The digestion time for salmon louse (*Lepeophtheirus salmonis*) in lumpfish (*Cyclopterus lumpus*)

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Abstract

Atlantic salmon aquaculture employs lumpfish as a control method to combat ectoparasites, given their unique cleaning behaviour. There are multiple studies which estimate the average number of salmon lice in the stomach contents of dissected lumpfish. However, these numbers cannot be used to assess the cleaning efficacy of lumpfish (e.g., the average number of lice consumed daily per lumpfish) without knowing the digestion time of lice in lumpfish. The aim of the study was to provide quantitative estimates of the degradation of salmon lice, through a blinded clinical study over a duration of seven days. Individually tagged lumpfish (45.8 g, SD ± 10.28) were randomly arranged in triplicate tanks ($n = 28$ per tank) and acclimatised for three days. Subsequently, lumpfish were fed using oral gavage dosing with counts of lice (0-6), feed pellets (0-6) or a combination of both. Lice used were recently captured and stored at -80 °C to prevent parasite transmission at the study location and photographed before and after digestion to estimate degradation. Samplings ranged from 6 h intervals during the first two days, to 24 h and eventually 48 h for the last two days. Analysis of salmon lice revealed an expected digestion time of 29 h while the median digestion time was estimated to 15 h at 9 °C. Pellets dissolved quickly and had no impact on the lice digestion time. The findings of this study can be used to estimate cleaning efficacy of lumpfish from stomach contents of salmon lice.

1 Introduction

Open net pen farming of Atlantic salmon (*Salmo salar*) uses cleaner fish as one of several control measures in an attempt to delay and avoid epidemics of ectoparasitic salmon lice (*Lepeophtheirus salmonis*) (Bjordal, 1990; Tully et al., 1996; Imsland et al., 2014a, 2014c; Skiftesvik et al., 2014). Wrasses, including goldsinny wrasse (*Ctenolabrus rupestris*) and ballan wrasse (*Labrus bergylta*), were the first fish tested as cleaner fish in salmon duoculture already in the 1980s (Bjordal, 1990; Deady et al., 1995; Tully et al., 1996). In 2010, a cottoid semi-pelagic teleost species (Davenport, 1985), namely the lumpfish (*Cyclopterus lumpus*), became the novel species of interest after anecdotal reports of wild juvenile individuals burglarising into net pens and cleaning farmed salmon. Later studies revealed significant reductions in numbers of salmon lice when lumpfish were deployed with salmon, both in small-scale (Imsland et al., 2014a) and commercial sized net pens (Imsland et al., 2018). Moreover, sea lice grazing of lumpfish has been investigated using large datasets involving counts of lice recovered in the digestive system from fish collected directly from commercial net pens during the production period (Boissonnot et al., 2022a; Imsland and Reynolds, 2022; Engebretsen et al., 2023).

There are to date two alternative approaches to assess the cleaning efficacy of lumpfish. One can estimate the cleaning efficacy indirectly by comparing sea lice infestation levels in cages with and without lumpfish. Through this approach, multiple studies reported efficient sea lice removal (Imsland et al., 2014a, 2014b, 2014c, 2016, 2018). However, a more indirect approach in a recent modelling study of all commercial Norwegian salmonid farms found small effects of cleaner fish (Barrett et al., 2020). A recent article by Imsland and Reynolds (2022) reviewed data and personal experiences from fish farmers from large-scale studies in Norway, Iceland, the Faroe Island and Scotland and concluded that lumpfish can reduce numbers of salmon lice and are susceptible for improved grazing effects through selective breeding and live feed conditioning prior to deployment at sea. Though it is clear that lumpfish graze on salmon lice, there are, to the authors knowledge, no studies which have attempted to quantify the effect of lumpfish defined as the expected number of salmon lice eaten per lumpfish per day. This emphasises the need for a better quantitative understanding of the cleaning efficacy.

A different approach for measuring cleaning efficacy is to investigate and count the presence of lice in the stomach contents of lumpfish, combined with assumptions on digestion time. Using stomach content alone, multiple studies have reported the proportion of lumpfish found with salmon lice in their stomach contents, ranging from 0 to 47% (Imsland et al., 2014a, 2015, 2016; Eliasen et al., 2018; Boissonnot et al., 2022a; Engebretsen et al., 2023). The counts of ingested lice per lumpfish varied between 0 and 120 and with means of 0.19 (Engebretsen et al., 2023) and 0.6 (Boissonnot et al., 2022a) based on datasets containing 25 000 and 2104 lumpfish, respectively. Note that Boissonnot et al. (2022) included both salmon lice and *Caligus elongatus*, while Engebretsen et al. (2023) only included salmon lice. The distribution of number of lice per lumpfish was skewed in both studies, where most of those that contained salmon lice only contained one louse, while only few lumpfish had consumed more than 100 lice.

However, both studies have highlighted an important missing factor when inferring the cleaning efficacy of lumpfish through analysing stomach contents. It is not enough to rely solely on the stomach content as a measure of their cleaning efficiency, as the duration for which salmon lice remain detectable in the stomach depends on their digestion time. Hence, by combining estimates of the mean number of salmon lice in the stomach contents of lumpfish and estimates of digestion time, we can estimate the mean cleaning efficacy of lumpfish. To the authors knowledge, there is currently no available data on the digestion time of salmon lice or other ectoparasites in stomach contents of lumpfish or other cleaner fish species commonly utilised in fish farming. Digestion time in teleosts is influenced by a range of factors, including species-specific adaptations, anatomical structures, and metabolism (Hidalgo et al., 1999; Rønnestad et al., 2013). Additionally, abiotic factors such as temperature have a significant impact, given that teleosts are ectothermic (Volkoff and Rønnestad, 2020). The gastrointestinal tract of teleosts is generally described as extending from the bucco-pharynx through the oesophagus, stomach, intestines, and anus (Rønnestad et al., 2013). In juvenile lumpfish, the intestines are approximately twice the length of the body, which is similar to the digestive system of herbivorous species (Banan Khojasteh, 2012). However, observations of lumpfish in their natural environment have shown that their diet includes a variety of organisms such as crustaceans, algae, and sessile species (Ingolfsson and Kristjansson, 2002). Hence, the consumption of crustacean during feeding is an expected dietary behaviour for the species, with the expectation of enzymatic digestion into assimilable macromolecules and subsequent absorption into the bloodstream (Hidalgo et al., 1999).

The aim of this study was to investigate the digestion time of salmon lice when consumed by lumpfish and to determine how long salmon lice are visually detectable in the stomach content.

