Aquaculture 561 (2022) 738720

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

How delousing affects the short-term growth of Atlantic salmon (*Salmo salar*)

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ARTICLE INFO

Keywords:
Atlantic salmon
Growth
Delousing
Salmon lice
Thermal growth coefficient
Salmon lice treatments

ABSTRACT

Infestations with salmon lice and subsequent salmon lice management is one of the most challenging and costly aspects of marine salmonid aquaculture. Both the handling and treatment, specifically non-medicinal treatment, against salmon lice cause stress and physical injuries to the host, the Atlantic salmon (*Salmo salar*). This in turn leads to reduced appetite and increased mortality. In this study, we have estimated the short-term growth loss of Atlantic salmon related to treatments (thermal, mechanical, hydrogen peroxide bath, freshwater bath and combination medicinal baths) for removal of salmon lice. To achieve this, we have obtained daily production data at cage-level from 2014 to 2019 from three large Norwegian aquaculture companies. We have used the registered feed-amount, number of fish and seawater temperature at cage level to calculate the thermal growth coefficient (TGC) of 635 fish-groups the week before a pre-treatment starvation period and the week after 2530 different treatments to estimate the reduction in TGC. We modelled this outcome using a mixed effect linear regression model, with treatment method as the main fixed effect of interest and fish weight, seawater temperature, smolt-age and year-class included as fixed effects. Results showed a period of suboptimal feeding and growth after all treatment methods, where non-medicinal treatment methods had a significantly larger negative effect on growth compared to medicinal treatments. The results also showed that timing of treatment played a role in the outcome of a treatment. The short-term biomass-loss in one cage following one non-medicinal treatment was estimated to 31,200 kg (average cage containing 150,000 fish weighing 3 kg, and seawater temperature of 10 °C). Thus, there could exist a potential for increased production in the Atlantic salmon aquaculture industry by reducing the number of delousing operations.

1. Introduction

Factors influencing growth are of great economic importance to the Norwegian salmonid aquaculture industry. For many years, infestations with salmon lice and salmon lice management have been one of the most challenging and costly aspects of Norwegian salmonid aquaculture, affecting both mortality and growth of Atlantic salmon.

Farmed Atlantic salmon live the first part of their lives in land-based freshwater sites. The fish are transferred to seawater sites in the fall the same year as they hatch (0-yearling), or in the spring the year after they have hatched (1-yearling). Groups of 150,000–200,000 salmon are stocked within open net-cages in seawater sites, where they live their on-growing period for about 1–1 ½ years until harvested. The sea-water sites are situated in one of 13 production zones on the Norwegian coast (NFD, 2017; Ådlandsvik, 2015).

The growth of Atlantic salmon is affected by several abiotic factors such as temperature, light, oxygen and salinity, and biotic factors such as fish size (age), access to and quality of feed and feeding regime (Brett, 1979). During the production at sea there is a substantial circannual and spatial variation in appetite and growth of Atlantic salmon, with a reduced growth during winter and increased appetite with increasing day-length (Aunsmo et al., 2014; Endal et al., 2000; Mørkøre and Rørvik, 2001; Nordgarden et al., 2003; Smith et al., 1993). Because Atlantic salmon is an ectothermic animal, appetite and growth are heavily

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https://doi.org/10.1016/j.aquaculture.2022.738720
Received 8 April 2022; Received in revised form 1 August 2022; Accepted 9 August 2022
Available online 13 August 2022

influenced by temperature. Growth will increase with increasing temperatures up to a threshold of about 12-19 °C, and then decrease with further increasing temperature (Handeland et al., 2008; Hevrøy et al., 2012; Jobling, 1997). The appetite and growth of Atlantic salmon also depend on weight, and the daily percentage growth will decline as the fish increases in size (Austreng et al., 1987; Brett, 1979; Jobling, 1997).

Keeping track of daily weight gain and growth rate of the farmed salmon is an important part of the production, and companies can use several different models to estimate both weight gain and growth rate. The most important parameter when estimating daily weight gain is the amount of feed given to the fish. In Norway, salmon are fed by appetite and the farmers use cameras to stop feeding when the fish no longer eat. The appetite is evaluated by swimming behaviour and visible pellets at a prior set water depth. The daily weight gain will then be estimated by the amount of feed given, number of fish and the biological feed conversion ratio (bFCR). The bFCR is temperature and size dependent, and companies can use different models for estimating bFCR. There are also several different models for estimating the growth rate of salmon, and one of the most applied formula for calculating growth rate, that adjust for both temperature and size, is the thermal growth coefficient (TGC) (Cho, 1990; Iwama and Tautz, 2011).

Another important part of the production of Atlantic salmon is treatment of ectoparasites, such as salmon lice and *Paramoeba perurans* (causative agent of amoebic gill disease [AGD]). Treatment against salmon lice and AGD are common stressors for farmed Atlantic salmon. As stress negatively affects the appetite and growth of Atlantic salmon, occurrence of treatments might also explain a varying growth rate during production (Madaro et al., 2015; McCormick et al., 1998). Norwegian legislation requires that if the average number of adult female lice per fish per sea site exceeds a defined limit, farmers need to take measures to reduce the number of lice below the limit (NFD, 2012). Normally they do this by treatment. During the production at sea, most salmon are therefore treated at least once, but usually several times for ectoparasites, in particular salmon lice. A treatment operation at a site may involve one, several or all cages. Delousing treatments are either non-medicinal or medicinal. The non-medicinal treatments are categorized based on the principle of delousing method. The principle of thermal treatment is to inactivate the lice by exposing salmon with lice with heated water (maximum 34 °C) for up to 30 s (Grøntvedt et al., 2015; Overton et al., 2018; Roth, 2016). In mechanical delousing, the lice are removed by either brushing or flushing the lice off the fish (Gismervik et al., 2017; Nilsen et al., 2010). Freshwater treatment of salmon lice and AGD involves bathing the fish in low salinity water to inactivate the parasite (Powell et al., 2015). Thermal, mechanical and freshwater treatments are performed in well-boats or in treatment chambers in specialised rigs, which requires crowding and pumping of the fish. The medicinal treatments are administered via feed or by bath. The fish can be treated in cage or well-boat by bathing them in active substances such as azametiphos, cypermethrin, deltametrin or hydrogen peroxide. Crowding the fish is needed regardless of the type of bath treatment. Thus, handling the fish by crowding and/or pumping is an inherent part of both the non-medicinal and medicinal treatment. This is a major stressor which causes growth reduction (Delfosse et al., 2021; Erikson et al., 2016).

Since all treatments are performed in closed compartments (being either a well boat, treatment chamber, or tarp around the cage), there is a risk of the water-quality deteriorating when the water volume and water exchange is reduced compared to the normal cage environment. To decrease the risk of reduced water quality, the salmon are starved prior to treatment to reduce the amount of faeces during the operation (Einen et al., 1998). The number of days of starvation is determined by seawater temperature, size and health of the fish (Anonymous, 2020).

