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Title: An evaluation of the impact of early infestation with the salmon louse Lepeophtheirus salmonis on the subsequent survival of outwardly migrating Atlantic salmon, Salmo salar L., smolts

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Keywords: marine survival; sea lice; Atlantic salmon

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Dear Peter,

The attached manuscript is based on data presented at the sea lice 2010 conference. The results are part of a study being carried out on the effects of sea lice infestation on the marine survival of outwardly migrating salmon smolts. The study has been ongoing for ten years now and the total number of ranched fish in the study groups (both treated and control) is approaching 250,000. The data presented is a time series of releases from the Marine Institute facility in Newport using the Burrishoole strain of ranched salmon.

I think the text of the paper speaks for itself, at least I hope it does, and we are hoping that if this publication is accepted it will be the first in a series covering different aspects of the ongoing study, including data sets from other ranched populations from different release sites.

I am the corresponding author and can be reached at 353 87 6993259 or by e-mail at dave.jackson@marine.ie.

Yours sincerely,

Dave

Dr David Jackson
**Responses to reviewers’ comments:**

The authors found the comments of the referees to be both constructive and helpful in improving the manuscript. We would like to thank them for taking the time to make such detailed comments and have tried to incorporate all suggested revisions in so far as practical. A detailed response is set out below:-

Reviewer #1: This is an incredible and extremely powerful long-term data set. The results are extremely clear in two important respects. The alarming 10-fold decline in returns is extremely clear. Equally clear is the marginal and irregular effect of SLICE in improving fish return. Clearly sea lice infestations for the period that the food additive lasts is having little effect on fish returns, with 2.3% being the largest difference between control and treated fish, and a significant difference being detected in less than half of the releases (in only 4 out of 10 trials).

I highly recommend that you make these minor changes.

1. Likely need to say how far off shore the fish are by the time SLICE wears off.

   **This has been done and two new references cited:** 700+ km in 7 weeks, well within the period of SLICE protection.

2. Line 184. You need to add the regression equations to all figure legends. Perhaps you can more clearly state the following in the results. The difference in the constant of these regression equations represents the effect of SLICE, which was significant. By removing this and showing a common gradient, you clearly demonstrate that the long term decline is common to treated and control fish.

   **Equations have been added and the text modified.**

Minor comments

I offer the following suggestions to correct typos and increase the message delivery.

**All the suggestions below have been addressed**

Abstract. I suggest deleting "many studies are attempting to elucidate potential causes for this decline." and run on the last two sentences.

The abstract should mention the study duration - 9 years.

Line 58. & = and

Line 59. remove extra fullstop

Line 67. therapeutant prior to release, the fish Line 80. (Moore et al, 2008). By comparing Line 92. 50 µg/kg/day Line 98. post-feeding Line 132. freshwater Line 164. 2001 treated group Line 167. percentage return was 2.38% (6.82% for the treated 4.44% for control) Line 170. I think this is 9 of 10 releases,
which should be stated. The average difference of these should also be stated - see above, which will be < 2.38%.

Line 196. Among the four releases where there was a significant improvement in returns as a result of SLICE treatment, the 2006 early release group (Table 2.) showed the greatest difference in percentage survival at 2.38%. However, the second release group in 2006.

Line 202. Start the paragraph with this sentence The results over the study period would suggest that the level of infestation pressure by L. salmonis experienced by the outwardly migrating smolts was not of a level to be a consistently significant source of additional marine mortality because no significant difference in survival rates was found between treated and unprotected groups.

Line 228. Over this nine year study period, there was a nearly 10-fold decrease in the percentage of salmon return. This dramatic and alarming as well as highly significant trend was observed..

Line 240. minor and irregular between (and even within) years component.

Reviewer #2: This is an important paper that deserves to be published. Some minor corrections and adjustments required are as follows:

a) insert Salmo salar L. in title and at first mention in text
b) Piggins & Mills 1985 reference missing
c) page 2, line 59, remove "the"
d) first paragraph in Discussion needs a little correction for typos and parentheses
e) reference list needs some correction and should be produced in accordance with journal requirements (Cotter reference out of sequence, standardise journal title abbreviations, etc.)