2 Material and methods

2.1 Ethical statement

The use of lumpfish for experimental purposes was accepted by the Norwegian Food Safety Authority (FDU #29562). All fish were carefully handled based on the Norwegian law on Regulation of Animal Experimentation (FOR-1996-01-15-23). All personnel involved in the study have previously completed the FELASA-C course, developed by the Federation of European Laboratory Animal Science Association. The experiment was planned and conducted using the ARRIVE guidelines (Kilkenny et al., 2010).

2.2 Research animals

2.2.1 Lumpfish

Hatchery reared lumpfish used in the study originated from the Namdal Rensefisk AS GEN2 selected strain. The strain is composed of roe collected from wild caught female broodfish from Trøndelag, Norway, and milt collected from captive male broodfish from the broodstock nucleus of Namdal Rensefisk AS and AquaGen AS. Lumpfish were fed with pellets based on standard recommendations given by a commercial feed producer (BioMar, Karmøy, Norway). All lumpfish were vaccinated with AMarine micro 3-1® (Pharmaq, Overhalla, Norway) and given 400 day-degrees immunisation. At the beginning of the experiment, the mean weight was 45.8 g, with a standard deviation (SD) of 10.3 g. This size represents fish that were ready to be sold and delivered to commercial use as cleaner fish. Also, all lumpfish used were juvenile individuals with no gonadal development, indicating no maturation.

2.2.2 Salmon lice

Salmon lice were collected from the fish farm location Nausttaren operated by Bjørøya AS in Osen, Trøndelag county, Norway. Collection occurred in November 2022 during a mechanical delousing procedure using a Hydrolicer ® system. The method of salmon lice removal entailed the use of pressurised water which physically detaches lice from the salmon skin. Lice were alive and just recently detached from the salmon when collected for experimental purposes. A random collection of different stages of salmon lice was quickly stored on dry ice and later stored in a -80 °C freezer. On the first day of the trial, lice were defrosted and kept at 0 °C, ready for oral gavage. Macro photography documentation and clinical inspection revealed that lice were still in good condition without any visual damage from the freezing process.

2.3 Experimental setup

Lumpfish (n = 84) were tagged three days prior to experimental start-up using Floy tag t-bars (Floy Tag and Mfg Inc, Seattle, USA) to allow 72 h acclimation to new tanks and recovery from the tagging procedure. Individuals were anesthetised with an 80 mg L^- tricaine (Pharmaq, Overhalla, Norway) exposure for 8 min, which induced a stop in swimming activity, loss of equilibrium, lack of responsiveness and shallow respiration (Skår et al., 2017). Tags were attached to the dorsal crest using a t-bar pistol. Three white tanks (1 m x 1 m x 1 m, 600 L) were installed outdoor at Namdal Rensefisk, providing access to filtered (100 µm) and disinfected (UV) water from 80 m depth. Flow was adjusted to 40 L min^{-1}. Daily measurements of water parameters (mean ± SD) included temperature (9.1 °C ± 0.2), dissolved oxygen (100.6% ± 1.0) and salinity (33.4 ppt ± 0.1). Tanks were covered with nets to keep potential predators away. Fish behaviour was observed twice a day during the acclimation period, and water quality monitored once a day. The fish were fed with 2% of total biomass per tank (2 mm dry feed pellets, BioMar, Karmøy, Norway) once on day 1 of acclimation, but then fasted for 48 h before experimental start up to ensure empty stomachs and intestines before oral gavage feeding.

The experiment started on 28 November 2022 and lasted for 7 days. On day 1, oral gavage was used to feed each fish (84 in total) with 0-6 salmon lice (*L. salmonis*) and/or 0-6 pellets (2 mm dry feed pellets, BioMar, Karmøy, Norway). The lumpfish were fed with either only pellets, only salmon lice, or a combination of salmon lice and pellets. The main purpose of including pellets was to investigate their effect on salmon lice digestion time in lumpfish. Since lumpfish in salmon duoculture are fed with pellets, it is important to know their impact on the salmon lice digestion time. Secondly, as a spill-over effect, the study design also potentially allowed us to assess the digestion time of these particular pellets in lumpfish. The lumpfish were fed with pellets and salmon lice according to the setup shown in Table 1, with two exceptions, where one lumpfish was given the wrong number of lice by accident, resulting in one additional lumpfish fed with two salmon lice, and one fewer lumpfish fed with six salmon lice. In cases with more than one salmon louse, the lumpfish were fed with both adult stages and motile stages of salmon lice. In cases with one salmon louse, the lumpfish were given either a motile stage or an adult female. The complete overview of the individual combinations of numbers of salmon lice at each stage and pellets are provided in the supplementary materials. The two different stages of salmon lice were used to study whether the digestion time depends on the type of salmon louse.

The oral gavage instrument consisted of a 0.5 L soft plastic bottle with an attached feeding tube (ENFIT feeding tube FG5 x 40 cm, Unomedical®, Lejre, Denmark). Lumpfish were anesthetised with 80 mg L^{-} tricaine for 8 min prior to placement of the feeding tube through the mouth and oesophagus into the stomach. The method was previously tested in a pilot study on deceased lumpfish (n = 6) to estimate the required feeding tube length and to test whether the feed reached the stomach. Using hand pressure to squeeze the bottle, salmon lice and pellets were dispatched from the tube and assumed placed in the stomach before the tube was pulled out. The oral cavity was inspected after the procedure to verify that both salmon lice and pellets were not present there. Fish recovered in aerated white buckets (20 L) to monitor behaviour and health 10 min after oral gavage. All fish recovered without showing indications of either distress or harm from the procedure. After recovering from anaesthesia, fish were distributed among the three tanks as described in Table 1. Filters at the tank bottom were inspected daily for the potential presence of lice. Two motile stage lice were found, one in tank 2 and one in tank 3 on day two of the experiment. Note that one lumpfish (containing six salmon lice) was recovered in the bottom of the tank after the experiment, and the analysis was thus performed on 83 lumpfish.

