Methods for tagging an ectoparasite, the salmon louse *Lepeophtheirus salmonis*

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ABSTRACT

Monitoring individuals within populations is a cornerstone in evolutionary ecology, yet individual tracking of invertebrates and particularly parasitic organisms remains rare. To address this gap, we describe here a method for attaching radio frequency identification (RFID) tags to individual adult females of a marine ectoparasite, the salmon louse *Lepeophtheirus salmonis*. Comparing two alternative types of glue, we found that one of them (2-octyl cyanoacrylate, *2oc*) gave a significantly higher tag retention rate than the other (ethyl 2-cyanoacrylate, *e2c*). This glue comparison test also resulted in a higher loss rate of adult ectoparasites from the population where tagging was done using *2oc*, but this included males not tagged and thus could also suggest a mere tank effect. Corroborating this, a more extensive analysis using data collected over two years showed no significant difference in mortality after repeated exposure to the *2oc* glue, nor did it show any significant effect of the tagging procedure on the reproduction of female salmon lice. The RFID tags were lost linearly over time, with the percentage of the living female population that retained their original tags declining by 0.28 percent.day^{-1}. In other words, tag retention was in general high, with a typical projected retention of about 89% after 30 days or 80% after 60 days, although one of the four batches of glue used, purchased from a different supplier, appeared to give significantly lower tag retention and with greater initial loss (70% and 53% respectively). Overall, we find that RFID tagging is a simple and effective technology that enables documenting individual life histories for invertebrates of a suitable size, including marine and parasitic species, and that it can be used over long periods of study.

Keywords: RFID tags, monitoring, individual identification, tagging effects, ectoparasites, parasites, *Lepeophtheirus salmonis*

Introduction

In the current biodiversity crisis, it is particularly important to understand how interacting species and in particular disease organisms respond to anthropogenic change (Hudson et al., 2006, Smith et al., 2009, Gupta et al., 2020). Disease ecology has developed as a major field over the past decades, revealing the scope and complexity of parasitism's effects in natural populations (Dobson et al., 2008, Poulin 2014). Comparatively little focus has been given to the selection that hosts exert on their parasites (Hudson et al., 2002). This is in part due to the study of parasite behaviour and evolution being limited to population-level data, as tracking individual parasites is notoriously difficult.

In non-parasitic species, tracking individuals across life stages has allowed researchers to study the causes and consequences of phenotypic variation within populations (Clutton-Brock & Sheldon 2010). Monitoring populations of uniquely marked individuals is a cornerstone of evolutionary ecology, as accessing individual life histories allows insight into microevolution, as well as informing conservation decisions (Clutton-Brock & Sheldon 2010, Charmantier et al., 2014, Sheldon et al., 2022, Pemberton et al., 2022).

Studies of individually marked vertebrates, especially birds and mammals, have traditionally been far more common than studies involving invertebrates (Sheldon et al., 2022) due to the difficulty in consistently identifying small, numerous, and mobile animals (Hagler & Jackson 2001, Streit et al., 2003, Rodríguez-Muñoz et al., 2019). A few notable exceptions include long-term longitudinal studies in natural populations of insects (e.g. field crickets, Rodríguez-Muñoz et al., 2019). Recent advances in radio-frequency identification (RFID) technology now also allow for the tagging and digital identification of more invertebrate species, including eusocial insects such as bees and ants (Streit et al., 2003, Robinson et al., 2014, de Souza et al., 2018, Nunes-Silva et al., 2019).

While individual tagging approaches will likely remain difficult for most internal parasites, ectoparasites living on the outer surfaces of their hosts are more easily accessed and thus have a greater potential for individual tracking using similar techniques to those currently used on free-living invertebrates, with similar limitations (Rataud et al., 2020).

Here we describe a method for tracking individual adults of an ectoparasite, the marine copepod *Lepeophtheirus salmonis* (salmon louse), using an RFID tagging system. *L. salmonis* became a major pest following the exponential growth of Atlantic salmon (*Salmo salar*) populations in marine intensive aquaculture facilities, where it experiences anthropogenic selection (Mennerat et al., 2010, Coates et al., 2021) and contributes to the decline of wild salmonid populations (Costello 2009, Vollset et al., 2016, Shephard & Gargan 2021). In this article, we detail our method for attaching RFID tags onto adult female *L. salmonis*, document the retention time of the tags, and test their effects on mortality and reproduction.

