

# Estimating cage-level mortality distributions following different delousing treatments of Atlantic salmon (*Salmo salar*) in Norway

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## Abstract

This retrospective descriptive study estimates cage-level mortality distributions after six immediate delousing methods: thermal, mechanical, hydrogen peroxide, medicinal, freshwater and combination of medicinal treatments. We investigated mortality patterns associated with 4 644 delousing treatment of 1 837 cohorts of farmed Atlantic salmon (*Salmo salar*) stocked in sea along the Norwegian coast between 2014 and 2017. The mortality is expressed as mortality rates. We found distributions of delta mortality rate within 1, 7 and 14 days after all six delousing treatments, using mortality rate within 7 days before treatments as baseline. The results show that we can expect increased mortality rates after all six delousing methods. The median delta mortality rates after thermal and mechanical delousing are 5.4 and 6.3 times higher than medicinal treatment, respectively, for the 2017 year-class. There is a reduction in the delta median mortality for thermal and freshwater delousing from 2015 to 2019. There is a wide variability in the mortality rates, in particular for thermal delousing. Our results suggest that the variability in delta mortality for thermal delousing has been reduced from the 2014 to 2017 year-class, indicating an improvement of the technique. However, a significant increase in the number of thermal treatments from 14 in 2015 to 738 in 2018 probably contributes to the overall increased mortality in Norwegian salmon farming.

## KEY WORDS

aquaculture, Atlantic salmon, delousing, mortality, salmon lice

## 1 | INTRODUCTION

There is a growing concern regarding increasing mortality of farmed Atlantic salmon (*Salmo salar*) in Norway (Bang Jensen et al., 2020; Tørud et al., 2019). In the seawater phase of the production, the total cumulated mortality of salmonid fish increased from 2014 to 2018, from 14.3% to 16.8% of the standing stock (Bang Jensen, Qviller, et al., 2020). The increasing mortality constitutes a serious welfare problem as well as an economic challenge. Treatment against salmon

lice by physical removal of the lice (so called non-medicinal treatments) has been discussed as one of the reasons for the increased mortality in the seawater phase of the production (Bang Jensen, Qviller, et al., 2020; Hjeltnes et al., 2019; Oliveira et al., 2021; Overton, Dempster, et al., 2018).

Salmonid aquaculture has struggled with salmon lice since its inception (Costello, 2009). The extensive use of lice treatments has a severe financial impact. The total cost of controlling salmon lice in Norway was estimated at 8.70% of the Norwegian industry's total

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value in 2011 (Abolofia et al., 2017), and NOK 5 billion per year in 2017 (Iversen et al., 2017).

Salmon lice *Lepeophtheirus salmonis* (Krøyer, 1837) are naturally occurring ectoparasites that can infest salmonid fish in salt water in the Northern hemisphere (Pike & Wadsworth, 1999). The parasite feeds on the mucus, skin and blood of the host, causing mechanical damage and osmotic stress. If the infection levels increase beyond the hosts ability to compensate, salmon lice can cause pathology and even be lethal to its host (Finstad et al., 2000; Pike & Wadsworth, 1999). However, in Norway the maximum permitted number of adult female lice per cultured fish ensures that pathology in the fish rarely occurs (NFD, 2012). To keep lice levels below the legal limit, farmers use different preventive measures as for example, cleaner fish or so called "lice skirts", but they are still dependent on measures for immediate delousing.

Over a long time period, large-scale reliance on a few chemotherapeutants to control salmon lice has resulted in a drift towards resistance (Denholm et al., 2002; Myhre Jensen et al., 2020). Today, salmon lice display reduced sensitivity and/or resistance towards all available chemotherapeutants, except benzoylphenyl ureas (Aaen et al., 2015; Helgesen et al., 2020). Around 2015, thermal and mechanical delousing methods were introduced as non-medicinal substitutes (Helgesen et al., 2020; Overton, Dempster, et al., 2018), and from 2015 to 2016, the industry rapidly shifted from medicinal to non-medicinal treatments. Currently, in Norway thermal and mechanical delousing are the most frequently applied treatment methods for immediate removal of salmon lice (Helgesen et al., 2020). In 2019, about 3.3 non-medicinal treatments occurred for each medicinal treatment event (Myhre Jensen et al., 2020), and of all immediate delousing treatments performed in 2019, 54% were thermal and 27% mechanical (Oliveira et al., 2021).

Previous studies and reports from field indicate both injuries and increased mortality after mechanical, thermal and hydrogen peroxide treatment (Hjeltnes et al., 2018; Overton, Dempster, et al., 2018; Overton, Samsing, et al., 2018). Less is known about mortality after freshwater treatment. Most of the immediate delousing methods involve crowding and pumping, which is stressful for the fish (Ashley, 2007). The principle of thermal treatment is to inactivate the lice by exposing salmon and lice to warm water for a short time (Grøntvedt et al., 2015; Roth, 2016). In mechanical delousing, the lice are removed by either brushing or flushing the louse from the fish (Gismervik et al., 2017; Nilsen et al., 2010). Exposing Atlantic salmon to warm water can lead to aversive reaction and injuries (Gismervik et al., 2019; Moltumyr et al., 2021; Nilsson et al., 2019).

Salmon mortality is a negative effect of delousing. To our knowledge, mortality after different delousing methods has not been quantified and compared on a cage level. Neither has the change over the past years been explored. There can be large variation in mortality between groups of fish within a site (Bang Jensen, Qviller, et al., 2020). Production data from aquaculture companies have a cage-level resolution, which makes it possible to describe variation between groups of fish when looking at mortality after delousing. If variability in mortality after delousing exists, a potential for reducing the mortality also exists. Knowledge of both the effect from treatment measures and their side effects in terms of biological and financial losses is

important when deciding on treatment strategies. Therefore, the aim of this study is to estimate the mortality distribution after different delousing treatments on a cage level to aid understanding the potential for reducing biological losses and provide a fundament for an up-to date cost estimation for salmon lice treatments.

## 2 | MATERIALS AND METHODS

### 2.1 | Study population

Salmonid farming commonly consists of a freshwater and a marine phase. Stocking of smolt in marine farms occurs in either the spring or the fall, and fish are kept in cages until slaughter, usually after 14–19 months. Fish stocked in the same year is here defined as a year-class. A marine farm is usually comprised of several cages, with a legal maximum limit of 200 000 fish per cage (NFD, 2008b). During the marine phase, groups of fish are often moved, and sometimes split or merged between cages within a farm. On occasion, they are also transferred between farms.

Five large Norwegian salmonid farming companies (companies with over > 20 farms operating in multiple production zones) were asked to provide data for this study. Three companies agreed. We choose to ask only large companies to provide data for this study to increase the chance of achieving a satisfying spatio-temporal distribution of the different delousing treatments.

We received data describing four year-classes of farmed Atlantic salmon stocked in sea at 295 farms (210 unique) along the Norwegian coast, from 2014 to 2017. The study period started in year 2014 and ended in 2019. During the study period there were 717 active farms along the coast. Since 2017 the Norwegian coast has been divided into 13 production zones, based on hydrographic properties and estimated capacity of production (Ådlandsvik, 2015; NFD, 2017).

