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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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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 interchangeably. 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 rationale for the tests. It's difficult to determine why both a sign test and chi-squared test 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 tests 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 hand, these lice populations were resistant 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.

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

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10
11 **Abstract**

12
13 The potential impact of sea lice infestation on outwardly migrating Atlantic salmon
14 smolts has been investigated by treating populations of ranched salmon, prior to
15 release, with a prophylactic sea lice treatment conferring protection from sea lice
16 infestation, for up to 9 weeks. Established populations of ranched Atlantic salmon
17 with well described rates of return were chosen to investigate the potential
18 contribution of early infestation with the salmon louse, *Lepeophtheirus salmonis* to
19 mortality in Atlantic salmon. Against a backdrop of a declining trend in survival rates
20 of Atlantic salmon many studies are attempting to elucidate potential causes for this
21 decline. Results from this study over a period of 9 years point to infestation with the
22 salmon louse (*L. salmonis*) as being a minor component of marine mortality in the
23 stocks studied.

24
25 **Key Words:** marine survival, sea lice, Atlantic salmon.

26
27 **Introduction**

28
29 Against a backdrop of long term declines in stock levels of Atlantic salmon (*Salmo*
30 *salar* L.) throughout its range, stock levels in Ireland and the NE Atlantic since the
31 1970s (ICES, unpubl. data) have given rise to serious concerns for the status of the
32 species. This has resulted in conservation measures being introduced and strengthened
33 to include restrictions in existing fisheries, closures of mixed stock fisheries and the
34 introduction of carcass tagging and quota systems (Ó Maoiléidigh *et al*, 2004). It has
35 also led to increased interest in gaining a better understanding of the factors
36 underlying the current trends (Peyronnet *et al*, 2007).

37
38 Significant declines in sea survival and reduced returns to the coast and rivers of
39 Atlantic salmon in recent decades have been recorded in Ireland (Salmon
40 Management Task Force Report (Anon., 1996); Ó Maoiléidigh *et al*, 2004). The
41 reasons for the reduced sea survival remains unclear and speculation has covered such
42 issues as global warming effects (Friedland *et al*, 2000; Friedland *et al*, 2005),
43 changes in locations or availability of prey species, loss of post-smolts as by-catch in
44 pelagic fisheries, increased fishing pressure, habitat changes and sea lice infestation
45 (Finstad *et al*, 2007). However, despite many years of study, processes contributing to
46 the high mortality of juvenile Atlantic salmon between ocean entry and the first winter
47 at sea remain poorly understood (Jones, 2009).

48
49 In order to investigate if lice infestations were a significant factor in early marine
50 mortality of Irish salmon smolts and to measure the inter annual variation in the

51 impacts of early lice infestations on sea survival the Marine Institute has been
52 undertaking a long term study of lice infestations in outward migrating salmon smolts.
53 The goal of this study is to attempt to measure the impact of early infestation of
54 outward migrating salmon smolts with the salmon louse, *Lepeophtheirus salmonis*
55 Krøyer in established ranched strains. The study is based at the Marine Institute
56 research facility in Burrishoole in County Mayo (Figure 1, location map), which is an
57 index catchment with full upstream trapping. The Burrishoole ranched stock is an
58 established strain which has been studied for over thirty years (Piggins & Mills, 1985)
59 and there is considerable historical data on it's marine survival and return rates.
60 Studies are also under way on ranched stocks at other locations in Ireland.

63 **Materials and Methods**

65 Experimental Design

67 By treating experimental batches of tagged fish with a prophylactic dose of SLICE, a
68 commercial sea lice therapeutant, prior to release the fish can be protected from
69 infestation with the salmon louse for up to nine weeks (Stone *et al*, 2000; Copley *et*
70 *al*, 2007). The active ingredient in SLICE is emamectin benzoate. It is an animal
71 medicine licensed for use in Ireland as a treatment for sea lice infestation in salmon.
72 Since 2001 the Marine Institute has released treated and control groups of
73 experimental ranched smolts over a number of years and recorded their subsequent
74 survival and return rates as grilse and multi sea-winter fish to the coast and their
75 release catchments. Treated fish are protected from sea lice infestation in their early
76 weeks in the sea and therefore can be expected to be free of any adverse impacts on
77 their survival related to early lice infestation. As salmon smolts are known to migrate
78 quickly out of the bays and into the open sea treated smolts will have moved well
79 offshore before the protective effects of the SLICE treatment have worn off. Studies
80 at Burrishoole have shown that salmon smolts have moved into coastal waters within
81 48 hours (Moore *et al*, 2008) and post smolt recapture data (Shelton *et al*, 1997;
82 Dadswell *et al*, 2010) has shown that smolts from the study area have travelled a
83 distance of over 700 kilometres in seven weeks and are in an area north of Scotland
84 and west of Norway. By comparing their survival and return rates with control fish,
85 which do not enjoy this protection it is possible to differentiate any additional
86 mortality associated with lice infestation in the first six to eight weeks post migration.

