# Review of “Methods for tagging an ectoparasite, the salmon louse *Lepeophtheirus salmonis*” by Alexius Folk and Adèle Mennerat

## General appreciation

It is a very interesting method. Especially since this kind of methodology paper is not that common, while they are necessary to avoid multiple research teams wasting time trying to develop the same approach. It is also beneficial for a data-demanding field to answer questions that could not be answered before concerning individual ecology and evolution of small species. Moreover, since the tag loss rate has been estimated, it could also be used in further Mark-Release-Recapture analyses, correcting the apparent survival.

However, before being published, I think there are a few points that need to be addressed.

## Major critics

Even though I called them “major critics”, they do not have a huge impact on the main outcomes of this paper.

One of the major critics I have is concerning the variation of retention rate according to the glue batch. However, the explanation concerning shelf and opening dates seems logical and appropriate. It is reassuring that in 2023, with more care given towards the opening date, the results were closer to those of 2021. It is therefore a good point that you give recommendations for *2oc* glue usage (best to prefer recent manufacturing dates and to avoid vials being opened for too long (more than 6 months)). One should however be careful with this unexpected variation that could still be due to the supplier; preliminary tests could be conducted to estimate the glue quality from different suppliers.

The other major critic I have concerns your analyses of retention time. For the analyses behind Figure 3, and Table 3, I would suggest setting the intercept as 1, since at day 0, 100% of the females had a tag. Moreover, for this kind of loss rate data, an exponential fit (Y = *a***b*^X, where *a* is the intercept and *b* is the retention rate between two consecutive days) would be best suited in contrast to a linear fit (Y = a + b*X), since it is not expected to follow a straight line, but rather a negative exponential curve tending asymptotically to 0, similarly to decay or survival rates; between each time interval you expect the same *proportion* of your Y axis being lost, not the same *amount* of Y. In this approach, you should fix a = 1 since it will fix the intercept as 1, as mentioned above. You could test the goodness of fit for those two approaches. However, even if your analyses were not the best suited for this data in my opinion, the conclusions are expected to remain the same: this method is promising.

## Comments on the introduction

I have a few comments regarding the introduction to give more background to the reader.

You have used post-smolt Atlantic salmon in this study, but would the results presented here be transferable to the other life stages of the Atlantic salmon (since detachment rates could be different on other life stages)? I would suggest mentioning which salmon life stage *L. salmonis* infects preferably in the introduction, as I suppose it prefers post-smolt individuals. This would support your methodological choices. In the same context, it would be interesting to know if (and how often) salmon lice change host in natural environments and if it occurred in your experiment.

It could also be interesting to note how long these adult lice usually live to have a comparison point for the efficiency of the retention of the tags; how much of the adult lifetime could be covered by these tags?

## Comments on the methodology

The methodology is generally very clear. Figure 1 and the video are very nicely appreciated.

In the video demonstration, the second black screen mentions "(Scanning chip and taking photo)", even though (if I understood correctly) the video then shows the final process to set the glue (lines 114 to 117). If it is the case, it should be adapted. Or perhaps you meant that you scanned the chip and took a photo between the two parts of the video. If it is the case, it should be explained a bit more clearly.

It would have been nice to have at least two tanks per glue type (and ideally per glue batch) to avoid pseudoreplication and be able to control for a tank (and glue batch) effect.

You could have added interactions between time and sex, as well as time and glue in the mortality test during the comparison of glue types. Even though no interaction seems to exist here, it could be best to test for it. Again, you should fix the intercept as 0.

Why not take into consideration the replaced tags and add the individuals as a random variable for the analysis of retention time? The time=0 would be the day when the chip is replaced. It could provide more data and give insight to the variation of retention time due to individuals (some could be more adapted for glue applications).

Line 216: For GLMs, you should check overdispersion, not normality and heteroscedasticity.

## Comments on the results and discussion

You show promising results. However, the analysis of retention time should be reconsidered as mentioned above.

For Figure 2b, I would represent the data as a proportion of dead lice rather than the number of dead lice, since the total number of lice in the different tanks were not the same.

Very interesting absence of effect of tagging on reproduction. The very anecdotical amount of potential blocked oviduct is very promising. Interesting apparent absence of effect of tagging on mortality as well. However, I am quite surprised with the latter since in the first experiment, lice had a higher mortality with *2oc*. Nonetheless, as you mentioned, this result is probably a tank effect since even males were affected even though you used very small amounts of glue in tanks of 500L, making the toxicity hypothesis very unlikely, since females, which were directly exposed to the compound, were dying less than males.

About the death of lice, are there any reports of salmon eating lice? Could it explain why sometimes you have individuals disappearing? If it is the case, having a different number of salmon in the tanks could produce a bias in the number of deaths.

## Specific comments

Lines 43-44: A more recent source than that dating from 2002 would be preferable to talk about the lack of information on a topic.

Line 135: “[...] the most effective [...]” instead of “[...] the more effective [...]” would be more appropriate.

Lines 142-143: “All lice at every step were recorded as being either male or female, and as being either tagged or untagged for the females.” would maybe a better phrasing.

Lines 175-176: You could maybe make it a bit more explicit that there were 216 instances of missing + nonfunctional tags. I first thought that the “/” separated the number of instances of both events, meaning that there would have been 4 missing tags and 216 nonfunctional tags. Maybe just write “[...] the date of the first check when the p-Chip was observed to be missing (212 instances) or nonfunctional (4 instances).”

Lines 201-206: I had problems understanding this section. I eventually managed to understand what you have done thanks to the discussion. I would suggest rephrasing these sentences.

Lines 217-218: I am not sure that “[…] to test prediction proportionality […]” are the correct terms to use.

Line 231: I think that you forgot the minus sign in front of 0.19.

Lines 308-309: The number of weeks does not seem to match with what was mentioned before in the manuscript.

And finally, a very small detail: remove lines 37, 88, 124, 212, 234, 241, as they are empty, and replace them with a spacing.