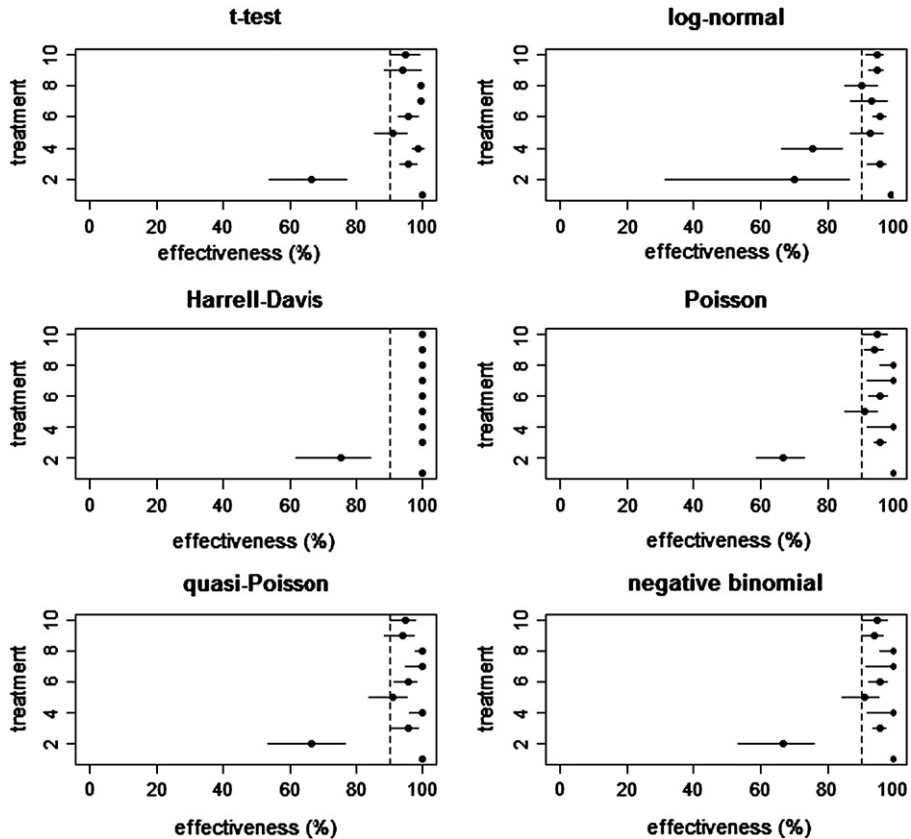


Fig. 3. Estimates and 95% CI of treatment effectiveness against *L. salmonis* treatment effectiveness for 10 treatment events aggregated at the site level. The confidence intervals were calculated using six different methods: *t*-test, log-normal, Harrell–Davis, Poisson, quasi-Poisson and negative binomial.

Table 2

Number of treatments against *L. salmonis* at pen level ($n = 24$) that were acceptable (A) at 90% effectiveness and unacceptable (U) according to the methods described in the text. Estimates and 95% CI were calculated with six methods.

Result	Method for calculation of CI					
	<i>t</i> -test ^a	log-normal	Harrell–Davis	Poisson	Quasi-Poisson	Negative binomial
A	14	7	19	8	11	8
U	9	17	5	16	13	16

^a The two-sample *t*-test could not compute a 95% confidence interval for treatment performed at Site 3 (Pen 6) on 10/1998.

4. Discussion

The continued use of parasitic treatments has resulted in the development and spread of resistance for common drugs such as organophosphates (Fallang et al., 2004; Jones et al., 1992; Tully and McFadden, 2000), avermectins (Jones et al., in review; Lees et al., 2008); pyrethroids (Sevatdal and Horsberg, 2003) and hydrogen peroxide (Treasurer et al., 2000). The growing awareness of drug resistance emergence in certain sea lice populations and the need for information on their occurrence and spread have increased the requirements for standardized methods of treatment evaluation.