### 2.2 | Data

Norwegian aquaculture farming companies register daily production data concerning stocked fish, mortality numbers, treatments, feeding, lice counts, environmental data etc. (NFD, 2008a). The data consist of cage-level historical production data related to salmon lice treatments and mortality, registered from 2014 to 2019. Two of the companies extracted the data directly from their production data monitoring system. AquaCloud, a digital database standard for the aquaculture industry in Norway (NCE Seafood Innovation, 2017), provided data on behalf of the third company. One company delivered accumulated production data related to a salmon lice treatment on the following different time periods: within 7 days before treatment, on the treatment day, and within 7 and 14 days after treatment. The other two companies supplied daily production data. Since registration of production data in AquaCloud started first of January 2017, production data concerning the 2015 and 2016 year-class from one company were incomplete. However, the year-class of 2014 was complete as AquaCloud used this as an initial trial data set.

The data included the following variables: production zone, company name, year-class, farm identification number, cage identification number, fish group number, date of delousing, method of delousing, active substance (in case of medicinal treatment), number of fish, estimated average weight of fish, and number of dead fish.

A key in understanding the dynamics of mortality is the possibility to follow the same cohort before and after treatment. In this study, a cohort is a group of fish defined by production zone, company, farm number and cohort identification number. When cohorts are deloused, they are often transferred from one cage to another. In these cases, tracking groups of fish before and after treatment based on cage number is not possible. At stocking the different groups of fish are usually designated a number in the production data. In more recent production data, this number serves as a consistent identification number, making it possible to track the same cohort from stocking in sea to harvesting. In the accumulated data set provided by one company, all the cohorts had a consistent identification number 7 days before and 14 days after treatment, but only the year-class of 2016 and 2017 had an identification number consistent from stocking to harvesting. In the two data sets with daily registration, several cohorts did not hold a consistent identification number and the fish group number designated to the cohorts in the production data, changed when the group transferred from one cage to another, and split or merged with other cohorts. In such cases, the high resolution of the daily production data often made it possible to detect and trace cohorts by comparing estimated average weight and number of fish in each cage before and after transfer. We traced the cohorts 7 days before to 14 days after treatment, or if feasible from stocking to harvest, and gave them a unique identification number. After this process, about 20% of the original identification numbers from one daily data set, and 50% from the other daily data set, remained.

Occasionally, there were movements of several groups of fish with approximately equal weights and numbers to different cages at the same day, or adjustment of weight and number in a group during handling/transfer. As a result, the weight and number before and after movement between cages is not always identical. Therefore, we cannot dismiss the possibility that some cohorts were not correctly traced. These examples also highlight the importance of consistent tracing procedures to exploit industry data entirely in high-resolution analyses.

Production data concerning species other than Atlantic salmon were excluded from the study data. Moreover, farms that were registered at the Directorate of Fisheries as brood stock, hatcheries or research farms were also excluded. Further, cohorts were excluded if they were moved from one farm to another, split or merged with other cohorts, or impossible to trace. We categorized the cohorts in the following weight-classes: <2kg, 2–3kg, 3–4 kg, 4–5kg and > 5 kg in order to describe delta mortality over different weight-classes.

### 2.3 | Salmon lice treatments

The production data included information on the date of treatment, cause for treatment, treatment method and active substance. Based on this information delousing treatments were divided into

six different categories as presented in Table 1. It was not possible to categorize delousing as treatment in cage or well boat, as recording of this information was not consistent. We did not expect that delousing with medicinal feed would give increased mortality (Veterinærkatalogen, 2020); therefore treatment with medicinal feed was not included in this study. Hydrogen peroxide and freshwater treatment against only amoebic gill disease (AGD) was excluded. Hydrogen peroxide and freshwater treatment against both AGD and salmon lice were included.

### 2.4 | Calculating mortality rate

Mortality during four different time periods: within 7 days before delousing and within 1, 7 and 14 days after delousing were set to ensure comparability of all the data included in the study and under the assumption that the effects of delousing would decrease after two weeks. Any extension after two weeks was not attempted, with the risk of accidentally including any subsequent delousing. During the four different time periods of interest, fish could leave the cohort for other reasons than death, for instance slaughter or sampling. A cohort was included in the study even if the entire cohort were slaughtered between 8 and 14 days after treatment. Premature withdrawal or entering of animals during the time period defines a dynamic cohort; hence, the appropriate incident measure of deaths is mortality rate (Mrate) (Toft et al., 2004).

We calculated the mortality rate for the four different time periods using Equation 1 (Toft et al., 2004). The time periods are expressed in number of days. Thus, the denominator is an approximation of number of fish days at risk, and Mrate is expressed in number of dead fish per fish-day.

$$\text{Mrate} = \frac{\text{number of dead fish during time period}}{\frac{\text{number of fish at risk at start} + \text{number of fish at risk at end}}{2} \times \text{time period}} \quad (1)$$

We set the mortality rate within 7 days before delousing as baseline mortality, and subtracted this from the mortality rate at 1, 7 and 14 days after delousing to find the change in mortality rate, referred to as delta mortality rate,  $\Delta\text{Mrate}$  (equation 2). The  $\Delta\text{Mrate}$  will have a positive sign in case of increased mortality after a delousing compared to the mortality prior to treatment, or negative in the opposite case.

$$\Delta\text{Mrate} = \text{Mrate}_{\text{post treatment}} - \text{Mrate}_{\text{prior treatment}} \quad (2)$$

### 2.5 | Data management and descriptive statistics

Data management and statistical descriptive analysis was performed in the statistical software package Stata ® SE 15.1 (StataCorp, College Station, Texas USA) and Microsoft ® Office Excel (bar diagrams). We produced tables, box plots and graphs with the calculated  $\Delta\text{Mrate}$  and its distribution over year, year-classes and weight-classes.

Categories ( <i>n</i> = 6) of delousing 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 (H <sub>2</sub> O <sub>2</sub> ) bath in cage or well boat
Medicinal bath	Medicinal bath in cage or well boat using one of the following active substances: a. Azamethiphos b. Cypermethrin c. Deltamethrin d. Imidaclorpid e. Other
Freshwater bath	Freshwater bath in cage or well boat
Combination	Treatment of the same cohort with two different delousing methods on the same day. Includes the following combinations: a. two different active substances b. hydrogen peroxide and medicinal bath

## 2.6 | Transformation of ΔMrate to number of dead fish

We used the distribution of ΔMrate obtained for each of the six different treatment categories, to find the median ΔMrate. We transformed the obtained median ΔMrate to number of dead fish to provide an example of the absolute median number of accumulated dead fish following the different delousing treatments. The accumulated number of dead fish 14 days after treatment, is calculated using Equations 3 and 4 (Toft et al., 2004). N<sub>t</sub> is the number of fish in the end of the period (t), N<sub>0</sub> is the number of fish at start set to 150 000 fish, e is Euler's number, Mrate is median ΔMrate 14 days after the six different treatment methods and t is time period which is 14 days. If ΔMrate is negative, this gives a negative number of accumulated number of dead, meaning fewer fish die after the treatment, than before.