Because of increased resistance of salmon lice to medicinal compounds, thermal and mechanical delousing methods were introduced as non-medicinal substitutes around year 2015 (K. O. Helgesen et al., 2020; Overton et al., 2018). From 2015 to 2016, the industry rapidly shifted from medicinal to non-medicinal treatments (Overton et al., 2018). Currently, in Norway, the non-medicinal treatment operations are the most frequently applied methods for immediate removal of salmon lice (K. O. H. Helgesen et al., 2022). However, these non-medicinal methods negatively affect fish survival, and Persson and co-workers identified handling of fish and delousing operations to be the predominant causes of mortality during the marine production phase (Oliveira et al., 2021; Overton et al., 2018; Persson et al., 2022; Sviland Walde et al., 2021). Both mortality and suboptimal growth are indicators for reduced animal welfare and causes an economic loss to the farmers (Aunsmo et al., 2010; Stien et al., 2013). To reduce mortality and ensure optimal growth it is important to estimate the effects different management and environmental factors have on mortality and growth. We have previously showed that mortality after the most commonly used methods -thermal and mechanical- is many times higher compared to medicinal treatments (Sviland Walde et al., 2021). However, to the authors' knowledge there are no studies estimating the effect of different treatment methods on appetite and growth after a delousing treatment. The aim of this study was therefore to estimate the immediate effect different delousing methods have on growth of Atlantic salmon.

2. Material and methods

2.1. Data

Norwegian salmonid farming companies record data of their production, such as number of stocked fish, average fish weight, feeding (type and amount), mortality, treatments, salmon lice counts, environmental data etc. on cage-level (NFD, 2008). For this study, three large Norwegian Atlantic salmon farming companies (companies with >20 sites operating in multiple production zones) provided daily data from their production databases. The dataset used for this study was similar to the data utilized in the study described in Sviland Walde et al., 2021, but one exception: accumulated data from one company was replaced by daily data at cage-level covering the same sites and time period from that company (Sviland Walde et al., 2021). Two of the companies extracted the data directly from their production data monitoring system. Data from the third farming company was collected through Aquacloud, a digital database service within the Norwegian aquaculture industry (NCE Seafood Innovation, 2017).

The study population consisted of four year-classes (2014–2017) stocked in 279 unique sites. Year-class was defined as fish stocked within the same year. This corresponds to eight generations, with generations defined as fish stocked in either spring or fall within the same year (spring 2014-fall 2017). The smolt-age was denoted as years from the start of feeding to sea transfer, which would be either 0-yearling or 1-yearling. The study period started in spring 2014 and ended in fall 2019. Thus, the length of the study period allowed for production of more than one year-class at a specific site. Number of production cycles was defined as the number of year-classes per site during the study period. A fish-group was defined as fish within the same generation stocked at a sea site within the same cage. During the marine phase, groups of fish were often transferred between cages, and on occasion split or merged with other groups of fish, or had been moved to a different site. Therefore, we traced fish-group movements between cages from stocking until harvest. One observation (one row) in the dataset corresponded to one production day of one fish-group in one cage belonging to a specific year-class at a specific site in a specific production area.

2.2. Treatments

The main variable of interest for growth loss was treatment method. Based on information regarding treatment method, treatment indication and active substance (for medicinal treatments) supplied in the production data, treatments were categorized into eight categories as listed in Table 1. Both freshwater and hydrogen peroxide were used to remove the causative agent for the disease amoebic gill disease (AGD) *Paramoeba perurans* in addition to salmon lice. Because we wanted to look at the effect following treatments, excluding AGD-treatment could bias the results. We therefor included these AGD-treatments. However, the holding time in freshwater to treat AGD was substantially shorter compared to treatment against salmon lice (Holan et al., 2017; Hytterød et al., 2017). The concentration of hydrogen peroxide is also lower when treating against AGD compared to treating against salmon lice (Hytterød et al., 2017; Veterinærkatalogen, 2022). Therefore, we separated these two treatment methods into four different categories based on treatment indication. Information regarding whether treatments were performed in well-boat or in cage was not consistent in the data and therefore not included. Most of the current delousing methods require crowding and/or pumping, and we regard such handling as an inherent part of the treatment method. We did not expect delousing with medicinal feed to cause decreased feed intake (Veterinærkatalogen, 2022b), therefore treatment with medicinal feed was not included in this study. We gave each treatment event in a fish-group a treatment number according to the sequential order of treatments as indicated by the date of treatment supplied in the production data.

2.3. Exclusion criteria

The dataset consisted of 1,022,472 observations prior to filtering. Production data concerning other production types than that of Atlantic salmon for food consumption were excluded from the study data (151,713 observations). In addition, production data for the 2015 and 2016 year-class (159,439 observations) from one company were excluded because registration of production data started first of January 2017 in the digital database, Aquacloud. In some cases, production data had been recorded after a cage had been emptied, with the only information in these rows being the date, fish-group number and zero biomass. These rows were deleted (102,321 observations). Fish-groups were excluded if they were moved from one site to another, split or merged with other fish-groups, or impossible to trace (213,830 observations). We also excluded entire groups of fish that had less than seven days between two subsequent treatments (33,455 observations), as we regarded the first of these two treatments as failed, and the effect of each treatment impossible to separate. In addition, groups of fish with no treatment operation during the production cycle were excluded (54,859 observations). After we had excluded fish-groups based on the above-mentioned exclusion criteria, the dataset consisted of 306,855 observations, from 97 unique sites and 124 production cycles. In total, 635 fish-groups were treated 2530 times.

Table 1
Categories ($n = 8$) of immediate treatment operations applied in farmed Atlantic salmon in three Norwegian companies from 2014 to 2019.

Categories of treatment operations	Description of category of delousing operation
Thermal	Non-medicinal treatment using heated seawater. Includes all treatments using: a. Optilice ® b. Thermolicer c. Heated seawater
Mechanical	Non-medicinal treatment using brushing or flushing. Includes all treatments using: a. FLS Avlusersystem b. Hydrolicer c. SkaMik d. Flushing or mechanical treatment
Hydrogen peroxide	Hydrogen peroxide (H2O2) bath in pen or well boat against salmon lice
Medicinal bath	Medicinal bath in pen or well boat using one of the following active substances: a. Azamethiphos b. Cypermethrin c. Deltamethrin d. Imidaclorid e. Other
Freshwater bath	Non-medicinal treatment using freshwater bath in pen or well boat against salmon lice
Combination medicinal	Medicinal treatment of the same cohort using the following two different combinations on the same day: a. two different active substances b. hydrogen peroxide and medicinal bath
Hydrogen peroxide AGD	Hydrogen peroxide (H2O2) bath in pen or well boat to treat amoebic gill disease (AGD)
Freshwater AGD	Freshwater bath in pen or well to treat amoebic gill disease

2.4. Calculating and describing growth

We estimated the daily weight gain and daily weight for each fish-group by eq. 1.1 and 1.2., and assumed all fish-groups converted feed with a fixed biological feed conversion rate (bFCR). The mean bFCR (=1.15) from one company was used as the fixed bFCR in the calculations. The recorded weight at first production day was defined as stocking weight. The close count was the number of fish in the fish-group at the end of the day. The feed amount was derived from the production data as kg/day/cage.

$$\text{Daily weight gain } [g] = 1000^{*}(\text{feed amount } [kg]/\text{close count}/\text{bFCR}) \quad (1.1)$$

$$\text{Estimated daily weight } (w_t)[g] = \text{stocking weight} + \sum_{i=1}^{n} \text{daily weight gain} \quad (1.2)$$

n = number of production days.