Methods

Salmon hosts and culturing of lice

To compare types of glue, document retention rates, and test the effect of RFID-tagging on *L. salmonis* mortality and reproduction, we infected naïve Atlantic salmon (Industrilaboratoriet, Bergen, Norway) using salmon louse larvae hatched from eggs sampled from a marine facility located at the Institute of Marine Research, Austevoll, Norway (glue comparison test), and from commercial salmon farms in Fosså, Rogaland county, Norway and Oppedal, Vestland county, Norway (glue retention time and impacts on mortality and reproduction). Salmon lice eggs were incubated in the lab for 14 days following protocol described in Hamre et al., (2009). The copepodites from those eggs were used to infect post-smolt Atlantic salmon housed in 1m x 1m, 500L tanks supplied with filtered and UV-treated seawater (flowrate, 2 - 6 L min^{-1}, temperature, 7.1 - 9.5 °C). The salmon hosts used in the initial glue comparison test were kept in two tanks containing 15 to 16 fish each, while those used in the other tests were kept in three pairs of tanks housing either 15 or five fish. Tanks with either 15 or 16 fish were infected by lowering the water level and adding 30 copepodites per fish on average (following e.g. Mennerat et al., 2017), while tanks with five fish were infected at full tank volume with an average of 15 copepodites per fish, as part of another, ongoing study. The tanks were equipped with sieves to filter outlet water and daily collect salmon lice that had detached from their hosts and been flushed out.

Handling and registration of salmon lice

From 60 days post-infection onwards, salmon hosts were individually netted and anesthetised until unresponsive in 1 mL L^{-1} metomidate and 0.3 mL L^{-1} benzocaine (glue comparison study), or later on with either these anesthetic compounds or with 120 mg L^{-1} tricaine mesylate (glue retention time and impacts on mortality and reproduction), after which they were inspected for salmon lice. All adult lice were carefully removed with fine curved forceps and placed onto a moistened paper label in a petri dish, after which the salmon was placed into a holding tank to recover. Female salmon lice were tagged with p-Chips using surgical glue (see below for details). Finally, tagged lice were registered by scanning their RFID tags, photographed, and placed back onto their original host, which was returned to its tank. Subsequent checks for lice followed a similar host capture and anesthesia procedure.

Attachment of RFID tags

For an ecdysozoan like *L. salmonis*, attaching RFID tags externally can only be done on adults, i.e., once they stop moulting. Adult female *L. salmonis* can be tagged by gluing an RFID tag onto the dorsal side of their abdomen (genital segment). To do so we used a 'p-Chip' microtransponder (p-Chip Corp., Princeton, NJ, U.S.A.), a 500 x 500 x 100µm RFID tag carrying a unique 9-digit identification number. The readable side of the tag contains a photocell that is powered by a laser on a tag-reading wand, which records the ID number as well as additional information as a comment (e.g. sample name), and logs all scans in a .csv file. In males, which are the smaller sex, the genital segment is too small for a p-Chip, and gluing the tag to the cephalothorax was found to be invariably and quickly fatal.

To prepare female salmon lice for tagging, we set all lice onto a paper label, wetted with cold seawater in a petri dish. The top of the genital segment was thoroughly dried with a cotton swab (**Figure 1a**), and a small amount of glue was applied to the center of the segment by touching it with the tip of a wooden toothpick (**Figure 1b**). The tag was placed facing upward onto the louse's genital segment using the tip of a clean, damp toothpick (**Figure 1c**), and an additional dot of glue was applied over the edges of the tag to encapsulate it (**Figure 1d**).

To set the glue and following the removal of any extruded egg matrices, cold seawater was either dripped onto the salmon louse, or the louse was gently dipped into the water, or both (**Figure 1e**). Any excess glue that might have detached upon contact with water was removed to prevent clumping around the female's oviducts. After letting the glue set for an additional 30 - 60 seconds, newly tagged female lice were squirted again with saltwater and then flipped onto their backs to be re-applied to their original host. With some experience, the RFID application process takes two to five minutes for one to nine lice aligned in the same Petri dish. Multiple lice may be prepared in this way simultaneously.

Due to the short transmission distance, p-Chips attached to salmon lice can only be read near the reading wand (< 1cm). Upon later checks of previously tagged lice, we removed them from their hosts, set them onto a wet paper label in a Petri dish, and brought them to the chip reader.

