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Response

Response to M Krkošek, C W Revie, B Finstad and C D Todd's comment on Jackson *et al.* 'Impact of *Lepeophtheirus salmonis* infestations on migrating Atlantic salmon, *Salmo salar* L., smolts at eight locations in Ireland with an analysis of lice-induced marine mortality'

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Jackson *et al.* (2013a) was published in Volume 36, Number 3 of the Journal of Fish Diseases. In the same issue of the journal there was an article describing a similar long-term study carried out in Norway. Both studies were large scale long-term field studies covering periods from 2001–2009 and 1997–2009 respectively. The Norwegian article, which had six authors (O T Skilbrei, B Finstad, K Urdal, G Bakke, F Kroglund & R Strand) from four research institutes in Norway, not only used the same statistical methodology to analyze its results it also reported similar findings. Skilbrei *et al.* (2013) reported that their "results are similar to those of Jackson *et al.* (2011a)" while Jackson *et al.* (2013a) reported that "the analysis carried out here on a much larger data set" support the conclusions of Jackson *et al.* (2011a). Jackson *et al.* (2013a) reported an absolute difference in risk of 1% and an odds ratio of 1.14:1 and Skilbrei *et al.* (2013) reported an odds ratio of 1.17.

The authors would like to point out that both Jackson *et al.* (2013a) and Skilbrei *et al.* (2013) were subject to full scientific peer review prior to publication. The claim by Krkosek *et al.* that we made methodological errors is not justified.

The primary analysis we presented, a logistic regression model to compare the difference in the (log) odds of a treated fish returning compared to a control, incorporating a random effect to model the degree of heterogeneity between river (e.g. release date environmental conditions) and adjusting for the significant year effect, is the correct approach when modelling a binary response variable arising from such a designed experiment (McCullagh & Nelder 1989; Pinheiro & Bates 2000; Zuur *et al.* 2009; Skilbrei, Finstad *et al.*, 2013).

Krkosek *et al.,* in their comment, present an analysis using weighted summary data at the *river* level and not at the *individual fish* level as in our analysis. Their use of the word 'paired' is misleading as the data do not represent a single sample with a pair of measurements per fish. Presumably, the use of the word 'paired' refers to within river comparisons. Their criticism that the 'paired' structure of the data was not utilized in our analyses is not correct. The Chi squared tests reported in our analysis (Jackson *et al.* 2013a, Table 1) are within river comparisons of the proportions surviving incorporating the 'paired' structure present in the design.

The analyses presented by Krkosek *et al.*, a weighted within river comparison of the difference in the percentage returning between treated and

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Study	Experimental Events	Experimental Total	Control Events	Control Total	OR	95%-CI	W(fixed)	W(random)
Delphi_01_BUR	1216.6	6392	984.8	6385	1.29	[1.18; 1.41]	11.4%	4.3%
Delphi_01_DEL	836.1	6368	892.2	6395	0.93	[0.84; 1.03]	9.4%	4.2%
Burr_01	565.1	5496	996.6	10039	1.04	[0.93; 1.16]	8.2%	4.2%
Burr_02	543.7	5960	542.3	5989	1.01	[0.89; 1.14]	6.2%	4.2%
Gowla_03	225.6	4955	20.4	4822	10.98	[7.00; 17.22]	0.5%	2.7%
Invermore_03	88.6	4589	37.7	4594	2.36	[1.61; 3.46]	0.7%	3.1%
Burr_03	471.1	4745	373.8	4587	1.24	[1.08; 1.43]	4.8%	4.1%
Gowla_04	164.6	4655	91.0	4699	1.85	[1.43; 2.40]	1.4%	3.6%
Invermore_04	105.4	4653	96.2	4671	1.10	[0.83; 1.46]	1.2%	3.6%
Erriff_04	101.8	4325	107.9	4229	0.92	[0.70; 1.21]	1.3%	3.6%
Burr_04	403.3	4437	398.2	4369	1.00	[0.86; 1.15]	4.6%	4.1%
Erriff_05	171.8	4659	8.4	4689	20.19	[10.28; 39.66]	0.2%	1.9%
Gowla_05	306.3	4583	317.8	4735	1.00	[0.85; 1.17]	3.7%	4.0%
Invermore_05	195.8	4716	111.2	4750	1.80	[1.42; 2.28]	1.7%	3.8%
Delphi_05	1038.4	8471	831.1	8893	1.36	[1.23;1.49]	10.4%	4.2%
Burr_05	253.0	3793	183.2	3867	1.44	[1.18; 1.75]	2.5%	3.9%
Lee_06	10.0	5207	10.0	5131	0.99	[0.42; 2.32]	0.1%	1.4%
Burr_06 May	326.0	4809	211.0	4779	1.57	[1.32; 1.88]	3.1%	4.0%
Screebe_06	157.0	10990	121.0	9618	1.14	[0.90; 1.44]	1.7%	3.7%
Delphi_06	477.9	10560	172.4	8788	2.36	[1.98; 2.82]	3.1%	4.0%
Burr_06	180.0	3907	334.0	8000	1.11	[0.92; 1.34]	2.8%	4.0%
Erne_06	70.0	5752	68.0	10357	1.86	[1.33; 2.60]	0.9%	3.3%
Burr_07	491.0	6746	440.0	6784	1.13	[0.99; 1.29]	5.4%	4.1%
Delphi_07	550.8	9451	567.4	9719	1.00	[0.88; 1.13]	6.6%	4.2%
Delphi_08 DEL	293.0	16346	183.0	10811	1.06	[0.88; 1.28]	2.8%	4.0%
Burr_08	169.0	10132	130.0	10224	1.32	[1.05; 1.66]	1.8%	3.8%
Burr_09	267.0	6881	300.0	6640	0.85	[0.72; 1.01]	3.4%	4.0%
Fixed effect model		**173578**		**178564**	**1.20**	**[1.16; 1.23]**	**100%**	**--**
Random effects model					**1.39**	**[1.23; 1.58]**	**--**	**100%**