As a measure of growth rate, we applied the formula for thermal growth coefficient (TGC) (Cho, 1992), and calculated the daily TGC for each fish-group by eq. 1.3. The end weight (*weight end)* was the estimated daily weight derived from eq. 1.2, and the starting weight (*weight start)* was the estimated weight the day before. Since we calculated the daily TGC, day degrees (DG) was equal to the daily registered seawater temperature. Daily temperature registrations were occasionally missing. In cases of missing temperature for scattered single days, the mean temperature was imputed from the temperature registered the day before and after. In a few cases, more than one week of coherent temperature-registrations were missing. Such cases were handled by assigning the corresponding weekly sea temperature for the site registered in the public database Barentswatch (www.barentswatch.no). If the weekly temperature was missing in Barentswatch, the temperature was linearly interpolated (extrapolated) using the command “epolate” in Stata (StataCorp, 2017a).

$$\text{daily TGC} = \frac{1000\ (\text{weight end})^{1/3} - (\text{weight start})^{1/3}}{\text{DG}} \quad (1.3)$$

$$\text{baseline TGC} = \sum_{i=1}^{n=5} \frac{\text{daily TGC}_{\text{pre-treatment starvation } i}}{n} \quad (1.4)$$

A treatment event was normally initiated by a period of starvation. We defined starvation as daily feed amount equal to zero in the production data. Due to daily variations in the TGC, we calculated the mean of the daily TGC for each fish-group during the last five days before a starvation related to a treatment event, and used this as a baseline (eq. 1.4). To reduce the risk of including a period of starvation from an earlier treatment in the baseline TGC, the period for calculating the baseline was restricted to maximum 14 days prior to the day of treatment. We subtracted the baseline TGC from the calculated daily TGC to find the daily change in TGC (daily ΔTGC) after treatment (eq. 1.5).

$$daily\ \Delta TGC = daily\ TGC\ post\ treatment - baseline\ TGC \quad (1.5)$$

We presented the distribution of daily ΔTGC over a 14-day period after a treatment event in the form of Tukey Box plots. Data management, descriptive statistics and statistical analysis were performed in the statistical software package Stata ® SE 15.1 (StataCorp, College Station, Texas USA) and in Microsoft ® Office Excel.

2.5. Statistical analysis

2.5.1. Outcome variable

The outcome variable in the statistical modelling was the mean of the daily ΔTGC seven days post treatment (abbreviated to ΔTGC) (eq. 1.6). The baseline comparison was thus the five day mean TGC prior to each treatment of each fish-group (eq. 1.4). The distribution of the outcome variable was visually assessed by histogram and box plots.

$$\Delta\mathrm{TGC} = \sum_{i=1}^{n=7} \frac{\mathrm{daily}\ \Delta\ \mathrm{TGC_i}}{\mathrm{n}} \quad (1.6)$$

2.5.2. Explanatory variables

In addition to the main explanatory variable of interest -treatment method- we selected other available production parameters in the dataset that could have a biological rationale for affecting growth. These were variables that described treatment (seawater temperature at treatment, number of days starved prior to treatment, treatment sequence number, number of weeks between treatments and treatment year), variables describing fish-groups (year-class, generation, smolt-age, weight at treatment), temporal variables (number of days and day-degrees from stocking to treatment, season, month and year) and spatial variables (production area and latitude).

The interaction between the explanatory variables and the outcome were assessed by building casual diagram using directed acyclic graphs (DAGitty v3.0), and by univariable analysis. We selected explanatory variables to be included in the final model building if they were regarded as possible confounders (guided by literature review and the casual diagram), or the *p*-value was below 0.2 in the univariable analysis. Some of the explanatory variables contained essentially the same information, and to avoid multicollinearity we selected which explanatory variable to be included in further model building based on criteria of biological plausibility and reliability of measurement.

2.5.3. Data structure and multivariable statistical model

To account for the clustered nature of production data, statistical modelling was performed with a mixed effect linear regression model with random intercepts using the mixed command in Stata (StataCorp, 2017b).

Different unconditional models (model without fixed effects) were tested, and the model with the most sensible biological rational clusters, highest intraclass correlation coefficient (ICC) and lowest AIC was selected as final unconditional-model. Interclass correlation coefficient (ICC) for each level was calculated as described in Rabe-Hesketh and Skrondal, 2008 (Rabe-Hesketh and Skrondal, 2008).

We included the fixed effects (explanatory variables) using a forward stepwise model building procedure, and variables were kept in the model if they were assessed as possible confounders and/or the p-value was below 0.01. The simplest model with the lowest AIC was preferred.

The residuals were visually assessed for the assumption of normality by q-norm and histogram plots and homoscedasticity by scatter plots. The reliability of the model was tested by randomly splitting the data in half, and the final mixed effect linear model was run on both halves.

The statistics calculated from both the overall estimation of the fitted mixed effect model and with specified values for the fixed effects (estimated margins) were graphically presented using the margins plot command in Stata. (Willams, 2012). A post hoc pairwise comparison across the levels of the treatment categories from the fitted model was done using the Stata margins command "pwcompare" (StataCorp, 2017c).

2.6. Calculating losses in grams

To provide an example of what the absolute number of weight gain loss in grams related to an individual treatment could be, we applied the estimated means for treatment method from the mixed effect linear regression to calculate the potential loss in grams per fish (eq. 1.7–1.11). The estimated mean was adjusted for weight category of 3-4 kg, temperature of 10 °C (i.e. the optimal growth temperature), and year-class of 2017. We calculated the end weight in a 14 day period for a standardized fish weighing 3 kg before treatment (weight start), being starved for 7 days (eq. 1.7), set the TGC prior treatment of 3.5 and added the adjusted estimated ΔTGC to calculate the end weight seven days after an individual thermal, mechanical and medicinal treatment (eq. 1.8). We compared the weight gain (eq. 1.10) of the standardized treated fish with a standardized fish of 3 kg, growing at a constant TGC of 3.5 the entire 14-day period (eq. 1.9) to find the difference (loss) in weight gain (eq. 1.11) for the treated fish vs. the non-treated fish. We could then estimate the potential growth loss for an average cage (eq. 1.12) and site (eq. 1.3).

$$weight_{end\ 7\ days\ starv} = \left[(\mathrm{weight_{start}})^{\frac{1}{3}} + \frac{\mathrm{TGCstarv} = 0}{1000} * (DG) \right]^3 \quad (1.7)$$

$$weight_{end\ 7\ days\ post\ treat} = \left[(\mathrm{weight_{end\ 7\ days\ starv}})^{\frac{1}{3}} + \frac{\mathrm{TGC\ prior\ treat} + \Delta\mathrm{TGC}}{1000} * (DG) \right]^3 \quad (1.8)$$

$$weight_{end\ no\ treat} = \left[(\mathrm{weight_{start}})^{\frac{1}{3}} + \frac{\mathrm{TGC\ prior\ treat}}{1000} * (DG) \right]^3 \quad (1.9)$$

$$weight\ gain = weight_{end} - weight_{start} \quad (1.10)$$

$$growth\ loss\ per\ fish = weight\ gain_{no\ treat} - weight\ gain_{treat} \quad (1.11)$$

$$growth\ loss\ per\ treat\ per\ cage = growth\ loss\ per\ fish^{*}\ number\ of\ fish\ in\ cage \quad (1.12)$$

$$growth\ loss\ per\ treat\ per\ site = growth\ loss\ per\ treat\ per\ cage^{*}\ number\ of\ cages\ per\ site \quad (1.13)$$

3. Results

3.1. Data

There were 717 active sites along the Norwegian coast during 2014–2019 (Oliveira et al., 2021). This dataset thus represented 13.5% of all active sites in the study period. Table 2 shows the overall frequency distribution for the eight different categories of salmon lice treatments

Table 2
Frequency distribution of treatment categories over year-classes (after applying exclusion criteria as explained in section 2.3).