Video demonstration:

http://paranthrope.w.uib.no/files/2023/08/licechip_procedure_folk_mennerat.mp4

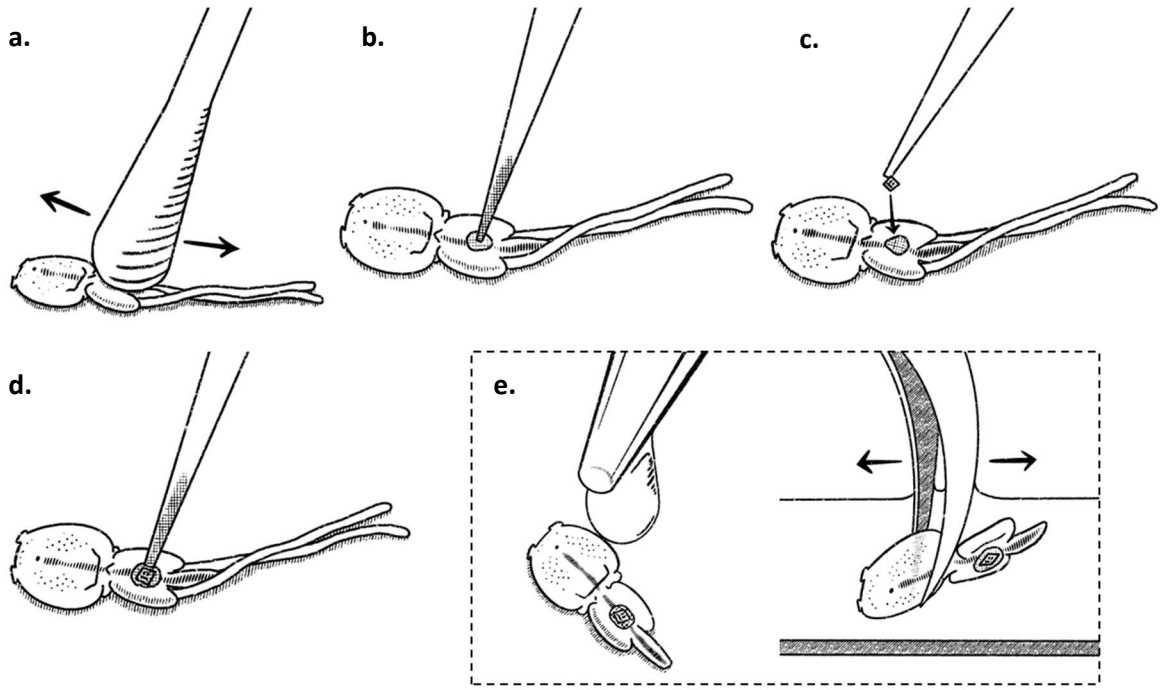

Figure 1 - Procedure for tagging female salmon lice with RFID tags. (a) Drying the genital segment with a cotton swab; (b) applying glue to the louse with a toothpick; (c) placing a p-Chip onto the louse with a damp toothpick; (d) applying glue over the top of the p-Chip; and then (e) dripping saltwater onto the louse and/or dipping it in saltwater to set the superglue. (Illustration A.Folk)

Comparison of two alternative types of glue

According to a preliminary search, two alternatives for glue seemed to be to 2-octyl cyanoacrylate (Surgi-Lock, hereafter 2oc) and ethyl 2-cyanoacrylate (Würth Klebfix, hereafter e2c). To determine the more effective glue, adult female salmon lice in the 15-fish tank (n = 62) were tagged with p-Chips using 2oc at 59 days post-infection, while those in the 16-fish tank (n = 79) were tagged using e2c at 66 days post-infection.

After the initial RFID-tagging and until the end of the experiment, the tank outlet filters were checked daily for dislodged and dead salmon lice. All fish were re-captured 21 days after initial tagging; salmon lice were sampled and registered following the procedure outlined above and placed back on their hosts. The fish were caught, the lice tallied, and their p-Chips scanned a final time 52 and 59 days post-tagging for the e2c group and the 2oc group, respectively. All lice at every step were recorded as being either male or female, and if female, as being either tagged or untagged. Since it was possible to capture all female salmon lice during the initial RFID-tagging and no new individuals were added after that, all untagged recaptured females were known to have been tagged previously. At the conclusion of the study, the salmon were humanely euthanised under full anesthesia. One salmon in the e2c group was instead euthanised 6 days prior to the end of the study due to a damaged fin.

Combining the daily checks of the outlet filters and the two recapture sessions, we obtained the numbers of tagged and untagged females for successive days of the study. Tag retention was compared between the two glue types using a generalised linear model (GLM) fitted with a binomial distribution, where the binomial response variable combined the number of tagged female salmon lice scored as successes and that of untagged females scored as failures. The explanatory variables used were glue type as a factor and time elapsed (in days) since initial tagging as a covariate, as well as the interaction between the two.

