Efficacy of emamectin benzoate against sea lice infestations of Atlantic salmon, *Salmo salar* L.: evaluation in the absence of an untreated contemporary control

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Abstract

The efficacy of emamectin benzoate (SLICE®) against sea lice infestations of Atlantic salmon, *Salmo salar* L., is typically assessed using untreated fish, or fish treated with alternative therapeutants, as controls. The State of Maine, USA, is currently under active management for the OIE-notifiable pathogen, infectious salmon anaemia virus (ISAV); consequently, neither control group is feasible in this region. Untreated salmon risk extensive damage from the ectoparasites, and threaten to increase vector-borne exposure or susceptibility of farms to ISAV; and the only treatment presently available in Maine is SLICE®. However, because sea lice infestations are unlikely to resolve spontaneously, and response to treatment occurs within weeks, use of a pretreatment baseline is a reasonable alternative for confirmatory studies. We evaluated SLICE® efficacy on Atlantic salmon farms in Cobscook Bay 2002–2005, in the absence of untreated controls, using pretreatment lice loads as a reference for calculation. Maximum efficacy ranged from 68% to 100% reduction from initial levels. Time-to-maximum efficacy ranged from 1 to 8 weeks after treatment initiation. Efficacy duration, measured between first reduction and first progressive rise in counts, ranged from 4 to 16 weeks.

Keywords: *Caligus* spp., efficacy, *Lepeophtheirus salmonis*, *Salmo salar*, SLICE®, treatment.

Introduction

Sea lice are copepod ectoparasites endemic to salmonid populations in the USA and elsewhere (Kabata 1979). Two genera are common to Atlantic salmon, *Salmo salar* L., in Maine: *Lepeophtheirus salmonis*, a species fairly specific to Atlantic salmon, and *Caligus* spp. known to parasitize over 80 different species of fish (Kabata 1979). *Lepeophtheirus salmonis* cause extensive dermal damage to host fish, and are considered potential vectors of bacterial and viral fish pathogens (Nylund, Bjorknes & Wallace 1991), including the OIE-notifiable disease infectious salmon anaemia (ISA) (Nylund, Wallace & Hovland 1993; Rolland & Nylund 1998). *Caligus* spp., though causing less skin damage to the host (MacKinnon 1993), may have farther-reaching consequences as they can be carried to, or from, a region on numerous species of wild fish (Kabata 1979; Bruno & Stone 1990; Revie, Gettinby, Treasurer & Rae 2002a).

The Maine salmon aquaculture industry, in partnership with Maine’s Department of Marine Resources and USDA’s Animal and Plant Health Inspection Service (APHIS), is currently striving to control ISA in the Cobscook Bay region of Maine, one of the most productive Atlantic salmon farming regions in the USA. One of the facets of the ISA control strategy (USDA APHIS Veterinary Services, Maine Department of Marine Resources and Maine Aquaculture Association 2002) is the adoption of an integrated pest management plan to minimize potential pathogen spread or increase in disease susceptibility due to sea lice (Nylund *et al.* 1991, 1993; MacKinnon 1993). SLICE®, an oral formulation of emamectin benzoate for the treatment of...
Efficacy evaluations typically rely on the availability of an appropriate, and ideally untreated, control group (ICH Expert Working Group 2001). Although entirely plausible in a laboratory setting, the maintenance of untreated cohorts for SLICE® efficacy evaluation is difficult in commercial marine operations. Lice infestations allowed to fester in a commercial open-water farm can subject farmed or wild salmonid populations (Johnson & Albright 1991; Piasecki & MacKinnon 1995). Consequently, commercial trials have been known to interrupt an efficacy study to treat controls with an established substitute parasiticide (Stone, Sutherland & Simmons 2002), rather than an INAD protocol. Counts categorize lice by genus, and *L. salmonis* are further differentiated by life-stage, including a category for chalimus (attached larval stages), non-gravid motile stages (preadults and non-gravid adults) and gravid females. The data are recorded by site managers or designated industry personnel trained in sea lice identification, overseen by the veterinarian responsible for the site, and compiled by USDA APHIS VS ISA programme staff in their function as SLICE® INAD monitors. Data are collected monthly whenever water temperatures reach 4 °C and biweekly to weekly when temperatures exceed 8 °C. Counts are discontinued below 4 °C.