2.4 Experimental sampling

The aim of the experiment was to estimate a continuous function for the probability of visually detecting the salmon lice over time. Hence, the lumpfish were sampled at different sampling points to record the stomach contents. In order to gain the most information, it was useful to sample lumpfish at early time points when the probability of detecting the salmon lice was assumed to be high, and at late time points when the probability of detecting the salmon lice was assumed to be low. However, there is most information if one is able to sample around the time points where the probability of detection changes the most. A total number of ten sampling time points were chosen for the study (n = 8 lumpfish in each sample, except for n = 12 lumpfish after 24 h and n = 7 at the final sampling). The study period was between Monday 28 November 2022 and Monday 5 December 2022. The sampling interval, starting from when the first lumpfish was given combinations of pellets and salmon lice was 8, 13, 21, 26, 37, 50, 74, 98, 122 and 170 h. Since the initial procedure involving oral gavage was conducted over a six-hour period, the time since feeding varied between the lumpfish individuals sampled at each sampling time point. Due to individual tagging, the exact number of minutes since feeding was nonetheless accounted for. For each sampling time, a random sample of 2-3 lumpfish were collected from each tank to detect any potential tank effect. Lumpfish were euthanised with a 10 min exposure of 500 mg L^{-1} tricaine and a blow to the head before stomachs and guts (from now on referred to as “stomach content”) were dissected and the content assessed. Measurements included time since feeding, weight, length, external health scores, liver colour and sex. Stomach content was quantified as number of (1) adult female salmon lice, (2) motile stages of salmon lice and (3) pellets. The persons in charge of the dissection and salmon lice count were unaware of the correct number of salmon lice and pellets fed, and the study was thus blinded. Salmon lice in the stomach contents were photographed and categorised, both before oral gavage and after dissection. Pictures were standardised using a white polystyrene photo box (30x30x30 cm) with an external light source (40 w light bulb) and a camera system including a Canon EOS R camera and a Canon EF-S 60mm f/2.8 macro USM lens. Manual settings were fixed at f/2.8, 1/1000 shutter speed, 1250 ISO and 4000 Kelvin.

2.5 Data curation

2.5.1 Classification of salmon lice

As the study was blinded, it could be that the number of salmon lice counted after sampling exceeded the initial number of salmon lice. Reassuringly, there were no such occurrences in our data set. All lice were quality controlled by personnel with expertise after each fish was dissected and the stomach content investigated. However, for three lumpfish, the number of salmon lice within a category exceeded the initial number of salmon lice of that category, indicating potential misclassification. For two of them, this was most likely due to misclassification, and the misclassified stage was reassigned to the other, valid category. For the third lumpfish, a comparison of the pictures of the salmon lice before and after digestion (Figure 1) clearly suggested that it had consumed a regurgitated motile louse free floating in the tank environment after the study was initiated. It was decided to interpret this as two adult female lice after digestion.

2.5.2 Salmon lice degradation

Based on data on the timespan since feeding for each individual lumpfish and on the pictures of stomach contents, it was possible to produce a quantitative measurement on the degradation of salmon lice. Degradation caused lice to become visually more transparent. Previous methods using imageJ™ have shown how black and white ratios in an image can be calculated from converting a digital colour image into a grayscale image and adjusting the threshold which decides which pixels turn dark or white (Staven et al., 2021, 2022). This method made it possible to estimate proportion of pixels below and above the threshold within a defined area in an image (Figure 2). The threshold in this case was manually tuned to 144 which caused the image to show the area of live material in the image (salmon lice) as dark pixels and the white background and/or the transparent areas of the salmon lice as white pixels. The ratio of black and white pixels and a measurement of the total area of each salmon louse was then used to calculate a percentage of transparency for salmon lice. Areas with glare were not included in the calculation. The total number of lumpfish stomach contents analysed (image before and after digestion) deviates from the total number of lumpfish used in the study due to the total absence of lice in stomachs where digestion had completely dissolved the salmon lice.

2.5.3 Welfare scores

Welfare was scored based on operational welfare indicators (OWIs) specifically developed for lumpfish (Boissonnot et al., 2023). This involved scoring (from 0-3) deformity, the caudal fin, other fins, skin damage, eye injury and cataract. The overall welfare score was used to determine the welfare status of the lumpfish (see table 2 in Boissonnot et al., (2023)). Internal assessments of liver scores were also included based on published methods developed by Eliasen et al., (2020).

2.6 Model for salmon lice digestion time

A binomial logistic regression model was used to estimate the probability of recovering salmon lice as a function of time. First, the full model containing all variables of interest was fitted. Hence, the expected probability of recovering salmon lice was modelled as a function of time since feeding (measured in minutes), where the probability over time was allowed to depend on lice category (adult females or other motile stages of salmon lice), the number of lice fed and the number of pellets fed, as

$$\mathrm{logit}(p_i) = \eta_i = \beta_0 + \beta_1 \log(x_{1i}) + \beta_2 x_{2i} \log(x_{1i}) + \beta_3 x_{3i} \log(x_{1i}) + \beta_4 x_{4i} \log(x_{1i}),$$

where

$$\mathrm{logit}(p_i) = \log\left(\frac{p_i}{1 - p_i}\right),$$

such that

$$p_i = 1/(1 + \exp(-\eta_i)),$$

and

$$Y_i \sim \mathrm{Bin}(n_i, p_i),$$

where Y_i is the number of either adult female or other motile salmon lice recovered in a lumpfish individual which had been fed n_i salmon lice of that category, Bin represents the binomial probability distribution, $\beta = (\beta_0, \beta_1, \beta_3, \beta_4)$ are the regression parameters which were estimated, x_{1i} is the time since feeding for observation i, x_{2i} is 1 if observation i is adult female, and 0 otherwise, x_{3i} is the total number of salmon lice fed for the lumpfish individual corresponding to observation i, and x_{4i} is the number of pellets fed for the lumpfish individual corresponding to observation i.

Secondly, the nonsignificant variables (at significance level 0.05) were removed and the estimated probability of recovering salmon lice over time from this model was reported. In addition, a model where the probability of recovering salmon lice was allowed to differ between the three study tanks was fitted to investigate whether there was a significant difference between the tanks. Note that for two of the observations, the number of pellets fed was missing. Hence, models including pellets fed were fitted without these two lumpfish. More details on model choice are provided in the supplementary material. The regression analysis was performed using the R software™ R.4.0.5, and the glm function implementation in the base package stats.

2.6.1 Estimated digestion time and number of daily removed salmon lice

The fitted probability of recovering a salmon louse Δt minutes after feeding was obtained first, as detailed above. The integral

$$F = \frac{1}{24 \cdot 60} \int_0^\infty p(\Delta t) d\Delta t,$$

is then the sum of the probabilities of observing a salmon louse fed at any prior time. We divide by $24 \cdot 60$ to obtain the probabilities per day instead of per minute. If a lumpfish on average consumes x salmon lice per day, then one expects to observe

$$y = \frac{x}{24 \cdot 60} \int_0^\infty p(\Delta t) d\Delta t$$

salmon lice in the stomach contents at a snapshot at time t. Hence, given an estimate of y, an estimate of the expected number of salmon lice consumed daily per lumpfish, x, can be obtained as

$$x = 24 \cdot 60 \cdot y / \int_0^\infty p(\Delta t) d\Delta t.$$

The integral was approximated by a sum using short time steps.

