

# Randomized clinical trial to investigate the effectiveness of teflubenzuron for treating sea lice on Atlantic salmon

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**ABSTRACT:** A double-blind, randomized control clinical trial was performed to investigate the effectiveness of teflubenzuron in controlling sea lice *Lepeophtheirus salmonis* on farmed Atlantic salmon *Salmo salar*. A total of 40 sea cages from 3 commercial cage sites in Atlantic Canada were used in this Good Clinical Practice (GCP) trial. The teflubenzuron was administered in the feed at a dosage of 10 mg kg<sup>-1</sup> biomass d<sup>-1</sup> for 7 d. Medicated and control cages were matched by site, cage size, and pre-treatment mean lice counts using cages as the unit of concern. Post-treatment lice counts and staging of developmental stages were performed at 1 and 2 wk after the end of treatment. Chalimus stages in medicated cages were significantly lower than in control cages at 1 wk (79% reduction in mean lice counts,  $p < 0.001$ ), and at 2 wk (53% reduction,  $p < 0.001$ ). Mobile (pre-adult and adult) stages were also significantly reduced in medicated cages at 1 wk (69% reduction,  $p < 0.01$ ), and at 2 wk (40% reduction,  $p < 0.01$ ) post-treatment, respectively. Teflubenzuron was proven effective for reducing lice burdens on salmon despite the low parasite levels experienced during the trial and the recruitment of lice from the untreated cages. The use of cage as the unit of concern was an important design component of this trial.

**KEY WORDS:** Sea lice · *Lepeophtheirus salmonis* · Teflubenzuron · Atlantic salmon · *Salmo salar* · Clinical trial · Good Clinical Practice

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## INTRODUCTION

*Lepeophtheirus salmonis* is an ectoparasitic copepod which has become a serious problem in recent years for Atlantic salmon *Salmo salar* producers in Atlantic Canada, particularly in New Brunswick. Significant economic losses have occurred due to infestations and control programs. In 1995, it was estimated that sea lice infestations resulted in an approximate \$20 million loss to the New Brunswick industry. This was primarily due to increased mortality, downgrading of carcass quality, and cost of treatments (MacKinnon 1997).

The current treatment options available in Canada are limited. Bath treatments with organophosphates (e.g. azamethiphos) or hydrogen peroxide are labour

intensive and pose a potential health risk to the applicators. Also, these treatments are generally only effective against the mobile stages of lice (Bruno & Raynard 1994, Roth et al. 1996). Ivermectin (Ivomec<sup>®</sup>, Merck) has, until recently, been the only oral treatment available, although it is not registered for food fish in any jurisdiction and only available using extra-label veterinary prescriptions (Burka et al. 1997). Due to its narrow safety margin, its 'off-label' prescription use, slow residue depletion rates and subsequent extended withdrawal time, ivermectin usage is often limited to use in fish that are 1 yr from harvest (i.e. first summer in sea cages). Emamectin, another oral formulation in the avermectin family, is currently being used through emergency drug release (EDR) but was not available at

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the time this trial was conducted (Stone et al. 1999, Armstrong et al. 2000).

Teflubenzuron is an orally administered chitin synthesis inhibitor. It has been shown to be effective against all stages of sea lice that undergo a molt, including the larval and pre-adult stages (Ritchie 1996, Branson et al. 2000, Ritchie et al. 2002). Teflubenzuron is approved as an in-feed treatment for sea lice control in Norway (under the trade name Ektobann), the UK and Ireland (under the trade name Calicide). Teflubenzuron is one of a group of compounds called the acylureas (benzophenylureas). These compounds act to disrupt the synthesis of chitin, a polysaccharide of particular importance to arthropods (Hassall 1990). Chitin is a long-chain polymer consisting of  $\beta$ -1,4-linked N-acetylglucosamine which, in the arthropod cuticle, is bound to a protein to form a glycoprotein (Hochachka 1973). Therefore these compounds affect those periods of the life cycle (larval and pre-adult stages in sea lice) where chitin is being formed and where its incorrect or insufficient production can lead to malformations of the exoskeleton; however, the precise mechanism of action is unknown (Hassall 1990).