The numbers of dead female and male salmon lice over successive days were estimated by adding a cumulative sum of lice found in the tank outlet filters, while also adding any quantity of lice that were missing during a population census. The number of living salmon lice corresponded to the numbers observed during the censuses, with lice known to be dead subtracted from daily estimates in between. The lice from one fish euthanised at day 46 (three females and two males) in the e2c tank were not tallied as

dead but were subtracted from the expected number of live lice after that point. Mortality was compared between the two glue types (e2c group: n = 56; 2oc group: n = 63 for untagged adult males; e2c group: n = 79; 2oc group: n = 62 for tagged adult females) using a GLM fitted with a binomial distribution. The binomial response variable combined the number of alive and dead salmon lice scored as successes and failures, respectively. Explanatory variables included glue type and sex as factors, and the number of days since tagging as a covariate.

Documenting retention time

Following our glue comparison test, we tagged hundreds of female salmon lice as part of other ongoing research. Our methods for tagging follow the procedure described above using 2-octyl cyanoacrylate (2oc), and the salmon lice are surveyed every six to 10 days. Here we use data collected during a period ranging from 11th January 2021 to 16th December 2022.

All female salmon lice (n = 948) were tagged at adulthood, usually after extruding their first clutch. On subsequent checks, adult females found without a readable p-Chip were tagged again and their IDs were determined by visually comparing them to pictures taken previously, as their pigmentation patterns are repeatable and unique (see **Figure SM1**). The time until tag loss is the number of days between the initial tagging date and the date of the first check when the p-Chip was observed to be missing (most instances of tag replacement) or nonfunctional (4/216 instances). For those lice that were re-tagged multiple times, only the retention of original p-Chips is considered here. The date of mortality was determined either by the date at which we found a tagged louse in the tank's outlet filter, or else as the date of the first weekly check when the louse was no longer observed.

Starting from day 0 (the date of tagging), the data were split into 7-day periods in which all adult females alive at that time were tallied as having either retained or lost their original p-Chip (overview in **Figure SM2**). To estimate the daily rate of tag loss, the proportion of living adult females retaining their original p-Chip in a 7-day period was linearly regressed against the number of days since the p-Chip was originally glued (setting this proportion to 1 for day 0, when the tagging occurred). This analysis was limited to periods with more than 20 living adult females. Due to suspected differences in glue batch quality, this analysis was repeated with females split based on whether they were initially tagged in 2021 (n = 599) or in 2022 (n = 349), where 2021 corresponds to the first two vials of Surgi-Lock 2oc (including that used for the glue comparison study), and 2022 corresponds to a third vial.

Effect of tagging on reproduction

To test whether the gluing of tags may have blocked the oviducts of females, we used a subset of female salmon lice that were tagged before (n = 55) or shortly after (n = 178) having extruded a first clutch of eggs. The number of egg matrices these females had extruded at the time of their initial tagging (0, 1 or 2) was compared to the maximum number they were observed to extrude within two observations (two weeks) after being tagged. Females that did not live through at least two observations or that were re-tagged within 15 days of their original tagging were excluded.

Effect of tagging on mortality

Due to the relative difficulty in determining the date of mortality for untagged salmon lice (as some of those found in the outlet filters were too damaged to be identified from pictures), we estimated the impact of gluing adult females with 2oc on their mortality by comparing females that were tagged once to those that were tagged more than once, with the reasoning that this constitutes a "second dose". To evaluate this, we selected the largest group of females from our dataset that were re-tagged the same number of days since their original tagging (seven days) and limited to lice in 15-fish tanks (n = 49), of which 11 were later re-tagged again. For comparison, we selected a cohort of females that were originally tagged on the same date and in the same tank as each of the selected re-tagged females and which were still alive seven days after their original tagging (n = 270). Note that the cohort was limited to females that were never re-tagged, which may exclude some longer-lived individuals re-tagged later in life.

To test whether multiple exposures to 2oc had long-term impacts on adult female mortality, we compared the number of days during which female lice lived after being re-tagged to that of females only tagged once using a Cox Proportional Hazards model. To test for shorter-term effects of the glue on female

mortality, we used a chi-square test to compare the numbers of dead and live females after 14 days, according to whether they had been re-tagged or not.

All statistical analyses and visualizations of the data used for this paper (Folk & Mennerat 2023) were performed in the statistical programming environment R (version 4.2.3, R Core Team 2023), using the R packages *broom* (Robinson et al. 2023), *survival* (Therneau 2023), and *tidyverse* (Wickham et al. 2019).