88 Fish Stocks

90 The stock used in the study is the Burrishoole grilse stock. This stock has been line
91 bred in an ongoing experimental ocean ranching programme since the early 1970s
92 (Piggins and Mills, 1985; Cotter *et al*, 2000). In each release experimental groups of
93 smolts were split into two approximately equal groups, one treated, one control. The
94 treated groups were administered SLICE as an in feed preparation at the rate of
95 50µg/kg/day for seven days. Treatment was completed approximately seven days
96 before the release date of the smolts. Control groups were fed either with food mixed
97 with a placebo or, in certain years, with untreated food.

99 Samples of treated food were retained and analysed to ensure appropriate inclusion
100 rates and samples of both treated and control fish was taken for flesh analysis. Fish

101 samples were taken two days post-feeding to ensure the guts were voided of
102 medicated feed. Flesh analysis for emamectin benzoate was carried out by accredited
103 laboratories to ensure a therapeutic dose was present in the treated groups prior to
104 release.

105

106 Tagging

107

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

119

120

121 Tag Recovery

122

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

134

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

141

142

143 Release Groups

144

145 Results for a total of 10 releases over a period of nine years, from 2001 to 2008 are
146 presented. There were two releases in each of 2006 and 2008. Details of release dates
147 and the size of groups are given in Table 1. Ranched release groups were released as
148 1+ year old smolts into Lough Furnace, a tidal lake immediately downstream of the
149 Marine Institute hatchery at Burrishoole, County Mayo.

150

151

152 Data Analysis

153

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

162

163

164 **Results**

165

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

175

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

180

181 Chi-squared tests of independence showed significant differences in treated and non-
182 treated returning and non-returning rates in four of ten instances (in 2003, $X^2=8.98$
183 $p<0.005$; in 2005, $X^2=13.70$ $p<0.001$; in 2006(i) $X^2=25.64$ $p<0.001$ and; in 2007 $X^2=$
184 $p<0.05$).

185

186 Clear declines in returns in both treated and non-treated batches were apparent over
187 the experimental time period, Figure 4. An ANCOVA was used to assess
188 relationships between these declining rates. Independently regression lines of the
189 declines in returns were extremely significant ($p>0.001$; $n= 10$ for each), however no
190 difference between the mean returns was found (analysis of variance, $n=20$), Figure 4.
191 A common regression of the two sets (Figure 5.) was extremely significant ($p< 0.001$;
192 $n=20$) though there was no difference between the rates of decline between treated
193 and non-treated returns ($n=20$) or between their instantaneous returns when corrected
194 to a common decline rate (Figure 6.) (Sokal & Rohlf (1995).

195

196

197 **Discussion**

198

199 While treatment with SLICE generally resulted in a higher percentage return than the
200 untreated control group (9 out of 10 cases, sign test) in the majority of releases, six

201 out of ten, this difference was not significant when compared against the expected
202 number of returns (contingency table chi-squared tests). In 2006 the early release
203 group (Table 2.) showed the greatest difference in percentage survival, which was
204 extremely significant, however the difference in return in the later release group
205 between treated and control batches was not significant. Over the period of the study
206 the relationships between rates of return for treated and control batches exhibit similar
207 trends.

208
209 The difference in percentage survival between the treated and control groups is not
210 significant (ANCOVA) but the fact that the treated groups have higher survival in
211 nine out of ten cases (sign test) is. These results are consistent with the expectation
212 that a reduction in potential parasite load on outwardly migrating smolts, such as
213 would be conferred by protection against sea lice infestation for a period of up to nine
214 weeks should contribute to increased fitness and survival potential. The results over
215 the study period would suggest that the level of infestation pressure by *L. salmonis*
216 experienced by the outwardly migrating smolts was not of a level to be a consistently
217 significant source of additional marine mortality because no significant difference in
218 survival rates was found between treated and unprotected groups.