Sea lice living on the fish during a treatment with teflubenzuron are affected by the compound. The chalimus and pre-adult stages are most susceptible, and morphologic examination of exposed lice shows damage to the cuticle (Ritchie 1996). Since acylurea compounds affect physiological processes that do not occur in vertebrates, they are considered to have a very high margin of safety (Hassall 1990). Orally administered chitin synthesis inhibitors are currently available for other species to control parasites. Lufenuron is used in dogs and cats as a treatment for fleas (Dryden et al. 1999).

Rigorous clinical field trials using production farms can be difficult to design and implement. There are many logistical constraints in blinding operators to the treatment allocation, and the farm management is often reluctant to even include negative controls. Randomly allocated pens may be inconveniently located, making them subject to treatment errors. Large numbers of individual fish in each experimental unit or pen will increase the potential for a significant financial loss if problems are encountered during the trial. Site managers resist delegating decisions on study populations to researchers, since their most immediate concerns will outweigh any longer term benefits that could be realized by the research. Clearly defined exit rules allowing the withdrawal of experimental animals from a study are necessary to assure compliance. Lack of site exposure to the disease of interest and the potential for negative control groups to experience some level of protection due to their proximity to treatment groups are other considerations when designing field trials in aquaculture.

The objective of this study was to rigorously assess the field effectiveness of teflubenzuron in the reduction of sea lice *Lepeophtheirus salmonis* burdens on Atlantic salmon nearing market size, using a randomized, double-blind clinical trial with cage groups as the unit of concern.

## MATERIALS AND METHODS

**Trial design.** This was a double blind randomized clinical trial done to Good Clinical Practice (GCP) standards. The study included a total of 40 cages of pre-market salmon in 3 commercial sea cage sites in the Bay of Fundy, New Brunswick, Canada, during the period August 6 to September 12, 1996. Pre-treatment samples were taken from all cages to determine lice infestation levels and to obtain the mean fish weight for biomass estimates. Medicated and control cages were matched by site, cage size, and average number of lice per fish prior to allocation. All cages received study feed for 7 d, then each cage was sampled twice to determine post-treatment lice levels: once at 6 to 7 d after the end of treatment, and again 13 to 14 d after the end of treatment. Post-treatment lice levels were then compared between medicated and control groups.

**GCP requirements.** GCP is a set of accounting and record-keeping principles for documentation and verification of what is done during a clinical trial that ensures that studies can be reconstructed at any time after completion of the study. It is the standard that should be followed when generating clinical trial data that are intended for submission to regulatory authorities (USFDA 1997).

An independent Quality Assurance (QA) team provided reviews, inspections, and audits at various points during this study. Prior to initiation of the trial, QA reviewed the protocol, standard operating procedures, and all data collection and record sheets. During the trial, inspections were made at each site during each phase of the study (pre-treatment sampling, treatment, and post-treatment sampling) to ensure that data were being collected and recorded as stated in the protocol. After data collection was completed, QA conducted a detailed audit of all data sheets to look for errors and inconsistencies. Data entry was also checked for errors prior to analysis. The final report was reviewed to ensure accuracy and completeness of analysis, documentation, and conclusions.

**Site descriptions.** Site 1 was located in Lime Kiln Bay in southeast New Brunswick. At the time of the study, there were twenty-eight  $12 \times 12$  m steel cages on site. Of these, 14 contained pre-market fish (approximately 3000 per cage) and were included in the trial; the rest held smolt, broodstock, or were empty.

Site 2 was located in Bliss Harbour, which is near Lime Kiln Bay. There were ten 70 m (circumference) polar circle cages at the site at the time of the trial. Each cage held approximately 20 000 pre-market fish. One of the cages was being harvested at the time of the trial; consequently only 8 of the cages could be included.

Site 3 was also located in Bliss Harbour. A total of 18 square steel cages of pre-market salmon were included in the trial (twelve 12 × 12 m cages containing approximately 3000 fish each, and six 15 × 15 m cages containing approximately 5000 fish each). There were also approximately 6 circular cages at the site, which were not included in the study. These contained smolts, broodstock, and some pre-market fish.