Currently, evaluation of treatment is carried out by comparing the point estimate of treatment effectiveness without confidence limits to a pre-specified fixed value. Evaluation of treatment efficacy in this manner has limitations such as uncertainty in the estimate of treatment efficacy. Hence, it is desirable to calculate the variability in efficacy as estimated, through the use of confidence intervals. A potential problem for the calculation of CIs is the lack of control or non-treated groups of fish, as this precludes a direct method for calculation of confidence intervals (Coles et al., 1992). Several solutions were proposed by Fieller and others, who developed theorems to calculate a confidence interval from a ratio of two means (Chaudhary and Stearns, 1996; Cimermanová, 2007).

We have calculated and compared six methods for calculation of generalized confidence intervals. The log-normal ratio produced the widest confidence interval and bootstrapping the narrowest intervals. These two and the *t*-test produce an estimate of treatment effectiveness that is slightly different from that calculated using the formula in Section 2.2, and therefore were considered inferior to the ones used for count data. The Poisson, quasi-Poisson and negative binomial are preferred because they are more appropriate for count data. All three methods give confidence intervals with similar characteristics.

Using CIs, we here propose basic guidelines for calculation and evaluation of topical treatments against *L. salmonis*, so that data on the effectiveness of treatments as well as on the epidemiology of drug resistance in sea lice can be more accurately determined.

In our data, the distributions of mobiles prior to treatment fit the Poisson distribution when the average number of mobiles is below ten mobiles per fish. This is in agreement with published studies in Norwegian locations (Heuch et al., 2011). Salmon lice can exhibit overdispersion (Heuch et al., 2011). However, overdispersion is likely to occur at site level due to large differences between levels of mobiles among pens (Heuch et al., 2011; Revie et al., 2007).

Overdispersion can cause underestimation of the standard error and confidence intervals which are too narrow, resulting in wrong inference for the treatment effect. Both the negative binomial and quasi-Poisson are commonly used for data with unbounded positive range whose sample variance is larger than the mean (Ver Hoef and Boveng, 2007). For this reason, we have selected the quasi-Poisson method. The quasi-Poisson produces parameter estimates equivalent to the Poisson, where the mean is equivalent to that obtained using

Table 3

Number of treatments against *L. salmonis* at site level ($n = 10$) that were acceptable (A) at 90% effectiveness and unacceptable (U) according to the methods described in the text. Estimates and 95% CI of treatment effectiveness were calculated with six methods.

Result	Method for calculation of CI					
	<i>t</i> -test	log-normal	Harrell–Davis	Poisson	Quasi-Poisson	Negative binomial
A	7	5	9	8	7	8
U	3	5	1	2	3	2

the Poisson and the variance is calculated as a multiplicative constant of the mean. This results in standard errors that are larger than those of the Poisson. Quasi-likelihood methods are based on generalized linear model principles and allow calculation of parameter estimates based on a specification of the mean and variance of the model observations without specification of an underlying log-like function (Hilbe, 2011).

Treatment effectiveness was calculated and compared at pen and site level with four different confidence levels. The proportion of acceptable treatments is larger at site compared to pen at the same confidence level. At pen level, disagreement was seen in four out of 24 treatments between the 70% and 95% confidence levels. In three out of four of these pens, treatments were unacceptable at the 95%, but acceptable at the 80%, and belonged to sites where treatment effect on the site and adjacent pens were also acceptable (at the 95% confidence level). In the fourth pen, treatment was acceptable only at the 70% level, but failed at higher confidence levels both on the site and pen. The best agreement between the treatment effect at site and pen level was achieved at the 90–95% and 80% confidence level, respectively. These results are not surprising considering that more data were available, and therefore, less uncertainty and higher precision were found on site versus pen treatments. In this case, it could be justified to use a high confidence level but the choice of confidence level should be dictated by the relative incentives for accuracy and precision in each field situation. Yaniv and Foster (1997) have suggested that the precision of uncertain judgments involves a trade-off between these two competing objectives. Selection of a particular method and confidence level should be further investigated due to the limited availability of data and the substantial differences in today's production management in aquaculture.

Treatment results are classified in two categories, acceptable and unacceptable. We have used a fixed margin for evaluation of treatments since the use of a placebo or a non-treatment group is not possible in field treatments. The expected treatment outcome was equal to or higher than a 90% reduction in mean abundance of mobiles after treatment, based on prior studies (Hart et al., 1997). A non-inferior margin was initially set at 90% as recommended in both the guidelines on the efficacy requirements for new parasiticides (CVMP, 2004; Marchiondo et al., 2007) and regulations set by the Norwegian Food Safety Authority (NFSA). The NFSA defines as failures those delousing treatments with effectiveness below 90% (NFSA, 2011) which must be reported, and further investigated to evaluate possible drug resistance (Directorate of Fisheries, 2009; NFSA, 2010b).