219
220 It is well recognised that large numbers of mobile *L. salmonis* can cause host
221 morbidity and death (Wagner *et al*, 2008) and natural levels of *L. salmonis* on wild
222 salmon returning to the Irish coast are known to have a mean abundance of more than
223 10 mobile lice per fish and a prevalence in excess of 90% (Copley *et al*, 2005).
224 Skilbrei and Wennevik (2006) found similar survival rates in treated and untreated
225 groups of smolts released in western Norway in May 2003 but found significantly
226 better survival in the treated group released in June of the same year. Glover *et al*
227 (2004) suggested that there may be a genetic susceptibility component to differences
228 in infestation rate observed between five different stocks of Atlantic salmon, three
229 wild and two farmed. Finstad and Jonsson (2001) have reported very large differences
230 between treated and untreated groups in Norway. They reported treated groups having
231 recapture rates of 0.9% as against 0.03% in unprotected fish. Differences of this
232 magnitude were not recorded in this study and minimum survival levels were always
233 in excess of 1%.

234
235 The highly significant trend observed in both treated and untreated groups of a decline
236 in percentage survival from values in the region of 10% survival in the 2001 releases
237 to values ranging from just over 2% to 1.07% in the 2008 releases is of great concern.
238 Both treated and control groups share a common rate of decline (Figure 5.) and there
239 is no difference in their returns when corrected to a common decline rate (Figure 6.)
240 clearly demonstrating that the long term decline rate is common to both groups. This
241 highly significant decline in marine survival over the study period is independent of
242 whether the fish were treated to protect against infestation with sea lice or not.

243 244 245 **Conclusions**

246
247 The results to date show a strong and significant trend in increasing marine mortality
248 of Atlantic salmon originating in the study area. They would also point to infestation
249 of outwardly migrating salmon smolts with the salmon louse (*L. salmonis*) as being a

250 minor and irregular component of marine mortality in the stocks studied and not being
251 implicated in the observed decline in survival rate.
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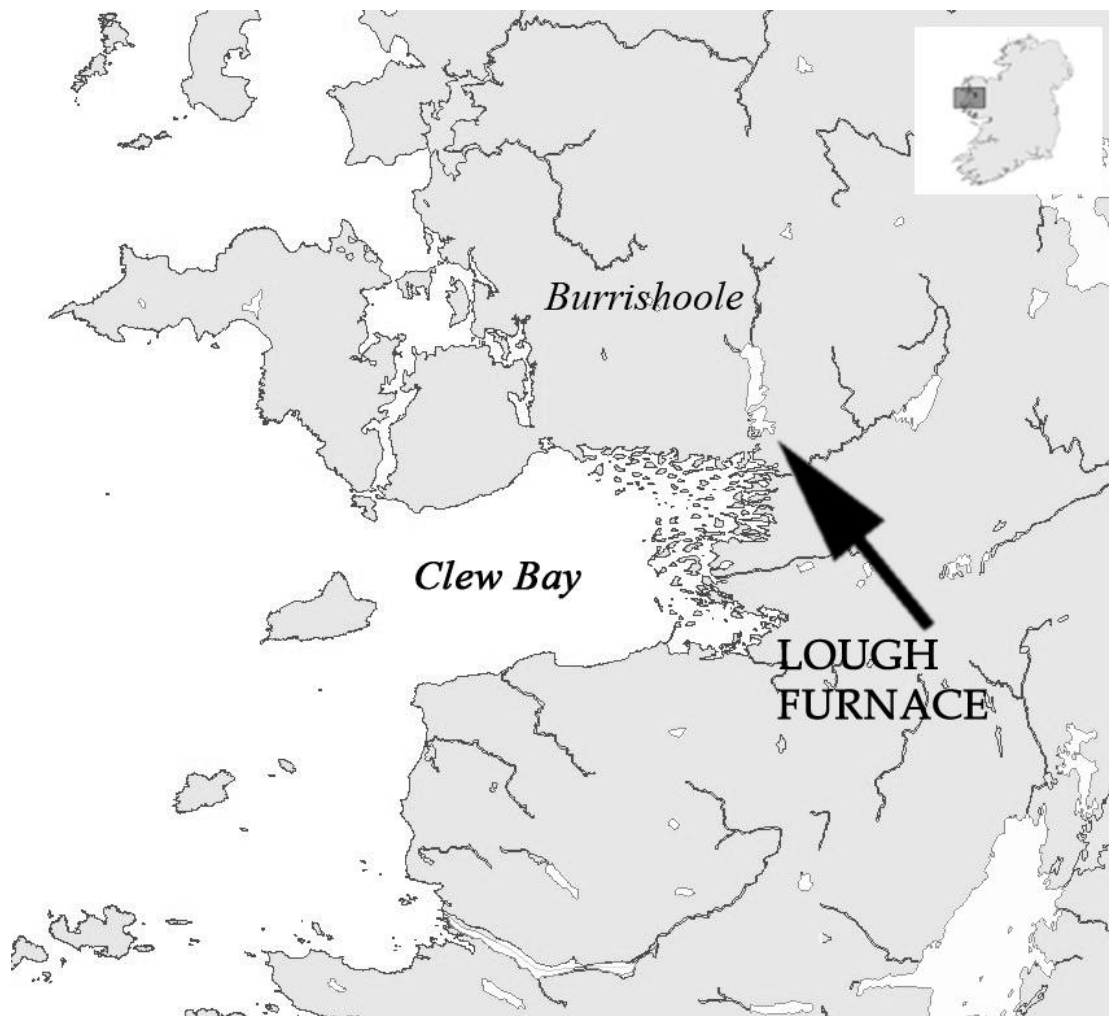