During the trial at Site 3 there was a rapid build up of lice numbers in some of the cages. Consequently, 3 matched pairs of cages were sampled on Day 4 after treatment to allow for azamethiphos bath treatments in control cages. These 6 cages were then removed from the trial (see subsection 'Early exit rules' described later). The remaining 12 cages stayed in the trial and were sampled as planned.

**Treatment.** There were 2 treatments: medicated diet and control diet. The medication was administered at a dosage of 10 mg of active ingredient kg<sup>-1</sup> biomass d<sup>-1</sup> for 7 d. The concentration of the teflubenzuron in the medicated feed was 2.0 kg t<sup>-1</sup>. The medicated and matched control feeds were fed at a rate of 0.5% biomass d<sup>-1</sup> to achieve the required dosage. Cage biomass was calculated using weight measurements from the pre-treatment samples and the inventory estimates available from farm records.

To ensure blinding of both workers and investigators, all of the treatment feed bags, both medicated and control, were identical except for a label which indicated the site number and cage letter identification, weight of feed in the bag, and the number of bags that cage was to receive each day. Each cage was also labelled with a sign indicating site number, cage letter, number of bags of study feed to be administered per day, and the total weight of study feed for each day. Although it was possible that site workers could detect a difference in smell of medicated feed, there was no indication that this occurred. Also, site workers were not involved in outcome assessments, so they could not influence measurements of lice burden even if they could detect a difference.

The fish were fed to satiation twice a day. If the predetermined amount of study feed (medicated and control feed) was insufficient to satiate the fish within a cage for the first feeding, non-study feed was used as a supplement. If the fish did not eat all of the study diet during the first feeding, the remaining study feed was given at the second feeding, prior to any non-study feed.

**Treatment allocation and blinding.** Within each site, matched pairs of cages were identified based on cage size (absolute match), then on pre-treatment mean total sea lice counts (closest value match). A list of matched pairs (identified only by site numbers and cage letters assigned by the investigators) was supplied to the feed company for random allocation (using a random numbers table) of 1 cage from each matched pair into the medicated group and 1 cage into the control group. The result was that investigators remained blinded to treatment allocation, with 50% of the cages at each site assigned to the medicated group and 50% of the cages at each site assigned to the control group, such that each cage group within the matched pair had comparable lice levels.

**Sampling.** Sampling was performed by crowding the fish and then capturing a small number of fish at a time with a dip net. Crowding was accomplished by either seining or by reducing the net size within the cage except in one circumstance when farm management deemed the extra handling as potentially stressful to the fish, a systematic random sample of dip nets was used to reduce the potential for sampling bias. Sampled fish were anesthetized in a 50 to 100 mg l<sup>-1</sup> tricaine methanesulfonate (TMS<sup>TM</sup>, Syndel) bath prior to the measurements.

The number of fish to be sampled from each pen for lice count measurements was determined by calculations using the formula  $n = 1.96^2 s^2/L^2$  (Martin et al. 1987) where  $n$  = sample size, 1.96 is the value of  $Z$  for 95% confidence in the estimate,  $s$  = standard deviation, and  $L$  = allowable error (i.e. precision of the estimate set at 20% of the mean for higher burdens and 40% of the mean for lower lice burdens). Estimates of SDs were obtained from lice count data from the previous summer. Data from 9 cages with relatively high lice numbers (mean, mn, lice burden of 38 lice per fish,  $s = 16$ ) and 9 cages with low lice numbers (mn = 1.8,  $s = 1.8$ ) were examined. Based on these calculations done separately for high versus low lice burdens and logistical constraints of field procedures, a sample size of 25 fish was set as the minimum acceptable sample size for lice counts. For greater precision, a sample size of 50 was attempted when possible. Sample sizes exceeded recommendations published later by Treasurer & Pope (2000) in which the purpose of sampling was for lice monitoring at the farm level, whereas this study attempted to detect a difference between treated and untreated cages.

The following measurements were made on each fish: (a) fork length, (b) weight, (c) a subjective score of sea lice damage, whereby 0 = no damage, 1 = increased mucus, 2 = small (<2 cm<sup>2</sup>) areas of superficial damage, 3 = large (>2 cm<sup>2</sup>) areas of superficial damage, 4 = small (<2 cm<sup>2</sup>) areas of deep damage (through

the epidermis), and 5 = large (>2 cm<sup>2</sup>) areas of deep damage. Counts of all lice were obtained at the farm on each sampled fish and were categorized in the following groups: (a) copepodids, (b) chalimus 1 and 2, (c) chalimus 3 and 4, (d) pre-adults, (e) non-gravid adults, (f) gravid females.