Heterogeneity: I-squared = 92.8%, tau-squared = 0.09, p < 0.001

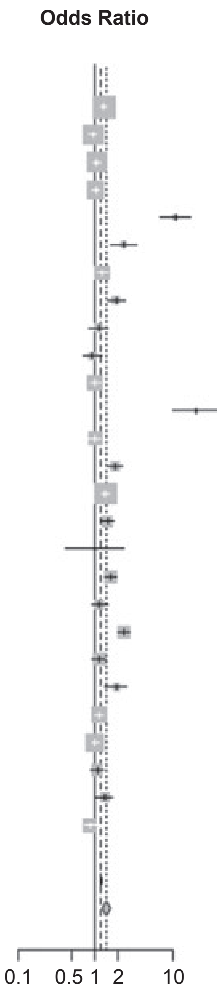

Figure 1 Meta –analysis used by Krkosek *et al.* when calculating the 1.39 odds ratio including weights (fixed and random effects) and the I^2 statistic (93%) not originally included in their letter to the Journal of Fish Diseases. Using a combined estimate base on a meta-analysis with such heterogeneity is highly questionable. The claim that the Odds Ratio is incorrect by a multiple of 30 is therefore based on flawed inference.

untreated fish, highlight the pitfalls of analyzing data using paired differences (regardless of transformation) and as a meta-analysis in the presence of considerable heterogeneity. Krkosek *et al.* present results based on a meta-analysis using summary data from Table 1 of our article. Meta-analysis is useful and valid when study summaries, rather than the raw data, are available for analysis and studies are comparable (i.e. homogeneous). The results from a meta-analysis are highly unreliable, however, when the variability between studies (i.e. heterogeneity) is large.

Heterogeneity is evident in a meta-analysis by a high value of the I^2 statistic and a meaningful difference between the fixed and random effect estimates. In the analysis presented by Krkosek *et al.* the I^2 statistic, the percentage of variation due to heterogeneity, was extremely high at 93% (Fig. 1) and the test for heterogeneity was highly significant ($P < 0.001$). Using a combined estimate base on a meta-analysis with such heterogeneity is highly questionable.

Krkosek *et al.* did not report heterogeneity (nor explore its source), which is a mandatory requirement for reporting meta-analyses as outlined by the PRISMA document (Preferred Reporting Items for Systematic Reviews and Meta-Analyses, http://www.prisma-statement.org/), an evidence-based minimum set of items for reporting in systematic reviews and meta-analyses.

The study weights used when calculating the 1.39 odds ratio for the random effect analysis (as quoted by Krkosek *et al.* for their justification of a '30 times ...' comment) are given in Fig. 1. The weight each river is given is driven by the heterogeneity as all rivers are weighted similarly and essentially by 'noise' and no longer weighted purely by sample size. The claim made by Krkosek *et al.* that the Odds Ratio is incorrect by a multiple of 30 is based on flawed inference.

One explanation for the source of heterogeneity is due to the significant change in percentage returning over time as reported in our article. Their statement about not adjusting for inter-annual fluctuations is indirectly stating that one should not look for sources of heterogeneity.

How best to report the results of a logistic regression, i.e. as an odds ratio estimating the multiplicative effect on the odds of a treated fish returning compared to a control or as a difference in percentage returns on an absolute scale is a valid question.

In our article, we reported both relative and absolute risk differences which is consistent with the CONSORT statement (Schulz, Altman & Moher 2010), which encompasses various initiatives to alleviate the problems arising from inadequate reporting of randomized controlled trials (Consolidated Standards Of Reporting Trials, http://www.consort-statement.org/).

In reporting the impact of lice infestation as a source of mortality in Atlantic salmon post smolts, it is important to recognize that over time the level of lice-induced mortality has remained relatively constant (Jackson *et al.* 2013a; Skilbrei *et al.* 2013) at approximately 1% or 10 fish in a thousand whereas the other mortality factors have increased substantially leading to a drop in the survival of Atlantic salmon over the study period from the region of 20% to <5% (Peyronnet *et al.* 2007; Jackson *et al.* 2011a,b; Jackson *et al.* 2013b).

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