	Year-class				
Treatment category	2014	2015	2016	2017	Total
Thermal	10	248	316	485	1059
Mechanical	0	99	133	122	354
Hydrogen peroxide	237	35	0	36	308
Medicinal bath	106	9	1	40	156
Freshwater bath	2	26	4	16	48
Combination medicinal	285	70	2	4	361
Hydrogen peroxide AGD	111	42	20	4	177
Freshwater AGD	6	4	4	53	67
Total	757	533	480	760	2530

over year-classes. The first treatment event took place May 2014 and the last in April 2019. More than half of all treatments were non-medicinal, where the main share were thermal treatments. The medicinal treatments mainly consisted of hydrogen peroxide and combinational treatments. A shift from medicinal to non-medicinal treatments dominating occurred between the 2014 and 2015 year-class (Table 2).

Table 3
The range and number of clusters at each level in the data structure. "Treatment sequence number" is number of clusters of fish-groups within the same site having the same treatment number. Treatment event is the cluster of treatments of fish-groups within the same site having the same treatment number.

			Number of clusters at level above	
	Level	Number of units	Mean	Range
Data structure	Site	97	–	–
	Treatment sequence number	571	5.8	1–11
	Treatment event	2530	4.4	1–18

3.2. Change in growth rate after treatment

The overall daily change in TGC 14 days after the 2530 treatment events is shown in Fig. 1 A. The median value for the daily change in TGC returned to the baseline level, i.e., the same appetite and growth rate as before starvation, after seven days. The greatest variation in the

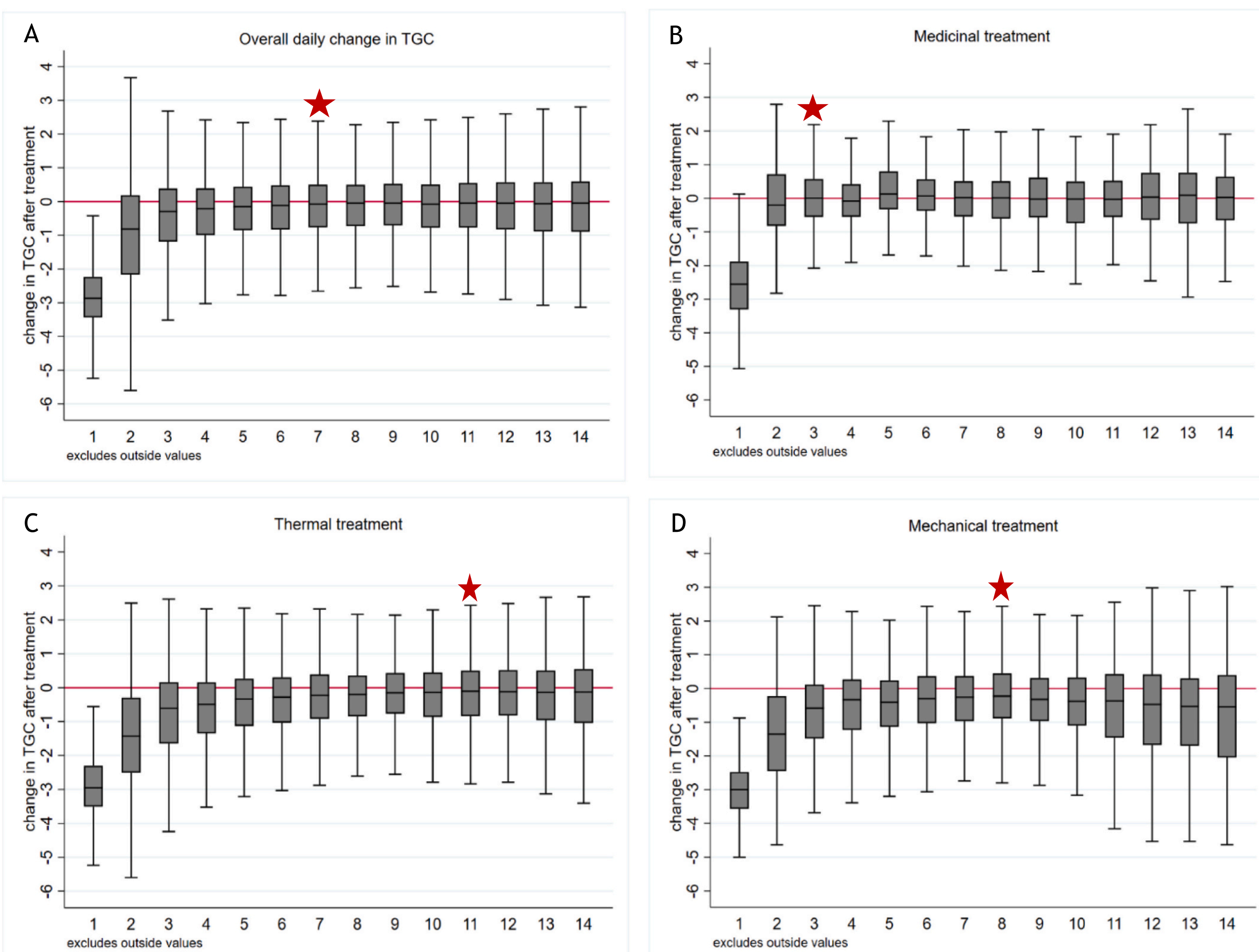

Fig. 1. Box plot showing change in daily thermal growth coefficient (TGC) 1–14 days after a treatment event overall for all treatments (A) and for medicinal (B), thermal (C) and mechanical (D) treatment. The red reference line indicates no change in TGC (=0) after treatment. A negative change in daily TGC thus indicates a reduction in the appetite and growth rate compared to the baseline TGC (given by the mean of daily TGC over a five-day period before starvation and treatment). The star shows the first day the median change in daily TGC is closest to the baseline TGC. Any outliers are excluded from the visual presentation, but not from the calculations of the box plots. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

daily change in TGC occurred at day 2. The daily change in TGC specifically for medicinal bath, thermal and mechanical treatment is shown in Fig. 1 B—D. For medicinal treatment, the daily change in TGC was back to baseline level after three days (Fig. 1B), whereas for thermal treatment this took about eleven days (Fig. 1C). For mechanical treatment, the daily change in TGC was closest to baseline level after eight days (Fig. 1D).