Pre-treatment weight samples were used to estimate treatment dosages for each site. The largest logistically feasible sample size during the pre-treatment sampling period was determined to be 100 fish per cage. The 25 to 50 fish on which lice count and other measurements were collected were obtained from a subsample of these 100 fish.

Two of the investigators were trained as lice counters. To help ensure consistency, only these 2 people performed lice counts and damage scoring during the study. Fish from the entire site were counted by the same person during each sample period to standardize comparisons.

**Environmental measurements.** Dissolved oxygen and water temperature were measured at a depth of 2.5 to 3 m each day that treatment feed was given, in each trial cage.

**Statistical analysis.** All data were entered into a Quattro Pro (Corel) spreadsheet then transferred to Stata statistical software package for analysis. Graphs were generated using Sigma Plot (Jandel, now SPSS).

The overall average numbers and SDs of individual stages of lice per fish were calculated by sampling period. The average number of lice per fish within each treatment group was calculated by stage and sampling period.

Assessment of lice count differences between medicated and control groups at each sampling period was the primary analysis of interest. Overall significance was assessed using random effects linear regression (also known as mixed effects model) with 'cage' as a random effect to adjust for clustering within cages, and 'site' as a fixed effect. For the purposes of this significance testing, the dependent variable was a log transformed lice count [ $\log(\text{count} + 1)$ ] to ensure that the residuals were approximately normally distributed. The general model for these regression analyses were:

$$y_{(i,j)} = \alpha + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_{3(j)} + u_{(j)} + e_{(i,j)}$$

where  $y_{(i,j)}$  is  $\log([\text{lice count on } i\text{th fish in } j\text{th cage}] + 1)$ ;  $\alpha$  is the intercept;  $x_1$  is a dummy variable for (site = 2);  $x_2$  is a dummy variable (for site = 3);  $x_{3(j)}$  is the treatment applied to  $j$ th cage;  $\beta_1$ ,  $\beta_2$ ,  $\beta_3$  are coefficients for fixed effects;  $u_{(j)}$  is the random effect of  $j$ th cage; and  $e_{(i,j)}$  is residual error.

Separate analyses were performed for: copepodids, chalimus 1 and 2, chalimus 3 and 4, pre-adult, adult, all chalimus (1 and 2 plus 3 and 4), mobiles (pre-adult

and adult), and total (all stages) at each time period (pre-treatment, 1st post-treatment, 2nd post-treatment).

For the calculation of the reduction in lice levels, medicated cages and control cages were compared at each post-treatment sampling period with the assumption that many extraneous variables would affect both treatment groups similarly due to random allocation of treatment within matched pairs. Percent reductions in the medicated group as compared to the control group within the same matched pair were calculated for each stage using  $[1 - (\text{mean lice burden in medicated}/\text{mean lice burden in control})] \times 100$ , and sampling period when a significant treatment effect was found.

Weight (kg), length (cm), and condition factor ( $\text{weight}/\text{length}^3 \times 10^5$ ) were also compared between the treatment groups using random effects linear regression with 'cage' as a random effect to adjust for clustering within cages, and 'site' as a fixed effect.

Damage scores were compared between treatment groups with a simple chi-square test. The comparison was based on a damage/no damage (score  $\geq 1$ /score = 0) classification due to the fact that so few fish had any observable damage, and those that did were nearly all had a score of 2.

The percent reduction observed in each medicated cage as compared to total number of lice in the matched control cages was tested to see whether percent reduction was a function of lice numbers. This relationship was evaluated by linear regression analysis with percent reduction (in each medicated cage as compared to its match at each post-treatment sampling period) as the dependent variable and total number of lice (in each control cage at each post-treatment sampling period) as the independent variable.

All models were evaluated by plotting combined (cage and fish level) residuals against predicted values to look for evidence of heteroscedasticity and/or outliers. Normal quantile plots of residuals were used to evaluate the assumptions of normality.