In order to compute the expected digestion time, its probability density is needed. As $p(t)$ is the probability of recovering a salmon louse t minutes after feeding, then $1 - p(t)$ is the cumulative probability density function for the digestion time. Hence, the probability density of the digestion time (i.e., probability density of not recovering a salmon louse due to complete digestion) was obtained by differentiation and normalisation of $1 - p(t)$. Note that the function $p(t)$ is always positive and hence there is never zero probability of detecting the salmon lice for any time since feeding (i.e., the upper limit of the integrals is ∞). However, this is biologically unrealistic, as we know that after some cut-off limit, it will not be possible to detect the salmon lice. We therefore need to set a maximal limit for the digestion time. However, it is not obvious what this limit should be.

2.7 Statistics on the salmon lice transparency

2.7.1 Modelling lice opacity versus time

In addition to estimating the probability of recovering lice over time, lice transparency data were similarly used to estimate lice opacity versus time since feeding. In order to obtain results that were comparable to the estimated probability of recovering lice versus time since feeding, we modelled the lice opacity instead of directly modelling the lice transparency data. We defined the lice opacity as $(100 - \text{Lice transparency})/100$, so that it was a number between 0 and 1. We then modelled the expected lice opacity for observation *I*, o_i, as

$$\text{logit}(o_i) = \beta_0 + \beta_1 \log(x_{1i}),$$

where β_0 and β_1 are parameters which we estimated, and x_{1i} is time since feeding for observation i (as before). We estimated the model by binomial quasilikelihood (McCullagh and Nelder, 2019), see supplementary material, using the quasibinomial option in the glm function, as the lice opacity observations were continuous numbers between 0 and 1 and not binary variables.

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3 Results

3.1 Salmon lice digestion time in lumpfish

The proportion of salmon lice recovered versus time since feeding is shown in Figure 3. There were four outliers, where lice were recovered after comparably long time since feeding. For all observations except these four, there were no lice recovered after 2175 min (36 h).

Firstly, the regression model where the probability of recovering a salmon louse over time depended on lice category and other stomach content (number of lice and pellets fed) was fitted. The effects of lice category, number of lice and number of pellets fed were all nonsignificant. However, the estimated effects were in the direction of slower digestion for adult females than other motile stages of lice, and slower digestion the more lice and pellets fed. For lice category and number of fed lice, the effects were borderline significant, with p-values around 0.1. The estimated model is shown in the supplementary materials. As these three variables were not significant, they were removed from the regression model, and hence a model containing only an intercept and the logarithm of time since feeding was fitted. The fitted probability of recovering a salmon louse is shown in Figure 4a. Note that the probability decreased rapidly with time. After 873 min (14.6 h) with approximate 95% confidence interval (599.5, 1158) min, corresponding to (10, 19.3 h), only 50% of the salmon lice were visually detected.

The parametric estimate for the probability of recovering a salmon louse after time since feeding, $p(t)$, was given by

$$p(t) = 1/(1 + \exp\bigl(-(8.995 - 1.328 \log t)\bigr),$$

where t denotes the time since feeding measured in minutes. Details on the estimated coefficients are provided in Table 2.

No significant effect of tank was found (estimated p-value of likelihood ratio test of 0.3).

3.1.1 Expected digestion time and estimated number of lice removed daily

When calculating the expected digestion time, a choice on whether to extrapolate the estimated probability of recovering salmon lice beyond the 7 days for which data were available had to be made. The estimated function had an unrealistically long tail, so it was not possible to extrapolate the function far beyond the time frame where data were available. Accordingly, a maximum digestion time of 14 days was assumed, for which the estimated probability was 1.5%. This resulted in an expected digestion time of 29 h. If the maximum digestion time had instead been set at 7, 10, or 20 days, the corresponding estimates would have been 24 h, 26 h, or 31 h, respectively.

The cumulative probability of observing a salmon louse ingested at any time in the past was found to be 1.39 (i.e., the integral F above), when assuming a maximum digestion time of 14 days. Hence, the estimated mean number of salmon lice consumed daily can be found by dividing the mean number of salmon lice per lumpfish in the stomach content by 1.39. The corresponding value for 7, 10, or 20 days was 1.22, 1.31, or 1.46, respectively. Hence, assuming an estimate of 0.19 salmon lice per lumpfish in average in the stomach content, the estimated expected number of salmon lice consumed daily per lumpfish was 0.14 when assuming a maximum digestion time of 14 days. Similarly, the weekly number of consumed salmon lice per lumpfish is estimated to 0.98 $(0.14 \cdot 7)$. Note that these are estimated effects per lumpfish, hence in order to obtain the total effect, one needs to multiply with the total number of lumpfish. For example, if there are 1000 lumpfish present, then the estimated expected number of salmon lice consumed daily for these lumpfish is 140. If the maximum digestion time had instead been set at 7, 10, or 20 days, the corresponding estimated number of salmon lice consumed daily per lumpfish would have been 0.16, 0.14, or 0.13, respectively.

3.1.2 Lice category and lice fed

Even though lice category and the number of lice fed were not significant, the estimated effects are reported here, as these were found to be borderline significant. As the effect of pellets was far from significant (p-value of 0.9), the number of pellets fed was not included. Hence, a model including all the terms except the interaction term between time since feeding and the number of pellets fed was fitted. The estimated coefficients are provided in Table 3.

The corresponding estimated expectations are provided in Figure 5 for specific choices of lice stage and number of lice fed. As expected, the estimate for the model which only contained the intercept and time since feeding lied between the other estimated curves.

3.2 Salmon lice opacity

The estimated lice opacity versus time since feeding is shown in Figure 4b. The uncertainty was larger than for the estimated probability of recovering a salmon louse, most likely due to fewer observations of lice opacity. The shapes were nonetheless similar. The table with the fitted coefficients is provided in the supplementary materials.

3.3 Pellet digestion time in lumpfish

Almost no pellets were recovered in the stomach contents. Pellets were found only in three of the lumpfish sampled, with times since feeding of 3 h, 3 h and 7 h. Hence, the digestion time for pellets was not possible to estimate, but it was clear that the digestion time for pellets was much shorter than the digestion time for salmon lice.

3.4 Mortality and welfare scores

No mortalities were observed in the three tanks containing lumpfish. Overall welfare scores from fish revealed that 47 lumpfish were categorised as with “good welfare” while the remaining 26 individuals were categorised as with “slightly reduced welfare”. Among these 26 individuals, caudal fin damage was the most common cause of increased welfare scores. The mean liver score was 3.92 and sex distribution was 53% females and 47% males.