3.3. Statistical analysis

The outcome for the statistical analysis was the mean of daily change in TGC in a seven-day period after a treatment event (ΔTGC). The outcome was normally distributed (mean = −0.726, maximum = 4.38 and minimum = −3.98).

3.3.1. Data structure

In Table 3, the final clustering of the data structure is described with mean and range of clusters per level above. In this dataset, when a treatment within a site was initiated, normally all fish-groups within the site were treated. This meant that when a treatment at a site occurred, most of the fish-groups that were treated had the same number of previous treatments, and approximately the same number of days had elapsed since the last treatment. The number of days between treatments of the different fish-groups within a site were on average 8 days, median 1 day apart. In the final structure, a site had on average 5.8 treatments, and when a treatment at a site was initiated, an average of 4.4 different fish-groups were treated, within an average 16-day (median 4-day) period. The cluster "treatment sequence number" was the treatment number for each fish-group within the same year-class and site. The final structure thus defined clusters of fish group within the same site where most of the fish-groups within the cluster "treatment sequence number" had been treated temporarily close in time and had the same previous amount of treatments.

We excluded production cycle and production area as levels, as there were too few production cycles within a site, and the variance of number of sites within production area were high. Within a site, there was a moderate correlation (ICC = 0.503) of the outcome of treatments performed averagely within the same month, median within the same week on different fish-groups sharing the same amount of previous treatments.

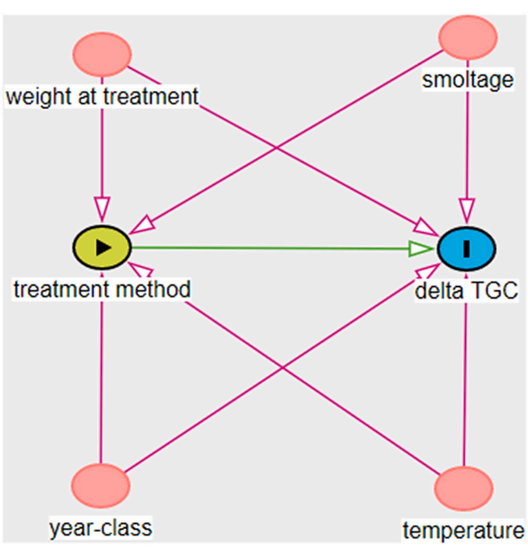

Fig. 2. A directed acyclic graph showing the proposed associations between the selected fixed effects and outcome (blue circle). We want to know the effect of treatment method (green circle) on growth rate, expressed as ΔTGC (blue circle) and adjust for the factors shown in red circles. The arrowed lines indicate casual path and their directions. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.3.2. Factors affecting change in growth after treatment

Descriptive statistics as number of treatment events per level for categorical values, and mean, median and range for continuous variables, in addition to the association (*p*-value) with the outcome variable (univariable analysis) for all potential explanatory variables are shown in Table A.1. The mean temperature at treatment day was 9.6 °C (median 9.2 °C, minimum 2.3 °C and maximum 19.2 °C).

3.3.3. Statistical model and estimations

The final model included the following fixed effect variables: treatment method, weight at treatment, year-class, smolt-age and temperature. Fig. 2 shows that year-class, weight at treatment, smolt-age and temperature were considered as confounders.

The full mixed effect linear regression model with both the fixed and random effects is shown in Table 4. There was a random intercept for site and treatment sequence number. The ICC at site level was 0.045, which meant there was 4.5% correlation between treatment events within the same site. However, there was 41% correlation between treatments of different fish-groups within the same site, with the same treatment sequence number. When including the fixed variables to the model, the total variance accounted for about 16% (R^2). For the proportion of the variance of the full model, 59% lied between treatments of different fish-groups within the same site, 37% between following treatments within

Table 4
Fixed and random effects in the fitted mixed effect linear model where outcome is change in TGC after treatment event. Coeff = coefficients, SE = standard error, CI = confidence interval, prop. Var. = proportion of the variance between levels, ICC = intracluster correlation. Treatments, weight-classes and year-classes sharing the same superscript letter are not significantly different at the 5% level.

	Coeff.	SE	95% CI		p-value	
Intercept	−0.777	0.147	−1.07	−0.49	<0.001	
Fixed effects						
Treatment method					<0.001	
Medicinal (baseline)[c]						
Thermal[a]	−0.415	0.111	−0.63	−0.20	<0.001	
Mechanical[a]	−0.394	0.121	−0.63	−0.16	0.001	
Hydrogen peroxide[bc]	−0.088	0.109	−0.30	0.13	0.419	
Freshwater bath[ab]	−0.365	0.160	−0.68	−0.05	0.023	
Combination[c]	0.059	0.112	−0.16	0.28	0.597	
Hydrogen peroxide AGD[bc]	−0.136	0.138	−0.41	0.13	0.323	
Freshwater AGD[bc]	−0.063	0.163	−0.38	0.26	0.699	
Weight					<0.001	
<1 kg (baseline)[b]						
1–2 kg[b]	−0.067	0.066	−0.20	0.06	0.309	
2-3 kg	−0.159	0.071	−0.30	−0.02	0.025	
3–4 kg[a]	−0.363	0.075	−0.51	−0.22	<0.001	
>4 kg[a]	−0.307	0.080	−0.46	−0.15	<0.001	
Yearclass					<0.001	
2014 (baseline)[a]						
2015	−0.379	0.104	−0.58	−0.17	0.002	
2016[a]	−0.134	0.110	−0.35	0.08	0.031	
2017[a]	−0.019	0.104	−0.22	0.18	0.733	
Smoltage					0.009	
1-yearling (baseline)						
0 yearling	0.159	0.061	0.04	0.28	0.009	
Temp	0.052	0.007	0.04	0.07	<0.001	
Random effects	Estimate	SE	95% CI		Prop. var	ICC
Site	0.172	0.042	0.107	0.279	0.045	0.045
Treatment sequence number	0.492	0.023	0.448	0.540	0.367	0.412
Residual	0.622	0.010	0.603	0.642	0.588	