All of the above have been addressed.

Reviewer #3: This is a very interesting study that adds to significant gaps in our understanding of possible near shore effects from sea lice on out-migrating juvenile salmon. One limitation to the paper, however, is difficulty following the statistical analysis used as discussed below. Provided better clarity can be provide to explain the analysis carried out I would not hesitate to recommend this paper for publication.

Specific comments are as follows:

Line 68: The paper notes fish can be protected "..for up to nine weeks". Yet in the abstract and other places (see line 82) other values are used (see line 82 ".six to eight weeks"; line 206 ".up to nine weeks).
This is somewhat confusing and the authors should determine a consistent interval for the purposes of their interpretation/study and that reported in the literature.

This has been standardized at up to 9 weeks.

Line 118 Tag Recovery. The concepts of "tags recovered" vs % survival are used interchangeable. This requires more explanation in the text since, presumably, it is assumed a fish does not survive if a tag is not recovered. Thus, what information can be provided to validate the recovery program?

The tag recovery programme is part of a national micro-tagging programme and a summary of the programme is given in the Tag Recovery paragraph. In addition details are given in Browne et al 1982 (ref cited) and Browne et al 1994 (new ref cited) and O’Maoileidigh et al 2004 (ref cited).

Line 151. Data Analysis. This is confusing. As written it would be difficult to replicate the statistical analysis. The authors need to provide more detailed information on how the analysis were carried out specifying response/tested variables - including the rational for the tests. It's difficult to determine why both a sign test and chi-squared tested were used to assess returns of treated and non-treated fish. Further, it's difficult to follow the interpretive relationship between these two tests. For example, why do the two test appear to contradict one another? The first paragraph of the discussion, line 193, is confusing. The use of multiple independent tests loses information and doesn't account for the larger experimental design and, as a result, the relationships between variables are not tested. One suggestion would be to consider a logistic regression (using recovery rate, treatment group (treated vs non treated), and year of release as variables).

New text has been added to clarify the data analysis.

Line 191 Discussion. Resistance to SLICE has been reported as an emerging issue in the literature. The paper assumes that farm-origin lice that might be affecting smolts heading out to sea are fully sensitive to SLICE. If, on the other head, these lice populations were resistance to SLICE, and were interacting with out-migrating salmon, it would affect the hypothesis being tested. The authors should comment.

Reduced sensitivity to SLICE was not an issue in Ireland during the period under study. The results of studies carried out by the authors (Copley et al 2007) during the course of this study verify this and are reported in the experimental design section. While reduced sensitivity to SLICE has been documented in Ireland recently it is limited to certain sites and SLICE is regularly and successfully used to control lice infestation on farmed smolts.

Figure 2 and Table 1: the authors should consider rationalizing data presentation here.

The authors wish to retain both as the data presented in each is pertinent to understanding the significance of the discussion and data analysis.
Figure 3 and Table 3 are redundant. It would be informative to indicate where significant differences exist if a figure is used.

There is no Table 3. Significant differences in return rates, with summary statistics, are given in the text in the results section & are indicated in figure 2. The authors wish to retain Figure 3 as it presents a good visual representation of the time series data without statistical treatment and clearly shows the extent of the decline in survival and how closely it is mirrored in both treated and control groups.
An evaluation of the impact of early infestation with the salmon louse
Lepeophtheirus salmonis on the subsequent survival of outwardly migrating
Atlantic salmon, Salmo salar L., smolts

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Abstract

The potential impact of sea lice infestation on outwardly migrating Atlantic salmon
smolts has been investigated by treating populations of ranched salmon, prior to
release, with a prophylactic sea lice treatment conferring protection from sea lice
infestation, for up to 9 weeks. Established populations of ranched Atlantic salmon
with well described rates of return were chosen to investigate the potential
contribution of early infestation with the salmon louse, Lepeophtheirus salmonis to
mortality in Atlantic salmon. Against a backdrop of a declining trend in survival rates
of Atlantic salmon many studies are attempting to elucidate potential causes for this
decline. Results from this study over a period of 9 years point to infestation with the
salmon louse (L. salmonis) as being a minor component of marine mortality in the
stocks studied.