A larger number of treatments would have been deemed to be “failures” if the confidence interval approach had been used rather than a point estimate of treatment effectiveness. Here the lower confidence limits were below the 90% effectiveness level in the majority of pen treatments. This result is not surprising considering that treatments performed with skirts are much less effective than with full tarpaulin (Corner et al., 2011; Fridell, 2009; SEARCH Consortium, 2006). It is also possible that treatments were limited to a group of pens instead of all pens on the site, which will negatively impact the effect of treatment both at pen and site level. Regardless,

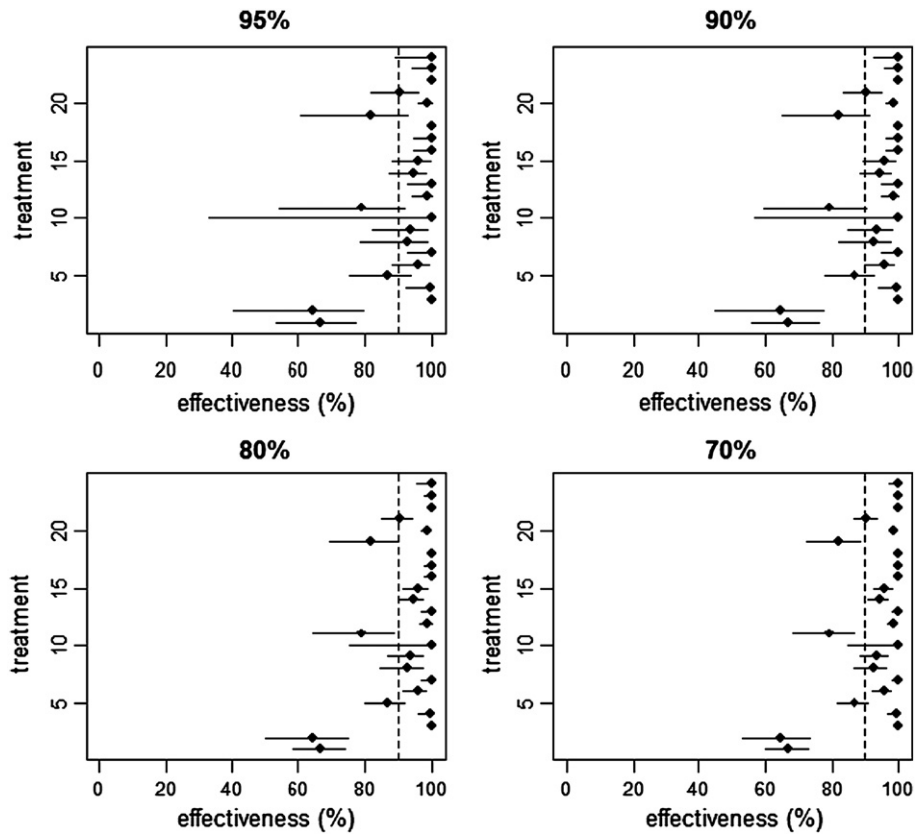


Fig. 4. Estimates of *L. salmonis* treatment effectiveness calculated with confidence levels of 70%, 80%, 90% and 95% in all treated pens ($n = 24$) using the quasi-Poisson method.