Emamectin benzoate was prescribed by the site-veterinarian and administered as a medicated feed (SLICE®), at 50 μg kg⁻¹ fish for 7 days. All cages on an affected site were treated simultaneously. Treatments, initiated after settlement, were aimed to commence before reaching thresholds of five *L. salmonis* (preadult or non-gravid motiles) per five fish or one gravid female *L. salmonis* per five fish. Actual timing occasionally varied with processing, manufacturing or shipping constraints.

Selection and description of treatment events

The exhaustive set of SLICE® treatments in the Cobscook Bay region (Fig. 1) from 2002 through 2005 included 13 farms (one farm was counted twice, because it completed two full production
cycles during the study period) and 39 SLICE® treatments. Treatments from this list were selected for the efficacy study if lice counts were monitored at least every other week for at least 8 weeks post-treatment. This criterion eliminated all (13) late autumn (November/December) treatments, and seven others that did not meet the specified count frequency, resulting in 19 treatments (from 11 different farms) for maximum efficacy and time-to-efficacy calculations. Consequently, the study covered treatments applied during July to October (comprising fish in their first or second summer/autumn in sea water), with average morning water temperatures (as recorded at the sites on count days) ranging from 10.3 to 13.1 °C (median 11.3 °C). Treatments were further selected for duration calculations if counts were monitored through a wane in efficacy as demonstrated by a resurgence of infestation. Thirteen of the 19 treatments met these duration criteria. Study farms held from 150 000 to 600 000 single-year class Atlantic salmon. Cages were either of steel-pen or polar circle structure and ranged in number from 8 to 40 per farm.

**Efficacy calculations**

Trends in lice infestation pressure were examined through descriptive statistics of average lice loads per fish at the time of treatment initiation and at biweekly intervals post-treatment initiation. Maximum per cent efficacy was defined as the maximum per cent reduction in lice from treatment initiation counts, and was calculated as

$$\text{Efficacy} = 100 - \frac{\text{lice}_{\text{post}}}{\text{lice}_{\text{pre}}} \times 100,$$

where lice_{pre} is the average lice count per fish at the peak of treatment response, and lice_{post} is the average lice count per fish at treatment initiation. Time-to-maximum efficacy was measured in weeks from treatment initiation. Efficacy duration was calculated as weeks from first reduction in counts from treatment initiation levels to first progressive rise in counts above 0.04 per fish for counts of gravid female lice and 0.2 per fish for all other counts (chalinus, non-gravid motiles, total *L. salmonis* and total *Caligus* spp.). Weeks without counts (for those treatments monitored biweekly rather than weekly) were included in duration estimates, as long as they
were flanked by counts (i.e. as long as counts were conducted at least biweekly).

SLICE® treatments are presumed to work on a per cent-reduction basis and most treatments achieved close to 100% efficacy at some point in the treatment cycle. However, identifying beginning and end dates for duration calculations by a target per cent reduction (from pretreatment loads) would have resulted in a moving target. For example, per cent-reduction endpoints calculated from heavily infested farms (i.e. with numerous lice per fish) would translate to higher endpoint lice counts than treatments initiated earlier in the life cycle of the infestation (i.e. with less lice per fish). To avoid this bias, we measured treatment duration as the number of weeks between the first consistent reduction below treatment-initiation counts, to the first progressive rise above peak efficacy counts, beyond a specified threshold level. This provided a standardized basis for comparison of duration across treatments in farms undergoing natural parasite challenge. Because some sites applied subsequent SLICE® treatments after a new lice settlement, but before the appearance of gravid female lice, the duration calculated for gravid female lice represents a conservative (minimum) estimate. The actual efficacy duration for gravid female lice may have been longer for some of these treatments.