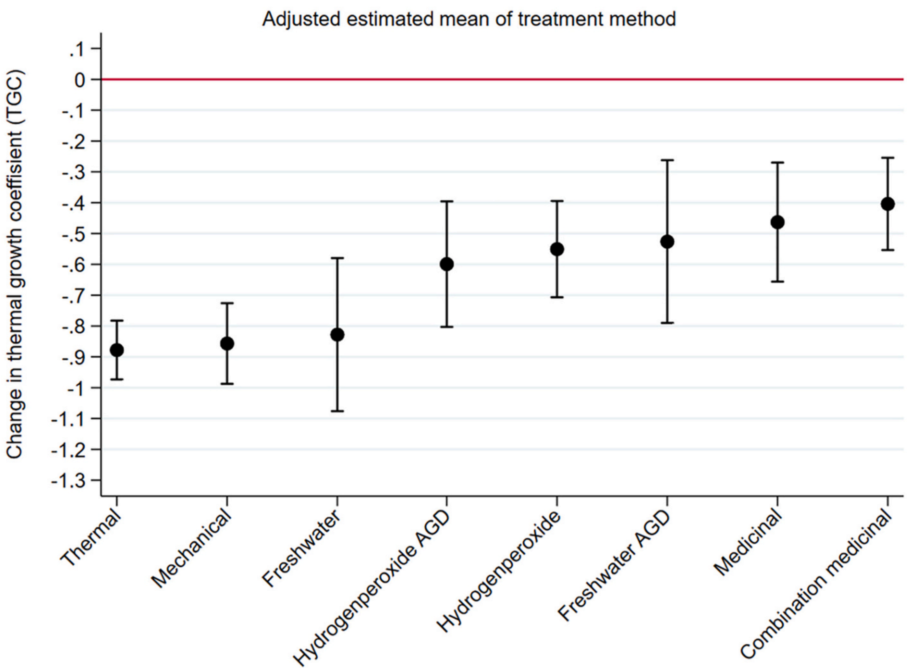

Fig. 3. Graph showing the estimated mean of the change in thermal growth coefficient (ΔTGC) from the fitted mixed effect linear regression model. The black circle for each treatment method represents the average value of the ΔTGC. The bars indicate 95% confidence intervals. The red reference line indicate the baseline TGC (given by the mean of daily TGC over a five-day period before starvation and treatment) and is equal to no change in TGC (=0) after treatment. A negative change in ΔTGC indicates a reduction in the appetite and thus growth rate. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the same site, and only 4.5% between different sites.

Treatment method had a significant negative effect on the change in growth rate seven days after treatment (Table 4 and Fig. 3). The post hoc pairwise comparison showed thermal, mechanical and freshwater treatments differed significantly from the baseline (medicinal treatment), with a larger effect of −0.415, −0.395 and − 0.365, respectively (Table 4 and Fig. 3).

The post hoc pairwise comparison also showed that the negative effect of the 2015 year-class were significantly different from the 2014 year-class, however the negative effect of year-classes decreased from 2015 to 2017, and were no longer significantly different compared to 2014 (Table 4). When fish exceeded three kg in weight, there was a significant negative effect on the change in TGC, compared to when the fish was under one kg (Table 4). 1-yearlings had a lower change in TGC compared to the 0-yearling (Table 4) and higher seawater temperature showed a positive effect on the change in TGC (Table 4).

When splitting the dataset randomly in two halves, the output of the final mixed effect linear regression model gave the same trends as for the entire dataset, except for freshwater treatments against salmon lice.

3.4. Calculating losses in grams

The estimated potential weight gain loss per fish seven days after treatment was highest for thermal (52.5 ± 6.0 g) and mechanical (51.5

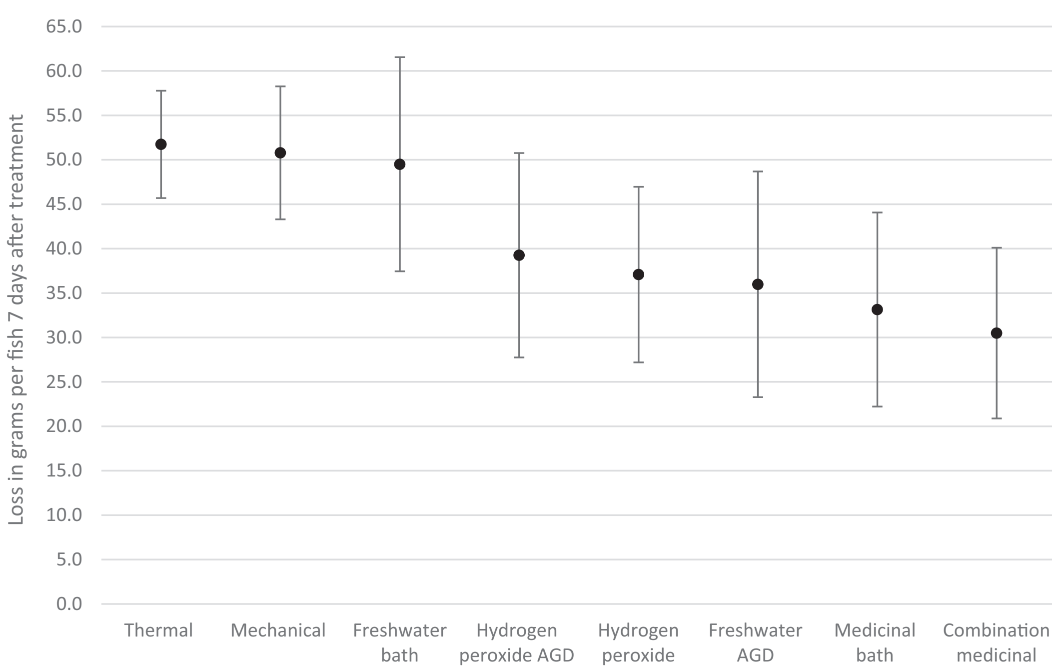

Fig. 4. Loss in weight (grams/per fish) related to each type of treatment method, when treating a standardized fish weighing 3 kg at a seawater temperature of 10 °C (i.e. the optimal growth temperature). The bars show the 95% confidence interval.

Table 5
The estimated weight gain for a fish-group from the 2017 year-class, treated at 3 kg at 10 °C seawater temperature. These figures are based on the post estimation from the mixed effect linear regression shown in Fig. 4. The 14-day period is divided into a 7-day period of starvation and the following 7-day period after a non-medicinal treatment. The treated fish-group is compared to a fish-group with the same starting weight of 3 kg and growing with a constant TGC of 3.5 at 10 °C.

	Estimated weight gain per fish	
	Non-medicinal treatment	No treatment
Pre treatment starvation period (7 days)	0 g	155
Post treatment weight gain (7 days)	52 ± 7.5 g	161
Total weight gain (14 days)	108 ± 7.5 g	316
Difference non-medicinal treatment vs non-treatment 7-day period post treatment	52 ± 7.5 g	
Difference non-medicinal treatment vs non-treatment 14-day period	208 ± 7.5 g	

± 7.5 g) treatments (Fig. 4).

Table 5 shows the estimated weight gain for a fish-group from the 2017 year-class, treated at 3 kg at 10 °C seawater temperature based on the post estimation from the mixed effect linear regression shown in Table 4 and Fig. 3. Thermal and mechanical treatments reduced the growth the week after treatment with about 52 g per fish compared to a non-treated group (Table 5). When a period of starvation of seven days was included, this added up to an average loss of 208 g per fish. About 25% of the loss in a two-week period was due to suboptimal growth the week after treatment, and the remaining 75% was due to the period of starvation prior to treatment. In this dataset, the average number of fish in a cage at treatment date was approximately 150,000 and average number of fish-groups (i.e. cages) per site 5. Thus, seven days of starvation and seven days of suboptimal appetite after a non-medicinal delousing operation, added up to a potential weight gain loss of about 31,200 kg of biomass per cage per non-medicinal treatment, corresponding to 156,000 kg biomass per site per non-medicinal treatment.