4 Discussion

Information on the digestion time of salmon lice in lumpfish is a key prerequisite for measuring cleaning efficacy as it allows to estimate the number of salmon lice eaten per time unit from an estimate of the number of salmon lice recovered in the stomach content. To the authors' knowledge, this is the first study that investigated the probability of recovering a salmon louse as a function of time since feeding had occurred. The only study that gave an indication of lumpfish digestion time was conducted by Imsland et al., (2019), reporting no salmon lice in the stomachs of 25% of lumpfish that had fed on sea lice from ice blocks 6 h after ingestion at 10-12 °C, which coincides with the findings of the present study.

As only a small proportion of lumpfish consume salmon lice, many observations are needed to assess cleaning efficacy of lumpfish from hands-on stomach content data. Due to large variance, it is also necessary to have observations from a wide range of localities. Based on a sample of 25 000 lumpfish from 80 localities in Norway, Engebretsen et al. (2023) reported an estimate of 0.19 salmon lice per lumpfish in the stomach contents. Note that the estimated number of salmon lice per lumpfish was also found to vary with external factors, and in particular with the lice abundance in the sea cages. Hence, for typically low numbers of salmon lice abundance, the mean number of salmon lice per lumpfish would be lower than 0.19, while it would be higher for typically high numbers of salmon lice abundance. Similarly, lumpfish weight is an important factor for salmon lice grazing (Imsland et al., 2016; Boissonnot et al., 2022a; Engebretsen et al., 2023). The mean number of salmon lice in small lumpfish (< 100 g) is therefore likely to be higher than 0.19, while it is expected to be close to 0 in large lumpfish (> 300 g). Other factors such as availability of other feed types in the water, production conditions (e.g., feeding frequency and type, availability of shelters and hides), welfare and weather conditions are also known to affect lice grazing efficacy (Eliasen et al., 2018; Imsland et al., 2020; Boissonnot et al., 2022a; Engebretsen et al., 2023). By assuming 0.19 salmon lice per lumpfish on average, a daily expected delousing effect of 0.14 salmon lice per lumpfish was estimated in the present study.

Seawater temperature may have a strong effect on lumpfish digestion time, as metabolism increases with temperature (Nytrø et al., 2014). It is also well known that digestion time in fish in general increases with temperature. For example, rainbow trout (*Oncorhynchus mykiss*) fed with pellets digested their entire stomach contents after 15 h at 22.5 °C and > 35 h at 4.5 °C under experimental conditions (He and Wurtsbaugh, 1993). In this study, expected digestion time of salmon lice was estimated at 9 °C, which is characteristic of mean seawater surface temperatures (3 m depth) in Norway in spring and autumn, in latitudes where lumpfish is commonly used (BarentsWatch database, URL: https://www.barentswatch.no/nedlasting/fishhealth/lice, accessed 09.05.2023).

According to the findings of newly conducted studies, the salmon industry strategically deploys more lumpfish into salmon net pens during autumn, winter, and spring while avoiding the summer season in regions with temperatures above optimal conditions (Reynolds et al., 2022; Sommerset et al., 2021, 2022; Boissonnot et al., 2023). The expected digestion time found in this study is therefore likely to be representative for lumpfish most of the time they spend in salmon cages. Lumpfish digestion time during winter, when mean seawater temperatures decrease to 5 °C in Norway (BarentsWatch database, URL: https://www.barentswatch.no/en/nedlasting/fishhealth/lice, accessed 09.05.2023), is expected to be longer.

4.1 Effect of salmon lice category

The present analysis investigated to what extent the probability of recovering a salmon louse was dependent on whether the lice were adult females or other motile stages. No significant difference for the different lice categories was found. However, the estimated effect was in the direction of adult females being detectable for longer time in comparison with the other lice stages. This is reasonable due to larger body size for adult females, which could delay the digestion time in comparison with smaller motile lice stages (Hamre et al., 2013). The most detectable and visible components of salmon lice is the cuticular exoskeleton, which is made mainly from polysaccharide chitin (Hamre et al., 2009). When salmon lice moult, the composition and texture of the new exoskeleton might differ from that of an adult female louse, which is likely to have a stronger exoskeleton. Thus, in a commercial setting where a majority of salmon lice are recognized as adult females in stomach contents, this experience can be explained by a longer digestion time for adult lice in general. If the digestion time for adult female lice is indeed longer than the digestion time for other motile stages, then one would expect a higher number of adult female lice than other mobile lice in the stomach contents of lumpfish, even if lumpfish did not have a preference for either lice category, given the same availability of the different types of salmon lice.

4.2 Effect of other stomach content

This study investigated the probability of recovering a salmon louse depending on the amount of food in the stomachs, through a total number of lice and a total number of pellets fed. This approach was used to resemble the access lumpfish have to pellets in a net pen and was thus of importance to investigate. No significant effects of these variables were found. However, the estimated effect in the direction of slower digestion with more lice and pellets fed, was as expected. The effect of pellets was clearly not statistically significant, while the effect of lice fed was borderline significant. This suggests that the added pellets did not affect the stomach concentrations of gastric juices, including hydrochloric acid, to an extent that affected the digestion time of salmon lice.

Almost no pellets were retrieved in the stomachs, while deteriorated and shapeless pellets were observed in the intestines in a few cases where exoskeletons were still detectable. Pellets are developed with properties facilitating quick digestion and absorption while a crustacean louse requires longer digestion time. Crustacean exoskeletons have previously been shown to remain in the stomach for longer periods of time compared to more digestible food (Hopkins and Larson, 1990). For example, the warm-temperate grouper *Mycteroperca microlepis* exhibited a gastric evacuation time for crab of 24 h and of sardine of 15 h at 28 °C (Berens and Murie, 2008). It is therefore not surprising that the sampling frequency of the present study, mainly designed for salmon lice, was not high enough to determine the digestion time for pellets.

4.3 Lice opacity

In addition to analysing whether salmon lice were present or not as a function of time since feeding, the digestion time of salmon lice in lumpfish was also analysed by examining the transparency (or, equivalently, opacity) of the salmon lice. This approach resulted in a smaller sample size, as lice transparency could only be measured in the cases where the lice were not fully digested, but the two approaches resulted in similar trends. When a copepod such as salmon louse comes in contact with the digestive enzymes in the stomach, its tissues get digested much faster than its exoskeleton, which is little digestible due to its composition (e.g., Conway et al., 1993, 1994). A gradual loss of observable pigmentation is therefore initiated, which eventually leaves the exoskeleton transparent.

Interestingly, the results of the present study may more generally imply that it can be challenging to visually separate between salmon lice and *C. elongatus* in lumpfish stomach content already within the first 24 h after consumption. This may be highly relevant for the salmon industry, which sometimes struggles with infestations from both species (Powell et al., 2018; Overton et al., 2019). Studies that assess stomach contents to investigate lumpfish delousing effect often need to properly identify both species (Imsland et al., 2018, 2021; Gentry et al., 2020). This is mostly done visually, using body shape, colour, and number of eyes as complementary parameters for species identification (Boissonnot et al., 2022b). This suggests that categorising of lice species should be carefully performed when investigating lumpfish stomach contents during commercial use. When impossible to differentiate between salmon lice and *C. elongatus*, those should be categorised as undetermined, as done in some studies (Eliasen et al., 2018; Boissonnot et al., 2022a).