**Lethal sampling.** Lethal sampling to collect tissue samples for ivermectin residue analysis and verification of lice counts and life cycle stages was done at Site 3. Ivermectin residues were a concern because most fish of this year class had received ivermectin treatments during the previous fall, and if residues had been present at sufficient levels, they may have affected study results. From each selected cage, 10 fish were euthanized by an overdose of anesthetic (TMS™) following routine sea lice counts at the site. Each fish was identified, placed in a plastic bag and stored on ice until a more detailed count could be repeated in the controlled conditions of the laboratory. Comparisons of post-anesthetic field counts versus laboratory counts were then made.

**Early exit rules.** Participating farms were encouraged to visually monitor all study cages for signs of lice build-up or skin damage. Farm staff remained blinded for this evaluation. If management decisions were made that involved treatment, a 48 h delay allowed sufficient time to sample all cages to be treated, and their matched pairs. These cages were then considered withdrawn from the study. Only Site 3 employed this early exit rule for 6 (3 plus their matches) cages.

## RESULTS

### Lice counts

The 3 study sites were combined for the analysis to assess the effectiveness of teflubenzuron. Differences between sites in overall lice levels were controlled by including 'site' as a fixed effect in the regression analysis models. The clustering of fish within a cage (i.e. fish within a cage more likely to have similar lice counts than fish in different cages) was controlled by including 'cage' as a random effect in the model. The regression diagnostics were considered acceptable and there was no consistent evidence of heteroscedasticity across the models. In general, the residuals conformed to a normal distribution, except that there were fewer large negative residuals than expected due to the truncation of the left hand of the distribution (i.e. no counts below zero).

Table 1 shows the average number of lice per fish in each treatment group at each sampling period and Fig. 1 presents this information graphically. Pre-treat-

ment levels have been included to show that there were no significant differences between treatment groups before treatment. The observed significance levels were derived from the random effects linear regression analysis. Percent reductions have been calculated where significant differences were found between treatment groups. The means shown are the averages of the non-adjusted (i.e. not log transformed) cage means. The effect of medication was statistically significant (as indicated by  $p < 0.05$ ) for all chalimus stages and pre-adults at the both post-treatment sampling periods. The effect on adults was statistically significant at the first post-treatment sampling period but not at the second.

A comparison of percent reduction and total number of lice, by matched pairs, indicated that as the average number of lice increased, the percent reduction increased. Linear regression analysis showed a significant relationship ( $p = 0.003$ ,  $r^2 = 0.31$ ).

### Damage scores

While there was a significant correlation between the number of lice and damage score ( $r = 0.33$ ,  $p < 0.01$ ), chi-square tests showed no difference in damage scores between medicated and control groups at each sampling period. The majority (68%) of the fish examined during the study had a damage score of 0 (no damage). Almost all of the rest (29%) had a damage score of 2 (small areas of superficial damage). Only 35 fish (0.8%) had damage scores of 3 or 4. There were no fish with a score of 5.

Table 1. *Lepeophtheirus salmonis* on *Salmo salar*. Comparison (mean lice fish<sup>-1</sup>) between medicated (Med.) and control groups at each sampling period. Lice counts, weight, length, and condition factor data are averages of arithmetic cage means within each group. Parentheses: SD. Pre-treatment and 1st post-treatment: calculations based on 20 cages in each treatment group; 2nd post-treatment: calculations based on 17 cages in each treatment group; Red.: reduction seen in medicated cages as compared to control cages (%), calculated only where a statistically significant difference was detected; Mobiles: includes pre-adult and adult stages; Condition factor:  $\text{weight}/\text{length}^3 \times 10^6$ . **Bold text:**  $p < 0.05$ ; p values were results of tests (random effects linear regression) on log transformed counts; thus the proportional reduction, rather than absolute means, was tested