Models were validated by visual inspection of the normality and heteroscedasticity of the residuals. For the Cox Proportional Hazards model, the package *survival* (Therneau 2023) was used to test prediction proportionality, and diagnostic plots were generated with the package *survminer* (Kassambara et al. 2021). Model results reporting for glm and lm functions used the package *report* (Makowski et al. 2023).

Results

Comparison of two alternative types of glue

Almost all females could be assigned as either having retained or lost their tag, with only two females in the 2oc group and four in the e2c group unaccounted for. Tag retention was significantly and negatively related to the number of days since initial tagging (glm; days since tagging: b = -0.03, 95% CI: -0.05 – -0.01, p = 0.002), and retention decreased significantly faster for lice glued with e2c than for those glued with 2oc (glm; glue type * days: b = -0.03, 95% CI: -0.06 – -3.84e-03, p = 0.024), although the main effect for glue type was not statistically significant (glm; glue type: b = -0.60, 95% CI: -1.63 – 0.35, p = 0.233; **Figure 2a, Table 1**).

The number of living salmon lice in all groups decreased significantly over time (glm; days since tagging: b = -0.04, 95% CI: -0.05 – -0.04, p < 0.001), and mortality was higher for males than for females (glm; sex: b = -0.34, 95% CI: -0.49 – 0.19, p < 0.001). Lice (both males and females) in the tank where the females were tagged using e2c experienced significantly lower mortality than those in the tank where females were tagged using 2oc (glm; glue type: b = 0.44, 95% CI: 0.28 – 0.60, p < 0.001; **Figure 2b, Table 2**).

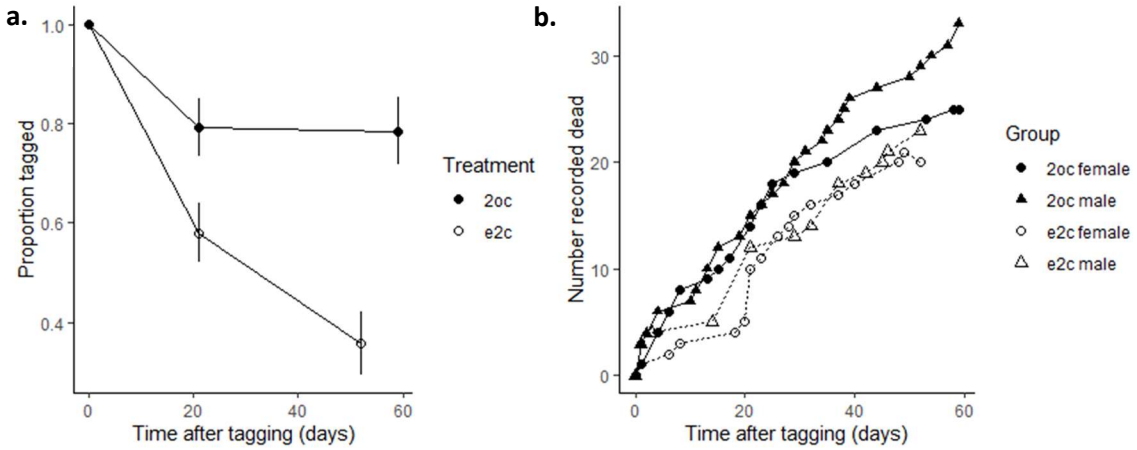

Figure 2 - Comparison of (a) RFID tag retention during census events with standard error based on population size and (b) mortality according to the type of glue used for tagging adult female salmon lice (e2c = ethyl 2-cyanoacrylate, 2oc = 2-octyl cyanoacrylate). Day 0 is the day when RFID tags were glued dorsally on the abdominal segment of adult female salmon lice. Males were not tagged but are included under the glue type used on females in the same tank.

	Estimate	SE	*z*	*P*
Intercept	2.759	0.408	6.752	1.45e-11
Glue type	-0.596	0.500	-1.192	0.2332
Days since tagging	-0.031	0.010	-3.109	0.002
Glue type x Days	-0.030	0.013	-2.262	0.0237

Table 1 - Generalised linear model parameter estimates of number of female salmon lice having retained their tag, as a function of glue type (e2c vs. 2oc) and number of days since initial tagging, using a binomial distribution. See also Figure 2a.