4.4 Limitations

There are potential sources of bias in the present study. In two cases, the number of counted salmon lice in a category exceeded the number of initial salmon lice of that category. This indicated that misclassifications in manually counted salmon lice had occurred, which may vary from person to person. This could potentially affect our result on the different digestion time for the different lice stages, and further implies as mentioned above, that it may be difficult to separate between salmon lice and *C. elongatus* in lumpfish stomach content.

Even though a pilot study was performed to estimate the necessary length of feeding tube and to test whether the method allowed to place the lice into the stomach, it is not possible to exclude the likelihood that not all lice reached the stomach and that some were placed in the oesophagus. In the pilot, nonetheless, all lice were recovered in the stomach which suggested that the method was reliable. It is uncertain if regurgitation among some individuals were related to lice placement, but regurgitation of consumed salmon lice is considered normal behaviour for lumpfish, also without the use of a feeding tube (Imsland et al., 2019). Regurgitated salmon lice could later have been ingested by other lumpfish, which would cause errors in the variable time since feeding in the observed data and could thus affect our estimated probability of recovering a salmon louse versus time since feeding by overestimation. This phenomenon could also potentially explain the four outliers, where lice in the stomach contents were found late in the experimental period. However, an attempt was made to control regurgitation by regularly checking filters in the experimental tanks for salmon lice, which resulted in detection of in total two salmon lice. Note also that the two salmon lice that were detected in the filters were not controlled for. Hence, it may be that the digestion time for the lumpfish which had initially been fed these two lice was wrongly underestimated. Using already euthanised lice could have impacted the digestion time. Nonetheless, the procedure of collecting and storing at -80 °C, did not cause any visual degradation of chitin, as seen in the macro images.

To estimate the expected digestion time, we needed to assume a maximum time for salmon lice to be possible to recover in the stomach contents. This was because our estimated function had an unrealistically long tail, likely affected by the four outliers. As shown in the results, the expected digestion time varies with maximum digestion time, and it is not clear what this maximum digestion time should be.

The use of frozen lice could also have impacted the digestion time to some degree. Since live lice were quickly frozen to -80 °C, the enzymatic degradation was miniscule. But the time lice were kept at 0 °C (from 0-6 h) during the feeding procedure should be accounted for. It is argued that the randomisation of lice in different lumpfish would spread this effect across all lumpfish and that the overall digestion time could vary only with hours due to this effect, in comparison with feeding live lice.

4.5 Future work

The digestion time of salmon lice in lumpfish was only studied for one temperature, 9 °C. Hence, as suggested above, future studies should investigate digestion time of salmon lice in lumpfish for other temperatures, in order to assess the temperature dependence.

The estimated daily number of salmon lice consumed by lumpfish was found by combining the estimated probability of recovering a salmon louse over time with the estimate of 0.19 salmon lice per lumpfish as found in Engebretsen et al. (2023). However, that estimate is an overall average of stomach content, and not conditional on different covariates. From a model of salmon lice in the stomach contents, it is possible to provide estimated cleaning effects of lumpfish under different operating conditions. In future work, this should be combined with estimates of digestion time of salmon lice in lumpfish for different temperatures.

With knowledge on the number of salmon lice removed by lumpfish per time unit, it is possible to investigate different lumpfish strategies, like for example the effect of different stocking densities of lumpfish per salmonid. This could be studied through simulation models of salmon lice infection over time, like those published in Aldrin et al. (2017) and Aldrin et al. (2019). In addition, estimating digestion time for pellets is relevant for future optimisation of feeding regimes in commercial salmon farms, and the authors encourage further controlled experiments with increased sampling frequency during the first 24 h after feeding.

5 Conclusion

In order to infer the cleaning efficacy from data on number of lice found in stomach samples of lumpfish, it is necessary to know the digestion time for salmon lice in lumpfish. In this study, the expected digestion time of salmon lice for lumpfish was found to be 29 h at 9 °C. The present study of the probability of recovering salmon lice in lumpfish over time is thus an important contribution to the critical issue of estimating the salmon lice cleaning efficacy of lumpfish. From an estimated expected number of salmon lice per lumpfish, the estimated expected number of salmon lice consumed per lumpfish per day resulting from the present study can be found by dividing by 1.39.

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7 Data availability

The experimental data obtained in this study are provided in the supplementary materials for reproducibility. The table shows the time since feeding, number of adult female salmon lice fed, number of other motile salmon lice fed, number of pellets fed, and number of adult female salmon lice, other motile salmon lice, and number of pellets recovered after dissection, and the calculated lice transparency.

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9 Figure legends

Figure 1. Salmon lice before (A) and salmon lice after (B) digestion. The lumpfish was fed with two adult female lice, while one adult female and one motile stage louse were found during inspection of the stomach content. This case suggested that the lumpfish had consumed a regurgitated motile louse floating in the tank environment after the study was initiated.

Figure 2. Illustration of a salmon lice analysed for change in black to white ratio of pixels, where white areas indicate degradation of the lice. At this threshold, a live salmon louse would have almost 100% cover in dark pixels. After a period of degradation, the area of white pixels became increasingly larger.

Figure 3. The proportion of lice recovered in the stomach data for the various times since feeding. The observations are coloured by number of lice fed.

Figure 4. Estimated digestion time. a) Estimated probability of recovering a salmon louse as a function of time since feeding, and b) estimated lice opacity versus time since feeding, with estimated 95% confidence bands.

Figure 5. Estimated digestion time for lice category and lice fed. The estimated expected probabilities of recovering a salmon louse for the two lice categories adult females and other motile, for lumpfish which had been fed with 1 or 6 salmon lice for each lice stage, together with the corresponding estimated probability for the model which only contained the intercept term and logarithm of time since feeding.