$$N_t = N_0 e^{-Mrate \cdot t} \quad (3)$$

$$N_0 - N_t = \# \text{dead} \quad (4)$$

## 3 | RESULTS

### 3.1 | Salmon lice treatments

The data set originally consisted of 6 131 delousing treatments of four year-classes of Atlantic salmon in 295 farms (210 unique) during

**TABLE 1** Categories (*n* = 6) of delousing operations applied in farmed Atlantic salmon in three Norwegian companies from 2014–2019

**TABLE 2** Overall frequency of treatments within the different categories from year 2014 to 2019. The treatment category "Combination" contains combination of two different medicinal baths or combinations of hydrogen peroxide bath and medicinal bath

Delousing category	Frequency	Per cent
Thermal	2 692	57.97
Mechanical	619	13.33
Hydrogen peroxide	445	9.58
Medicinal bath	198	4.26
Freshwater bath	172	3.70
Combination	518	11.15
Total	4 644	100

2014–2019. After exclusion of cohorts that were split or merged, or not possible to trace, the data set consisted of 4 644 delousing treatments (N) (Table 2) in 214 farms (159 unique) in 1 837 cohorts consisting of about 24.7 billion fish. The first delousing occurred in May 2014 and the last in April 2019.

Table 2 shows the overall frequency distribution of treatments for the six delousing categories. More than half of the treatments are thermal delousing (58%), while the categories "medicinal baths" and "combination" constitutes 15%. Table 3 shows the frequency distributions of treatment categories from 2014 to 2019. Most treatments are performed in 2015–2018, since the data are gathered per year-class. From 2015 to 2016, there is a pronounced

shift in the frequency distributions of treatments. In 2015, half of the total amount of treatments is combination, over 30% hydrogen peroxide baths and only 2.6% of the total amount of treatments is non-medicinal (thermal and mechanical). In 2016, the non-medicinal treatments make up 78% of the total amount of treatments, and 54% of the total amount constituted of thermal treatments. There is also an increased use of freshwater baths, and at the same time a decrease in hydrogen peroxide baths and combination treatments. The main share of treatments continues to be non-medicinal treatments from 2017 to 2019. Table 4 shows the frequency distribution of treatment categories over year-classes. In the 2014 year-class the categories "hydrogen peroxide baths" and "combination" make up the main share of treatments, and a few experimental tests of thermal treatments. In the 2015 year-class non-medicinal treatments make up 75% of the total amount of treatments. The main share of treatments continues to be non-medicinal in the 2016 and 2017 year-classes.

The treatments took place in 159 unique farms in 10 of 13 production zones in Norway (Table 5). Treatment events are registered in production zone 1–9 and 11, but most of the treatments in the data are in production zone 3–7 (Table 5).

### 3.2 | Mortality

The N, mean, median, minimum, maximum and standard deviation of the mean for the baseline mortality Mrate within 7 days before treatment and  $\Delta Mrate$  within 1, 7 and 14 days after treatment for all six categories over years and year-classes are shown in Tables S1 and S2. There is a discrepancy in N between 1 and 7 to 14 days after treatment because the data set from one of the companies did not contain the number of dead one day after treatment, but rather the number of dead at treatment day. There is also a slightly different N between 7 and 14 days after treatment for thermal, mechanical and freshwater bath, because all fish in some cohorts were slaughtered between 8 and 14 days after treatment.

Figure 1 shows that the median  $\Delta Mrate$  for thermal treatment is the highest of all six treatment categories one and two weeks

post-treatment. The median  $\Delta Mrate$  one day after treatment is highest for mechanical delousing. The median  $\Delta Mrate$  increases one week after thermal, hydrogen peroxide and freshwater treatment and then declines the following week. For mechanical, medicinal and combination treatment, the median  $\Delta Mrate$  has a slight decrease over the two-week period after treatment.

For all categories, the mean has a greater positive value than the median for all periods, which implies that there are some cohorts that experience very high mortality (positive outliers), contributing to a positive skewness (Tables S1 and S2). For example, in 2017 year-class (Table S2) the maximum  $\Delta Mrate$  two weeks after a thermal delousing was 0.0102928 compared to the median  $\Delta Mrate$  of 0.0003772.

For all six categories, the variability in  $\Delta Mrate$  is the highest for thermal, hydrogen peroxide and freshwater treatment. For all methods, the variability is reduced two weeks after treatment.

Further, we wished to explore if there has been a change in the  $\Delta Mrate$  over the past years and year-classes, especially for the newer delousing methods, thermal and mechanical. Here we have chosen to represent the change in number of treatments and  $\Delta Mrate$  both as yearly change (Figure 2) and as change over year-class (Figure 3). It is important to note that when looking at these results different year-classes, and thus size of fish, is represented in the different years.

For thermal treatments, the median  $\Delta Mrate$  shows a yearly decline from 2015 to 2018, followed by a rise in 2019 (Figure 2a). When looking at  $\Delta Mrate$  for thermal treatment over year-class (Figure 3a), there is a decrease from year-class 2014 to 2017. For mechanical delousing, there is an increase in median  $\Delta Mrate$ , both over the years (Figure 2b) and year-classes (Figure 3b). Hydrogen peroxide baths show no apparent pattern of change in the median  $\Delta Mrate$  over year (Figure 2c) and year-class (Figure 3c). For medicinal bath, the median  $\Delta Mrate$  declines with respect to both year (Figure 2d) and year-class (Figure 3d). For freshwater baths (Figure 2e), there is a decrease in median  $\Delta Mrate$  one and two weeks after treatment from 2016 to 2018. For combination treatment, there is an increase in the median  $\Delta Mrate$  over both year (Figure 2f) and year-class (Figure 3f). From year-class 2015 to 2016, this increase is pronounced; however, this is also the year-classes with the lowest N of this treatment method.

**TABLE 3** Frequency of treatments within the different categories from year 2014 to 2019. The treatment category "Combination" contains combination of two different medicinal baths or combinations of hydrogen peroxide bath and medicinal bath. The data are gathered per year-class

Delousing category	Year						Total
	2014*	2015	2016	2017	2018	2019*	
Thermal	0	14	436	1 446	738	58	2 692
Mechanical	0	9	194	243	173	0	619
Hydrogen peroxide	29	297	49	41	29	0	445
Medicinal bath	1	94	33	16	54	0	198
Freshwater bath	0	6	54	87	25	0	172
Combination	16	452	40	10	0	0	518
Total	46	872	806	1 843	1 019	58	4 644

\*Year 2014 represents only treatments of first year in sea of year-class 2014. Year 2019 represents only treatments of the last year in sea of year-class 2017. First treatment is in May 2014 and last in April 2019.

The average estimated weight of fish in the cohorts at the time of treatment ranged from 0.119 kg to 6.68 kg. Figure 4 shows box plot of the  $\Delta M$ rate one week after treatment, over different weight-classes for different delousing methods. Thermally delousing salmon between 4-5kg and freshwater bath of salmon under 2kg have the highest median  $\Delta M$ rate of all weight-classes and treatment methods. For freshwater baths of salmon under 2kg, the positive skewness and large positive variation imply some treatments with large mortalities. For thermal, mechanical and hydrogen peroxide treatment, the median  $\Delta M$ rate rise for each kg up to 4kg.