	Pre-treatment			1st post-treatment				2nd post-treatment			
	Med.	Control	p	Med.	Control	p	Red.	Med.	Control	p	Red.
Copepodids	2.1 (2.0)	2.1 (1.9)	0.980	0.9 (0.5)	1.2 (0.6)	0.140		1.4 (1.6)	1.0 (1.2)	0.206	
Chalimus 1 and 2	1.8 (1.6)	1.9 (1.4)	0.337	<b>1.1 (1.2)</b>	<b>5.0 (4.2)</b>	<b>&lt;0.001</b>	<b>78</b>	<b>1.1 (0.9)</b>	<b>2.4 (1.7)</b>	<b>&lt;0.001</b>	<b>54</b>
Chalimus 3 and 4	0.6 (0.4)	0.6 (0.4)	0.983	<b>0.2 (0.2)</b>	<b>1.1 (0.9)</b>	<b>&lt;0.001</b>	<b>82</b>	<b>0.3 (0.2)</b>	<b>0.5 (0.4)</b>	<b>&lt;0.001</b>	<b>40</b>
Pre-adult	3.5 (1.6)	3.0 (1.2)	0.317	<b>1.7 (2.0)</b>	<b>6.5 (7.3)</b>	<b>&lt;0.001</b>	<b>74</b>	<b>0.5 (0.5)</b>	<b>0.9 (1.0)</b>	<b>0.001</b>	<b>44</b>
Adult	0.6 (0.4)	0.6 (0.3)	0.750	<b>1.3 (1.4)</b>	<b>2.8 (3.7)</b>	<b>0.016</b>	<b>54</b>	0.4 (0.6)	0.6 (0.7)	0.444	
Gravid	1.7 (0.8)	1.4 (0.6)	0.221	2.4 (1.3)	2.4 (1.4)	0.942		2.1 (1.5)	2.4 (1.8)	0.192	
All chalimus	2.4 (1.9)	2.5 (1.6)	0.417	<b>1.3 (1.5)</b>	<b>6.1 (5.0)</b>	<b>&lt;0.001</b>	<b>79</b>	<b>1.4 (0.9)</b>	<b>3.0 (1.9)</b>	<b>&lt;0.001</b>	<b>53</b>
Mobiles	4.1 (1.8)	3.6 (1.4)	0.331	<b>2.9 (3.2)</b>	<b>9.3 (10.8)</b>	<b>0.002</b>	<b>69</b>	<b>0.9 (1.0)</b>	<b>1.5 (1.6)</b>	<b>0.032</b>	<b>40</b>
Total	10.3 (5.0)	9.6 (4.5)	0.583	<b>7.6 (5.4)</b>	<b>19.0 (15.4)</b>	<b>&lt;0.001</b>	<b>60</b>	<b>5.8 (4.2)</b>	<b>7.9 (5.1)</b>	<b>0.010</b>	<b>27</b>
Weight (kg)	3.0 (0.4)	3.1 (0.3)	0.172	3.3 (0.6)	3.2 (0.5)	0.469		3.5 (0.5)	3.5 (0.4)	0.606	
Length (cm)	61.1 (1.7)	61.7 (1.9)	0.175	63.0 (2.3)	62.5 (2.7)	0.496		64.5 (2.0)	64.1 (2.4)	0.607	
Condition factor	1.32 (0.10)	1.30 (0.08)	0.307	1.30 (0.10)	1.29 (0.09)	0.871		1.29 (0.11)	1.30 (0.08)	0.819	



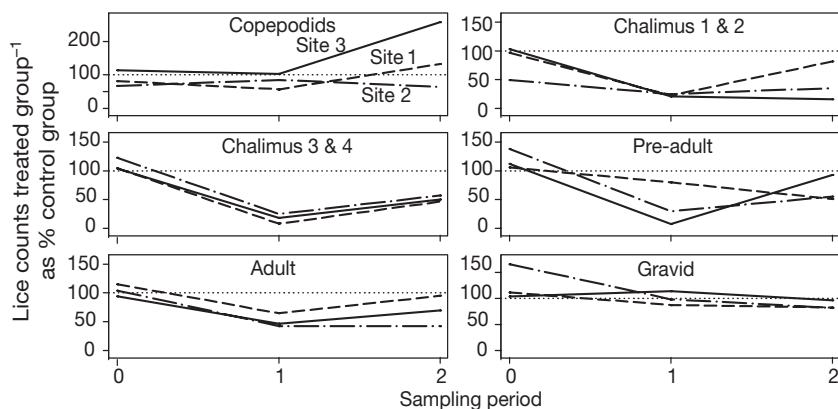


Fig. 1. *Lepeophtheirus salmonis* on *Salmo salar*. Number of lice per fish by life cycle stage and site expressed as a percentage of control group during the clinical trial. Sampling periods for pre- and 1st post-treatment: 20 cages in each treatment group. Sampling period 2nd post-treatment: 17 cages in each treatment group, due to early removal of 3 cages