4. Discussion

The results from this study showed a significant negative effect of treatment against salmon lice and AGD on the mean change in TGC 7 days after treatment (Table 4 and Fig. 3). Thermal, mechanical and freshwater treatments against salmon lice had a significantly larger negative effect, compared to medicinal treatments (Table 4 and Fig. 3). The main source of the unexplained variation was attributed to the actual treatment event and there was a moderate correlation of the outcome of treatments within the same site, when treatments were performed close in time (Table 4).

4.1. Measurement of growth

The TGC is a widely used formula for predicting growth in production planning, since it adjusts for both seawater temperature and size of fish. TGC has been shown to be independent of temperature within a temperature range of 7.5-16 °C (Jobling, 2003). However, outside this range, the TGC will give erroneous results (Jobling, 2003). Temperature at treatment day approximated a normal distribution in this dataset, with a mean of 9.6 °C, and a range of 2.3–19.2 °C. Thus, the main share of the observations were within the area where TGC is a suitable measure for growth.

In addition to temperature and size, several unmeasured spatial and temporal factors influences growth both within and between fish-groups, such as occurrence of disease, seasonal variations, breed and environmental differences (light, water quality etc.). These factors are not adjusted by the TGC. To correct for the erroneous results obtained for the observations outside the temperature range for TGC, and to some extent try to adjust for these unmeasured factors, we applied the mean change in TGC in a 7-day period after treatment (ΔTGC) as the outcome for the mixed effect linear regression. If the ΔTGC was equal to zero, this implied the same growth rate the week after treatment as the growth rate the week before the pre-treatment starvation period (baseline TGC) began. A negative ΔTGC implied a lower growth rate the week after treatment compared to the baseline.

To calculate the daily TGC the biological feed conversion ratio (bFCR) is necessary. The true bFCR was not known since bFCR is an estimated value in the production data. In addition, the bFCR models used by different companies vary. Therefore, a simple approach was used where the same bFCR was assumed for all fish-groups during their entire production cycle. This approach did not adjust for the size dependency of bFCR and could cause a small underestimation of the treatment effect on growth.

It is also possible that the feed amount did not perfectly reflect the appetite of the fish. However, this was largely accounted for by analysing the change in growth rate. In addition, since feed constitutes for about 50% of the production cost (Iversen et al., 2017) the farmers have a strong incentive for reducing feed-spill.

One disadvantage of using the change in TGC as an outcome variable was that we might have adjusted for effects related to the prior treatment. This matter was reflected in the univariable analysis when we added weeks between treatments as a fixed effect (Table A.1). In this model, decreasing the number of weeks between treatments showed a positive effect on the ΔTGC after treatment (Table A.1). This made no sense, as the fish would have less time to recover from the last treatment. The reason for this became apparent when the outcome variable was changed from ΔTGC to baseline TGC in a univariable analysis with weeks between treatment as fixed effect (Table A.2). This analysis showed a significant reduction in the baseline when there was less than two weeks between two following treatments (Table A.2). This indicated an additive effect of treatments closer than two weeks. In addition, it showed that the negative effect on the baseline most likely lead to a decrease in ΔTGC, since decreasing number of weeks had a positive effect when the outcome variable was ΔTGC. Even if the variable "weeks between treatment" was statistically significant, it could not be added as an explanatory variable to the full model, because it caused a false positive effect in the estimation of ΔTGC.

In this dataset there were few freshwater treatments against salmon lice ($n = 48$). When the reliability of the dataset was tested by split-sample analysis, the above mentioned was probably the reason why freshwater treatments were not significantly different from medicinal treatment. We found this to be true in one of the split-samples, opposed to the results from the other sample, and the full dataset.

4.2. The effect of treatment methods on growth

The aim of the study was to estimate the effect of different treatments on growth rate after a treatment event.

As the causal diagram (Fig. 2) shows, we included weight of fish, seawater temperature, smolt-age and year-class as fixed effects to control for possible confounding (Fig. 2). The coefficient of the main explanatory variable of interest -treatment method- will thus be interpreted as the conditional total effect on the outcome variable (ΔTGC) at any given level of the other fixed effects, whereas the coefficients of the confounding variables are interpreted differently (Westreich and Greenland, 2013). For temperature, for example, the coefficient in Table 4 is interpreted as the controlled direct effect of temperature on the outcome when treatment method is held fixed at a given level. This blocks the temperature effect on treatment method. Similar interpretation is valid for the coefficients of the variables fish weight, year-class and smolt-age.

The descriptive statistics showed, as expected, that treating Atlantic salmon against salmon lice or AGD had a negative effect on the daily change in growth rate (Fig. 1A). Overall, the decrease in growth rate lasted about seven days, as the median of fish groups were back to baseline growth rate (ΔTGC = 0) seven days after a treatment event (Fig. 1A). It took longer for the non-medicinally treated fish-groups (Fig. 1C-D) to return to base-level growth rate compared to medicinally treated groups (Fig. 1B). The descriptive statistics also indicated a great variation in the daily change in growth the week after treatment (Fig. 1A-D). The statistical analysis and post hoc pairwise comparison between treatments showed some of this variance was explained by treatment method, and there was a significant negative effect on growth when treated non-medicinally or with freshwater bath compared to treatments with hydrogen peroxide, medicinal or combination medicinal bath (Table 4).

Stress and injuries caused by both handling and the treatment method itself, might be one explanation for the reduction in growth rate after all treatment methods. The variation both between and within treatment methods, could be explained by differences in the handling procedures associated with treatments. These procedures include length of crowding time, pumping equipment, type of well-boat and thermal/mechanical treatment rig. One explanation for the significantly larger negative effect of non-medicinal treatments could be that these treatments were stressful and caused injuries. This is supported by previous studies and reports from the field that indicate both injuries, increased mortality and decreased resistance to infections after especially thermal and mechanical treatments (Hjeltnes et al., 2018; Overton et al., 2018; Persson et al., 2022; Sviland Walde et al., 2021). Fig. 1A also showed a great variation in the daily change in growth specifically the second day after a treatment. This might be explained by some treatment events extending into day two, instead of ending on day one. Alternatively, there can be different feeding strategies between companies and sites.

Sea temperature and fish weight are adjusted for in the calculation of TGC to make it feasible with growth comparisons between fish groups in both space and time. However, the sea temperature or fish weight may itself be associated with the outcome variable. In the final model, seawater temperature had a statistically significant positive effect on the outcome after a treatment event, implying a greater reduction in growth rate after treatments at lower temperatures relative to treatments at higher temperatures. In regards to thermal treatments, there will be a higher difference between the temperature in the treatment chamber and in the sea if the seawater temperature is low. A higher delta temperature could lead to a larger temperature shock. This could be more stressful for the fish, since exposing Atlantic salmon to warm water lead to aversive reactions and injuries (Gismervik et al., 2019; Moltumyr et al., 2021; Nilsson et al., 2019; Overton et al., 2018). The possible additional stress of thermally treating at lower seawater temperatures could explain decreased growth at low seawater temperatures. Another explanation could be additional stress caused by a higher risk of secondary infections with *Moritella viscosa* and *Tenacibaculum* spp. in injuries caused by handling and treatment (especially mechanical treatment) as the wound healing process takes longer at lower seawater temperatures (Andrews et al., 2015; Sommerset et al., 2020).