Associations with explanatory variables

Unvariable associations between explanatory variables and time-to-maximum efficacy were evaluated using Spearman correlation or Mann–Whitney tests. Explanatory variables examined at treatment initiation included weeks since stocking of the grow-out site, mean number of *L. salmonis* gravid females on the site, mean number of *L. salmonis* gravid females at all active sites in the bay, presence of *Caligus* spp. and average water temperature (during treatment).

Results

Median lice loads per fish at treatment initiation (*n* = 19 treatments) were 0.8 (range 0.2–4.8) for total *L. salmonis*, 0.2 (range 0–2.1) for gravid female *L. salmonis*, 1.2 (range 0.2–9.4) for preadult/ non-gravid *L. salmonis*, 1.2 (range 0–3.2) for chalimus *L. salmonis* and 0.1 (range 0–1.0) for total *Caligus* spp. Box plots of *L. salmonis* and *Caligus* spp. loads over time (Figs 2 & 3, respectively) show a relatively rapid decline in estimated abundance followed by an eventual resumption of infestation.

Maximum per cent efficacy and time-to-maximum efficacy were calculated for each of the different stage categories (chalimus, non-gravid motiles and gravid females) of *L. salmonis* and for *Caligus* spp. (Table 1). Maximum efficacy achieved was very high, with median rates approaching 100%, and the median time to this maximum effect ranged from 2 to 4 weeks post-treatment initiation. *Caligus* spp. responded first, with median time-to-maximum effect at 2 weeks. *Lepeophtheirus salmonis* were 1–2 weeks behind, with median time-to-maximum effect for gravid female lice at 3 weeks and other stages at 4 weeks. Efficacy duration was calculated for the smaller subset of sites that were monitored...
through the entire efficacy cycle (Table 2). Median duration of efficacy, calculated on the subset of treatments that continued lice counts through re-infestation, ranged from 7 to 11 weeks. Again, Caligus spp. (10 weeks) and gravid female L. salmonis (11 weeks) showed the strongest response.

We also evaluated associations with time-to-maximum efficacy. The only statistically significant Spearman correlation ($P < 0.05$) related to weeks in sea (a proxy for age of fish) at treatment initiation (positive correlations with time-to-maximum efficacy for total $L.\ salmonis$ $P = 0.02$, preadults/non-gravid lice $P = 0.02$ and gravid females $P = 0.01$). Time-to-maximum per cent efficacy for non-gravid motile $L.\ salmonis$ in populations with gravid females (median 4, min 2, max 8) at the time of treatment initiation varied from populations without gravid female lice (median 3, min 1, max 6). However, these differences were not statistically significant (Mann–Whitney $P > 0.05$). The presence of Caligus spp. and average water temperatures (during treatment) did not correlate with efficacy parameters.

**Discussion**

The calculated efficacy parameters corroborate other studies showing a strong treatment response to SLICE$^\circledR$ (emamectin benzoate) treatments ($n$) for sea lice ($Lepeophtheirus salmonis$ & Caligus spp.) on farmed Atlantic salmon in the Cobscook Bay region of Maine, USA, 2002–2005 through the entire efficacy cycle (Table 2). Median duration of efficacy, calculated on the subset of treatments that continued lice counts through re-infestation, ranged from 7 to 11 weeks. Again, Caligus spp. (10 weeks) and gravid female $L.\ salmonis$ (11 weeks) showed the strongest response.

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**Table 1** Maximum efficacy (%) and time-to-maximum efficacy (weeks from treatment initiation) of SLICE$^\circledR$ (emamectin benzoate) treatments ($n$) for sea lice ($Lepeophtheirus salmonis$ & Caligus spp.) on farmed Atlantic salmon in the Cobscook Bay region of Maine, USA, 2002–2005