Figure 1. Burrishoole catchment and Lough Furnace (smolt release site), West coast of Ireland.

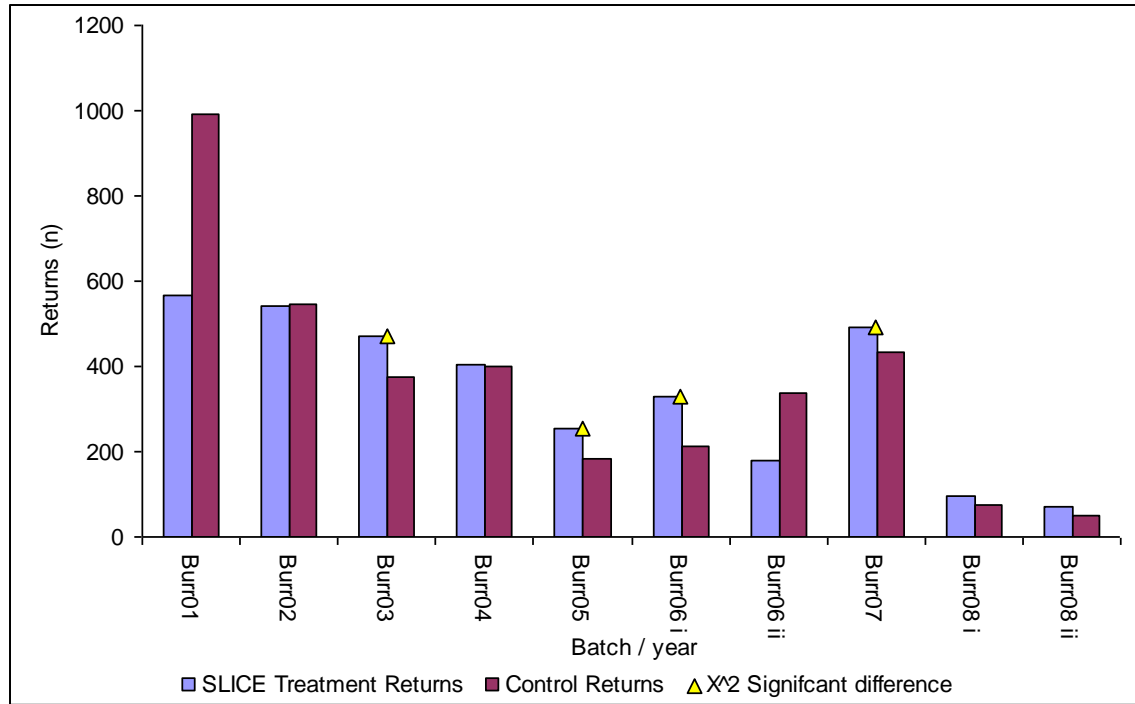


Figure 2. Time series, numbers of fish returning from treated and control groups.