The statistical analysis also showed an increasing negative effect on growth rate as the fish grows. This could be due to the increased force necessary to lift and transport the fish in the treatment rig, and thereby increased force on the fish itself or some treatment methods simply were not suitable for larger fish. Another explanation could be an accumulating effect of several management operations during the production. It is also shown that freshwater treatment of salmon <1 kg was associated with higher mortality compared to freshwater treatment of salmon >1 kg (Sviland Walde et al., 2021). Thus, treatments performed at different temperatures and weight at treatment might give different effects on growth after treatment.

Seawater temperature and fish weight could also have an effect on the choice of the treatment method. It is for example preferable to avoid treatments with hydrogen peroxide at high seawater temperatures, and mechanical treatments at low seawater temperatures (Anonymous, 2020; Veterinærkatalogen, 2022).

The variable year-class served as a proxy for time, and thus reflected the shift from medicinal to non-medicinal treatments, and development in treatment methods. The significantly negative effect of the 2015 year-class compared to the 2014 year-class, might be explained by the introduction of and shift to non-medicinal treatment methods. However, the statistical analysis showed this negative effect was reduced over time. The same trend was observed in Sviland Walde et al., 2021, where the median value and the variation in mortality after thermal treatment methods was reduced over the year-classes (Sviland Walde et al., 2021). An explanation for the reduction of this negative effect over the year-classes might be improvements of the non-medicinal treatment methods over time.

Regarding the smolt-age, there are essential differences between a 0 and 1-yearling, for example time and size of stocking. It was therefore biological sensible to adjust for smolt-age in the model.

4.3. Sources of variation

Most of the unexplained variation in the statistical model was attributed to individual treatment events (58.8%) and treatments performed close in time at a site (36.7%). Little variation was found between different sites (Table 4). This suggests that change in growth after a treatment event was influenced primarily by factors affecting the individual treatment event (e.g., weather conditions, crowding, management of the delousing unit) and factors affecting the fish-groups within a site equal in time (for example environmental conditions or disease status). This further indicated that timing of the treatment and treatment type was of importance for the growth rate after a treatment. We therefore suggest that future studies, with aims of investigating risk factors for the outcome from lice treatments, should gather high-resolution data within the period of treatment describing fish-group characteristics (such as health-status), environment, handling (such as crowding time), in cage or well-boat treatment, chamber temperature with regards to thermal treatment, and management.

4.4. Calculating losses in grams

The estimated adjusted means from treatment method from the mixed effect linear regression model showed a potential short-term loss in weight gain of 52 ± 7.5 g per fish seven days after an individual non-medicinal treatment event compared to a non-treated fish (Table 5). In addition to the lost growth potential caused by the treatment, the pre-treatment starvation period causes a reduction in the growth potential that should be considered when assessing the total effect of treatments on growth. In this study, when including a seven-day period of starvation, 75% of the loss in weight gain was due to the starvation period. The recommended period of starvation for a 3–4 kg salmonid at a seawater temperature of 10 °C is 3–4 days (Anonymous, 2020). In this dataset, the median and average number of starvation days prior to a treatment event, was six days. However, this was based on a cut-off value at maximum seven days of starvation even though some fish-groups in the dataset were starved more than seven days, as we regarded starvation period lasting longer than seven days as unintended. (Anonymous, 2020). Even though the main share of the potential biomass-loss was due to starvation, the period of suboptimal growth the week after treatment was also an important contributor to the overall loss. The estimations in this study was based on feed amount and appetite, and did not include loss of biomass during starvation or a possible negative effect on feed conversion (Einen et al., 1998). The estimation was thus a conservative estimate of the short-term effect of an individual treatment on growth.

Even though the median part of the fish-groups showed a reduction in growth rate that lasted about seven days post treatment, some fish-groups had a positive ΔTGC seven days after treatment (Fig. 1A-D). One possible reason for this could be overfeeding, but it could also indicate increased appetite or compensatory growth. We did not have the ability to investigate the long-term effect on end-harvest weight, since we did not have harvest data. Even though there was quite a substantial loss in potential growth after treatment, Atlantic salmon have great potential to catch up lost growth if they have the time to recover (Hvas et al., 2022). In our study, starvation followed by a non-medicinal treatment reduced the potential weight gain on average by 208 g per fish in a 14-day period. For the fish-groups that experience less than two weeks between treatments, the reduction in weight gain would probably be larger. The number of days between treatments during the production cycle was a median of 38 days and average of 55 days between two treatments. From the last treatment until harvest, there was a median of 54 days and mean of 80 days. Within the first two weeks after an individual treatment, the median part of the fish groups did not show signs of an increased growth rate compared to the growth rate before treatment, ΔTGC >0 (Fig. 1A-D). In a study by Hvas et al., 2022, it was shown after about 3 months of refeeding and growing at a temperature range of 10-16 °C the size gap of 544 g between a fish group starved eight weeks, and the control group were minor (Hvas et al., 2022). Thus, it seems possible that the main part of the fish-groups in our study did not have enough time between treatments (average four treatments during the production cycle) and after last treatment until harvest, to compensate for the growth loss. In addition, other factors affecting appetite and growth such as disease may extend the required time for full compensatory growth. Further studies are needed to investigate the long-term effect of delousing treatments on growth and the extent of compensatory growth between delousing treatments, in addition to the effect treatment might have on bFCR.

In this paper, we have demonstrated how the estimates from the model can be transformed to calculate the potential weight gain loss in grams/fish of one specific treatment, at one specific time. Assuming the fish did not compensate for the lost growth, this would mean a potential extra biomass of 31,200 kg per cage per non-medicinal treatment, if it was possible to avoid this one non-medicinal treatment. If a site contained five cages (average in this dataset), this would add up to 156,000 kg biomass per site per non-medicinal treatment. In support of this, a recent study shows the mean harvesting weight has decreased since 2012, which corresponds with the onset of the current non-medicinal delousing methods (Barrett et al., 2022).

Results from this study could further be included when modelling economic costs of different strategies to combat salmon lice in the aquaculture industry.

5. Conclusion

In this study, the immediate effect of delousing operations on growth of Atlantic salmon post treatment has been estimated. This was achieved by using daily production data on cage level. The results suggest that all treatment methods reduce growth rate seven days after treatment, where thermal and mechanical treatments have a significantly larger negative effect on growth rate compared to medicinal treatments. The results further indicate that timing of the treatment and treatment type is of importance for the growth rate after treatment. If the number of delousing operations, especially non-medicinal operations, could be reduced, this study indicate a potential for an increased growth and a more efficient aquaculture industry.

Data availability statement

Data are subjected to third party restrictions.

Declaration of Competing Interest

Jostein Mulder Pettersen is affiliated with Pharmaq AS, a pharmaceutical company supplying products to salmon production. Magnus Vikan Røsæg is affiliated with SalMar Farming AS, a Norwegian salmon producer.

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The authors do not have permission to share data.

Acknowledgements

This work has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No.101000494 (DECIDE). We would like to thank the aquaculture companies and Aquacloud for supplying production data. A special appreciation is extended to Haakon Christopher Bakka at Norwegian Veterinary Institute for providing useful inputs and help regarding statistical analysis.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2022.738720.

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