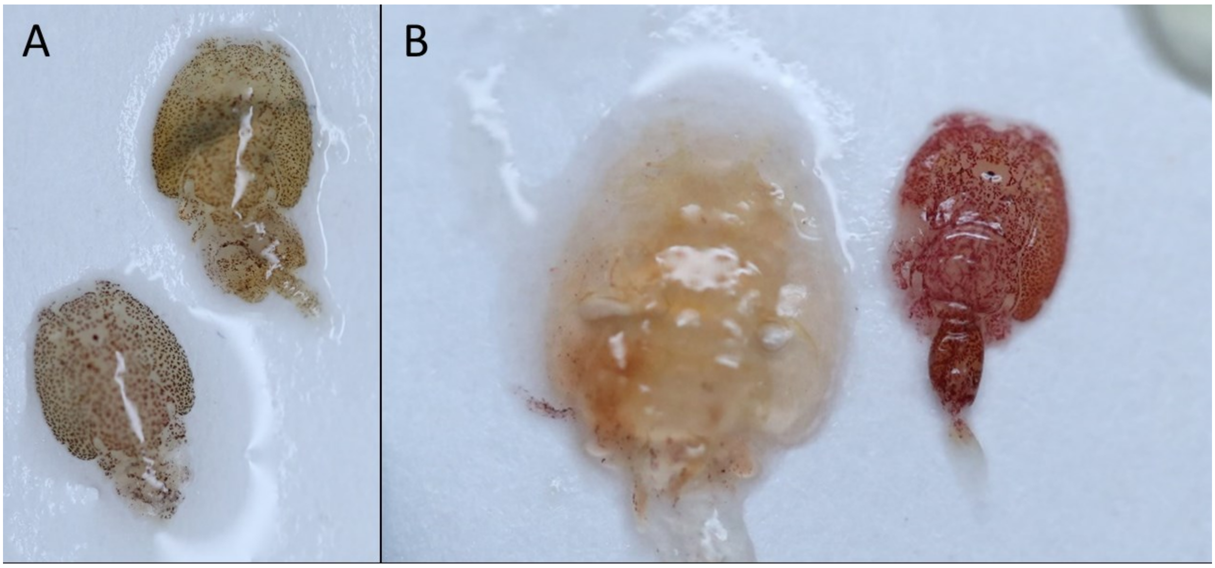

Figure 1. Staven et al.

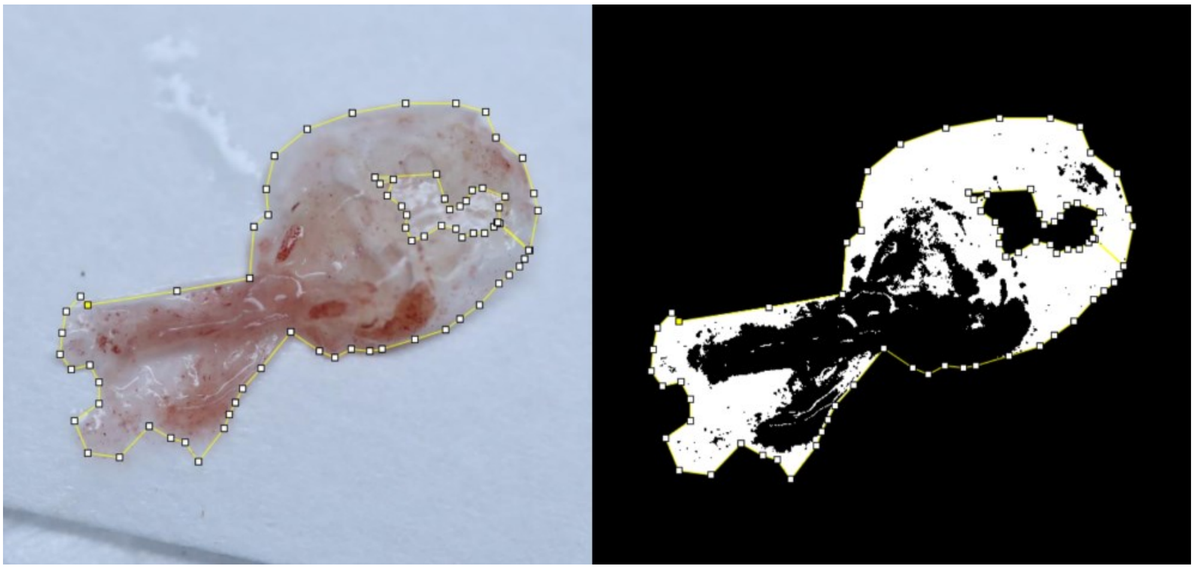

Figure 2. Staven et al.

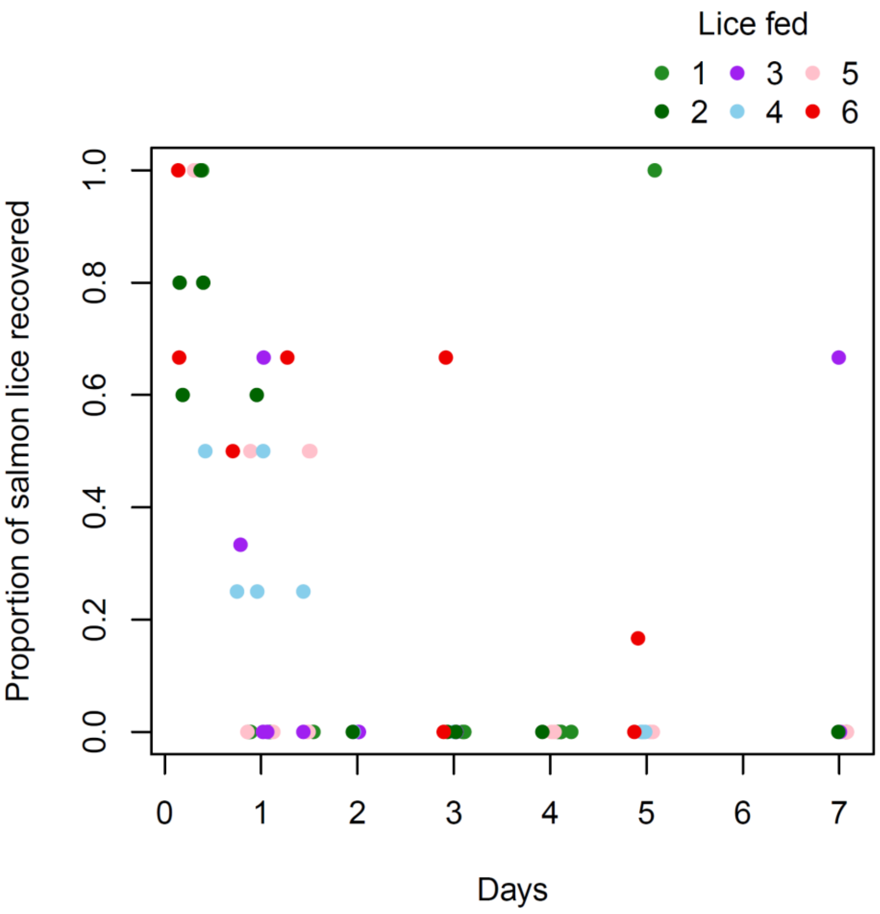

Figure 3. Staven et al.