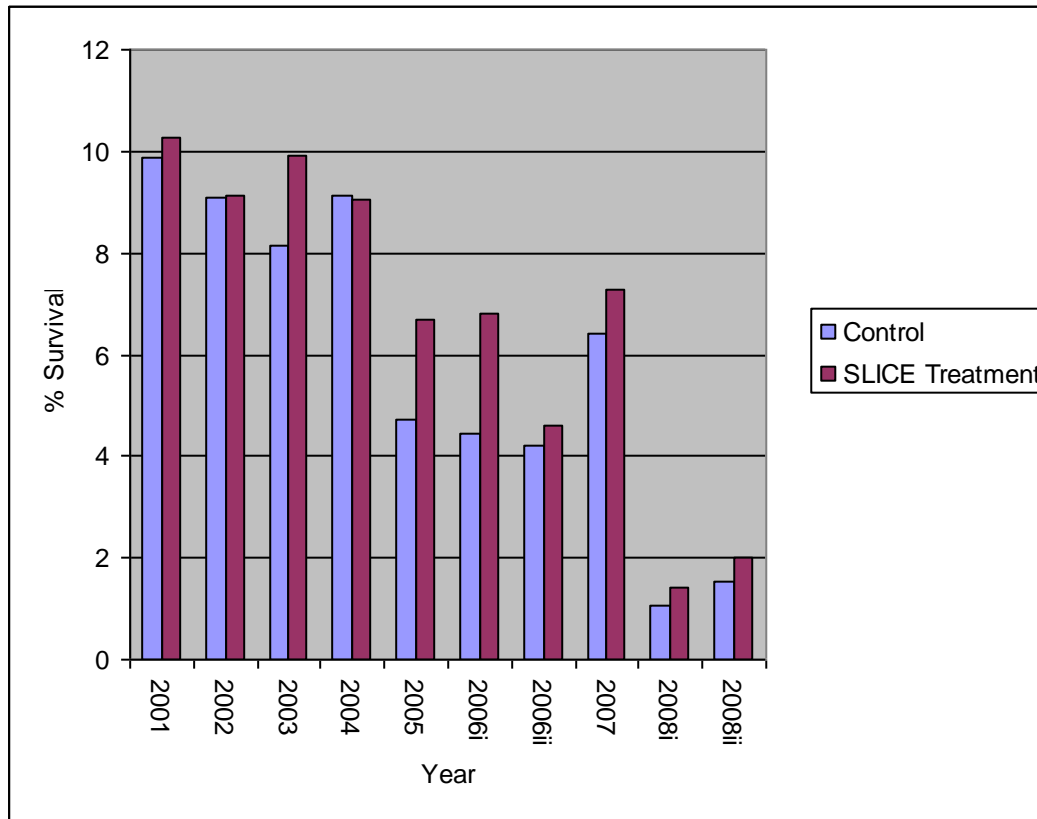


Figure 3. Time series, percentage survival of treated and control groups.