<table>
<thead>
<tr>
<th>Stage</th>
<th>$n$</th>
<th>Max per cent efficacy</th>
<th>Time-to-max efficacy</th>
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<tbody>
<tr>
<td></td>
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<tr>
<td>Total $L.\ salmonis$</td>
<td>19</td>
<td>97</td>
<td>4</td>
</tr>
<tr>
<td>Chalimus</td>
<td>18</td>
<td>100</td>
<td>4</td>
</tr>
<tr>
<td>Preadult/non-gravid</td>
<td>19</td>
<td>100</td>
<td>4</td>
</tr>
<tr>
<td>Gravid females</td>
<td>12</td>
<td>100</td>
<td>3</td>
</tr>
<tr>
<td>Total Caligus spp.</td>
<td>10</td>
<td>100</td>
<td>2</td>
</tr>
</tbody>
</table>

**Table 2** Duration of efficacy (weeks) of SLICE$^\circledR$ (emamectin benzoate) treatments ($n$) for sea lice ($Lepeophtheirus salmonis$ & Caligus spp.) on farmed Atlantic salmon in the Cobscook Bay region of Maine, USA, 2002–2005

<table>
<thead>
<tr>
<th>Stage</th>
<th>$n$</th>
<th>Efficacy duration</th>
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<tbody>
<tr>
<td></td>
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</tr>
<tr>
<td>Total $L.\ salmonis$</td>
<td>13</td>
<td>7 6 10</td>
</tr>
<tr>
<td>Chalimus</td>
<td>13</td>
<td>8 6 9</td>
</tr>
<tr>
<td>Preadult/non-gravid</td>
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</tr>
<tr>
<td>Gravid females</td>
<td>7</td>
<td>11 9 12</td>
</tr>
<tr>
<td>Total Caligus spp.</td>
<td>7</td>
<td>10 8 16</td>
</tr>
</tbody>
</table>

Treatments on fish that had accrued more weeks from stocking in the marine environment tended to require more time to reach maximum effect. As all but one of the cohorts were stocked in the spring of a year (the other was stocked in the autumn), this parameter provides a relative, though indirect, indication of fish age. This effect, however, may also be an artefact of variability in biomass estimates. Population growth is difficult to track in marine grow-out farms, and under-estimates of fish size could result in a reduced drug delivery per actual kg of fish. Natural populations of lice may vary with season and temperature (Boxaspen 1997; McKenzie, Gettinby, McCart & Revie 2004). However, water temperatures did not vary greatly in our study and did not correlate to any degree with treatment efficacy.

Field efficacy calculations would have been improved by consistent counts at weekly, rather than biweekly or longer, intervals; and relatively low chalimus counts suggest a need for improved detection at early life stages in the field. The current sea lice monitoring protocol also discontinues counts at temperatures below $4\,^\circ\mathrm{C}$, which reduces follow-up duration for treatments initiated in late autumn or winter. Altering the protocol to encourage counts for at least 8 weeks following a treatment, regardless of temperature, would almost...
double the number of treatments available for future efficacy calculations.

Comparison with baseline loads at treatment initiation is an important alternative for evaluation of therapeutic efficacy in situations where untreated controls can compromise animal welfare or concurrent disease management. A baseline-controlled approach is best justified if the course of disease is predictable and unlikely to spontaneously resolve, and if the response to treatment is fairly immediate (ICH Expert Working Group 2001). If ethical and contextual constraints preclude a randomized controlled trial, the combined findings of earlier trials and observations should influence the design of confirmatory studies. Earlier trials of emamectin benzoate in Atlantic salmon show a strong and relatively rapid therapeutic response (Stone et al. 2000a,b; Ramstad et al. 2002; Treasurer et al. 2002), and suggest that infestation is unlikely to resolve in the absence of treatment (MacKinnon 1993; Revie et al. 2002b). In addition, sea lice incidence varies with location (Murray, Amundrud & Gillibrand 2006) and because all cages on a farm are often treated at the same time to try to break the life cycle, disease intensity at the time of treatment is not entirely uniform throughout a site. Consequently, baseline-controlled studies should provide reliable confirmation of SLICE efficacy in field conditions. Further, by matching each treatment response to its pretreatment infestation pressure, baseline comparisons may even improve the accuracy of calculated efficacy parameters over that of unmatched designs.

References


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