	Estimate	SE	*z*	*P*
Intercept	2.587	0.100	25.661	< 2e-16
Sex	-0.340	0.078	-4.353	1.34e-5
Glue type	0.437	0.080	5.447	5.14e-8
Days since tagging	-0.044	0.002	-19.642	< 2e-16

Table 2 - Generalised linear model parameter estimates of the mortality over time for tagged female salmon lice and untagged male salmon lice as a function of sex (males vs. females), the glue type used on the females in that tank (e2c vs. 2oc), and the number of days since initial tagging. The model was fitted using a binomial distribution. See also Figure 2b.

Documenting retention time

During this study (from 11th January 2021 to 16th December 2022), 216 of 948 p-Chips glued using 2oc were replaced (i.e., 22.7%); of these, four were replaced due to loss of function, while the remaining 212 had detached from the louse. Expressed as the percent of living females retaining their original tags, we found a rate of loss of -0.27 percent.day^{-1} for the first 161 days after tagging (*percent retaining* = 90.4 - 0.27 x *days post-tagging*; **Figure 3**). Time until tag loss ranged from 0 to 150 days, while the longest time that a louse retained its original p-Chip in our study was 263 days (until death).

Comparing the two vials of glue used in 2021 to the one used in 2022, all the same brand but purchased from different suppliers, we found that in 2021, 117 of 599 p-Chips were lost (i.e., 19.5%), while 99 of 349 p-Chips were lost in 2022 (i.e., 28.4%). A linear regression of the proportion of living females retaining their original tags showed significantly lower retention for the batch in 2022, with both a lower intercept (97.2 % in 2021 and 86.3% in 2022; lm; year: b = -0.11, p < 0.001; **Figure 3, Table 3**), as well as a significantly faster rate of decrease in the proportion of retained tags (-0.28 percent.day^{-1} in 2021 and -0.56 percent.day^{-1} in 2022; lm; year * days post-tagging: b = -2.77e-3, p < 0.001).

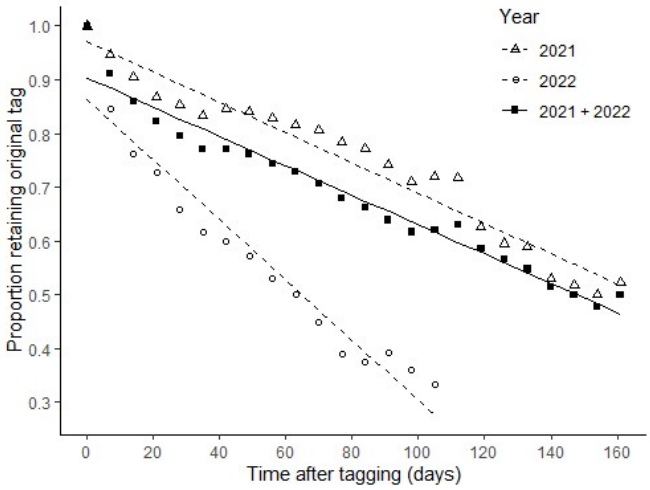

Figure 3 - The proportion of living female salmon lice retaining their original RFID tag decreased over consecutive 7-day intervals, starting from the date of initial tagging (day 0, n = 948) up to 161 days (n = 22). The linear regression line for all females is shown in solid black. There was evidence of batch differences in the glue used, since the rate of decrease in RFID tag retention for one vial used in 2022 (day 0, n = 349) was significantly higher than for two vials used in 2021 (day 0, n = 599). The linear regression lines for these subgroups are shown as dashed lines.

	Estimate	SE	*t*	*P*
Intercept	0.972	0.016	60.572	< 2e-16
Days since tagging	-0.0028	0.0002	-16.515	< 2e-16
Year	-0.109	0.025	-4.319	1.18e-4
Year x Days	-0.0028	0.0004	-7.762	3.38e-9

Table 3 - Linear regression parameter estimates of the proportion of living female salmon lice retaining their original RFID tag as a function of the year during which they were tagged (2022 vs. 2021) and the number of days since initial tagging, with the proportion at day 0 set to 1. See also Figure 3.

Effect of tagging on reproduction

Of the 233 female salmon lice checked for differences in egg extrusion before and after attachment of a p-Chip, all lice were observed to be able to extrude eggs from at least one oviduct except for two females that were never observed to produce eggs. Of the lice glued before they extruded eggs (n = 55), 51 extruded a pair of egg matrices within two weeks, while two extruded only one egg matrix, and two extruded zero. Of the lice glued after they had extruded two egg matrices (n = 172), 164 continued to extrude pairs, while six extruded only one egg matrix and two did not extrude any eggs in that 2-week period. For a subset of females glued after they had extruded only one egg matrix (n = 6), two continued to extrude only one, while four extruded pairs.