### Ivermectin analysis

The ivermectin levels found in the lethally sampled fish were well below what would be found in recently treated fish. The samples averaged  $2.4 \text{ ppb} \pm 1.6 \text{ SD}$ . Ivermectin treated fish have ivermectin residues in the liver which are much higher ( $459 \text{ ppb} \pm 103 \text{ SEM}$ , with a half life of 98 d) (Kennedy et al. 1993). As a result, it was concluded that previous ivermectin treatments did not affect the study.

### Environmental measurements

At Site 1 the water temperature during the medication period averaged  $12.3^\circ\text{C} \pm 0.4 \text{ SD}$ , with a minimum of  $11.7^\circ\text{C}$  and a maximum of  $13.1^\circ\text{C}$ . Dissolved oxygen levels averaged  $7.5 \text{ mg l}^{-1} \pm 0.5 \text{ SD}$ , with a minimum of  $6.5 \text{ mg l}^{-1}$  and a maximum of  $8.9 \text{ mg l}^{-1}$ . At Site 2 the water temperature during the medication period averaged  $12.0^\circ\text{C} \pm 0.1 \text{ SD}$ , with a minimum of  $11.8^\circ\text{C}$  and a maximum of  $12.3^\circ\text{C}$ . Dissolved oxygen levels averaged  $8.1 \text{ mg l}^{-1} \pm 0.3 \text{ SD}$ , with a minimum of  $7.4 \text{ mg l}^{-1}$  and a maximum of  $8.8 \text{ mg l}^{-1}$ . At Site 3 the water temperature during the medication period averaged  $12.1^\circ\text{C} \pm 0.2 \text{ SD}$ , with a minimum of  $11.7^\circ\text{C}$  and a maximum of  $12.3^\circ\text{C}$ . Dissolved oxygen levels averaged  $7.0 \text{ mg l}^{-1} \pm 0.4 \text{ SD}$ , with a minimum of  $6.1 \text{ mg l}^{-1}$  and a maximum of  $7.8 \text{ mg l}^{-1}$ .

### DISCUSSION

The effect of medication was most evident in the molting stages of lice, namely the chalimus and pre-

adult stages. At 1 wk after the end of treatment there was a 79% reduction for chalimus stages and a 69% reduction for mobile (pre-adult and adult) stages in medicated as compared to controlled cages. This was lower than results reported by Ritchie (1996) who found up to 95% effectiveness against chalimus and pre-adult stages with teflubenzuron treatments in sea cages in Norway, though the time after treatment was not reported. Our results were also somewhat lower than the 85.8% reductions reported by Branson et al. (2000). The effect of treatment had reduced at 14 d after the end of treatment, but it was still significant with a 53 and 40% reduction in chalimus and mobiles, respectively, compared to control cages.

Reductions seen in this study may well underestimate the potential effect of teflubenzuron treatment. The proximity of negative control cages at the same site may have provided a ready supply of lice for transfer to the medicated cages. Ritchie (1997) demonstrated that mobile stages are able to transfer between fish in the same cage as well as between fish in different cages. Increased recruitment rates of mobile lice due to neighboring untreated cages should not occur under normal circumstances if an effective treatment was used in all cages at the same site. The fact that there was no significant difference in the number of copepodids between the test groups at either post-treatment sampling suggests there was continuous copepodid recruitment from gravid lice into the treated cages in both the treated and control groups.

The relatively low levels of lice infestations may have contributed to an underestimate of effect simply because the range between medicated and control groups was so narrow. Had pre-treatment lice levels been higher, the observed magnitude of decrease in the medicated group may have been larger. This was supported by the findings that, within matched pairs, as the average number of lice increased, the percent reduction increased.