Key Words: marine survival, sea lice, Atlantic salmon.

Introduction

Against a backdrop of long term declines in stock levels of Atlantic salmon (Salmo
salar L.) throughout its range, stock levels in Ireland and the NE Atlantic since the
1970s (ICES, unpubl. data) have given rise to serious concerns for the status of the
species. This has resulted in conservation measures being introduced and strengthened
to include restrictions in existing fisheries, closures of mixed stock fisheries and the
introduction of carcass tagging and quota systems (Ó Maoiléidigh et al, 2004). It has
also led to increased interest in gaining a better understanding of the factors
underlying the current trends (Peyronnet et al, 2007).

Significant declines in sea survival and reduced returns to the coast and rivers of
Atlantic salmon in recent decades have been recorded in Ireland (Salmon
Management Task Force Report (Anon., 1996); Ó Maoiléidigh et al, 2004). The
reasons for the reduced sea survival remains unclear and speculation has covered such
issues as global warming effects (Friedland et al, 2000; Friedland et al, 2005),
changes in locations or availability of prey species, loss of post-smolts as by-catch in
pelagic fisheries, increased fishing pressure, habitat changes and sea lice infestation
(Finstad et al, 2007). However, despite many years of study, processes contributing to
the high mortality of juvenile Atlantic salmon between ocean entry and the first winter
at sea remain poorly understood (Jones, 2009).

In order to investigate if lice infestations were a significant factor in early marine
mortality of Irish salmon smolts and to measure the inter annual variation in the
impacts of early lice infestations on sea survival the Marine Institute has been
undertaking a long term study of lice infestations in outward migrating salmon smolts.
The goal of this study is to attempt to measure the impact of early infestation of
outward migrating salmon smolts with the salmon louse, *Lepeophtheirus salmonis*
Krøyer in established ranched strains. The study is based at the Marine Institute
research facility in Burrishoole in County Mayo (Figure 1, location map), which is an
index catchment with full upstream trapping. The Burrishoole ranched stock is an
established strain which has been studied for over thirty years (Piggins & Mills, 1985)
and there is considerable historical data on it’s marine survival and return rates.
Studies are also under way on ranched stocks at other locations in Ireland.

Materials and Methods

Experimental Design

By treating experimental batches of tagged fish with a prophylactic dose of SLICE, a
commercial sea lice therapeutant, prior to release the fish can be protected from
infestation with the salmon louse for up to nine weeks (Stone *et al*, 2000; Copley *et
al*, 2007). The active ingredient in SLICE is emamectin benzoate. It is an animal
medicine licensed for use in Ireland as a treatment for sea lice infestation in salmon.
Since 2001 the Marine Institute has released treated and control groups of
experimental ranched smolts over a number of years and recorded their subsequent
survival and return rates as grilse and multi sea-winter fish to the coast and their
release catchments. Treated fish are protected from sea lice infestation in their early
weeks in the sea and therefore can be expected to be free of any adverse impacts on
their survival related to early lice infestation. As salmon smolts are known to migrate
quickly out of the bays and into the open sea treated smolts will have moved well
offshore before the protective effects of the SLICE treatment have worn off. Studies
at Burrishoole have shown that salmon smolts have moved into coastal waters within
48 hours (Moore *et al*, 2008) and post smolt recapture data (Shelton *et al*, 1997;
Dadswell *et al*, 2010) has shown that smolts from the study area have travelled a
distance of over 700 kilometres in seven weeks and are in an area north of Scotland
and west of Norway. By comparing their survival and return rates with control fish,
which do not enjoy this protection it is possible to differentiate any additional
mortality associated with lice infestation in the first six to eight weeks post migration.