### 3.3 | Actual numbers of dead fish due to treatment

Figure 5 shows the median  $\Delta M$ rate transformed to the accumulated number of dead fish the first two weeks after delousing in a cage holding  $N = 150\,000$  salmon (close to the median population size in the data set which is 143 000). There is a variation in median mortality between the different delousing methods. In this study medicinal bath have the lowest and thermal treatment the highest median  $\Delta M$ rate. When calculating mortality within two weeks after treatment using median  $\Delta M$ rate of the 2017 year-class, 146/ 150 000 salmon died after medicinal delousing treatments, compared to 790

**TABLE 4** Frequency of treatments within the different categories from year-class 2014 to 2017. The treatment category "Combination" contains combination of two different medicinal baths or combinations of hydrogen peroxide bath and medicinal bath

Delousing category	Year-class					Total
	2014	2015	2016	2017		
Thermal	14	481	1 449	748		2 692
Mechanical	0	169	284	166		619
Hydrogen peroxide	324	52	32	37		445
Medicinal bath	114	13	21	50		198
Freshwater bath	5	55	85	27		172
Combination	419	91	8	0		518
Total	876	861	1 879	1 036		4 644

**TABLE 5** Frequency of treatments within the different categories over production zones in Norway. The treatment category "Combination" contains combination of two different medicinal baths or combinations of hydrogen peroxide bath and medicinal bath

Delousing category	Production zone										Total
	1	2	3	4	5	6	7	8	9	11	
Thermal	47	207	272	523	398	790	272	133	19	31	2 692
Mechanical	1	3	16	7	54	337	125	62	7	7	619
Hydrogen peroxide	0	70	177	61	52	60	25	0	0	0	445
Medicinal bath	15	97	4	32	11	25	0	3	0	11	198
Freshwater bath	0	2	8	0	19	137	6	0	0	0	172
Combination	0	0	41	17	110	309	35	0	6	0	518
Total	65	380	522	654	669	1 672	465	205	34	49	4 644

/150 000 after thermal and 928/150 000 after mechanical treatment of the same year-class. There is also a variation in median mortality between the year-classes within the same delousing methods. In 2014 year-class almost 4,000 salmon died within the first two weeks after thermal delousing, compared to 790 in the 2017 year-class.

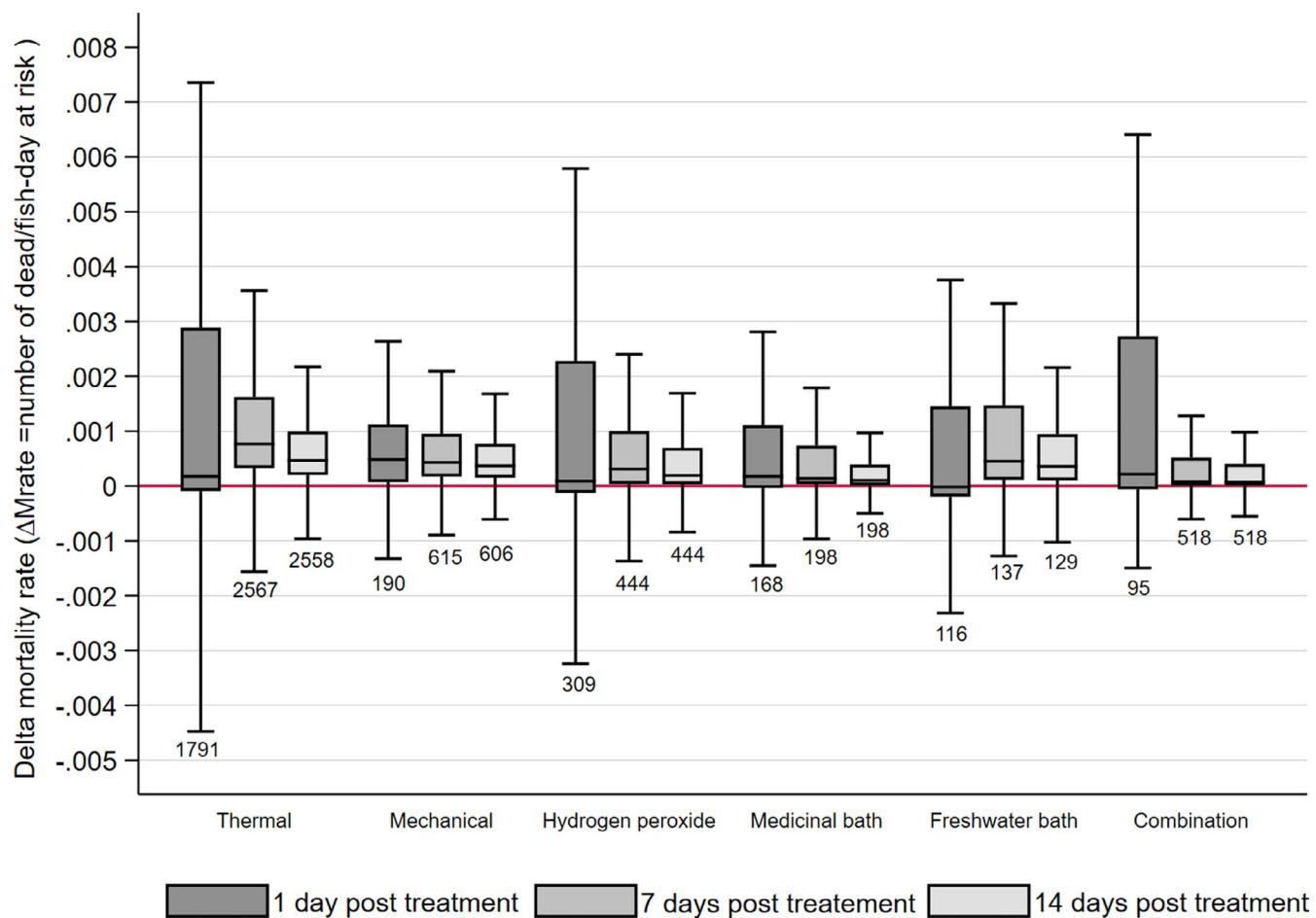
## 4 | DISCUSSION

The results show that we can expect elevated mortality after all of the six different delousing methods. There is variation in median delta mortality between the categories, where the overall median delta mortality after thermal and mechanical delousing is the highest of all methods. Median delta mortality decreases within two weeks after delousing, but not to the level before delousing for thermal, mechanical, hydrogen peroxide and freshwater treatments. Median delta mortality evolves differently between the delousing methods. We also find wide variability in delta mortality within the different delousing methods.

The results demonstrate that we can expect increased mortality after all six delousing methods (Figure 1), where the overall median delta mortality is the highest for thermal and mechanical delousing. This corresponds to the findings in a recent study done by Oliveira et al. (2021) where the most detrimental factor among all the mortality determinants analysed was the use of non-medicinal treatments for sea-lice.

The wide variability in delta mortality within, and variation between, the treatment methods could be associated with the delousing method itself, the health condition of the fish, environmental factors, or managerial factors at farm or company level.