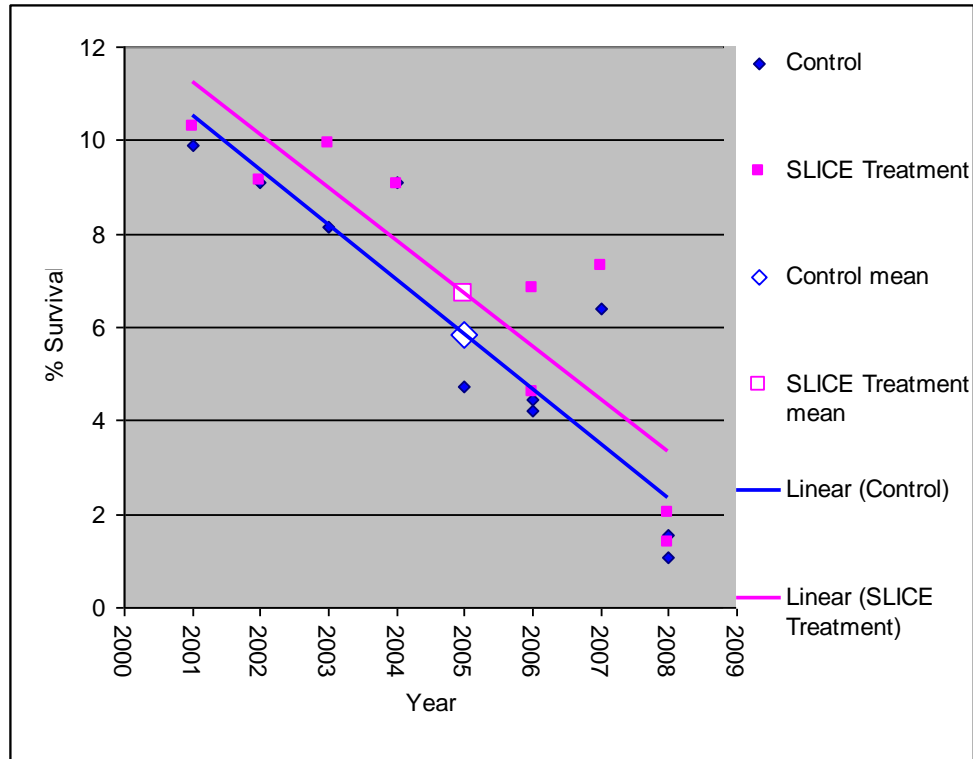


Figure 4. Time series of treated ($y = -1.132x + 12.383$; $r^2 = 0.774$) and control ($y = -1.166x + 11.691$; $r^2 = 0.811$) groups with their respective mean returns over the time period and regression lines fitted.

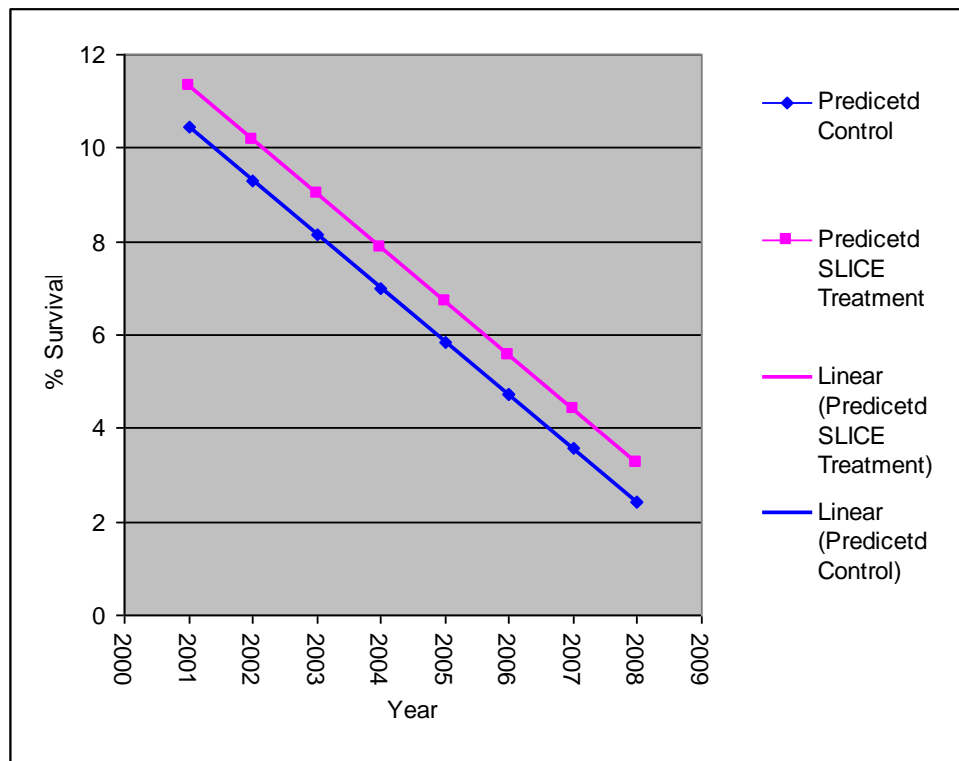


Figure 5. Regressions of time series of treated ($y = -1.149x + 12.467$; $r^2 = 1.0$) and control ($y = -1.149x + 11.606$; $r^2 = 1.0$) groups fitted to a common slope by an ANCOVA.