Fish Stocks

The stock used in the study is the Burrishoole grilse stock. This stock has been line
bred in an ongoing experimental ocean ranching programme since the early 1970s
(Piggins and Mills, 1985; Cotter *et al*, 2000). In each release experimental groups of
smolts were split into two approximately equal groups, one treated, one control. The
treated groups were administered SLICE as an in feed preparation at the rate of
50µg/kg/day for seven days. Treatment was completed approximately seven days
before the release date of the smolts. Control groups were fed either with food mixed
with a placebo or, in certain years, with untreated food.

Samples of treated food were retained and analysed to ensure appropriate inclusion
rates and samples of both treated and control fish was taken for flesh analysis. Fish
samples were taken two days post-feeding to ensure the guts were voided of medicated feed. Flesh analysis for emamectin benzoate was carried out by accredited laboratories to ensure a therapeutic dose was present in the treated groups prior to release.

Tagging

Experimental batches of fish were all tagged with coded wire tags. Pre-smolts were microtagged according to the methods of Browne (1982). Each magnetised microtag had a specific code which identified the release group and stock of the fish. A 1 mm long magnetised tag, etched with a specific batch code was injected into the nose cartilage of the juvenile fish. The code identifies the origin and release circumstances of any fish subsequently recaptured. All fish were anaesthetised when tagged. The adipose fin was removed to facilitate the identification of these fish in the recovery programme. A quality control check was made on the tagged fish to ensure that the tag has been correctly magnetised. Tagging mortality and tag loss were also estimated and subsequent analyses were based on the numbers of fish migrating rather than the number of fish tagged.

Tag Recovery

Information on capture location and return data of the experimental groups was gathered as part of an ongoing Irish national coded wire tag recovery programme (Browne et al, 1994; Ó Maoilédigh et al, 2004). Catches from coastal commercial fisheries (drift nets, draft nets, etc.) were monitored at 15 major salmon landing ports in Ireland. These fisheries operate between May and July inclusive and catches were scanned consistently during this period. Over 50% of the catch landed in Ireland is sampled for tags each year. The number of tagged salmon taken in these fisheries (raised data) was estimated by multiplying the actual number of tagged salmon in each area by the ratio of the total declared salmon landings in these areas to the sample size examined. An adjustment for non-catch fishing mortality due to losses from nets and non-reporting of catches was also applied.

Complete upstream and downstream trapping facilities at the Marine Institute hatchery, situated on the Burrishoole river system in Co. Mayo, ensured an accurate count of the numbers of tagged adult salmon returning to the hatchery location. The number of fish entering the river was derived from total trap data and angling for the Burrishoole system. For fresh water, the percentage return was calculated using the actual number of tags recovered divided by the number of fish migrating.

Release Groups

Results for a total of 10 releases over a period of nine years, from 2001 to 2008 are presented. There were two releases in each of 2006 and 2008. Details of release dates and the size of groups are given in Table 1. Ranched release groups were released as 1+ year old smolts into Lough Furnace, a tidal lake immediately downstream of the Marine Institute hatchery at Burrishoole, County Mayo.
Data Analysis

A sign test was calculated on the observed returns of treated and non-treated salmon over the entire test period to determine if treatment improved potential of salmon returning. Two way contingency tables were used to calculate expected returns for comparison against observed returns for each yearly pair of treatment and control batches using the chi-squared test. To investigate in more detail changes in the number of returns of treated and untreated salmon over the experimental period, and the differences and similarities in these changes between the treated and untreated batches, an analysis of co-variance (ANCOVA) was used (Sokal & Rohlf, 1995).

Results

The actual numbers of returning fish recovered for each experimental release is shown in Figure 2. Percentage survival for the same groups is shown in Figure 3. A trend of decreasing survival rates in both treated and control groups over time can be clearly observed. Percentage survival ranged from a maximum of just over 10% in the 2001 release treated group (10.28%) to a minimum of just over 1% in the 2008 early release control group (1.07%). The maximum difference in percentage survival between treated and control groups (2.38%) was recorded in the early release group in 2006 when the percentage return for the treated group was 6.82% as against 4.44% in the control group. Percentage survival rates for all groups are outlined in Table 2.