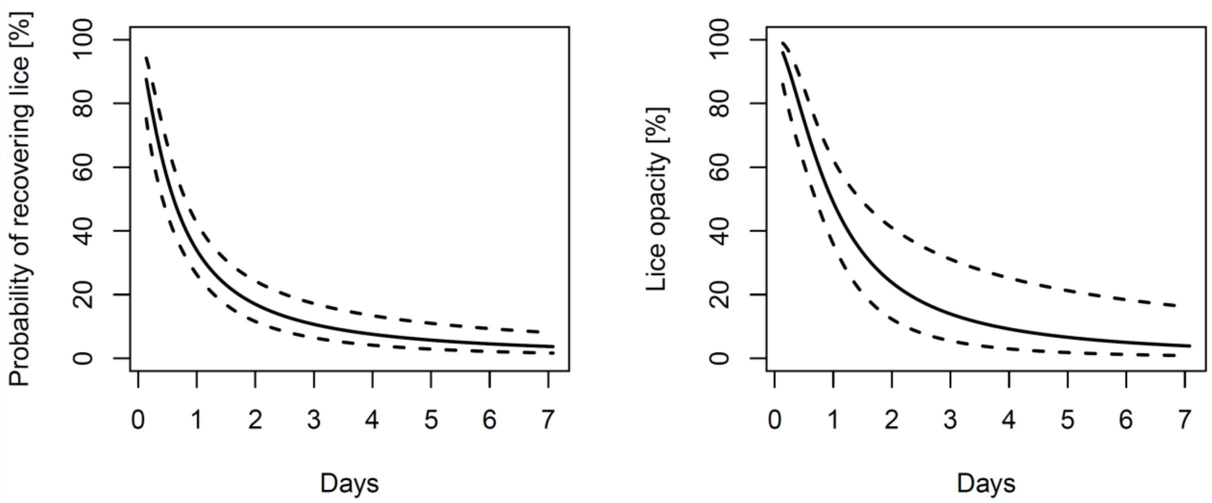

Figure 4. Staven et al.

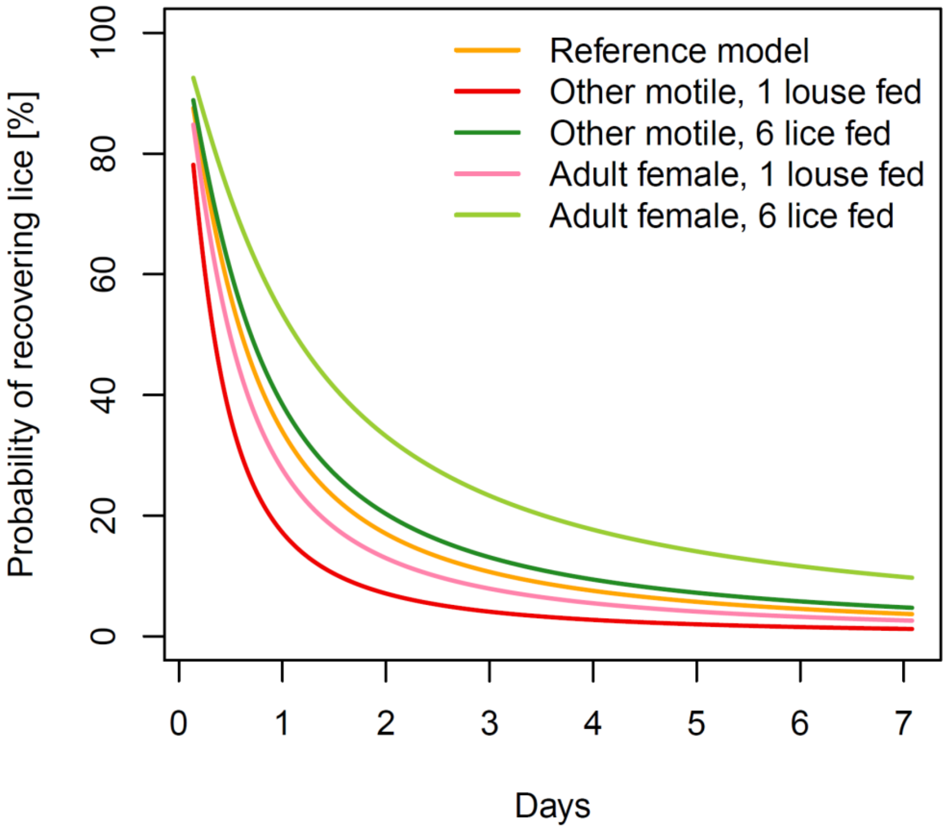

Figure 5. Staven et al.

10 Table legends

Table 1. A matrix illustrating the number of salmon lice and pellets given to different individuals of lumpfish using oral gavage. Each number (“1”) represents an individual lumpfish. Black numbers were assigned to tank 1, orange numbers to tank 2 and blue numbers to tank 3 to account for any tank effect. For example, two lumpfish were fed with two pellets and three salmon lice, and these two lumpfish were put in two different tanks (the “black” and “blue” tank in the table).

Table 2. Estimated coefficients and standard deviations for the model for the probability of recovering a salmon louse which only contains an intercept and logarithm of time since feeding.

Table 3. Estimated coefficients and standard deviations for the model for the probability of recovering a salmon louse which contains an intercept term, logarithm of time since feeding, and interaction effects with lice category and number of lice fed.

Table 1. Staven et al.

Number of salmon lice

Number of pellets

	0	1	2	3	4	5	6
0	0	1+1+1	1+1+1	1+1	1+1	1+1	1+1
1	1+1+1	1+1	1+1	1+1	1+1	1+1	1+1
2	1+1+1	1+1	1+1	1+1	1+1	1+1	1+1
3	1+1	1+1	1+1	1	1	1	1
4	1+1	1+1	1+1	1	1	1	1
5	1+1	1+1	1+1	1	1	1	1
6	1+1	1+1	1+1	1	1	1	1

Table 2. Staven et al.

Covariate	Parameter	Estimate	Standard error	P-value
Intercept	β_0	8.995	1.405	$1.54 \cdot 10^{-10}$
$\log t$	β_1	-1.328	0.191	$3.77 \cdot 10^{-12}$

Table 3. Staven et al.

Covariate	Parameter	Estimate	Standard error	P-value
Intercept	β_0	8.936	1.431	$4.25 \cdot 10^{-10}$
$\log t$	β_1	-1.475	0.2111	$2.78 \cdot 10^{-12}$
Adult female	β_2	0.08383	0.05123	0.1018
Number of lice fed	β_3	0.03024	0.01693	0.0741