Effect of tagging on mortality

We found no significant difference in long-term survival for females that were re-tagged seven days after their original tagging compared to those that were only tagged once (Cox Proportional Hazard, n = 319: HR = 0.94, 95% CI: 0.69 – 1.28, p = 0.693). After 14 days, 32 of 49 re-tagged and 175 of 270 non-re-tagged females were still alive. A chi-square test found no significant difference in short-term mortality between these groups (X^2 = 6.65e-30, df = 1, p = 1).

Discussion

We compared two types of glue for attaching p-Chip RFID tags to adult female salmon lice. We found that 2-octyl cyanoacrylate (Surgi-Lock, 2oc) resulted in a significantly higher retention rate than ethyl 2-cyanoacrylate (Würth Klebfix, e2c). Mortality was found to be significantly higher for adult lice (both males and females) in the tank where females were glued with 2-octyl cyanoacrylate than in the tank where females were glued with ethyl 2-cyanoacrylate. This result was surprising as males are not tagged and thus not in direct contact with the glue. Even though this might be due to 2-octyl cyanoacrylate having a certain level of toxicity that might also have affected the males living in the same tank (same water), we cannot rule out a mere tank effect, as the two types of glue were compared in distinct tanks where the salmon hosts might have experienced slightly different levels of stress or behaved otherwise differently, causing the lice to detach from their hosts at different rates.

During a more extensive period of testing, we further investigated the impact of 2-octyl cyanoacrylate on female salmon louse mortality by selecting a subset of females that had been re-tagged seven days after their initial tagging and comparing these to a cohort of females, from the same tanks and at a similar stage of maturity, that had never been re-tagged. We reasoned that if 2-octyl cyanoacrylate were toxic, then this would represent a “double dose” within a relatively short period of time. There was no significant difference in the remaining lifespan of these two groups. We also tested for increased mortality in the two weeks immediately following the two consecutive weeks of exposure but did not find evidence of increased mortality in this period either. Additionally, 2-octyl cyanoacrylate remained workable for a longer period in open air than ethyl 2-cyanoacrylate, preventing accidental adhesions of tools. Given the purpose and scale of our planned research, the longer tag retention and better tractability of 2-octyl cyanoacrylate were substantial enough to justify using it in subsequent experiments.

We found that gluing had little apparent impact on the extrusion of eggs for female salmon lice. The majority of lice (219/233) that were glued after producing zero, one, or two egg matrices (strings) went on to produce a full pair, regardless of the initial number. Closer examination of pictures of the two lice that never extruded egg matrices revealed that one of them had eggs in its ovaries that could not be extruded

and died shortly after, while for the other one, the ovaries remained empty. Of the eight females that went from extruding two egg matrices to extruding zero or one, four resumed producing pairs while the other four died shortly after, one of those with glue residue visible around the oviduct. A small subset of lice appeared to either naturally extrude egg matrices from only one oviduct or to produce matrices that naturally detached shortly after extrusion. Glue residue was visible for three females tagged prior to extruding eggs and that later extruded from only one side, amongst which one resumed producing a pair of matrices shortly after. For those females that could be recaptured within two weeks, our study did not detect a significant effect of tagging on their further survival or reproduction. All in all, our study did not find strong evidence for any impact of the tagging procedure on vital or reproductive functions.

Over a longer period and including all batches of glue, we found that p-Chips glued to the abdominal segment of adult female salmon lice with Surgi-Lock 2oc were lost steadily over time after an initial week one decrease, with a generally high percentage of the living population retaining their original tag (about 82% after 30 days, or 74% after 60). However, we also found that this rate may be affected by the age of the glue both in terms of shelf date and time since opening, and that there may also be differences in batch quality. After noticing an increase in lost p-Chips after opening a new vial of Surgi-Lock 2oc at the start of 2022, which was then also used for longer after opening, and comparing the retention rate for females initially tagged in 2021 to those tagged in 2022, we found a higher rate of decrease for tag retention in 2022. Subsequently, while we have not extended the analysis further, switching to a fourth vial in 2023 with a later manufacturing date resulted in a noticeably large decrease in the number of tags replaced each week. This suggests that the vial used in 2022 was atypical, and that the normal retention rate for this glue may be closer to the regression line for 2021 (i.e. 89% retention after 30 days and 80% retention after 60; **Figure 3, Table 3**). In addition, it was the authors' impression that retention decreased as more time elapsed since opening a vial, so for the sake of caution we would advise using vials of glue that are less than six months old.