A sign test was calculated on observed returns of treated and non-treated salmon ($n=10$). In nine instances a greater proportion of treated than non-treated salmon returned, which represents a significant departure from the expected binomial equality at $p < 0.05$.

Chi-squared tests of independence showed significant differences in treated and non-treated returning and non-returning rates in four of ten instances (in 2003, $X^2=8.98$ $p<0.005$; in 2005, $X^2=13.70$ $p<0.001$; in 2006(i) $X^2=25.64$ $p<0.001$ and; in 2007 $X^2 = p<0.05$).

Clear declines in returns in both treated and non-treated batches were apparent over the experimental time period, Figure 4. An ANCOVA was used to assess relationships between these declining rates. Independently regression lines of the declines in returns were extremely significant ($p>0.001$; $n=10$ for each), however no difference between the mean returns was found (analysis of variance, $n=20$), Figure 4. A common regression of the two sets (Figure 5.) was extremely significant ($p< 0.001$; $n=20$) though there was no difference between the rates of decline between treated and non-treated returns ($n=20$) or between their instantaneous returns when corrected to a common decline rate (Figure 6.) (Sokal & Rohlf (1995).

Discussion

While treatment with SLICE generally resulted in a higher percentage return than the untreated control group (9 out of 10 cases, sign test) in the majority of releases, six
out of ten, this difference was not significant when compared against the expected number of returns (contingency table chi-squared tests). In 2006 the early release group (Table 2.) showed the greatest difference in percentage survival, which was extremely significant, however the difference in return in the later release group between treated and control batches was not significant. Over the period of the study the relationships between rates of return for treated and control batches exhibit similar trends.

The difference in percentage survival between the treated and control groups is not significant (ANCOVA) but the fact that the treated groups have higher survival in nine out of ten cases (sign test) is. These results are consistent with the expectation that a reduction in potential parasite load on outwardly migrating smolts, such as would be conferred by protection against sea lice infestation for a period of up to nine weeks should contribute to increased fitness and survival potential. The results over the study period would suggest that the level of infestation pressure by *L. salmonis* experienced by the outwardly migrating smolts was not of a level to be a consistently significant source of additional marine mortality because no significant difference in survival rates was found between treated and unprotected groups.

It is well recognised that large numbers of mobile *L. salmonis* can cause host morbidity and death (Wagner et al., 2008) and natural levels of *L. salmonis* on wild salmon returning to the Irish coast are known to have a mean abundance of more than 10 mobile lice per fish and a prevalence in excess of 90% (Copley et al., 2005). Skilbrei and Wennevik (2006) found similar survival rates in treated and untreated groups of smolts released in western Norway in May 2003 but found significantly better survival in the treated group released in June of the same year. Glover et al. (2004) suggested that there may be a genetic susceptibility component to differences in infestation rate observed between five different stocks of Atlantic salmon, three wild and two farmed. Finstad and Jonsson (2001) have reported very large differences between treated and untreated groups in Norway. They reported treated groups having recapture rates of 0.9% as against 0.03% in unprotected fish. Differences of this magnitude were not recorded in this study and minimum survival levels were always in excess of 1%.

The highly significant trend observed in both treated and untreated groups of a decline in percentage survival from values in the region of 10% survival in the 2001 releases to values ranging from just over 2% to 1.07% in the 2008 releases is of great concern. Both treated and control groups share a common rate of decline (Figure 5.) and there is no difference in their returns when corrected to a common decline rate (Figure 6.) clearly demonstrating that the long term decline rate is common to both groups. This highly significant decline in marine survival over the study period is independent of whether the fish were treated to protect against infestation with sea lice or not.