All six delousing categories presented, require handling prior to treatment. Depending on method and duration of handling (crowding, pumping etc.), handling can cause stress, panic, injuries, decreased immunity and death (Ashley, 2007). Thus, the additional stress related to treatment handling, could itself cause increased mortality. If the delousing methods require different degrees of handling prior to treatment, this could explain some of the differences in mortality between methods. For example, farmers can delouse with hydrogen peroxide, medicinal and freshwater bath, in either cage or



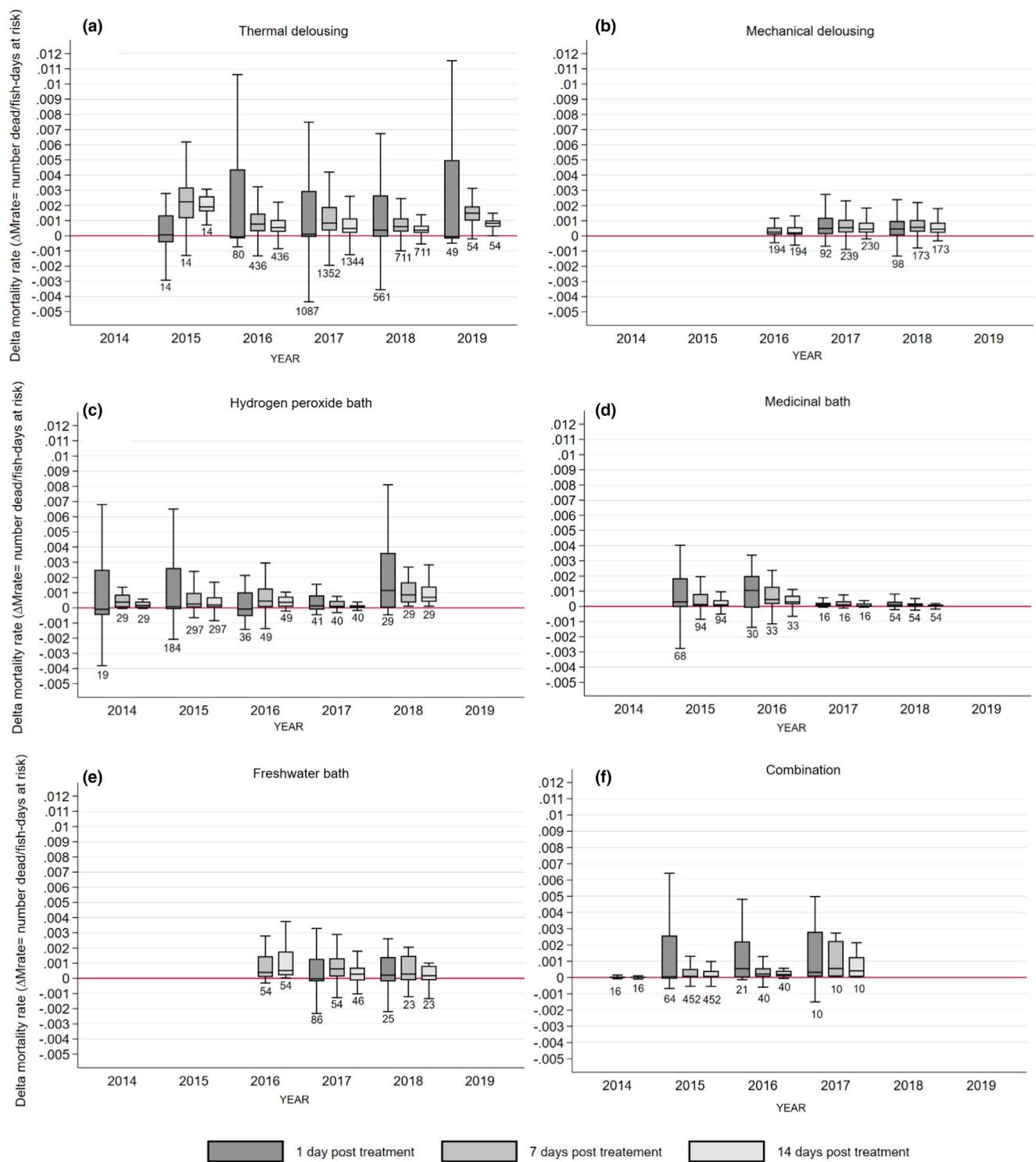
**FIGURE 1** Boxplots showing delta mortality rate ( $\Delta M\text{rate}$ ) and its development 1, 7 and 14 days after six different categories of delousing treatments (thermal, mechanical, hydrogen peroxide, medicinal, freshwater and combination treatments). The treatment category "Combination" contains combination of two different medicinal baths or combinations of hydrogen peroxide bath and medicinal bath. The solid line within the coloured box indicates the median, and the coloured boxes indicate the  $\Delta M\text{rate}$  of 50% of the treatments. The number beneath the box is  $N$  = number of treatments. The solid red reference line ( $\Delta M\text{rate} = 0$ ) shows mortality rate after delousing equal to the baseline mortality rate within 7 days before treatment. Note that the  $\Delta M\text{rate}$  can be negative, which implies a reduction in mortality rate after delousing. The outliers are excluded from the visual presentation, but not the calculation [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

well boat. Performing a treatment in a well boat will require more handling than in-cage treatment.

Another explanation for the increased mortality after delousing and the differences in median delta mortality between the methods could be the degree of distress the delousing methods themselves inflict on the fish. In this study, both thermal and mechanical treatments have the highest median delta mortality. In a survey, fish health personal reported that they had experienced both thermal and mechanical treatments to cause a larger increase in mortality compared to medicinal and freshwater treatment (Hjeltnes et al., 2019). In the same survey, they also reported panic and episodes of poor water quality in treatment chamber and injuries after thermal treatment. In laboratory trials, exposing salmon to warm water caused rapid swimming and vigorous aversive reaction, a panic behaviour that can cause injuries in closed systems (Gismervik et al., 2019; Molsumyr et al., 2021; Nilsson et al., 2019). Less is known about how mechanical treatment affects fish. Gismervik et al. (2017), however,

described industrial mechanical treatment having different effects on fish welfare and mortality, where a significant increase in bleeding from gills and scale loss were observed.