It is possible that some copepodids attached and then molted between the end of the treatment period and the first post-treatment sampling period. Thus, the sampling interval of 7 d used in this study may not have detected maximum reductions.

Since adult lice do not molt, it was not expected that a chitin-inhibitor would have any noticeable effect on this stage. However, during the treatment period, some of the female adults likely matured into gravid females (i.e. developed egg strings), while the maturation of pre-adults into adults would have been inhibited by

the treatment. This may explain the reduction in average adult lice observed in this study.

Teflubenzuron was more effective against the chalimus stages than the mobile stages. This is in contrast to the bath treatments currently available. Azamethiphos, an organophosphate bath treatment which was the primary compound used for sea lice infestations in Canada during the study period, is more effective on the adult and pre-adult stages than the chalimus stages (O'Halloran & Hogans 1996, Roth et al. 1996). Similarly, hydrogen peroxide, another bath treatment available to farmers, is effective only against these mobile stages (Treasurer & Grant 1997).

Using the early exit rules previously established with the farmers, 3 pairs of cages from Site 3 were removed after the first post-treatment sampling period. Bath treatments (azamethiphos) were then administered to 1 cage from each pair which, when the treatment allocation was revealed, all turned out to be control cages. The primary impact of this on the study was a subsequent reduction in the number of cages available for the second post-treatment sampling period. This reduction in sample size may have reduced the power to detect a difference between medicated and control cages. Another potential impact on the study results is that the chemicals released from these bath treatments may have affected lice populations in other cages at the site.

It can be seen from Table 1 that lice counts dropped between the first and second post-treatment sampling periods. This reduction occurred in all cages at Site 2 and Site 3, but the reason for this reduction was unknown. The only unusual events that occurred during that interval was the bath treatment of the 3 cages at Site 3, and a very large rainfall. It is possible that chemical treatment drift from the treated cages at this site (or neighbouring sites) adversely affected the lice in these cages. However, the treatments at this site were done while the tidal currents were moving away from the other cages. Even if the currents had been directed towards the other cages, the chemical should have been diluted and in contact with the fish for a much shorter time than required for a standard treatment. Therefore these bath treatments should not have affected the study cages. Also, it would be very unexpected that the rainfall could have so dramatically reduced the number of lice. Brocklebank (1995) reported that incessant rains will remove lice from salmon; however, this rain lasted less than 2 d. No other farmers in the area observed similar reductions (anecdotal reports), but there was no on-going lice recording system to monitor changes. Similar unexplained reductions have been described by others, however (Branson et al. 2000).

It was hoped that the damage scores would provide a useful measure of the effect of lice on the fish, and

indirectly, a measure of treatment effect. For this purpose, though, the scoring system used during this trial proved not to be very useful. The damage score of 1 (increased mucus) proved difficult to assess because it was very subjective, and may have been more of an indication of sexual maturity (grilse), rather than lice damage.

While there were significant reductions in most stages, the treatment still left a sizeable population of lice, predominately adults and gravid females, on the fish population in medicated cages. This indicates that teflubenzuron may be most useful as a component of a sea lice management program which includes an initial bath treatment to remove mature lice followed by periodic teflubenzuron treatments to keep lice levels low and the lice population immature. This is especially important considering that as the lice mature, the stress occurring in the fish increases (Bowers et al. 2000). The combination of a chitin synthesis inhibitor (lufenuron) with an adulticide (pyrethrin) has been shown to be very successful in the control of fleas on dogs and cats (Dryden et al. 1999). It is also likely that repeated teflubenzuron treatments over time would eventually result in reductions in the adults and gravid females due to a combination of natural attrition and reduced recruitment from immature stages.

This trial evaluated the short-term effectiveness of teflubenzuron following one treatment using a randomized, double-blind clinical trial using negative control cages and GCP standards. Such clinical trials using cages as the unit of concern have rarely been used to assess salmon farm disease control methods.

*Acknowledgements.* This study was funded by Moore-Clark Co. (St. Andrews, Canada) and the New Brunswick Salmon Growers Association.

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*Editorial responsibility: Jay F. Levine,  
Raleigh, North Carolina, USA*

*Submitted: November 1, 2002; Accepted: September 7, 2005  
Proofs received from author(s): May 1, 2006*