One potential disadvantage of p-Chip use is that it relies on fine motor skills, but when tagging is done as described here, it appears to be a robust yet simple technology that allows for individual studies of ectoparasites, including those living in seawater like *L. salmonis*. More generally, our procedure for RFID tagging with microtransponders could easily be adapted for other aquatic and terrestrial species and be used as a relatively rapid and cost-effective method (compared to molecular approaches) for identifying invertebrates traditionally not considered suitable for individual studies.

Ethical statement

All procedures adhered to "the Regulation on the Use of Animals in Research" under the Norwegian Food Safety Authority, and the Atlantic salmon in this study were used according to a permit from the Norwegian animal research authority Mattilsynet to A. Mennerat (FOTS id 24917).

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Data, scripts, code, and supplementary information availability

Data, scripts, and code are available online: https://doi.org/10.5281/zenodo.8304839 (Folk & Mennerat 2023).

Conflict of interest disclosure

The authors declare that they comply with the PCI rule of having no financial conflicts of interest in relation to the content of the article.

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Appendix

Supplementary materials

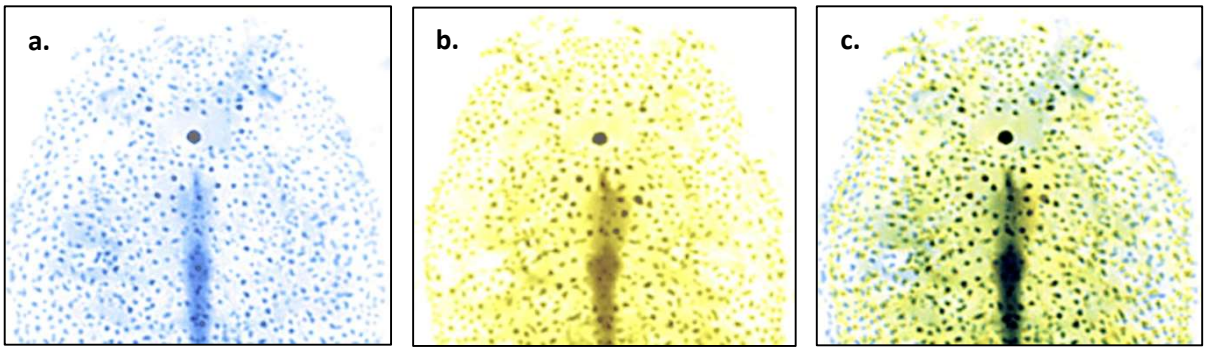

Figure SM1 - An illustration of conserved pigmentation pattern in adult female salmon lice. The same female is shown here (a) in blue at 61 days post-infection and (b) in yellow at 271 days post-infection. The central dark stripe is the gut. In (c) the two images are overlaid; areas in black thus indicate a high degree of overlap.

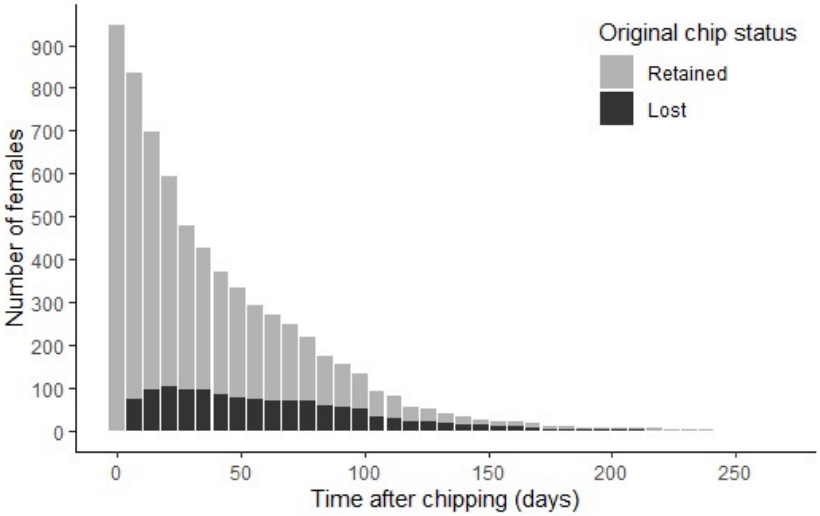

Figure SM2 - The number of living female salmon lice in 7-day intervals from the date of initial tagging (day 0, *n* = 948) up to 266 days (*n* = 1); the colours indicate whether females had lost their original RFID tag (black) or retained it (grey).