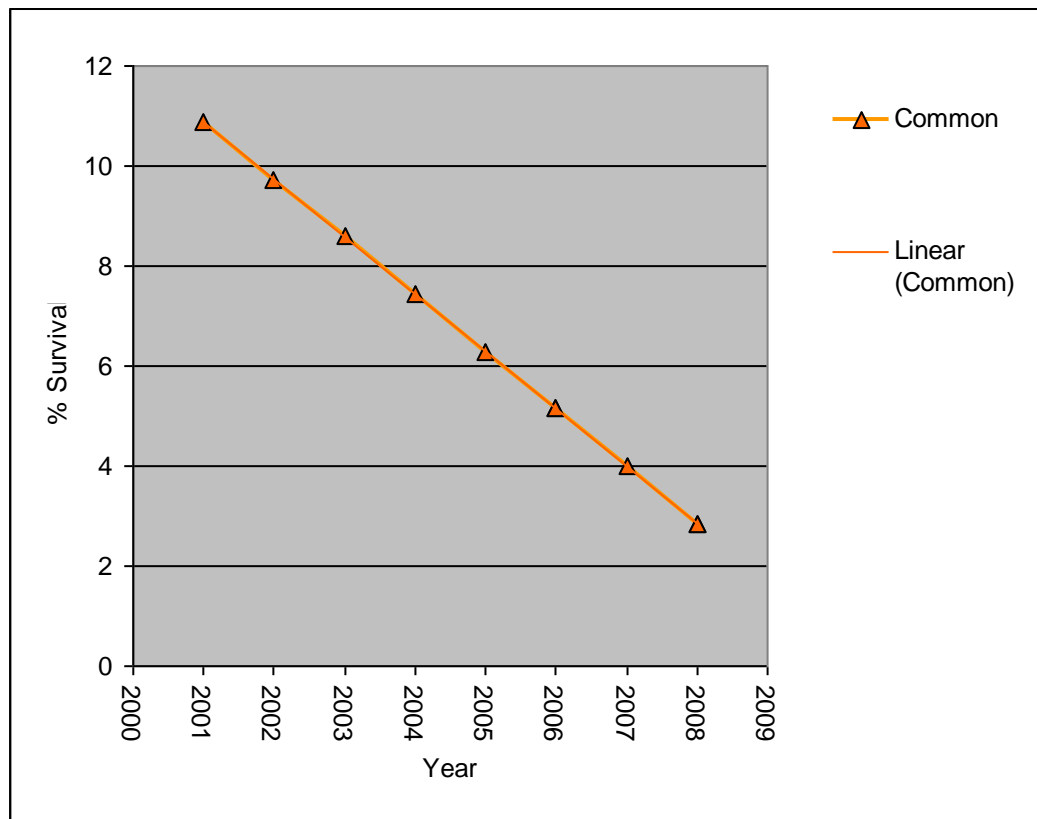


Figure 6 . Common regression slope and intercept as defined by an ANCOVA of treated and control groups ($y = -1.149x + 12.037$; $r^2 = 1.0$).

Location of Release	Release date	Control (n)	Treated (n)
Lough Furnace	03/5/2001	10039	5496
Lough Furnace	01/5/2002	5989	5960
Lough Furnace	01/5/2003	4587	4755
Lough Furnace	29/4/2004	4369	4437
Lough Furnace	28/4/2005	3867	3793
Lough Furnace	26/4/2006	4779	4809
Lough Furnace	04/5/2006	8000	3907
Lough Furnace	24/4/2007	6795	6746
Lough Furnace	29/4/2008	6832	6719
Lough Furnace	06/5/2008	3392	3413

Table 1. Details of release dates, and numbers migrating for all 10 groups.

Location of Release	Release date	Control % survival	Treated % survival
Lough Furnace	03/5/2001	9.88	10.28
Lough Furnace	01/5/2002	9.10	9.12
Lough Furnace	01/5/2003	8.15	9.93
Lough Furnace	29/4/2004	9.11	9.07
Lough Furnace	28/4/2005	4.71	6.67
Lough Furnace	26/4/2006	4.44	6.82
Lough Furnace	04/5/2006	4.21	4.61
Lough Furnace	24/4/2007	6.40	7.29
Lough Furnace	29/4/2008	1.07	1.40
Lough Furnace	06/5/2008	1.53	2.02

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