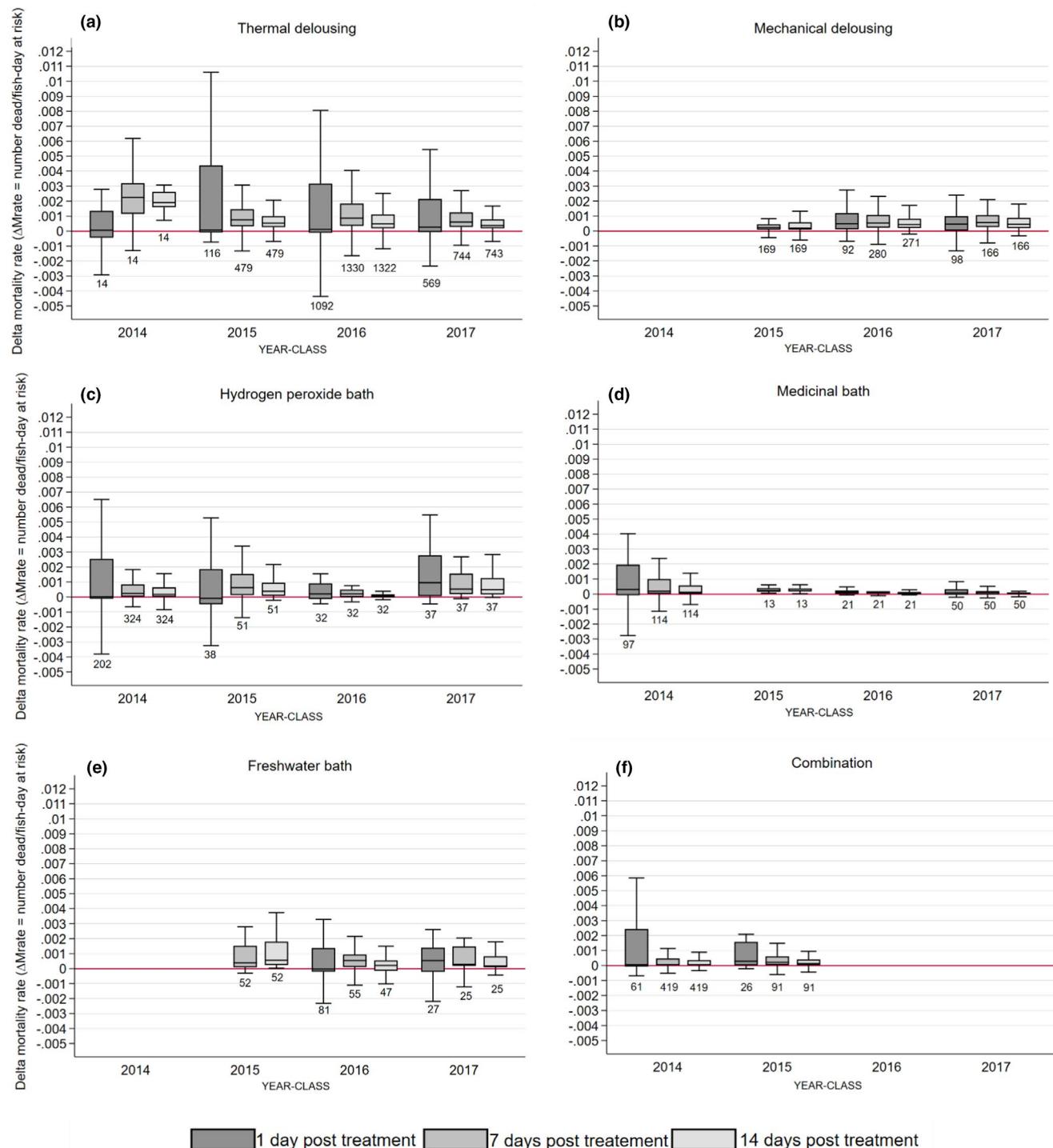
Regarding all delousing methods, median delta mortality generally decreases after two weeks, but not to the level it was prior to treatment. We also observed a difference between the delousing methods in progression of median delta mortality from one to 14 days after treatment. For thermal, hydrogen peroxide and freshwater delousing, the fish die with an increasing rate from day two to seven. The following week, the rate then declines. On the contrary, the median rate for mechanical, medicinal and combination treatment have a slight decrease within the first week and further decrease the following week. The reason for this delayed effect after treatment could be damages inflicted on the fish through handling and treatment method itself. In this study, treatments against only AGD are excluded. However, freshwater and hydrogen peroxide treatments are in some cases applied to



**FIGURE 2** Boxplots show yearly change in delta mortality rate ( $\Delta M\text{rate}$ ) 1, 7 and 14 days after thermal (a), mechanical (b), hydrogen peroxide bath (c), medicinal bath (d), freshwater bath (e) and combination treatments (f). The treatment category "Combination" contains combination of two different medicinal baths or combinations of hydrogen peroxide bath and medicinal bath. Boxplots are made the same way as for Figure 1. The solid red reference line ( $\Delta M\text{rate} = 0$ ) shows mortality rate after delousing equal to the baseline mortality rate within 7 days before treatment. Categories with <10 treatments are not shown. The outliers are excluded from the visual presentation, but not the calculation [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

treat against salmon lice and AGD at the same time. Thus, one explanation for the increasing mortality rate seen after hydrogen peroxide and freshwater could be poor gill health. Depending on

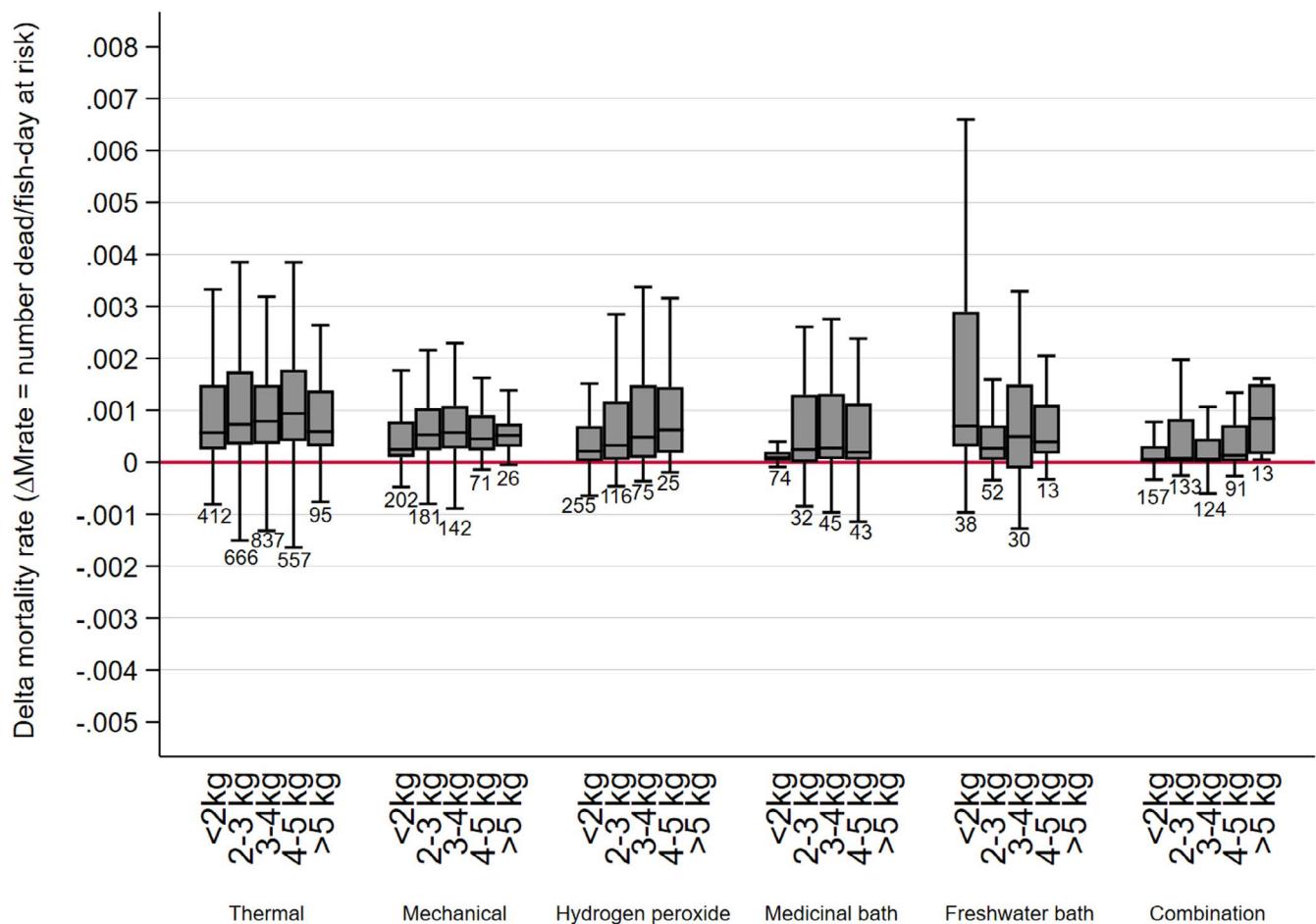
the degree of poor gill health the mortality could be immediate or delayed.