**Conclusions**

The results to date show a strong and significant trend in increasing marine mortality of Atlantic salmon originating in the study area. They would also point to infestation of outwardly migrating salmon smolts with the salmon louse (*L. salmonis*) as being a
minor and irregular component of marine mortality in the stocks studied and not being implicated in the observed decline in survival rate.
References


Copley, L., P. O’Donohoe, S. Kennedy, D. Tierney, O. Naughton, F. Kane, D. Jackson and D. McGrath, 2007. Lice infestation pressures on farmed Atlantic Salmon smolts (Salmo salar L.) in the west of Ireland following a SLICE (0.2% emamectin benzoate) treatment. Fish Vet. Journal (9), 10-21.


Figure 1. Burrishoole catchment and Lough Furnace (smolt release site), West coast of Ireland.
Figure 2. Time series, numbers of fish returning from treated and control groups.
Figure 3. Time series, percentage survival of treated and control groups.
Figure 4. Time series of treated ($y = -1.132x + 12.383; r^2 = 0.774$) and control ($y = -1.166x + 11.691; r^2 = 0.811$) groups with their respective mean returns over the time period and regression lines fitted.
**Figure 5.** Regressions of time series of treated ($y = -1.149x + 12.467; r^2 = 1.0$) and control ($y = -1.149x + 11.606; r^2 = 1.0$) groups fitted to a common slope by an ANCOVA.
Figure 6. Common regression slope and intercept as defined by an ANCOVA of treated and control groups ($y = -1.149x + 12.037; r^2 = 1.0$).
<table>
<thead>
<tr>
<th>Location of Release</th>
<th>Release date</th>
<th>Control (n)</th>
<th>Treated (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lough Furnace</td>
<td>03/5/2001</td>
<td>10039</td>
<td>5496</td>
</tr>
<tr>
<td>Lough Furnace</td>
<td>01/5/2002</td>
<td>5989</td>
<td>5960</td>
</tr>
<tr>
<td>Lough Furnace</td>
<td>01/5/2003</td>
<td>4587</td>
<td>4755</td>
</tr>
<tr>
<td>Lough Furnace</td>
<td>29/4/2004</td>
<td>4369</td>
<td>4437</td>
</tr>
<tr>
<td>Lough Furnace</td>
<td>28/4/2005</td>
<td>3867</td>
<td>3793</td>
</tr>
<tr>
<td>Lough Furnace</td>
<td>26/4/2006</td>
<td>4779</td>
<td>4809</td>
</tr>
<tr>
<td>Lough Furnace</td>
<td>04/5/2006</td>
<td>8000</td>
<td>3907</td>
</tr>
<tr>
<td>Lough Furnace</td>
<td>24/4/2007</td>
<td>6795</td>
<td>6746</td>
</tr>
<tr>
<td>Lough Furnace</td>
<td>29/4/2008</td>
<td>6832</td>
<td>6719</td>
</tr>
<tr>
<td>Lough Furnace</td>
<td>06/5/2008</td>
<td>3392</td>
<td>3413</td>
</tr>
</tbody>
</table>

**Table 1.** Details of release dates, and numbers migrating for all 10 groups.
<table>
<thead>
<tr>
<th>Location of Release</th>
<th>Release date</th>
<th>Control % survival</th>
<th>Treated % survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lough Furnace</td>
<td>03/5/2001</td>
<td>9.88</td>
<td>10.28</td>
</tr>
<tr>
<td>Lough Furnace</td>
<td>01/5/2003</td>
<td>8.15</td>
<td>9.93</td>
</tr>
<tr>
<td>Lough Furnace</td>
<td>28/4/2005</td>
<td>4.71</td>
<td>6.67</td>
</tr>
<tr>
<td>Lough Furnace</td>
<td>26/4/2006</td>
<td>4.44</td>
<td>6.82</td>
</tr>
<tr>
<td>Lough Furnace</td>
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<td>4.21</td>
<td>4.61</td>
</tr>
<tr>
<td>Lough Furnace</td>
<td>29/4/2008</td>
<td>1.07</td>
<td>1.40</td>
</tr>
<tr>
<td>Lough Furnace</td>
<td>06/5/2008</td>
<td>1.53</td>
<td>2.02</td>
</tr>
</tbody>
</table>

Table 2. Details of percentage survival for all 10 groups.