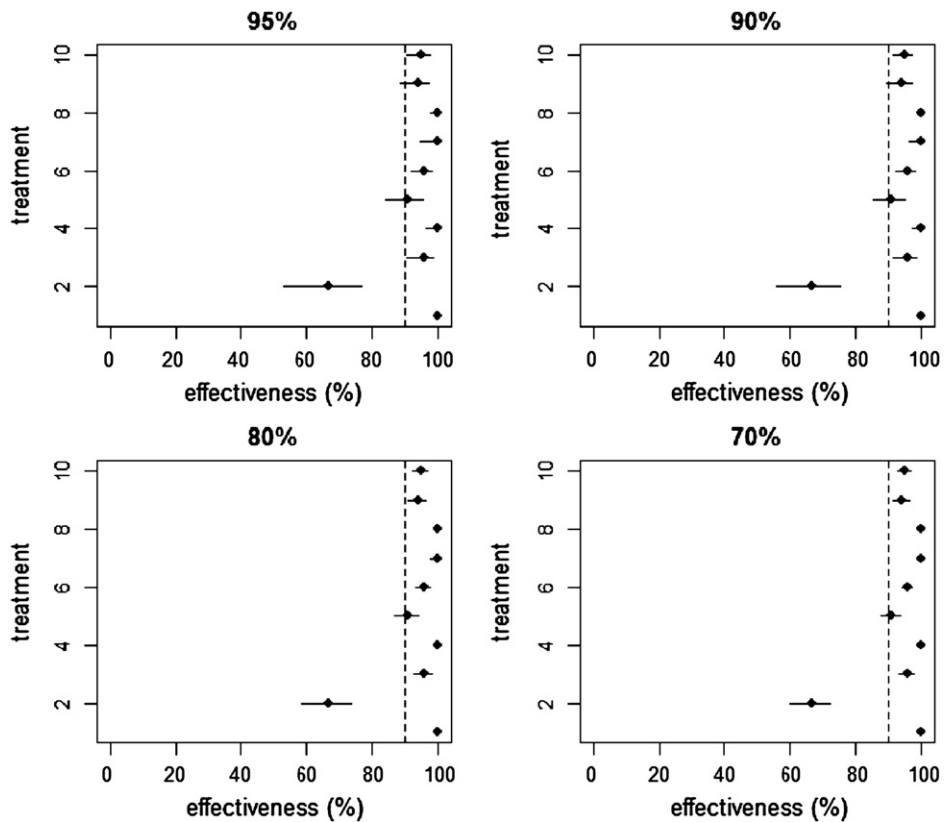


Fig. 5. Estimates of *L. salmonis* treatment effectiveness calculated with confidence levels of 70%, 80%, 90% and 95% in treatments for all sites ($n = 10$) using the quasi-Poisson method.

Table 4

Number of treatments against *L. salmonis* at pen level ($n=24$) that were acceptable (A) and unacceptable (U) calculated with the “quasi-Poisson” method and using four different confidence levels.

Result	95%	90%	80%	70%
A	11	12	14	15
U	13	12	10	9

Table 5

Number of treatments against *L. salmonis* at farm level ($n=10$) that were acceptable (A) and unacceptable (U) calculated with the “quasi-Poisson” method and using four different confidence levels.

Result	95%	90%	80%	70%
A	7	7	8	8
U	3	3	2	2

analysis of the factors related to treatment failure is beyond the scope of this study.

Finally, calculation of the treatment at site level was obtained by aggregating counts of mobiles from the available pens. The proportion of treatment failures was higher at pen than site level due to the average effect, and smaller variance from adding multiple pens and using a larger sample size. Sample size is a major determinant on the width of the confidence interval. Hence, appropriate sample sizes need to be calculated for estimations of effectiveness. Recommendations around sample size are challenging and beyond the objectives of this study, as additional theoretical (statistical power, for example) and practical issues (feasibility or cost associated with sampling, for example), that are not discussed in this study, need to be taken into consideration.

In this study, counts of mobiles were only available for a small proportion of pens on each site, and consequently, evaluation of treatment effect is likely not representative for the whole site as the sample of pens used was neither sufficient nor randomly selected. Hence, evaluation of the treatment at site level should include preferably all pens on the site to reduce the bias associated with the selection of a group of pens. This is achievable in Norway where large scale aquaculture uses fewer pens per site and monitoring of sea lice levels is performed in all pens at biweekly intervals.

In conclusion, it is more informative to provide measures of treatment effect together with confidence intervals. This will be useful for the early detection of changes in drug sensitivity and also for identifying technical problems associated with the application of baths. The proposed method can elucidate the current situation regarding the effectiveness of different delousing treatments in Norway and provide the basis for setting guidelines which are practical but also robust thus meeting the requirements of regulatory authorities and other stakeholders.

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