**FIGURE 3** Boxplots show change over year-classes 2014–2017 in delta mortality rate ( $\Delta M\text{rate}$ ) after thermal (a), mechanical (b), hydrogen peroxide bath (c), medicinal bath (d), freshwater bath (e) and combination treatments (f). The treatment category "Combination" contains combination of two different medicinal baths or combinations of hydrogen peroxide bath and medicinal bath. Boxplots are made in the same way as for Figure 1. The solid red reference line ( $\Delta M\text{rate} = 0$ ) shows mortality rate after delousing equal to the baseline mortality rate within 7 days before treatment. Categories with < 10 treatments are not shown. The outliers are excluded from the visual presentation, but not the calculation [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

For all categories, the mean delta mortality has a greater positive value than the median, which implies that there are some cohorts that experience very high mortality after delousing, contributing to a positive skewness. For example, in the 2017 year-class the maximum delta

mortality rate after a thermal delousing corresponds to 19 961/150 000 salmon that died within two weeks after treatment. The median delta mortality rate corresponds to 790/150 000. The very high delta mortality and variability in mortality within the delousing treatments could



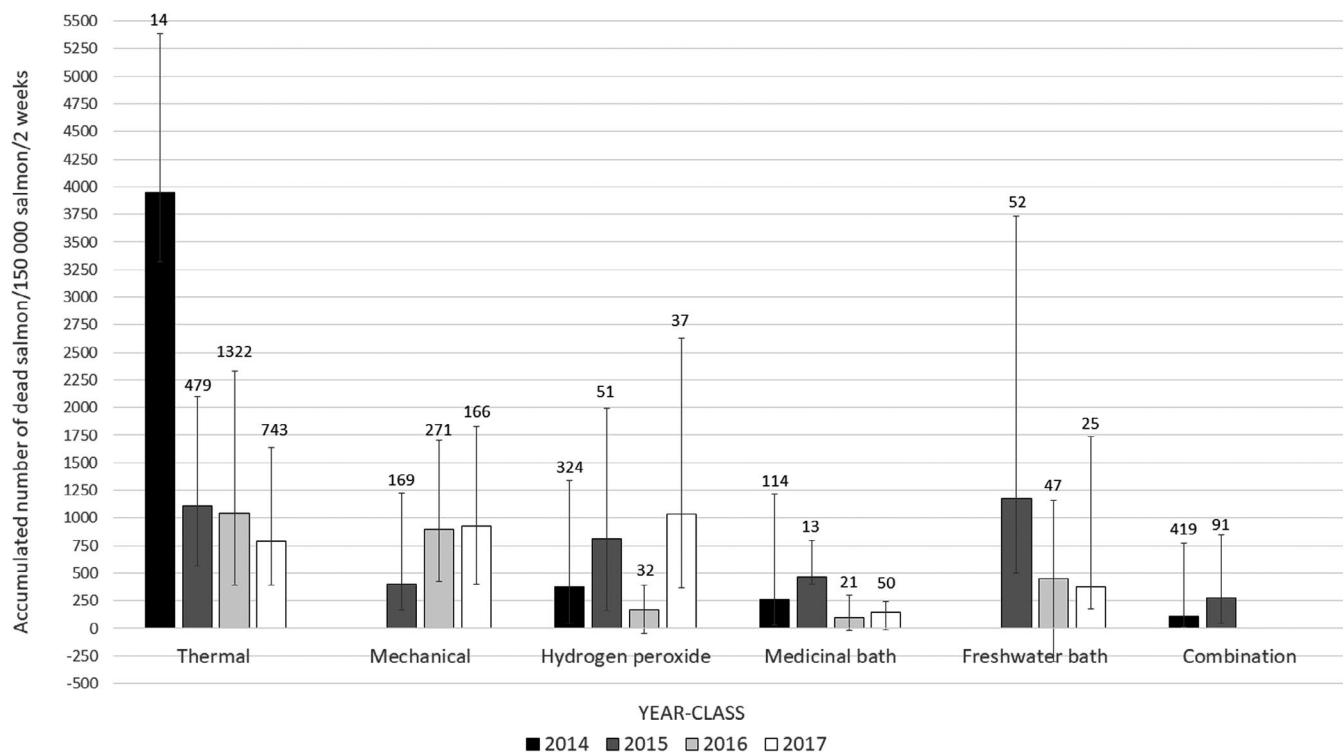
**FIGURE 4** Boxplots show change over weight-classes: <2 kg, 2-3 kg, 3-4 kg, 4-5 kg and >5 kg in delta mortality rate ( $\Delta Mrate$ ) 7 days after thermal, mechanical, hydrogen peroxide, medicinal, freshwater and combination treatments. The treatment category "Combination" contains combination of two different medicinal baths or combinations of hydrogen peroxide bath and medicinal bath. Boxplots are made in the same way as for Figure 1. The solid red reference line ( $\Delta Mrate = 0$ ) shows mortality rate after delousing equal to the baseline mortality rate within 7 days before treatment. The outliers are excluded from the visual presentation, but not the calculation [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

be due to poor health/diseases, environmental factors (lack of oxygen), treatment failure, differences in skill and treatment rigs etc. Identifying such risk factors may prevent episodes of high mortality.

An explanation for the variability in delta mortality within especially the newly introduced delousing methods could be improvements in knowledge, and management of treatment method. Implementing new methods is a continuous process of learning, evaluation and improvement. With increased experience, it is likely that technical equipment is adapted to decrease mortality after treatment. It is also likely that most companies have procedures to externalize experience made by farmers, related to for example handling or crowding fish prior to treatment. Regarding mechanical delousing, however, this study shows an increase in median delta mortality and no apparent change in variability in delta mortality over year (Figure 2) and year-classes (Figure 3). On the contrary, for thermal and freshwater treatment, we observe a decrease in both median and variability in delta mortality rate over the years and year-classes, with one exception. From year 2018 to 2019 there is an increase in median delta mortality after thermal treatment. Thermal

treatment was introduced as delousing method in 2015, and we observe a yearly decrease in median delta mortality until 2018 and then an increase from 2018 to 2019. However, looking at change in delta mortality over year-class there is a consistent decrease from 2016 to 2017 year-class. It is important to remember this data set ends with the 2017 year-class. Therefore, 2019 represents only the second year of the 2017 year-class, which consist of mainly large fish, and this probably bias the results in year 2019. However, this increase in median delta mortality from year 2018 to 2019 might suggest that larger fish are more at risk of dying after thermal delousing. Therefore, we investigated delta mortality and fish weight.

The results of this study indicate that mortality increase with increasing weight up to 4 kg for all treatment methods, except freshwater. It is interesting that freshwater bath of salmon under 2 kg have one of the highest median delta mortalities. One explanation could be that subjecting salmon adapted to seawater to an abrupt transfer to freshwater will be more stressful to a young salmon (Powell et al., 2015). Salmon between 4-5kg that have been thermally deloused have the largest delta mortality within



**FIGURE 5** Accumulated number of dead fish 14 days after thermal, mechanical, hydrogen peroxide, medicinal bath, freshwater bath and combination of delousing treatments in an initial population of 150 000 before treatment. The treatment category "Combination" contains combination of two different medicinal baths or combinations of hydrogen peroxide bath and medicinal bath. The error bars show the interquartile range (25%–75%). Number above error bars are N = number of treatments. Categories with < 10 treatments are not shown

and between all treatment categories. Thus, difference in weight might explain some of the variability in delta mortality within the delousing methods.

Weight increase with number of months in sea, and number of months are shown to be significant factors determining the level of mortality in sea phase aquaculture of salmonid fish (Bang Jensen, Qviller, et al., 2020). By examining mortality in cohorts slaughtered from 2014 to 2018, Bang Jensen, Qviller, et al. (2020) found that median mortality rate increased from the fourth to the 15th month across all years. Kilburn et al. (2012) found that mortalities due to pancreas disease are generally observed in larger fish (2–5 kg), later in the production cycle. Risk of developing heart and skeletal muscle inflammation increased with increasing months at sea (Kristoffersen et al., 2013). Risk of developing cardiomyopathy syndrome increased with the number of days at sea (Bang Jensen et al., 2020). Thus, one possible explanation for median delta mortality increasing with increasing weight might be larger fish being more at risk of having these detrimental underlying diseases than younger fish.

Occurrence of underlying diseases that do not necessarily cause death unless extra stress is inflicted on the fish such as through delousing, can also explain variability in delta mortality after delousing. If health prior to treatment is poor, but mortality rate is low, this could result in a larger change in delta mortality after delousing, compared to a situation where prior treatment health is poor and mortality higher. Another explanation for the increased delta mortality in larger fish could be that these have been through several

treatments, and there could be a build-up effect of repeated exposure to treatments.

Region (production zone) is also shown to be significant factors determining the level of mortality in sea phase aquaculture of salmonid fish (Bang Jensen, Qviller, et al., 2020). Regional variation in farm density and thus differences in host-density-dependent diseases might explain some of the variation seen in this study. Another explanation could be regional environmental differences, in for example sea temperature. Regional differences could also be one reason for the observed smaller variability in delta mortality after mechanical treatment compared to thermal treatment, as half of the mechanical delousing are in production zone 6. Preference could also be an explanation if mechanical treatment is only preferred in certain circumstances.

In this study, the most often used delousing methods are thermal (58%) and mechanical (13%). Variability and median delta mortality after thermal treatment has decreased from the 2015 year-class to the 2017 year-class, suggesting an improvement of the method. However, there is an increase in the number of thermal treatments from 14 in 2015 to 738 in 2018. Also, medicinal baths were the most commonly used delousing method until 2015 (Overton, Dempster, et al., 2018). If we compare medicinal baths to thermal treatment of the 2017 year-class, 146/150 000 salmon die within two weeks after medicinal bath. On the contrary, 790/150 000 and 928/150 000 salmon die within two weeks after thermally and mechanically treating the same

year-class. This is a substantial increase. Also, if we use the median delta mortality for thermal treatment and the 2 692 thermal treatments in this data set, this adds up to over 2 million Atlantic salmon have died after thermal treatment from 2015 to 2019. This is a huge welfare and economic burden.

The present data set contains a large number of treatments for the most frequently applied delousing methods. The analysis of this data set also confirms the reported national yearly reduction of medicinal treatments and paradigm shift from medicinal to non-medicinal delousing treatments in Norway (Aaen et al., 2015; Helgesen et al., 2020; Myhre Jensen et al., 2020; Overton, Dempster, et al., 2018). The substantial increase in total number of treatments from year 2016 to 2017 (Table 3) and from year-class 2015 to 2016 (Table 4) is mainly due to missing production data from year-class 2015 and 2016 from one company. Since AquaCloud started registration of production data 01.01.2017, main part of production data from the 2015 year-class and the first year in sea from the 2016 year-class from one company is missing.

During the study period, the study population represents 214 farms (159 of them unique farms). In the period from 2014–2017 there were 717 active farms along the Norwegian coast (Oliveira et al., 2021), which means the data set represents about 22% of all active farms during that period. The data set might not reflect the actual distribution of treatments over production zones. There could also be differences in mortality between different companies and this may affect the mortality distributions presented here. Since the data from this study are only from large multi-farm companies, the results might not be representable for smaller companies. A recent study show that large (>20 locations) companies on average have a lower production loss (Pincinato et al., 2021). This does not necessarily imply that the described delta mortality in this study will be higher for smaller companies. Notably, in Pincinato et al. (2021), some of the best performing companies and farms were found in the group with the smallest companies.

The most important result from this study is the described wide variability and positive skewness in delta mortality, showing a potential for improvement. Compared to the other delousing methods the overall median delta mortality for thermal and mechanical delousing are the highest. Here, we have discussed if the wide variability in delta mortality within especially thermal treatment and variation between the treatment methods, can be associated with the delousing method itself, the health condition of the fish, environmental factors, or managerial factors. However, to make inference on causality more variables need to be included in the data set, and statistical modelling, such as multilevel regression would be required.

There is a growing concern regarding increasing mortality of farmed Atlantic salmon in Norway. If the numbers in the present study are explained by the delousing method itself and are representative for the entire Norwegian aquaculture salmon population, the shift from medicinal to non-medicinal delousing probably contributes to the overall increase in Norwegian salmon farming. Knowledge of both the effect from treatment measures and their side effects is important when deciding on treatment strategies.

Salmon mortality is a direct negative effect of delousing. Few options for immediate delousing are available to the farmers, and the most commonly used methods cause higher mortality than the ones that previously dominated. Thus, finding the risk factors for mortality after thermal and mechanical treatments should be a top priority, together with alternative solutions of both managing and regulating salmon lice in Norway.

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## CONFLICT OF INTEREST

None of the authors have any conflicts of interest.

## DATA AVAILABILITY STATEMENT

Data are subject to third party restrictions.

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